INTRODUCTION

The important and complex role that pathogens play in the ecology and conservation of wildlife populations has been the subject of several recent reviews (e.g. Daszak et al. 2000; Deem et al. 2001; Cleaveland et al. 2002). Of particular interest are those diseases that pose a threat to the persistence of sensitive, threatened, or endangered species (Thorne and Williams 1988; McCallum and Dobson 1995). Pathogens can affect host populations either directly, through mortality of individuals, or indirectly by a variety of mechanisms such as reducing fecundity or altering predator avoidance behavior. However, while many of the most noteworthy pathogens and parasites of wild animals may lead to death of the individual, host mortality as the end result of infection is probably the exception rather than the rule (Yuill 1987). Basic epidemiological theory suggests that host-pathogen coevolution will lead to moderate levels of pathogen virulence in a manner than maximizes transmission potential. A highly virulent pathogen that infects a host and causes mortality before that host becomes capable of transmission will generally be selected against. Therefore, direct mortality of the host should only occur when host mortality facilitates pathogen transmission or when the pathogen is a generalist, capable of infecting a variety of host species (Yuill 1987; Aguirre and Starkey 1994).

One notable exception to the host-pathogen coevolution model involves introduced pathogens, which often cause high mortality in naïve host populations. Two classic examples of introduced diseases causing high mortality in wildlife populations are rinderpest in African ungulates and avian malaria in Hawaiian land birds. Rinderpest caused the death of millions of domestic and wild ungulates in Africa at the end of the

19th century and is considered to be responsible for altering the distribution of many large herbivores on the African continent (Scott 1981). Introduced avian malaria was likely involved in the decline and extinction of some native Hawaiian avifauna in historic times and continues to negatively impact bird populations in the Hawaiian Islands today (van Riper et al. 1986). Another disease that has been widely introduced and is now of conservation concern for many species of mammals is plague.

The etiologic agent of plague is *Yersinia pestis*, a Gram-negative coccobacillus of the family Enterobacteriaceae that is transmitted by the bite of infected fleas (Siphonaptera) (Perry and Fetherston 1997). Transmission of *Y. pestis* can also, less commonly, be accomplished by direct contact with the blood of infected animals, inhalation of infected respiratory droplets, or through consumption of infected animal tissues. Plague is a zoonotic disease, one that is maintained in wild populations of animals, with humans acting only as incidental hosts (Perry and Fetherston 1997). In nature, *Y. pestis* continuously circulates among hosts and primarily affects wild rodents, although many other groups of wild and commensal mammals have been shown to harbor infection (Barnes 1982).

Y. pestis likely evolved in Asia and has since been successfully introduced onto all continents except Antarctica and Australia. In North America, the presence of *Y. pestis* was first identified ~1900, arriving in Pacific Coast ports via infected rats (*Rattus* sp.) on ships from Asia (Eskey and Haas 1940). By 1908 the disease had spread to native wild rodents outside San Francisco, California (Wherry 1908) and subsequently underwent a rapid range expansion into the interior West, where by ~1950 the range of plague in the United States covered 15 states (Barnes 1993). Since then the range of the

disease in the United States has remained relatively stable, today encompassing most areas west of the 100th meridian (Cully et al. 2000).

In North America, concern over plague has centered around its effects on prairie dogs (*Cynomys* spp.), which are uniformly susceptible to the disease (Cully and Williams 2001). Prairie dogs are burrowing rodents that occupy short- and mixed-grass prairie habitats of the Great Plains and intermountain basins in western North America. Several authors contend that prairie dogs play a keystone role in prairie ecosystem processes by altering grassland habitat structure through their burrowing activity and by providing critical food and shelter resources to a suite of associated species (Kotliar et al. 1999; Miller et al. 2000). Although once abundant, prairie dogs now inhabit only a fraction of their historic range. Of the five species of prairie dogs in North America, two are currently listed as either threatened (the Utah prairie dog, *C. parvidens*) or endangered (the Mexican prairie dog, *C. mexicanus*) under the Endangered Species Act (Miller and Cully 2001). The United States Fish and Wildlife Service [USFWS] recently reviewed a petition to list the black-tailed prairie dog (*C. ludovicianus*) as threatened and found that threatened status was warranted but precluded by higher priority actions (USFWS 2000). In this finding they identified plague as the greatest threat to the long-term persistence of black-tailed prairie dogs.

This study was initiated in response to several years of epizootic plague activity among populations of the black-tailed prairie dog in Phillips County, Montana. Phillips County contains the most extensive populations of prairie dogs in the state and is also a reintroduction site for the endangered black-footed ferret (*Mustela nigripes*), an obligate associate of prairie dogs. Two other species of conservation concern that occur in

association with prairie dogs in Phillips County are the burrowing owl (*Athene cunicularia*) and mountain plover (*Charadrius montanus*). Wildlife managers have few tools with which to manage or mitigate the effects of plague on prairie dogs. The purpose of this study was to gain a better understanding of what species are involved in plague ecology in this region and how infection is maintained between periods of epizootic activity among prairie dogs.

Chapter 1. Comparison of Small Mammal and Flea Communities at Sites With and Without a History of Sylvatic Plague in Phillips County, Montana.

Abstract. I compared small mammal and flea communities between prairie dog colonies with a history of plague, prairie dog colonies with no known history of plague, and "off-colony" sites where plague history was unknown. I also evaluated the effect of host sex, host age, collection year, plague history, and habitat on flea loads of small mammals. Small mammal species composition was essentially identical at colonies with and without a history of plague, but all the same species (except for prairie dogs) and several additional species were captured off colonies in a variety of habitats. Deer mice were least abundant at sites with a history of plague during both years of the study. Only deer mice and prairie dogs were captured in sufficient numbers for analyses of flea loads. Flea loads were significantly higher on prairie dogs from colonies with no history of plague and flea loads on deer mice were higher on prairie dog colonies (regardless of plague history) than at off-colony sites. Site plague history and coarse-scale habitat association appeared to be the most significant factors influencing prevalence of flea loads on deer mice, the most abundant small mammal host in the study area. Patterns of flea abundance and small mammal species composition suggest that areas of diverse habitat associated with the Missouri River corridor may be most suitable for the persistence of plague in southern Phillips County.

Introduction

Yersinia pestis, the etiologic agent of plague, is unique in its ability to infect such a great diversity of host species, essentially any mammal, although rodents are considered to be of primary importance in plague epizootiology. In general, the sylvatic (wild animal) cycle of *Y. pestis* infection is characterized by relatively stable periods of activity where it circulates at low levels within the enzootic or "maintenance" host community, followed by explosive epizootics involving one or more species of epizootic or "amplifying" host that often experience high mortality (Poland and Barnes 1979). Epizootic host mortality effectively "amplifies" the disease by creating a surplus of infected, host-seeking fleas in the environment. In western North America, these epizootic hosts include species of prairie dogs (*Cynomys*), ground squirrels (*Spermophilus*), woodrats (*Neotoma*), and chipmunks (*Tamias*) (Barnes 1993). Plague-

associated die-offs among prairie dogs can be particularly dramatic, with mortality often approaching 100% in colonies of Gunnison's (*C. gunnisoni*) and black-tailed (*C. ludovicianus*) prairie dogs. The high degree of sociality and high population densities seen in prairie dogs leads to frequent contact between individuals, facilitating flea exchange and pathogen transmission (Cully and Williams 2001). This exacerbates the inherently high susceptibility to plague characteristic of these species. Several studies have confirmed the amplifying role played by prairie dogs, as *Y. pestis*-positive fleas and/or sera from sympatric hosts are often only taken during and immediately after an epizootic among prairie dogs (see Barnes 1993; Cully et al. 1997)

Fleas show considerable heterogeneity in the role they play in the maintenance and transmission of the disease. However, while only about 30 species of flea are proven plague vectors, all should be considered biologically able to transmit the bacterium under the right conditions. Following a blood meal from a host of sufficient bacteremia, the bacteria multiply in the stomach of the flea and within a few days may completely block the proventriculus (foregut), inhibiting successful subsequent feeding attempts. As the flea attempts to feed, ingested blood enters the proventriculus but is then regurgitated, along with a quantity of bacteria, as a result of the blockage. In this way a new host may be inoculated with the plague bacterium (Perry and Fetherston 1997). Furthermore, largely because *Y. pestis*-infected fleas have been recovered from rodent burrows many months after an epizootic, several authors (Olsen 1981; Barnes 1982; Cully and Williams 2001) have acknowledged that fleas probably also play an active role in the maintenance of plague, in addition to their role as a vector.

Barnes (1993) and Piesman and Gage (2000) listed several aspects of flea ecology that are important to the maintenance and transmission of *Y. pestis* in a natural setting. Among other factors they cited flea density/abundance and degree of host-specificity as important. The degree of host-specificity exhibited by fleas varies by taxa and circumstance. While some fleas are generalist in their host preferences, most show some proclivity for one species or a group of biologically or ecologically related species of hosts (Thomas 1988; Lewis 1998). For example, it is not uncommon for prairie dogs to carry fleas that are typical parasites of ground squirrels of the genus *Spermophilus*, the closest extant relative of prairie dogs (Pizzimenti 1975) that occupy a similar ecological niche. Flea host-specificity often breaks down in the absence of a living preferred host, facilitating interspecific exchange of fleas which might otherwise not occur.

Because they suffer such high mortality as a result of plague epizootics, prairie dogs serve as a very good "sentinel" for the presence of *Y. pestis*. Land managers in Phillips County have monitored and mapped prairie dog colonies since 1979 such that good data now exist on the location and year of plague epizootics there. The situation in Phillips County provides a unique opportunity to compare attributes of small mammal and flea communities between sites with and without a history of plague, with the goal of identifying any differences that may predispose some areas to plague epizootics. Host associations of fleas in Montana were reviewed by Jellison and Senger (1973), however no quantitative studies of this group have come from Montana and very few specimen records exist from Phillips County. Given that plague has become such an important consideration in prairie dog management and conservation (Cully and Williams 2001) and Phillips County is the state's stronghold for prairie dogs, an understanding of host-

flea ecology here may aid in future management actions that aim to mitigate the negative effects of plague on prairie dogs and associated species.

The specific objectives of this study were to 1) compare small mammal abundance and community composition between sites with a history of plague, sites with no history of plague, and sites where plague history was unknown; 2) describe host-flea relationships in the study area; 3) compare prevalence and intensity of flea burdens on hosts between the three types of sites; and 4) evaluate the relative importance of several intrinsic (host sex, age class) and extrinsic (plague history, year, habitat) factors affecting flea loads. I also review the literature on flea collections from prairie dogs, focusing on interspecific exchange of fleas between associated mammals (potential reservoir hosts for *Y. pestis*) and prairie dogs. Results of serologic tests for antibody against *Y. pestis* among my study populations and PCR analysis of fleas for the presence of *Y. pestis* and *Bartonella* will be reported in Chapter 2.

Study Area

The study took place in southern Phillips County, Montana (Figure 1). Shrub and grassland habitats typical of the northern Great Plains predominated, with major vegetation components being big sagebrush (*Artemisia tridentata*) and greasewood (*Sarcobatus vermiculatus*) in shrub-dominated areas and western wheatgrass (*Agropyron smithii*), blue grama (*Bouteloua gracilis*), needle-and-thread (*Stipa comata*), and green needlegrass (*Stipa viridula*) in the grasslands. Plains prickly pear (*Opuntia polyacantha*) and fringed sagewort (*Artemisia frigida*) were common herbaceous understory plants. 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

Figure 1. The Study Area: southern Phillips County, Montana. Prairie dog colonies in red.

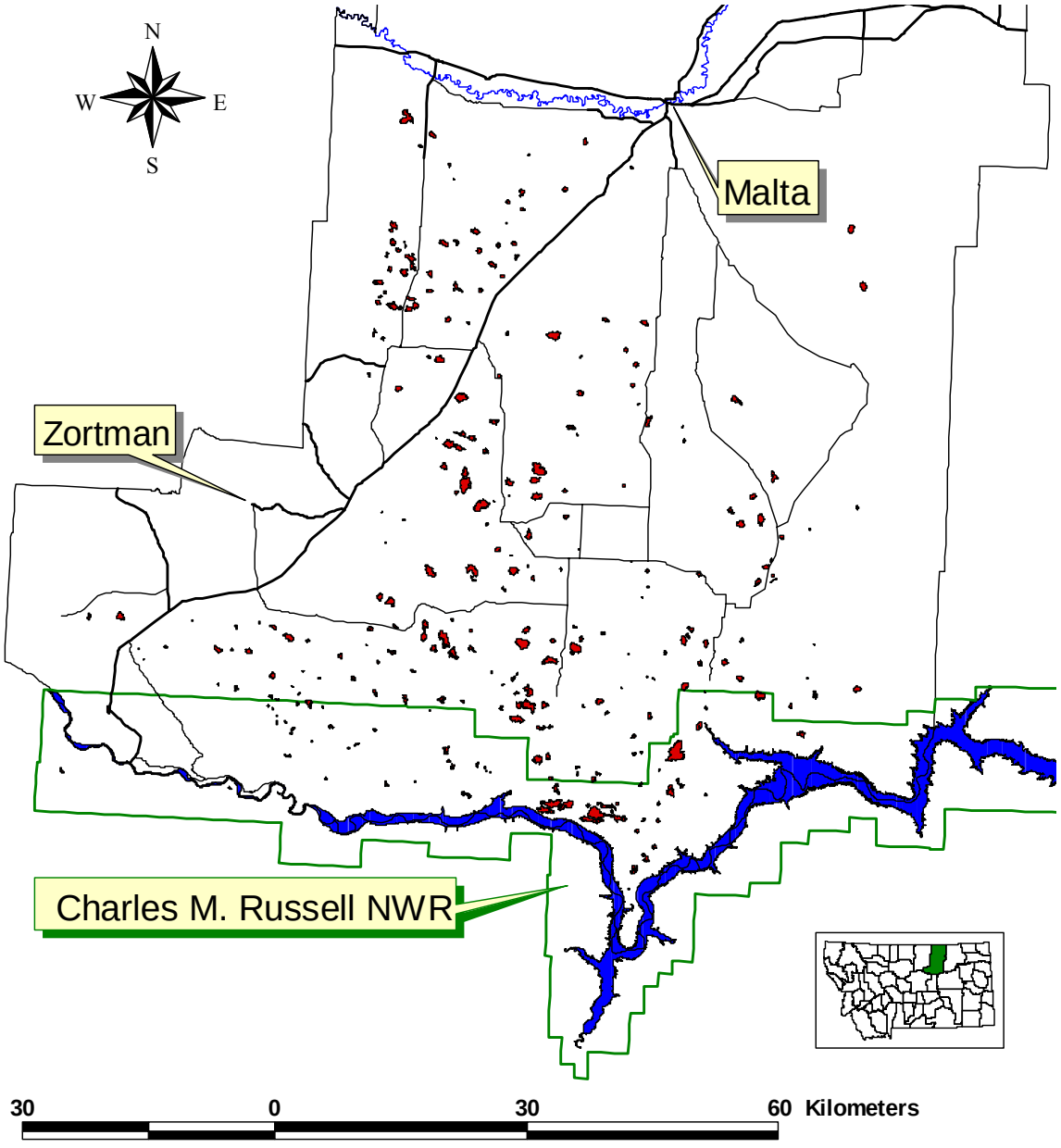
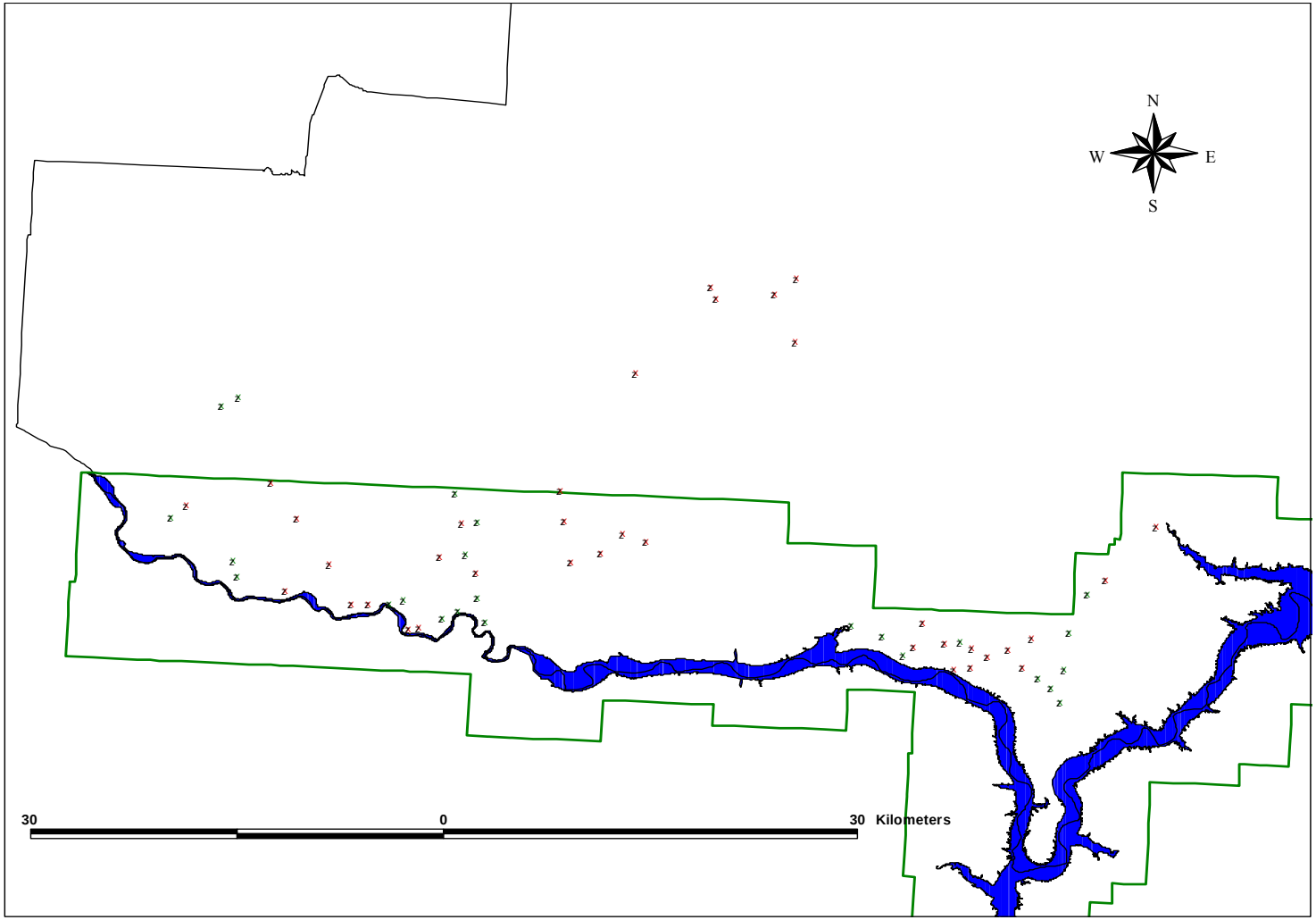


Figure 2. Locations of mammal trapping grids in southern Phillips County, Montana. June – August 2002 & 2003. Points in red were sampled in 2002 and 2003; points in green were sampled in 2003 only.



(*Pseudotsuga menziesii*), and Rocky Mountain juniper (*Juniperus scopulorum*). Stands of plains cottonwood (*Populus deltoides*) and willows (*Salix* spp.) occurred on bottomlands adjacent to the river. Elevations of study sites were between 740 and 1,050m. The area was a mosaic of federal, state, and private land ownership and supported ~300 active black-tailed prairie dog colonies. 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 (Figure 2).

Materials and Methods – Small Mammal Trapping

I identified three types of sites for study during June – August 2002 and 2003: 1) sites with no known history of plague, 2) sites with a history of plague, and 3) sites where plague history was unknown. Sites with no known history of plague were prairie dog colonies that had been continually active since plague epizootics among prairie dogs were first observed in Phillips County in 1992. Sites with a history of plague were also prairie dog colonies and 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. Some colonies with a history of plague had natural recolonization of prairie dogs subsequent to the epizootic, some received translocated prairie dogs in an effort to reestablish colonies by CMR personnel (see Dullum 2001), and some still had no prairie dogs present. Finally, "off-colony" sites had no sentinel species such as the prairie dog to indicate whether plague had been present and thus plague history was essentially unknown. All

off-colony sites were located >500m from the edge of the nearest prairie dog colony and all sites were separated by >500m to insure independence.

While *Y. pestis* has not been isolated from prairie dogs or their fleas in Phillips County, we (biologists working in Phillips County and I) are confident that die-offs attributed to plague were in fact plague epizootics for three reasons. First, all of these colonies were protected from poisoning and all but three were protected from recreational shooting during the time when die-offs occurred. Shooting pressure on the remaining colonies was very low or absent. Second, no other disease has yet been identified that causes such high mortality in prairie dogs (Barnes 1993). Third, antibody to *Y. pestis* has been consistently found in coyotes (*Canis latrans*) and badgers (*Taxidea taxus*) in the study area (R. Matchett, USFWS, unpublished data).

I chose sites non-randomly to satisfy conditions of plague history and to represent several different habitats in the study area. I sampled 36 sites in 2002 and 60 sites (36 resampled from 2002 and 24 new) in 2003. Five sites sampled in 2002 and scheduled to be resampled in 2003 were treated with an insecticide, Delta Dust (.05% Deltamethrin), in June of 2003 (prior to sampling) to reduce flea populations as part of the black-footed ferret reintroduction program on CMR. All five sites were prairie dog colonies with no history of plague and the insecticide was applied into all prairie dog burrows at the treatment colonies at a quantity of 4g/burrow. Flea collections from hosts at these five sites are reported in Table 2 but excluded from all statistical analyses.

Each study site consisted of a 10 x 10 grid of 100 Sherman live-traps (H.B. Sherman, Tallahassee, Florida) with 10m spacing and 20 Tomahawk live-traps (Tomahawk Live Trap Co., Tomahawk, Wisconsin) placed at prairie dog burrows on

colony sites and systematically throughout the grid on off-colony sites. I baited Sherman traps with rolled oats and Tomahawk traps with a mixture of corn, oats, and barley. Traps were open for four consecutive days and nights at each site. I determined species, sex, age (juvenile or adult), weight, and reproductive condition for each captured animal. I marked all animals by clipping a small patch of fur from the back and then released them at the site of capture following flea collection.

I report minimum small mammal population densities as the number of animals per hectare and determined this by dividing the total number of unique individuals at a site by the effective trapping area of the grid. I estimated effective trapping area by adding to the actual area of the grid a boundary strip equal to one half the mean maximum distance moved by a species (Wilson and Anderson 1985). This approach provides only a relative measure of density and figures reported should be interpreted as minimum population densities because capture probabilities were not estimated and are assumed to be <1.00 over the course of four trap-nights. I did not explicitly estimate population size of small mammals at each site because capture rates were low enough at many sites to preclude a formal estimate from being made with traditional mark-recapture techniques. I also did not estimate densities for prairie dogs because Tomahawk traps intended for prairie dogs were placed at locations to maximize trap success (at active burrow entrances) rather than systematically. Trapping and handling protocols adhered to all pertinent guidelines established by the Animal Care and Use Committee (1998) of the American Society of Mammalogists and were approved by the Institutional Animal Care and Use Committee at the University of Montana.

Materials and Methods – Flea Collection and Identification

I collected fleas from hosts by brushing the animal with a commercially available flea comb over a white enamel pan. The entire body was combed as many times as needed until no more fleas were observed. I anesthetized most animals with isoflurane ("IsoFlo" Abbot Laboratories, North Chicago, Illinois or "IsoSol" Halocarbon Laboratories, River Edge, New Jersey) prior to flea collection to facilitate blood sampling as part of the associated serologic survey. Fleas were either refrigerated or frozen in vials containing 2% NaCl solution with a small amount (<0.01%) of Tween 80 until identifications could be made. I also sampled fleas from prairie dog burrows by attaching a square piece of white flannel cloth to the end of a flexible plumber's snake. In this method, the cloth is extended as far into the burrow as possible, left for several seconds, and then retrieved. I placed burrow sampling cloths in plastic bags and froze them overnight to kill captured fleas; then I collected the fleas and placed them in vials as above. I classified burrows as either active or inactive based on the presence/absence of fresh digging and/or fresh droppings. The number of burrows sampled per colony was 100 in 2002 and 50 in 2003, or as many as possible for very small or inactive colonies.

I consulted three different references when making flea identifications (Hubbard 1947; Furman and Catts 1982; Holland 1985) and adopted current taxonomic revisions from Lewis (2000, 2002). I preserved a male and female voucher specimen (when available) of each species by clearing the flea in 10% KOH solution, dehydrating it, and mounting it in Canada balsam.

Materials and Methods – Habitat Sampling

In 2003, I quantified habitat attributes at each trapping grid by estimating percent cover of bare ground, litter, woody debris, lichen, forb, cactus, grass, and shrub at 20 randomly placed 1x1m quadrats. I estimated percent cover for each attribute as <5%, 5-25%, 25-50%, 50-75%, and 75-100%. I then calculated total percent cover for the site by averaging the mid-points of the cover estimate for each quadrat (Daubenmire 1959). At sites with trees, I counted the number of stems of each species within 5m of the center point of the quadrat and converted counts to stems/ha. I also recorded dominant species of shrub and tree at each quadrat.

Data Analysis

I compared small mammal minimum population densities between the three types of sites using a one-way ANOVA with Tukey pair-wise comparisons and between years using *t*-tests. The two basic measures I used to quantify parasite burdens on hosts were prevalence and intensity (Rózsa et al. 2000). Prevalence is simply the proportion of hosts carrying ≥ 1 flea and intensity is the mean or median number of fleas per infested host. I only used data collected from the first capture of an individual animal in analyses. I also tested whether the observed frequency distribution of flea burdens on hosts was significantly different from random by employing an index of dispersion (Wilson et al. 2002): $I_D = (s^2/\text{mean})(n-1)$, where s^2/mean is the variance-to-mean ratio and n is the number of hosts examined. This index is then compared to the Chi-square distribution with (n-1) degrees of freedom.

I used chi-squared tests to compare prevalence of flea parasitism on hosts between sites (i.e. plague, no plague, off-colony) and nonparametric Mann-Whitney and Kruskal-

Wallis tests to compare flea load intensities on hosts between sites. I then incorporated site/plague history into logistic regression models to evaluate the relative importance of several factors as predictors of flea prevalence. All model parameters were categorical and included host sex, host age (juvenile or adult), sampling year, plague history, and region. I then used these same factors to test for differences in flea load intensity using the Mann-Whitney test. The "region" parameter arose from a relationship I observed in the 2002 data that suggested that flea loads may be higher in some parts of the study area than others. Specifically, flea loads appeared to be higher at sites in the western portion of the study area that were in close proximity to the more heterogeneous as well as more mesic habitats of the Missouri Breaks and river corridor. I used the presence of forested cover as an indicator of heterogeneous/mesic habitat (Figure 3) and these forested areas were usually associated with steep coulees interspersed with areas of shrub/grass-dominated habitat and eroded badlands, creating a dynamic habitat mosaic that stood in contrast to the more homogeneous character of the eastern and northern "upland" study sites. The term "breaks" is used to describe the topographic and habitat features described here and will be used from here on in reference to sites associated with the forested/heterogeneous habitats therein. I chose a buffer distance of 500m and identified sites within 500m of forested habitat as "breaks" sites and sites >500m from forested habitat as "upland" sites using remotely sensed land cover data (Fisher et al. 1998) and ArcView GIS v3.2 software.

Another useful metric of flea burden is the total flea index (Gage 1999) which is the mean number of fleas per host examined and combines aspects of both prevalence and intensity. I used simple linear regression to test for a relationship between the total

Figure 3. Location of mammal trapping grids in relation to areas of forested cover (green shading) in southern Phillips County, Montana. Sites colored red are prairie dog colonies with a history of plague, sites colored yellow are colonies with no history of plague, and off-colony sites are colored black.

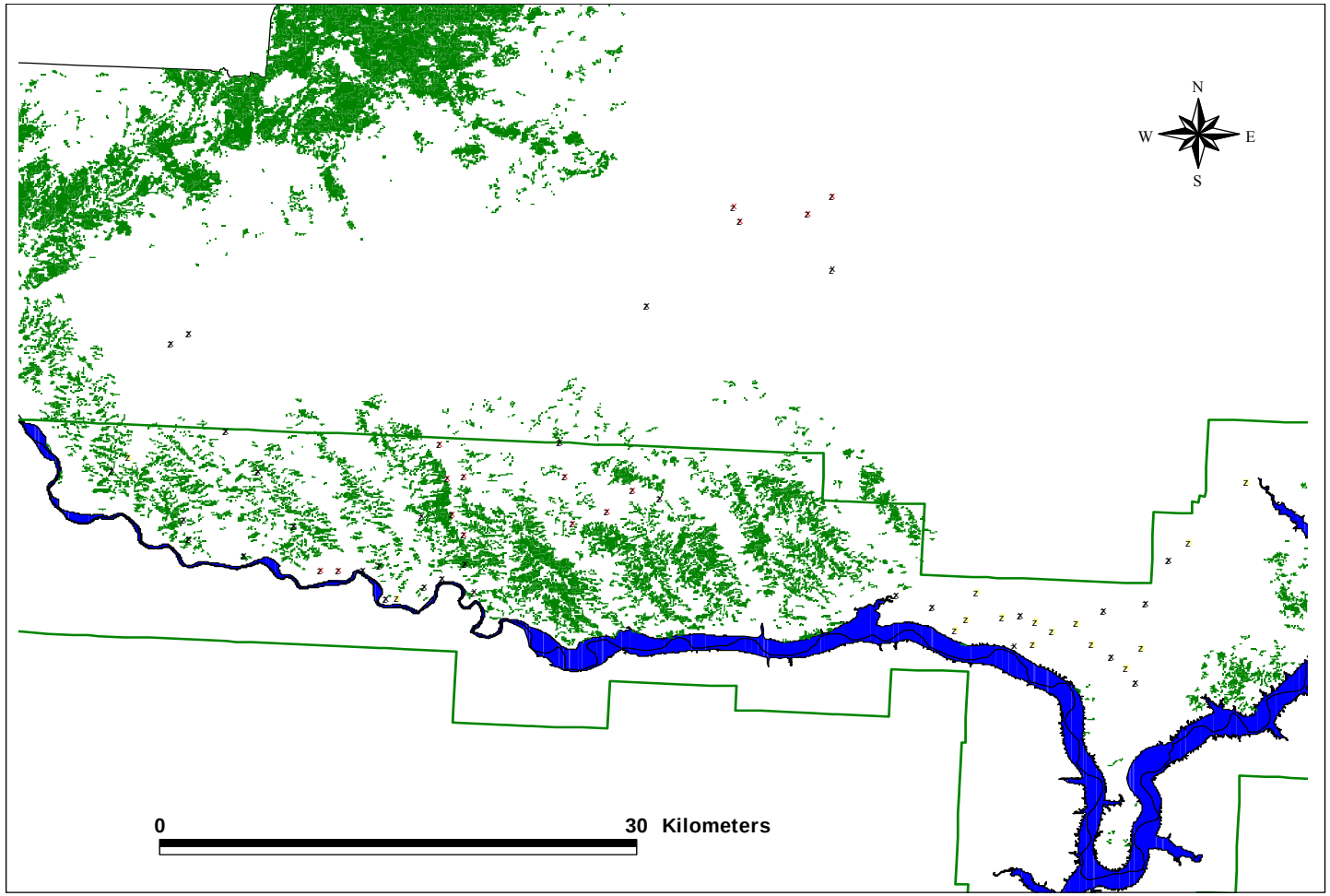


Table 1. Number and species of mammals captured in Phillips County, Montana. June – August 2002 & 2003.

Species	Individuals	Recaptures	Total Captures
Black-tailed Prairie Dog (*Cynomys ludovicianus*)	290	80	370
Striped Skunk (*Mephitis mephitis*)	1	0	1
Prairie Vole (*Microtus ochrogaster*)	15	3	18
Unknown Vole* (*Microtus* sp.)	11	2	13
Bushy-tailed Woodrat (*Neotoma cinerea*)	4	0	4
Northern Grasshopper Mouse (*Onychomys leucogaster*)	26	5	31
Olive-backed Pocket Mouse (*Perognathus fasciatus*)	13	6	19
Deer Mouse (*Peromyscus maniculatus*)	1,057	1,178	2,235
Western Harvest Mouse (*Reithrodontomys megalotis*)	12	2	14
Merriam's Shrew (*Sorex merriami*)	1	0	1
Desert Cottontail (*Sylvilagus audubonii*)	26	7	33
Least Chipmunk (*Tamias minimus*)	4	0	4

*Unconfirmed identification: either *M. ochrogaster* or *M. pennsylvanicus*.

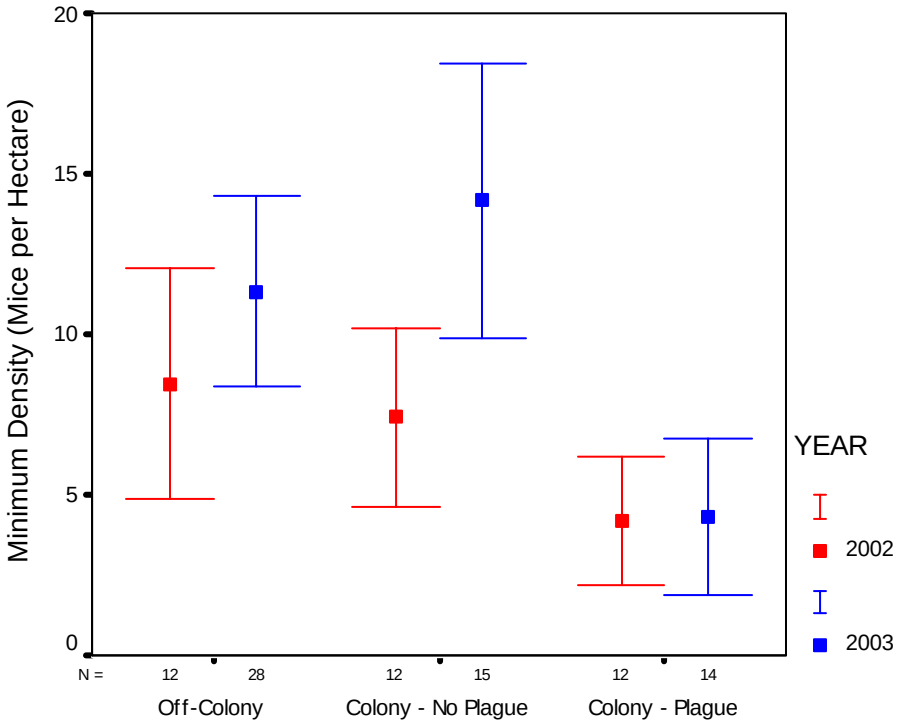
flea index for sympatric populations of prairie dogs and associated small mammals and for a relationship between the total flea index for a host species and minimum population density. I only included sites at which ≥5 hosts were sampled. I performed all statistical analyses using SPSS v11.5 and tests were considered significant at $p<.05$.

Results

Small Mammals

A total of 1,460 individual small mammals representing 11 species were captured throughout the study (Table 1). Deer mice (*Peromyscus maniculatus*) and black-tailed prairie dogs accounted for 92.3% of all captures and were the only two species caught in sufficient numbers for statistical analyses. Deer mice were captured at all sites except one, which was a wet meadow occupied by voles (*Microtus* sp.) and western harvest mice (*Reithrodontomys megalotis*). However, these two species were sympatric with deer mice at other sites. Aside from a prairie vole (*M. ochrogaster*) taken at one prairie dog colony with a history of plague, small mammal species composition at colonies with and without a history of plague were identical. Species found in association with prairie dogs included deer mice, northern grasshopper mice (*Onychomys leucogaster*), desert cottontails (*Sylvilagus audubonii*), and the one prairie vole. Although none were trapped as part of this study, evidence of northern pocket gopher (*Thomomys talpoides*) activity was present at colonies with and without a history of plague, as well as at off-colony sites. The one specimen of Merriam's shrew (*Sorex merriami*) is notable as it is only the tenth specimen known from Montana and the second from Phillips County (Foresman 2001). This specimen was taken in a Sherman trap in habitat with 6-28% cover of big sagebrush, 28-50% grass cover, 1-5% forb cover, and 21-39% bare ground. This is very

Figure 4. Mean minimum density of deer mice (*Peromyscus maniculatus*) by year and site category in Phillips County, Montana. June – August 2002 & 2003. Bars represent 95% confidence intervals.



similar to the sagebrush-grass habitat from which MacCracken et al. (1985) reported taking one *S. merriami* in Carter County, Montana.

Figure 4 shows means and 95% confidence intervals for minimum deer mouse density by site and year. In 2002 minimum densities were highest at off-colony sites ($\bar{x}$ = 8.47 mice/ha) and lowest at plague sites ($\bar{x}$ = 4.20), although the differences between sites were not significant (F=2.93, p=.067). In 2003 minimum densities were significantly higher at off-colony ($\bar{x}$ = 11.34) and no plague ($\bar{x}$ = 14.16) sites than at plague sites ($\bar{x}$ = 4.32; F=7.65, p≤.010). Minimum densities were higher at all three types of sites in 2003 than in 2002 and with all sites combined densities were significantly higher in 2003 ($\bar{x}$ = 10.36) than in 2002 ($\bar{x}$ = 6.69; *t*=2.53, p=.013).

Table 2. Host-flea associations in Phillips County, Montana. June – August 2002 & 2003.

Host-Flea Associations

A total of 3,158 fleas representing 16 species were collected from eight species of host and prairie dog burrows (Table 2). No fleas were collected from olive-backed pocket mice (*Perognathus fasciatus*) or the one Merriam's shrew, and collection was not attempted from the one striped skunk (*Mephitis mephitis*). The most abundant flea collected, *Aetheca wagneri*, also had the widest host distribution, being collected from six host species as well as prairie dog burrows. Likewise, the most abundant host species examined, deer mice, harbored the greatest number of flea species (10). All were typical parasites of deer mice except for one specimen each of *Corrodopsylla curvata*, a flea commonly found on shrews, *Eumolpianus eumolpi*, a chipmunk flea, and *Foxella ignota*, a pocket gopher flea. Northern grasshopper mice also carried *F. ignota*.

Three species of flea were collected from prairie dogs: *Oropsylla hirsuta*, *O. tuberculata*, and *Pulex simulans*. These are common parasites of black-tailed prairie dogs throughout their range and were recovered only from prairie dogs and their burrows and not from any other host. In fact, there was no relationship between flea loads on sympatric populations of deer mice and prairie dogs (r^2=.027, p=.589; Appendix I), and no flea species were common to prairie dogs and any other host species, although some fleas typical of other hosts were recovered from burrows. The number of *O. hirsuta* collected from prairie dogs at colonies with a history of plague was significantly more than expected (χ^2=203.13, p<.001) based on the overall distribution of the three species of flea collected from prairie dogs. The majority of fleas collected from prairie dogs at colonies with a history of plague (63.6%, 96/151) were *O. hirsuta*, whereas *O. hirsuta*

Table 3. Number of hosts examined and total flea burdens on small mammals in Phillips County, Montana. June – August 2002 & 2003.

				Intensity		
Host Species	n	Prevalence(%)	Range	Median	Mean	SD[a]
Cynomys ludovicianus	216	75	0 - 54	6	8.29	8.33
Microtus sp.	21	67	0 - 11	2	3.36	2.98
Neotoma cinerea[b]	4	100	7 - 43	21.5	23.25	18.98
Onychomys leucogaster	24	75	0 - 11	2	3.06	2.73
Perognathus fasciatus	9	0	0	--	--	--
Peromyscus maniculatus	831	54	0 - 25	2	2.63	2.56
Reithrodontomys megalotis	10	30	0 - 2	1	1.33	0.58
Sylvilagus audubonii	21	57	0 - 7	1	2.33	2.27
Tamias minimus	3	33	0 - 1	1	1.00	--

[a]Standard deviation

[b]Two individuals of this species had very high flea burdens (36 and 43 collected) and some fleas escaped during the collection process. Therefore, range and intensity figures presented are *underestimates*.

comprised only 11.9% (123/1,031) of fleas collected from prairie dogs at sites with no history of plague (Appendix II). A summary of burrow sampling effort for each year of the study is given in Appendix III.

Flea Abundance

Prevalence, range, and intensity of flea burdens for hosts are reported in Table 3. Prevalence of flea parasitism ranged from 0% for olive-backed pocket mice to 100% for bushy-tailed woodrats (*Neotoma cinerea*). Woodrats also had the highest mean and median intensity of the hosts examined. Frequency distributions of flea loads are given for prairie dogs and deer mice in Figures 5 and 6, respectively. Both showed a highly aggregated distribution which was significantly different from random ($p<.001$). This type of aggregation is typical of most macroparasite distributions (Wilson et al. 2002) and indicates that a minority of hosts carried the majority of the flea burden.

Figure 5. Frequency distribution of flea loads for black-tailed prairie dogs (*Cynomys ludovicianus*) in Phillips County, Montana. June – August 2002 & 2003.

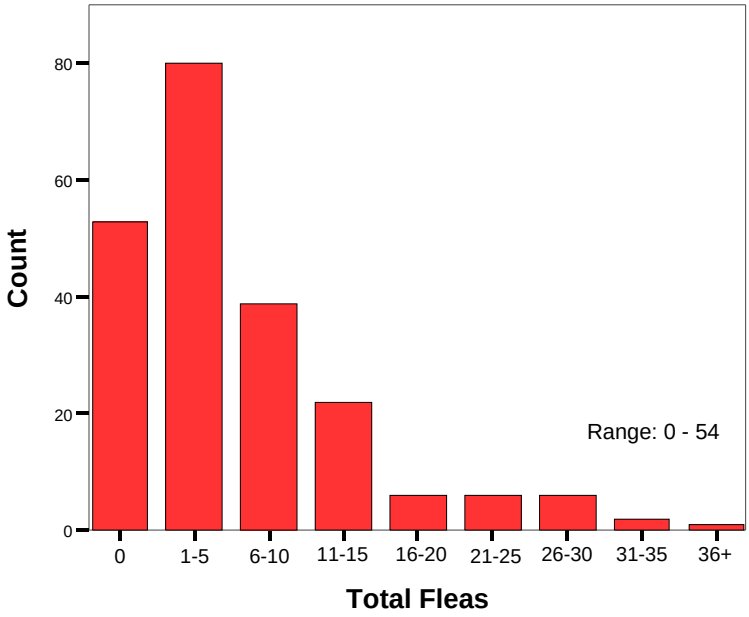
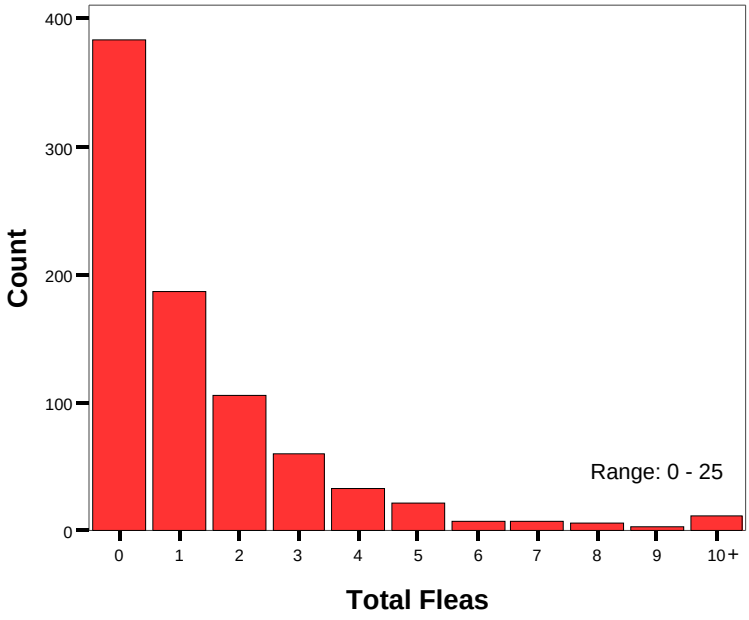


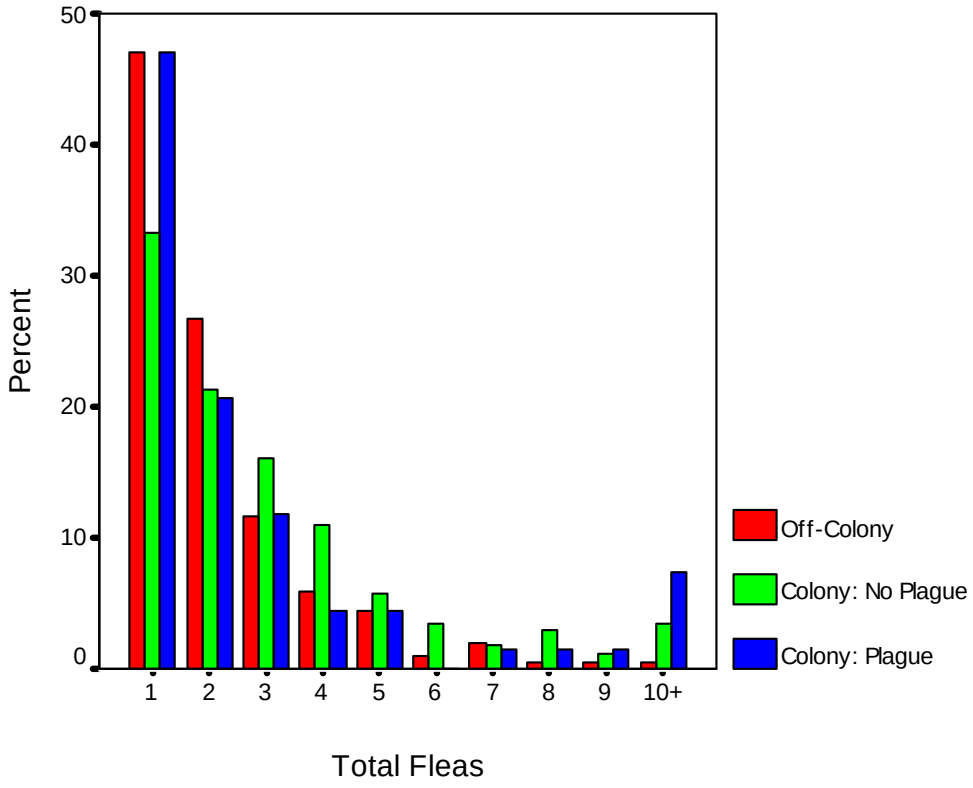
Figure 6. Frequency distribution of flea loads for deer mice (*Peromyscus maniculatus*) in Phillips County, Montana. June – August 2002 & 2003.



For prairie dogs, both prevalence (χ^2=20.96, p<.001) and intensity (*U*=1360, p=.001) of flea loads were significantly higher on colonies with no history of plague than on colonies with a history of plague (Appendix IV). Most prairie dog colonies with a history of plague were in close proximity to the breaks habitats, which resulted in the "plague history" and "region" variables being significantly correlated (Spearman coefficient=.688, p<.001). Because of this correlation, separate logisitic regression models were run incorporating these two parameters to avoid effects of collinearity. In each model (Appendix V) site and region were significant predictors of prevalence (p<.001), as was age class (adults were more likely to carry fleas than juveniles, p≤.003). Intensity of flea burdens were also higher on adult prairie dogs than on juveniles (*U*=2714.5, p=.048).

Prevalence (χ^2=34.87, p<.001) and intensity (*H*=14.63, p=.001) of flea loads were significantly different between the three types of sites for deer mice. Pair-wise comparisons showed that prevalence was significantly higher on prairie dog colonies (regardless of plague history) than at off-colony sites (p≤.003). Intensity was also higher on prairie dog colonies (no plague: $\bar{x}$ =3.00, median = 2; plague: $\bar{x}$ =3.18, median = 2) than at off-colony sites ($\bar{x}$ =2.15, median = 2; Appendix IV). However, the only statistically significant difference was between no plague and off-colony sites (*U*=14002, p<.001). As with prairie dogs, two logistic models were considered (Appendix V) and again site and region were significant predictors of prevalence (p≤.002). The only other significant parameter was host sex (p=.036) in the first model. In addition to differences in flea intensity on deer mice by site plague history, deer mice from the breaks habitats carried higher intensity of flea loads (*U*=22209, p=.028) than deer mice from upland

Figure 7. Percent frequency distribution of flea loads on deer mice (*Peromyscus maniculatus*) carrying at least one flea upon first capture in Phillips County, Montana. June – August 2002 & 2003. Each flea load category is separated by site of capture and bars represent the percent of individuals from each category carrying that number of fleas.



sites. There were also differences due to sex and age ($p \leq .041$), with adult males carrying the highest average flea burden. There was no relationship between flea loads on deer mice and deer mouse minimum population density ($r^2=.017$, $p=.319$; Appendix I).

Qualitatively, the overall distributions of flea load intensity on deer mice from the three different types of sites show some interesting patterns (Figure 7). Mice sampled from off-colony sites tended to have most individuals in the lowest flea load categories and very few in the highest categories, while those sampled from prairie dog colonies had a stronger representation in the tail of the distribution. Although the sample size of

individuals from the highest categories was too small to test for significance, the difference between sites in the highest (10+) category is striking. The percent of animals carrying 10+ fleas was more than twice as high at prairie dog colonies with a history of plague (7.4%, 5/68) than at colonies with no plague history (3.4%, 6/174) and only one of 206 (0.5%) deer mice carrying at least one flea upon first capture at off-colony sites harbored ≥10 fleas.

Discussion

Deer mice are often the most abundant small mammal found in grassland and shrub-steppe habitats of the northern Great Plains (Hingtgen and Clark 1984; Agnew et al. 1986; Rauscher and Kissell 1996; Anderson and Williams 1997). Likewise, deer mice were the most abundant and, at some sites, the only small mammal present in this study. Relative abundance of small mammals associated with prairie dog colonies and nearby off-colony sites has been examined (O'Meilia et al. 1982; Agnew et al. 1986) but only one study (Anderson and Williams 1997) has compared small mammal abundance between prairie dog colonies with and without plague. Anderson and Williams (1997) found no difference in deer mouse populations between plague-positive and plague-free sites. I found no difference in minimum deer mouse density between colonies with and without a history plague in 2002 but found significantly higher minimum deer mouse densities on colonies with no history of plague in 2003 (Figure 4). Anderson and Williams (1997) based their comparisons on whether plague was active at the time of trapping whereas I based comparisons on historical occurrence of plague among prairie dogs. In both years of my study minimum deer mouse densities on colonies with a history of plague were lowest of the three types of sites examined. Habitat sampling

indicated no significant differences in ground cover between colonies with and without a history of plague that may have influenced these numbers. While small mammal populations can fluctuate widely, these estimates are three-month averages over a period of two summers – the time of year when most plague epizootics occur in this region. If these data are representative of long-term trends in small mammal abundance associated with the different types of sites, then factors other than density of associated mammalian hosts may be of greater importance in affecting the course of plague epizootics among prairie dogs.

It is interesting to note the complete separation of flea species taken from black-tailed prairie dogs and associated mammals in this study. Of 252 prairie dogs examined, none carried fleas common to any other host, and of nearly 500 associated small mammals examined, none carried a flea species recovered from prairie dogs. This is remarkable, as fleas common to other hosts were taken from prairie dog burrows, densities of associated small mammals were often high on prairie dog colonies (up to 30 individuals/ha), and nearly every small mammal taken on colony sites immediately retreated to a prairie dog burrow upon release (B. Holmes, personal observation). The use of prairie dog burrows for escape cover during daily activity, or even as permanent residence once abandoned, is likely common practice for species such as deer mice and grasshopper mice that regularly occur in sympatry with prairie dogs, providing an ideal environment for interspecific exchange of fleas and the potential for vector-borne pathogen transmission. A literature review of flea collections from prairie dogs of all species (Table 4) indicates that exchange of fleas between associated mammals and prairie dogs is indeed relatively rare. Excluded from this review of atypical or accidental

Table 4. Literature review of flea collections from prairie dogs (*Cynomys* spp.), highlighting the occurrence of fleas which are not typical parasites of the ground dwelling sciurids *Cynomys* and *Spermophilus*. Also highlighted are fleas that are generalist in their host preferences, sometimes found on prairie dogs, but not obligate parasites of ground-dwelling sciurid rodents.

flea occurrences on prairie dogs are records of fleas which are typically found on ground squirrels of the genus *Spermophilus*. As mentioned above, because prairie dogs and ground squirrels are ecologically and phylogenetically closely related, exchange of fleas between these animals is not remarkable. Furthermore, with respect to plague transmission, most ground squirrels (like prairie dogs) show high mortality when exposed to *Y. pestis* and are generally considered to be epizootic hosts, facilitating the spread of the plague organism during epizootics but unimportant in the maintenance of the disease during interepizootic periods (Poland and Barnes 1979). Like this study, Lechleitner et al. (1968) found no fleas common to prairie dogs and any other host species and suggested that flea-borne transmission of *Y. pestis* infection from a competent reservoir host into a population of prairie dogs is an improbable event. Data from this study and from a review of published flea records from prairie dogs support this supposition.

Pulex simulans is generally considered to be a poor plague vector (Hopla 1980) while fleas of the genus *Oropsylla*, of which *O. hirsuta* and *O. tuberculata* were collected from prairie dogs in this study, are considered to be relatively important plague vectors (Barnes 1993). The relative abundance of *O. hirsuta* was much higher at prairie dog colonies with a history of plague than at colonies with no history of plague in this study. However, it is unclear whether higher relative abundance of *O. hirsuta* may have influenced the course of past plague epizootics or if it may be an effect of past plague epizootics. For instance, the observed difference may represent an "equilibrium" situation where microclimatic conditions naturally favor higher relative abundance of *O. hirsuta* at these sites. Because *O. hirsuta* is a capable vector, a higher relative abundance of this species on prairie dogs may facilitate intraspecific transmission of *Y. pestis* once

the pathogen enters a prairie dog colony. On the other hand, the observed distribution may be the result of past plague epizootics which have somehow selected against *P. simulans*, resulting in the higher relative abundance of *O. hirsuta* currently observed. I am unable to distinguish between these two scenarios. However, this is a pattern worth further exploration and illustrates the importance of considering flea species composition in addition to abundance.

Prairie dogs sampled from colonies with a history of plague had significantly lower prevalence and intensity of flea burdens than prairie dogs sampled from colonies with no plague history. This difference may be an artifact of management activities and explained by the fact that many of these plague sites had prairie dogs present as a result of translocation by CMR personnel rather than by natural recolonization, although some degree of immigration may have occurred subsequent to releases. Processing of prairie dogs for release in Phillips County includes treatment with insecticide to kill fleas (R. Matchett, USFWS, personal communication), so with limited or no natural immigration of prairie dogs from discrete source populations, reestablishment of pre-epizootic flea levels may be slow to occur. However, prairie dog releases occurred in 1997 – 1999 on CMR and I sampled these populations in 2002 and 2003. Karhu and Anderson (2000) found that flea populations had returned to pre-treatment levels within one year after treatment of wild prairie dog colonies with pyriproxyfen, an insecticide. If flea burdens observed on prairie dogs from colonies with a history of plague do represent fully reestablished, "climax" flea populations, then this would suggest that different local-scale factors are influencing the flea burdens observed on prairie dogs and deer mice, as there

was no relationship between flea loads on sympatric populations of these two species (Appendix I).

That deer mice sampled from prairie dog colonies (regardless of plague history) had higher prevalence and intensity of flea burdens than those sampled off colonies is also difficult to explain. I have already shown that mice were not acquiring prairie dog fleas, even though these fleas were relatively abundant on prairie dogs and in burrows at colony sites. In addition, flea load differences between sites were not confounded by host density, as there was no relationship between minimum deer mouse density and total flea index. One possibility is that mice occupying prairie dog colonies may be using abandoned prairie dog burrows for permanent residence. The microclimate in these burrows, which is characterized by high relative humidity and low temperature fluctuations (Hoogland 1995), probably provides an environment more favorable for the development and survival of fleas than would occur on the surface or in shallower burrows typically excavated by deer mice (Reynolds and Wakkinen 1987). While there were some differences in prevalence and intensity of flea loads between the sexes and between age classes for deer mice, there was no significant difference in age-sex structure of populations sampled at the three types of sites which might confound these results. Also, because both sexes and age classes should be represented in any persistent population of mammalian hosts, differences in flea loads due to sex or age class should not be of functional importance in predisposing some areas to plague epizootics.

In addition to the presence of a preferred host, the distribution and abundance of ectoparasites may be influenced by both abiotic (e.g. temperature, humidity) and biotic (e.g. habitat) factors of the host's environment. Seasonal effects on the abundance of

fleas have been well-described (Schwan 1986; Lang 1996) and are likely driven by changes in temperature and precipitation/humidity. The importance of habitat associations in determining flea species composition and abundance has been shown in several studies (Benton and Miller 1970; Berseth and Zubac 1987; Krasnov et al. 1997). Lindsay et al. (1999) also showed a significant difference between habitat types in the abundance of immature *Ixodes scapularis* ticks. I evaluated the effects of coarse-scale habitat association (the "region" variable) on flea loads for prairie dogs and deer mice. Because flea loads on prairie dogs were likely confounded by management actions, as described above, I focus here on deer mice. Both prevalence and intensity of flea loads were significantly higher on deer mice sampled from breaks sites than from the more homogenous upland sites. These differences associated with habitat are probably proximate effects, with precipitation, humidity, and/or soil characteristics promoting flea development and survival in these areas. No matter what the ultimate factors that cause these differences, that deer mice occupying these more heterogeneous/mesic habitats carried greater flea loads has important implications for the maintenance of a vector-borne disease such as sylvatic plague.

Maintenance of *Y. pestis* infection is thought to be dependent upon continued circulation among competent hosts (Barnes 1993; Perry and Fetherston 1997). Because transmission dynamics are driven by the frequency of infective flea bites, the hosts most important in transmission are expected to be those supporting the greatest number of vectors (Perkins et al. 2003). Conditions that favor higher flea loads on an abundant and widespread species that is considered to be a competent host of plague, such as the deer mouse, therefore become mechanistically significant. Moreover, in addition to

supporting deer mouse populations with higher flea loads, the breaks habitats supported populations of all the same potential host species found elsewhere in the study, but with two additional species: bushy-tailed woodrats and least chipmunks (*Tamias minimus*). Woodrats and chipmunks (and their associated flea fauna) are known to be important in the ecology of sylvatic plague in many areas throughout the western United States (Barnes 1982; Gage et al. 1995). In most plague foci these species have traditionally been considered to be epizootic hosts, although Davis et al. (2002) regularly found seropositive Merriam's chipmunks (*T. merriami*) and dusky-footed woodrats (*N. fuscipes*) throughout 17 years of testing in California.

The concept of landscape epidemiology was developed by Pavlovsky (1966) and embodies the idea that complex interactions between wildlife (host), vector, habitat, and climate result in spatially structured systems where disease agents are more likely to persist in some areas than others. These natural foci (or nidi) are areas in which pathogen transmission and/or maintenance is favored by the right combination of the above-mentioned factors. Several authors (Olsen 1981; Gage et al. 1995; Biggins and Kosoy 2001) have proposed that the factors most likely to support permanent plague foci involve several host species co-occurring in areas of diverse or patchy habitats. To date, this idea has prevailed over the concept of monohostality for maintenance of sylvatic plague in North America. The breaks habitats associated with the Missouri River in Phillips County may, then, provide the right set of conditions for the enzootic maintenance of plague and serve as a source area for epizootic plague to radiate out from. Factors implicating these areas are: 1) extensive plague epizootics among prairie dogs on CMR have occurred among colonies in close proximity to the breaks while those colonies

removed from these diverse habitats (in the UL Bend area) have not experienced epizootics, 2) the breaks habitats are more diverse and patchy than adjacent "upland" areas, supporting populations of woodrats and chipmunks, species of known epizootiological importance in other plague foci, and 3) vector populations on the most common small mammal host, the deer mouse, are higher in the breaks habitats than in other areas.

Interpretation of these results should be done with caution. Data presented here are strictly correlational and two years of serologic and PCR surveillance for evidence of *Y. pestis* infection among these same small mammals has resulted in no positives (see Chapter 2). There are prairie dog colonies within the breaks which have not experienced plague epizootics while other colonies in Phillips County, far removed from the breaks habitats, have. I do not intend to suggest that enzootic plague cannot be maintained in structurally homogenous environments or in areas where mammalian diversity is low, only that more complex habitats may provide better conditions for maintaining a pathogen with such a wide host range. Evidence of persistent, circulating *Y. pestis* infection among a population of hosts (one species or several) is needed in order to implicate an area in the long-term maintenance of sylvatic plague.

Chapter 2. Survey for Evidence of *Yersinia pestis* and *Bartonella* Infection Among Small Mammals and Their Fleas in Phillips County, Montana.

Abstract. The means by which sylvatic plague is maintained between epizootics involving highly susceptible species such as prairie dogs and ground squirrels are poorly understood. The most extensive populations of black-tailed prairie dogs in Montana occur in Phillips County where plague epizootics have significantly reduced populations at some colonies while other colonies have apparently not been affected. During June – August 2002 and 2003, I collected blood samples and fleas from small mammals at prairie dog colonies with a history of plague, prairie dog colonies with no history of plague, and "off-colony" sites where plague history was unknown. Blood samples were screened for antibody against *Yersinia pestis*, the etiologic agent of plague, and fleas were screened for the presence of *Y. pestis* and *Bartonella* spp. with PCR. No blood samples or fleas were positive for *Y. pestis* but a small number of fleas (1.4% of flea pools tested) taken from deer mice, coyotes, and a black-tailed prairie dog were *Bartonella*-positive. It appears that *Y. pestis* infection is rare or absent in the small mammal populations sampled and that infection does not persist in prairie dog colonies that have previously been affected by epizootics. To date there is no evidence that *Bartonella* spp. are pathogenic to wildlife. However, *Bartonella* infection appears to be established in several prairie dog colonies where there is currently a population of endangered black-footed ferrets. Further investigation into the possible affects of *Bartonella* on ferrets and their main prey base, prairie dogs, should be considered.

Introduction

The maintenance of plague (*Yersinia pestis*) infection in the wild depends on a complex set of interactions between host, vector, pathogen, and environmental factors. How these factors interact to support permanent or semi-permanent foci of plague in nature is poorly understood. The sylvatic (wild animal) cycle of *Y. pestis* infection is characterized by relatively stable periods of enzootic activity where it 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. Conditions favoring the persistence of plague in a landscape nearly always involve

several potential mammalian hosts of varying susceptibility and their associated fleas (Gage et al. 1995).

Over 200 species of mammals worldwide, and at least 76 in the United States, are known to become infected with *Y. pestis* in the wild, including all four species of prairie dogs (*Cynomys* spp.) in the United States (Barnes 1993; Cully 1993). To date, there is no evidence that plague has affected populations of the Mexican prairie dog (*C. mexicanus*, Treviño-Villarreal et al. 1998). Individual species vary greatly in their susceptibility to the disease, and a number of challenge studies have been undertaken, usually involving rodents, to determine levels of susceptibility and, accordingly, what role different host species may play in the epidemiological cycle of plague in nature (e.g. Holdenried and Quan 1956; Quan et al. 1985; Thomas et al. 1988). In general, species range from the highly resistant kangaroo rats (*Dipodomys* spp.) to those that show a relatively moderate and/or heterogeneous response to infection such as deer mice (*Peromyscus maniculatus*) and voles (*Microtus* spp.) to those that exhibit a uniformly high mortality, including prairie dogs and ground squirrels (*Spermophilus* spp.) (Holdenried and Quan 1956; Gage et al. 1995).

Persistence of the plague bacterium within a landscape may take several forms. One potential scenario is that certain areas may accommodate the right combination of host, vector, and environmental interactions which support persistent infection once the organism is initially introduced. Once host and/or vector populations and environmental conditions reach a critical threshold the cycle may shift to epizootic mode, thereby involving more susceptible species, seeding new areas as permanent foci, or eventually running its course and receding to the original focal area. Another possibility is that

plague is ubiquitous across the landscape, generally present at low levels, whereby observed epizootics among "sentinel" species (those such as prairie dogs or ground squirrels that are diurnal and readily apparent to humans) are improbable events which belie constant plague activity in an area. Meyer and Eddie (1938:334), working in California, suggested this type of interaction, stating that "sylvatic plague persists probably indefinitely in an area once invaded." They took this position after isolating *Y. pestis* from fleas collected from "the same colony or series of burrows" where they had found plague-positive ground squirrels 20 years prior. Prairie dogs often recolonize "plagued-out" colonies within one or two years following epizootics (Menkens and Anderson 1991; Anderson and Williams 1997; 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 a plague epizootic again the year after recolonization. Whether these cases in which the same colonies experience plague again after one or several years represent a continued presence of infection among hosts in that area or a reintroduction of *Y. pestis* from surrounding areas is not known.

The genus *Bartonella* is a group of Gram-negative bacteria that are intracellular blood parasites. Some of these are known human pathogens: *B. quintana* is transmitted by the human body louse (*Pediculus humanus*) and causes trench fever; *B. henselae* is transmitted by the cat flea (*Ctenocephalides felis*) and causes cat scratch disease; and *B. bacilliformis*, the etiologic agent of verruga peruana and Oroyo fever in South America, is transmitted by sand flies of the genus *Lutzomyia* (Schwartzman 1996; Piesman and Gage 2000). Additional *Bartonella* strains, many of which have not yet received specific

designation, have been isolated from wild animals and/or their ectoparasites (Breitschwerdt and Kordick 2000). However, unlike *Y. pestis*, little is known about the potential role of *Bartonella* spp. as pathogens of wildlife. Collinge et al. (2001) have proposed to use data on *Baronella* occurrence as a model for plague transmission dynamics within and among prairie dog colonies. *Bartonella* occurs naturally in prairie dogs and has also been isolated from prairie dog fleas, suggesting flea-borne transmission in this system (Stevenson et al. 2003). Because *Bartonella* often occurs at higher prevalence than *Y. pestis* among prairie dogs and associated mammals (M. Kosoy, CDC, unpublished data), this organism may prove to be useful in describing general patterns of vector-borne disease transmission in this system in the absence of plague.

This survey was initiated in response to several years of epizootic plague activity among black-tailed prairie dogs (*C. ludovicianus*) in Phillips County, Montana. Plague epizootics among prairie dogs were first detected in Phillips County in 1992 and have resulted in significant decreases in population size of prairie dogs in some areas while other areas have apparently been unaffected (R. Matchett, USFWS, unpublished data). The purpose of this study was to describe the prevalence of *Y. pestis* infection among small mammals and their fleas and to address several aspects of the ecology and maintenance of sylvatic plague in southern Phillips County. First, I wanted to test whether there was a difference in *Y. pestis* antibody prevalence between sites with a history of plague, sites with no history of plague, and sites where plague history was unknown. This would address the question of whether, once present, *Y. pestis* infection remains at low, enzootic levels in an area. Second, because plague epizootics at my study sites occurred from 1992 to 2001 (one to 11 years prior to sampling), I had the

opportunity to test whether sites with a more recent history of plague were more likely to still have detectable levels of *Y. pestis* infection compared to sites where epizootics had occurred long ago. Related to this, I was able to test whether there was some threshold period of time that *Y. pestis* infection is likely to persist in an area following an epizootic among prairie dogs. Lastly, I describe the prevalence of *Bartonella* infection in the fleas of mammalian hosts from southern Phillips County. This is the first report of *Bartonella* infection in wildlife populations in Montana.

Materials and Methods – Sample Collection

Descriptions of the study area, small mammal trapping, and flea collection methods are given in detail in Chapter 1. I collected blood and flea samples from small mammals at 60 sites during June – August 2002 and 2003. Fifteen sites were prairie dog colonies with no history of plague, 15 were prairie dog colonies with a history of plague occurring from 1992 to 2001, and 30 were "off-colony" sites (≥500m from the edge of the nearest prairie dog colony). I sampled 36 sites in 2002 (12 no plague, 12 plague, 12 off-colony) and all 60 sites (36 from 2002 resampled) in 2003 (Appendix VI). I anesthetized individual animals with isoflurane ("IsoFlo" Abbot Laboratories, North Chicago, Illinois or "IsoSol" Halocarbon Laboratories, River Edge, New Jersey) prior to blood and flea sampling. The anesthetic comes in liquid form and I placed several drops onto cotton inside a film canister with holes in the lid. I then placed the film canister into either a plastic bag or a homemade PVC induction chamber where the animal received the anesthesia. I collected blood samples of ~200 µl from the retro-orbital sinus of smaller animals (mice, voles, woodrats) using micro-hematocrit tubes (Chase Scientific, Rockwood, Tennessee) as described by Stone (1953). This method of blood collection is

fast and Douglass et al. (2000) found no difference in handling mortality for bled and un-bled animals using this technique. For larger animals (prairie dogs, cottontails), I collected ≥200 µl of blood by clipping a hindfoot toenail to induce bleeding.

In 2002, I immediately placed whole blood samples on ice and then stored them in a conventional (-20°C) freezer upon return from the field. I transported samples to the University of Montana, Missoula, MT on dry ice at the end of the field season and stored them at -70ºC until diagnostics were done. In 2003, I centrifuged most blood samples the day of collection to separate off serum which was then stored as above. I collected the remainder of samples onto individual Nobuto filter papers (Advantec MFS, Pleasanton, California) which were air-dried, placed in paper envelopes, and stored at room temperature (Wolff and Hudson 1974).

Materials and Methods – Sample Diagnostics

All laboratory diagnostics were performed at the Centers for Disease Control and Prevention, Division of Vector-Borne Infectious Diseases, Plague Section, Fort Collins, Colorado. Serologic analyses generally followed protocols described by Chu (2000). I screened serum samples for the presence of antibody against *Y. pestis*-specific Fraction 1 (F1) antigen using either competitive enzyme-linked immunosorbent assay (cELISA) or passive hemagglutination assay (PHA). I screened all Nobuto strips using PHA. I did not perform serologic analyses for *Bartonella* because prior research has shown that serology performs poorly in detecting *Bartonella* infection (Kosoy et al. 1997). Also, due to time constraints, I did not attempt to culture *Bartonella*.

Following identification, I placed fleas into pools of one to 10 individuals corresponding to the same species, host, date, and site of capture. I used a multiplex

Table 1. Number and source of whole blood and serum samples collected in southern Phillips County, Montana. June – August 2002 & 2003.

Source	# Blood/Serum Samples
Cynomys ludovicianus	107
Microtus sp.	21
Neotoma cinerea	4
Onychomys leucogaster	24
Peromyscus maniculatus	787
Reithrodontomys megalotis	4
Sylvilagus audubonii	14
Tamias minimus	3
Total	964

polymerase chain reaction (PCR) assay to screen flea pools for the presence of *Y. pestis* and *Bartonella* as described by Stevenson et al. (2003). This assay targets specific DNA sequences that code for portions of the *pla* and *gltA* genes of *Y. pestis* and *Bartonella*, respectively. A positive result for a pool means that one to all of the fleas in that pool contained an infected blood meal. I did not record the presence/absence of a blood meal in the gut of fleas before testing, so some unfed fleas undoubtedly were screened for these agents.

Results

The source and number of samples collected for serologic analysis is given in Table 1. A total of 964 whole blood or serum samples were collected from eight different host species during June – August 2002 and 2003. None of these samples were positive for antibody against *Y. pestis* by cELISA or PHA.

Figure 1. Location of *Bartonella*-positive flea samples collected in southern Phillips County, Montana. June – August 2002 & 2003. Points in red represent mammal trapping grids where positive samples were collected. Points in green are grids where no positive samples were collected.

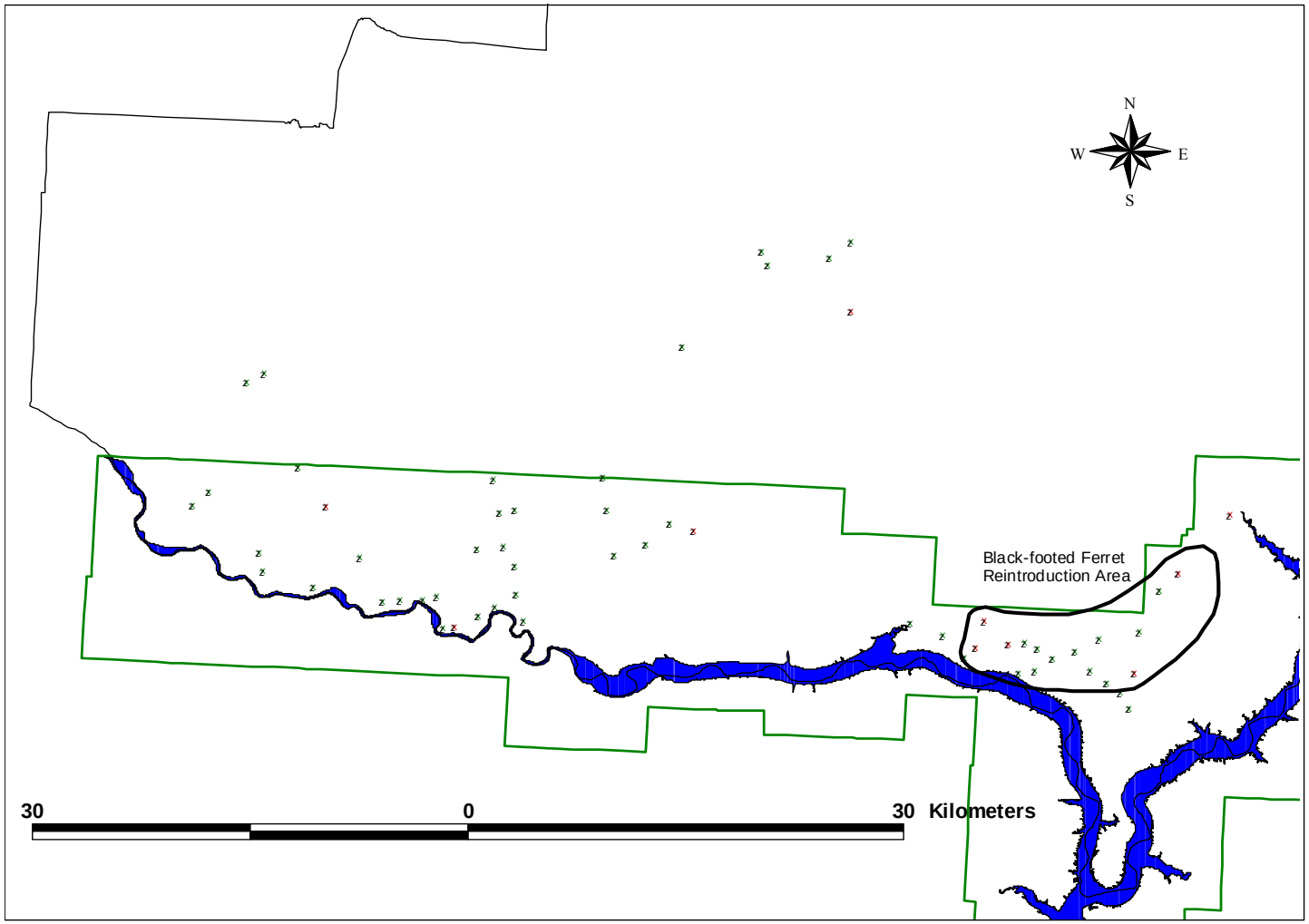


Table 2. *Bartonella*-positive flea pools taken from mammals in southern Phillips County, Montana. June – August 2002 & 2003.

Year	Date	Host	# Fleas	Species	Site
2002	10 July	*Peromyscus maniculatus*	1	*Aetheca wagneri*	Sagebrush
	10 July	*Peromyscus maniculatus*	1	*Aetheca wagneri*	Sagebrush
	11 July	*Peromyscus maniculatus*	3	*Aetheca wagneri*	North Hawley
	22 July	*Peromyscus maniculatus*	1	*Aetheca wagneri*	Long-X
	22 July	*Cynomys ludovicianus*	1	*Oropsylla tuberculata*	Valentine
	15 August	*Peromyscus maniculatus*	1	*Aetheca wagneri*	Dry Fork Creek
2003	3 June	*Peromyscus maniculatus*	4	*Aetheca wagneri*	Rock Creek
	5 July	*Peromyscus maniculatus*	1	*Aetheca wagneri*	Camp Knowles
	16 July	*Peromyscus maniculatus*	1	*Aetheca wagneri*	Bell Ridge #5
	18 July	*Peromyscus maniculatus*	5	*Aetheca wagneri*	East Legg
	27 August	*Canis latrans*	5	*Pulex simulans*	Wilderness
	27 August	*Canis latrans*	4	*Pulex simulans*[a]	Sagebrush
	27 August	*Canis latrans*	4	*Pulex simulans*[a]	Sagebrush
	27 August	*Canis latrans*	6	*Pulex simulans*[b]	North Hawley
	27 August	*Canis latrans*	5	*Pulex simulans*[b]	North Hawley

[a,b]Pools with the same superscript indicate samples came from the same animal.

In addition to fleas collected from small mammals as a part of this study, four fleas from two black-footed ferrets and 24 fleas from three coyotes (*Canis latrans*) were submitted by CMR biologist R. Matchett for testing. A total of 451 pools representing 1,435 fleas collected in 2002 and 643 pools representing 1,598 fleas collected in 2003 were screened for the presence of *Y. pestis* and *Bartonella*. Of 1,094 flea pools tested, none were positive for *Y. pestis* and 15 (1.4%) were positive for *Bartonella* (Figure 1). *Bartonella*-positive fleas were present in both years of the study and were collected from deer mice, coyotes, and one black-tailed prairie dog (Table 2). Broken down by species, 1.3% (9/676) of flea pools from deer mice, 0.3% (1/259) of pools from prairie dogs, and 100% (5/5, representing three individuals) of flea pools taken from coyotes were positive for *Bartonella*.

Discussion

Plague epizootics among prairie dogs are well documented (Lechleitner et al. 1968; Fitzgerald 1970; Rayor 1985; Ubico et al. 1988; Menkens and Anderson 1991; Cully et al. 1997). Once the plague organism is introduced into a prairie dog colony, intraspecific contact between individuals probably becomes the most important means of transmission. However, the means by which infection is maintained between epizootics and introduced into prairie dog populations are still poorly understood. I found no evidence of *Y. pestis* infection in small mammals or their fleas during two field seasons of surveillance. This result suggests one of three possible scenarios. First, active *Y. pestis* infection may have been completely absent from the study area during the two summers of fieldwork and no small mammals sampled as part of this effort had ever been exposed and, thus, were seronegative to *Y. pestis*. A second possibility is that there was some low level of enzootic plague activity but I was unable to detect it because my sample size was too small, animals did not produce antibodies, or mortality was high among exposed animals such that only unexposed animals were represented in my sample. A third possible scenario is that persistent *Y. pestis* circulation does occur in southern Phillips County but in localized areas that were not sampled as part of this study. The most conservative interpretation of these negative data is that *Y. pestis* infection is not widespread among small mammals in southern Phillips County. Furthermore, I found no evidence that *Y. pestis* infection persists at sites with a history of plague or that these sites were any more likely to produce plague-positive samples (sera or fleas) than sites with no history of plague or off-colony sites where plague history was unknown.

The only apparent plague epizootic among prairie dogs that I observed in Phillips County occurred at colony B048 during a preliminary season of fieldwork in summer 2001. In this case, I trapped small mammals and collected fleas from burrows within approximately two weeks of the epizootic which eliminated all but two or three observed individual prairie dogs from a colony of ~220 acres. No mammals were trapped on two 0.81ha trapping grids established at the site, but two pools of six fleas each recovered from burrows tested positive with a PCR-based procedure used by a field team from the U.S. Army Medical Research Institute of Infectious Diseases. The fleas were not identified prior to testing and the assay and equipment used to make the positive determination were in the development phase. Because no field samples were verified by established procedures in the laboratory, these can only be described as tentative positives. Flea and serum samples collected at this site one and two years later were all negative.

Two previous studies (Lechleitner et al. 1968; Cully et al. 1997) 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 addition, Lechleitner et al. (1968) found only one of 108 deer mice 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, a pattern that may be generalizeable to Phillips County. The B048 colony described above had a small number of prairie dogs

present in 2002 (one year after the epizootic) and fleas collected from burrows in the same areas where probable plague-positive fleas had been collected in 2001 were all negative. In 2003 a number of discrete areas within the colony had prairie dogs present and again no positive samples were found.

These and other studies have also illustrated how few animals are found seropositive even during an active plague epizootic among prairie dogs, and that routine serosurveillance of small mammals associated with prairie dogs has proven to be ineffective in identifying potential reservoir hosts (Lechleitner et al. 1968; Ubico et al. 1988; Cully et al. 1997; Treviño-Villarreal et al. 1998). In contrast, Davis et al. (2002) found five different species of rodent seropositive for plague during 13 of 17 years of testing in a relatively small (5.5ha) area in California. What these observations suggest is that a prairie dog colony *per se* is not an ideal focal area for the long-term maintenance of *Y. pestis* infection. The general scenario involving prairie dogs has been that the plague organism is introduced into a population of prairie dogs, high mortality occurs among these animals, large numbers of infected fleas become available in the burrows which seek out new hosts, additional species sympatric with prairie dogs become involved, and then the epizootic runs its course such that no infection is evident within one to two years after the epizootic ceases. An important difference between these prairie dog colonies where plague infection is short-lived and areas such as Chuchupate Campground in California (Davis et al. 2002) where plague infection is persistent is the diversity of habitats in a localized area and the associated diversity of host species, principally rodents. It is worth mentioning that the majority of human plague cases in the United States over the past 30 years (~80%) have occurred in the greater Four Corners area of

the southwestern United States (Gage et al. 1995), a region with the highest rodent diversity in North America (Feldhamer et al. 1999).

I found evidence of *Bartonella* spp. in the fleas of deer mice, coyotes, and a black-tailed prairie dog, although prevalence was low (1.4% of flea pools tested). Positive flea samples imply *Bartonella* bacteremia in the host from which the fleas were collected, however the flea could have acquired infection from a different host and subsequently come into contact with the host from which it was collected. In Colorado, Stevenson et al. (2003) found 13.1% of prairie dog fleas containing bloodmeal remnants positive for *Bartonella* and also found 8.8% of fleas tested coinfected with *Bartonella* and *Y. pestis*. Although flea-borne transmission of rodent-associated *Bartonella* spp. has not been proven, data from the current study and from Stevenson et al. (2003) suggest a flea vector.

Sequence analyses have shown that several distinct strains of *Bartonella* may be present in a given geographic area at any one time (Kosoy et al. 1997; Ying et al. 2002; Stevenson et al. 2003). In fact, Kosoy et al. (1997) isolated three phylogenetically distinct *Bartonella* strains from one species (the cotton rat, *Sigmodon hispidus*) at a single site in Georgia. Whether these different strains represent different species of *Bartonella* is not known. It is also unclear what role these different *Bartonella* spp. may play as pathogens of wildlife. To date, the only evidence of possible pathogenic effects of *Bartonella* in wildlife is a study by Boulouis et al. (2001) who found that *Bartonella birtlesii* of wild rodent (*Apodemus* sp.) origin reduced reproductive fitness in laboratory mice.

The implications of *Bartonella* infection in rodents and coyotes in southern Phillips County are not known. *Bartonella*-positive fleas were collected from coyotes and rodents at the same sites, although in successive years. Future sequence analyses of isolates from these animals are planned and should reveal whether *Bartonella* isolates from coyotes are similar to rodent isolates, suggesting a possible oral route of transmission via consumption of infected small mammals, or whether these coyotes are infected with a canid-associated *Bartonella* similar to *B. vinsonii* subsp. *berkhoffii* found in coyotes elsewhere (e.g. Chang et al. 1999). Also worth mention is that many of the *Bartonella*-positive flea pools were collected from prairie dog colonies where there are currently populations of black-footed ferrets or that are within the ferret recovery area on CMR (Figure 1). Persistent *Bartonella* infection may be present in these areas, however, nothing is known of possible pathogenic effects in the black-footed ferret, an endangered species. As such, future screening of ferrets, prairie dogs (their main prey base), and associated fleas for the presence of *Bartonella* is warranted.

CONCLUSIONS AND RECCOMENDATIONS

In this study I used data on the historic occurrence of sylvatic plague epizootics among black-tailed prairie dogs to evaluate differences in small mammal communities, flea communities, and disease prevalence associated with prairie dog colonies with a history of plague, prairie dog colonies with no history plague, and off-colony sites where plague history was unknown. I found no evidence of *Yersinia pestis* infection in small mammals or their fleas during two field seasons of surveillance, indicating that *Y. pestis* was either absent or occured at very low prevalence across much of the landscape in southern Phillips County during the time period sampled. Furthermore, I found no evidence that *Y. pestis* persists in prairie dog colonies which have previously experienced plague epizootics. Data from this and other studies suggest that, following an epizootic among prairie dogs, persistent *Y. pestis* infection recedes to (as yet, uncharacterized) focal areas in which conditions are more suitable for the long-term maintenance of this pathogen.

It is still unclear which species of small mammals are involved in maintaining sylvatic plague in Phillips County. Given their numerical dominance, and the fact that they were the only species of small mammal present at many sites with a history of plague in this study, it is hard to imagine a scenario where deer mice are not involved in plague epizootiology in this area. Deer mice show a heterogeneous response to challenge with *Y. pestis* and have a high reproductive rate, two factors that Poland and Barnes (1979) identified as important characteristics of enzootic hosts. Many studies investigating the ecology of plague associated with prairie dogs find that deer mice are the most abundant associated small mammal and, consequently, often rely on

seroprevalence in deer mice as an indicator of plague activity (Lechleitner et al. 1968; Ubico et al. 1988; Cully et al. 1997; Luce et al. 1997; Chapter 2, this thesis). However, routine surveillance of these small mammals has resulted in few positives and little information gained regarding inter-epizootic maintenance of *Y. pestis*. None of these studies have found persistent infection in a population of deer mice as has been shown for California voles (*Microtus californicus*) by Kartman and Hudson (1971). Also, there is no good data on how long detectable antibodies remain in deer mice in a natural setting, although it is assumed to be approximately the life of the animal (~1 year in the wild). Quantifying patterns of antibody production and persistence in deer mice is a major research need if studies will continue to use this species as an indicator of plague prevalence.

Routine serosurveillance of carnivores such as coyotes and badgers has been more successful in producing positive serology results (Messick et al. 1983; Gese et al. 1997; Dyer and Huffman 1999; Arjo et al. 2003). The most likely mechanism of infection for these predators, which are usually asymptomatic, is via consumption of infected prey. Arjo et al. (2003) felt that the difference in serum antibody prevalence between two populations of coyotes in Utah was due to differences in prey species composition associated with different habitats. A surveillance program that incorporates carnivore serosurveys to indicate probable areas of enzootic plague in rodents, followed by a focused survey of small mammals may prove to be effective in identifying potential plague foci.

In Chapter 1, I described the potential role of mammalian (specifically rodent) biodiversity in facilitating plague persistence. I suggest, as others have (e.g. Gage et al.

1995; Ostfeld and Keesing 2000; Biggins and Kosoy 2001), that areas with a higher diversity of hosts are more suitable for the maintenance of sylvatic plague than areas of low host diversity. While small mammal species composition was essentially the same at prairie dog colonies with and without a history of plague in this study, colonies with a history of plague often occurred near or directly adjacent to the heterogeneous, forested habitats of the Missouri Breaks. The increased structural diversity of these habitats provides a greater breadth of available niches and, thus, supports a greater diversity of rodent species. No paper has yet explicitly addressed the role of mammalian biodiversity in the ecology of sylvatic plague, although several have alluded to its importance. Further investigation into this as one of the major factors influencing the ecology and distribution of plague in North America should be considered.

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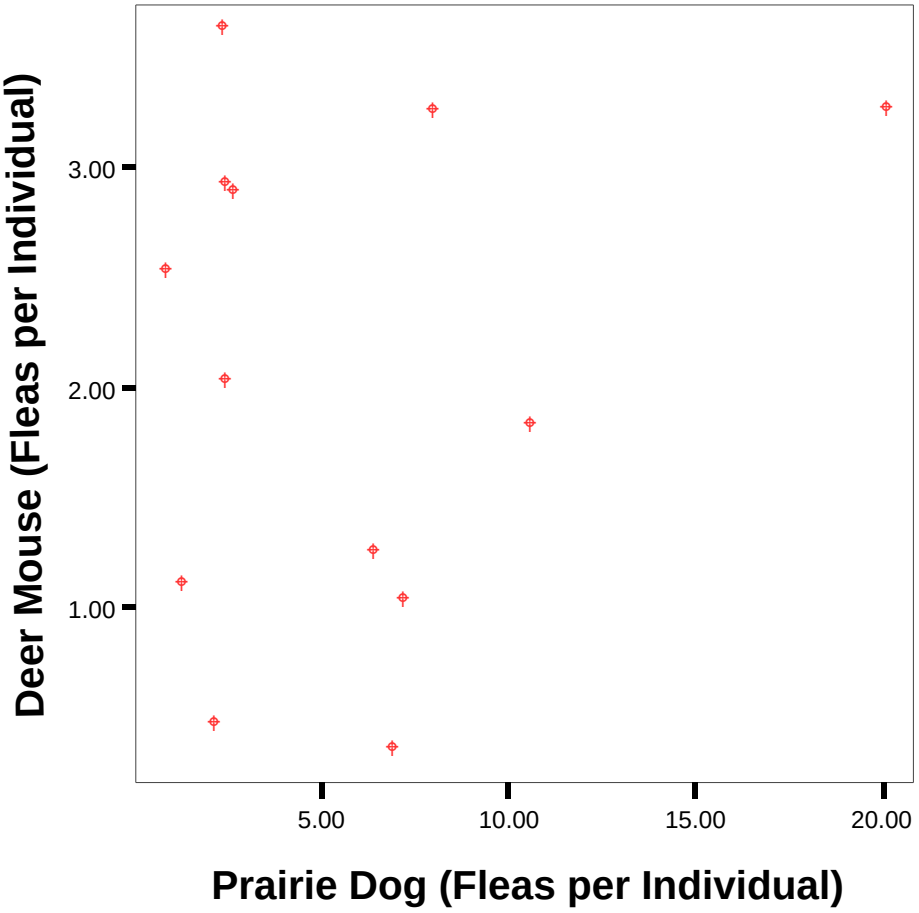
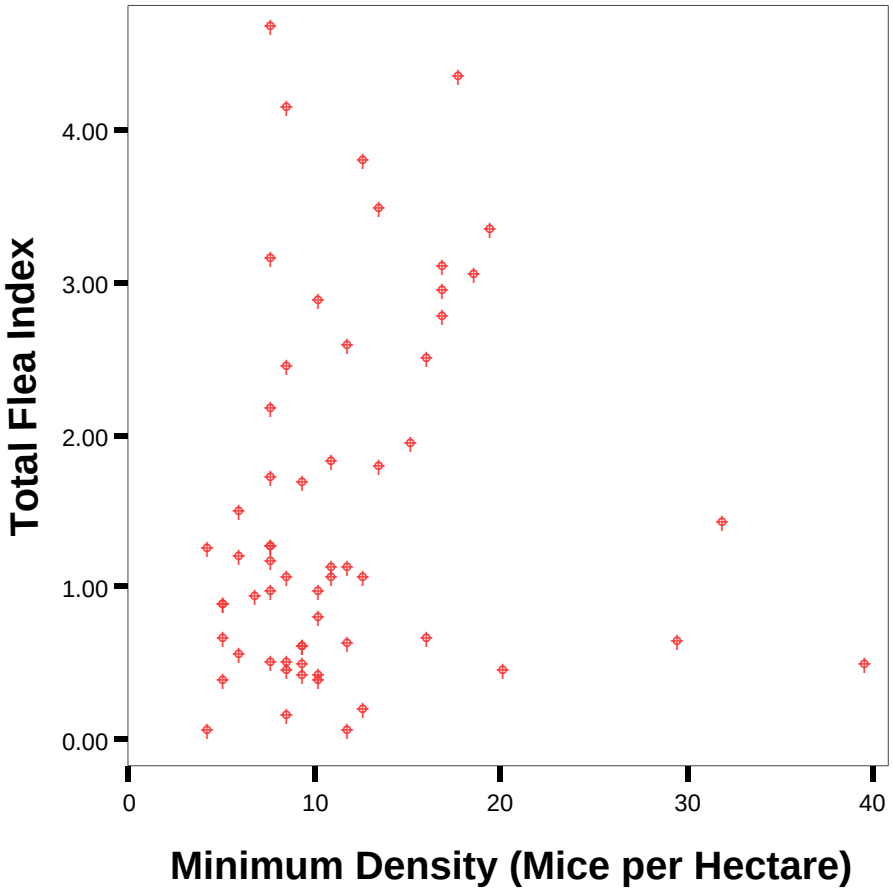
Appendix I

Scatterplot of total flea index for sympatric populations of deer mice (*Peromyscus maniculatus*) and black-tailed prairie dogs (*Cynomys ludovicianus*) in Phillips County, Montana. June – August 2002 & 2003. Each point represents a site where ≥5 deer mice and ≥5 prairie dogs were sampled.



Appendix I (continued)

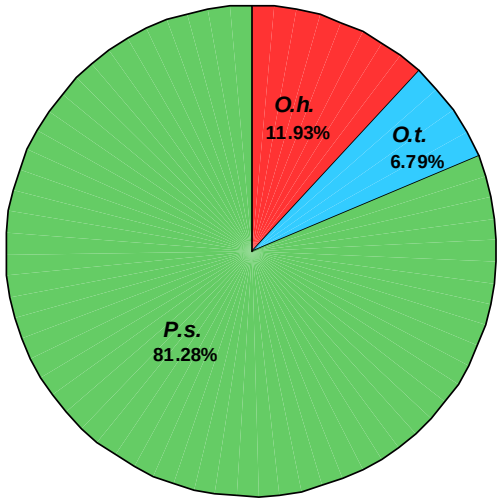
Scatterplot of minimum population density vs. total flea index for deer mice (*Peromyscus maniculatus*) in Phillips County, Montana. June – August 2002 & 2003. Each point represents a site where ≥5 deer mice were sampled.



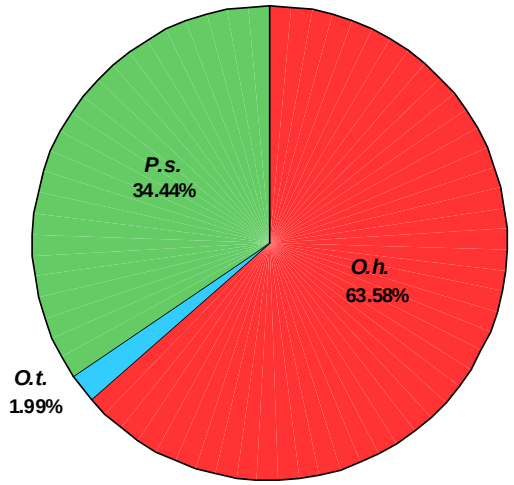
Appendix II

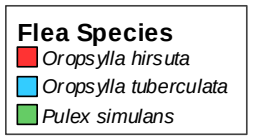
Species composition of fleas collected from black-tailed prairie dogs (*Cynomys ludovicianus*) at colonies with and without a history of sylvatic plague in Phillips County, Montana. June – August 2002 & 2003. Fleas of the genus *Oropsylla* are considered competent vectors of plague while the flea *Pulex simulans* is considered a poor vector of plague.

Prairie Dog Fleas Collected at Colonies *Without* History of Plague
n = 1,031 fleas



Prairie Dog Fleas Collected at Colonies *With* History of Plague
n = 151 fleas





Appendix III

Burrow sampling summary. June – August 2002.

Colony	Date	# Burrows Sampled	# Burrows w/Fleas	# Active	# Inactive	% Burrows w/Fleas	Total Fleas
Camp Charlie	6/17/2002	94	0	0	0	0	0
Rock Creek	6/20/2002	100	21	21	0	21	46
Wavy	6/24/2002	100	5	5	0	5	6
Dump Town	6/25/2002	20	0	0	0	0	0
Sagebrush	7/3/2002	100	7	6	1	7	7
North Hawley	7/5/2002	100	13	13	0	13	15
Wilderness	7/19/2002	100	7	6	1	7	8
Airport	7/19/2002	100	2	2	0	2	2
Long-X	7/21/2002	100	6	6	0	6	7
Legg Well	7/23/2002	100	3	3	0	3	5
Sharptail	7/27/2002	8	0	0	0	0	0
Big Snowy	7/27/2002	100	2	2	0	2	4
North Manning Corral	7/28/2002	100	2	2	0	2	3
South Dead Calf	7/28/2002	100	3	2	1	3	10
East Robinson	7/29/2002	100	9	9	0	9	26
Valentine	7/31/2002	100	17	17	0	17	27
Main Locke	7/31/2002	100	13	13	0	13	15
Squat Resevoir	8/11/2002	14	0	0	0	0	0
B101	8/13/2002	100	2	2	0	2	2
B048 North	8/15/2002	100	2	1	1	2	2
B048 South	8/19/2002	100	0	0	0	0	0
B049	8/20/2002	100	1	1	0	1	1
Small Town	8/23/2002	50	3	3	0	6	3
South Locke	8/23/2002	50	6	6	0	12	7
Total		2036	124	120	4	6.09	196

Appendix III (continued)

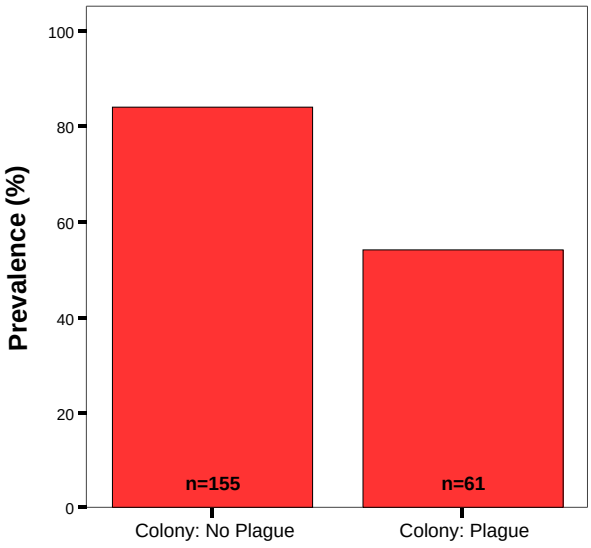
Burrow sampling summary. June – August 2003.

Colony	Date	# Burrows Sampled	# Burrows w/Fleas	# Active	# Inactive	% Burrows w/Fleas	Total Fleas
North Hawley	6/20/2003	50	3	3	0	6	4
South Locke*	6/20/2003	50	0	0	0	0	0
Main Locke*	6/21/2003	50	0	0	0	0	0
Valentine	6/22/2003	50	4	4	0	8	4
Dump Town	6/25/2003	18	0	0	0	0	0
Rock Creek	6/25/2003	50	2	2	0	4	3
Camp Charlie	6/26/2003	50	15	15	0	30	38
Wavy	6/26/2003	50	2	2	0	4	2
Sagebrush	7/2/2003	50	4	4	0	8	6
Legg Well*	7/2/2003	50	0	0	0	0	0
Big Snowy	7/5/2003	50	0	0	0	0	0
Sharptail	7/5/2003	4	1	0	1	25	3
Squat	7/5/2003	2	0	0	0	0	0
East Robinson	7/6/2003	50	5	4	1	10	7
Small Town*	7/6/2003	50	0	0	0	0	0
Wilderness	7/6/2003	50	11	10	1	22	20
East Legg	7/16/2003	50	1	1	0	2	1
SE Legg 222	7/16/2003	50	2	2	0	4	2
Airport*	7/19/2003	50	0	0	0	0	0
Long-X	7/20/2003	50	12	12	0	24	16
North Manning Corral	7/20/2003	50	3	3	0	6	5
South Hawley	7/29/2003	50	11	9	2	22	39
Manning BLM	8/2/2003	48	1	0	1	2	1
South Manning Corral	8/2/2003	50	0	0	0	0	0
South Dead Calf	8/13/2003	50	2	2	0	4	3
B101	8/21/2003	50	4	4	0	8	7
B049	8/21/2003	50	1	1	0	2	1
B048 South	8/21/2003	50	3	3	0	6	4
B048 North	8/21/2003	50	1	0	1	2	1
Total		1322	88	81	7	6.66	167

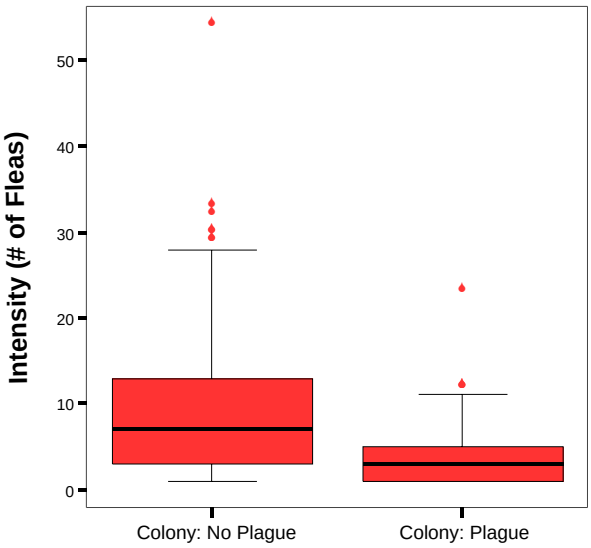
*Colonies treated with Delta Dust (.05% Deltamethrin) insecticide @ 4g/burrow in June 2003 – prior to burrow sampling.

Appendix IV

Prevalence of flea parasitism on black-tailed prairie dogs (*Cynomys ludovicianus*) by site in Phillips County, Montana. June – August 2002 & 2003.

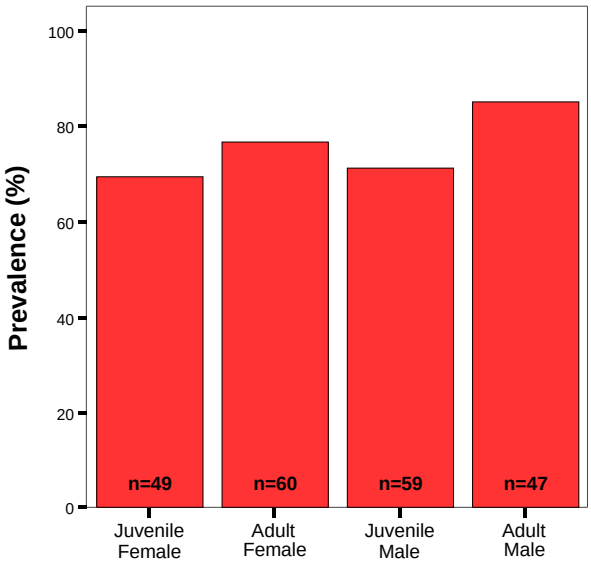


Box-plots of flea burdens on black-tailed prairie dogs (*Cynomys ludovicianus*) by site in Phillips County, Montana. June – August 2002 & 2003. Bolded lines represent the median, boxes represent the interquartile range (IQR), whiskers represent data points within ±1.5X the IQR, and asterisks are outliers.

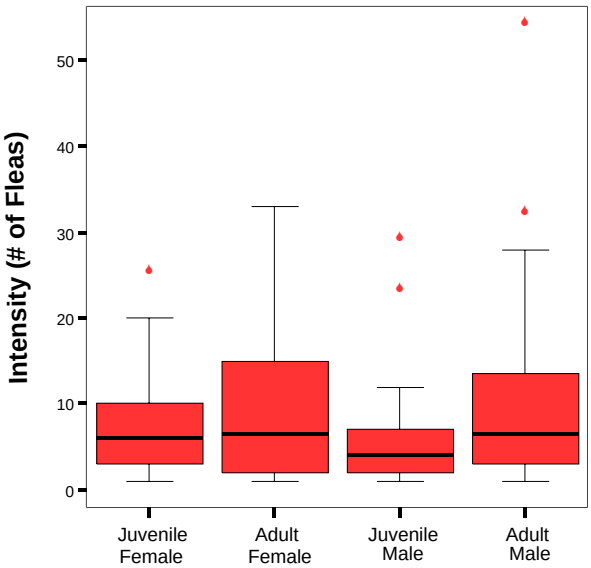


Appendix IV (continued)

Prevalence of flea parasitism on black-tailed prairie dogs (*Cynomys ludovicianus*) by age-sex class in Phillips County, Montana. June – August 2002 & 2003.

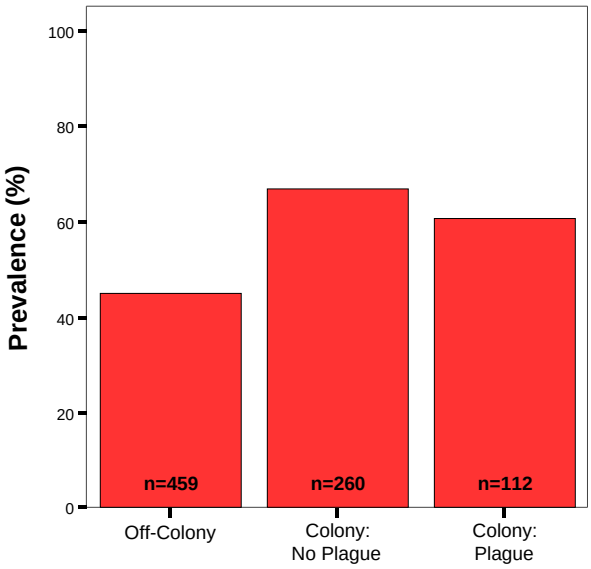


Box-plots of flea burdens on black-tailed prairie dogs (*Cynomys ludovicianus*) by age-sex class in Phillips County, Montana. June – August 2002 & 2003. Bolded lines represent the median, boxes represent the interquartile range (IQR), whiskers represent data points within ±1.5X the IQR, and asterisks are outliers.

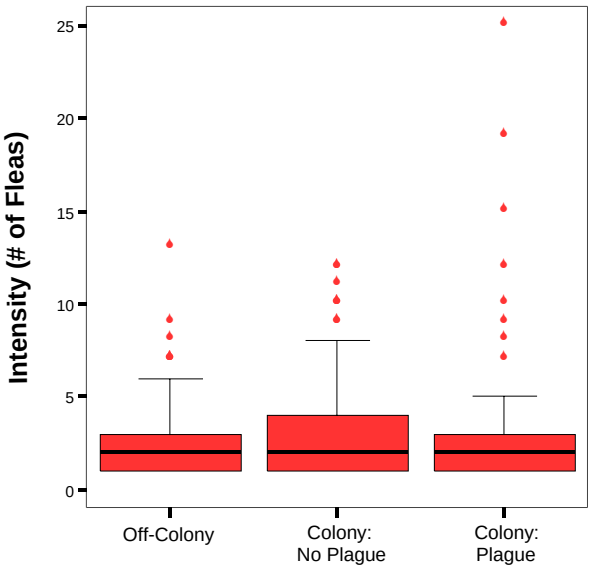


Appendix IV (continued)

Prevalence of flea parasitism on deer mice (*Peromyscus maniculatus*) by site in Phillips County, Montana. June – August 2002 & 2003.

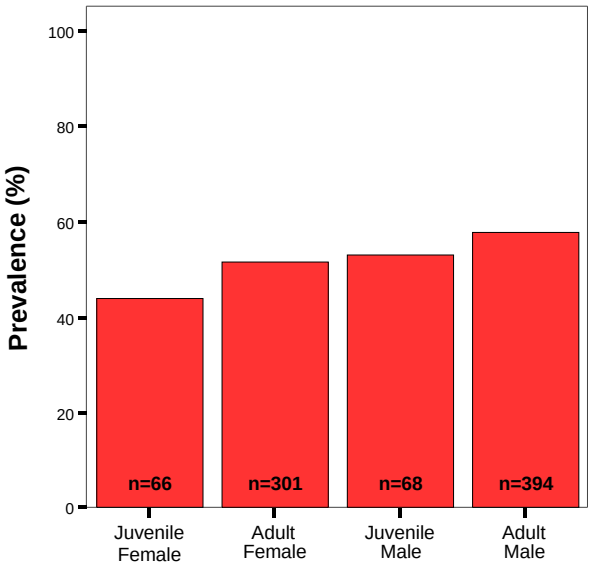


Box-plots of flea burdens on deer mice (*Peromyscus maniculatus*) by site in Phillips County, Montana. June – August 2002 & 2003. Bolded lines represent the median, boxes represent the interquartile range (IQR), whiskers represent data points within ±1.5X the IQR, and asterisks are outliers.

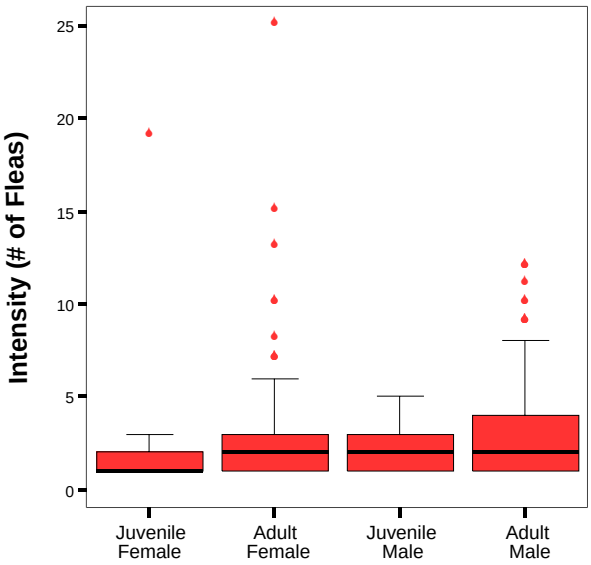


Appendix IV (continued)

Prevalence of flea parasitism on deer mice (*Peromyscus maniculatus*) by age-sex class in Phillips County, Montana. June – August 2002 & 2003.



Box-plots of flea burdens on deer mice (*Peromyscus maniculatus*) by age-sex class in Phillips County, Montana. June – August 2002 & 2003. Bolded lines represent the median, boxes represent the interquartile range (IQR), whiskers represent data points within ±1.5X the IQR, and asterisks are outliers.



Appendix V

Logistic regression model summary for prevalence of flea parasitism on black-tailed prairie dogs (*Cynomys ludovicianus*) in Phillips County, Montana. June – August 2002 & 2003.

Variable[a]	Coefficient	SE	Odds Ratio	p-value
Model 1				
Year	-0.016	0.346	0.98	0.962
Site	1.880	0.388	6.55	<0.001
Sex	0.253	0.346	1.29	0.465
Age Class	1.165	0.387	3.21	0.003
Constant	0.896	0.175	--	--
Model 2				
Year	-0.034	0.342	0.97	0.922
Region	-1.687	0.383	5.41	<0.001
Sex	0.234	0.343	1.26	0.495
Age Class	1.215	0.387	3.37	0.002
Constant	1.088	0.172	--	--

[a]Coefficient, odds ratio, and significance are compared against a reference category for each variable. Reference categories are: Year=2002, Site=plague, Sex=female, Age Class=juvenile, and Region=upland.

Logistic regression model summary for prevalence of flea parasitism on deer mice (*Peromyscus maniculatus*) in Phillips County, Montana. June – August 2002 & 2003.

Variable[a]	Coefficient	SE	Odds Ratio	p-value
Model 1				
Year	0.134	0.156	1.14	0.390
Site (No Plague)	0.950	0.165	2.59	<0.001
Site (Plague)	0.671	0.218	1.96	0.002
Sex	0.303	0.144	1.36	0.036
Age Class	0.243	0.195	1.28	0.211
Constant	0.198	0.108	--	--
Model 2				
Year	-0.021	0.151	0.98	0.888
Region	0.441	0.142	1.55	0.002
Sex	0.247	0.142	1.28	0.081
Age Class	0.307	0.192	1.36	0.109
Constant	0.065	0.099	--	--

[a]Coefficient, odds ratio, and significance are compared against a reference category for each variable. Reference categories are: Year=2002, Site=off-colony, Sex=female, Age Class=juvenile, and Region=upland.

Appendix VI

Prairie dog colonies included in survey for *Yersinia pestis* and *Bartonella* in southern Phillips County, Montana. June – August 2002 & 2003.

Prairie Dog Colony - No Plague	Prairie Dog Colony - Plague (year)
Airport	B048 North (2001)
*East Legg	B048 South (2001)
Legg Well	B049 (2001)
Long-X	B101 (2000)
Main Locke	Big Snowy (1994/1995)
North Hawley	East Robinson (1994)
Rock Creek	Sharptail (1994)
Sagebrush	Squat Resevoir (1994)
*SE Legg 222	Camp Charlie (1992)
Small Town	Dump Town (1992)
*South Hawley	*Manning BLM (1992)
South Locke	*North Dead Calf (1992)
Valentine	North Manning Corral (1992)
Wavy	South Dead Calf (1992)
Wilderness	*South Manning Corral (1992)

*Colonies sampled in 2003 only. All other colonies were sampled in 2002 & 2003.