COMMUNITY DYNAMICS OF RODENTS, FLEAS AND PLAGUE ASSOCIATED WITH BLACK-TAILED PRAIRIE DOGS

by

BALA THIAGARAJAN

B. Sc., University of Madras, India, 1999
M.S., Pondicherry Central University, India, 2001

AN ABSTRACT OF A DISSERTATION

Submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Division of Biology
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ABSTRACT

Black-tailed prairie dogs (*Cynomys ludovicianus*) are epizootic hosts for plague (*Yersinia pestis*); however, alternate enzootic hosts are important for the maintenance of the pathogen. We determined small rodents and prairie dog associations and quantified rodent and flea relationships in the presence and absence of prairie dog colonies and plague. We identified potential alternate hosts and flea vectors for the maintenance and transmission of plague in the prairie ecosystem. This is the first multi-year study to investigate associations between prairie dogs, rodents and fleas across the range of the black-tailed prairie dog. Few rodent species associated with black-tailed prairie dogs and were found to be highly abundant on colonies. Rodent species implicated in plague were present at study areas with and without plague. *Peromyscus maniculatus* and *Onychomus leucogaster*, two widely occurring species, were more abundant in areas with a recent history of plague. Flea community characteristics varied within each study area in the presence and absence of prairie dogs. Based on flea diversity on rodents, and the role of rodents and fleas in plague, we identified *P. maniculatus* and *O. leucogaster* and their associated fleas, *Aetheca wagneri, Malareus telchinus, Orchopeas leucopus, Peromyscopsylla hesperomys*, and *Pleochaetis exilis* to be important for the dynamics of sylvatic plague in our study areas. *Peromyscus maniculatus* and *O. leucogaster* were consistently infected with *Bartonella* spp., another blood parasite. Presence of prairie dog fleas on other rodents at both off and on prairie dog colonies suggests the potential for intra and interspecific transmission of fleas between rodent hosts, and between other small rodents and prairie dogs.

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Approved by:

Major Professor
Dr. Jack F. Cully, Jr.

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Black-tailed prairie dogs (*Cynomys ludovicianus*) are epizootic hosts for plague (*Yersinia pestis*); however, alternate enzootic hosts are important for the maintenance of the pathogen. We determined small rodents and prairie dog associations and quantified rodent and flea relationships in the presence and absence of prairie dog colonies and plague. We identified potential alternate hosts and flea vectors for the maintenance and transmission of plague in the prairie ecosystem. This is the first multi-year study to investigate associations between prairie dogs, rodents and fleas across the range of the black-tailed prairie dog. Few rodent species associated with black-tailed prairie dogs and were found to be highly abundant on colonies. Rodent species implicated in plague were present at study areas with and without plague. *Peromyscus maniculatus* and *Onychomus leucogaster*, two widely occurring species, were more abundant in areas with a recent history of plague. Flea community characteristics varied within each study area in the presence and absence of prairie dogs. Based on flea diversity on rodents, and the role of rodents and fleas in plague, we identified *P. maniculatus* and *O. leucogaster* and their associated fleas, *Aetheca wagneri, Malareus telchinus, Orchopeas leucopus, Peromyscopsylla hesperomys*, and *Pleochaetis exilis* to be important for the dynamics of sylvatic plague in our study areas. *Peromyscus maniculatus* and *O. leucogaster* were consistently infected with *Bartonella* spp., another blood parasite. Presence of prairie dog fleas on other rodents at both off and on prairie dog colonies suggests the potential for intra and interspecific transmission of fleas between rodent hosts, and between other small rodents and prairie dogs.

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DEDICATION

To

My Parents

Radha and Thiagarajan

For everything

PREFACE

Each chapter was written in the format of a journal manuscript. Hence, some information is repeated among chapters. Differences in formatting among the chapters reflect respective formats of the journals targeted for submission.

Chapter 1

INTRODUCTION

Of the widely recognized threats to global biodiversity and ecosystem functioning, infectious diseases of wildlife play an important role (Daszak *et al.* 2000). Diseases have long been recognized as one of the potential limiting factors of wildlife populations and pose a serious threat to the persistence of endangered species (McCallum and Dobson 1995). Of particular interest are introduced pathogens that can cause high mortality in many native animal species. Wildlife diseases also have a potential risk of spillover or crossover infection in domestic animal and/ or human populations. Understanding the dynamics of disease and its ecology is imperative and has implications for management of species of conservation concern. Epidemics of infectious diseases may be an important population regulator in mammals (Elton 1925) and the occurrence and localization of disease are determined by a variety of factors relating to the host, vectors and pathogens (Wobeser 1994). For vector-borne diseases, interactions between populations of host species are important for the distribution of the vectors and transmission of the pathogens.

Plague is a vector borne zoonosis, introduced in the United States and was first identified around 1900 on the West Coast. *Yersinia pestis,* the etiologic agent of plague, is a gram-negative coccobacilus (Enterobacteriaceae) that is transmitted to mammals by flea bites, direct contact, or inhalation of respiratory droplets from an infected animal (Ewald 1994, Gage *et al.* 1995). Sylvatic plague is vectored by several flea species that occur primarily on vertebrate hosts (Thomas 1988, Anderson and Williams 1997, Cully *et al*. 1997). In addition to flea-borne transmission among overlapping populations and species of rodents, *Yersinia pestis* might be

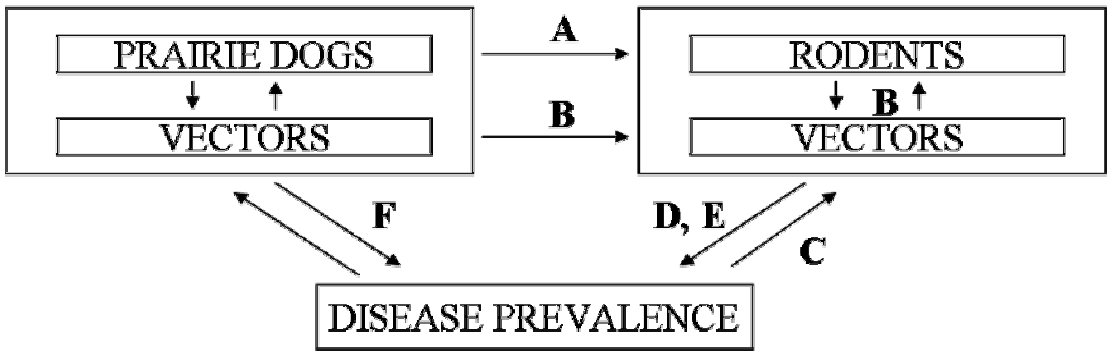
spread over considerable distances by other mammals, especially rodent consuming carnivores that can become infested with infected rodent fleas and transport these insects from one area to another (Poland and Barnes 1979). Plague causes large population reductions in rodents of several species within its native and introduced ranges (Barnes 1993). Plague usually appears in the US in epizootics in colonial wild rodents such as prairie dogs or other ground squirrels, colonies of which are greatly reduced or eradicated.

Black-tailed prairie dogs (*Cynomys ludovicianus)* occupy only 2% of their former range as a result of grassland conversion to crop agriculture, fragmentation of the habitat, poisoning, and sylvatic plague (USFWS 2000). Prairie dogs infected with plague suffer close to 100% mortality (Cully *et al.* 1997, Cully and Williams 2001). Prairie dogs are an epizootic host for plague but alternate hosts and their vector dynamics are also thought to be important for disease prevalence. Black-tailed prairie dogs may influence the abundance and distribution of many native plant and animal species, including several species that act as alternate hosts for sylvatic plague (Cully *et al.* 1997, Gage *et al.* 1995).

Black-tailed prairie dogs may have a key effect on the densities and community composition of alternate small rodent hosts and may have a strong influence on the small rodents that might act as a reservoir of diseases. Black-tailed prairie dogs are considered a keystone species and ecosystem engineers because of their role in the alteration of the vegetation structure and the wide diversity of species that is associated with the colonies (Kotliar 2000, Collinge *et al.* 2006). Prairie dog burrows provide habitat for rodents and also act as refugia for fleas. Prairie dog burrows are also important as off-host environment for fleas (Gage and Kosoy 2005).

Understanding of host-vector relationships is essential to understand the role of arthropod vectors as well as mammalian reservoirs in the maintenance of various diseases. This study focused on multiple components of the host-vector-pathogen system and their role in disease transmission and maintenance, over a broad geographical area, with study areas distributed over the range of the black-tailed prairie dog and in the presence and absence of plague. The distribution of plague in the United States has been more or less stable since 1950, and the 100th meridian is approximately the eastern boundary for plague (Cully *et al.* 1997). Another component of this study was to determine whether differences in host and vector dynamics play a role in maintaining this current boundary of plague.

Figure 1.1: Conceptual model of the study



Our study investigated relationships between the key elements of the host-vector-pathogen system. In Chapter 1, indicated by arrow 'A' in the conceptual model, we evaluated the association of rodents with the black-tailed prairie dog colonies and the variation of this association in areas with and without a known history of plague. We discuss the differences in rodent community structure and composition and how these differences could play a role in disease prevalence. The objectives of this chapter were to (i) quantify the variation of rodent

species composition and abundance in the presence of prairie dogs in areas with and without a known history of plague and (ii) identify rodent species that could be important for the maintenance and transmission of plague in the prairie dog ecosystem.

The second chapter explores the host-vector relationships of rodents and fleas that are associated with the black-tailed prairie dogs (arrow 'B' in the model). We quantified the rodent-flea relationship by estimating prevalence, probability of infestation, flea load, and mean intensity of fleas on rodents and the variation of these characteristics in the presence of black-tailed prairie dogs. The third chapter analyses the differences of rodent-flea communities in areas with and without a known history of plague (arrow 'C' in the model). Our objective in this chapter was to identify rodent-flea complexes that are important for the maintenance and transmission of plague on the landscape.

Black-tailed prairie dogs in one of the study areas, Thunder Basin National Grassland in Wyoming, underwent a plague epizootic between 2000 and 2001 and we evaluated disease prevalence in this area. In chapter 4, indicated by arrow 'D' in the model, we discuss the prevalence of the plague bacterium in rodents and their fleas associated with the black-tailed prairie dogs (arrow 'F' in the model) and identify potential enzootic hosts for plague.

As plague is hard to detect unless immediately after prairie dog die-offs (Gage 1999), we used *Bartonella*, an intracellular hemotropic parasite, that exhibits similar mode of transmission as plague, as a surrogate to understand the role of rodents and fleas in diseases. In Chapter 5, indicated by arrows 'E' and 'F' in the model, we discuss the prevalence of *Bartonella* in rodents and fleas associated with the black-tailed prairie dogs and the species that are important for disease transmission and maintenance.

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Chapter 2

APPARENT EFFECTS OF BLACK-TAILED PRAIRIE DOGS *(CYNOMYS LUDOVICIANUS)* ON RODENT ASSEMBLAGES IN AREAS WITH DIFFERING PLAGUE HISTORIES *

Abstract

Black-tailed prairie dogs (*Cynomys ludovicianus*) are epizootic hosts for plague (*Yersinia pestis*) in western North American grasslands; however, the mechanism by which the bacterium is maintained on prairie dog colonies is not well understood and alternate enzootic hosts such as ground squirrels, mice, and rats are likely necessary for the maintenance of this pathogen. We evaluated the distribution and abundance of rodent species that are associated with black-tailed prairie dogs and that are thought to be important for plague dynamics. We compared the effect of prairie dogs on species composition, richness, diversity, and abundance of rodents in grasslands where plague has recently occurred with those where plague has not been recorded. Rodent species richness was generally higher at off-colony grids where prairie dogs were absent than on prairie dog colonies. Diversity and evenness were similarly higher at off-colony sites than on prairie dog colonies. Abundance of particular rodent species varied among the study areas and in the presence or absence of prairie dogs and plague. *Onychomys leucogaster* was more abundant on prairie dog colonies; other common species including *Chaetodipus hispidus, Perognathus flavus* and *Spermophilus tridecemlineatus* were more abundant in the surrounding grasslands. Species that have been implicated in plague were present in study areas with and

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* Authors: Bala Thiagarajan[1], Jack F. Cully Jr.[1, 2], Thomas M. Loughin[3], Chris Ray[4], and Sharon K. Collinge[4, 5]

[1]Division of Biology, Kansas State University; [2]United States Geological Survey, Kansas Co-operative Fish and Wildlife Research Unit, USGS, Kansas State University; [3]Department of Statistics, Kansas State University, Manhattan, KS; [4]Department of Ecology and Evolutionary Biology and [5]Environmental Studies Program, University of Colorado, Boulder, CO.

without a history of plague activity. Thus, the variation in the rodent assemblages does not seem to limit the presence of plague. *Peromyscus maniculatus* was captured in all of the study areas and showed variable association with prairie dogs in the presence of plague. *Peromyscus maniculatus* was less abundant on colonies that had high abundance of *O. leucogaster* and more abundant in areas with low abundance of *O. leucogaster*. *Peromyscus maniculatus* and *O. leucogaster*, two widely occurring species, were more abundant in areas with a recent history of plague and may be important in the maintenance of plague.

Keywords: abundance, Poisson rate regression, species richness, evenness, species diversity, sylvatic plague.

Introduction

Emerging pathogens of wildlife pose a serious threat to the conservation of global diversity, by changing both numerical abundance and the genetic composition of wildlife populations (Altizer *et al*. 2003, Daszak *et al.* 2000). Epidemics of infectious diseases may regulate mammal populations (Elton 1925, Thorne and Williams 1988, McCallum and Dobson 1995, Daszak *et al*. 2003), and wildlife diseases may spill over or cause crossover infection of domestic animals and/or humans (Daszak *et al.* 2000). The occurrence and localization of a disease are determined by a variety of factors, including the host(s), the causative agent, and the environmental factors (Wobeser 1994). For vector-borne zoonotic diseases, the interactions between populations of host species may strongly influence the spread of pathogens and their vectors.

Plague, caused by the bacterium *Yersinia pestis*, is one of the most widespread bacterial zoonotic diseases in the United States (Walker *et al.* 1996). Plague has been reported in at least 76 species of mammals in the United States, and causes large population reductions in several species of rodents (Barnes 1993). Plague outbreaks are thought to be related to the interactions of different rodent species and the diversity of species seems to influence the maintenance of plague (Biggins and Kosoy 2001). Sylvatic plague in wild rodents is transmitted by several flea species (Thomas 1988, Anderson and Williams 1997, Cully *et al*. 1997). The distribution of plague in the United States has been more or less stable since 1950, and the 100th meridian is approximately the eastern boundary for plague (Cully *et al.* 2006).

Many rodent species of conservation concern (Hafner *et al*. 1998) occur within the range of plague in North America (Barnes 1982). Plague often appears in the United States in epizootics in colonial wild rodents such as prairie dogs or other ground squirrels, colonies of which are either greatly reduced or eradicated by the disease. Prairie dog colonies infected with plague suffer close to 100% mortality (Cully *et al.* 1997, Cully and Williams 2001) and this has been identified as an important factor in shaping prairie dog populations in the future (USFWS 2000, Cully *et al.* 2006). Some of the ecological consequences of plague in prairie dogs are local extirpation of colonies, reduced colony size, increased variance in local population sizes and increased distances between colonies (Cully and Williams 2001, Johnson 2005).

Prairie dogs have often been considered a keystone species in the mixed and short grass prairie ecosystems, because of their role in altering the physical and vegetation structure and because of the wide variety of species that are associated with prairie dog colonies (Miller *et al.* 1994, Kotliar 2000, Ray and Collinge 2006, Collinge *et al. in press*). Black-tailed prairie dogs

(*Cynomys ludovicianus*) influence the abundance and distribution of many native plant and animal species, including several species that may act as alternate hosts for sylvatic plague (Gage *et al.* 1995, Cully *et al.* 1997). Prairie dogs are highly susceptible to plague and their presence may amplify plague epizootics, but the exact mechanism(s) by which the plague bacterium is maintained on prairie dog colonies in between epizootics is not understood. More resistant rodent hosts are presumed important for enzootic maintenance and transmission of the pathogen (Poland and Barnes 1979, Cully 1993, Biggins and Kosoy 2001, Gage and Kosoy 2005).

The diversity and community composition of host species may affect the prevalence of pathogens, as in Lyme disease (LoGiudice *et al.* 2003). Black-tailed prairie dogs have key effects on the densities and community composition of alternate rodent hosts, and may have a strong influence on rodent species that are purported to be reservoir hosts of plague (Collinge *et al*. *in press,* Cully *et al. submitted*). Some species of rodents have developed varying degrees of resistance to plague and likely serve as reservoirs of this disease (Hudson *et al*. 1964, Lang and Wills 1991, Pollitzer 1954). Populations of deer mice (*Peromyscus maniculatus*) show variable resistance to plague (Holdenried and Quan 1956, Gage and Kosoy 2005). Deer mice are considered to be a primary reservoir for plague (Nelson and Smith 1976, Larson *et al*. 1996). Thomas *et al.* (1988) considered *Onychomys leucogaster,* the northern grasshopper mouse to be a potential alternate host for plague. Few studies have investigated the role of small rodents in maintenance and transmission of plague (Nelson and Smith 1976, Thomas *et al.* 1988, Larson *et al.* 1996) and their relationship to prairie dogs, but so far no study has identified the alternate hosts that act as reservoirs of plague in the black-tailed prairie dog system.

Local extirpation of prairie dogs creates a mosaic of grassland plant communities in different successional stages and a broad range of habitat for other animals (Stapp 1998). Hence, in addition to prairie dogs, the history of plague in a given geographical area may contribute to the variation in the rodent community. The rodent community could also vary in composition and abundance over the landscape as a result of heterogeneity among habitats. Several studies have compared the communities of birds, mammals and vascular plants in the presence of prairie dogs and in the surrounding grasslands (Coppock *et al.* 1983, Ceballos *et al.* 1999, Winter *et al*. 1999, Lomolino and Smith 2003), but few studies have compared the relative abundance and rodent species composition on prairie dog colonies and in the surrounding grasslands (but see O' Meilia *et al*. 1982, Agnew *et al*. 1986). Some communities have been positively influenced by the prairie dogs, with increased species richness and/or diversity, while others have experienced negative or no effect of their presence.

The current study aimed to identify relationships between black-tailed prairie dogs and rodent species in the presence and absence of plague on the landscape, and to identify the most likely species that could serve as alternate hosts for the maintenance of plague. In this paper, we explored the effects of the presence of prairie dogs on rodent communities by comparing rodent communities on prairie dog colonies and in surrounding grasslands. We addressed the temporal and spatial dynamics of rodent species, favored and excluded on prairie dog colonies and examined patterns of individual rodent species on and off prairie dog colonies. We identified the variation of rodent species composition and abundance in the presence of prairie dogs in areas with and without a known history of plague. Based on our findings, we identified rodent species that could be important for the maintenance and transmission of plague in the prairie dog ecosystem.

Study Design and Methods

We conducted our research at five study areas spread across four states in the mid-western US and one additional area in Mexico (Fig. 2.1). Of these areas, Thunder Basin National Grasslands in Wyoming (TB), Cimarron National Grassland in Kansas (CIM) and Comanche National Grassland in Colorado (COM) have a known history of plague. Plague has not been reported in Wind Cave National Park (WC) and Badlands National Park (BAD) in South Dakota, and Chihuahua Grasslands near Janos in Mexico (JAN). Areas with a known history of plague are those from which animals have tested positive for *Y. pestis* or *Y. pestis* antibodies and are referred to as 'plague areas'. Areas without known histories of plague are referred to as 'no-plague areas'. Rodents were trapped at Cimarron between 2000 and 2004; at Comanche between 2001 and 2004; at Thunder Basin between 2002 and 2004; and at Badlands, Wind Cave and Janos between 2001 and 2003. The number of grids that were trapped in these areas varied with year (Table 2.1).

To assess the influence of prairie dogs on the distribution and abundance of rodents, we established paired trapping grids in each area, including 'on-colony grids' positioned within prairie dog colonies and 'off-colony grids' situated in grasslands 500-2000 m from prairie dog colonies. Off-colony grids were paired with on-colony grids based on similarities in topography and vegetation. Each grid consisted of 49 trap stations (7x7), with a single Sherman live trap (H. Sherman Traps, Tallahassee, FL) at each station. Stations were separated by 20 meters. The traps were opened and baited with oatmeal in the late afternoon and were checked for captures the next morning. They were left closed during the daytime. Each grid was trapped for three consecutive nights on two separate occasions each year, once in May-June and once in July-August. The rodents trapped were identified to species and sex, marked with a numbered ear

tag, and standard measurements of total length, tail length, hind foot length and ear pinna length were taken. All rodents were released at their capture sites after data collection. The research methods were approved by the Institutional Animal Care and Use Committee of Kansas State University.

Field guide to the mammals of North America (Burt and Grossenheider 1962) and Mammals of North America (Kays and Wilson 2002) were used to identify rodents. Rodents that were not identified to species included one *Neotoma*, one *Reithrodontomys*, two *Microtus* species and two *Peromyscus* species. All of these were excluded from analyses.

Statistical Analyses

We estimated species richness at both off- and on-colony sites using the jackknife estimator as proposed by Burnham and Overton (1979), because the number of species observed varied and not all species were caught consistently over the years. Estimates were computed using software COMDYN (Hines *et al*. 1999), designed specifically for the study of communities. Shannon diversity indices and Shannon evenness indices were estimated from the actual captures as described by Magurran (1988) using the software Bio-Dap (Thomas 2000). The mean species richness, Shannon diversity and Shannon evenness indices were compared between off- and on-colony sites in each of the study areas using paired *t-tests*.

To determine whether the abundance of rodents varied between rodent species and with the presence of prairie dogs, we modeled the capture per trap effort as the response variable against rodent species and the presence or absence of prairie dogs using a Poisson rate regression (Agresti 1996) on SAS version 8.2 (SAS/STAT Institute, Cary, NC). Type III likelihood ratio

tests were used to test for main effects and interactions. When the association between prairie dogs and rodent species was determined to vary significantly across rodent species within an area, the effect of prairie dog presence was determined for each rodent species using a chi-square test with 1 degree of freedom (χ^2). We used the number of captures per trap effort as an estimate of abundance. Species captured five times or less in each study area were not included in the statistical analyses for that study area because samples were too small to allow detection of effects due to the presence of prairie dogs. The cut-off for significance was set at 0.1 to accommodate the high variability in capture data. Only individual captures and not recaptures (within years) were included in the analyses.

Poisson rate regression was also used on data from 2001-2003 to test if the history of plague in the area had an effect on the abundance of rodents and their association with the black-tailed prairie dogs. The mean capture per trap effort for each rodent species was modeled against the presence of plague (areas TB, COM, CIM) and absence of plague (areas BAD, WC, JAN), presence or absence of prairie dog colonies and rodent species. When capture rates differed between off- and on-colony grids across species, the comparison for each species was performed using a separate chi-square test with 1 degree of freedom.

Results

We captured 6452 rodents belonging to 23 species over the period of study across all six areas during ~76,000 trap nights. Of these, 29% of captures (1896 individuals) were recaptures; however, less than 1% of individuals were recaptures between years. We used 4556 individual captures in the analyses. Capture and recapture rates varied among years (Fig. 2.2a). The species composition and the number of rodents captured varied across study areas (Table 2.2).

Within each study area, there was variation in the number of captures among years and between off- and on-colony grids (Fig. 2.2b).

Estimated species richness was higher at off-colony grids at Wind Cave and Janos (Fig. 2.2c). The mean abundance did not change with the observed species richness for either off- or on-colony grids ($r = 0.711$, $p = 0.113$ and $r = 0.313$, $p = 0.547$ respectively; Fig. 2.2d). Shannon diversity was significantly different between off- and on-colony grids for all study areas ($p < 0.1$) and with the exception of Thunder Basin, off-colony grids were more diverse than on-colony grids. At Thunder Basin, on-colony grids had significantly higher species diversity than off-colony grids (Fig. 2.2e). The highest diversity was found at off-colony grids at Cimarron and the lowest diversity was seen in on-colony grids at Wind Cave. Evenness was also significantly different between off- and on-colony grids in all study areas except Janos. At Badlands, Wind Cave, Cimarron and Comanche, Shannon evenness was higher at off-colony grids but at Thunder Basin, on-colony grids had higher evenness of species distribution (Fig. 2.2f).

Rodent associates of prairie dogs: Within each study area, the difference in capture rates between off- and on-colony grids varied significantly across rodent species (all $p < 0.001$). *Peromyscus maniculatus* was the only species captured within all study areas (Table 2.2). Among the other widely distributed species, *O. leucogaster* was absent from Wind Cave and had low abundance at Janos. The hispid pocket mouse (*Chaetodipus hispidus*) was absent only at Thunder Basin and the thirteen-lined ground squirrel (*Spermophilus tridecemlineatus*) was absent only at Janos.

Areas without a known history of plague

Badlands: Abundance of three of the five species analyzed varied between off- and on-colony grids (Fig. 2.3a). *Onychomys leucogaster* and *P. maniculatus* were significantly more abundant at on-colony grids ($\chi^2 = 18.57$, $p < 0.001$; $\chi^2 = 96.67$, $p < 0.001$ respectively) and S. *tridecemlineatus* was significantly more abundant at off-colony grids ($\chi^2 = 3.84$, $p < 0.05$).

Wind Cave: Fewer species (n = 4) were captured here than in other study areas. *Peromyscus maniculatus* and *S. tridecemlineatus* were the only species captured at on-colony grids. *Microtus ochrogaster* was caught only in 2003 and along with *C. hispidus* was present at off-colony grids only (Fig. 2.3b). *Peromyscus maniculatus* was significantly more abundant at on-colony grids ($\chi^2 = 140.97$, $p < 0.001$) *and S. tridecemlineatus* were significantly more abundant at off-colony grids ($\chi^2 = 4.97$, $p = 0.026$).

Janos: Eight rodent species were significantly more abundant at off-colony grids than at on-colony grids (Fig. 2.3c). Three of these, *O. leucogaster, P. maniculatus* and *Peromyscus leucopus* were found only at off-colony grids. *Chaetodipus penicilatus* and *Neotoma albigula* were also found only at off-colony grids. *Spermophilus spilosoma* was only captured at on-colony grids; however, they were seen in off-colony grids as well (Cully, *personal observation*).

Areas with a known history of plague

Thunder Basin: Of the 10 species captured, 8 were found on both off- and on-colony grids (Fig. 2.4a). *Dipodomys ordii* and *O. leucogaster* were significantly more abundant at on-colony grids ($\chi^2 = 21.93$, $p < 0.001$; $\chi^2 = 13.32$, $p < 0.001$ respectively) and *P. maniculatus* was significantly more abundant on the surrounding grasslands ($\chi^2 = 4.0$, $p < 0.046$).

Cimarron: All 10 species included in the analysis were present on both on-colony and off-colony grids (Fig 2.4b). *Chaetodipus hispidus* ($\chi^2 = 58.73$, $p < 0.001$), *D. ordii* ($\chi^2 = 14.50$, $p < 0.001$), *Perognathus flavus* ($\chi^2 = 4.83$, $p = 0.028$), *Reithrodontomys megalotis* ($\chi^2 = 7.00$, $p = 0.008$) and *Reithrodontomys montanus* ($\chi^2 = 12.75$, $p < 0.001$) were more abundant at off-colony grids. Only *O. leucogaster* was significantly more abundant at on-colony grids ($\chi^2 = 151.58$, $p < 0.001$). Cimarron was the only area at which the abundance of *P. maniculatus* did not vary with the presence of prairie dogs.

Comanche: All of the species captured were present on both on-colony and off-colony grids (Fig. 2.4c). *Onychomys leucogaster* was significantly more abundant at on-colony grids ($\chi^2 = 56.24$, $p < 0.001$) and *C. hispidus* and *P. maniculatus* were more abundant at off-colony grids ($\chi^2 = 19.17$, $p < 0.001$; $\chi^2 = 44.73$, $p < 0.001$ respectively).

Comparison of rodent associates in relation to plague history: Five species that were present in both plague and no-plague areas and at off- and on-colony grids were analyzed to see if broad spatial patterns in abundance varied with presence or absence of plague or prairie dogs. For all of these species – *C. hispidus, O. leucogaster, P. flavus, P. maniculatus* and *S. tridecemlineatus*, the effect of prairie dogs on rodent abundance depended significantly on plague history (plague history*prairie dog interaction, $p < 0.001$ for all comparisons; Fig. 2.5). *Chaetodipus hispidus, O. leucogaster and S. tridecemlineatus* were significantly more abundant at both off- and on-colony grids in plague areas than no-plague areas ($p < 0.001$). *Perognathus flavus* was significantly more abundant in no-plague areas due to high numbers at Janos ($p < 0.1$). *Onychomys leucogaster* was significantly more abundant at on-colony grids in plague areas than

no-plague areas ($p < 0.001$); *C. hispidus* and *S. tridecemlineatus* were more abundant at off-colony grids in plague areas ($p < 0.1$); however, *P. flavus* was more abundant at on-colony grids in areas without plague. Only *P. maniculatus* was significantly more abundant at off-colony grids in plague areas ($p < 0.001$) and significantly more abundant at on-colony grids in no-plague areas ($p < 0.001$).

Discussion

Our goal was to determine associations between rodents and black-tailed prairie dogs in the presence and absence of plague in order to identify potential alternate hosts for the maintenance of plague in the prairie ecosystem and to see if differences in assemblages would explain why plague has not moved further east since it arrived at the western Great Plains during the 1940s. This is the first multi-year study to investigate associations between prairie dogs and rodents across the range of the black-tailed prairie dog.

Abundance of rodents fluctuated widely between years and varied with the presence of prairie dogs and plague history among the different study areas. Rodent abundances were similar within off- and on-colony grids except at Badlands and Wind Cave, where significantly more rodents were captured within on-colony grids. This result could be related to the absence of plague in South Dakota, or to other factors influencing the ecology of the region that contains these two neighboring grasslands. In our other study areas, including the plague-free study area in Mexico, prairie dog colonies did not support a higher abundance of rodents than the surrounding grasslands.

The community measures of species richness, diversity and evenness varied between study areas and with the presence of prairie dogs. Although previous studies have found lower rodent species richness on prairie dog colonies than in surrounding grasslands (O'Meilia *et al.* 1982, Agnew *et al.* 1986, Mellink and Madrigal 1993), in the current study rodent species richness was lower on colonies in only two (Wind Cave and Janos) of six study areas. A previous study at Badlands found higher species richness at off-colony grids (Agnew *et al.* 1986), a result at odds with the current study. Two of the rodent species that were not captured at on-colony grids by Agnew *et al.* (1986), *C. hispidus* and *M. ochrogaster* were captured at on-colony grids in the present study. However, *R. megalotis*, captured at off-colony grids by Agnew *et al.* (1986) were not captured during this study and all species captured were present at both off- and on-colony grids.

Shannon diversity was significantly different between off- and on-colony grids in every study area. Janos was similar to Cimarron and Comanche in diversity of off-colony grids and to all plague areas in diversity of on-colony grids. The diversity of Thunder Basin off-colony grids was similar to Badlands and Wind Cave off-colony grids and the diversity at on-colony grids was similar to Cimarron, Comanche and Janos. Although prairie dog colonies were known to support fewer species, there was variation in the species composition between colonies and surrounding grasslands (Kotliar *et al.* 1999). In our study, three species – *Mus musculus*, *Sigmodon hispidus,* and *Spermophilus spilosoma* were caught only at on-colony grids, in at least one area. However, none of these species were unique to prairie dog colonies, as they were trapped at off-colony grids in other study areas or were observed in the surrounding grasslands. Thus, presence of prairie dog colonies on the landscape did not enhance the diversity of rodents.

Species evenness was higher at off-colony grids at Badlands, Wind Cave, Cimarron and Comanche, higher at on-colony grids at Thunder Basin and not different between off- and on-colony grids at Janos. Abundance of *P. maniculatus* at Thunder Basin increased during the period of study and the increasing dominance of this species reduced evenness at both off- and on-colony grids in this area as its population grew. Based on diversity, Janos was similar to Cimarron and Comanche; Thunder Basin was different from Cimarron and Comanche, and had comparable indices to Badlands and Wind Cave. Given this variation in community characteristics across our entire study area, the role of rodent diversity appears not to be important for the dynamics of plague on a broad spatial scale in the black-tailed prairie dog system, but could still be important for the persistence of the pathogen at smaller spatial scales.

The associations between rodents and prairie dogs varied among study areas. There were distinctions between the northern and southern grasslands in the relative abundance and association of rodents with black-tailed prairie dogs. Few species of rodents had significant associations with black-tailed prairie dogs and were found to be highly abundant on colonies. *P. maniculatus* was at least 5 times as abundant as any other species at Badlands, Wind Cave and Thunder Basin. This species is often recorded as the most abundant small mammal on the grasslands of the Northern Great Plains (Anderson and Williams 1997, Cully *et al.* unpublished data). In our study, deer mice were more abundant at on-colony grids and had a significant association with the prairie dogs at Badlands and Wind Cave, but at Thunder Basin, they were more abundant at off-colony grids. They were also more abundant at off-colony grids at Comanche, but did not vary in the presence of prairie dogs at Cimarron and were not present at on-colony grids at Janos. Previous studies in South Dakota showed that *P. maniculatus* was

more common on black-tailed prairie dog colonies (Agnew *et al.* 1986) and our study found the same there, but we showed that this association was variable across the range.

Peromyscus maniculatus were more abundant at on-colony grids in no-plague areas and more abundant at off-colony grids in plague areas in our study. However, in Boulder County, Colorado, a plague area, a companion study showed that the abundance of *P. maniculatus* was significantly higher at on-colony grids than off-colony grids (Ray and Collinge 2006, Cully *et al.* submitted). Among the plague areas, Thunder Basin and Comanche had a significantly lower abundance of *P. maniculatus* at on-colony grids. Thunder Basin has been undergoing a plague epizootic since 2001 (Cully, *unpublished data*) and susceptibility to plague with increased mortality could be a reason for the lower abundance of *P. maniculatus* at on-colony grids. We also observed that the abundance of *P. maniculatus* has increased annually at Thunder Basin since 2002 and its increasing dominance is responsible for the decreasing evenness at this area (Bala *et al*. unpublished).

Onychomys leucogaster have been known to associate positively with black-tailed prairie dogs possibly due to their preference for disturbed areas, suitable habitat in the form of burrows or food supply (Choate and Terry 1974, Stapp 1997a). Our study supports this finding as we observed and a significant association of *O. leucogaster* with black-tailed prairie dogs is seen throughout their range overlap. *Onychomys leucogaster* were also more abundant in plague areas than no-plague areas. Individuals of this species have been known to develop resistance to plague, confirmed by the presence of antibodies to *Yersinia pestis* specific F1 antigen (Thomas *et al*.1988, Stapp *pers. comm*, Bala *et al.* unpublished) and have been considered to be a potential alternate host for plague (Thomas *et al.*1988, Biggins and Kosoy 2001).

In areas with high abundance of *O. leucogaster*, *P. maniculatus* was found in low abundance and in areas with high *P. maniculatus* abundance, *O. leucogaster* abundance was low. This large scale pattern has not been reported before and it is likely that both species might be playing a similar role with respect to plague in the different areas. Stapp (1997b) showed that the abundance of *P. maniculatus* declined in areas where *O. leucogaster* increased and attributed this to predatory interference; hence at Cimarron and Comanche, the abundance of *P. maniculatus* may be regulated by the high abundance of *O. leucogaster* on prairie dog colonies. This reciprocal abundance pattern could also be due to direct or apparent competition between these rodents and might be important in structuring rodent communities on prairie dog colonies (Ray and Collinge 2006).

Species of rodents that are abundant at off-colony grids may also be important for disease dynamics. *Chaetodipus hispidus* was highly abundant at off-colony grids at Comanche, Cimarron and Janos. This species was not captured at Thunder Basin and occurred in low abundance at Badlands and Wind Cave. *Spermophilus tridecemlineatus* was significantly more abundant at on-colony grids at Badlands and Wind Cave but showed no variation with the presence of black-tailed prairie dogs in plague areas. They were more abundant in plague areas than no-plague areas. Ground squirrels in general are considered to be susceptible to plague (Barnes 1982, Davis *et al.* 2002). Cully *et al.* (1997) reported the disappearance of thirteen-lined ground squirrels shortly before a plague epizootic among Gunnison's prairie dogs in the Moreno valley of New Mexico and considered them likely to play a role in the transmission of plague there. *Perognathus flavus* was highly abundant at off-colony grids at Janos and at on-colony grids at Cimarron and Comanche. Rodents that are abundant in the surrounding grasslands could

play a role in interspecific transmission of the plague pathogen and could be important for introducing plague to the prairie dog colonies. Also, rodents that are found in low abundance, such as *Neotoma* sp., sometimes could harbor multiple flea species in high density, and could also play a role in maintaining plague (Murray and Barnes 1969, Davis *et al.* 2002).

Areas with a known history of plague had higher species richness and higher abundance of rodents that are likely enzootic hosts, than areas without a known history of plague, except Janos. We do not attribute the patterns of rodent abundance and species composition to plague directly, but suggest the possibility that the pattern we observed could be important for disease maintenance. The eastern boundary of plague in the United States may or may not be due to the geographical changes in the rodent species dynamics. Heterogeneity in the rodent community may also have implications for the ectoparasite community that infect these animals and in turn, the transmission of vector-borne pathogens (Stanko *et al.* 2002, Holt *et al.* 2003). It is imperative to evaluate the multiple interactions of rodent species and their ectoparasites, particularly fleas, to understand the dynamics of plague in the prairie dog system.

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Table 2.1: Number of paired grids trapped on prairie dog colonies and the surrounding grasslands in each study area in different years. * Trapped only once in this year.

Area \ year	2000	2001	2002	2003	2004
Badlands		4	4	4	
Wind Cave		4	4	4	
Janos		8	8	8 *	
Cimarron	9	8	8	6	6
Comanche		8 *	8	6	6
Thunder Basin			6	6	6

Table 2.2: Species captured in the study areas. Values indicate the number of individual animals trapped over the period of study.

Species / Area (off-colony/ on-colony)	*BAD*		*WC*		*JAN*		*TB*		*CIM*		*COM*	
	off	on	off	on	off	on	off	on	off	on	off	on
Chaetodipus hispidus (CHAEHISP)	7	3	2	-	11	2	-	-	192	63	51	13
Chaetodipus penicillatus	-	-	-	-	5	-	-	-	-	-	-	-
Dipodomys merriami (DIPOMERR)	-	-	-	-	204	10	-	-	-	-	-	-
Dipodomys ordii (DIPOORDI)	-	-	-	-	1	-	48	8	108	58	5	8
Dipodomys spectabilis (DIPOSPEC)	-	-	-	-	128	85	-	-	-	-	-	-
Lemmiscus curtatus (LEMMCURT)	-	-	-	-	-	-	5	1	-	-	-	-
Microtus ochrogaster (MICROCHR)	7	3	7	-	-	-	1	1	-	-	-	-
Mus musculus	-	-	-	-	1	-	-	-	-	-	-	1
Neotoma albigula (NEOTALBI)	-	-	-	-	17	-	-	-	1	3	-	-
Neotoma micropus (NEOTMICR)	-	-	-	-	-	-	-	-	10	9	3	2
Onychomys leucogaster (ONYCLEUC)	4	38	-	-	6	-	37	77	108	411	47	163
Onychomys torridus (ONYCTORR)	-	-	-	-	12	10	-	-	-	-	-	-
Perognathus fasciatus (PEROFASC)	-	-	-	-	-	-	5	3	-	-	-	-
Perognathus flavescens	-	-	-	-	-	-	-	-	2	-	2	-
Perognathus flavus (PEROFLAV)	-	-	-	-	101	30	-	-	37	20	16	9
Peromyscus boylii	-	-	-	-	-	-	-	-	-	-	1	-
Peromyscus leucopus (PEROLEUC)	-	-	-	-	7	-	1	2	5	2	1	2
Peromyscus maniculatus (PEROMANI)	36	212	62	322	22	-	527	464	132	117	94	13
Reithrodontomys megalotis (REITMEGA)	-	-	-	-	-	-	1	-	14	1	5	1
Reithrodontomys montanus (REITMONT)	-	-	-	-	-	-	-	2	32	8	7	3
Sigmodon hispidus	-	-	-	-	-	-	-	-	4	1	-	1
Spermophilus spilosoma	-	-	-	-	-	4	-	-	-	-	-	-
S. tridecemlineatus (SPERTRID)	20	9	10	1	-	-	33	28	28	27	36	29

Figure Legends

Figure 2.1: Map showing the historical range of the black-tailed prairie dogs (BTon-colony), counties that are positive for plague (source: CDC), and the study areas.

Figure 2.2: a) Total number of rodents captured: individual and recaptures during the period of study and the number of paired grids (n) trapped in that year in all study areas. b) Number of rodents captured by year. Grey symbols represent off-colony grids and black symbols represent on-colony grids. c) Estimated number of species using program COMDYN at off- and on-colony grids in each study area. Error bars indicate 95% estimated confidence limits. d) Correlation between number of species caught and abundance. Error bars indicate 90% estimated confidence limits. e) Mean Shannon Diversity, over the period of study. Error bars indicate 90% estimated confidence limits. f) Mean Shannon Evenness, over the period of study. Error bars indicate 90% estimated confidence limits. All comparisons marked with an asterix (*) are significantly different ($p<0.1$).

Figure 2.3: Estimated mean number of individuals with 90% confidence limits in areas without plague – a) Badlands, b) Wind Cave and c) Janos. Refer Table 2.2 for species name.

Figure 2.4: Estimated mean number of individuals with 90% confidence limits in areas with plague – a) Thunder Basin, b) Cimarron and c) Comanche. Refer Table 2.2 for species name.

Figure 2.5: Numbers of individuals captured per grid over three years at off- and on-colony grids in plague and no-plague areas. Error bars indicate 90% estimated confidence limits.

Figure 2.1

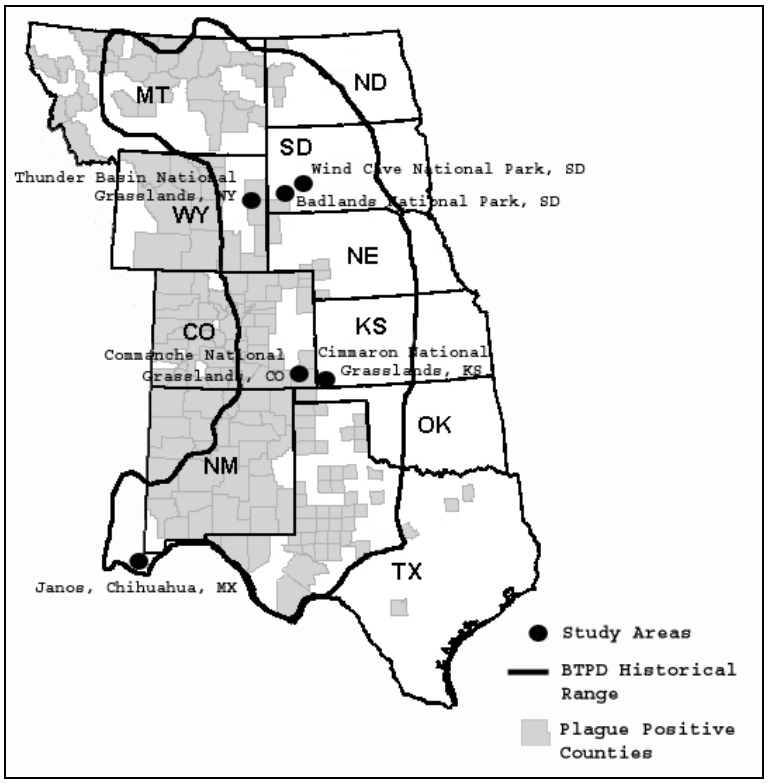

MT
ND
SD
Wind Cave National Park, SD
Thunder Basin National Grasslands, WY
Badlands National Park, SD
WY
NE
KS
CO
Commanche National Grasslands, CO
Cimmaron National Grasslands, KS
OK
NM
TX
Janos, Chihuahua, MX
Study Areas
BTPD Historical Range
Plague Positive Counties

Figure 2.2

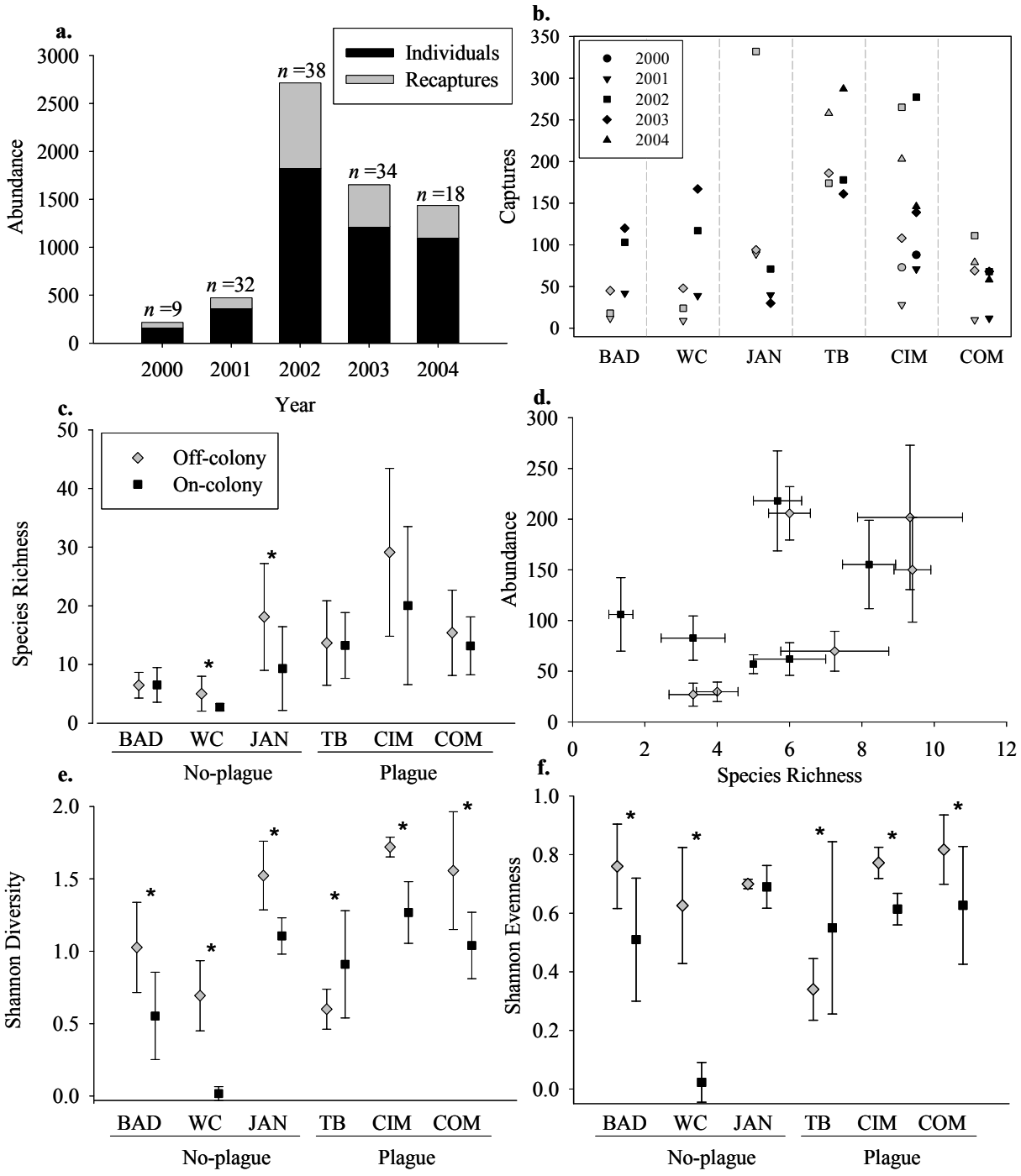

a.
Individuals
Recaptures
Abundance
Year
n =9
n =32
n =38
n =34
n =18
2000
2001
2002
2003
2004
b.
Captures
BAD
WC
JAN
TB
CIM
COM
c.
Off-colony
On-colony
Species Richness
No-plague
Plague
d.
Abundance
Species Richness
e.
Shannon Diversity
f.
Shannon Evenness

Figure 2.3

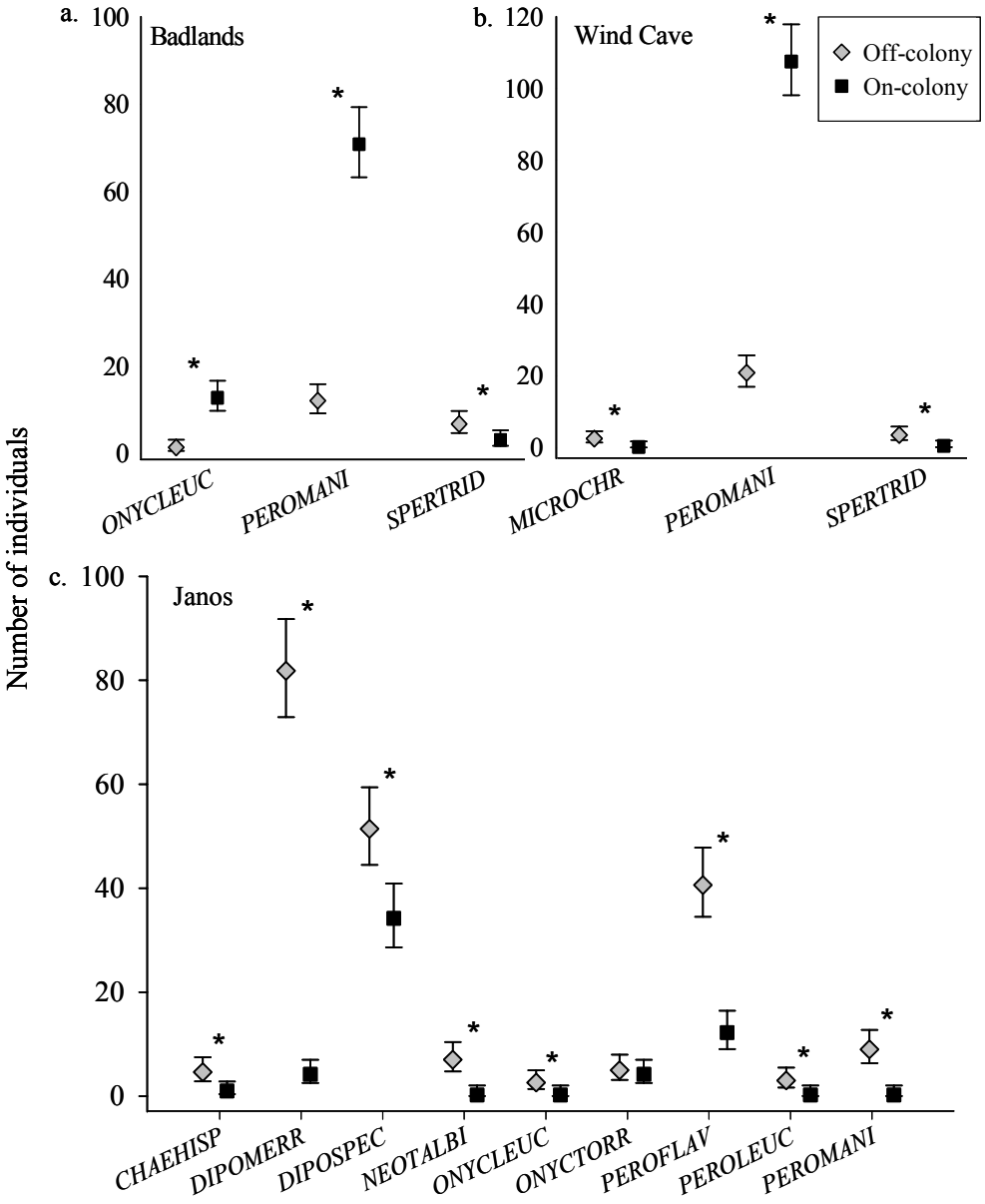

a.
Badlands
b.
Wind Cave
Off-colony
On-colony
c.
Janos
Number of individuals
0
20
40
60
80
100
120
ONYCLEUC
PEROMANI
SPERTRID
MICROCHR
CHAEHISP
DIPOMERR
DIPOSPEC
NEOTALBI
ONYCTORR
PEROFLAV
PEROLEUC

Figure 2.4

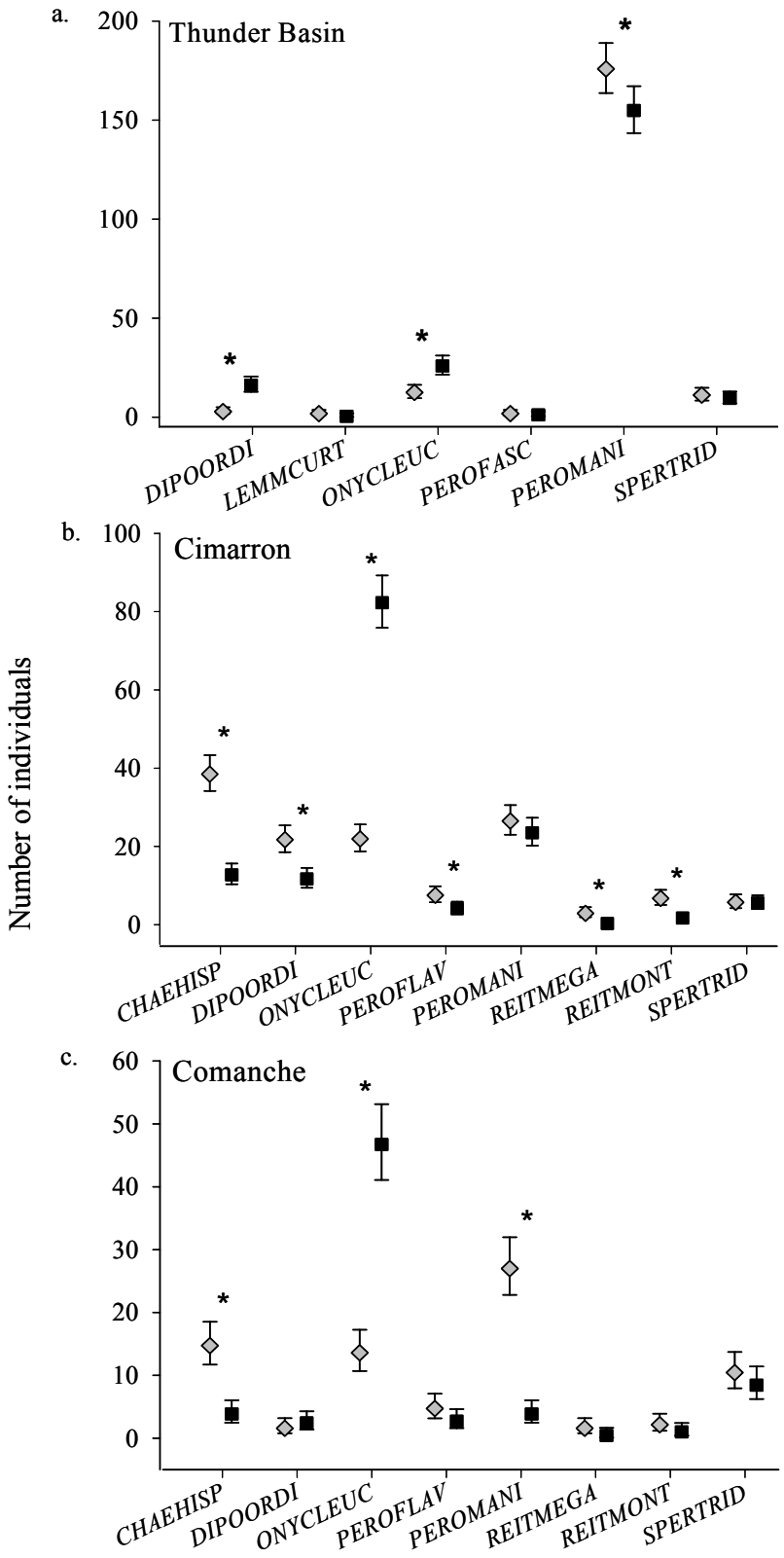
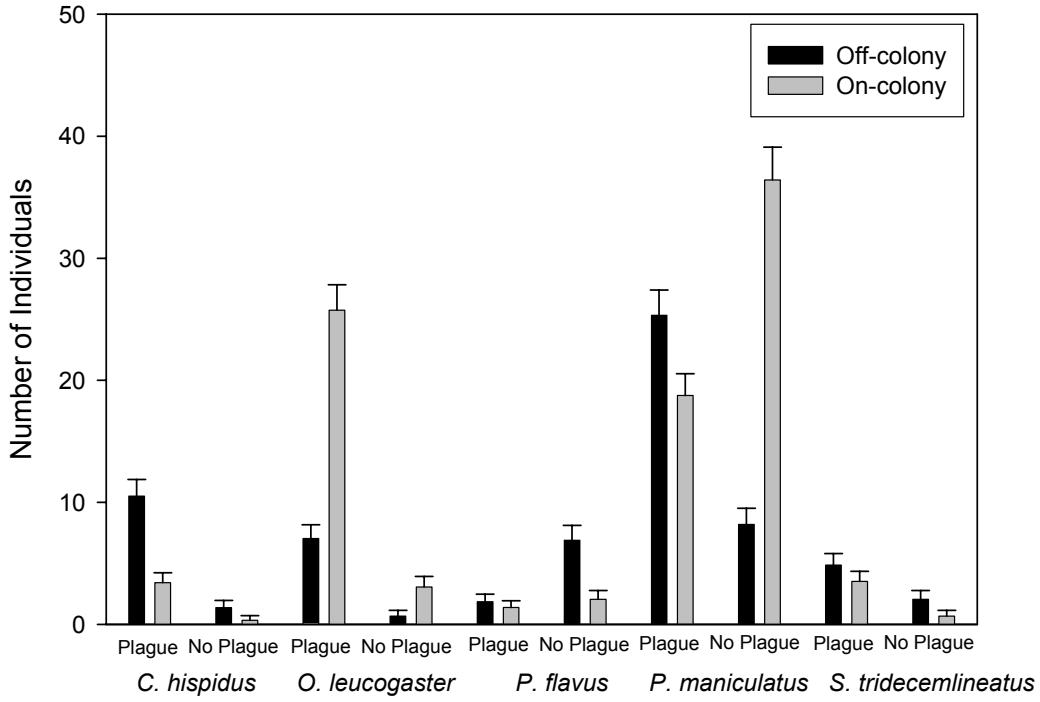

a.
Thunder Basin
200
150
100
50
0
DIPOORDI
LEMMCURT
ONYCLEUC
PEROFASC
PEROMANI
SPERTRID
b.
Cimarron
100
80
60
40
20
0
Number of individuals
CHAEHISP
DIPOORDI
ONYCLEUC
PEROFLAV
PEROMANI
REITMEGA
REITMONT
SPERTRID
c.
Comanche
60
50
40
30
20
10
0
CHAEHISP
DIPOORDI
ONYCLEUC
PEROFLAV
PEROMANI
REITMEGA
REITMONT
SPERTRID

Figure 2.5


Off-colony
On-colony
Number of Individuals
50
40
30
20
10
0
Plague
No Plague
Plague
No Plague
Plague
No Plague
Plague
No Plague
Plague
No Plague
C. hispidus
O. leucogaster
P. flavus
P. maniculatus
S. tridecemlineatus

Chapter 3

GEOGRAPHIC VARIATION IN FLEA-RODENT RELATIONSHIPS IN THE PRESENCE OF BLACK-TAILED PRAIRIE DOG COLONIES*

Abstract

Plague is a vector-borne zoonosis transmitted between fleas and their rodent reservoirs. As a first step towards understanding the role of fleas and other rodents in plague epizootics in black-tailed prairie dogs, we characterized the relationship between fleas and their rodent hosts in the presence of prairie dog colonies and compared them to adjacent assemblages away from colonies. We evaluated the rodent-flea relationship by quantifying prevalence, probability of infestation, flea load, and intensity of fleas on rodents. As prairie dog burrows are thought to provide refugia for fleas, we hypothesized that prevalence, flea load, and intensity would be higher for rodents that are associated with black-tailed prairie dog colonies. Rodents were trapped at off- and on-colony grids, resulting in the collection of 4509 fleas from 1430 rodents in six study areas. The rodent community composition varied between these study areas. Flea species richness was not different between prairie dog colonies and the surrounding grasslands ($p = 0.883$) but was positively correlated with rodent species richness ($p = 0.055$). Prairie dog colonies did not increase the prevalence of fleas ($p > 0.10$). Flea loads on rodents did not vary between off- and on-colony grids at three of the study areas ($p > 0.10$). Based on the prevalence, infestation rates, and flea loads, we identified *Peromyscus maniculatus, Onychomys leucogaster,*

* Authors: Bala Thiagarajan[1], Jack F. Cully Jr.[1, 2], Thomas M. Loughin[3], John A. Montenieri[4], and Kenneth L. Gage[4]
[1]Division of Biology, Kansas State University, [2]Kansas Co-Op Fish and Wildlife Research Unit, USGS, Kansas State University, Manhattan, KS, [3]Department of Statistics, Kansas State University, [4]Vector Ecology Lab, DVBID, Centers For Disease Control and Prevention, Fort Collins, CO.

and two *Neotoma* species as important rodent hosts for fleas and *Aetheca wagneri, Orchopeus leucopus, Peromyscopsylla hesperomys, Pleochaetis exilis,* and *Thrassis fotus* as the most important fleas associated with these rodents. Prairie dog colonies did not seem to facilitate transmission of fleas between rodent hosts, and the few rodent- flea associations exhibited significant differences between off- and on-colony grids.

Keywords: Fleas, Rodents, Generalized linear mixed models, prairie dogs, prevalence, intensity, flea load, infestation.

Introduction

Rodents are one of the most successful and diverse groups of mammals, being found in a wide variety of habitats. Rodents are also important sources of infection for various viral, rickettsial and bacterial pathogens that cause disease in humans. Rodent-borne diseases in humans include plague, typhus, spotted fever, Hantavirus and Lyme disease. Many of these diseases are transmitted by arthropod vectors infesting rodent reservoirs. At present, our lack of understanding about the ecology of these diseases limits our ability to prevent them in humans and limit their impacts on wildlife populations (Woodhouse *et al*. 2001, Dobson and Foufopoulos 2001).

Fleas (Order Siphonaptera), are ectoparasites of warm-blooded vertebrate hosts, especially mammals, which are primary hosts for 94% of the known flea species. Some fleas are the only significant vectors of plague, which is caused by the bacterium *Yersinia pestis* (Hubbard 1947, Thomas 1988, Anderson and Williams 1997, Cully *et al*. 1997). The normal hosts of this disease are certain rodent species, many which are highly susceptible to the disease and often

experience severe population reductions in areas where plague is native or has been introduced (Barnes 1993). Most human plague cases involve contact with fleas of wild or commensal rodents. In the United States, plague usually becomes apparent only when epizootics greatly reduce or eradicate colonies of wild rodents such as prairie dogs or ground squirrels. Black-tailed prairie dogs infected with plague suffer close to 100% mortality (Cully and Williams 2001) and this has been identified as the single greatest factor likely to affect prairie dog populations in the future (Knowles and Knowles 1984, Hoogland 2006).

Black-tailed prairie dogs (*Cynomys ludovicianus*) are considered a keystone species and ecosystem engineer because of their role in the alteration of the vegetation structure and the wide diversity of species that are associated with the colonies (Kotliar 2000, Collinge *et al.* in press). Prairie dog burrows provide habitat for rodents and are important as an off-host environment for fleas, providing ideal conditions for maintaining large flea populations (Cully and Williams 2001, Gage and Kosoy 2005). Prairie dogs may also affect the distribution and abundance of fleas; in one study, flea abundance and flea species richness were higher on rodents captured from prairie dog colonies (Collinge *et al*. In press).

Host-specificity of fleas is variable and although most fleas are rarely host-specific (Whittaker 1968), some species have one or two preferred hosts of a particular genus or family whereas others will feed on any available vertebrate host (Stark 1958, Traub and Starcke 1980, Clover *et al.* 1989, Thomas 1996). Hosts also differ in their ability to harbor fleas; some hosts harbor only one flea species whereas others may harbor multiple flea species (Traub and Starcke 1980, Thomas 1996, Caro *et al.* 1997). Most mammals have several species of fleas which they readily share. The flea-host relationship may show a complex dynamic in relation to vector-

borne diseases, particularly within social or colonial species, such as prairie dogs (Alexander 1974, Hoogland and Sherman 1976). Krasnov *et al.* (2004a) showed a positive relationship between species diversity of fleas and mammal hosts.

Ectoparasites, such as fleas, are not only influenced by their host characteristics, but also by the characteristics of their off-host environment (Marshall 1981, Combes 2001). For example, Krasnov *et al.* (2004b) showed that the species richness of ectoparasites is affected little by the parameters of the host body and to a greater extent by the parameters related to the host environment, reflecting the importance of the role of environment in mediation of flea-host relationships and the survival and development of the non-parasitic immature stages of the flea life cycle. There is a constant exchange of fleas between animals living in close proximity and this interchange increases the possibility of spread of pathogens from one flea-host complex to another. Knowledge of host-vector relationships in the prairie dog system will improve our understanding of how community structure influences the potential for disease emergence. This in turn provides insights for disease management and conservation of threatened species such as black-tailed prairie dogs and black-footed ferrets (Cully and Williams 2001, Miller and Cully 2001).

Quantification of host-parasite associations from field data, and research on relative abundances and spatial and temporal patterns of host and vector distribution is a fundamental step towards understanding host-parasite and host-parasite-pathogen dynamics (Cumming 2004). In this study we sought to characterize the relationship of fleas and their rodent hosts from data collected on a wide geographic scale, within study sites across the current range of the black-tailed prairie dogs. We enumerated the number of flea species collected from different rodent

hosts from prairie dog colonies and from the surrounding grasslands, and tested for relationships between species richness of fleas and their rodent hosts. Rodent species varied in their abundance and in the presence of prairie dog colonies in our study areas (Chapter-1) and hence we expected the rodent-flea relationship to vary among the study areas.

We quantified the rodent-flea relationship by estimating the prevalence, probability of infestation, flea load, and mean intensity of fleas on rodents. The prevalence, defined as the proportion of rodents that were infested with fleas among all rodents captured, is predicted to vary with rodent species in the study areas. We tested whether this probability of infestation by fleas and flea load (the number of fleas per infested rodent host) differed among different rodent species. We estimated the mean intensity as the number of individuals of a given flea species per infested rodent host (Rozsa *et al.* 2000) as a means to characterize the relationship of each flea species collected from a study area with its rodent host. Given that prairie dog burrows provide an ideal off-host environment for fleas and could influence the transmission of fleas between rodents, we hypothesized that (1) Species richness of fleas would be higher on prairie dog colonies (2) Prevalence of fleas would be higher on prairie dog colonies than the surrounding grasslands (3) Probability of infestation of a rodent would be higher on prairie dog colonies and (4) Flea loads would be higher on prairie dog colonies. We also identified spatial patterns in flea-host relationships and geographical variation in abundance of fleas.

Study Design and Methods

Study sites: We trapped rodents in five areas in the mid-western US and one area in Mexico. At each of these areas, rodents were trapped on prairie dog colonies and in the surrounding grasslands where prairie dog colonies were absent. The study areas were - Wind Cave National

Park (Wind Cave) and Badlands National Park (Badlands) in South Dakota, Thunder Basin National Grasslands in Wyoming (Thunder Basin), Cimarron National Grassland in Kansas (Cimarron), Comanche National Grassland in Colorado (Comanche) and Chihuahua Grasslands near Janos in Chihuahua, Mexico (Janos), all of which are within the distribution range of the black-tailed prairie dogs (Fig. 3.1). Rodents were trapped for this study at Cimarron and Comanche between 2001 and 2004; at Thunder Basin between 2002 and 2004; and at Badlands, Wind Cave and Janos between 2001 and 2003. We trapped different number of grids across years in these areas (Table 3.1). Grids on prairie dog colonies are hereafter termed as 'on-colony grids' and grids in the surrounding grasslands are hereafter termed as 'off-colony grids'. The off-colony grids were selected based on their similarity to on-colony grids in vegetation and topography, and were between 500-2000 m from on-colony grids. Each grid was trapped for three consecutive nights on two separate occasions each year, once in May-June and once in July-August.

Rodent and flea sampling: Each trapping grid had 49 trap stations (7X7) at 20m intervals and rodents were trapped with a single Sherman live-trap at each station (H. Sherman Traps, Tallahassee, FL). The traps were opened in late afternoon, baited with oatmeal and checked the following morning. All captured rodents were moved to a processing site and were ear-tagged, sexed, identified to species, and examined for ectoparasites. Animals recaptured within the same trapping session were not reprocessed for fleas. Rodents and their fleas were anaesthetized with a mixture of isofluorane and oxygen using a vaporizer (SurgiVet, Waukesha, WI). After anaesthetization, rodents were held over a white plastic tray and combed vigorously for fleas, with a plastic toothbrush (Gage 1999). Additional fleas from the plastic bag used in handling the animal and the anaesthetizing jar were collected. Fleas collected from each animal were

preserved in a vial with 2% saline solution and sent to the Centers for Disease Control and Prevention (CDC) in Fort Collins. We limited the number of rodents examined during a trap session in each species to 10 individuals on each grid in 2003 and 2004. All rodents were released at their capture sites after data collection. The research methods were approved by the Institutional Animal Care and Use Committee of Kansas State University.

Flea identification: All fleas were identified in the Flea-borne Diseases Laboratory at CDC, Fort Collins, CO. Fleas were identified to species and sexed under a dissecting microscope (Hubbard 1947, Stark 1958, 1970, Furman and Catts 1982) and current taxonomic revisions follow Lewis (1990, 1993, 2000, 2002). Voucher specimens of all flea species were collected and are stored at CDC.

Statistical Analyses:

Species richness

We compared the flea species richness in each study area between off- and on-colony grids using paired t-tests combining all years to test for differences in the presence and absence of black-tailed prairie dog colonies. To test the relationship between host diversity and parasite diversity, we used a correlation analysis on the total number of rodent species captured across years versus the total number of flea species collected across years at both off- and on-colony grids, in all areas.

Probability of infestation

We estimated the probability of infestation by fleas for rodents based on observed prevalence to account for sampling efforts and variability in rodent capture. For each area, we modeled the proportion of individuals infested with fleas against rodent species, grid (off- or on-

colony), and their interaction using mixed-effects logistic regression (Proc Glimmix in SAS v9.1; SAS Institute, Cary, NC) with year as a random effect. When this model provided a poor fit (large deviance / df ratio), we added random year*rodent and / or year*grid interactions to further reduce overdispersion, until deviance / df ratios were reasonably close to 1. Otherwise, when the estimated variance component for year was not positive, it was removed from the model and a fixed effects model was used. We used a type III likelihood ratio test ($\alpha = 0.1$; Agresti 1996) to backward-eliminate non-significant fixed effects from the model. Because sparse data adversely affects the fit of models and subsequent inferences, species that had no more than 3 total captures in the study area were not included in the statistical analyses. We also added a constant of 0.01 to flea numbers to aid model fitting when zero counts interfered with the model fit and interpretation.

Flea load

For each area, we modeled the flea load against rodent species and grid using a Poisson rate regression (Proc Glimmix) with year as a random effect. Random effects modeling and backward-elimination of fixed effects were carried out as above. We estimated the flea load for all rodent species infested with fleas. We tested for significant differences between off- and on-colony flea loads for rodents present at both treatments using pairwise comparisons.

Intensity of fleas

For each area, we estimated the mean intensity of fleas of each species on rodents for both off and on-colony grids using a Poisson rate regression (Proc Glimmix) with year as a random effect. Random effects modeling was carried out as above except that (a) rodent*year and flea*year random effects were added as needed, and (b) modeling was done separately by grid

type as not all fleas and rodents were present at both grids, which interfered with model convergence.

Results

We captured 6,291 rodents over four years. Of these, 1,430 rodents had 4,509 fleas. Twenty flea species were collected from 18 rodent species (Table 3.2).

***Flea species richness**:* Flea species richness was variable among the study areas. Across areas, there was no overall significant difference in flea species richness between off- and on-colony grids ($t = 0.1546$, $df = 5$, $p = 0.883$; Fig 3.2a). We observed lower flea species richness at off-colony grids at Badlands and Comanche, and we observed higher flea species richness at off-colony grids at Wind Cave, Janos and Thunder Basin. At Cimarron, we did not observe any difference in flea species richness between off- and on-colony grids. The highest number of flea species was observed at Thunder Basin, which along with Cimarron and Comanche had higher flea species richness at on-colony grids than the other areas. Five flea species were caught from rodents only at on-colony grids - *Opisocrostis t. tuberculatus*, *Rhadinopsylla* spp. on *O. leucogaster* from Thunder Basin, *Opisodaysis keeni* on *P. maniculatus*, *Oropsylla montana* on *C. hispidus* from Janos and *Pulex simulans* on *O. leucogaster* from Cimarron. However, these species were collected only once and were not common at on-colony grids.

***Relationship between host and parasite diversity**:* There was a weak linear relationship between flea species richness and rodent species richness ($r = 0.564$, $p = 0.05$, $df = 10$; Fig 3.2b), when both off- and on-colony grids were considered. However, the relation was not statistically significant when off- and on-colony grids were tested separately ($r = 0.568$, $df = 5$ and $r = 0.616$,

df = 5 respectively), even though the strength of the relationship was almost the same in all cases. Low sample size (n=6) limited the power of the tests. All rodent species caught at Badlands, Thunder Basin and Cimarron had fleas. Three of the four rodent species caught at Wind Cave, nine of the thirteen species at Janos and ten of the fourteen species at Comanche had fleas.

***Prevalence of fleas**:*

Based on the observed prevalence of fleas on rodents, we estimated the probability of infestation for each rodent species at each study area. There was no significant difference in the probability of infestation by fleas on rodents between off- and on-colony grids in any of the study areas (all $p > 0.10$). At Badlands and Wind Cave, rodents did not differ significantly in their probability of infestation ($F_{4, 15} = 1.35$, $p = 0.2969$ and $F_{2, 7} = 1.19$, $p = 0.36$ respectively). Cimarron and Janos had the highest probability of infestation of rodents while Badlands had the lowest.

At Badlands, the estimated probability of infestation ranged between 0.20 and 0.50 (Fig. 3.3a). *Microtus ochrogaster* had the lowest probability and *O. leucogaster* had the highest probability of infestation by fleas. This is the only area where *P. maniculatus* had a higher probability of infestation than the mean infestation probability of all rodents. At Wind Cave, the probability of infestation ranged from 0.30 to 0.58 (Fig. 3.3b) and there was no significant difference in infestation among rodent species. *Spermophilus tridecemlineatus* had the highest infestation probability. At Thunder Basin, the probability of infestation by fleas varied among rodent species ($F_{9, 24} = 4.31$, $p = 0.002$) and ranged between 0.27 and 0.65 (Fig. 3.3c). *Onychomys leucogaster* had the highest infestation probability (0.65). At Cimarron, the

infestation probability ranged from less than 0.10 to 0.78 (Fig 3.3d). *Onychomys leucogaster, P. leucopus* and *N. micropus* had high probabilities of infestation. At Comanche, the range was between 0.10 and 0.65 (Fig 3.3e), with *O. leucogaster* having the highest infestation probability. Janos had the widest range of infestation probabilities ranging from 0.01 to 0.91 (Fig 3.3f). *Onychomys torridus*, *O. leucogaster* and *N. albigula* had infestation probabilities higher than the mean. Overall, at Badlands, Thunder Basin and Comanche, *O. leucogaster* had the highest probability of infestation by fleas. At Janos, though the density of *O. leucogaster* was low, they had a higher infestation probability.

Flea load: The mean number of fleas per infested host did not vary between off- and on-colony grids at Badlands, Wind Cave and Thunder Basin and varied between off- and on-colony grids at Cimarron, Comanche and Janos. Flea loads also did not vary among rodent species at Badlands and Thunder Basin (Table 3.3).

At Badlands, *P. maniculatus* had the highest mean flea load; *Microtus ochrogaster* had the highest flea load at Wind Cave and *Lemmiscus curtatus* had the highest flea load at Thunder Basin (Fig. 3.4a, 3.4b and 3.4c respectively). At Cimarron, *O. leucogaster* and *N. micropus* had a significantly higher flea load at on-colony grids than off-colony grids ($t = -7.01$, $df = 1$, $p < 0.0001$ and $t = -1.76$, $df = 1$, $p = 0.0887$ respectively; Fig 3.5a). At Comanche, the flea load of *O. leucogaster* was significantly higher at on-colony grids ($t = -3.96$, $df = 1$, $p = 0.0014$; Fig 3.5b). *Neotoma micropus* had the highest flea load at both off- and on-colony grids. At Janos, *N. albigula* had the highest flea load at both off- and on-colony grids (Fig 3.5c).

Mean intensity of fleas: The best-fit model to estimate the mean intensity of flea species on rodents varied in their random effects among areas and between grids within areas (Table 3.4).

Badlands: Eight species of fleas were collected from five rodent species. Of these, only four flea species were collected from off-colony grids, whereas all eight species were collected from on-colony grids (Table 3.5). *Peromyscopsylla hesperomys* had the highest intensity among all rodents and was highly intense on *O. leucogaster* at off-colony grids and on *C. hispidus* at on-colony grids. *Aetheca wagneri* was intense on *P. maniculatus* at both off- and on-colony grids, but more prevalent on rodents at off-colony grids. *Thrassis fotus* was highly intense on *O. leucogaster* at off-colony grids. Among rodents, *O. leucogaster* had seven species of fleas at on-colony grids with *P. hesperomys* being the most abundant. *Peromyscus maniculatus* was infested with all four species at off-colony grids, with *Aetheca wagneri* being the most abundant.

Wind Cave: We collected seven species of fleas from four rodents (Table 3.6). Of these seven species, *Oropsylla hirsuta* was only found on *P. maniculatus* at on-colony grids. *Aetheca wagneri* was intense on *P. maniculatus* at on-colony grids. *Peromyscus maniculatus* harbored all seven species of fleas captured in Wind Cave, two of which were present only at on-colony grids.

Thunder Basin: We collected 14 species of fleas from this area, with 12 species from off-colony grids and 11 species from on-colony grids (Table 3.7). Five of these species were found in very low intensity and not included in Table 3.7 are: *Rhadinopsylla* spp and *Opisocrostis t. tuberculatus* with a mean intensity of 0.06 from *O. leucogaster* from on-colony grids; *Orchopeas sexdentatus*, *Callistopsyllus deuterus* and *Epitedia wenmanni*, with 0.02 intensity from *P. maniculatus* from off-colony grids. *Aetheca wagneri* was collected from 9 of the 10 rodent species. *Peromyscus leucopus* at off-colony grids, and *Lemmiscus curtatus* and *Perognathus fasciatus* at both off- and on-colony grids had high mean intensities. Mean intensity of *Thrassis*

fotus was high on *S. tridecemlineatus* at both grids. *Peromyscus maniculatus* had 12 species of fleas, with *A. wagneri* being the most intense. *Lemmiscus curtatus* had the highest flea intensity of any rodent in the area. *Onychomys leucogaster* had high intensity of *A. wagneri* on-colony and *Foxella ignota* at off-colony grids.

Cimarron: We collected 10 species of fleas from Cimarron. Among these, *A. wagneri* was found only at off-colony grids on *D. ordii,* and *Pulex simulans* was found on *O. leucogaster* at off-colony grids. Five flea species were most prevalent – *O. hirsuta*, *O. sexdentatus*, *O. leucopus*, *P. exilis* and *T. fotus* (Table 3.8). *Orchopeus leucopus* and *P. exilis* were found on 10 of the 13 rodent species captured. *Oropsylla hirsuta* was intense on *R. megalotis* at off-colony grids and on *S. tridecemlineatus* at on-colony grids. Intensity of *Orchopeus leucopus* was highest on *N. micropus* at both off- and on-colony grids; it was also intense on *Sigmodon hispidus* at off-colony grids. *Orchopeus sexdentatus* was highest on *N. albigula* and *N. micropus* at both off- and on-colony grids. *Pleochaetis exilis* was highest on *N. micropus* at on-colony grids and was also intense on *O. leucogaster* at both grid types. *Thrassis fotus* was highest on *P. flavus* on off-colony grids. Among rodent species, *O. leucogaster* had nine flea species, with *P. exilis* being the most intense. *Peromyscus maniculatus* had low flea prevalence with *O. leucopus* being the most intense. Both *N. albigula* and *N. micropus* had fewer flea species, but had higher mean intensity of fleas.

Comanche: We collected nine flea species at this area and four of them were particularly common – *O. hirsuta*, *O. leucopus*, *P. exilis* and *T. fotus* (Table 3.9). *Oropsylla hirsuta* was intense on *N. micropus* and *O. leucogaster* at on-colony grids; *O. leucopus* was intense on *M. ochrogaster* and *N. micropus* at off-colony grids. Intensity of *Orchopeus sexdentatus* and *P.*

exilis were high on *N. micropus* at both grid types and *T. fotus* intensity was high on *S. tridecemlineatus* at both grids. *Chaetodipus hispidus, O. leucogaster* and *P. maniculatus* had high intensity of *P. exilis*. *Peromyscus maniculatus* also had high intensity of *A. wagneri* and *P. hesperomys* at on-colony grids. *Spermophilus tridecemlineatus* had high intensity of *O. hirsuta, P. exilis* and *T. fotus* at off-colony grids; *P. flavus* had only *P. exilis* at off-colony grids.

Janos: Nine species of fleas were collected from Janos, of which four species were found only at off-colony grids (Table 3.10). *Anomiopsyllus nudatus* was intense on N. albigula at on-colony grids; *Echidnophaga gallinacea* was found on 4 species of rodents and was intense on *N. albigula* at off-colony grids and on *O. torridus* at on-colony grids. *Meringis arachis* was intense on *D. spectabilis* at both off- and on-colony grids and *P. exilis* was intense on *O. torridus* at both grids. Among rodents, *O. torridus* had 5 flea species with *P. exilis* exhibiting the highest intensity. *Onychomys torridus* had high intensity of *E. gallinacea* at on-colony grids and *O. leucopus* at off-colony grids. *Neotoma albigula* had 5 flea species at off-colony grids in contrast to only *A. nudatus* at on-colony grids.

Discussion

Our goal in this study was to quantify rodent-flea relationships in several grasslands and to determine the effect of prairie dog colonies on the distribution and abundance of fleas harbored by various rodent species. Abundance of fleas and flea-host relationships exhibited a geographic variation and were different between the study areas. There was no significant difference in flea species richness between off- and on-colony grids. Prairie dog colonies did not influence the probability of infestation or flea load on rodents in Badlands, Wind Cave or Thunder Basin.

Flea species richness varied among study areas and is related to the rodent community structure in the area. The presence of prairie dog colonies on the landscape does not appear to play a detectable role in enhancing the diversity of flea assemblages among the study areas. Some of the causes for variation in the flea community structure and the abundance of fleas between study areas could be landscape heterogeneity, habitat differences, and contrasting rodent species abundances over the geographical range. The linear relationship between flea species richness and rodent species richness indicates that parasite diversity in our study areas increases with host diversity, as shown in other similar studies (e.g., Krasnov *et al.* 2004a, Krasnov *et al.* 2005).

Flea prevalence was lower than 50% on most rodent species. *Onychomys leucogaster* had consistently high flea prevalence at both off- and on-colony grids in all areas where it was present; flea prevalence was higher at on-colony grids in Thunder Basin, Cimarron and Comanche. Though *O. leucogaster* has been reported to have high diversity of flea species (Thomas 1988), this is the first study to report high prevalence of fleas on *O. leucogaster* on prairie dog colonies. Holdenried and Morlan (1955) reported 31% flea prevalence in *O. leucogaster* from Sante Fe County, New Mexico. Woodrats (*N. albigula* and *N. micropus*) also had higher prevalence of fleas, although their abundance was low. Woodrats are known to have high prevalence of fleas due to their nesting habitats (Holdenried and Morlan 1955, Davis *et al.* 2002). Rodent nests are a primary breeding ground for fleas (Holdenried *et al*. 1951, Ryckman 1971, Bursten *et al.* 1997) and are also the foci for host, flea, and pathogen interaction (Graves *et al.* 1974). Woodrats are known to have high infestation rates (Davis *et al.* 2002) and are also known to carry plague (Holdenried and Quan 1956, Murray and Barnes 1969, Biggins and Kosoy 2001). *Peromyscus maniculatus*, one of the most common rodents on the northern Great

Plains and the only rodent present at all study areas had low prevalence at both off- and on-colony grids in the study areas. Other studies have shown similar low prevalence rates (<50%) for *P. maniculatus* (Holdenried and Morlan 1955, Cully *et al.* 1997, Davis *et al.* 2002). The prevalence rates for *S. tridecemlineatus* were also low, although other members of this genus have been shown to have high flea infestation rates (e.g., 79% in *S. beecheyi*; Davis *et al*. 2002).

The probability of infestation in rodents did not vary between off- and on-colony grids, although rodent species varied in infestation probability at each of the study areas. This shows that prairie dog colonies do not facilitate the infestation of rodents by fleas. *Onychomys leucogaster* consistently had high probability of infestation in all the study areas, both off and on colonies. Other rodents with high infestation probabilities were – *S. tridecemlineatus, N. albigula, N. micropus*, and *O. torridus*.

Flea load did not vary between off- and on-colony grids at the northern grassland study areas – Badlands, Wind Cave, and Thunder Basin; however, it differed between grid types at Cimarron, Comanche, and Janos, the southern grasslands. At Badlands, Wind Cave, and Thunder Basin, rodents that did not have the highest flea prevalence had higher flea loads – *P. maniculatus* at Badlands, *M. ochrogaster* at Wind Cave and *L. curtatus* at Thunder Basin. However, in the southern grasslands, rodents that had high flea prevalence also had high flea loads at both off- and on-colony grids. Flea loads were higher at on-colony grids when they were significantly different between off- and on-colony grids. *Neotoma* spp. and *O. leucogaster* had significantly higher flea loads at on-colony grids at Cimarron. At Comanche also, *O. leucogaster* had significantly higher flea loads at on-colony grids. Given that all of these rodent species are implicated in plague activity associated with prairie dog colonies and are considered as potential

enzootic hosts for plague, it is not surprising that flea loads are higher at on-colony grids (Cully *et al*. 1997, Anderson and Williams 1997, Cully and Williams 2001, Lang 1996, Davis *et al.* 2002, Collinge *et al.* In press).

From the mean intensity estimates, some fleas including *A. wagneri, O. hirsuta, O. leucopus, O. sexdentatus, P. hesperomys, P. exilis* and *T. fotus* were shared between multiple hosts. Similarly, several species of rodents were infested by multiple flea species, including *O. leucogaster, P. maniculatus,* and *Neotoma* species. The highest number of flea species in the study was collected from *P. maniculatus* at Thunder Basin (n=12) and the highest number of rodent species (n=10) were infested by *P. exilis* at Cimarron. Most of the rodent species shared some fleas in all the study areas.

Although we expected the flea-host relationship to be different between off- and on-colony grids due the importance of off-host environment in flea-rodent relationships (Krasnov *et al.* 1997), the characteristics of the flea-rodent relationships were affected more by the characteristics of the host species and geographical location than by the presence of prairie dog colonies. The effect of prairie dog colonies was seen only for a few species of rodents and their fleas and no uniform effect of prairie dog colonies was seen among all the study areas. Nevertheless, variation in the flea load of some rodents on prairie dog colonies might be important in the ecology of vector-borne diseases and further analyses based on the history of plague in these study areas and the presence of a pathogen might help elucidate the rodent-flea complexes that are critical in disease maintenance and transmission.

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Table 3.1: Number of paired grids trapped on prairie dog colonies and the surrounding grasslands in each study area in different years. * Trapped only once in this year.

Area \ year	2001	2002	2003	2004
Badlands	4	4	4	
Wind Cave	4	4	4	
Janos	8	8	8 *	
Cimarron	8	8	6	6
Comanche	8 *	8	6	6
Thunder Basin		6	6	6

Table 3.2: Flea species and rodent species caught and their codes used in tables and figures.

Flea code	Flea species	Rodent Code	Rodent Species
AEWA	*Aetheca wagneri*	CHAEHISP	*Chaetodipus hispidus*
ANNU	*Anomiopsyllus nudatus*	DIPOMERR	*Dipodomys merriami*
CADE	*Callistopsyllus deuterus*	DIPOORDI	*Dipodomys Ordii*
ECGA	*Echidnophaga gallinacea*	DIPOSPEC	*Dipodomys spectabilis*
EPWE	*Epitedia wenmanni*	LEMMCURT	*Lemmiscus curtatus*
FOIG	*Foxella ignota*	MICROCHR	*Microtus ochrogaster*
MATE	*Malaraeus telchinus*	NEOTALBI	*Neotoma albigula*
MEAR	*Meringis arachis*	NEOTMICR	*Neotoma micropus*
MEDI	*Meringis dipodomys*	ONYCLEUC	*Onychomys leucogaster*
MEPA	*Meringis parkeri*	ONYCTORR	*Onychomys torridus*
OPKE	*Opisodaysis keeni*	PEROFASC	*Perognathus fasciatus*
OPTU	*Opisocrostis t. tuberculatus*	PEROFLAV	*Perognathus flavus*
ORHI	*Oropsylla hirsuta*	PEROLEUC	*Peromyscus leucopus*
ORLE	*Orchopeas leucopus*	PEROMANI	*Peromyscus maniculatus*
ORMO	*Oropsylla montana*	REITMEGA	*Reithrodontomys megalotis*
ORSE	*Orchopeas sexdentatus*	REITMONT	*Reithrodontomys montanus*
PEHE	*Peromyscopsylla hesperomys*	SIGMHISP	*Sigmodon hispidus*
PLEX	*Pleochaetis exilis*	SPERTRID	*Spermophilus tridecemlineatus*
PUSI	*Pulex simulans*		
RHAD	*Rhadinopsylla spp.*		
THFO	*Thrassis fotus*		

Table 3.3: Poisson Rate regression best fit models for estimating flea loads on rodent species at off- and on-colony grids at each study area.

Area	Grids	Model	Random Effect	Test Statistic	P-value
Badlands	Both	Rodent	Year	$F_{4, 11} = 0.76$	0.5727
Wind Cave	Both	Rodent	-	$\chi^2_{7} = 6.97$	0.0306
Thunder Basin	Both	Rodent	Rodent*year	$F_{9, 6} = 1.99$	0.2077
Cimarron	Both	Rodent*grid	Year	$F_{9, 30} = 2.56$	0.0255
Comanche	Both	Rodent*grid	Year	$F_{4, 14} = 2.58$	0.0833
Janos	Both	Rodent*grid	Year	$F_{3, 8} = 3.35$	0.0762

Table 3.4: Poisson Rate regression best fit models for estimating the mean intensity of fleas on rodent species at off- and on-colony grids at each study area.

Area	Grids	Model	Random Effect	Test Statistic	P-value
Badlands	Off	Flea*rodent	-	$\chi^2_5 = 14.92$	0.0107
	On	Flea*rodent	Year	$F_{2, 10} = 9.44$	0.0050
Wind Cave	Both	Flea*rodent	-	$\chi^2_3 = 23.35$	0.0001
Thunder Basin	Off	Flea*rodent	Rodent*year	$F_{8, 4} = 25.60$	0.0035
	On	Flea*rodent	Rodent*year	$F_{12, 3} = 12.27$	0.0312
Cimarron	Off	Flea*rodent	Year	$F_{16, 19} = 4.28$	0.0016
	On	Flea*rodent	Flea*year	$F_{19, 17} = 7.73$	0.0001
Comanche	Off	Flea*rodent	Flea*year	$F_{14, 8} = 2.30$	0.1195
	On	Flea*rodent	Flea*year	$F_{8, 5} = 5.13$	0.0442
Janos	Off	Flea*rodent	Flea*year	$F_{5, 3} = 9.85$	0.0443
	On	Flea*rodent	Year	$F_{2, 2} = 2.32$	0.3009

Table 3.5: Estimated Mean Intensity of fleas on infested rodents at off- and on-colony grids at Badlands. (Refer to Table 3.2 for species codes)•

Off-colony	**Flea species**							
Rodent	AEWA	EPWE	FOIG	ORHI	ORLE	PEHE	PLEX	THFO
CHAEHISP	1							
MICROCHR					**1**	**1**		
ONYCLEUC	**1**					***3***		**1**
PEROMANI	***1.42***				0.33	0.17		0.17
SPERTRID	1					0.2		***1.85***
On-Colony								
CHAEHISP						1.16		
MICROCHR					**1.16**			
ONYCLEUC	0.38		0.16	**0.4**	0.12	0.54	0.16	0.4
PEROMANI	***1.7***	0.06		0.06		0.4		
SPERTRID								0.8

• Bold values in each column represent the rodent with the highest intensity of the flea species; Italicized values for each row represent the highest flea intensity for that rodent. Multiple values highlighted in the same row or columns are not significantly different.

Table 3.6: Estimated Mean Intensity of fleas on infested rodents at off- and on-colony grids at Wind Cave. (Refer to Table 3.2 for species codes)[*]

Off-colony	**Flea species**						
Rodent	AEWA	EPWE	MATE	ORHI	ORLE	PEHE	THFO
MICROCHR			**2.00**		***5.33***		
PEROLEUC							
PEROMANI	**1.79**	0.08	0.31			0.31	0.04
SPERTRID	*1.00*				0.50		
On-colony							
MICROCHR							
PEROLEUC			1.00				
PEROMANI	**2.29**	0.04	0.36	0.02	0.04	0.10	0.08
SPERTRID						*1.00*	

[*] Bold values in each column represent the rodent with the highest intensity of the flea species; Italicized values for each row represent the highest flea intensity for that rodent. Multiple values highlighted in the same row or columns are not significantly different.

Table 3.7: Estimated Mean Intensity of fleas on infested rodents at off- and on-colony grids at Thunder Basin. (Refer to Table 3.2 for species codes)•

Off-colony	Flea Species								
Rodent	**AEWA**	**FOIG**	**MATE**	**MEPA**	**ORHI**	**ORLE**	**PEHE**	**PLEX**	**THFO**
DIPOORDI	*1.61*								
LEMMCURT	***3.49***		***6.35***						
MICROCHR			0.95						
ONYCLEUC	0.93	***1.09***		0.20			0.32		0.34
PEROFASC	***2.42***					0.35			
PEROLEUC	**4.36**								
PEROMANI	***2.42***	0.02	0.03	0.01	0.02	0.06	0.10	0.02	0.08
REITMEGA	1.61								
REITMONT									
SPERTRID	0.95						0.20		***2.64***
On-colony									
DIPOORDI	*0.82*	0.25		*0.54*			0.10		0.13
LEMMCURT	***3.50***					*1.00*			
MICROCHR									
ONYCLEUC	***1.22***	0.08		0.07			***0.97***	0.20	0.25
PEROFASC	***2.19***						1.00		
PEROLEUC									
PEROMANI	***2.44***	0.03	0.05	0.02	0.03	0.03	0.21		0.09
REITMEGA									
REITMONT	1.24								
SPERTRID	0.68		0.22						***1.56***

• Bold values in each column represent the rodent with the highest intensity of the flea species; Italicized values for each row represent the highest flea intensity for that rodent. Multiple values highlighted in the same row or columns are not significantly different.

Table 3.8: Estimated Mean Intensity of fleas on infested rodents at off- and on-colony grids at Cimarron. (Refer to Table 3.2 for species codes)•

Off-colony Rodent	Flea Species								
	AEWA	**FOIG**	**MATE**	**ORHI**	**ORLE**	**ORSE**	**PEHE**	**PLEX**	**THFO**
CHAEHISP				0.51		0.09		0.81	0.48
DIPOORDI	0.61				0.30			1.05	
MICROCHR					1.20				
NEOTALBI						***3.40***			
NEOTMICR					***7.30***	**2.29**	0.15	0.61	
ONYCLEUC		0.08			0.33	0.42		***2.73***	0.21
PEROFLAV					0.91				**1.20**
PEROLEUC					**1.50**			0.30	0.30
PEROMANI			0.09		1.08	0.50	0.10	0.52	0.10
REITMEGA				***3.65***				**0.91**	
REITMONT					0.70			0.50	
SIGMHISP					**2.74**				
SPERTRID				0.77				0.70	0.77

On-colony

CHAEHISP				0.17	0.17	0.12		*0.79*	0.24
DIPOORDI	0.61			0.22				*1.14*	
NEOTALBI				0.30	***1.86***	***3.92***			
NEOTMICR					***3.93***	***1.84***		***3.56***	
ONYCLEUC		0.01	0.15	**0.76**	0.07	0.02	0.01	***3.16***	0.05
PEROFLAV					0.74			**1.26**	
PEROLEUC								1.34	
PEROMANI				0.06	*0.70*	0.13	0.14	0.51	0.13
REITMONT					1.31			1.42	
SPERTRID				***2.72***	0.37			*1.58*	0.18

• Bold values in each column represent the rodent with the highest intensity of the flea species; Italicized values for each row represent the highest flea intensity for that rodent. Multiple values highlighted in the same row or columns are not significantly different.

Table 3.9: Estimated Mean Intensity of fleas on infested rodents at off- and on-colony grids at Comanche. (Refer to Table 3.2 for species codes)*

Off-colony	**Flea species**								
Rodent	**AEWA**	**FOIG**	**MATE**	**ORHI**	**ORLE**	**ORSE**	**PEHE**	**PLEX**	**THFO**
CHAEHISP					0.25		0.34	0.79	0.50
DIPOORDI									
MICROCHR					***3.63***				0.81
NEOTMICR					***2.19***	***1.50***		***5.33***	
ONYCLEUC		0.18		0.10	0.14			***1.74***	0.15
PEROFLAV								**1.19**	
PEROMANI	0.17			0.09	0.22	0.11	0.13	*0.69*	0.38
REITMEGA								1.41	
REITMONT					0.25			0.40	
SPERTRID	0.33			**0.83**	0.15			0.74	***1.76***
On-colony									
CHAEHISP								0.92	
DIPOORDI					0.55			0.79	
NEOTMICR				**1.38**		1.00		***2.71***	
ONYCLEUC	0.06	0.05	0.09	***1.45***	0.06		0.05	***1.76***	0.23
PEROMANI	**1.15**				0.55		**1.67**	*1.23*	
SPERTRID				0.16	0.34			0.51	***0.74***

* Bold values in each column represent the rodent with the highest intensity of the flea species; Italicized values for each row represent the highest flea intensity for that rodent. Multiple values highlighted in the same row or columns are not significantly different.

Table 3.10: Estimated Mean Intensity of fleas on infested rodents at off- and on-colony grids at Janos. (Refer to Table 3.2 for species codes)[*]

Off-colony	**Flea species**								
Rodent	**ANNU**	**ECGA**	**MEAR**	**MEDI**	**OPKE**	**ORLE**	**ORMO**	**ORSE**	**PLEX**
CHAEHISP							1.00		
DIPOMERR			0.52						
DIPOSPEC		0.39	***1.47***						0.16
NEOTALBI	0.23	***7.68***				**1.67**		***2.15***	0.24
ONYCLEUC			***1.03***						***2.19***
ONYCTORR			0.49	0.20				0.40	***4.31***
PEROFLAV									
PEROLEUC			0.52						
PEROMANI					0.50	***2.68***			1.00
On-colony									
CHAEHISP									
DIPOMERR									
DIPOSPEC		*2.00*	0.86						0.20
NEOTALBI	**4.00**								
ONYCLEUC									
ONYCTORR		***3.00***	0.50						***2.50***
PEROFLAV	1.00	1.00						1.00	

[*] Bold values in each column represent the rodent with the highest intensity of the flea species; Italicized values for each row represent the highest flea intensity for that rodent. Multiple values highlighted in the same row or columns are not significantly different.

Figure Legends

Figure 3.1: Map of the mid-west, showing the historical range of the black-tailed prairie dogs and our study areas.

Figure 3.2: a) Species richness of fleas, collected from rodents at off- and on-colony grids, from the six study areas. b) Relationship between rodent species richness and flea species richness. Solid line represents the linear regression. Dashed lines indicate 90% confidence limits and the dotted lines indicate predicted intervals. Grey circles represent off-colony grids and dark circles represent on-colony grids.

Figure 3.3: Probability of infestation by fleas estimated using logistic regression for all rodent species. Error bars indicate 90% confidence limits. a) Badlands, b) Wind Cave, c) Thunder Basin, d) Cimarron, e) Comanche, and f) Janos (Refer to Table 3.2 for species codes). Reference line represents the mean probability of infestation for that area. The rodents are arranged in increasing order of their probability of infestation and rodents on the right end of the x-axis have the highest infestation probabilities.

Figure 3.4: Mean flea load on rodent species estimated using Poisson rate regression. Error bars indicate 90% confidence limits. a) Badlands, b) Wind Cave, and c) Thunder Basin (Refer to Table 3.2 for species codes). Reference line represents mean flea load for that area. The rodents are arranged in increasing order of their flea loads and rodents on the right end of the x-axis have the highest flea loads.

Figure 3.5: Mean flea load on rodent species from off- and on-colony grids estimated using Poisson rate regression. Error bars indicate 90% confidence limits. a) Cimarron, b) Comanche, and c) Janos (Refer to Table 3.2 for species codes). Reference line represents mean flea load for that area. Asterisks represent significant pairwise differences at the 0.1 level. The rodents are arranged in increasing order of their flea load at on-colony grids and rodents on the right end of the x-axis have the highest flea loads at on-colony grids and rodents with fleas from only off-colony grids are on the left end.

Figure 3.1

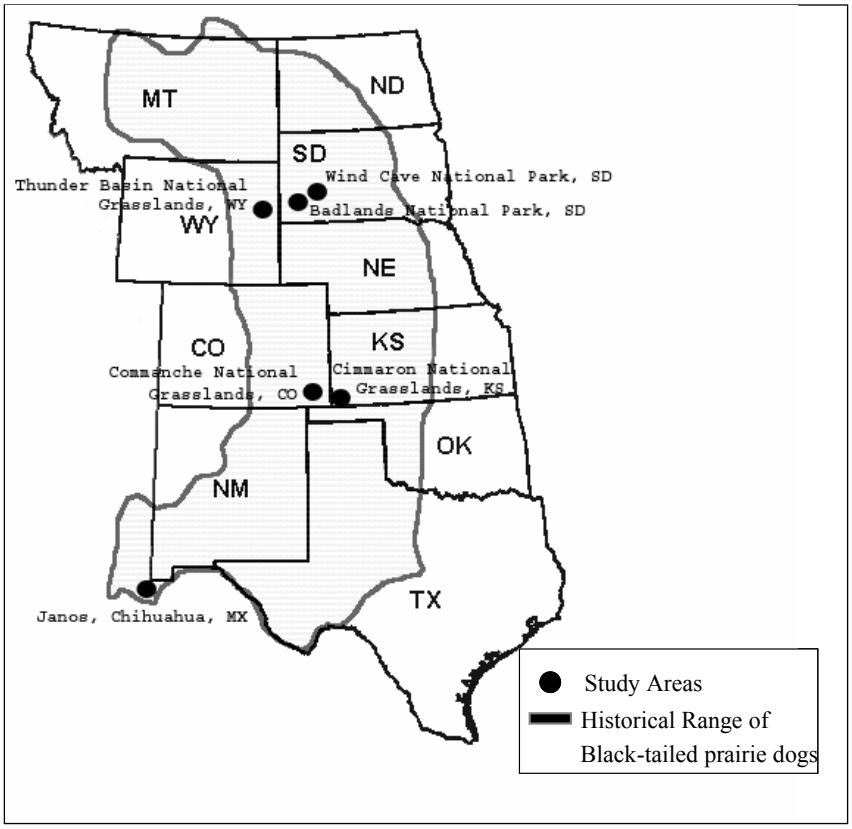

MT
ND
SD
Wind Cave National Park, SD
Thunder Basin National Grasslands, WY
Badlands National Park, SD
WY
NE
CO
KS
Comanche National Grasslands, CO
Cimmaron National Grasslands, KS
OK
NM
TX
Janos, Chihuahua, MX
Study Areas
Historical Range of Black-tailed prairie dogs

Figure 3.2

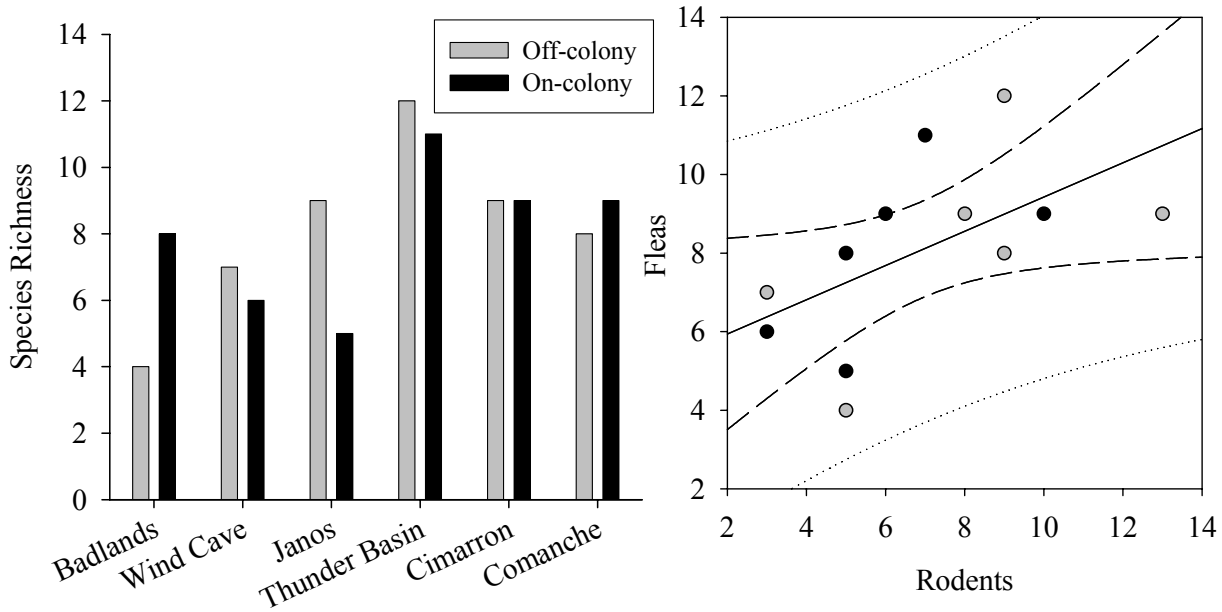

Species Richness
Off-colony
On-colony
Badlands
Wind Cave
Janos
Thunder Basin
Cimarron
Comanche
Fleas
Rodents

Figure 3.3

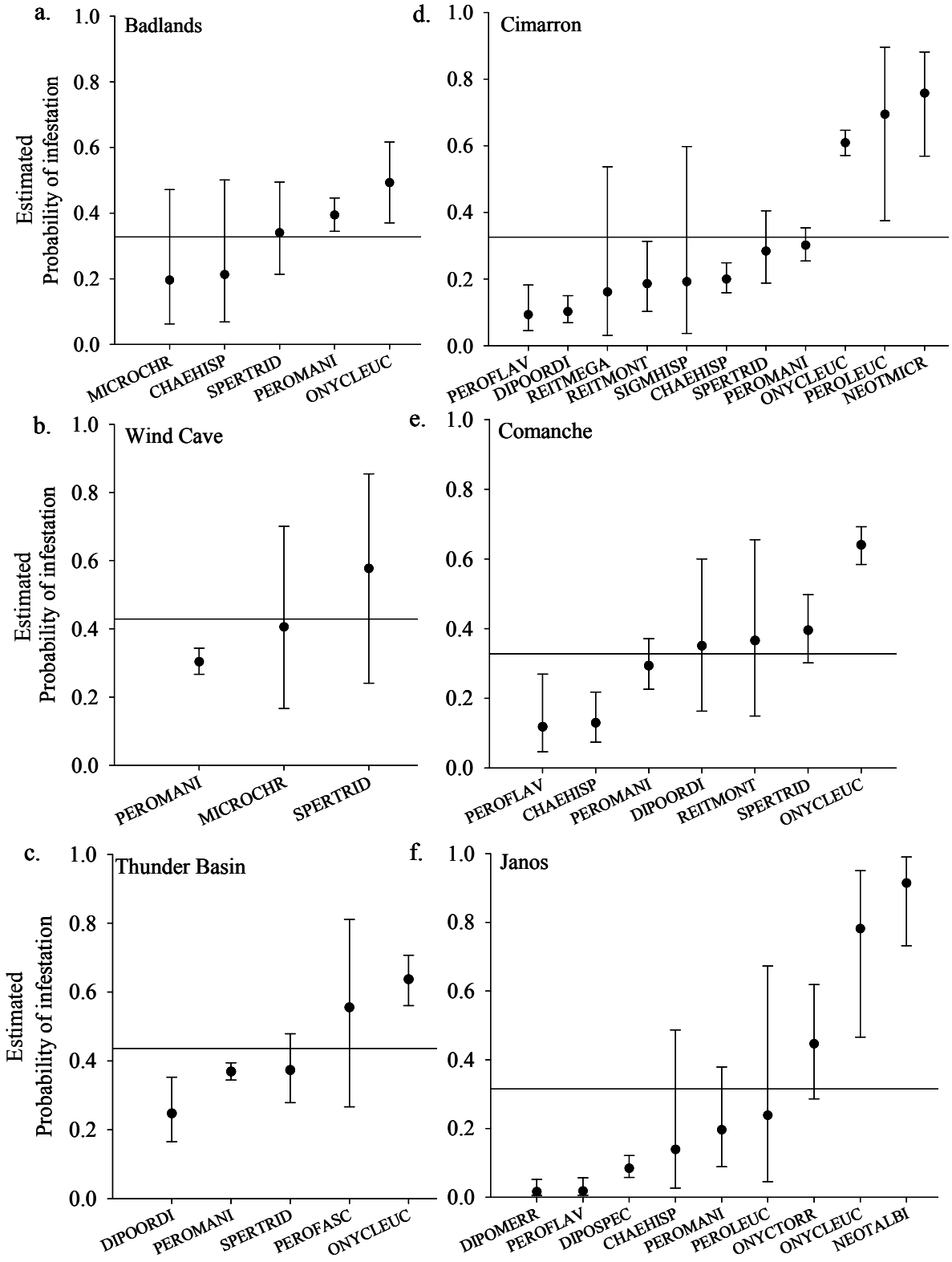

a.
Badlands
Estimated Probability of infestation
1.0
0.8
0.6
0.4
0.2
0.0
MICROCHR
CHAEHISP
SPERTRID
PEROMANI
ONYCLEUC
d.
Cimarron
PEROFLAV
DIPOORDI
REITMEGA
REITMONT
SIGMHISP
CHAEHISP
SPERTRID
PEROMANI
ONYCLEUC
PEROLEUC
NEOTMICR
b.
Wind Cave
PEROMANI
MICROCHR
SPERTRID
e.
Comanche
PEROFLAV
CHAEHISP
PEROMANI
DIPOORDI
REITMONT
SPERTRID
ONYCLEUC
c.
Thunder Basin
DIPOORDI
PEROMANI
SPERTRID
PEROFASC
ONYCLEUC
f.
Janos
DIPOMERR
PEROFLAV
DIPOSPEC
CHAEHISP
PEROMANI
PEROLEUC
ONYCTORR
ONYCLEUC
NEOTALBI

Figure 3.4

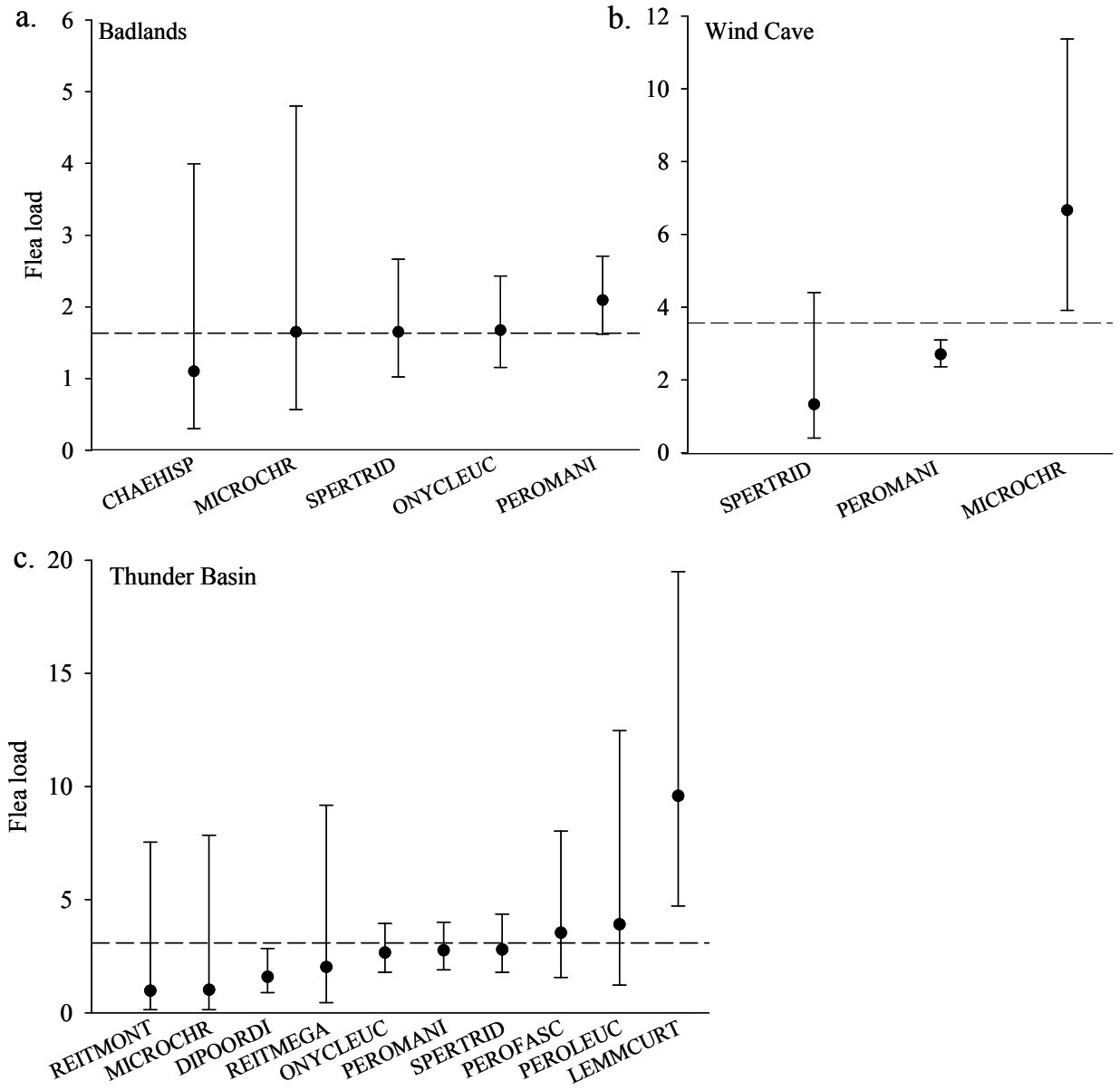
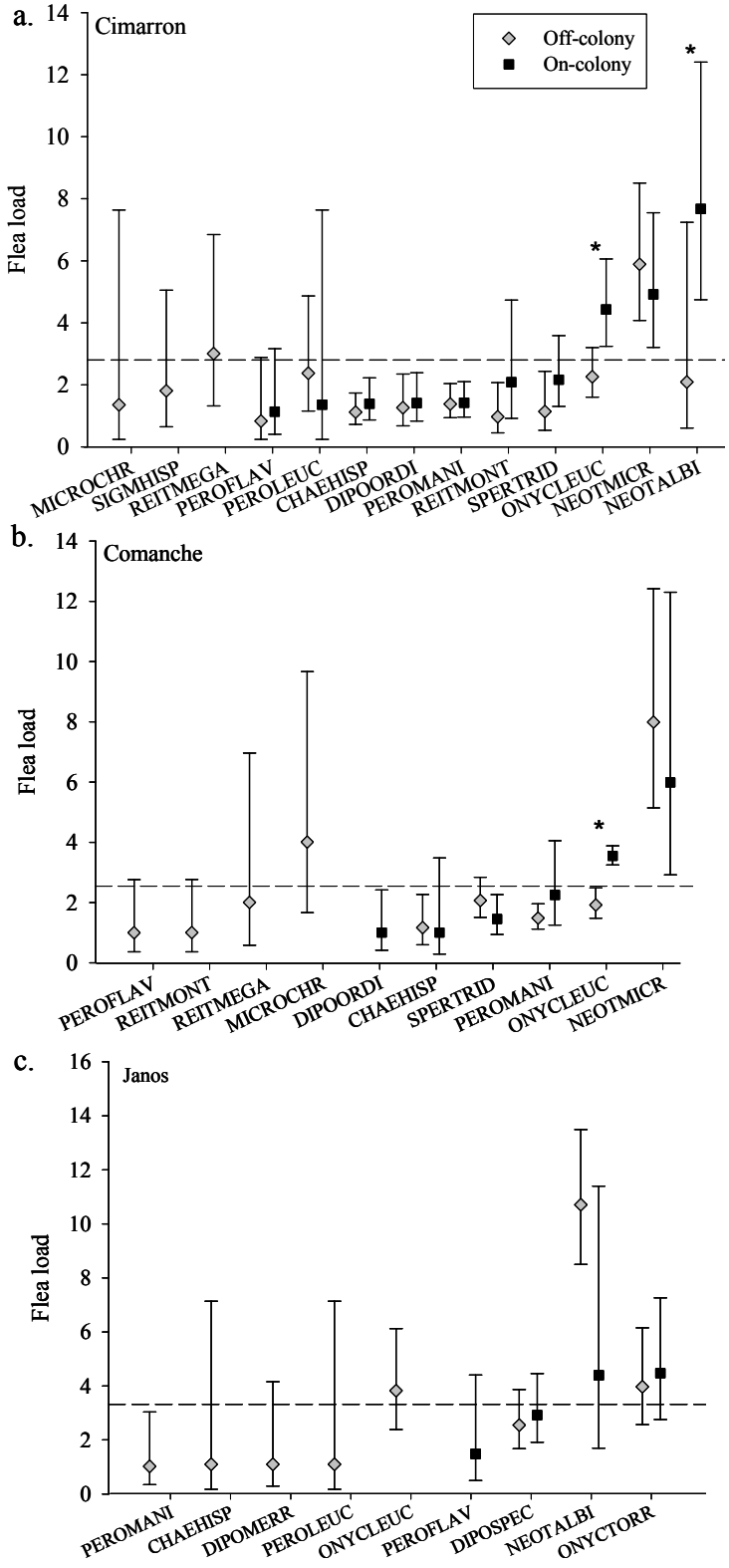

a.
Badlands
Flea load
0
1
2
3
4
5
6
CHAEHISP
MICROCHR
SPERTRID
ONYCLEUC
PEROMANI
b.
Wind Cave
0
2
4
6
8
10
12
SPERTRID
PEROMANI
MICROCHR
c.
Thunder Basin
Flea load
0
5
10
15
20
REITMONT
MICROCHR
DIPOORDI
REITMEGA
ONYCLEUC
PEROMANI
SPERTRID
PEROFASC
PEROLEUC
LEMMCURT

Figure 3.5


a.
Cimarron
Off-colony
On-colony
Flea load
MICROCHR
SIGMHISP
REITMEGA
PEROFLAV
PEROLEUC
CHAEHISP
DIPOORDI
PEROMANI
REITMONT
SPERTRID
ONYCLEUC
NEOTMICR
NEOTALBI
b.
Comanche
Flea load
PEROFLAV
REITMONT
REITMEGA
MICROCHR
DIPOORDI
CHAEHISP
SPERTRID
PEROMANI
ONYCLEUC
NEOTMICR
c.
Janos
Flea load
PEROMANI
CHAEHISP
DIPOMERR
PEROLEUC
ONYCLEUC
PEROFLAV
DIPOSPEC
NEOTALBI
ONYCTORR

Chapter 4

FLEA - HOST RELATIONSHIPS IN THE BLACK-TAILED PRAIRIE DOG AND PLAGUE ECOSYSTEM *

Abstract

Plague, a rodent-borne bacterial disease transmitted by fleas, causes large population reductions in black-tailed prairie dogs (*Cynomys ludovicianus*). The aim of this study was to identify rodent-flea complexes that might be important in the transmission and maintenance of plague in the prairie dog ecosystem and the possible species crossover routes for plague that might be shown by fleas. We evaluated the flea-host relationship by quantifying the proportion of fleas present on their primary and secondary hosts. We trapped rodents on prairie dog colonies and the surrounding grasslands, where colonies were absent and collected fleas from rodents in six areas. The rodent and flea community varied among these study areas. We identified *Peromyscus maniculatus* and *Onychomys leucogaster* and their associated fleas, *Aetheca wagneri, Orchopeas leucopus, Peromyscopsylla hesperomys,* and *Pleochaetis exilis* to be important for the dynamics of sylvatic plague in our study areas. Although these rodents and fleas have been implicated in plague, their presence also in areas without a known history of plague suggests that the current distribution of plague is not limited by the distribution of these rodents or their fleas. Prairie dog fleas were present on rodents at both off- and on-colony grids and suggest the potential for intra and interspecific transmission of fleas between rodent hosts,

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* Authors: Bala Thiagarajan[1], Jack F. Cully Jr.[1, 2], Thomas M. Loughin[3], and Kenneth L. Gage[4] [1]Division of Biology, Kansas State University, [2]Kansas Co-Op Fish and Wildlife Research Unit, USGS, Kansas State University, [3]Department of Statistics, Kansas State University, Manhattan, KS, [4]Vector Ecology Lab, DVBID, Centers For Disease Control and Prevention, Fort Collins, CO.

and between rodents and prairie dogs. The rodent-flea relationship varied across a latitudinal gradient.

Keywords: Siphonaptera, *Yersinia pestis*, rodents, host-specificity, reservoir, vector, prairie dogs

Introduction

Fleas, belonging to the order Siphonaptera, are ectoparasites of mammals and birds. They are important vectors of such rodent-borne infectious diseases as plague and murine typhus. Flea species vary in their specificity for hosts, and though most flea species are rarely host-specific (Whittaker 1968), some species have one or two preferred hosts from particular genus or family, whereas others will feed on any available vertebrate host (Stark 1958, Clover *et al.* 1989, Thomas 1996). Hosts also differ in their ability to harbor fleas. Some hosts harbor only one flea species while others may harbor multiple flea species (Thomas 1996). Most mammals have several species of fleas, which they readily share. The interactions between fleas and hosts are important in the dynamics of vector-borne diseases and have implications for disease management and conservation.

Plague is a rodent-borne bacterial zoonosis, caused by *Yersinia pestis*. The disease is transmitted by fleas and about 31 species of fleas are known to be plague vectors (Perry and Fetherston 1997). Plague has been reported in at least 76 species of mammals in the United States and is maintained in nature through transmission between fleas and certain rodent hosts (Barnes 1993). The distribution of plague in the United States has been more or less stable since 1950, and the 100th meridian is approximately the eastern boundary for plague (Cully *et al.*

2000). Plague causes large population reductions and appears as epizootics in colonial species such as prairie dogs (*Cynomys* spp.) and ground squirrels (*Spermophilus* spp.; Barnes 1993). Prairie dog colonies infected with plague suffer close to 100% mortality (Cully *et al.* 1997, Cully and Williams 2001).

Several rodent species are known to develop varying degrees of resistance to plague and some are considered as potential enzootic hosts for the maintenance and transmission of plague (Gage and Kosoy 2005). *Peromyscus maniculatus* (deer mouse) and *Onychomys leucogaster* (northern grasshopper mouse) are considered as potential reservoirs for plague (Quan and Kartman 1956, Nelson and Smith 1976, Larson *et al*. 1996, Thomas *et al.* 1988). Prairie dogs influence the abundance and species composition of rodents by altering the vegetation and their burrowing activities and thereby the flea assemblages on these rodents (Collinge *et al.* 2006). Prairie dog burrows provide an important off-host environment for the fleas and can maintain large populations of fleas (Cully and Williams 2001, Gage and Kosoy 2005). Burrows may increase the diversity of fleas and also facilitate exchange of fleas between various rodents and between prairie dogs and other rodents. Prairie dog colonies play an active role in the interaction of rodents and fleas, thereby potentially affecting the dynamics of diseases such as plague.

In addition to transmitting plague, fleas also have been suggested to be important in the maintenance of plague (Kartman *et al.* 1962, Olsen 1981, Barnes 1982). Densities of fleas on rodents and the host-specificity of these fleas are considered to be important in the dynamics of plague (Barnes 1993, Piesman and Gage 2000, Gage and Kosoy 2005). In the presence of plague and the absence of primary rodent hosts as a result of plague-related mortality, fleas might become less host specific and be common on secondary rodent hosts. We expect that

reduced host-specificity of fleas has the potential to increase the rate of interspecific transmission of plague, within the small rodents and between small rodents and prairie dogs. Rodents that are potential alternate hosts for plague are generalist hosts with multiple flea species and one or more important flea vectors, promoting the transmission of the pathogen (Gage and Kosoy 2005). In addition to flea-borne transmission among overlapping populations and species of rodents, *Y. pestis* might be spread over considerable distances by other mammals, especially rodent consuming carnivores that can become infested with infected rodent fleas and transport these insects from one area to another (Poland and Barnes 1979).

Understanding the role of fleas and their populations has been identified as key to understanding plague outbreaks (Murray 1971). Knowledge of rodent-flea relationships is not only essential for understanding the dynamics of the disease, but might also have broad implications for comprehending the epidemiology of human plague. This disease is transmitted to humans most frequently in the United States by the bites of wild rodent fleas (Perry and Fetherston 1997). This is the first study on a broad spatial scale across a latitudinal gradient to examine rodent-flea ecology on prairie dog colonies with and without a known history of plague. In this study we evaluated the impact of plague history of areas on rodent-flea relationships in the presence or absence of black-tailed prairie dog colonies. Our objectives were to identify the rodent-flea complexes that may be important for the interspecific transmission of plague and to consider whether differences in species composition of rodents and fleas contribute to the current boundary of plague.

Study Design and Methods

Study sites

We trapped rodents on prairie dog colonies and in the surrounding grasslands where prairie dog colonies were absent, in five areas in the mid-western US and one area in Mexico. The study areas were - Wind Cave National Park (Wind Cave) and Badlands National Park (Badlands), South Dakota, Thunder Basin National Grassland, Wyoming (Thunder Basin), Cimarron National Grassland, Kansas (Cimarron), Comanche National Grassland, Colorado (Comanche) and Chihuahua Grasslands near Janos, Mexico (Janos). Of these areas, Thunder Basin, Cimarron and Comanche have a known history of plague. Plague has not been reported from Wind Cave, Badlands or Janos. Areas with a known history of plague are those from which animals or humans have tested positive for *Y. pestis* or *Y. pestis* antibodies and are referred to as 'plague areas' and areas without known histories of plague are referred to as 'no-plague areas'. All study areas were within the distribution range of the black-tailed prairie dog (Fig. 1).

Rodents were trapped for this study at Cimarron and Comanche between 2001 and 2004; at Thunder Basin between 2002 and 2004; and at Badlands, Wind Cave and Janos between 2001 and 2003. We trapped rodents on paired grids including 'on-colony grids' positioned within prairie dog colonies and 'off-colony grids' situated in grasslands 500-2000 m from prairie dog colonies. Off-colony grids were paired with on-colony grids based on similarities in topography and vegetation. Each grid was trapped for three consecutive nights on two separate occasions each year, once in May-June and once in July-August.

Rodent and flea sampling

Each trapping grid consisted of 49 trap stations (7x7), with a single Sherman live-trap at each station (H. Sherman Traps, Tallahassee, FL), separated by 20 meters. The traps were opened in late afternoon, baited with oatmeal and checked the following morning. All captured rodents were moved to a processing site and were ear-tagged, sexed, identified to species, and examined for ectoparasites. Animals recaptured within the same trapping session were not re-processed for fleas. Rodents were anaesthetized with a mixture of isofluorane and oxygen using a vaporizer (SurgiVet, Waukesha, WI). After anaesthetization, rodents were held over a white plastic tray and combed vigorously for fleas, with a plastic toothbrush (Gage 1999). Additional fleas from the plastic bag used in handling the animal and the anaesthetizing jar were collected. Fleas collected from each animal were preserved in a vial with 2% saline solution and sent to the Centers for Disease Control and Prevention (CDC) in Fort Collins, CO. We limited the number of rodents examined in each species to 10 individuals on each grid in 2003 and 2004. All rodents were released at their capture sites after data collection. The research methods were approved by the Institutional Animal Care and Use Committee of Kansas State University.

Flea identification

All fleas were identified at the Flea-Borne Diseases Laboratory, CDC. Fleas were identified to species and sexed under a dissecting microscope (Hubbard 1947, Stark 1958, 1970, Furman and Catts 1982) and current taxonomic revisions follow Lewis (1990, 1993, 2000, 2002). Voucher specimens of all flea species were collected and are stored at CDC. Fleas that were present on its primary rodent host were considered as host-specific for that rodent species and those fleas that were present on their secondary rodent hosts were considered to be non-specific for that rodent species.

Statistical Analysis

We enumerated the number of flea species present at each area and calculated the prevalence of fleas for rodent species as the proportion of individuals captured that were infested with fleas. We calculated the mean intensity of each flea species, as the number of fleas of each species per infested rodent, for both off- and on-colony grids. Fleas were classified into two groups, based on whether they were present on their primary rodent host species or other rodent species according to Hubbard (1947; Table 4.1). We estimated the probability of fleas being present on their primary hosts (π) using logistic regression (Proc Genmod; SAS v9.1, SAS Institute, Cary, NC; Agresti 1996) by rodent species and presence of prairie dog colonies in each study area. We tested for significant differences between off- and on-colony grids using pairwise comparisons.

Results

We captured 6,291 rodents during four years. Of these, 1,430 rodents had 4,509 fleas. Twenty flea species were collected from 18 rodent species (Chapter 2). The number of flea species varied among the study areas and between off- and on-colony grids.

Flea composition and abundance varied among rodent species, within rodent species among areas and with presence of prairie dog colonies (Chapter 2). At Badlands, where *P. maniculatus* was the most abundant rodent (Chapter 1), fleas that were largely host-specific for this rodent – *Aetheca wagneri* and *Peromyscopsylla hesperomys* were the most abundant and also present on 3 of 4 other rodent species (Table 4.2). Similarly, at Wind Cave, *Orchopeas leucopus*, which primarily infects *Peromyscus spp.* was most abundant on *P. maniculatus*, but also occurred on *Microtus ochrogaste* and *S. tridecemlineatus* (Table 4.3). At Janos, where

Dipodomys spp. were most abundant, *Echidnophaga gallinacea* and *Pleochaetis exilis* were the most abundant fleas on rodents at both off- and on-colony grids. *Aetheca wagneri* was absent at Janos and *O. leucopus,* another *Peromyscus* –specific flea, was collected from *N. albigula* and *P. maniculatus* at off-colony grids (Table 4.4). At Thunder Basin, *A. wagneri* was present on all rodent species infested with fleas except *M. ochrogaster* and was abundant on the one *P. leucopus* caught at off-colony grids and on *Lemmicus curtatus* at both off- and on-colony grids (Table 4.5). At Cimarron and Comanche, where *P. maniculatus* and *O. leucogaster* were the most abundant rodents, their fleas, *O. leucopus* and *Pleochaetis exilis*, respectively, were the most abundant fleas at both off- and on-colony grids (Table 4.6 and 4.7).

Prevalence of fleas and flea loads on rodents varied between rodent species and off- and on-colony grids (Table 4.8, 4.9). At Badlands, *O. leucogaster* had high prevalence at both off- and on-colony grids. At on-colony grids, *P. maniculatus* had higher number of fleas per infested rodent than other species. At Wind Cave, *M. ochrogaster* had fleas at off-colony grids but were absent at on-colony grids. At on-colony grids, though the prevalence of fleas on *P. maniculatus* was low, infested mice had high flea loads. At Janos, *Neotoma albigula* had a high prevalence of fleas and flea load and most of the other rodent species also had high flea loads. At Thunder Basin, *L. curtatus*, present in low numbers, had high prevalence and high flea loads at both off- and on-colony grids. *Onychomys leucogaster* had high prevalence and flea load, but *P. maniculatus* and *S. tridecemlineatus* had low prevalence and high flea load. At Cimarron, *N. micropus* and *O. leucogaster* had high prevalence of fleas and flea loads at both off- and on-colony areas. However, *P. maniculatus* and *P. leucopus* had high prevalence and flea load at off-colony grids and *Reithrodontomys montanus* and *S. tridecemlineatus* had higher prevalence and flea loads at on-colony grids. At Comanche, *N. micropus, O. leucogaster* and *S.*

tridecemlineatus had high prevalence and flea loads at both off- and on-colony grids and *P. maniculatus* had high flea loads at on-colony grids only.

The probability of fleas being present on their primary rodent hosts (π, Table 4.1) varied among study areas. Many of the rodent species infested including *Chaetodipus hispidus, M. ochrogaster, L. curtatus, Perognathus* spp., *Reithrodontomys* spp., *Sigmodon hispidus* and *Dipodomys* spp did not have any flea species to which they were primary hosts. *Dipodomys ordii* had specific fleas only at Thunder Basin on-colony grids ($\pi = 0.36$). *Neotoma albigula* had specific fleas at Cimarron ($\pi = 0.99$ at off-colony grids and $\pi = 0.68$ at on-colony grids) and a low probability of specific fleas at Janos ($\pi = 0.17$ at off-colony grids and $\pi = 0.002$ at on-colony grids).

For rodents that are present at most of the study areas and had high flea prevalence and flea loads, we compared the variation in the probability of fleas being present on rodents that were considered to be their primary hosts. *Spermophilus tridecemlineatus* had varying proportions of specific and non-specific fleas across study areas (Fig. 2a). Overall, they had a higher mean probability of being infected with specific fleas at off-colony grids in plague areas and a higher probability of hosting specific fleas at on-colony grids at Badlands, a no-plague area. At Badlands and Thunder Basin, *O. leucogaster* had a very low probability of specific fleas, at both off- and on-colony grids (Fig. 2b) and were less abundant in these areas (Chapter 1). In the southern grasslands, at Cimarron and Comanche, *O. leucogaster* had a significantly higher proportion of specific fleas at the off-colony grids than on-colony grids. The other common rodent, *P. maniculatus*, trapped and infested with fleas at off- and on-colony grids in all our study areas, also showed a longitudinal gradient in the proportion of specific fleas. At

Badlands, Wind Cave and Thunder Basin, they had a very high probability of specific fleas at both off- and on-colony grids (Fig. 2c). Probability of host-specific fleas on *P. maniculatus* was higher at off-colony grids at Cimarron and at on-colony grids at Comanche.

One of the interesting results of this study was the collection of varying intensities of prairie dog fleas from other rodents in all of our study areas (Fig. 3). At Badlands, the common prairie dog flea, *Oropsylla hirsuta*, was present on both *O. leucogaster* and *P. maniculatus* at on-colony grids. The mean intensity of *O. hirsuta* was significantly higher on *O. leucogaster* than *P. maniculatus* ($t = 2.21$, $df = 10$, $p = 0.052$). They were also collected from *P. maniculatus* at Wind Cave on-colony grids. At Thunder Basin, they were collected at both off- and on-colony grids from *P. maniculatus*. We also collected one *Opisocrostis tuberculatus tuberculatus*, another prairie dog flea from an *O. leucogaster* collected from an on-colony grid at Thunder Basin. *Oropsylla hirsuta* was present on six species of rodents at on-colony grids and three species of rodents at off-colony grids at Cimarron, with significantly higher intensity on *R. megalotis* than *C. hispidus* and *S. tridecemlineatus* from off-colony grids ($p = 0.006$). They were also present at high intensities on *S. tridecemlineatus* at on-colony grids ($p = 0.044$). We also collected one *Pulex simulans*, which are common on prairie dogs in some areas, from *O. leucogaster* at an off-colony grid. At Comanche, *O. hirsuta* were collected from *N. micropus, O. leucogaster* and *S. tridecemlineatus* at on-colony grids, with *O. leucogaster* having a significantly higher mean intensity than *S. tridecemlineatus* ($p = 0.082$). They were also collected from *O. leucogaster, P. maniculatus* and *S. tridecemlineatus* at off-colony grids with no significant differences among these rodent species (all $p > 0.1$).

Discussion

The goal of this study was to evaluate the history of plague on the rodent-flea relationships in the presence of black-tailed prairie dogs and identify complexes/routes that might be important in the interspecific transmission of plague between prairie dogs and other rodents and among rodents. There was a high degree of variation in the composition of rodents and fleas at both off- and on-colony grids and in areas with and without a known history of plague.

Our results show that fleas are rarely completely host specific, and most of the fleas in our study areas were present on more than one rodent species. Similarly, rodent hosts often harbored multiple species of fleas, not only those that are highly specific for the rodent. Almost all rodent species captured had more than one species of fleas. There was a high proportion of interspecific exchange of flea species and it was not limited by the presence of prairie dog colonies. Many of the flea species we collected, that were common on rodents, have been implicated in plague. These include *A. wagneri, M. telchinus, O. hirsuta, O. t. tuberculatus, O. leucopus, O. sexdentatus, P. exilis* and *T. fotus* (Eskey and Haas 1940, Burroughs 1947, Kartman *et al.* 1962, Lechleitner *et al.* 1968, Cully *et al.* 1997, Anderson and Williams 1997, Cully *et al.* 2000). Several of the flea species that were rare, such as *Oropsylla montana, P. simulans* and *Rhadinopsylla* spp. have also been implicated in plague (Eskey and Haas 1940, Mead 1963, Fagerlund *et al.* 2001). Among the rodents captured, *P. maniculatus, O. leucogaster, Neotoma* spp., *L. curtatus*, and *S. tridecemlineatus* have been implicated in plague (Johnson *et al*. 1948, Quan and Kartman 1956, 1962, Thomas *et al*. 1988, Biggins and Kosoy 2001, Davis *et al.* 2002). Given the presence of rodent and flea species that were implicated in plague in areas with and

without plague, the current boundary of plague does not seem to be limited by the distribution of rodents or their fleas.

Flea assemblages of rodents changed with the study areas and we see replacements of fleas on hosts in different areas. In the northern grasslands, where the abundance of *P. exilis*, a specific flea of *O. leucogaster* is low, *O. leucogaster* harbored high intensities of *A. wagneri*, the deer mouse flea. The relative number of flea species on each rodent species remained high within each study area, irrespective of off- or on-colony grids, but the flea loads on these rodents varied between off- and on-colony grids and also among the different study areas.

The proportions of specific and non-specific fleas on some potential enzootic hosts were influenced by host distribution and abundance, than by history of plague at our study areas. For *O. leucogaster*, the proportion of its specific fleas was higher in the southern grasslands, compared to the northern grasslands. For *P. maniculatus*, the proportion of specific fleas was higher in the northern grasslands than the southern grasslands. Similar to the distribution and abundance of these rodents (Chapter 1), their flea distribution and composition were influenced by the geography but may also be affected by host distribution. Hence, these rodents and their flea complexes may complement each other in the dynamics of plague and plague dynamics may not be constrained by these relationships.

Interactions between rodents and fleas are important for the dynamics of plague (Davis *et al.* 2002). In California, the California ground squirrel *(Spermophilus beecheyi)* and its flea (*O. montanus*) are thought to be responsible for maintaining plague in this region (Davis 1999, Lang and Wills 1991, Lang 1996). Among our plague areas, we could identify rodents and fleas that are likely to be important in plague dynamics. At Thunder Basin, three species of rodents could

be important for plague: *P. maniculatus, O. leucogaster* and *L. curtatus* (sagebrush vole). *Peromyscus maniculatus* had a very high proportion of specific fleas, such as *A. wagneri, M. telchinus, O. leucopus* and *P. hesperomys*, and also hosted a wide diversity of fleas (Table 4.4 - 4.9). Among these, were at least 6 flea species have been known to transmit plague and most of these fleas were also present on *O. leucogaster*; *L. curtatus* also had three deer mouse flea species that are known to transmit plague (Johnson *et al*. 1948, Table 4.7). Given that these rodents have been reported to exhibit varying degrees of resistance to plague and also harbor plague vectors, there is little doubt that they are likely to be important in plague dynamics. At Cimarron and Comanche, *O. leucogaster* and *P. maniculatus*, harbored the largest number of fleas and also shared a high proportion of fleas with other rodents (Table 4.8, 4.9). *Peromyscus maniculatus* had the highest intensity of *O. leucopus*, which was also found in high intensities on most rodents in these areas; similarly, *O. leucogaster* had a high intensity of *P. exilis*, which was also found on almost all other rodent species.

In all of our plague areas, voles (*Microtus* spp.), which have been identified as important for the dynamics of plague in California ground squirrel (Miles *et al.* 1957), lacked fleas at on-colony grids and had low intensities of *M. telchinus* and *O. leucopus* at off-colony grids. Hence, the role of these voles in plague dynamics on black-tailed prairie dog colonies is not clear. However, sagebrush voles (*L. curtatus*) are thought to be the principal reservoir of sylvatic plague in Washington (Johnson *et al*. 1948) and among the six flea species known from sagebrush voles in this area, two are known plague vectors (Egoscue 1966, O'Farrell 1975). In our study areas, this species was present only at Thunder Basin, and was infested with three species of fleas- *A. wagneri, M. telchinus* and *O. leucopus*, all of which are known to vector plague and have been reported from other species of voles, as well as species of *Peromyscus*

(Hubbard 1947, Stark 1958). *Neotoma* species also harbored multiple flea species in high numbers and have been known to play a role in maintaining plague, by harboring fleas in their nests (Murray and Barnes 1969, Davis *et al.* 2002). Hence, based on the prevalence, intensity, and host-specificity of the fleas in our study areas, *P. maniculatus, O. leucogaster, L. curtatus, N. micropus,* and *S. tridecemlineatus* are rodents that could be potential enzootic hosts for plague.

This is the first study to report the collection of prairie dog fleas (*O. hirsuta*) from potential enzootic rodent species including *P. maniculatus* and *O. leucogaster*, at off-colony grids. These species of fleas are considered to be highly host-specific on prairie dogs (Hubbard 1947, Stark 1958) and are usually not present on rodents that are abundant on prairie dog colonies. The presence of these species on rodents at off-colony grids, suggest sharing of prairie dog fleas among rodents and the movement of fleas and rodents between prairie dog colonies and the surrounding grasslands. These fleas, *O. hirsuta* and *O. t. tuberculatus*, are important vectors for plague (Barnes 1993) in the prairie dog system. All of our off-colony grids are located at least 500 m away from the nearest prairie dog colony and presence of prairie dog fleas on rodents at off-colony grids suggest a higher exchange of flea species between rodents and their movement from colonies than previously known. Presence of prairie dog fleas on rodents at on-colony grids suggests that there are routes of transmission of plague between prairie dogs and rodents, through infected fleas.

Overall, *P. maniculatus* and *O. leucogaster* showed great flea diversity, and harbored a high proportion of fleas known to vector plague. Although most other rodent species captured did not have host-specific fleas, they might be important in the interspecific transmission of

plague. Rodent that are distributed in only some areas, but had high flea prevalence and intensity, such as woodrats and sagebrush voles might be important for the persistence and transmission of the pathogen locally. Potential rodent hosts of plague and their fleas are present on both prairie dog colonies and in the surrounding grasslands and that these species could maintain plague in low levels during prairie dog interepizootic periods. These rodent-flea complexes could also serve as routes for intra- and interspecific transmission of plague.

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Table 4.1: Specific and non-specific flea species collected from rodents in the study areas, classified according to Hubbard (1947).

Rodent Species	Specific fleas	Non-specific fleas
Chaetodipus hispidus		*A. wagneri, O. hirsuta, O. leucopus, O. montana, O. Sexdentatus, P. hesperomys, P. exilis, T. fotus*
Dipodomys spp.	*M. arachis, M. parkeri*	*A. wagneri, E. gallinacea, F. ignota, O. hirsuta, O. leucopus, T. fotus P. hesperomys, P. exilis,*
Lemmiscus curtatus		*A. wagneri, M. telchinus, O. leucopus*
Microtus ochrogaster		*M. telchinus, O. leucopus, P. hesperomys, T. fotus*
Neotoma spp.	*O. sexdentatus*	*A. nudatus, E. gallinacea, O. hirsuta, O. leucopus, P. hesperomys, P. exilis,*
Onychomys spp.	*P. exilis*	*A. wagneri, F. ignota, M. telchinus, M. arachis, M. parkeri, P. simulans, O. t. tuberculatus,O. hirsuta, T. fotus, O. leucopus, O. sexdentatus, P. hesperomys, Rhadinopsylla spp.*
Perognathus spp.		*A. wagneri, A. nudatus, E. gallinacea, O. leucopus, O. sexdentatus, P. exilis, P. hesperomys, T. fotus*
Peromyscus spp.	*A. wagneri, O. keeni E. wenmanni, M. telchinus, O. leucopus, P. hesperomys*	*E. gallinacea, F. ignota, O. hirsuta, O. sexdentatus, P. exilis, T. fotus*
Reithrodontomys spp.		*A. wagneri, O. hirsuta, O. leucopus, P. exilis*
Sigmodon hispidus		*O. leucopus*
Spermophilus tridecemlineatus	*T. fotus*	*A. wagneri, M. telchinus, O. leucopus, P. exilis, P. hesperomys*

Table 4.2: Number of rodents infested (N) and the number of fleas collected from each rodent species at off- and on-colony grids at Badlands.

Rodents	*C. hispidus*		*M. ochrogaster*		*O. leucogaster*		*P. maniculatus*		*S. tridecemlineatus*	
Grid	Off	On	Off	On	Off	On	Off	On	Off	On
Flea \ N	1	1	1	1	2	19	12	86	7	3
A. wagneri	1				1	7	17	153	2	
E. wenmanni								5		
F. ignota						1				
O. hirsuta						4		2		
O. leucopus			1	1		1	2			
P. hesperomys		1	1		3	10	1	34	1	
P. exilis						3				
T. fotus					1	4	1		13	3

Table 4.3: Number of rodents infested (N) and the number of fleas collected from each rodent species at off- and on-colony grids at Wind cave.

Rodents	*M. ochrogaster*		*P. maniculatus*		*S. tridecemlineatus*	
Grid	Off	On	Off	On	Off	On
Flea \ N	3		34	82	2	1
A. wagneri			61	188	2	
E. wenmanni			2	1		
M. telchinus	4		10	28		
O. hirsuta				1		
O. leucopus	16		10	1	1	
P. hesperomys			1	8		1
T. fotus				2		

Table 4.4: Number of rodents infested (N) and the number of fleas collected from each rodent species at off- and on-colony grids at Janos.

Rodents	*C. hispidus*		*D. merriamis*		*D. spectabilis*		*N. albigula*		*O. leucogaster*		*O. torridus*		*P. maniculatus*		*P. flavus*	
Grid	Off	On	Off	On	Off	On	Off	On	Off	On	Off	On	Off	On	Off	On
Flea \ N	1		2		10	8	16	1	5		6	4	3	1		2
A. nudatus							3	4								1
E. gallinaceae					3	16	115					6				1
M. arachis			2		20	6			7		5	1				
M. dipodomys											1					
O. keeni													1			
O. leucopus							13						1			
O. sexdentatus							28				2					1
O. montanus	1															
P. exilis					1	1	2		11		14	1	1			

Table 4.5: Number of rodents infested (N) and the number of fleas collected from each rodent species at off- and on-colony grids at Thunder Basin.

Rodents	*D. ordii*		*L. curtatus*		*O. leucogaster*		*P. fasciatus*		*P. leucogaster*		*P. maniculatus*		*S. tridecemlineatus*	
Grid	Off	On	Off	On	Off	On	Off	On	Off	On	Off	On	Off	On
Flea \ N	2	12	3	1	21	52	1	1	1		191	175	13	10
A. wagneri	4	9	13	4	19	65	6	5	4		486	476	12	7
E. wenmanni											1			
F. ignota		2			30	1					2	1		
M. telchinus			20								6	7		1
M. parkeri		6			3	3					1	1		
O. hirsuta											1			
O. leucopus				1			1				10	1		
O. sexdentatus											1	3		
P. hesperomys		1			5	51		2			19	39	2	
P. exilis						8					1			
T. fotus		1			5	10					8	7	35	12

Table 4.6: Number of rodents infested (N) and the number of fleas collected from each rodent species at off- and on-colony grids at Cimarron.

Rodents	*C. hispidus*		*D. ordii*		*N. albigula*		*N. micropus*		*O. leucogaster*		*P. flavus*		*P. leucopus*		*P.maniculatus*		*R. megalotis*		*R. montanus*		*S. tridecemlineatus*	
Grid	Off	On	Off	On	Off	On	Off	On	Off	On	Off	On	Off	On	Off	On	Off	On	Off	On	Off	On
Flea \ N	26	17	7	9	1	3	9	6	45	232	2	3	4	1	35	34	1		5	2	5	8
A. wagneri			2																			
F. ignota									2	2												
M. telchinus										10					1							
O. hirsuta	8	5		2		2				234						3	4				2	2
O. leucopus		2	1			5	48	6	6	8	1	1	5		29	23			4	1		1
O. sexdentatus	1	2			2	15	22	7	2	2					3	3						
P. hesperomys							1			2					1	1						
P. exilis	16	11	7	14			4	20	112	846		2	1	1	17	18	1		2	4	2	14
T. fotus	6	3							1	21	1		1		1	3					2	1

Table 4.7: Number of rodents infested (N) and the number of fleas collected from each rodent species at off- and on-colony grids at Comanche.

Rodents	*C. hispidus*		*D. ordii*		*N. micropus*		*O. leucogaster*		*P. flavus*		*P.maniculatus*		*S. tridecemlineatus*	
Grid	Off	On	Off	On	Off	On	Off	On	Off	On	Off	On	Off	On
Flea \ N	6	2		4	2	1	24	111	3		27	4	15	11
A. wagneri								2			3	1	2	
F. ignota							2	2						
M. telchinus								3						
O. hirsuta						1	1	148			1		5	1
O. leucopus	1			1	4		2	2			6	1	2	3
O. sexdentatus					3	1					1			
P. hesperomys	1							6			2	5		
P. exilis	2	2		3	9	4	38	216	3		20	2	11	5
T. fotus	3						3	15			7		11	7

Table 4.8: Prevalence (Number of rodents infested with fleas) and Intensity (Number of fleas per infested rodent) at off- and on-colony grids in no-plague areas. N is the total number of rodents captured.

Rodent	Off-colony			On-colony		
	N	Prevalence %	Intensity	N	Prevalence %	Intensity
Badlands						
O. leucogaster	4	50	2.50	38	50	1.58
P. maniculatus	36	33	1.75	212	41	2.26
S. tridecemlineatus	20	35	2.29	7	43	1.00
Wind Cave						
M. ochrogaster	7	43	6.67			
P. maniculatus	62	55	2.47	322	25	2.80
Janos						
D. spectabilis	114	9	2.40	85	9	2.88
N. albigula	17	94	10.06	1	100	4.00
O. leucogaster	6	83	3.60			
O. torridus	12	50	3.67	10	40	4.25
P. maniculatus	19	16	1.00	1	100	6.00

Table 4.9: Prevalence (Number of rodents infested with fleas) and Intensity (Number of fleas per infested rodent) at off- and on-colony grids in no-plague areas. N is the total number of rodents captured.

Rodent	Off-colony			On-colony		
	N	Prevalence %	Intensity	N	Prevalence %	Intensity
Thunder Basin						
D. ordii	5	40	2.00	48	25	1.58
L. curtatus	3	100	11.00	1	100	5.00
O. leucogaster	37	57	2.95	77	68	2.69
P. maniculatus	527	36	2.82	464	38	3.06
S. tridecemlineatus	33	39	3.77	28	36	2.00
Cimarron						
C. hispidus	158	16	1.19	56	30	1.35
D. ordii	94	7	1.43	44	20	1.78
N. micropus	10	90	8.33	9	67	5.50
O. leucogaster	98	46	2.73	352	66	4.85
P. leucopus	5	80	1.75	2	50	1.00
P. maniculatus	118	30	1.49	110	31	1.50
R. montanus	28	18	1.20	4	50	2.50
S. tridecemlineatus	22	23	1.20	20	40	2.25
Comanche						
C. hispidus	42	14	1.17	4	50	1.00
D. ordii				8	50	1.00
N. micropus	3	67	8.00	2	50	6.00
O. leucogaster	45	53	1.92	163	68	3.55
P. maniculatus	93	29	1.48	12	33	2.25
S. tridecemlineatus	32	47	2.07	29	38	1.45

Figure Legends

Figure 4.1: Map of the mid-west, showing the historical range of the black-tailed prairie dogs, plague positive counties and our study areas.

Figure 4.2: Estimated probability of fleas that are present on their primary host (specific) in the study areas a) *S. tridecemlineatus* b) *O. leucogaster* and c) *P. maniculatus*. The proportion of fleas that are not specific to these rodents is (1 – probability of specific fleas). Error bars indicate 90% confidence limits and asterisks represent significant pairwise differences at the 0.1 level. BAD = Badlands, WC = Wind Cave, JAN = Janos, TB = Thunder Basin, CIM = Cimarron, COM = Comanche.

Figure 4.3: Estimated mean intensity of prairie dog fleas on rodents at off- and on-colony grids in the study areas. All prairie dog fleas are *O. hirsuta*, with the exception of one *O. t. tuberculatus* (marked with **). Error bars indicate 90% confidence limits and || indicates upper confidence limits approaching 10.

Figure 4.1

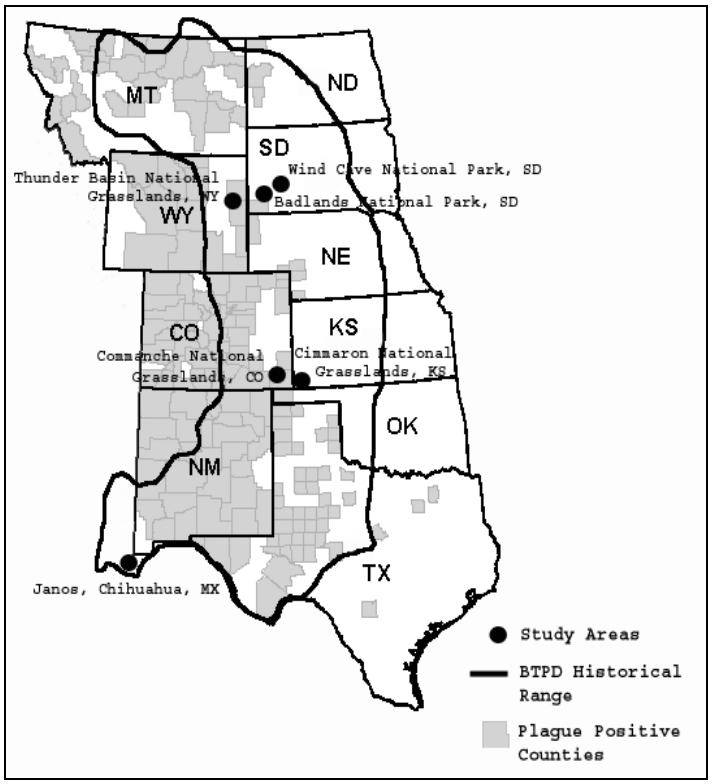

MT
ND
SD
Wind Cave National Park, SD
Thunder Basin National Grasslands, WY
Badlands National Park, SD
WY
NE
KS
CO
Comanche National Grasslands, CO
Cimmaron National Grasslands, KS
OK
NM
TX
Janos, Chihuahua, MX
Study Areas
BTPD Historical Range
Plague Positive Counties

Figure 4.2

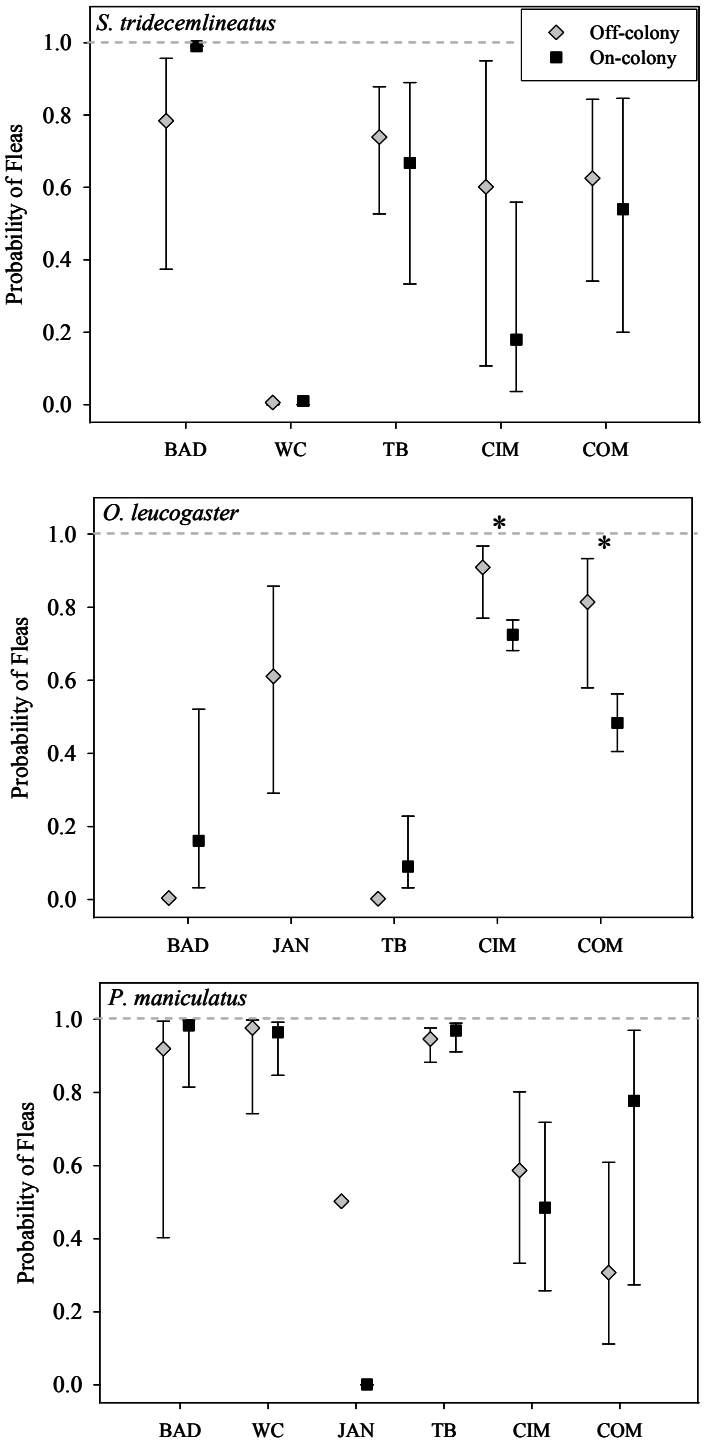
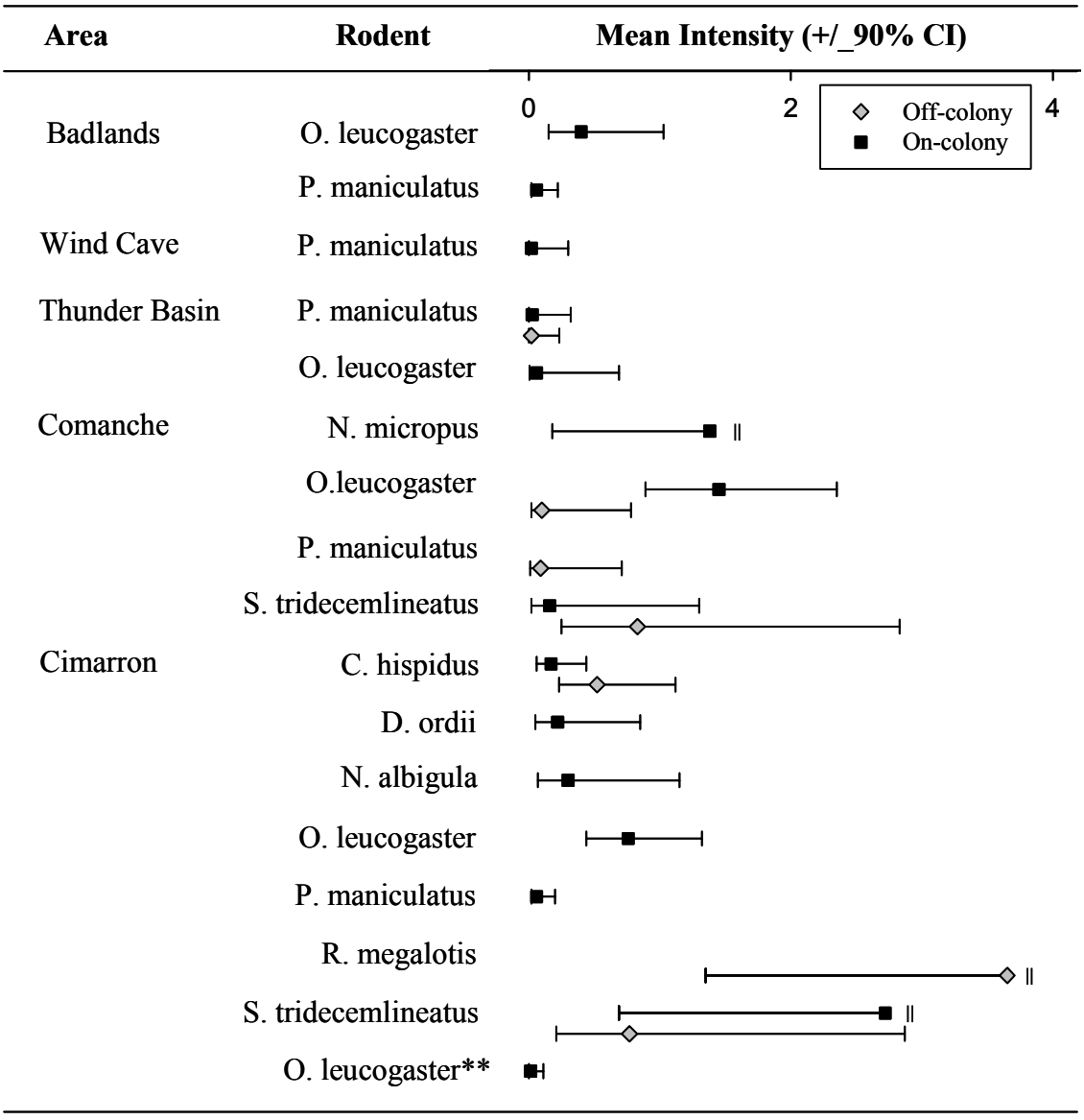

S. tridecemlineatus
Off-colony
On-colony
Probability of Fleas
1.0
0.8
0.6
0.4
0.2
0.0
BAD
WC
TB
CIM
COM
O. leucogaster
*
*
Probability of Fleas
1.0
0.8
0.6
0.4
0.2
0.0
BAD
JAN
TB
CIM
COM
P. maniculatus
Probability of Fleas
1.0
0.8
0.6
0.4
0.2
0.0
BAD
WC
JAN
TB
CIM
COM

Figure 4.3


Area
Rodent
Mean Intensity (+/_90% CI)
0
2
4
Off-colony
On-colony
Badlands
O. leucogaster
P. maniculatus
Wind Cave
P. maniculatus
Thunder Basin
P. maniculatus
O. leucogaster
Comanche
N. micropus
O.leucogaster
P. maniculatus
S. tridecemlineatus
Cimarron
C. hispidus
D. ordii
N. albigula
O. leucogaster
P. maniculatus
R. megalotis
S. tridecemlineatus
O. leucogaster**

Chapter 5

PREVALENCE OF *YERSINIA PESTIS* IN RODENTS AND FLEAS ASSOCIATED WITH BLACK TAILED PRAIRIE DOGS *(CYNOMYS LUDOVICIANUS)* AT THUNDER BASIN NATIONAL GRASSLAND, WYOMING*

Abstract

Rodents and their fleas that are associated with prairie dogs are considered important for the maintenance and transmission of the bacterium (*Yersinia pestis*) that causes plague. Our goal was to identify rodents and fleas that were involved in a plague epizootic in black-tailed prairie dogs at Thunder Basin National Grassland. We collected blood samples and ectoparasites from rodents trapped at off- and on-colony grids at Thunder Basin National Grassland. Blood samples were tested for antibodies to *Y. pestis* F-1 antigen and fleas were tested for the presence of the bacterium. Among the 1421 fleas tested between 2002 and 2004, only one prairie dog flea, *Oropsylla hirsuta*, collected from a *Peromyscus maniculatus* in 2002, tested positive for *Y. pestis*. Of the 493 blood samples tested from 2003 and 2004, two *Onychomys leucogaster*, tested positive for *Y. pestis* antibodies in summer 2004. All three samples were collected from on-colony grids, shortly after a plague epizootic occurred. This study confirms that plague is hard to detect in rodents and fleas associated with prairie dog colonies unless samples are collected immediately after a prairie dog die-off.

Keywords: Plague, rodents, fleas, epizootic, seropositive, prevalence

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* Authors: Bala, Thiagarajan[1], Jack F. Cully Jr.[2], Ying Bai[3], and Kenneth L. Gage[3]

[1]Division of Biology, [2]Kansas Co-Operative Fish and Wildlife Research Unit, USGS, Kansas State University, Manhattan, KS, [3]Vector Ecology Lab, DVBID, Centers for Disease Control and Prevention, Fort Collins, CO.

Introduction

Plague is a vector-borne zoonotic disease, caused by the gram-negative bacterium *Yersinia pestis* (Enterobacteriaceae). Plague causes large population reductions in rodents of several species within its native and introduced ranges (Barnes 1993). *Yersinia pestis* is transmitted to animals by infected flea-bites, direct contact, or inhalation of respiratory droplets from an infected animal (Gage *et al.* 1995, Webb *et al*. 2006). Sylvatic plague is thought to be primarily maintained among wild rodent populations and is transmitted by several flea species that occur on vertebrate hosts (Thomas 1988, Anderson and Williams 1997, Cully *et al*. 1997). Sylvatic plague is most devastating for colonial species of rodents such as prairie dogs and ground squirrels and all species of prairie dogs are susceptible to the disease (Perry and Fetherston 1997). Plague causes close to 100% mortality in infected colonies (Cully *et al.* 1997). Prairie dogs are highly susceptible to plague and their presence may amplify plague epizootics; however, other rodent hosts are presumed important for enzootic maintenance and transmission of the pathogen (Poland and Barnes 1979, Cully 1993, Biggins and Kosoy 2001, Gage and Kosoy 2005).

Our objective was to identify rodent and flea species that may be important in the transmission and maintenance of plague in the black-tailed prairie dog system. We hypothesized that species of rodents that are strongly associated with the presence of black-tailed prairie dogs, act as reservoirs of plague, or are infested with a wide diversity of flea species would be the most likely maintenance hosts for the interspecific transmission of *Y. pestis* in the black-tailed prairie dog and the associated rodent community. Antibodies to *Y. pestis* provide evidence that an animal has been exposed and survived the infection. Because plague is rare in rodents and fleas unless samples are taken immediately after a die-off (Gage 1999), we tested this hypothesis on

samples collected from rodents trapped on prairie dog colonies and surrounding grasslands at Thunder Basin National Grassland, Wyoming, which has been undergoing a plague epizootic since 2000-2001 (Byer 2001).

Study Design and Methods

Thunder Basin National Grassland is located in northeastern Wyoming (105.0°W, 43.6°N; Fig 5.1) in the Powder River Basin between the Black Hills and the Big Horn Mountains. The grassland is interspersed with private, state and federal lands. It covers about 231,481 ha, and the elevation ranges between 1000 and 1500 m with a semi-arid climate. The vegetation is complex with many common prairie species and is dominated by Western wheatgrass (*Agropyron smithii*), blue grama (*Bouteloua gracilis*) and big sagebrush (*Artemesia tridentata*). In 1999, prior to plague, prairie dog colonies covered at least 6597 ha at Thunder Basin (Cully and Johnson 2005, *in litt.*).

We trapped rodents at Thunder Basin on 6 grids on prairie dog colonies and 6 grids positioned 500 – 2000 m away from the nearest prairie dog colony in the surrounding grasslands. Each grid consisted of 49 trap stations (7x7), with a single Sherman live trap (H. Sherman Traps, Tallahassee, FL) at each station. Stations were separated by 20 meters. Traps were opened and baited with oatmeal in the late afternoon and were checked for captures the next morning. They were left closed during the daytime. Each grid was trapped for three consecutive nights on two separate occasions each year, once in May-June and once in July-August.

All captured rodents were moved to a processing site and were ear-tagged, sexed, identified to species, and examined for ectoparasites. Animals recaptured within the same

trapping session were not re-processed. Rodents were anaesthetized with a mixture of isofluorane and oxygen using a vaporizer (SurgiVet, Waukesha, WI). After anaesthetization, rodents were held over a white plastic tray and combed vigorously for fleas with a plastic toothbrush (Gage 1999). Additional fleas from the plastic bag used in handling the animal and the anaesthetizing jar were collected. Fleas collected from each animal were preserved in a vial with 2% saline solution and sent to the Flea-Borne Disease Laboratory at the Centers for Disease Control and Prevention (CDC) in Fort Collins, CO for identification. We limited the number of rodents examined of each species to 10 individuals on each grid in 2003 and 2004. We collected blood samples (200 µl) from a retro-orbital sinus of each of the rodents captured in 2003 and 2004 and each blood sample was coated onto a nobuto strip per the manufacturer's instructions (Advantec MFS, Dublin, CA). The strips were then air-dried and stored individually in marked envelopes prior to processing for serology. All rodents were released at their capture sites after data collection. The research methods were approved by the Institutional Animal Care and Use Committee of Kansas State University.

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

Results

We captured 1773 rodents in 3 years and of these, 529 were recaptures. The number of individual rodents of each species captured varied among years (Fig 5.2). We tested 493 blood samples from 2003 and 2004, and 1428 fleas from 2002- 2004 for *Y. pestis*. The rodent species and the number of blood samples and fleas tested are given in Tables 5.1 and 5.2 respectively. Among the fleas tested for *Y. pestis*, an *Oropsylla hirsuta* female, from a *P. maniculatus* male at an on-colony grid in 2002 tested positive. From the serologic testing, two *O. leucogaster* from the same prairie dog colony tested positive with a 1:512 titer for *Y. pestis* antibodies. Both *O. leucogaster* and *P. maniculatus* harbored a wide diversity of fleas at both off- and on-colony grids (Table 5.3). All other samples were negative for *Y. pestis*.

Discussion

Interspecific transmission of plague is vital in maintaining plague among the various alternate hosts if more than one species act as reservoirs and for the transmission of plague from alternate small rodent hosts to the prairie dogs. In this study, we tested rodents and their fleas for *Y. pestis* or antibodies to *Y. pestis* to identify potential enzootic rodent host, flea vector, and the rodent - flea complexes that might be important for the maintenance of plague.

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

Yersinia pestis infected fleas have been collected from prairie dogs and rodents or from prairie dog burrows, usually during epizootics, and some infected fleas persist in burrows after an epizootic for as long as a year (e.g., Lechleitner *et al.* 1968, Cully *et al.* 1997). These two studies also tested rodents for *Y. pestis*, and Lechleitner *et al.* (1968) found one seropositive deer mouse and Cully *et al.* (1997) found seropositive prairie dogs. In the present study, we collected a *Y. pestis* -infected prairie dog flea from a deer mouse in 2002 and captured two seropositve *O. leucogaster* in 2004. The deer mouse with the *Y. pestis* positive flea was captured twice subsequent to the collection of flea and only one flea was present on this rodent. Given that only this prairie dog flea was positive for *Y. pestis* among 362 fleas tested from Thunder Basin in 2002, it seems most probable that the flea changed hosts after the prairie dogs died of plague. Presence of this infected flea on a deer mouse shows one potential mechanism and route for interspecific transmission of *Y. pestis* through fleas between prairie dogs and rodents. We did not detect any *Y. pestis* positive fleas in 2003 or 2004.

Persistence of plague on the landscape was confirmed by the seroconversion of the two *O. leucogaster* captured during 2004. The reciprocal titer value of 1:512 probably indicates either an anamnestic immune response to repeated exposure or perhaps a recent exposure to the *Y. pestis* antigen. One of the individuals was captured during the first trapping session (6/25/2004) in 2004 and the other during the second (7/27/2004), on the same prairie dog colony. *Onychomys leucogaster* had a very strong positive association with the black-tailed prairie dogs at Thunder Basin (Cully and Bala unpublished data) and the presence of resistant individuals strengthens their implicated role in plague dynamics.

Onychomys leucogaster associated with the prairie dogs at Thunder Basin, had a wide diversity of flea species with high flea loads (Table 5.3), and was the only species that tested positive for *Y. pestis* antibodies. Hence, among the rodents captured, this species was the most likely reservoir host for plague, a finding that is in agreement with previous studies (e.g., Thomas *et al.* 1988, Webb *et al*. 2006). *Peromyscus maniculatus* may also play an important role in the maintenance and or transmission of plague as it was present in higher numbers than other rodents, harbored a very high flea diversity, and was present at high densities both off- and on-colonies. Because deermice were also abundant and widespread away from prairie dog colonies, they provided continuity among populations on the landscape that might support disease transmission. At other areas where plague is present but grasshopper mice are not, deermice are a potential maintenance host (Cully et al., submitted).

Evidence of plague was rare among rodents and fleas at Thunder Basin. Given that only 3 among ~2000 samples were positive for plague, we do not yet understand the mechanisms involved in the maintenance and transmission of plague on the landscape apart from prairie dogs. This study reaffirms that plague is hard to detect in rodents and fleas unless samples are collected immediately after a prairie dog die-off (Gage 1999).

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Table 5.1: Number of blood samples of various rodent species collected on Nobuto strips and tested for antibodies to Y. pestis F-1 antigen by passive hemagglutination assay from off- and on-colony grids.

Rodent Species	2003		2004	
	Off-colony	On- Colony	Off-colony	On- Colony
Dipodomys ordii		4	2	10
Lemiscus curtatus			3	1
Microtus ochrogaster		1		
Onychomys leucogaster	8	29	12	14
Peromyscus maniculatus	94	73	104	108
Reithrodontomys megalotis			1	
Spermophilus tridecemlineatus	16	6	1	5
Thomomys talpoides		1		
Total	**118**	**114**	**123**	**138**

Table 5.2: Number of fleas collected (number of rodents infested) from rodent species that were tested for presence of *Y. pestis* by multiplex PCR, from off- and on-colony grids.

Rodent Species	**2002**		**2003**		**2004**	
	Off-colony	**On-colony**	**Off-colony**	**On-colony**	**Off-colony**	**On-colony**
D. ordii		14 (8)		1 (1)	4 (2)	4 (3)
L. curtatus					33 (1)	5 (3)
M. ochrogaster			1 (1)			
O. leucogaster	37 (10)	51 (16)	12 (4)	63 (22)	13 (7)	26 (14)
P. fasciatus					7 (2)	7 (2)
P. leucopus	4 (1)					
P. maniculatus	128 (45)	86 (41)	132 (64)	135 (51)	279 (82)	314 (83)
R. megalotis					2 (1)	
R. montanus		1 (1)				
S. tridecemlineatus	37 (6)	6 (2)	11 (6)	8 (4)	1 (1)	8 (4)

Table 5.3: Flea species and the number of fleas collected from *O. leucogaster* and *P. maniculatus* that were tested for the presence of *Y. pestis*, from off- and on-colony grids. Refer Table 5.2 for numbers of *O. leucogaster* and *P. maniculatus* infested with fleas.

Rodent Species	Flea Species	2002		2003		2004	
		Off-colony	On-colony	Off-colony	On-colony	Off-colony	On-colony
O. leucogaster	*A. wagneri*	6	13	4	36	1	1
	F. ignota	25		4		2	
	M. parkeri	1	1		2		
	O. tuberculata		1			1	9
	P. hesperomys	4	26		16		
	P. exilis		7		1		
	Rhadinopsylla spp.		1				
	T. fotus	1	2	4	8	6	5
P. maniculatus	*A. wagneri*	111	72	117	119	258	285
	E. wenmanni	1				1	
	C. deuterus			2			
	F. ignota	2	1				
	M. telchinus	1		2	5	3	2
	M. parkeri				1	1	
	O. hirsuta	1	1				
	O. leucopus			1		9	3
	O. sexdentatus	1					
	P. hesperomys	6	7	6	8	7	24
	P. exilis	1					
	T. fotus	4	5	4	2		

Figure Legends

Figure 5.1: Map of Thunder Basin National Grassland, showing paired trapping grids. Grey diamonds represent off-colony grids and black squares represent on-colony grids.

Figure 5.2: Number of rodent species and individuals captured from off- and on-colony grids between 2002 and 2004. Grey symbols represent off-colony grids and black symbols represent on-colony grids.

Figure 5.1:

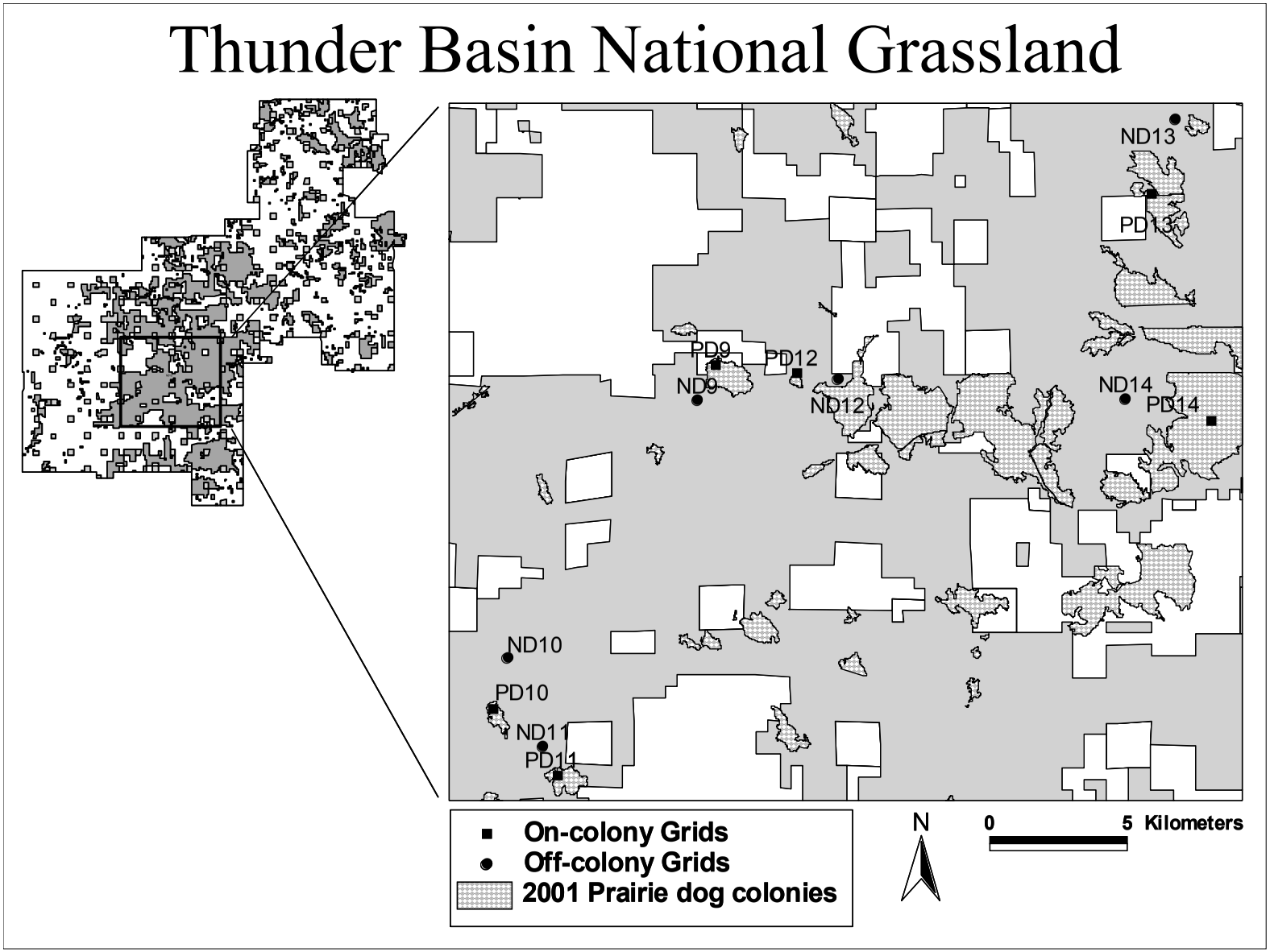
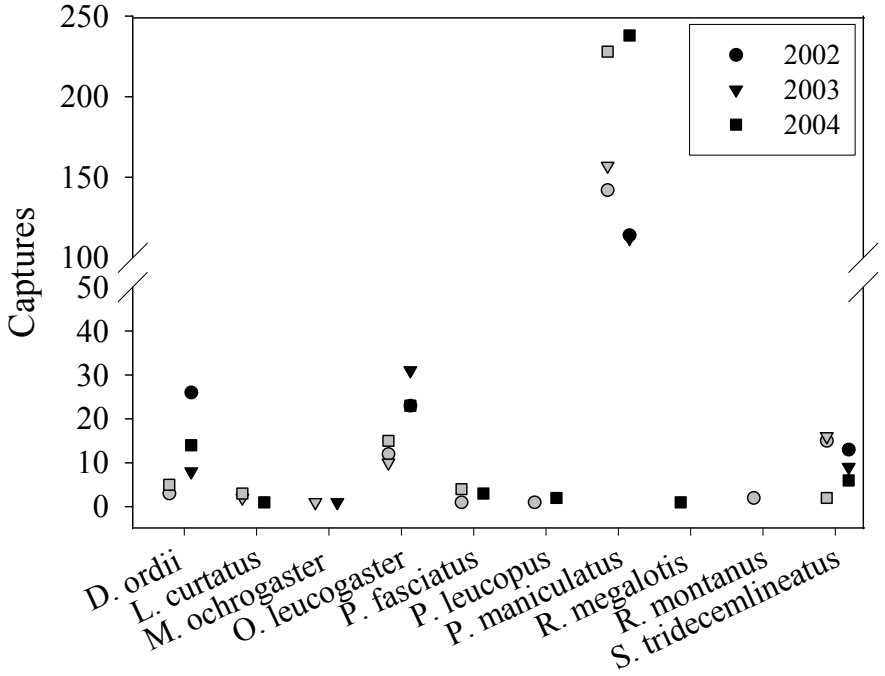

Thunder Basin National Grassland
ND13
PD13
PD9
PD12
ND9
ND12
ND14
PD14
ND10
PD10
ND11
PD11
On-colony Grids
Off-colony Grids
2001 Prairie dog colonies
N
0
5 Kilometers

Figure 5.2:


Captures
250
200
150
100
50
40
30
20
10
0
2002
2003
2004
D. ordii
L. curtatus
M. ochrogaster
O. leucogaster
P. fasciatus
P. leucopus
P. maniculatus
R. megalotis
R. montanus
S. tridecemlineatus

Chapter 6

PREVALENCE OF *BARTONELLA* SPECIES IN RODENTS AND FLEAS ASSOCIATED WITH BLACK-TAILED PRAIRIE DOGS [*]

Abstract

Plague is a flea-transmitted rodent-borne bacterial disease that causes large population reductions in black-tailed prairie dogs (*Cynomys ludovicianus)*. As plague is rare to detect in rodents and fleas unless immediately after a prairie dog die-off, we used the prevalence of *Bartonella* as a surrogate for plague to identify potential rodent reservoirs and their fleas that are important for the maintenance and transmission of bacteria in the prairie dog ecosystem. *Bartonella* species are intracellular hemotropic parasites associated with many zoonotic diseases. They are common among rodents and are thought to be transmitted by arthropod vectors. We tested blood samples and fleas from rodents captured off- and on- prairie dog colonies and evaluated the distribution and prevalence of *Bartonella* in rodents and fleas. Prevalence of *Bartonella* in rodents varied between 37% and 65% among the study areas. Prevalence in fleas was low (6%). Eight rodent species and nine flea species were positive for *Bartonella*. *Peromyscus maniculatus* and *Onychomys leucogaster* were frequently infected with *Bartonella*. These species may play an important role in the maintenance and transmission of diseases. Because of the wide diversity of rodents and fleas that were infected with *Bartonella*, there is a high potential for the maintenance and transmission of *Bartonella* between rodents and fleas.

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[*] Authors: Bala Thiagarajan[1], Jack F. Cully Jr.[1, 2], Thomas M. Loughin[3], Ying Bai[4], Michael Kosoy[4], and Kenneth L. Gage[4]. [1]Division of Biology, Kansas State University; [2]United States Geological Survey, Kansas Co-operative Fish and Wildlife Research Unit, USGS, Kansas State University; [3]Department of Statistics, Kansas State University, Manhattan, KS; [4]Vector Ecology Lab, DVBID, Centers For Disease Control and Prevention, Fort Collins, CO.

Further sequence analyses of the isolates of *Bartonella* in rodents and fleas are important for using *Bartonella* to elucidate the potential role of these rodents and fleas in plague ecology.

Keywords: Plague, reservoir, *Peromyscus maniculatus*, *Onychomys leucogaster*, Zoonoses.

Introduction

Sylvatic plague caused by the bacterium *Yersinia pestis*, causes large population reductions in black-tailed prairie dogs (*Cynomys ludovicianus*; Barnes 1993, Cully *et al.* 1997). *Yersinia pestis* is very rarely detected in the black-tailed prairie dog system unless samples are collected immediately after a prairie dog die-off (Gage 1999, Chapter 5) and significant gaps exist in our knowledge of the reservoir and enzootic hosts for plague in the black-tailed prairie dog system. Rodents including *Peromyscus maniculatus* and *Onychomys leucogaster* as well as their fleas are considered to be potential enzootic hosts for plague. Prairie dog burrows provide habitat for rodents and also act as refugia for fleas. They are important as off-host environment for fleas, providing ideal conditions for maintaining large flea populations (Cully and Williams 2001, Gage and Kosoy 2005).

In this study, we used the prevalence of Bartonella as a surrogate for *Yersinia pestis* to identify potential routes of transmission of bacteria in the prairie dog ecosystem, as they are common in both rodents and fleas. *Bartonella* species are vector transmitted, blood-borne, intracellular, gram-negative bacteria, and several species are pathogens of humans and other mammals. All species of *Bartonella* induce hemotropic infections in the reservoir hosts and are primarily transmitted between mammalian hosts by hematophagous arthropods. Various *Bartonella* species and genotypes infect a wide variety of mammalian hosts including rodents

(Anderson and Neuman 1997, Hoar *et al.* 2003, Kosoy *et al.* 2004, Maillard *et al.* 2004) and are important as emerging human pathogens (Chomel *et al.* 1996, Anderson and Neuman 1997, Breitschwerdt and Kordick 2000). Many animal reservoirs and a few species of arthropods have been implicated in the transmission of *Bartonella* (Regnery *et al.* 1992, Kosoy *et al.* 2003, Bown *et al.* 2004). Arthropods including sand flies, body lice, fleas and ticks have been known to transmit *Bartonella* (Alexander 1995, Maurin and Raoult 1996, Chomel *et al.* 1996, Schouls *et al.* 1999). Some species of *Bartonella* that have implications for human health are linked to rodent reservoirs (Birtles *et al.* 1995, Welch *et al.* 1999, Ellis *et al.* 1999).

Several studies have addressed the prevalence of *Bartonella* in rodents (Ellis *et al.* 1999, Stevenson *et al.* 2003, Kosoy *et al.* 2004), and *Bartonella* spp. have been found to be prevalent among mice and rats. Kosoy *et al.* (1997) detected ~50% *Bartonella* prevalence in rodent populations in the southeastern U.S and Hofmeister *et al.* (1998) detected a new species of *Bartonella* in the blood of *Peromyscus leucopus*. More recently, Kosoy *et al.* (2003) identified *B. washoensis*, a species previously identified from a human case of myocarditis, in samples collected from ground squirrels. Overall, available evidence indicates a high prevalence of *Bartonella* among rodents (Kosoy *et al.* 2004, Birtles *et al.* 2001). There is also seasonal variation in the prevalence of these bacteria in their rodent nests. The studies of Kosoy *et al.* (1997) and Kosoy *et al.* (2004) also indicate that individuals of particular host species could be infected with multiple strains within host populations and individuals.

Bartonella are primarily transmitted by arthropod vectors in addition to undergoing vertical and horizontal transmission in some rodent hosts. The role of fleas in the transmission of various bartonellae is not clear and few studies have tested fleas for the presence of *Bartonella*

(Parola *et al.* 2003, Stevenson *et al.* 2003, Durden *et al.* 2004). Fleas become infected with *Bartonella* after taking a blood meal from infected hosts (Stevenson *et al.* 2003) and few species of fleas are identified as competent vectors (Chomel *et al.* 1996, Bown *et al.* 2004, Durden *et al.* 2004).

Studies of *Bartonella* in rodents and fleas could provide insights on the ecology of plague and other diseases that have similar modes of transmission. As species of *Bartonella* occur in the same host-vector community and exhibit similar modes of flea-borne transmission as *Y. pestis*, but are found much more commonly than plague (Kosoy, *unpub. data*), it could provide a surrogate to answer certain questions about plague transmission. Our objective in this study was to evaluate the distribution, prevalence, and spatial variation of *Bartonella* in rodent species and their fleas that are associated with the black-tailed prairie dogs. We evaluated rodents and their fleas for their potential to act as reservoirs for *Bartonella* and plague from study sites across the geographical range of the black-tailed prairie dog.

Study Design and Methods

This study was conducted at five areas in four states in the mid-western US and one additional area in Mexico (Fig. 6.1). Three of these areas- Thunder Basin National Grasslands in Wyoming, Cimarron National Grassland in Kansas, and Comanche National Grassland in Colorado have a known history of plague. Plague has not been reported in Wind Cave National Park and Badlands National Park in South Dakota, or Chihuahua Grasslands near Janos in Mexico. Areas with a known history of plague are those from which animals or humans have tested positive for *Y. pestis* or *Y. pestis* antibodies at some time in the past, and are referred to as 'plague areas'. Areas without known histories of plague are referred to as 'no-plague areas.'

Rodents were trapped at Cimarron between 2001 and 2004; at Comanche between 2001 and 2004; at Thunder Basin between 2002 and 2004; and at Badlands, Wind Cave and Janos between 2001 and 2003. The number of grids that were trapped in these areas varied among years.

To assess the influence of prairie dogs on the prevalence of *Bartonella* in rodents and fleas, we established paired trapping grids in each area, including 'on-colony grids' positioned within prairie dog colonies and 'off-colony grids' situated in grasslands 500-2000 m from prairie dog colonies. Off-colony grids were paired with on-colony grids based on similarities in topography and vegetation. Each grid consisted of 49 trap stations (7x7), with a single Sherman live trap (H. Sherman Traps, Tallahassee, FL) at each station, separated by 20 meters. The traps were opened and baited with oatmeal in the late afternoon and were checked for captures the next morning. They were left closed during the daytime. Each grid was trapped for three consecutive nights on two separate occasions each year, once in May-June and once in July-August.

All captured rodents were moved to a processing site where they were ear-tagged, sexed, identified to species, and examined for ectoparasites. Rodents recaptured within the same trapping session were not re-processed for fleas. Rodents were anaesthetized with a mixture of isofluorane and oxygen using a vaporizer (SurgiVet, Waukesha, WI). After anaesthetization, rodents were held over a white plastic tray and combed vigorously for fleas, with a plastic toothbrush (Gage 1999). Additional fleas from the plastic bag used in handling the animal and the anaesthetizing jar were collected. Fleas collected from each animal were preserved in a vial with 2% saline solution and sent to the Centers for Disease Control and Prevention (CDC) in Fort Collins for identification. We collected blood samples from the rodents from the retro-

orbital sinus (200 μl) in 2003 in a vial and stored them at -20 degrees prior to testing at CDC. All rodents were released at their capture sites after data collection. The research methods were approved by the Institutional Animal Care and Use Committee of Kansas State University.

Field guide to the mammals of North America (Burt and Grossenheider 1962) and Mammals of North America (Kays and Wilson 2002) were used to identify rodents. Fleas were identified to species and sexed under a dissecting microscope (Hubbard 1947, Stark 1958, 1970, Furman and Catts 1982) and current taxonomic revisions follow Lewis (1990, 1993, 2000, 2002). Voucher specimens of all flea species were collected and are stored at CDC.

Rodents from 2003 were tested for *Bartonella* by culturing the blood samples on rabbit blood agar and *Bartonella*-like isolates were confirmed by a PCR assay using *glt*A primers as described elsewhere (Kosoy *et al.* 2003). Flea samples were tested for the presence of the *Bartonella* by a multiplex PCR assay that tested for *Y. pestis* and *Bartonella,* and used the same *glt*A primers as were used in PCR analyses of the rodent blood samples (Stevenson *et al.* 2003). In this study, we restricted the analysis to detecting presence of *Bartonella* and did not identify the different strains.

Statistical Analysis

For each area, we modeled the proportion of rodents infected with *Bartonella* against rodent species, grids (off- or on-colony) and their interaction, by a logistic regression model (Proc Genmod in SAS v9.1; SAS Institute, Cary, NC). We used a type III likelihood ratio test ($\alpha = 0.1$, Agresti 1996) to determine whether the probability of infection among rodent species varied by the presence or absence of prairie dog colonies. Similarly, we modeled the proportion of fleas infected with *Bartonella* against flea species, rodent hosts, grids and their interaction, by

a logistic regression model (Proc Genmod in SAS v9.1; SAS Institute, Cary, NC). We used a type III likelihood ratio test ($\alpha = 0.1$, Agresti 1996) to determine whether the probability of infection among fleas varied by the rodent species, and presence or absence of prairie dog colonies. We estimated the probability of infection by *Bartonella* in rodents and fleas by a logistic regression (Proc Genmod in SAS v9.1; SAS Institute, Cary, NC).

Results

Bartonella in rodents

We examined 804 rodents belonging to 16 species for presence of *Bartonella* from 2003. Of these, 416 (52%) were found positive for *Bartonella*. The number of rodents tested and the prevalence of *Bartonella* varied between off- and on-colony grids, among each of the study areas (Table 6.1). The prevalence of *Bartonella* in rodents varied among the study areas, with Cimarron having the highest prevalence of 65%, followed by Thunder Basin with 52%. Badlands, Wind Cave and Comanche had 43%, 45% and 41% prevalence respectively. Janos had a low prevalence of 37%, but this could be due to the small number of samples tested (n = 16). Eight of the rodent species tested negative for *Bartonella* and include *Chaetodipus hispidus* (n = 22), *Dipodomys spectabilis* (n = 2), *Lemmiscus curtatus* (n = 1), *Mus musculus* (n = 1), *Perognathus flavus* (n = 2), *Reithrodontomys megalotis* (n = 1), *R. montanus* (n = 4) and *Spermophilus spilosoma* (n = 1).

The probability of infection in the positive rodent species varied by the interaction of rodent species and presence of prairie dogs only at Cimarron ($\chi^2 = 13.76$, df = 7, $p = 0.0556$). There was no effect of rodent species – prairie dog interaction in the other study areas (all $p > 0.1$). At Badlands, only *O. leucogaster* and *P. maniculatus* were caught in sufficient numbers for

statistical analyses and *P. maniculatus* had a 50% probability of being infected (n = 90; Fig. 6.2a) and *O. leucogaster* had a 30% probability of infection (n=25; Fig. 6.2a). At Wind Cave also, *P. maniculatus* was the only rodent caught in sufficient numbers for statistical analyses and had a high probability of infection (Fig. 6.2b). At Thunder Basin, *O. leucogaster*, *P. maniculatus* and *S. tridecemlineatus* were infected at both off and on-colony grids and *D. ordii* was infected at on-colony grids (Fig. 6.2c). At Janos, only 5 of the 16 individuals tested were positive for *Bartonella* and there was high variation in the probability of infection (Fig. 6.2d). At Comanche, *O. leucogaster* had the highest probability of infection at both off- and on-colony grids, with >60% prevalence at on-colony grids (n = 45; Fig. 6.2e). Similarly at Cimarron, *O. leucogaster* had the highest probability of infection and almost all tested positive for *Bartonella* at both off- and on-colony grids (n = 126; Fig. 6.2f). At on-colony grids they had 85% prevalence and all *O. leucogaster* tested at off-colony grids (n = 37) were positive for *Bartonella*. Cimarron also had the largest number of rodent species infected (n = 6) with *Bartonella*. *P. maniculatus* were not infected in Comanche and had low infection probability at Cimarron.

Bartonella in fleas

We tested 3315 fleas belonging to 15 species for *Bartonella* from 2001, 2002 and 2004. Because of problems processing a portion of the fleas from 2003, only 365 of the 1189 were included for that year. Of the 3680 total fleas tested, 258 were positive for *Bartonella*. Nine of the 15 flea species tested positive for *Bartonella*. Few species, including *Anomiopsyllus nudatus* (n = 28), *Foxella ignota* (n = 28), *Malareus telchinus* (n = 36) and *Peromyscopsylla hesperomys* (n = 106) that were common on rodents, did not have any positive fleas. The number of fleas

tested and the prevalence of *Bartonella* varied between off- and on-colony grids, among each of the study areas (Table 6.2). The overall prevalence of *Bartonella* in fleas was 6%.

There was no effect of off- or on-colony grids in the prevalence of *Bartonella* in fleas by rodent species or flea species at any of the study area (all $p > 0.1$). Testing for the effect of rodent species on the presence of *Bartonella*-infected fleas by each area, we found that there was variation among rodent species in the prevalence of *Bartonella* infected fleas only at Janos ($\chi^2 = 17.08$, df = 7, $p = 0.017$) and Thunder Basin ($\chi^2 = 21.23$, df = 6, $p = 0.001$). We estimated the probability of having *Bartonella*-infected fleas for all rodents at these two study areas. At Janos, *Dipodomys spectabilis* had a 0.14 probability of having *Bartonella*-infected fleas, *O.leucogaster* had a 0.1 probability. At Thunder Basin, *D. ordii* had a 0.26 probability of having *Bartonella*-infected fleas and *S. tridecemlineatus* had a 0.1 probability. All other rodents in these two areas had <0.05 mean probability of having *Bartonella*-infected fleas. At Badlands, rodents had a 0.05 mean probability of having infected fleas. At Wind Cave, this probability was 0.01. Cimarron and Comanche had 0.33 and 0.17 mean probability of infected fleas on rodents respectively.

Probability of infection by *Bartonella* varied among flea species at Janos ($\chi^2 = 18.88$, df = 7, $p = 0.009$), Thunder Basin ($\chi^2 = 27.75$, df = 12, $p = 0.006$), Cimarron ($\chi^2 = 36.22$, df = 9, $p < 0.001$), and Comanche ($\chi^2 = 48.64$, df = 7, $p < 0.001$). There was no variation in the probability of *Bartonella* infection in fleas at Badlands ($p = 0.627$) or Wind Cave ($p = 0.455$). At Badlands, *Aetheca wagneri* (probability = 0.056) and *Thrassis fotus* (probability = 0.048) were infected with *Bartonella*. At Wind Cave, only *A. wagneri* was positive for *Bartonella* (probability = 0.032). At Janos, among *Bartonella*-positive fleas, *Meringis arachis* had a high probability of infection (Fig. 6.3a). At Thunder Basin, *M. parkeri* had the highest infection probability of all 5

fleas that tested positive (Fig. 6.3b). At Cimarron and Comanche, five species of fleas were positive for *Bartonella* and *Pleochaetis exilis* had the highest mean infection probability at Cimarron and *Orchopeas sexdentatus* had the highest mean infection probability at Comanche (Figs. 6.3c and 6.3d respectively).

We compared the prevalence of *Bartonella* in fleas with the presence of *Bartonella* in rodents from which the fleas were collected. In 2003, only part of the flea samples tested provided reliable results. This includes 31 *Bartonella* positive fleas among 365 tested. 14 rodents that were positive for *Bartonella* in 2003 also had fleas that tested positive (n = 16; Table 6.3). Of these 14 rodents, 8 were positive for *Bartonella* at the time the positive fleas were collected.

Discussion

In this study, we evaluated the distribution and prevalence of *Bartonella* among rodents and their fleas, associated with the black-tailed prairie dogs. Prevalence of *Bartonella* in rodents varied among the study areas and were among the highest that have been reported in this system. Similar studies in rodents elsewhere have shown varying degrees of *Bartonella* prevalence. In the United Kingdom, rodents were shown to have 62.2% prevalence for *Bartonella* (Birtles *et al.* 2001) and the prevalence was 42.25% in southeastern United States (Kosoy *et al.* 1997). Rodent communities near Saskatoon, Saskatchewan, Canada had 57% prevalence of *Bartonella* (Jardine *et al.* 2005). In Southern China, prevalence of *Bartonella* in rodent communities ranged from 26.7% to 46.7% (Ying *et al.* 2002).

Two species of rodents, *P. maniculatus* and *O. leucogaster* were consistently infected with *Bartonella* and had high infection probabilities. These two rodents were found in high

abundance in the study areas, had high flea loads and probability of infestation by fleas (Chapters 1, 2). Based on their distribution and their vector prevalence, we previously considered them to be important enzootic hosts for the maintenance and transmission of plague in the black-tailed prairie dog ecosystem (Chapters 3, 4). Given that *Bartonella* and plague have similar modes of transmission by flea vectors, the high prevalence of *Bartonella* in these rodents adds new evidence to our assessment of the importance of these species in disease dynamics. There was a wide diversity of rodents that were infected with *Bartonella* over the broad geographical range of our study. Kangaroo rats (*Dipodomy*s spp), Woodrats (*Neotoma* spp) and ground squirrels (*Spermophilus* spp) also showed high probability of infection.

Variation in *Bartonella* prevalence in rodents, but not fleas, in the presence of prairie dogs only at Cimarron is not surprising given that few species of rodents differed between off- and on-colony grids and there was no variation in flea loads between off- and on-colony grids (Chapters 1, 2).

Preference by vectors for host species is important for the transmission of bacteria and parasites. At each of our study areas, fleas that were infected with *Bartonella* were specific for hosts that were also positive for *Bartonella*. Fleas that were infected include *A. wagneri*, *P. exilis*, *O. sexdentatus*, *Meringis* spp., and *T. fotus*. Hosts of these fleas, specifically *Peromyscus* spp., *Onychomys* spp., *Dipodomys* spp., *Neotoma* spp., and *Spermophilus* spp., were also infected with *Bartonella*. In a similar study at Philips County, Montana, *A. wagneri* from *P. maniculatus*, *O. tuberculata* from a Prairie dog and *Pulex simulans* from *Canis lutrans* were positive for *Bartonella* (Holmes 2003). As many of these flea species are shared between rodent hosts (Chapter 2), there is high potential for transmission of the parasite between various rodents. At

Janos, Cimarron and Comanche, prevalence of *Bartonella* in both *O. leucogaster* and its *P. exilis* fleas shows the importance of this rodent-flea complex in zoonoses that could also be important for *Y. pestis*. Though the prevalence in the vector community was low, further sequence analyses of isolates from the positive samples are expected to provide more information on the host specificity, sharing and cross-over infection by *Bartonella* among rodents and their fleas.

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Table 6.1: Number of blood samples from rodents that were tested the number of samples positive from off- and on-colony grids at each of the study area.

	Off-colony		On-colony	
Area	**Tested**	**Positive**	**Tested**	**Positive**
Badlands	36	18	93	38
Wind Cave	35	12	62	32
Janos	7	4	9	2
Thunder Basin	111	66	109	49
Cimarron	99	62	127	85
Comanche	60	9	56	39
Total	**348**	**171**	**456**	**245**

Table 6.2: Number of fleas tested and number of fleas positive from off- and on-colony grids at each of the study area from 2001, 2002 and 2004.

	Off-colony		On-colony	
Area	**Tested**	**Positive**	**Tested**	**Positive**
Badlands	22	5	138	6
Wind Cave	46	1	67	7
Janos	202	7	26	0
Thunder Basin	540	32	517	15
Cimarron	243	17	1094	92
Comanche	106	13	314	30
Total	**1159**	**75**	**2156**	**150**

Table 6.3: Number of *Bartonella* positive fleas from positive rodents in 2003.

Area	Grid	Rodent Species	# of rodents	Flea Species	# of fleas
Janos	Off-colony	*O. leucogaster*	1	*P. exilis*	4
		O. torridus	1	*M. arachis*	1
Cimarron	Off-colony	*O. leucogaster*	2	*P. exilis*	3
	On-colony	*O. leucogaster*	2	*P. exilis*	2
Comanche	On-colony	*O. leucogaster*	4	*P. exilis*	5
		O. leucogaster	1	*O. hirsuta*	1

Figure Legends

Figure 6.1: Map of the mid-west, showing the historical range of the black-tailed prairie dogs and our study areas.

Figure 6.2: Estimated mean probability of infection by Bartonella for rodent species at each of the study area. Error bars indicate 90% confidence intervals. a) Badlands b)Wind Cave c) Thunder Basin d) Janos e) Comanche and f)Cimarron. Rondet species are: MICROCHR – *Microtus ochrogaster,* ONYCLEUC – *Onychomys leucogaster*, PEROMANI – *Peromyscus maniculatus*, DIPOORDi – *Dipodomys ordii*, SPERTRID – *Spermophilus tridecemlineatus*, ONYCTORR – *Onychomys torridus*, NEOTMICR – *Neotoma micropus*, PEROLEUC – *Peromyscus leucopus*.

Figure 6.3: Estimated mean probability of infection by Bartonella in fleas at four of the study areas. Error bars indicate 90% confidence intervals. a) Janos b) Thunder Basin c) Cimarron and d) Comanche.

Figure 6.1

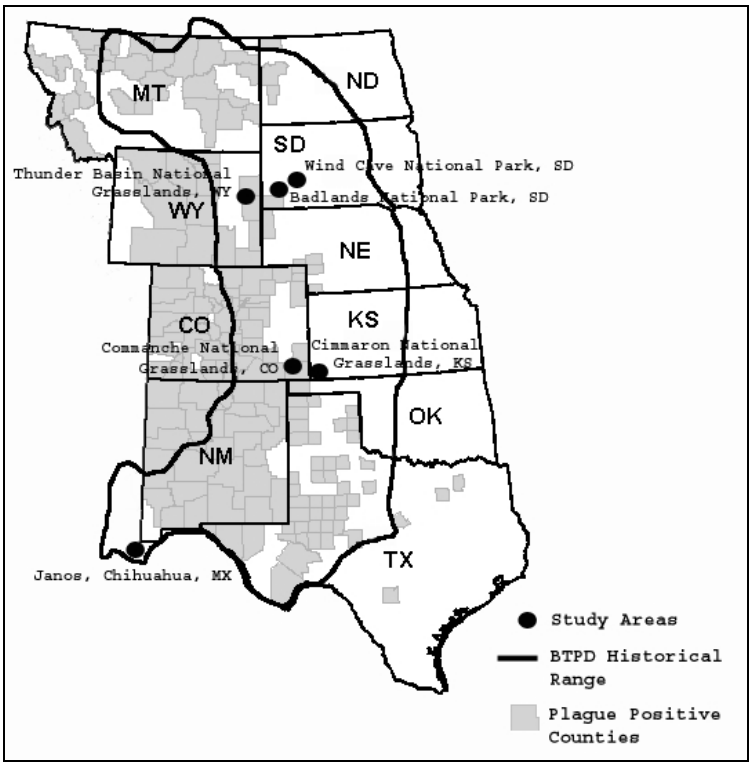

MT
ND
SD
Wind Cave National Park, SD
Thunder Basin National Grasslands, WY
Badlands National Park, SD
WY
NE
KS
CO
Comanche National Grasslands, CO
Cimmaron National Grasslands, KS
OK
NM
TX
Janos, Chihuahua, MX
Study Areas
BTPD Historical Range
Plague Positive Counties

Figure 6.2

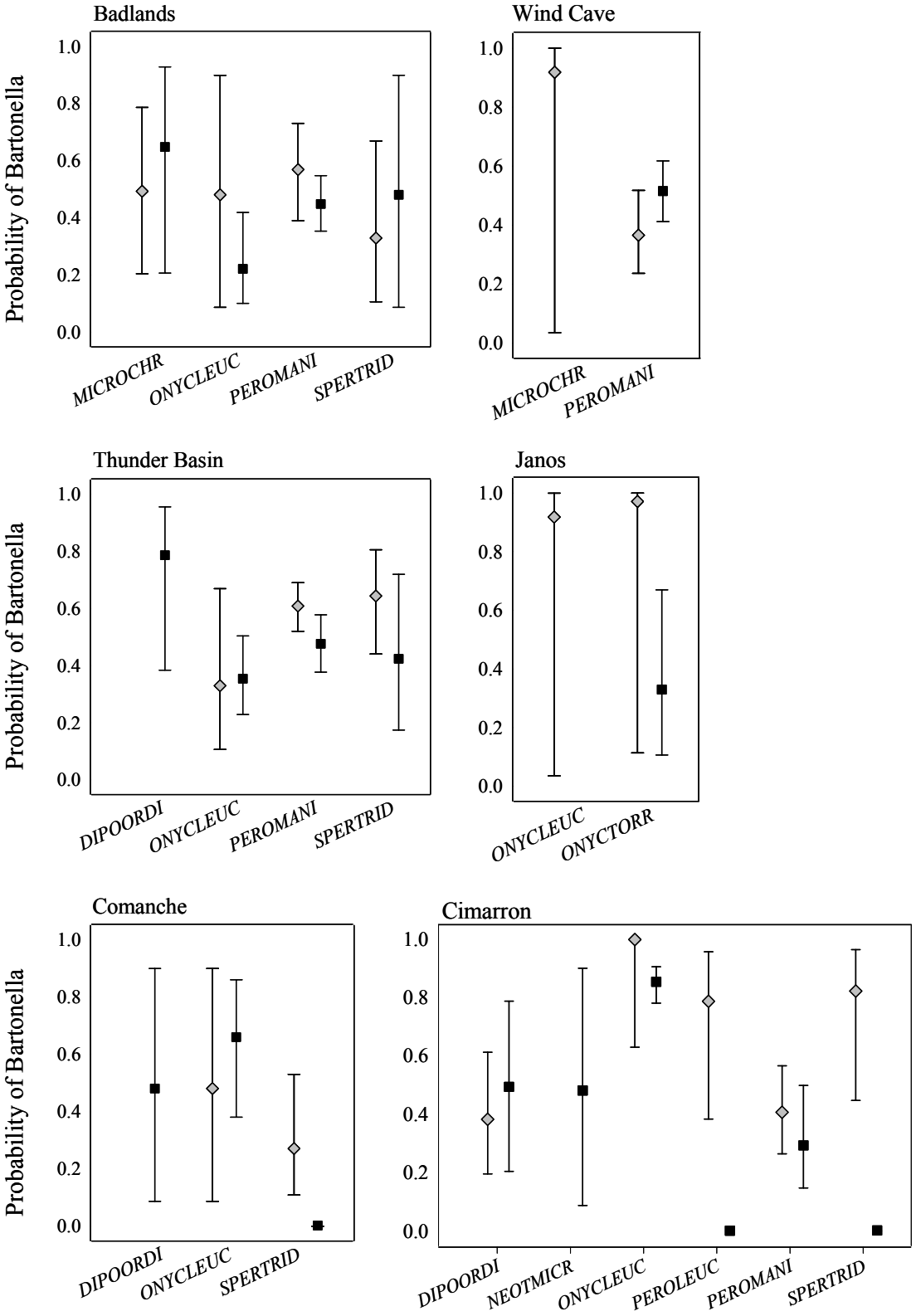
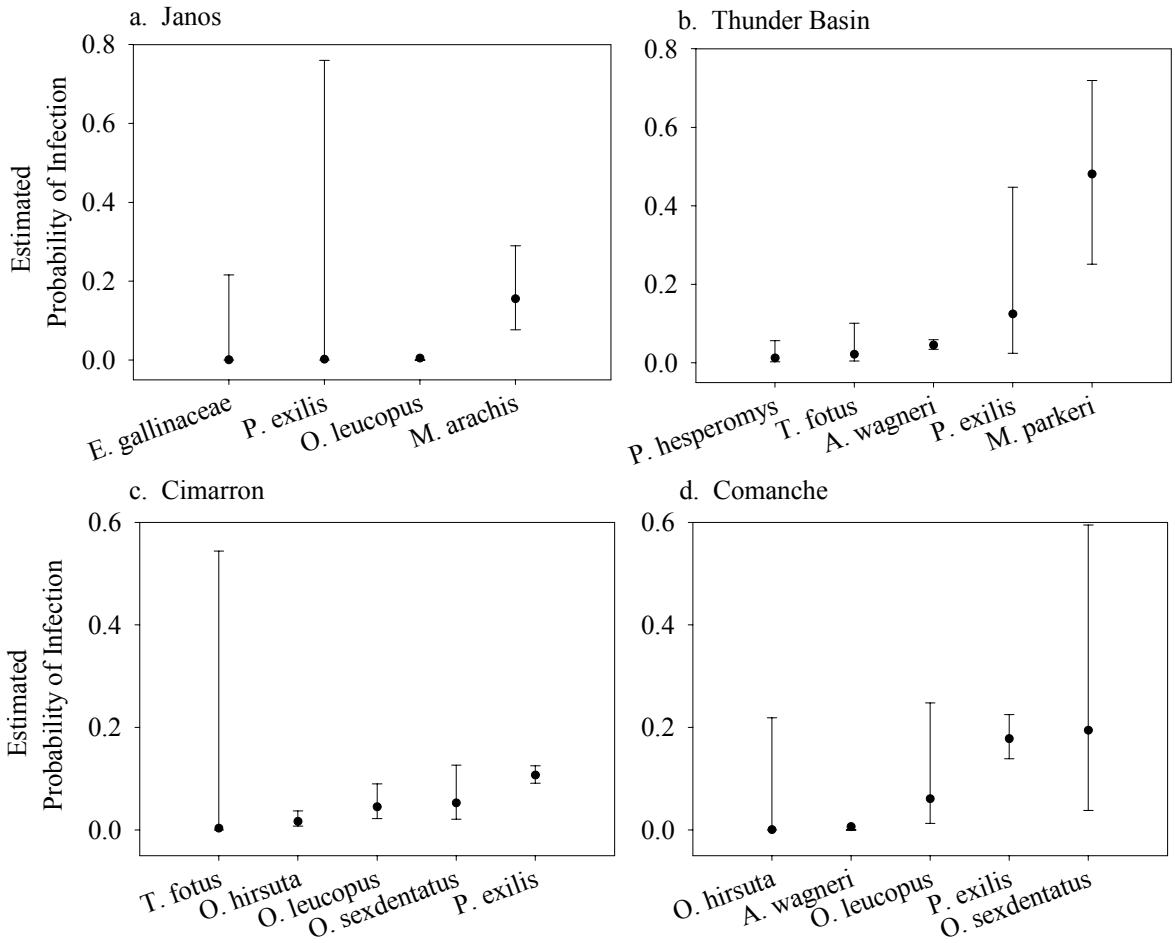

Badlands
Wind Cave
Thunder Basin
Janos
Comanche
Cimarron
Probability of Bartonella
1.0
0.8
0.6
0.4
0.2
0.0
MICROCHR
ONYCLEUC
PEROMANI
SPERTRID
DIPOORDI
ONYCTORR
NEOTMICR
PEROLEUC

Figure 6.3


a. Janos
b. Thunder Basin
c. Cimarron
d. Comanche
Estimated Probability of Infection
0.0
0.2
0.4
0.6
0.8
E. gallinaceae
P. exilis
O. leucopus
M. arachis
P. hesperomys
T. fotus
A. wagneri
P. exilis
M. parkeri
T. fotus
O. hirsuta
O. leucopus
O. sexdentatus
P. exilis
O. hirsuta
A. wagneri
O. leucopus
P. exilis
O. sexdentatus

CONCLUSION

The host-vector-pathogen relationships are essential components to understand the dynamics of plague and have immediate consequences for the management of prairie dogs and their habitat. This study addressed several gaps in our understanding of the dynamics of sylvatic plague in the black-tailed prairie dog system in North America. Our objectives were to: (i) identify the enzootic rodent hosts, which act as maintenance systems for *Yersinia pestis* in the black-tailed prairie dog system, (ii) identify the potential for interspecific and intraspecific transmission of plague in the black tailed prairie dog / rodent community, and (iii) identify rodent-flea complexes that are important in the transmission and maintenance of plague. We evaluated the distribution and abundance of other rodent species and their fleas that are associated with the black-tailed prairie dogs and their importance for plague dynamics.

The species composition and abundance of rodents varied between the presence and absence of black-tailed prairie dogs. Prairie dogs did not have the same interaction with all rodent species and with the same rodent species in different areas. This highlights the unique role of prairie dogs in each of the grassland study areas. Prairie dogs did not increase species richness of rodents in the study areas and the diversity and evenness of rodents associated with prairie dog colonies compared to grasslands without prairie dog colonies was variable across the study areas.

Prairie dog colonies did not seem to facilitate the transmission of fleas between rodent species. Flea species richness was not different between prairie dog colonies and the surrounding grasslands. Prairie dog colonies did not increase the prevalence of fleas and flea loads on rodents did not vary between off- and on-colony grids at three of the study areas. Rodents that were

associated with the black-tailed prairie dogs such as *Onychomys leucogaster* and *Peromyscus maniculatus* could be potential enzootic hosts for plague. These rodent species harbored high diversity of fleas, both on and away from prairie dog colonies. Overall, flea loads and probability of infestation by fleas were higher on *O. leucogaster*, along with *Neotoma micropus*, *N. albigula*, *Lemmiscus curtatus* and *Spermophilus tridecemlineatus* than other rodent species. *Peromyscus maniculatus* and *O. leucogaster* had high mean intensities of flea species in almost all of the study areas. These rodent species harbored important flea vectors such as *Aetheca wagneri, Orchopeas leucopus, O. sexdentatus, Pleochaetis exilis* and *Thrassis fotus*, which have been implicated in plague.

Prairie dog fleas were found on rodents, at both off- and on-colony grids and along with multiple flea species on rodents, shows that there are potential routes for intra- and interspecific transmission of plague and other vector-borne diseases among other rodents and between other rodents and prairie dogs. Based on the association of rodents with the black-tailed prairie dogs, flea assemblages, flea prevalence and infestation probability on rodents and their flea load and mean intensity of flea species, we identified *O. leucogaster* and *P. maniculatus* as the two important rodent species for plague maintenance and transmission in our study areas. This was confirmed by the presence of a plague-positive flea on a *P. maniculatus* and two *O. leucogaster* that were seropositive for *Yersinia pestis* antibodies at Thunder Basin National Grassland after a plague-epizootic.

The prevalence of another intracellular blood parasite, *Bartonella* species, in rodents and fleas, did not vary with the presence of black-tailed prairie dogs and the distribution of *Bartonella* is widespread. We showed that *O. leucogaster* and *P. maniculatus* and their fleas

were consistently infected with *Bartonella* and had high infection probabilities. The high prevalence of *Bartonella* in these two species adds strength to our assessment on the importance of these two species in plague dynamics. The presence of plague in the study areas did not seem to be limited by the rodent or flea community structure and composition.

The study emphasized that rodent-flea dynamics are important for the prevalence of disease in the black-tailed prairie dog system and the data we have collected will make an important contribution to the modeling of emerging and resurgent diseases. The study also helped to identify further gaps in our understanding of the mechanisms involved in the maintenance and transmission of plague on the landscape.