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Fleas, Hosts and Habitat: What can we predict about the spread of vector-borne zoonotic diseases?

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FLEAS, HOSTS AND HABITAT: WHAT CAN WE PREDICT ABOUT THE SPREAD OF VECTOR-BORNE ZOONOTIC DISEASES?

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ABSTRACT

FLEAS, HOSTS AND HABITAT: WHAT CAN WE PREDICT ABOUT THE SPREAD OF VECTOR-BORNE ZOONOTIC DISEASES?

MEGAN M. FRIGGENS

Vector-borne diseases of humans and wildlife are experiencing resurgence across the globe. I examine the dynamics of flea borne diseases through a comparative analysis of flea literature and analyses of field data collected from three sites in New Mexico: The Sevilleta National Wildlife Refuge, the Sandia Mountains and the Valles Caldera National Preserve (VCNP). My objectives were to use these analyses to better predict and manage for the spread of diseases such as plague (*Yersinia pestis*).

To assess the impact of anthropogenic disturbance on flea communities, I compiled and analyzed data from 63 published empirical studies. Anthropogenic disturbance is associated with conditions conducive to increased transmission of flea-borne diseases. Most measures of flea infestation increased with increasing disturbance or peaked at intermediate levels of disturbance. Future trends of habitat and climate change will probably favor the spread of flea-borne disease.

Rodents, including Gunnison's prairie dogs (*Cynomys gunnisoni*), were trapped for three years (2004-2006). Blood and flea samples were tested for the presence of plague and another bacterial pathogen, *Bartonella*. I conduct two analyses with this data. The first examines prairie dogs and their flea communities in the VCNP. Prairie dogs experienced a plague epizootic in fall 2004, after which we found plague positive fleas and positive antibody titers in three prairie dogs. We noted an increased tendency for flea exchange opportunities in the spring before flea abundance peaked. Spring conditions, which favor presence and exchange of certain flea species, may be just as important for determining plague outbreaks as the summer conditions, which lead to build up in flea populations.

In the second analyses, I found 38% of the rodents of 30 species and 60% of fleas of 24 species positive for *Bartonella*. *Bartonella* infections typically lasted two months and the prevalence of *Bartonella*. Changes in prevalence related to host density and environmental gradients, point to the importance of both fleas and rodents in *Bartonella* transmission cycles.

This research shows environment influences the risk of flea-borne disease spread. It is likely that future trends of habitat and climate change will favor the spread of flea-borne diseases, including plague and *Bartonella*.

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Table of Contents

LIST OF TABLES ..... **vii**
LIST OF FIGURES ..... **viii**
CHAPTER 1: INTRODUCTION **12**
Literature Cited 22
CHAPTER 2: ANTHROPOGENIC DISTURBANCE AND THE TRANSMISSION OF FLEA-BORNE DISEASES **27**
Abstract 29
Introduction 30
Materials and Methods 32
Results 38
Discussion 40
Acknowledgements 46
References 47
CHAPTER 3: FLEA ABUNDANCE, DIVERSITY, AND PLAGUE IN GUNNISON'S PRAIRIE DOGS (*CYNOMYS GUNNISONI*) AND THEIR BURROWS IN MONTANE GRASSLANDS IN NORTHERN NEW MEXICO. **58**
Abstract 60
Introduction 62
Materials and Methods 64
Results 69
Discussion 72
Acknowledgments 77
Literature Cited 79
CHAPTER 4: FLEA-BORNE TRANSMISSION OF *BARTONELLA* IN THREE RODENT AND FLEA COMMUNITIES IN NEW MEXICO **87**
Abstract 89
Introduction 90
Materials and Methods 91
Results 102
Discussion 106
Acknowledgments 113
References 115
CHAPTER 5: DISCUSSION AND CONCLUSIONS **127**
Literature Cited 132
LIST OF APPENDICES **134**

LIST OF TABLES

Table 1. Studies which report fleas collected from both animals and burrows on Gunnison's (GPD), White- (WTPD), and Black-tailed prairie dogs (BTPD) colonies. 20

Table 2.1. Continental distribution and biome classification of sites used in comparative analysis of anthropogenic disturbance and flea vector assemblage characteristics 53

Table 2.2 A-C. Significant ($P \leq 0.05$) effects (X) for mixed model analysis of disturbance level (Low, Intermediate and High disturbance) and Biome (Forest, Desert, Grassland/ Savanna and Mediterranean) on mammal and flea communities surveyed in 63 studies. **D.** Significant difference across disturbance classes within Forest biomes. **E-F.** Significant differences among Biomes within each level of disturbance class..... 54

Table 3.1. Flea species and number collected from Gunnison's prairie dog burrows, prairie dogs (GPD), *Cynomys gunnisoni*, and golden mantled ground squirrels (GMGS), *Spermophilus lateralis*, caught in the Valles Caldera National Preserve in northern New Mexico, 2004-2006. . 83

Table 4.1. Prevalence of *Bartonella* spp in rodents and their fleas collected from 3 sites in New Mexico. Though *Bartonella* was found in 30 rodent species only those with more than 10 captures are listed here. 119

Table 4.2. List of *Bartonella* positive fleas collected from rodents captured at three sites in New Mexico 121

LIST OF FIGURES

Figure 2.1. Pearson correlation analysis for variables calculated from 63 studies conducted around the world. Scatter plots with loess (locally weighted scatterplot smoothing) lines are displayed above the diagonal and r values for significant associations (values in bold represent $P<0.0001$, otherwise $0.0009<P<0.03$) are displayed below the diagonal. Stars indicate significant associations after variables were standardized for sampling effort and log transformed, but the plots reflect raw data. Descriptions of variables can be found in the text..... 55

Figure 2.2. Mean values for small mammal and flea variables from 63 studies categorized into three anthropogenic disturbance classes (low, intermediate, high) and four biomes (Forest ——, Grassland/Savanna —·—·—, Desert ·····, Mediterranean — — —). P-values are for F-tests from Generalized Linear Model analysis of the overall model (y is a function of Disturbance class and Biome) and analyses of disturbance class within each Biome. Vertices without a common letter indicate statistically significant difference ($P\leq0.05$ using Tukey-Kramer multiple comparison methods) among disturbance levels of that biome. Significant differences among biomes within a disturbance class are noted with circle symbols (significant differences are indicated by different fill shades)..... 56

Figure 2.3. Mean values for flea measures in small mammal communities from 63 studies categorized into three anthropogenic disturbance classes (low, intermediate, high) and four biomes (Forest ——, Grassland/Savanna —·—·—, Desert ·····, Mediterranean — — —). P-values are for F-tests from Generalized Linear Model analysis of the overall model (y is a function of Disturbance class and Biome) and analyses of disturbance class within each Biome. Vertices without a common letter indicate statistically significant difference ($P\leq0.05$ using Tukey-Kramer multiple comparison methods) among disturbance levels of that biome. Significant differences among biomes within a disturbance class are noted with circle symbols (significant differences are indicated by different fill shades..... 57

Figure 3.1. Location of three study sites in the Valles Caldera National Preserve in northern New Mexico. Outlines indicate perimeter of colony area that was the focus of trapping efforts and burrow sweeps from May 2004 until September 2006. One colony, El Cajete, contained areas where prairie dog burrows were blocked at the time of study (hatched polygons). 84

Figure 3.2. A) Mean abundance (Number of fleas/Host Individual ± SE) of fleas collected from prairie dogs captured from two colonies in the Valles Caldera National Preserve during six collection periods from 2004-2006. B) Mean abundance (Number of fleas/Host Individual ± SE) of fleas collected from prairie dog burrows sampled from three colonies in the Valles Caldera National Preserve during six collection periods from 2004-2006. Letters signify significant differences ($p<0.05$) among sampling periods for each site, where those points which share a letter are not different across sampling periods. 85

Figure 3.3. A) Prevalence (Number of infested individuals/Total individuals collected) of fleas collected from prairie dogs captured from two colonies in the Valles Caldera National Preserve

during six collection periods from 2004-2006. B) Prevalence (Number of infested burrow sweeps/Total sweeps) of fleas collected from prairie dog burrows sampled from three colonies in the Valles Caldera National Preserve during six collection periods from 2004-2006. Letters signify significant differences ($p<0.05$) among sampling periods for each site, where those points which share a letter are not different across sampling periods. 86

Figure 4.1. Number of recaptured animals that were *Bartonella* positive, negative, or with a loss or gain of infection. Left hand figures represent the infection status of animals caught for 2, 3 and 4 sequential months and right hand figures represent the infection status of animals caught every other month over 3, 4 and 5 month periods. 123

Figure 4.2. Density of rodents and prevalence of *Bartonella* caught on webs trapped twice each year from May 2004 through May 2007 at three sites in New Mexico. Figures display results of a generalized linear model analysis of density-prevalence-trapping period relationships. Placitas had significant season and density effects. Trapping period*Density was significant for Sevilleta rodents. Prevalence of *Bartonella* in Valles Caldera was influenced Density, Elevation Density*Elevation and Density*sampling period effects. Not all sites were trapped at all time periods and is reflected in these figures. Bars represent standard deviation. 125

Figure 4.3. Seasonal patterns of rodent capture (standardized to animals/100 trap nights), and *Bartonella* prevalence in rodent blood and flea samples. Months were divided into seasons according to their climatic similarities, where winter is December, January, February (three coldest months), Spring is March, April, and May, Summer is June, July, and August, and Fall is September, October and November. 125

Figure 4.4. Monthly prevalence of *Bartonella* in rodents and their fleas capture from 2 sites. *Perognathus flavus* and *Peromyscus leucopus* were capture at both sites, whereas *P. truei* and *P. boylii* were not. 126

DEDICATION

To the memory my Grandmother, Beverly Watson FitzPatrick (aka Alex Sienna), a headstrong, free thinking, and independent woman. I am lucky to have had her influence in my life.

PREFACE- This dissertation is comprised of three articles submitted to peer-reviewed journals for publication. Each article represents the culmination of one aspect of my dissertation research and is meant to substitute for chapters that might appear in a more traditional dissertation. This alternative format has the following conventions. First, the format of each article varies according the specific journal to which it was submitted. Second, there are some points of redundancy. In particular, the field methods are repeated in chapters 2 and 3 as is some of the introductory material used for all three articles. For this reason, and in order to minimize further redundancy, I have not included a separate methods chapter. A complete review of the study design and laboratory work is presented in Chapter 4 and detailed description of the prairie dog survey is presented in Chapter 3. Chapter 2 contains a comprehensive description of the methods used for my comparative analysis of flea communities. Third, the introduction and discussion review the major points relevant to these chapters and are designed to provide the setting for and bring together the major point of each individual chapter. A comprehensive review of the relevant literature is contained within the body of each chapter. Finally, the articles contained within this dissertation are the culmination of work and ideas from many collaborators. Though I am responsible for the conception and analysis of these manuscripts, each manuscript has or will have coauthors and, as such, I use words “we” rather than “I” where the overall product has resulted from contributions made by multiple individuals.

CHAPTER 1: INTRODUCTION

Humans have the single greatest impact of any species on the world. Among the most critical consequences of our large-scale modification of natural systems are the effects on biological systems and, in particular, diseases (Daszak et al. 2001; Patz et al. 2000; Wilcox and Gubler 2005). Pathogens both zoonotic and anthropogenically derived are emerging and reemerging due to an increasingly interconnected world, changes in habitat, and human encroachment into the remaining few wild areas (Wilcox and Colwell, 2005). These factors have to lead to increase in the transmission rates and incidence of diseases that are detrimental to both humans and wildlife. Wildlife already under pressure from direct habitat loss and degradation are threatened by increases in the incidence and severity of disease because of the indirect effects of these changes on host-pathogen interactions (Crowl et al. 2008; Daszak et al. 2001; Deem et al. 2001). Of particular concern for both human and wildlife health, is the collective effect of anthropogenic disturbance on vector borne diseases (Koontz and Daszak 2005). Vectors have free-living life stages and, thus, are influenced by anthropogenic changes in both environmental and host habitats.

Human activities, such as agricultural or forestry practices that change site microclimate (relative humidity, soil temperature), and anthropogenic changes in seasonal temperature and precipitation regimes directly affect vector survivorship, development and feeding rates (Harvell et al. 2002; Patz et al. 2000; Daszak et al. 2001; Keesing et al. 2006). Anthropogenic disturbances also have the potential to change the availability, density and susceptibility of hosts to pathogens and vectors,

and thus indirectly influence the spread and persistence of disease within an ecosystem (Patz et al. 2000; Daszak et al. 2001; Keesing et al. 2006). Human disturbance processes have led to the recent range expansions of many vector-borne pathogens including Lyme disease, malaria, dengue fever, tick-borne encephalitis, yellow fever, West Nile fever and plague (Harvell et al. 2002).

Plague, caused by the bacteria *Yersinia pestis*, is a good example of a zoonotic disease that has continues to perpetuate and spread to new populations due to human activity. *Yersinia pestis* has a long and dramatic history in human populations beginning at least as early as 542 A. D. where it is suspected to have caused the Justinian plague, which lasted 60 years and left 100 million dead (Poland et al., 1994). However, this pathogen is probably most famous for its role in the Black Death in Medieval Europe that killed nearly a quarter of the population. We have just emerged from a third pandemic that began in the late 1880's and continued well into the 1990's. This pandemic originated in China and quickly reached Hong Kong, where it was disseminated around the world on rat-infested ships. It was during this time that plague was introduced to the U.S. Though it appears to have made contact at several ports in the U. S., the fateful arrival of the Nippon Maru to San Francisco in 1899 resulted in the establishment of plague in native California ground squirrel populations (Link, 1955; Adjemian et al., 2007). From that point, *Yersinia pestis* made its way east eventually reaching as far as Texas by 1950. Today, plague is well established in wild rodents throughout the western half of the U.S.

Prairie dogs (*Cynomys* spp.) have been particularly hard hit by the introduction of plague (Gage and Kosoy, 2005; Cully and Williams, 2001). Prairie

dogs are important members of grassland systems and are considered a keystone species of North American grasslands because they modify the landscape in such a way that benefits other species (Kotliar et al., 1999). Hunting, eradication programs, habitat loss due to agriculture, and the introduction of plague have reduced prairie dogs species to less than 2% of their historic range (Millar et al., 1994). Prairie dogs are extremely susceptible to plague because they have no innate immunity and live in large colonies with elaborate burrow systems that favor reproduction and survival of the flea vector (Cully and Williams, 2001; Gage and Kosoy, 2005). Plague causes mortality rates in excess of 95% in exposed prairie dog colonies (Cully and Williams, 2001). A number of studies have identified prairie dogs themselves and their fleas as short term reservoirs of plague (Girard and Wagner et al., 2004; Webb et al., 2006; Wilder et al., 2008), but far less is known about how plague is maintained within the environment over the long term. Whereas, many of the threats facing prairie dogs can be managed, plague remains one of the single greatest threats to the survival of the prairie dog.

Plague requires two things in order to perpetuate: 1) Susceptible animals that become bacteriaemic and succumb to infection thereby becoming a source of infection to flea vectors, and 2) A flea vector that is itself susceptible to infection (Christie, 1982; Gage and Kosoy, 2005). The sylvatic lifecycle of plague in the U.S. is divided into two parts: the first involves an enzootic or maintenance host and the second an epizootic or amplification host (Poland et al., 1994; Gage and Kosoy, 1995). The enzootic host is typically described as having a high reproductive rate and variable response to plague. Enzootic species are able to persist as a viable host for

plague by replacing or minimizing its losses due to the disease. In contrast, the epizootic host is quite susceptible to plague and experiences high mortality and widespread outbreaks when exposed to *Y. pestis*. Outbreaks in epizootic hosts can amplify the presence of plague in an environment because the dying animals leave behind a large number of infectious fleas and are a source of infection themselves. However, these outbreaks are short-lived and, because of the widespread mortality typical of epizootics, are not a viable means for the long-term maintenance of plague within the environment. Prairie dogs are a classic example of an epizootic host and several rodent species associated with prairie dogs have been implicated as enzootic hosts, including grasshopper mice (*Onychomys* spp.) on black-tailed prairie dog towns (*C. ludovicianus*) and *Peromyscus* spp. in white-tailed (*C. leucurus*) and Gunnison's prairie dog towns (*C. gunnisoni*) (Gage et al., 1995; Thiagarajan et al., 2008). Fleas are also critical to the perpetuation of plague in the environment. Though plague can be transmitted directly between individuals, this manifestation, know as pneumonic plague, is highly virulent and kills the infected host in 1-3 days (Poland et al., 1994). Thus, directly transmitted plague is short lived and self-limiting, whereas the flea transmitted form, bubonic plague, is less pathogenic and moves more slowly within and between individuals. Over 150 flea species are known to transmit plague though they vary considerably in the efficiency in which they do this (Gratz, 1999). Some fleas may act as short-term reservoirs of plague and in one instance *Y. pestis* was found in a flea from a prairie dog burrow nearly one year after a prairie dog epizootic that eliminated the entire colony (Lechleitner et al., 1968). Despite a number of studies and observations regarding plague outbreaks in prairie dog towns

(Table 1), we have yet to identify the specific rodent and flea species responsible for maintaining plague in the habitats of the Southwestern U.S. nor do we know how plague is introduced into prairie dog colonies.

Fleas are ubiquitous parasites of small mammals and are vectors for a number of diseases that affect humans including plague (*Yersinia pestis*) and the *Rickettsia* organisms that cause murine typhus and Rocky Mountain fever (Gage, 1995). The presence and abundance of fleas is linked to the likelihood and spread of flea-borne disease like plague and are closely tied to the presence and abundance of their hosts (Lorange, 2005; Eisen et al. 2006; Krasnov et al. 2006a). In general, plague is more likely to spread in a population where flea species are highly susceptible to *Y. pestis* and have low host specificity (Gage and Kosoy, 2005). However, low efficiency vectors that exhibit some resistance to plague may actually be quite important to spread of the disease if they are common in the environment (Kartman et al., 1962; Lechleitner et al., 1968, Eisen et al., 2006). Indeed flea abundance is positively related to flea species vector potential for plague (Krasnov et al., 2006) and abundance and prevalence of flea species is an important determinant of *Y. pestis* transmission in particular (Eisen et al., 2006; Lorange, 2005). In addition, infection by *Y. pestis* may break down flea host specificity such that an infected flea is more likely to attempt to feed and thereby infect a greater number of host species than an uninfected flea. Thus, flea-borne disease spread is a function of the characteristics of the flea communities and these characteristics, in turn, are influenced by host availability and microclimate preferences.

The objectives of this dissertation project were to explore the mechanisms that influence the spread of vector borne diseases and apply this knowledge to plague cycles in the Western U.S. particularly with respect to prairie dogs. To do this, rodents including Gunnison's prairie dogs (*Cynomys gunnisoni*) were surveyed in three locations in New Mexico over the course of 3 years. During these surveys, we collected blood and fleas and tested these samples for the presence of two bacterial pathogens, *Yersinia pestis* and *Bartonella*. Plague is difficult to detect outside of prairie dog epizootics, which limits our knowledge of the pathogen-vector-host system. Therefore, in this dissertation, I focus on the underlying dynamics of flea borne diseases with three distinct analyses. The first chapter presents a comparative analysis of fleas and flea communities surveyed from around the globe and asks how anthropogenic habitat disturbance affects the likelihood of disease exchange by fleas. Plague is globally distributed and maintained in a variety of host flea systems (Gratz, 1999). In central Asia, plague is primarily maintained in Great Gerbil, *Rhombomys opimus*, populations and fleas. *Yersinia pestis* utilizes a number of hosts in Africa including rats, gerbils, meriones and wild mice. Plague has established urban cycles in both Vietnam and Madagascar where it is found in the rat, *Rattus rattus* and its flea *Xenopsylla cheopis*. In the United States, plague cycles are typically described as a two-cycle system involving enzootic and epizootic hosts. Several studies of plague outbreaks link certain weather conditions to increased incidence of sylvatic plague (Stapp et al., 2004; Collinge et al., 2005; Pole and Chan, 2006; Snäll et al., 2008). Seasonal changes in plague levels in rodent populations are mediated through precipitation and temperature regimes that have a direct effect on flea vector

populations (Collinge et al., 2005; Stenseth et al., 2006; Park, 2007). However, climate explains only part of the pattern of plague outbreaks. The single most important factor leading to plague outbreaks in human populations is contact with infected wildlife. Increase contact may be due to climate events that lead to population increases in rodent reservoirs (Stenseth et al., 2006), but may also be due to human encroachment into new habitats or, as hypothesized in this chapter, by changes in the rodent and flea communities due to human activity. The third and fourth chapters of this dissertation are dedicated to the analysis of the field data collected as part of this research project. In Chapter 3, I explore the dynamics of plague outbreaks in Gunnison's prairie dogs in the Valles Caldera National Preserve. This work adds to knowledge gained by other studies, which have examined plague in prairie dog towns (Table 1.1). Finally, Chapter 4 examines the infection dynamics of *Bartonella*, another bacterial pathogen that infects the erythrocytes of a diversity of mammals, in an attempt to identify the primary mechanisms driving the spread of flea-borne diseases in rodents. *Bartonella* is also considered an emerging disease (Azad et al., 1997; Boulouis et al., 2005) and has several characteristics that make it an ideal candidate for studies of vector borne disease. *Bartonella* is common in rodent species where it is most likely transmitted by fleas (Bown et al., 2004). Though many *Bartonella* species are implicated in human disease (Greub and Raoult, 2003), it appears to have little immunological consequences for its wild rodent hosts (Chomel et al., 2003, Boulouis et al., 2001). Therefore, *Bartonella* is common within rodents and rodent populations are not prone to extinction due to *Bartonella*, which allows us to collect ample data for analysis of flea versus host-mediated mechanisms of

pathogen transmission. It is the hope that such studies will inform the approach of studies on other, more difficult to detect, vector borne zoonotic disease such as plague, caused by the bacterium *Yersinia pestis*. Though a number of studies (Kosoy et al., 1997, 2004a,b; Jardine et al., 2006; Bai et al., 2007a; 2008; Reeves et al., 2005, 2007; Birtles et al., 2001; Holmberg et al., 2003; Telfer et al., 2007a,b; Stevenson et al., 2003; Morway et al., 2008) have contributed considerably to our understanding of the ecology of particular species, we lack a cohesive view on the nature of *Bartonella* infections in rodents and flea vectors. In particular, the specific mechanisms influence the transmission of this pathogen remains unknown. Therefore, this chapter is largely dedicated to describing the life cycle of *Bartonella* in rodents at the three study sites of this research.

Table 1.1. Number of prairie dogs, burrows and fleas sampled in six studies on Gunnison's (GPD), White- (WTPD), and Black-tailed prairie dogs (BTPD) colonies. *Asterisks indicate plague positive samples.

Study citation	Ecke and Johnson, 1950		Lechleitner et al., 1968		Cully et al., 1997		Ubico et al., 1988		Anderson and Williams, 1997		Holmes et al., 2006		This Study (Chapter 3)	
Location	Colorado		Colorado		New Mexico		Wyoming		Wyoming		Montana		New Mexico	
Species/Sample	GPD	BURR	GPD	BURR	GPD	BURR	WTPD	BURR	WTPD	BURR	BTPD	BURR	GPD	BURR
Number sampled	na	na	59	2700	61	467	32	165	208	2161	107	na	130	280
Aetheca wagneri				1		1*[z]	1[z]		4[z]	2[z]		11[z]		
Catallagia decipiens						4				8				2
Cediopsylla inaequalis									2					
Hystrichopsylla gigas dippiei	X*						1	6	1					
Monopsylla vison					1									
Neopsylla inopina							15	43*	38*	49*				
O. hirsuta	X			1	339*	662*					208	147	586*	120*
O. idahoensis		45	15*	85*			8*	24*	57*	25*			28	20
O. labis	9	82	19*	445*	58	289*	54*	314*	38*	54*				

Study citation	Ecke and Johnson, 1950		Lechleitner et al., 1968		Cully et al., 1997		Ubico et al., 1988		Anderson and Williams, 1997		Holmes et al., 2006		This Study (Chapter 3)	
Location	Colorado		Colorado		New Mexico		Wyoming		Wyoming		Montana		New Mexico	
Species/Sample	GPD	BURR	GPD	BURR	GPD	BURR	WTPD	BURR	WTPD	BURR	BTPD	BURR	GPD	BURR
O. tuberculata cynomuris[1]	63*	5*	54*	11033*	36	9*	85*	248*	49*	52*			14	8
O. t. tuberculata[1]											68	40	5	5*
Peromyscopsylla hesperomys												1		
Pulex sp.									1		867	159		
Rhadinopsylla fraterna[3]							4	3*	12	9				
R. sectilis[2]					1		2	5						
Thrassis bacchi					9[y]	13*[y]								
T. pandorae								6	2[w]	8*[w]				
Total Fleas	151	208	299	12540	505	978	202	649	795	475	1143	358	633	167

[1] *Opisocrotstis t. cynomuris=Oropsylla t. cynomuris* [2] *Micropsylla sectilis=Rhadintopsylla sectilis*; [3]*Rectofrontia fraternal=Rh. fraterna*;; [V] Reported as *P. simulans*; [W] Reported as *Oropsylla pandorae*; [X]reported as *Oropsylla tuberculata;* [Y]Reported as *Oropsylla bacchi*; [Z] Reported as *Monopsylla wagneri*. "na" = not available

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CHAPTER 2: ANTHROPOGENIC DISTURBANCE AND THE TRANSMISSION OF FLEA-BORNE DISEASES

PREFACE- This chapter is formatted for publication within *Oecologia*.

ANTHROPOGENIC DISTURBANCE AND THE TRANSMISSION OF FLEA-BORNE DISEASES

Abstract: Anthropogenic disturbance may lead to the spread of vector-borne diseases through effects on pathogens, vectors, and hosts. Identifying the type and extent of vector response to habitat change will enable better and more accurate management strategies for anthropogenic disease spread. I compiled and analyzed data from published empirical studies to test for patterns among flea and small mammal diversity, abundance, several measures of flea infestation, and host specificity in 70 small mammal communities spanning 5 biomes and 3 levels of human disturbance: 1) remote/wild areas; 2) agricultural areas; and, 3) urban areas. Ten of 12 mammal and flea characteristics showed a significant effect of disturbance category (6 traits), biome (4), or both (2). Six variables had a significant disturbance by biome interaction. For mammal-flea communities in forest habitats (39 of the 70 communities), disturbance affected all 12 characteristics. Overall, flea and mammal richness were higher in remote versus urban sites. Most measures of flea infestation, including percent of infested mammals and fleas/mammal and fleas/mammal species increased with increasing disturbance or peaked at intermediate levels of disturbance. In addition, host use increased, and the number of specialist fleas decreased, as human disturbance increased. Of the three most common biomes (forest, grassland/savanna, desert), deserts were most sensitive to disturbance. Finally, sites of intermediate disturbance were most diverse and exhibited characteristics associated with increased disease spread. Anthropogenic disturbance was associated with conditions conducive to increased transmission of flea-borne diseases.

Introduction:

Anthropogenic habitat disturbance disrupts ecosystem processes in ways that can affect zoonotic disease dynamics (Daszak et al. 2001; Patz et al. 2000; Wilcox and Gubler 2005 and references therein). Human population growth and coinciding increases in urbanization, agricultural intensification, and encroachment into wild areas are directly linked to the emergence of many zoonotic diseases in human populations (Wilcox and Colwell, 2005). Recent increases in the incidence and severity of disease within wildlife species have been attributed to a variety of interacting factors including habitat loss and degradation, animal and pest introductions and increased connectivity between populations (Crowl et al. 2008; Daszak et al. 2001; Deem et al. 2001). Of particular concern for both human and wildlife health, is the collective effect of anthropogenic disturbance on vector borne diseases (Koontz and Daszak 2005). Vectors have free-living life stages and, thus, may respond to anthropogenic changes in both environmental and host habitats.

Human activities, such as agricultural or forestry practices that change site microclimate (relative humidity, soil temperature), and anthropogenic changes in seasonal temperature and precipitation regimes directly affect vector survivorship, development and feeding rates (Harvell et al. 2002; Patz et al. 2000; Daszak et al. 2001; Keesing et al. 2006). Anthropogenic disturbances also have the potential to change the availability, density and susceptibility of hosts to pathogens and vectors, and thus indirectly influence the spread and persistence of disease within an ecosystem (Patz et al. 2000; Daszak et al. 2001; Keesing et al. 2006). Human disturbance processes have led to the recent range expansions of many vector-borne

pathogens including Lyme disease, malaria, dengue fever, tick-borne encephalitis, yellow fever, West Nile fever and plague (Harvell et al. 2002).

Fleas are ubiquitous parasites of small mammals and are the primary vector for a number of diseases that affect humans including plague (*Yersinia pestis*) and *Rickettsia* spp. such as murine typhus and Rocky Mountain fever (Gage, 1995). Human induced habitat change can affect small mammals (Tikhonova et al. 2006) and flea-borne mammal diseases (Azad et al. 1997) but does not always lead to increased disease incidence (Collinge et al. 2005). The presence and abundance of fleas are directly linked to the likelihood and spread of flea-borne disease like plague and are closely tied to the presence and abundance of their hosts (Lorange, 2005; Eisen et al. 2006; Krasnov et al. 2006a). Disease transmission is also more likely when fleas exhibit low host specificity (i.e. parasitize a diversity of host species) (Gage and Kosoy, 2005). Thus, the overall effect of disturbance on disease spread is a culmination of individual effects on host-parasite interactions, habitat dependencies of host and flea species, and host specificity of fleas. For instance, anthropogenic disturbance decreases mammal community diversity (Tikhonova et al. 2006), and should lead to decreased flea diversity. However, diversity loss may favor common host species, which tend to harbor more flea species (Egoscue, 1976) and lead to an increase in overall flea abundance.

To understand how fleas and flea-borne diseases might be impacted by human disturbance, we analyze flea community dynamics and flea host utilization patterns in relation to disturbance intensity in a large sample of published studies conducted across the globe and in a variety of habitats. We interpreted the resulting correlations

in light of current theory regarding habitat change and vector parasites. Our objectives were to answer the questions: 1) Does anthropogenic disturbance affect flea diversity, abundance and host specificity; and, 2) What does this mean for long-term persistence of fleas and flea borne pathogens in a changing world?

Materials and Methods:

Data Compilation: We searched Scisearch, CSA biological abstracts, Scirus, the Defense Pest Management Information Analysis Center Literature Retrieval System (Armed Forces Pest Management Board—LRS http://lrs.afpmb.org/rlgn_app), and Google scholar using the following search terms and combination of these terms: flea(s), rodents, small mammals, vector, habitat/habitat change, parasite, flea/parasite assemblage, abiotic and biotic, anthropogenic disturbance/ change, disease, plague, climate, murine typhus, flea-borne, vector borne, rickessia. We found additional articles in the literature cited sections of these papers.

We retained only those studies that 1) attempted to collect all fleas from animals captured in surveys that targeted the entire small mammal community, 2) live-trapped animals, 3) actively collected fleas (by brushing, etc.), 4) described the location and habitat of trapping locale, and 5) included numerical data for each flea and host species. Fleas are known to abandon dead hosts and thus studies of kill-trapped mammals are likely to underestimate true flea abundance and diversity (Murray, 1957). These criteria yielded a sample of 63 studies reporting small mammal flea surveys for 70 distinct sites across the world (Table 1; Appendix S1 in Supporting Information).

Classification Schemes: We assigned each field site to one of five vegetation-based biomes and one of three disturbance levels (Table 1). We usually used latitude and longitude to classify each site. If these data were not provided, we used city search engines, Google Earth, travel sites, web pages and scientific articles on other studies that used the same plots. The vegetation classifications used in this analysis were condensed versions of those presented by Olson et al. 2001. The Olson *et al.* (2001) terrestrial ecoregion and biome data are available in interactive form and as a GIS database from the World Wildlife Fund website (www.worldwildlife.org/science). This classification scheme identifies the dominant natural vegetation type based on latitude, soil conditions, elevation, and climate regime, but ignores human land use. We condensed the 14 biomes of Olsen's classification system as follows: Tropical, Temperate, and Boreal forests were condensed into Forests; Temperate grassland/savannas and Tropical grassland/savannas were classified into Grassland/Savannahs; Deserts, Arid Shrublands, and Arid steppe biomes were classified as Deserts; Mediterranean and Chaparral were merged; and Alpine and Tundra were merged. Canopy cover was the primary characteristic used to distinguish between forest and grassland/savanna ecoregions and precipitation regime (xeric versus mesic habitats) was the primary characteristic used to distinguish between grassland/savanna and desert categories in situations where sites existed in an intermediate type biome (e.g. those described as woodland or shrubland). Five sites existed within a mosaic type landscape or had a study site description that differed from the biome classification. In these instances, we considered the size and nature of the habitat patch when assigning biome. We assigned the Olson classification to three of

these studies (Achuthan et al. 1971; Nava et al. 2004; Hastriter et al. 2004) and we used the author's description or a new classification for the remaining two studies. For example, one study in Huambo, Angola (Linardi, *et al.*, 1994) fell with the Grassland/Savanna biome, but was described as a forest in the paper. Angola is dominated by grasslands but has distinct forest patches at high elevations (McGinely, 2008). Because these montane forests are considered relics of a moist forest biome that once dominated the region, we classified this study as a forest. Conversely, Shayan and Rafinejad, 2006 conducted surveys of several sites in Iran, which encompassed three ecoregions: Zagros Mountain forest steppe, Nubo sindian desert and semi-desert, and central Persian desert basin (http://www.nationalgeographic.com -Terrestrial Ecoregions-). Though the authors cite forest and meadow habitats, we categorized these surveys within grassland/savanna category to represent more accurately the steppe like nature of most of the study sites, which for the most part lacked a continuous canopy cover.

We used the GIS database of the Olson terrestrial ecoregions from the WWF website to assign a biome to each site. We used ArcView to open the database and then saved the file as a zipped .kml (Keyhole Markup Language) file, or .kmz file, for use in Google Earth. We used the anthropocentric biome map created by Ellis and Ramankutty (2008) in Google Earth to assign disturbance level to each study site. The map shows classification assignments conducted at 5 arc minute (5'=0.0833˚ or ~86km^2 at equator) and is available in interactive form from Encyclopedia of Earth, viewable maps in Google Earth and Microsoft Virtual Earth (www.eoearth.org/article/Anthropogenic_biome_maps) or in GIS format (Ecotope.org).

Ellis and Ramankutty (2008) define four major anthropocentric biomes, namely wild lands, rangelands, croplands and urban zones; these were further subdivided by population density and other factors to create 18 distinct habitat types. We used a simplified version of their scheme to recognize three disturbance levels: 1) Low disturbance sites were relatively **wild** or remote habitats that may include light human populations; 2) Intermediate included **agricultural** areas, rural villages, and pastures; and, 3) High disturbance areas were **urban** or densely populated areas. For studies published after 1990, the disturbance class assignments were based directly on the output of the Ellis and Ramankutty (2008) map, which is projected for conditions in 2005. For studies that occurred before 1990, we used Ellis and Ramankutty (2008) for initial classification and cross-checked this classification with the original study description as well as other data including census information, news articles, or other descriptions of the area near the time of the study. Using these methods, we reclassified six sites. One site characterized by Ellis and Ramankutty (2008) as intermediate (Walton and Hong 1976) was reclassified as urban and another "intermediate" site (Davis et al. 2002) was reclassified as wild because the study areas were too small to be mapped at the scale of the anthropocentric biome map. Four intermediate sites (Campos et al. 1985; Chenchijtikul et al. 1983; Coutrip et al. 1973; Graves et al. 1974; Poorbaugh and Gier 1961) were wild at the time of study but had converted to agriculture by 2005.

Most studies reported data for small mammal surveys conducted at multiple sites within an area. Where possible we pooled data from multiple surveys within a single biome and disturbance level. Seven studies (Adler et al. 2001; Bengtson et al.

1986; Chenchijtikul et al. 1983; Heisch et al. 1953; Liat et al. 1980; and Sunstsov et al. 1997) reported surveys from more than one disturbance class. For these studies, each distinct survey was analyzed as an independent sample, yielding three sites for Heisch *et al.* (1953) and two sites for each of the other studies.

***Hypothesis Testing and Statistical Analysis*:** Small mammal and flea richness (number of species), number of small mammal or flea individuals collected, prevalence (percent of hosts parasitized), intensity of infection (mean number of fleas/parasitized mammal), flea burden (mean number of fleas/mammal) and flea species burden (mean flea species/mammal) were calculated for each host species within each site. We used the average of prevalence, intensity and flea burden values calculated for each species within a site to test for differences among communities. Though these measures are typically used to compare parasite infections between host species, they also describe the overall infection characteristics of each community. We used the average number and proportion (number infested/number potential host species) of host species used by each flea species within each site as a measure of the breadth of flea host selectively (niche breadth) at each site. Finally, the proportion of flea species infesting just one host or three or more host species were used as the proportion of specialist or generalist flea species present, respectively.

Relationships between log transformed mammal and flea variables, standardized for sampling effort, were assessed with Pearson's correlation analysis using a Bonferroni adjusted alpha level for multiple tests (PROC CORR, SAS 9.2). Standardizing for sampling effort (number of mammals sampled) was appropriate because many previous studies note positive associations between number of

mammals captured and measures of diversity (e.g., Holdenried et al. 1951, Nava et al. 2003, Vasquez et al. 2005; Stanko 2002, Krasnov et al. 2004a, b, 2007, and Watve and Sukumar, 1995 for mammal number-flea richness and Krasnov et al. 2004b, Stanko et al. 2002, and Morrone and Gutiérrez, 2005 for mammal richness-flea richness relationships). Previous studies suggest that the number of mammals trapped is correlated with flea burden and abundance both positively (Kotti and Kovalesky, 1996; Krasnov et al. 2004b, 2007; and Zhonglai and Yaoxing, 1997) and negatively (Krasnov et al. 2006a; Stanko et al. 2002 and Schwan, 1986). Similarly, in our review, total number of mammals captured was significantly ($P < 0.05$) correlated with mammal and flea richness (r = 0.31 and 0.44, respectively), mammal diversity (r = -0.29), fleas collected (r = 0.85), flea species burden (r = 0.44), and number of host species infested (r = 0.30) (Online Resource 2). Though we did not find an association between number of hosts captured and flea prevalence, others have shown both positive (Lindsay and Galloway, 1997; Bossi et al. 2002) and negative relationships (Schwan, 1986). Therefore, we used hosts captured as a covariate in all analyses to minimize confounding the effect of capture effort with the effect of human disturbance and habitat.

We used generalized linear model (PROC GLIMMIX, SAS 9.2) analysis with a negative binomial distribution and log link to test for disturbance level and biome effects on number of mammal and fleas collected, richness, intensity, burden, and flea species burden. A negative binomial distribution is appropriate for count data with overdispersion (Little et al. 2002) and was consistent with the distributions of our data. We only analyzed data collected from the four dominant biomes (Forest, Desert,

Grassland/Savanna, and Mediterranean) because Alpine/Tundra habitats were not represented in all disturbance classes. We used PROC GLIMMIX analysis with a binomial distribution and logit link to test for disturbance and biome effects on prevalence of hosts infested, proportion of specialists, generalists and host species infested. Tukey adjusted tests of means were used to identify pair-wise differences between disturbance classes or biomes for significant model variables. We also ran an analysis as described above to test for differences among disturbance classes within the most prevalent biome, forest, as well as to look for specific differences among biomes within each disturbance level.

Results:

My sample of 63 studies included 70 sites (Table 2.1 and Appendix 1). These studies described flea communities from 23 high (urban) disturbance, 22 intermediate (agricultural), and 25 low (wild) disturbance sites. Sites were located on six continents with Asia and North America hosting the majority of study locations. Forest (both deciduous and rainforest) was the most well represented biome, followed by deserts and grasslands.

Mammal and flea richness were positively correlated with each other (Fig. 2.1). Flea number was positively correlated with flea burden, prevalence and intensity of infestation. Measures of flea infection (prevalence, intensity, flea burden) were positively correlated with one another (Fig. 1). Proportion of host specialist at each site was negatively correlated with the proportion of generalists ($r= -0.50$) and the average number of hosts/flea species ($r= -0.59$).

Disturbance level

Disturbance was a significant predictor for six of the 12 variables related to mammals and fleas; a significant interaction between disturbance class and biome also affected six variables (Table 2.2). Disturbance class had a stronger influence than biome on both small mammal community variables, whereas flea community variables were more commonly explained by biome or by the interaction term (Table 2.2). Averaged across biome, richness peaked in intermediate disturbance (agricultural) classes. Two of three abundance measures (number fleas, flea burden) were greatest in urban sites, whereas number of mammals captured was significantly greater in wild locations (Figs. 2.2 and 2.3). Two measures of infection, prevalence and proportion of host species used, were significantly higher in urban sites and three measures, number of host species used, intensity, and flea species burden, were greatest in agricultural sites (Figs. 2.2 and 2.3). Mean proportions of generalist and specialist fleas were greatest in agricultural sites (Fig. 2.3).

Within forest biomes, all 12 variables differed significantly among disturbance classes (Table 2.2). Number of mammals and fleas collected were significantly higher in urban sites, whereas most other variables were significantly greater in agricultural sites (Figs. 2.2 and 2.3).

Biome

Biome was the primary factor explaining observed changes in the total fleas collected at a site and significantly affected prevalence, proportion of host species infested, and proportion of host specialists at a site (Table 2.2). Most measures of

infestation were relatively low with little variation across biomes for low disturbance sites, but as disturbance increased, infestation increased also, with great variation among biomes (Figs. 2.2 and 2.3; Table 2.2). The prevalence of infested mammals showed the greatest degree of significant divergence across biomes. Forests had a significantly greater number of mammals and proportion of specialist fleas in wild sites and higher flea burden in agricultural sites, as compared to other habitats (Figs. 2.2 and 2.3). Deserts had a significantly higher number of fleas and higher prevalence than any other biome, and fleas infested a greater proportion of available host species in deserts versus other biomes (Figs. 2.2 and 2.3). Deserts also had a much lower proportion of specialists, particularly in high disturbance sites. Mediterranean sites had the greatest flea diversity and showed distinct trends with respect to the proportion of generalist, specialists and flea burden (Fig. 2.2).

Discussion:

There were clear and statistically significant associations between anthropogenic disturbance and mammal and flea community structure. Most measures of flea infestation increased with increasing disturbance (Figs. 2.2 and 2.3) and variables associated with increased risk of disease spread and transmission, in particular number of mammals and fleas collected, prevalence and intensity of infestation (Neito, et al. 2007; Krasnov et al. 2006a; Hawlena et al. 2007), increased significantly as disturbance increased. Because we used “total mammals” as an offset (covariate) in linear model analysis, the variable “total fleas” is equivalent to the flea index (fleas/capture), a measure commonly used to quantify flea infestation levels and associated with an increased likelihood of plague outbreaks (Hawlena *et al.*, 2007).

The influence of disturbance on mammal and flea characteristics was most evident in analyses restricted to the forest biome (Table 3), probably reflecting greater statistical power as sample size increased. Like Wilcox and Gubler (2005) and Tikhonova *et al.* (2006), we found that richness and diversity (Shannon's H, data not shown but trends and significance tests mirrored those produced with richness measures) of mammal communities decreased with increasing anthropogenic disturbance. Our analysis extends this pattern, in that human disturbance also reduces richness and diversity of flea communities when comparing wild and urban sites.

It is generally accepted that increased anthropogenic activity leads to decreased ecosystem heterogeneity and stability (*sensu* Wilcox and Gubler, 2005; Bradley and Altizer, 2006), which has several repercussions for disease transmission. In particular, changes in diversity can have many consequences for flea community structure with direct implications for disease spread. First, ecosystem simplification can favor host species that are natural reservoirs or good intermediate hosts for zoonotic disease (LoGuidice et al. 2003). Commonly, these host species are habitat generalists that benefit from disturbance related declines in abundance of habitat specialists (Keesing et al. 2006). In addition, these generalist host species often carry more diverse flea communities and higher flea loads (number of fleas/host), both of which are associated with increased disease transmission (Egoscue, 1976). Second, increases in the densities of generalist host species favors transmission of vectors and their pathogens (Egoscue, 1976; Keesing et al. 2006; Wilcox and Gubler, 2005). Third, disturbance can also favor generalist vector species, which are important determinants for the spread of zoonotic disease among wildlife populations due to

their tendency to feed from a variety of taxa (Molyneux, 2003; Gettinger and Ernest, 1995). For this reason, increased abundance of generalist vectors is strongly associated with increased parasite transmission (Gettinger and Ernest, 1995) and incidence of disease outbreaks in both human and wildlife population (Neito et al. 2007; Hawlena et al. 2007). In addition, at least one study found that fleas with broad host spectrums (infest multiple host species) tended to be good plague vectors (Krasnov et al. 2006), and thus there could be additional inherent characteristics of a generalist species that predispose them to be good disease vectors.

When comparing remote and urban sites, this study showed trends of diversity consistent with ecosystem simplification and flea host use became more generalized as disturbance increased. Specifically, the proportion of generalist flea species (excluding Mediterranean communities) and the average number and proportion of host species infested by each flea species increased with increasing disturbance (Fig. 3), whereas the number of specialists decreased (except in Mediterranean communities). Our analysis cannot suggest whether these trends reflect an evolutionary mechanism (generalists are better adapted for dealing with disturbance), or an ecological mechanism (specialist lost with loss of their host species). Nonetheless, it is clear that fleas in more disturbed site tend to infect a greater number of species. In addition, flea exchange among hosts is known to increase with the percentage of hosts infested (Bossard, 2006), and prevalence increased with greater disturbance in this study. Clearly anthropogenic activity can potentially increase disease risk through changes in flea host utilization patterns.

Flea host specificity was measured in two ways in this study: 1) by quantifying individual flea species host utilization or the number or host species used versus available at each site; and, 2) by classifying flea species according to the number (1 or greater than 3) of host species parasitized. A number of studies have examined the relationship between various measures of host specificity and environmental or host community characteristics. Many found that habitat type and the physical characteristics of habitat affect how fleas use hosts (Cole and Koepke, 1947; Krasnov et al. 2004a; Trpis, 1994; Chandrahas and Krishnaswami, 1971; and Castleberry et al. 2003). In contrast, Poulin (1998), in his review of specificity patterns of small mammal parasites, considered host traits such as density, lifespan, diversity of habitats used, and social structure most important in determining the host breadth of parasite species (e.g., Poulin et al. 2006). Poulin's view is supported by Krasnov *et al.* (2004c, 2006c) who found specialization negatively related to host body size and abundance. Our analysis only found a significant relationship between flea specificity and disturbance or host variables when measuring the number or proportion of host species used rather than quantifying fleas as specialists or generalist. It may be that our definition of specialists and generalist were limited (raw species counts versus an index). Host phylogeny, which was not addressed in this review, may have also affected our results (e.g. Felsenstein, 1985, but see Guègan et al. 2005). The proportion of hosts used by a flea species was significantly and negatively related to the host availability (mammal richness) (Fig. 1) indicating that fleas did not increase their host species spectrum linearly with host species availability. In analysis of disturbance effects, average number of host species used

and mammal richness trends correspond, but the proportion of host species used is clearly tied to disturbance (Fig. 2.3). Thus, the trend for broader host species utilization with increasing disturbance does not relate solely to host or flea diversity.

Many infection parameters peaked at sites of intermediate disturbance (Fig. 2.2 and 2.3). Most notably, the intensity of infestation, average number of hosts species utilized by flea species, and flea burden were significantly higher in intermediate disturbance sites (Fig. 2.2 and 2.3). Sites of intermediate disturbance can be important areas for disease exchange and emergence because they contain peridomestic mammal species, which readily carry disease between wild reservoir hosts and the commensal mammal species that live in proximity to humans. Indeed, plague in humans is commonly associated with the presence of peridomestic mammal species (Perry and Featherston, 1997). In this study, intermediate disturbance sites contained the greatest number of host and flea species, which may reflect the merging of domestic, peridomestic and wild mammal communities. Therefore, these sites provide not only greater opportunity for vector exchange between reservoir and commensal mammals, but also exhibit characteristics commonly associated with both increase vector exchange and disease transmission.

Biome was associated with both the magnitude and direction of the observed effects of disturbance on flea communities (Figs. 2.2 and 2.3). Forest and Mediterranean sites were most diverse, whereas grassland/savanna and desert sites contained the fewest species, which may reflect a relationship between habitat complexity and species richness. Deserts appeared to be more sensitive to disturbance than other biomes (Figs. 2.2 and 2.3). Mammals in deserts also had higher prevalence

and carried more fleas per individual than other sites. The tendency for high flea burden may be a result of the relatively low diversity and richness of fleas in desert sites, which could lead to a predominance of generalist species that tend to be more abundant within communities (Krasnov et al. 2004c). This tendency is also reflected in a much lower proportion of specialists in deserts relative to other habitats (Fig. 2.3). The degree and type of disturbance may be an important factor in how a system responds to disturbance. For instance, grassland to agriculture transitions are less dramatic than a forest to agriculture transitions with respect to overstory structure and species exchange and, therefore, grassland communities may be more tolerant to this particular change. These differences might explain why grassland /savanna communities appear to be the less susceptible to disturbance related changes in host and flea community characteristics (Figs. 2.2 and 2.3).

Global warming is predicted to lead to range expansions of many arthropod vector species (particularly in regions of reduced frost occurrence) and increase the frequency of vector borne disease outbreaks (Githeko et al. 2000; Epstein, 2001; Harvell et al. 2002). However, because higher temperatures reduce adult survivorship, population density of vector species could decrease and lead to lower disease transmission rates (Harvell et al. 2002). In addition, local climatic conditions (or biome) are likely to play an important role in determining disease emergence (Lafferty, 2009). While the ultimate effects of global warming remain to be seen, this study presents clear evidence for the important role of habitat disturbance in increasing flea-borne disease risk.

Anthropogenic disturbance favors several conditions conducive to flea-borne disease spread, namely higher infestation levels, greater flea abundance, and greater host utilization. Disturbance also facilitates greater flea exchange and higher flea infestation levels through its effect on diversity, which may favor generalist host and vector species. Disturbed habitats may play an important role in facilitating the range expansion of vectors predicted by global warming scenarios (Cummings and Van Vuren, 2006). Those regions that are already destabilized are most prone to the negative consequences of such expansion, whereas range expansions may be more limited in areas less affected by disturbance due to the presence of natural checks and balances, which reduce the conditions that promote flea exchange. Thus, preservation of functional and diverse ecosystems may be an effective strategy for limiting zoonotic disease spread.

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Table 2.1. Continental distribution and biome classification of sites used in comparative analysis of anthropogenic disturbance and flea vector assemblage characteristics

	Level of Disturbance		
	High[2,2-23]	**Intermediate**[24-45]	**Low**[46-70]
Number of Studies	23	22	25
Continent			
Africa [8, 10, 12, 13, 18, 31, 32, 38, 40, 42, 63, 69]	5	5	2
Asia[1, 2, 4, 9, 11, 14, 16, 17, 19, 22, 24, 28, 29, 36, 37, 41, 43-46, 65]	10	9	2
Australia(Oceana) [6]	1	-	-
Europe [26, 33, 50, 62]	-	2	2
North America [3, 5, 7, 15, 20, 21, 47, 48, 52-57, 59-61, 64, 66-68,70]	6	-	16
South America [23, 49, 51, 58]	1	6	3
Biome			
Forest[3-7, 11-12, 15-17, 19, 21-25, 27-30, 33, 36-37, 43-46, 49-50, 52, 54, 58, 60-61, 64, 66]	16	12	11
Grassland/Savanna[13, 31- 32, 34-35, 39-41, 53, 63, 68]	1	7	3
Desert[1-2, 8- 10, 18, 47-48, 59, 65, 67, 69, 70]	6	1	7
Chapparal[20, 42, 55, 56]	1	1	2
Tundra[26, 57, 62]	-	1	2

[2]Achuthan and Chandrahas, 1971; [3]Bakr *et al*., 1996; [4]Carrion, 1930; [5]Chenchijtikul *et al*, 1983; [6]Cole and Koepke, 1946; [7]Cole and Koepke, 1947; [8]Deguisti and Hartley, 1965; [9]Gaadoub *et al*., 1982; [10]Geevarghese *et al*, 1998; [11]Khalid *et al*., 1992;[12]Liat *et a*l, 1980; [13]Linardi *et al*., 1994; [14]Njunwa, 1989; [15]Renapurkar *et al*., 1971; [16]Rumreich, 1945; [17]Saxena, 1987; [18]Singchai *et al*., 2003; [19]Soliman *et al*., 2001; [20]Suntsov et al, 1997; [21]Trimble and Shephard, 1935;[22]Vogel, 1935;[23]Walton and Hong, 1976;[24]Wilson de Carvalho *et al*, 2001;[25]Adler *et al*., 2001;[26]Barros-Battesti *et al*., 1998;[27]Bengtson *et al*., 1986;[28]Bittencourt and Rocha, 2003;[29]Chenchijtikul *et al*, 1983;[30]Durden and Page, 1991;[31]Hastriter *et al*.., 2001;[32] Eads and Campos, 1983;[33]Heisch *et al*., 1953;[34]Jurik, 1983;[35]Lareschi and Iori, 1998;[36]Lareschi *et al*., 2003;[37]Liat *et al*, 1980;[38]Luyon and Salibay, 2007;[39]Mahdi *et al*., 1971;[40]Nava, Lareschi and Voglino, 2003;[41]Schwan, 1986;[42]Shayan and Rafinejad, 2006;[43]Shepard *et al*., 1983;[44]Suntsov et al, 1997;[45]Woo *et al*., 1983;[46]Adler *et al*., 2001;[47]Allred, 1968;[48]Anderson and Williams, 1997;[49]Beaucournu *et al*, 1998;[50]Bengtson *et al*.,1986;[51]Bossi *et al*., 2002;[52]Buckner, 1964;[53]Campos *et al*, 1985;[54]Clark and Durden, 2002;[55]Coultrip et al, 1973;[56]Davis et al, 2002;[57]Eads and Campos, 1983; [58]Gettinger and Ernest, 1995;[59]Grave *et al*, 1974; [60]Haas *et al*, 1973; [61]Harrison, 1954; [62]Hastriter *et al*, 2004;[63]Heisch *et al*., 1953;[64]Holdenried and Morlan, 1956;[65]Krasnov *et al*., 1997;[66]Medina *et al*, 2006; [67]O'Farrell; [68]Poorbaugh and Gier, 1961; [69]Shoukry *et al*., 1993; [70]US Army Env. Hygiene Agency, 1978-1980. See also Online Resource 1.

Table 2.2. A-C. Significant ($P \leq 0.05$) effects (X) for mixed model analysis of disturbance level (Low, Intermediate and High disturbance) and Biome (Forest, Desert, Grassland/ Savanna and Mediterranean) on mammal and flea communities surveyed in 63 studies. **D.** Significant difference across disturbance classes within the Forest biome. **E-F.** Significant differences among Biomes within each level of disturbance class.

	Overall Model			D. Disturbance in Forest habitat [b]	Biome within Disturbance Class [b]		
Variable (no. obs.)	A. Disturbance	B. Biome	C. Interaction		E. Low	F. Intermediate	G. High
No. Mammals Captured (67)	X		X	X		X	X
No. Mammal spp. [a] (67)	X			X			
Prevalence [a] (40)	X	X	X	X	X	X	X
Intensity [a] (41)	X			X			
Flea Burden* (63)			X	X		X	X
Flea spp. Burden [a] (65)				X			
Proportion Infested host spp. (65)	X	X	X	X			X
No. Fleas [a] (67)		X		X			X
No. Flea spp. [a] (67)				X			
Number host spp./Flea spp. [a] (65)	X			X			
Proportion flea specialists (67)		X	X	X	X	X	X
Proportion flea generalists (67)			X	X			X

[a] Log of the number of mammals captured/site was used as an offset variable.
[b] Tukey's Least Significant Difference (LSD) was used to control for Type I error.

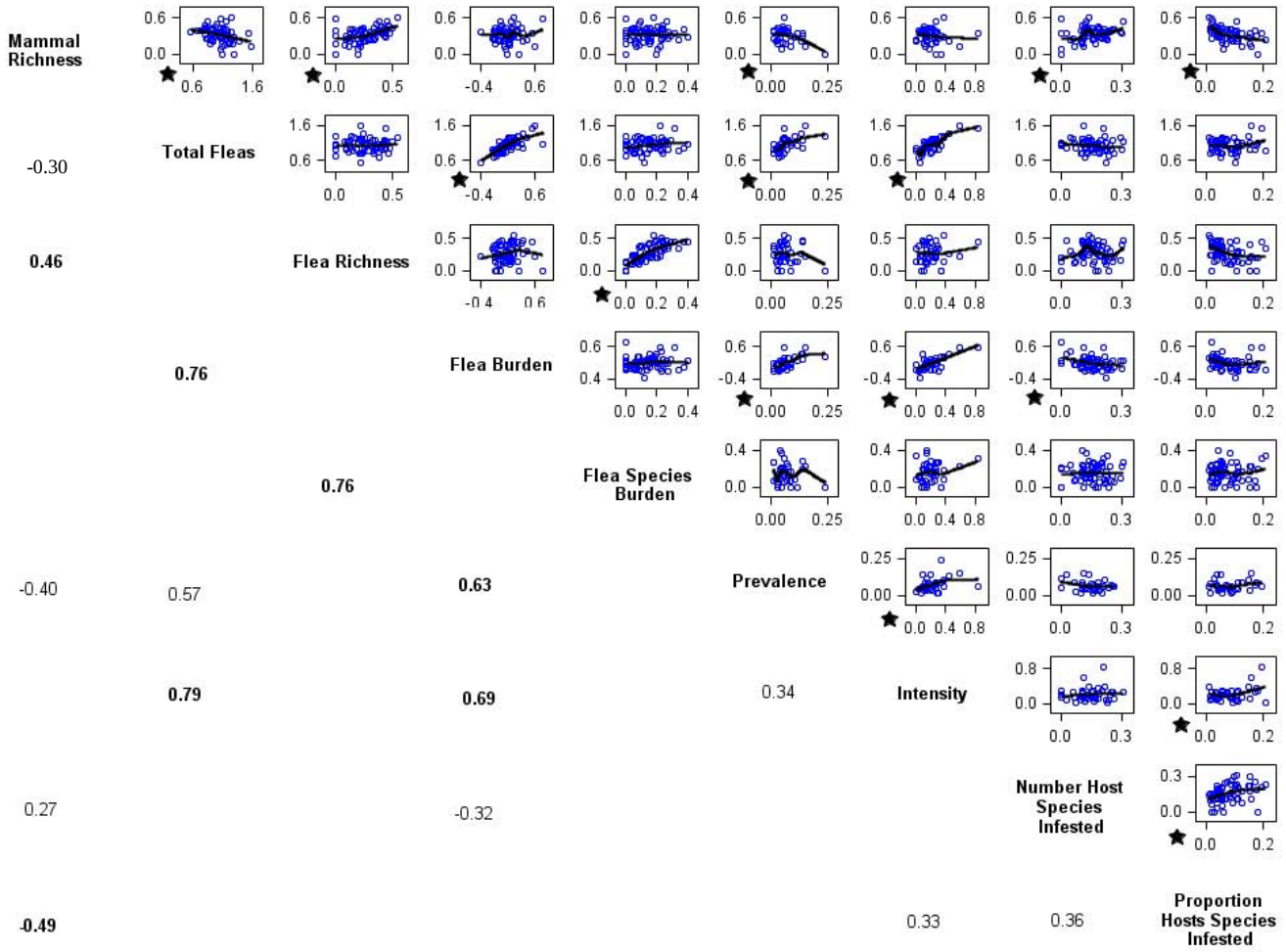

Figure 2.1. Pearson correlation analysis for variables calculated from 63 studies conducted around the world. Scatter plots with loess (locally weighted scatterplot smoothing) lines are displayed above the diagonal and r values for significant associations (values in bold represent $P<0.0001$, otherwise $0.0009<P<0.03$) are displayed below the diagonal. Stars indicate significant associations after variables were standardized for sampling effort and log transformed, but the plots reflect raw data. Descriptions of variables can be found in the text.

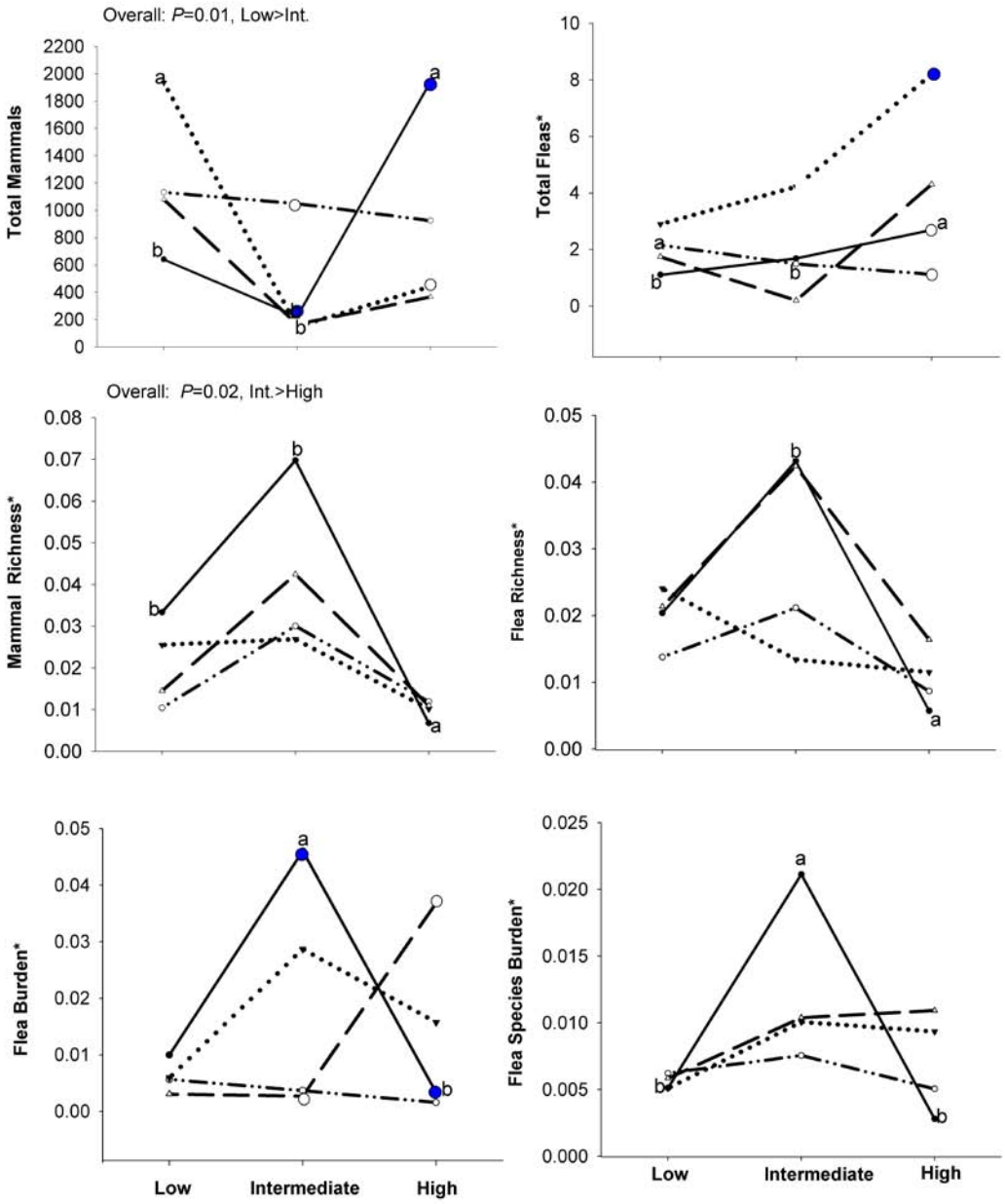

Figure 2.2. Mean values for small mammal and flea variables from 63 studies categorized into three anthropogenic disturbance classes (low, intermediate, high) and four biomes (Forest ——, Grassland/Savanna —·—·—, Desert ·····, Mediterranean — — —). P-values are for F-tests from Generalized Linear Model analysis of the overall model (y is a function of Disturbance class and Biome) and analyses of disturbance class within each Biome. Vertices without a common letter indicate statistically significant difference ($P \leq 0.05$ using Tukey-Kramer multiple comparison methods) among disturbance levels of that biome. Significant differences among biomes within a disturbance class are noted with circle symbols (significant differences are indicated by different fill shades).

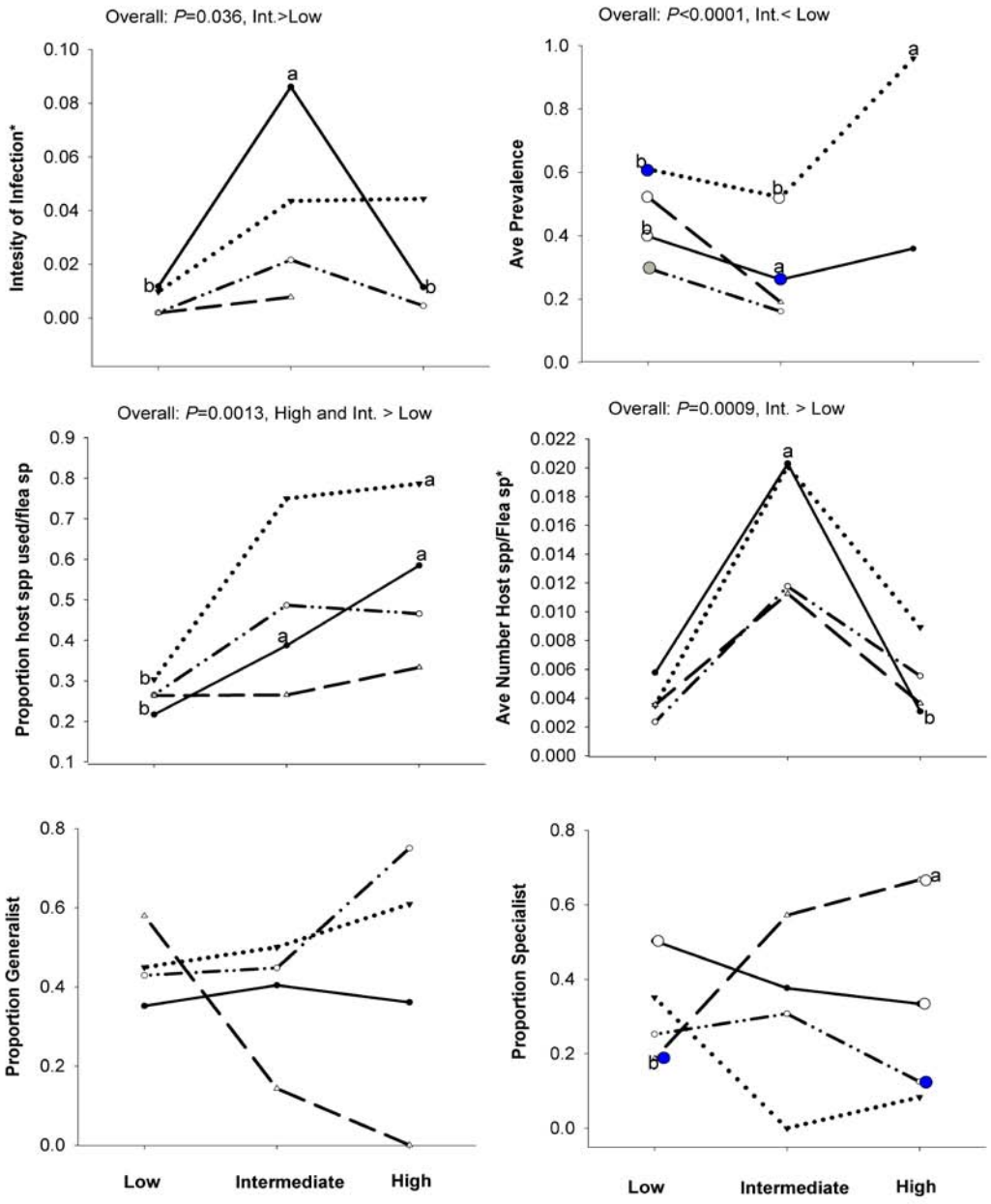

Figure 2.3. Mean values for flea measures in small mammal communities from 63 studies categorized into three anthropogenic disturbance classes (low, intermediate, high) and four biomes (Forest ——, Grassland/Savanna —·—·—, Desert ·····, Mediterranean — — —). P-values are for F-tests from Generalized Linear Model analysis of the overall model (y is a function of Disturbance class and Biome) and analyses of disturbance class within each Biome. Vertices without a common letter indicate statistically significant difference ($P \leq 0.05$ using Tukey-Kramer multiple comparison methods) among disturbance levels of that biome. Significant differences among biomes within a disturbance class are noted with circle symbols (significant differences are indicated by different fill shades.

CHAPTER 3: FLEA ABUNDANCE, DIVERSITY, AND PLAGUE IN GUNNISON'S PRAIRIE DOGS (*CYNOMYS GUNNISONI*) AND THEIR BURROWS IN MONTANE GRASSLANDS IN NORTHERN NEW MEXICO.

PREFACE- This chapter had been formatted for publication in the Journal of Wildlife Disease where it was published in the April, 2010 issue as “Flea abundance, diversity, and plague in Gunnison's prairie dogs (*Cynomys gunnisoni*) and their burrows in montane grasslands in northern New Mexico “by Megan M. Friggens, Robert R. Parmenter, Michael Boyden, Paulette L. Ford, Ken Gage, and Paul Keim.

FLEA ABUNDANCE, DIVERSITY, AND PLAGUE IN GUNNISON'S PRAIRIE DOGS (*CYNOMYS GUNNISONI*) AND THEIR BURROWS IN MONTANE GRASSLANDS IN NORTHERN NEW MEXICO.

Abstract: Plague, a flea-transmitted infectious disease caused by the bacterium *Yersinia pestis*, is a primary threat to the persistence of prairie dog populations (*Cynomys* spp.). In this paper, we report the results of a three-year survey (2004-2006) of fleas taken from Gunnison's prairie dogs (*Cynomys gunnisoni*) and their burrows in montane grasslands located in the Valles Caldera National Preserve in New Mexico. Our primary objectives were to describe these flea communities and identify flea and rodent species important to the maintenance and transfer of plague. We trapped prairie dogs and conducted burrow sweeps at three colonies in the spring and summer of each year. One hundred and thirty prairie dogs and 51 golden mantled ground squirrels (*Spermophilus lateralis*) were captured over 3,640 trap nights and 320 burrows were swabbed for fleas. Five flea species were identified from prairie dogs and ground squirrels and four were identified from burrow samples. *Oropsylla hirsuta* was the most abundant species found on prairie dogs and in burrows. *Oropsylla idahoensis* was most common on ground squirrels. Two colonies experienced plague epizootics in the fall, 2004. Plague positive fleas were recovered from burrows (*Oropsylla hirsuta* and *O. tuberculata tuberculata*) and a prairie dog (*Oropsylla hirsuta*) in the spring of 2005 and summer of 2006. Three prairie dogs collected in the summer of 2005 and 2006 had positive antibody titers. We found a significant surge in flea abundance and prevalence, particularly within burrows, following plague exposure. We noted an increased tendency for flea exchange opportunities in the spring before *O. hirsuta* reached its peak population. We hypothesize that spring conditions, which favor presence and exchange of certain flea species, may be

just as important for determining plague outbreaks as the summer conditions, which lead to build up in *O. hirsuta* populations.

Introduction:

Plague is an infectious vector-borne disease caused by the bacterium *Yersinia pestis* and transmitted between mammals by fleas (Biggins and Kosoy, 2001). Since its introduction to the United States around 1899-1900, sylvatic plague has become established in native rodent species and contributed to the precipitous decline of endemic prairie dog (*Cynomys* spp.) populations (Gage and Kosoy, 2005; Cully and Williams, 2001). Prairie dogs are particularly susceptible to plague because they have no innate immunity and live in large colonies with elaborate burrow systems that favor reproduction and survival of the flea vector (Cully and Williams, 2001; Gage and Kosoy, 2005). Mortality rates in excess of 95% in exposed prairie dog colonies (Cully and Williams, 2001) affect not only prairie dogs, but also prairie dog dependent species like the black-footed ferret, *Mustela nigripes* (Houston et al., 1986). In addition, prairie dog epizootics can amplify plague in an area by releasing large numbers of infected fleas into the environment. Predators that are attracted to the sick and dead prairie dogs can become infected by consuming these animals or as a result of being bitten by their fleas. Even more importantly, predators can spread potentially infectious fleas to other sites, including previously unaffected prairie dog colonies, thereby contributing to the local spread of plague (Lechenleitner et al., 1968). Although we have yet to identify the reservoir host and flea vector species important to the maintenance and spread of plague within the United States, it is clear that prairie dog fleas are able to perpetuate plague among colony members over the course of the epizootic (Webb et al., 2006; Wilder et al., 2008). However, there is little evidence to suggest that these fleas, or the prairie dogs themselves, can contribute to long-term plague maintenance cycles. Thus, research

focused on the factors that precede and lead to prairie dog plague epizootics must aim to identify other host and flea species that maintain plague in the ecosystem and, ultimately, transfer plague to prairie dog colonies.

Several rodent species have been proposed as enzootic or maintenance hosts, including grasshopper mice (*Onychomys* spp.) on black-tailed prairie dog towns (*C. ludovicianus*) and *Peromyscus* spp. in white-tailed (*C. leucurus*) and Gunnison's prairie dog towns (*C. gunnisoni*) (Gage et al., 1995; Thiagarajan et al., 2008). Others have provided evidence for maintenance cycles that involve soil or flea stages (Lechleitner et al., 1968; Ayyadurai et al., 2008; Eisen et al., 2008). The most likely long-term scenario involves several factors and relies on a suite of animals, all connected by flea vectors (Biggins and Kosoy, 2001). In general, flea species are more likely to spread plague if they have a high susceptibility to *Y. pestis* and exhibit low host specificity (Gage and Kosoy, 2005). However, low efficiency vectors may actually be quite important to spread of plague if they are common in the environment (Kartman et al., 1962; Lechleitner et al., 1968, Eisen et al., 2006). Indeed flea abundance is positively related to flea species vector potential for plague (Krasnov et al., 2006) and abundance and prevalence of flea species is an important determinant of *Y. pestis* transmission in particular (Eisen et al., 2006; Lorange, 2005).

In this paper, we report the results of a three-year survey of flea populations taken from Gunnison's prairie dogs and their burrows in montane grassland habitats located in the Valles Caldera National Preserve (VCNP) in New Mexico. We also analyzed flea and prairie dog blood samples for the presence of plague. Our primary objectives were to describe the flea communities within the VCNP prairie dog towns, compare animal and

burrow flea loads across years and seasons in sites with and without plague, and identify flea species, which may be important to the maintenance or transfer of plague within this population.

Materials and Methods:

Site descriptions- The study was conducted on the Valles Caldera National Preserve, Sandoval County, New Mexico. The study areas were located in montane grassland habitat, with elevations ranging between 2,460 m and 2,640 m. Annual precipitation averages 638 mm, with approximately 45% falling during the summer monsoon season (July-September). Mean annual temperature is 4.5° C, with mean July temperatures of 15° C and mean January temperatures of -5.3° C. Three grassland habitats with prairie dog towns were selected for sampling (Fig. 3.1): Redondo Meadow **(**N 35˚51'33", W 106˚36'15"), El Cajete (N 35˚50'18", W 106˚33'33"), and Valle Grande (N 35˚51'21", W 106˚29'29"). The Redondo Meadow site (2,459 m) vegetation was dominated by *Bouteloua gracilis, Potentilla hippiana, Erigeron flagellaris, Artemisia carruthii,* and *Polygonum douglasii*. Soils on this site were classified as fine, smectitic, superactive, frigid, Vertic Argialboll (Lyquilar series). The El Cajete site (elevation 2,638 m) vegetation was dominated by *Bromus inermis, P. hippiana, Taraxacum officinale, Erigeron flagellaris*, and *Achillea millefolium*. Soils on this site were ashy, glassy, frigid, Vitrandic Argiustoll (Jarmillo series). The Valle Grande site (elevation 2,590 m) vegetation was dominated by *Festuca arizonica, Koelaria macrantha, Poa pratensis, Muhlenbergia montana, P. hippiana, Carex* spp., and *Antennaria rosea*. Soils on this site were classified as loamy over ashy-pumiceous, mixed over glassy, superactive, frigid Vitrandic Argisutoll (Vallande series).

Colony surveys- We live-trapped prairie dogs and conducted burrow sweeps during spring (May-June) and summer (August-September) each year. Two colonies, El Cajete and Valle Grande, were not sampled in 2004. Each colony lay in a valley bottom (Fig 3.1a) and was isolated from other colonies by mountain ranges, which prevented movement of prairie dogs between sites. During the first trapping session at each site, we marked and took GPS coordinates of active burrows. We determined activity by the presence of scat, scratching, and/or flies at each burrow entrance. We updated these burrow characteristics at the start of each trapping period. If burrows appeared abandoned during this time, we chose new burrows to trap from the immediate area and processed these new burrows as described above. Two methods were used to estimate prairie dog densities: Active count (Severson and Plumb, 1998) and burrow survey transects (Biggins et al., 1993). Active count has been shown to be an effective method of population estimation in both black- and white-tailed prairie dogs (Fagerstone and Biggins, 1986; Menkens et al., 1990; Severson and Plumb, 1998). In 2004, we attempted to quantify prairie dogs by counting the number of above ground animals within a 300 x 300 m bounded area at 15 minute intervals over a 3 h period. However, after two days, we found ourselves unable to make accurate sightings due to vegetation structure and intermittent vehicle traffic in the area, the latter which negatively affected prairie dog activity patterns. Therefore, we decided to approximate relative colony size by estimating burrow density with a belt-transect method. Though criticized (Powell et al., 1994; Hoogland, 1995; Severson and Plumb, 1998), this method has been used successfully to estimate relative differences between towns and was found to correlate positively with prairie dog density in at least one study (Johnson and Collinge, 2004). To conduct burrow surveys,

we used a Trimble© GPS unit (Trimble Navigation Ltd, 2009, Worldwide) to track our path as we walked the perimeter of each colony and to calculate area based on that perimeter. We marked the boundary with pin flags and then walked a series of randomly placed 100 x 2 m transects until we had covered 10% of the colony area. We counted and classified (active or not) all burrows that fell within 2 m swath of the transect line by carrying a 2 m piece of polyvinyl chloride (pvc) pipe perpendicular to the transect and parallel to the ground as we walked along a 100 m measuring tape.

Rodent trapping-Two to four Tomahawk® live traps (Size #70, Tomahawk Live Trap Company, Tomahawk, WI, USA) were set around each burrow for a total of 76-104 traps per colony. Traps were baited with a combination of rolled oats and sweet feed and wired open for at least 4 days prior to trapping to acclimatize prairie dogs to traps. Prairie dogs were trapped for three consecutive days following the prebaiting period. Traps were opened and baited before sunrise (approx. 0530 hr) each morning and checked between 0830-0930 hr. Depending on capture success and weather conditions, traps were sometimes left open and checked every 45 minutes until 1200 hr. At a processing station removed from the trapping site, each prairie dog was weighed to the nearest gram with a Pesola® scale (Pesola AG, Rebmattli, Switzerland), sexed, and given a uniquely numbered ear tag (Gey Band and Tag Co., Norristown, PA, USA). Animals were processed with the aid of canvas cones described in Hoogland (2005). Blood samples were collected by clipping a toenail on a rear foot just distal to the quick and blotting the blood onto a Nobuto filter strip (Toyo Roshi Kaisha, Ltd., Tokyo, Japan). Toenails were thoroughly cleaned with alcohol pads before clipping and treated with a sulfur compound (styptic) after clipping to prevent infection. Nobuto strips were air dried and put into an

envelope for short-term storage. Fleas were collected by holding each animal over a plastic basin containing a 20 x 20 cm felt cloth and thoroughly brushing the fur with a flea comb and/or toothbrush until all observed fleas had fallen into the basin. Fleas were then collected from the felt cloth, placed into a labeled cryovial and flash frozen in liquid nitrogen (-70˚C). After processing, prairie dogs were returned to their trap and released at the site of capture. Prairie dogs were processed only during the first capture of each trapping period. Animal handling procedures were approved by the Animal Care and Use Committee of the University of New Mexico (04MCC002). Blood and flea samples were processed under the guidelines and standardized protocols for the safe handling of biohazard material where appropriate (Mills et al., 1995; Keim lab, NAU, Flagstaff, Arizona and CDC, Ft Collins, Colorado unpublished protocols)

Burrow sampling-Fleas were collected from burrows just prior to, or following, prairie dog trapping. Twenty burrows were swabbed at each site and during each season by attaching a white flannel cloth (20 x 20 cm) to the end of a plumber's snake and extending the cloth down into burrow to depth of at least 1 m. After 30 seconds, the cloth was removed, put into a 1 gal plastic zipper-seal bag, sealed and placed in a cooler with dry ice. Burrow sweep cloths were kept frozen until examination. Each felt cloth was carefully examined for ectoparasites using a dissecting microscope or magnifying glass. All ectoparasites were stored in labeled cryovials and kept frozen until laboratory analysis.

Flea Identification- Fleas were examined under a dissecting microscope and identified to genus, species or subspecies according to Furman and Catts (1982), Hubbard (1947) and Lewis (2002). Voucher specimens of each flea species were deposited in the Division of

Vector-borne Infectious Diseases of the Center for Disease Control and Prevention in Fort Collins, Colorado.

***Plague tests*-** Blood samples were analyzed for the presence of F1 plague antibodies using a standard passive hema-agglutination (PHA) test (Williams et al., 1976; Chu, 2000). Briefly, blood samples were eluted overnight in a 1M Sodium Borate solution and a 25ul volume of the eluant was used for PHA analysis. Positive samples were confirmed with passive hema-inhibition (PHI) tests. Seropositive results were recorded as reciprocal titers, denoting the concentrations as determined by titration. Reciprocal titers below 1:32 were considered nonspecific and not positive.

Fleas were examined for the presence of plague using a multiplex PCR reaction as described in Stevenson et al. (2007). This analysis targets a region of the plasminogen (pla) activator gene of *Yersinia pestis* (478-basepairs). Most fleas were analyzed individually for the presence of *Y. pestis* DNA. However, for nine hosts and two burrows that yielded >25 fleas only the first five individuals were analyzed individually. The remaining fleas (104 fleas) were analyzed in pools of 2 to 5 fleas each. DNA was obtained from the first 30 fleas by triturating individual fleas in 100ul BHI (Becton Dickenson, Sparks, MD, USA). The remaining fleas were processed with a DNA extraction procedure described in Allender et al. (2004). Following processing, 2.5ul of triturate or extracted DNA was used for PCR analysis.

***Statistical Analysis*-** We did not compare flea abundance and prevalence across the sites because we surveyed colonies that were accessible by vehicle rather than randomly selecting among all available colonies in the VCNP. We assessed differences in flea

abundance and flea prevalence between sampling periods, seasons (spring vs. summer), and years across all sites and within each site using generalized linear model analysis (PROC GLIMMIX, Statistical Analysis Software, SAS 9.2). For comparisons across all sites, we included colony as a random effect in our model. Prevalence data was analyzed with a binomial distribution and logit link, whereas abundance data was analyzed with a Gaussian distribution and log link. Statistical significance was set at $P < 0.05$ and Tukey adjusted p-values were used to reduce the likelihood of Type I errors.

Results:

The Redondo Meadow colony encompassed approximately 14 ha with an average of 273 active burrows/ha, El Cajete was 15 ha with 60 active burrows/ha, and Valle Grande was 2.7 ha with 120 burrows/ha.

One hundred and thirty prairie dogs were captured over 3,640 trap nights (including 22 recaptures). The majority (107 including 10 recaptures) were from Redondo meadow, 22 (including 1 recapture) were from Valle Grande, and none were caught in El Cajete. In addition, 51 golden mantled ground squirrels (*Spermophilus lateralis*) were captured from El Cajete (40 including 5 recaptures) and Valle Grande (11 including 1 recapture). Voucher specimens of three *S. lateralis* were deposited in the Museum of Southwestern Biology, University of New Mexico, Albuquerque, New Mexico (NK143015, NK 143434, and NK143450).

Prairie dogs were abundant in Redondo during 2004 (72 captures), but few animals emerged from burrows the following spring, 2005. By summer of 2005, many burrows began showing obvious signs of decline, and capture success was low (5

animals). The population appeared to be in recovery in 2006 and we captured 10 and 15 prairie dogs in the spring and summer, respectively. El Cajete was reported to have a large and active population of prairie dogs in 2004 (pers. comm., R.R. Parmenter). When trapping began there in spring of 2005, over 100 burrows were surveyed and, though open, very few appeared to be occupied by prairie dogs. Many additional burrows had blocked entrances. Trapping efforts yielded no prairie dogs, but 25 golden mantled ground squirrels were caught in traps laid around prairie dog burrows. No prairie dogs and only 15 squirrels were captured from El Cajete in 2006. Valle Grande had small but stable populations of prairie dogs and ground squirrels over the course of this study, with 14 and eight prairie dog captures and six and five squirrel captures for 2005 and 2006, respectively.

We collected 633 fleas from prairie dogs, 167 fleas from prairie dog burrows, and 66 fleas from golden mantled ground squirrels (Table 3.1). Golden mantled ground squirrels were not sufficiently sampled for further analysis. Prevalence and abundance were positively correlated across sites and years for both prairie dogs (r^2=0.61, P=0.007, df=9) and burrows (r^2=0.63, P=0.0007, df=13). Trends in flea abundance and prevalence between prairie dogs and burrows were similar in Redondo Meadow prior to plague, but did not correspond after plague outbreaks. Trends in flea abundance and prevalence were not similar for prairie dogs and burrows at other sites.

Across all sites, mean abundance (number fleas/sample ± StDev) and prevalence (% infested) were 4.89 ±8.31 and 65% for prairie dogs and 0.62±2.79 and 18% for burrow samples. Annual flea abundance on prairie dogs was significantly greater in 2006 (6.4±7.74) than 2005 (5.2±10.4) and 2004 (4.1±7.74) (P = 0.04, df =3). Annual

abundance was greatest in 2005 (1.0±4.1) and lowest in 2006 (0.32 ±1.13) for burrows ($P < 0.05$, df =5). Prevalence was highest in 2005 (78% and 3%) and lowest in 2004 (58% and 13%) for both prairie dogs and burrows, respectively ($P < 0.05$ for both, df= 3 or 5). Averaged across sites and years, abundance and prevalence of fleas in prairie dogs was more than 2 times greater in the summer than in the spring collections (5.9±8.1 vs. 2.52±8.35 and 78 vs. 35%, respectively). In burrows, summer prevalence and abundance were lower than spring prevalence and abundance (0.41±2.1 vs. 0.83±3.34 and 13 vs. 24%, respectively).

Significant trends in flea abundance and prevalence for each sampling period within each site are noted in Figures 3.2 and 3.3. Prevalence of fleas in burrows was significantly greater in 2005 than other years for both Redondo Meadow and El Cajete. Valle Grande showed a significantly greater overall prevalence of fleas in the spring versus summer (Fig. 3.2b). Prior to the suspected plague outbreak (fall/winter of 2004), flea abundance and prevalence were lower in spring than summer for both prairie dogs and burrows in Redondo (Figs. 2a-b, 3a-b). However, flea abundance was significantly higher in burrows in the spring following plague exposure in Redondo Meadow (Figs. 3.2b). With the exception of 2005, where prevalence was 100% for both sampling periods, the prevalence of flea infested prairie dogs increased significantly from spring to summer in Redondo Meadow (Fig. 3.2a).

Seven flea species were identified from the VCNP colonies (Table 3.1). *Oropsylla hirsuta* was the most abundant species found on prairie dogs and in burrows. Overall, flea diversity was higher during spring versus summer sampling (3 vs. 2 and 3 vs. 2.3 species for prairie dogs and burrows, respectively). Species specific trends in seasonality were

apparent among the fleas though patterns did not correspond between animals and burrows (Table 3.1). Plague positive fleas were recovered from two burrows (2-*Oropsylla hirsuta*, 1-*O. t. tuberculata*) and one prairie dog (1-*Oropsylla hirsuta*) from Redondo Meadow during the spring, 2005. Three prairie dogs collected in the summer of 2005 had positive antibody titers: one from Redondo Meadow with a titer of 1:256 and two from Valle Grande each with titers of 1:1024. Plague was detected again in the summer of 2006 in fleas (1-*O. hirsuta*) recovered from a burrow in El Cajete, and from a prairie dog (14-*O. hirsuta*) captured in Redondo Meadow. Two prairie dogs from Redondo Meadow (one recapture) showed positive titers (1:512 and 1:2048) to plague in the spring of 2006.

Discussion:

During this study, plague epizootics occurred in two of three colonies. Though we found plague antibodies in two prairie dogs captured at Valle Grande, there were no other overt signs, such as infected fleas or prairie dog die-off, of a plague epizootic in this colony. In contrast, the prairie dogs inhabiting Redondo Meadow and El Cajete experienced severe population declines and both fleas and prairie dogs were found with recent exposure to plague. We were only able to detect plague immediately following the prairie dog population crash, an observation that is similar to those reported for other Gunnison's colonies (Lechleitner et al., 1968), but stands in contrast to trends reported for white-tailed prairie dogs (Anderson and Williams, 1997).

We found that the number of fleas per host and per infested host and burrow were higher in plague-affected than non-affected colonies. Anderson and Williams (1997)

found significantly higher numbers of fleas in plague-affected versus non-affected white-tailed prairie dog colonies. We collected more fleas from a greater proportion of burrows and prairie dogs during the season of an epizootic and the season immediately following it than the years prior to or following an epizootic. In El Cajete, where prairie dogs were essentially eliminated, flea abundance declined following plague die-off (Fig. 3.2). In contrast, we did not detect a significant decline in the flea populations of Redondo Meadow where prairie dogs were in recovery. Indeed, by 2006, two years after the epizootic, Redondo Meadow (recovering population) and Valle Grande (stable population), showed similar seasonal patterns in flea abundance and prevalence, as compared to El Cajete (Figs. 2 and 3), a trend consistent with recovery at other prairie dog towns (Lechenleitner et al., 1968). Significant declines in flea abundance following plague die offs have been attributed to reduced host populations (Salkeld and Stapp, 2008) and subsequent low survival among off-host flea populations exposed to desiccation and possibly high temperatures (Gage and Kosoy, 2005). A similar density dependent interaction may have fostered the increase in flea abundance in the summer of 2004, just prior to plague related die offs (Figs. 3.2 and 3.3). Interestingly, most other studies have reported spring-time surges in flea populations prior to outbreaks, which corresponds more with the typical annual patterns in flea abundance seen in this study and elsewhere (Anderson and Williams, 1997; Cully et al., 1997; Stenseth et al., 2006). Whatever the cause, it seems likely that the increase in prevalence and abundance of fleas in the summer of 2004 was a key precursor to plague outbreak in this colony.

The seasonal trends in flea species composition in the VCNP were very similar to those reported elsewhere: *O. t. cynomuris* populations peaked in early spring (Cully et al.,

1997; Salkeld and Stapp, 2008), *O. idahoensis* populations peaked in midsummer (Anderson and Williams, 1997) and *O. hirsuta* numbers were greatest during mid- or late summer seasons (Salkeld and Stapp, 2008). Again, the only exception was seen in 2004, just prior to the epizootic.

Oropsylla hirsuta and *O. t tuberculata* were the primary fleas involved in prairie dog plague epizootics in the VCNP. *Yersinia pestis*-infected *O. hirsuta* were collected from both prairie dogs and burrows and plague infected *O. t. tuberculata* were collected from burrows. These flea species were widespread in the VCNP colonies and readily parasitize both golden mantled ground squirrels (*Spermophilus lateralis*) and prairie dogs. *Oropsylla hirsuta* has been implicated in the spread of plague in prairie dog towns (Cully and Williams, 2001) and appears to be the most important with respect to supporting fast moving transmission during the epizootics commonly reported to occur in prairie dog colonies (Ubico et al., 1988; Cully et al., 1997). This is the first report of plague in *O. t. tuberculata* collected from prairie dog burrows though its sister species, *O. t. cynomuris* is commonly infected with plague (Ecke and Johnson, 1950; Lechenleitner et al., 1968, Cully et al., 1997; Ubico et al., 1988, Anderson and Williams, 1997, Holmes et al., 2006). The separation of *O. t. tuberculata* and *O. t. cynomuris* into distinct taxa is not supported by all authorities, despite the general recognition that the latter come primarily from prairie dogs and former from ground squirrels (Lewis, 2002). Since most studies distinguish between *O. t. cynomuris* (vs. *O. t. tuberculata*) it seems reasonable to preserve this level of classification in this study. We do note, however, that *O. t. tuberculata* have been recovered by others on prairie dogs captured in the western U.S.,

including in northeastern Utah on *C. leucurus* (Stark, 1958) and on *C. gunnisoni* in the relatively high elevation South Park region of central Colorado (Ecke and Johnson,1950).

Ground squirrels may play a role in transferring infected fleas between reservoir host species (Lechleitner et al., 1968; Anderson and Williams, 1997). In addition, ground squirrels and prairie dogs often share flea species and exchange between these hosts is particularly evident during plague outbreaks (Ecke and Johnson 1950; Anderson and Williams, 1997; Cully and Williams, 2001). In the VCNP, ground squirrels were abundant on prairie dog towns, readily used prairie dog burrows, and ground squirrel associated fleas found in prairie dog burrows were positive for plague. Thus, the presence of ground squirrels and the ready transfer of fleas between ground squirrels and prairie dogs provides an increased the risk of plague exposure to prairie dogs to the likely detriment of the colonies in the VCNP.

Burrows are important habitat for fleas and have a demonstrated role in plague dynamics in the VCNP. In general, non-prairie dog fleas were more abundant and prevalent in burrows during spring seasons in both this and other Gunnison's colonies in NM (Cully et al., 1997). We saw clear shifts in the prevalence and abundance of both *O. hirsuta* and *O. idahoensis* from burrow to prairie dogs as summers progressed (Table 3.1). In contrast, *O. idahoensis* were equally present on their ground squirrel hosts during spring and summer (Table 3.1). Therefore, it appears that burrows provide favorable conditions for early season population increases in both prairie dog and non-prairie dog associated species. In addition, the capacity for *O. idahoensis* to successfully utilize burrows and readily parasitize prairie dogs is a strong indication that prairie dog burrows act not only as refugia to off-host populations of prairie dog fleas, but may foster flea

exchange among hosts. This may also be a mechanism for flea exchange between prairie dogs and other species such as the American badger, black-footed ferrets, and burrowing owls known to inhabit prairie dog burrows (Hoogland, 2005 and references therein).

Fleas are thought to play an important role in the maintenance of plague over time and are the primary mechanism by which plague is transmitted among hosts (Gage and Kosoy, 2005). At least two prairie dog fleas have been found in burrows and infected with plague up to a year after an epizootic (Lechleitner et al., 1968), implicating a significant role in plague maintenance cycles. However, the low resistance of prairie dogs to plague means that these fleas are unlikely to maintain plague in any kind of enzootic cycle. On the other hand, burrows harbor plague infected fleas of many species for at least many months following the occurrences of epizootics (Lechleitner et al., 1968; Cully et al., 1997; Ubico et al., 1988; Anderson and Williams, 1997; Holmes et al., 2006). Also, the immigration of other rodent species into areas that have previously experienced plague epizootics (as reported by Ecke and Johnson, 1950 and Lechleitner et al., 1962) would allow fleas to continue to transmit plague to new animals. The findings of this study support the conclusion of Cully and Williams (2001) that prairie dog burrows provide ample opportunity for the interspecific spread of *Y. pestis* between prairie dogs and other animals. In addition, recent evidence for the persistence of plague in soil may point to a more direct role of burrows in providing refuge for plague pathogens (Ayyadurai et al., 2008; Eisen et al., 2008).

Environmental conditions which differentially favor flea species or favor flea reproduction will influence the spread and intensity of epizootics (Rayor, 1985; Ubico et al., 1998). The ready exchange of cool weather flea species like *O. t. tuberculata, O. t.*

cynomuris, and *O. idahoensis*, between ground squirrel and prairie dog hosts and the tendency for *O. hirsuta*, a warm weather species, to support explosive epizootics in prairie dogs towns suggests that seasonal shifts in flea species composition influences the persistence and spread of plague. A mild winter followed by early onset of spring might allow larger populations of fleas to persist in burrows and increase the likelihood of disease exchange between prairie dogs and other mammals which utilize prairie dog burrows. Similarly, an early onset of summer conditions might favor early emergence and population increases of *O. hirsuta*, creating conditions that readily support the spread of plague epizootics. Therefore, warming trends that shorten winters and lead to longer summers might increase the probability of both exchange and build up of flea species that transmit plague and lead to more epizootics (Stenseth et al., 2006). Thus far, studies that compare prairie dog epizootics with respect to weather have not considered the species-specific implications of weather effects on prairie dog fleas (Stapp et al., 2004; Stenseth et al., 2006).

In conclusion, prairie dog burrows are an important component of plague cycles as a source for infectious off-host fleas, a site of flea exchange, and potentially by harboring the plague pathogen itself in the soils of the burrows. In addition, the ready exchange of fleas between ground squirrels and other species, in particular, prairie dogs, effectively increases prairie dog exposure to fleas and flea-borne pathogens and the likelihood of interspecific flea transfer in areas where *Spermophilus* and *Cynomys* coexist.

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Addendum Note: The significant increase in prevalence of fleas on prairie dogs and burrows following plague outbreak is likely a result of the sudden loss of host animals. The flea population is suddenly focused on a few remaining individuals and are more prone to collection by burrow sweeps.

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Table 3.1 Flea species and number collected from Gunnison's prairie dog burrows, prairie dogs (GPD), *Cynomys gunnisoni*, and golden mantled ground squirrels (GMGS), *Spermophilus lateralis*, caught in the Valles Caldera National Preserve in northern New Mexico, 2004-2006.

Sample	Flea Species	Spring	Summer	Total
Burrow	*Catallagia decipiens*	1	1	2
(n=280)	*Oropsylla hirsuta*	85	47	132
	Oropsylla idahoensis	16	4	20
	Oropsylla t. cynomuris	7	1	8
	Oropsylla t. tuberculata	5	--	5
Total		114	53	167
GPD	*Oropsylla hirsuta*	79	507	586
(n=130)	*Oropsylla idahoensis*	5	23	28
	Oropsylla t. cynomuris	14	--	14
	Oropsylla t. tuberculata	3	2	5
Total		101	532	633
GMGS	*Eumolpianus e. cyrturus*	3	2	5
(n=51)	*Opisodaysis enoplus*	--	1	1
	Oropsylla hirsuta	--	9	9
	Oropsylla idahoensis	21	29	50
	Oropsylla t. tuberculata	1		1
Total		25	41	66
Grand Total		240	626	866

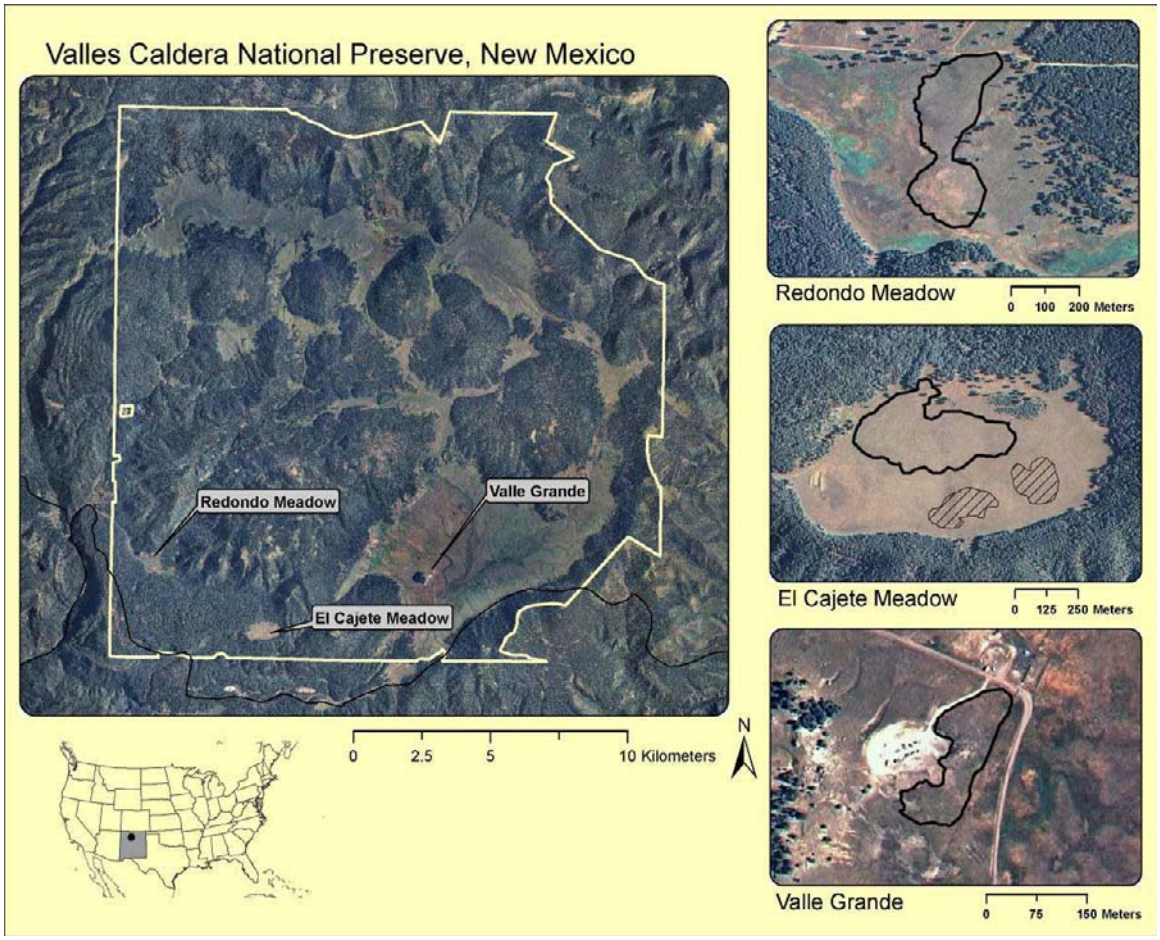

Figure 3.1. Location of three study sites in the Valles Caldera National Preserve in northern New Mexico. Outlines indicate perimeter of colony area that was the focus of trapping efforts and burrow sweeps from May 2004 until September 2006. One colony, El Cajete, contained areas where prairie dog burrows were blocked at the time of study (hatched polygons).

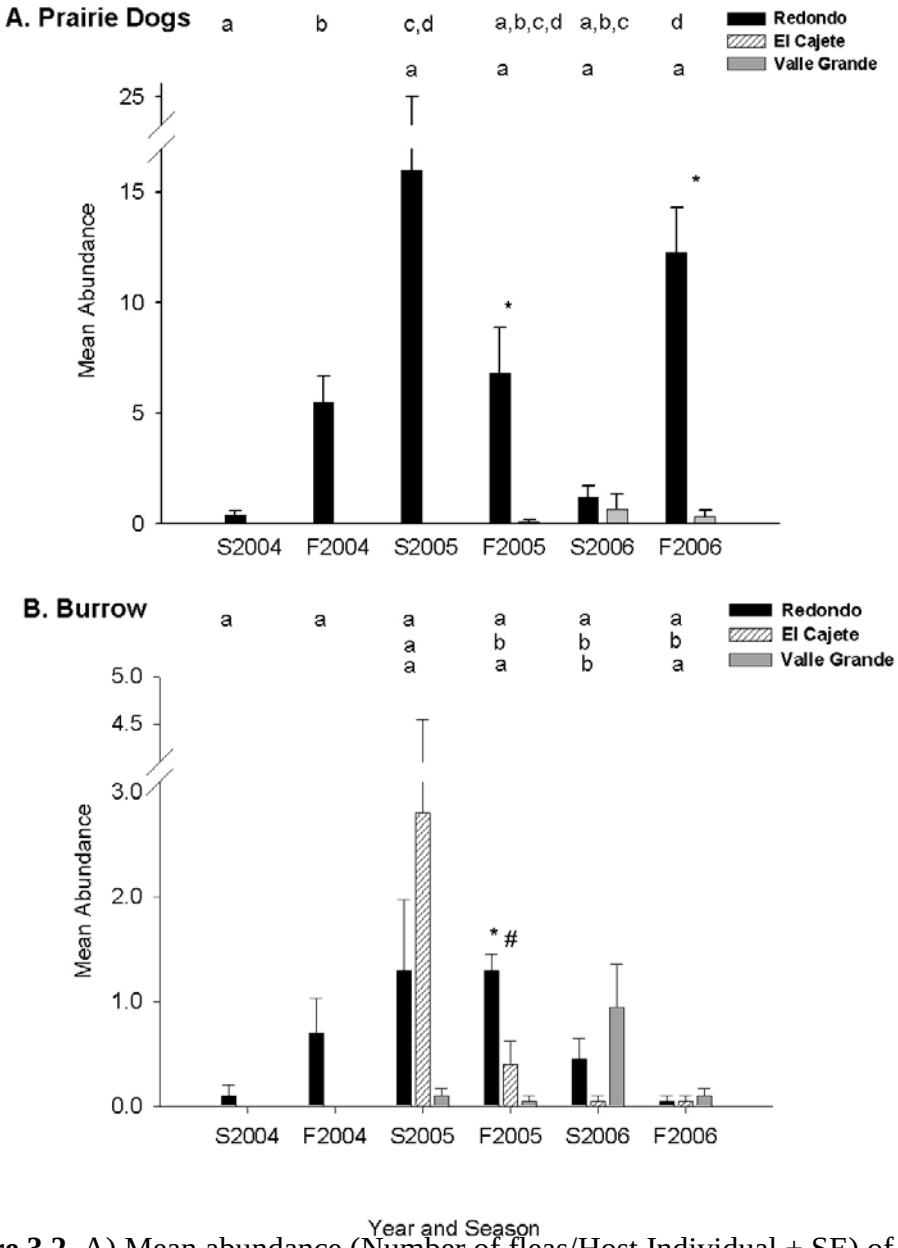

Figure 3.2. A) Mean abundance (Number of fleas/Host Individual ± SE) of fleas collected from prairie dogs captured from two colonies in the Valles Caldera National Preserve during six collection periods from 2004-2006. B) Mean abundance (Number of fleas/Host Individual ± SE) of fleas collected from prairie dog burrows sampled from three colonies in the Valles Caldera National Preserve during six collection periods from 2004-2006. Letters signify significant differences ($p<0.05$) among sampling periods for each site, where those points which share a letter are not different across sampling periods.

*Significant difference ($p<0.05$) between Redondo and Valle Grande. #Significant difference ($p<0.05$) between Redondo and El Cajete.

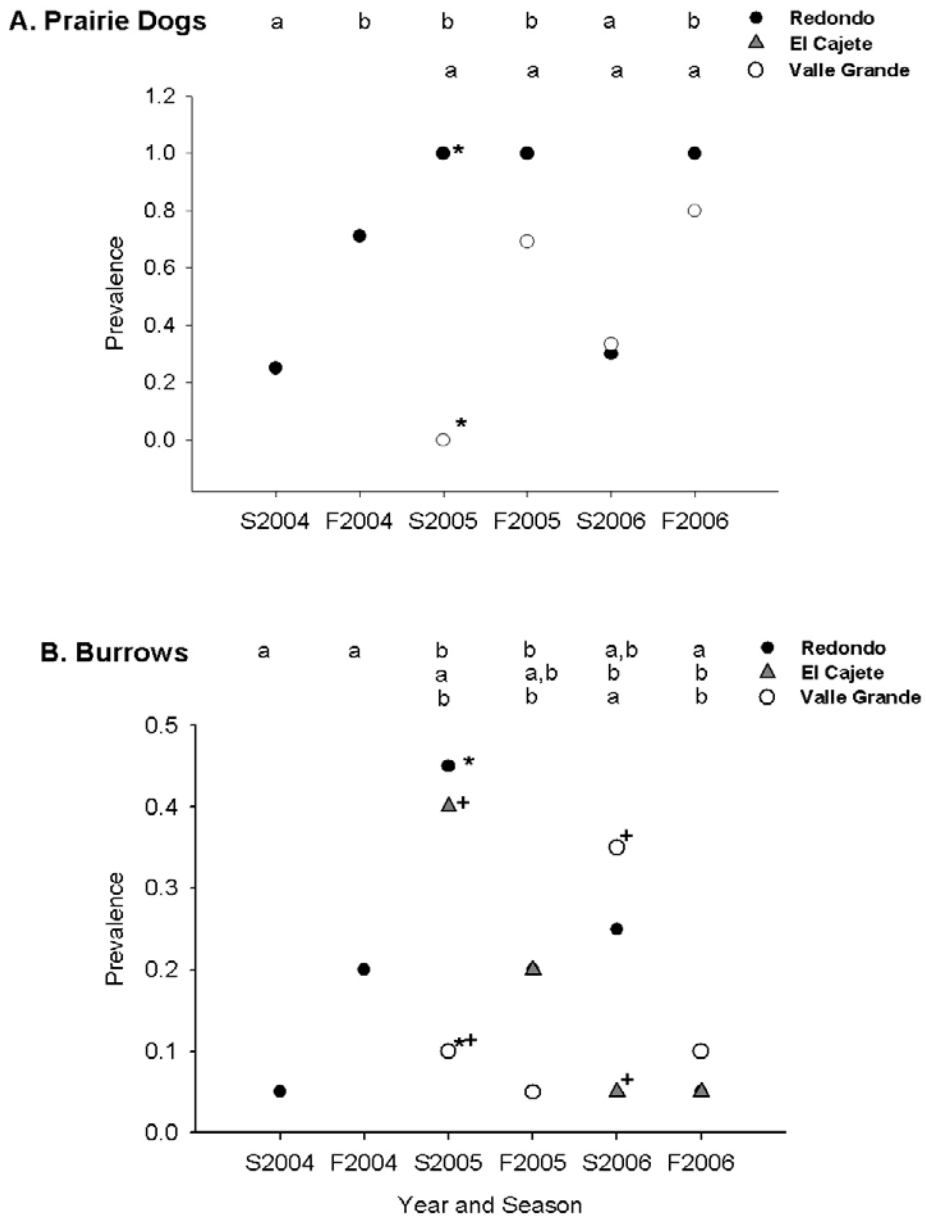

Figure 3.3. A) Prevalence (Number of infested individuals/Total individuals collected) of fleas collected from prairie dogs captured from two colonies in the Valles Caldera National Preserve during six collection periods from 2004-2006. B) Prevalence (Number of infested burrow sweeps/Total sweeps) of fleas collected from prairie dog burrows sampled from three colonies in the Valles Caldera National Preserve during six collection periods from 2004-2006. Letters signify significant differences ($p<0.05$) among sampling periods for each site, where those points which share a letter are not different across sampling periods.

*Significant difference ($p<0.05$) between Redondo and Valle Grande.$^{+}$Significant difference ($p<0.05$) between El Cajete and Valle Grande.

CHAPTER 4: FLEA-BORNE TRANSMISSION OF *BARTONELLA* IN THREE RODENT AND FLEA COMMUNITIES IN NEW MEXICO

PREFACE- This chapter has been formatted for publication within The Journal of Vector Borne and Zoonotic Disease. However, the final submitted manuscript is likely to be considerable shorter than that presented here.

ECOLOGY OF *BARTONELLA* IN THREE RODENT AND FLEA COMMUNITIES IN NEW MEXICO.

Abstract: A number of rodent-borne bacteria in the genus *Bartonella* are agents of human disease. *Bartonella*, transmitted between rodent hosts by fleas, are considered an emerging pathogen. We explore the natural course of *Bartonella* infections in rodents at three sites in New Mexico: the Valles Caldera National Preserve (VCNP); the Sevilleta National Wildlife Refuge (NWR); and the Sandia Mountains. We analyze, using Spearman correlations and generalized linear models, the site level characteristics of *Bartonella* infections in rodent and flea communities. Rodents (*n*=3,515) were sampled for *Bartonella* from May 2004 to May 2007. Overall, 38% of rodents and 30 rodent species were positive for *Bartonella*. Prevalence was lowest at the Sevilleta NWR (24.6%) and highest at the VCNP (53%). Fleas (*n*=827) were collected from these rodents and 478 (60%) fleas of 24 species were positive for *Bartonella*. *Bartonella* infections typically lasted two months, though three animals tested positive for three consecutive months. The prevalence of *Bartonella* corresponded with rodent density at each site though the nature of this relationship changed with season and elevation. Analysis of temporal patterns of *Bartonella* infection found no significant effect of sampling period, though sites were significantly different. *Bartonella* is a dynamic parasite that appears to maintain a steady cycle of infection in wild rodent species. Changes in prevalence related to host density and environmental gradients point to the importance of both rodent and flea-mediated transmission mechanisms in *Bartonella* cycles.

Introduction

The mechanisms driving the spread of vector-borne zoonotic diseases are often difficult to identify due to the complexity of the vector-host-pathogen system. Changes in the abundance or susceptibility of hosts and vector communities have far reaching and often unpredictable consequences for vector borne diseases. Vector-borne pathogens dominate lists of emerging diseases (Githeko et al., 2000; Epstein, 2001) and include the bacterial pathogen *Bartonella*, which causes a number of human diseases including cat scratch disease, endocarditis, and bacillary angiomatosis (Azad et al., 1997; Boulouis et al., 2005).

Bartonella is a gram negative fastidious bacterium that infects the erythrocytes of a diversity of mammal species (Breitschwerdt and Kordick, 2000). *Bartonella* is common in rodent species, where it is most likely transmitted by fleas (Bown et al., 2004). Though this blood-borne pathogen appears to have little immunological consequences for its wild rodent hosts (Chomel et al., 2003, Boulouis et al., 2001), many *Bartonella* species are implicated in human disease (Greub and Raoult, 2003). In particular, *B. elizabethae, B. grahamii, B. vinsonii*, and *B. washoensis*, naturally found in rodent species within the U.S., have been associated with cases of endocarditis and uveitis in humans (Jacomo et al. 2002). A number of studies have described *Bartonella* species in the rodents (e.g., Kosoy et al., 1997; Jardine et al., 2006; Bai et al., 2007a; 2008; Reeves et al., 2005, 2007) and fleas (Stevenson et al., 2003; Reeves et al., 2005, 2007; Morway et al., 2008) of North America. In addition, many analyses have addressed the seasonal and density dependent changes in the prevalence of *Bartonella* in rodent species. (Birtles et al., 2001; Holmberg et al., 2003; Kosoy et al., 2004a,b; Jardine et al., 2006; Telfer et al., 2007a,b;

Bai et al., 2008). Though these studies have contributed considerably to our understanding of the ecology of particular species, we lack a cohesive view on the nature of *Bartonella* infections in rodents and flea vectors. In particular, the specific mechanisms that influence the transmission of this pathogen remain unknown.

Such studies are important not only to understand the spread of *Bartonella* infections, but also may contribute to understanding other, more difficult to detect, vector-borne zoonotic bacterial diseases, such as plague (caused by the bacterium *Yersinia pestis*). Plague continues to threaten humans and wildlife in the Western U.S. and remains elusive to efforts to identify the means by which it is maintained in wild animal populations. Of particular interest in studies of *Bartonella* is the relative influence of flea dependent versus vertebrate host dependent transmission dynamics.

In this paper, we present the first of a two-part analysis of *Bartonella* infections in rodents from three mountain ranges in New Mexico. We analyze, at the site level, characteristics of *Bartonella* infections in rodent and flea communities. Our primary objectives are to: 1) Describe the course of *Bartonella* infections in rodent species at each site; 2) identify the potential flea vectors of *Bartonella*; 3) examine the relationship between host density and parasite prevalence; and 4) assess seasonal patterns in infection prevalence. By comparing trends seen in all three sites, we identify the degree to which fleas and rodent population dynamics influence the spread of *Bartonella*.

Materials and Methods

This study represents part of an effort to track the movement of three rodent-borne pathogens, Hanta virus, plague (*Yersinia pestis*) and *Bartonella,* along elevational

gradients (EID grant # 0326757). This study was conducted on three mountain ranges within New Mexico: the Sandia Mountains in the Cibola National Forest, Bernalillo County, NM (N 35˚ 16' 45.5087, W 106˚ 23' 51.1680), the Los Pinos Mountains in the Sevilleta National Wildlife Refuge (NWR), Soccoro County, NM (N 34˚ 23' 43.8432, W 106˚ 34' 0.4800), and the Jemez Mountains of the Valles Caldera National Preserve (VCNP), Sandoval County, NM (N 35˚ 52' 2.8524, W 106˚ 35' 37.6441). This effort employed two trapping scheme. In the first, we used transects to live-trapped rodents each month at each site, unless snowpack was present, along an elevational gradient from grassland habitats to upper montane forest habitats. Transects consisted of 2 rows of 10 Sherman© live traps (LFA and SFA Folding Traps, H.B. Sherman Traps, Tallahassee, FL) placed 10 m apart (20 traps/transect). Transects were located 100-400 m apart along an elevational gradient at each site. In the second, we used a web arrangement to live-trapped rodents biannually. Each web contained 144 Sherman traps set in a radial pattern with a diameter of 200 m (see Parmenter et al., 2003). Webs were placed at upper and lower elevations at the Sevilleta and VCNP and approximately mid-elevation at the Sandia mountain site. The current analysis is not concerned with rodent movement patterns and uses the transect data to explore temporal trends in the prevalence of *Bartonella* and the web data to explore the relationship between rodent density and prevalence of *Bartonella*.

Site descriptions

***Sandia Mountains*-** The primary habitat vegetation of this site is pinyon-juniper woodland consisting primarily of Pinyon pine (*Pinus edulis*), one seed juniper (*Juniperus monosperma*) and blue grama grass (*Bouteloua gracilis*). Annual precipitation of study

site is 314 mm, with most moisture falling during the summer monsoon season. Mean annual temperature is 12.8° C, with mean July temperature of 19.9° C and mean January temperature of 1.5° C.

Sevilleta NWR - Lowland habitats are characterized as Chihuahuan desert grasslands graduating to midlevel Juniper Savanna habitat and to Pinyon-Juniper woodland at upper elevations. Dominate vegetation in desert grasslands included burrograss (*Scleropogaon brevifolius*), sand dropseed (*Sporobolus cryptandrus*) and black grama (*Bouteloua eriopoda*). One seed-juniper (*Juniperus monosperma*), honey mesquite (*Prosopis gland µlosa*) are dominate in Savanna habitats. The pinyon juniper woodland is dominated by Colorado pinyon (*Pinus edulis*), one seed juniper (*Juniperus monosperma*) with various grass species including blue, hairy, sideoats and black grama species (*Bouteloua gracilis,B. hirsuta, B. curtipendula* and *B. eriopoda*), and purple threeawn (*Aristida purpurea*). Annual precipitation of study site is 242 mm, with about 60% falling during the summer monsoon season. Mean annual temperature is 12.9° with mean July temperature of 20.5° C and mean January temperature of 1.5° C.

Valles Caldera National Preserve - Habitats ranged from meadows dominated by various grass and forb species (*Bouteloua gracilis, Potentilla hippiana, Erigeron flagellaris, Artemisia carruthii, Polygonum douglasii, Bromus inermis, Taraxacum officinale, Achillea millefolium, Festuca arizonica, Koelaria macrantha, Poa pratensis, Muhlenbergia montana,* and *Antennaria rosea)* to midrange mixed conifer forests dominated by Ponderosa pine (*Pinus ponderosa*), Douglas-fir (*Pseudotsuga menziesii var glauca)*, white fir (*Abies concolor*) and Spruce-fir forest with quaking aspen (*Populus tremuloides*), Engelmann spruce (*Picea engelmannii*) and Corkbark fir (*Abies arizonica*)

at highest elevations. Annual precipitation averages 638 mm, with approximately 45% falling during the summer monsoon season (July-September). Mean annual temperature is 4.5° C, with mean July temperatures of 15° C and mean January temperatures of -5.3° C.

Rodent Captures

Monthly Rodent collections, 2004-2007- At the Sevilleta NWR, 15 transects were set from 1595 to 1971 m. At the VCNP, 15 transects covered a range of elevation of 2462 to 3200 m. At the Sandia Mountains site, 15 transects were placed along a gradient ranging from 1761 to 1939 m. Small mammals were trapped for three consecutive nights. Traps were baited with sweet feed (multi-grain mix with molasses) and left open each evening and checked each morning at dawn. Each captured animal was identified to species, ear-tagged (Gey Band and Tag Co, Norristown, PA, USA), measured (ear length, right hind foot, total length, tail length) and weighed to the nearest gram with a Pesola ® scale (Baar, Switzerland). Small animals (<10grams) were toe-clipped in lieu of ear tags.

Blood samples were obtained from rodents via an occipital puncture with a heparenized capillary tube. Whole blood samples were either instantly frozen in liquid nitrogen or blotted onto Nobuto filter strips (Toyo Roshi Kaisha, Ltd., Tokyo, Japan). Each Nobuto strip was air dried and placed in a manila envelope for short term storage. Whole blood samples were kept at -20º C until processed in the laboratory, and Nobuto strips were largely stored at room temperature (though some were stored at 4º C).

During certain trapping periods, the first 10 individuals of each rodent species were actively searched for fleas. Otherwise, fleas were collected when observed in the fur

of an animal, but an active search may or may not have been conducted. Fleas were picked or brushed from fur of animal and then placed into a labeled cryovial and flash frozen in liquid nitrogen (-70˚C). After processing, rodents were returned to their trap and released at the site of capture. All animals were processed as described above only during the first capture of each trapping period. Animal handling procedures were approved by the Animal Care and Use Committee of the University of New Mexico (04MCC002). Blood and flea samples were processed under the guidelines and standardized protocols for the safe handling of biohazard material where appropriate (Mills et al., 1995; Keim lab, NAU and CDC, Fort Collins, unpublished protocols).

Biannual rodent population density estimates – We trapped rodents at nine webs, three at high elevation sites, three at lower elevation, and three on prairie dog towns in the VCNP. We trapped rodents on seven webs, three at high elevation sites, three at lower elevation, and one on a prairie dog colony at the Sevilleta NWR. We trapped rodents on 3 webs place approximately mid-elevation at the Sandia mountain site. Therefore, rodent densities were calculated for high, intermediate and low elevation locations for the Sevilleta and VCNP. Webs were sampled in spring and autumn 2004-2006, and spring of 2007. Trapping methods and animal processing followed the guidelines noted above.

Prairie Dog trapping- Prairie dogs at two sites, Sevilleta NWR and the VCNP were trapped during spring (May-June) and summer (August-September) of each year. Detailed methods are described elsewhere (Friggens et al., 2010). Briefly, one colony at the Sevilleta NWR and three colonies at the VCNP were trapped for three consecutive nights during each trapping session (Spring/Fall). This trapping either preceded or followed the web trapping meant to capture other small mammal species. We trapped

prairie dogs for effect and place traps near active burrows rather than using a standard formation. Two to four Tomahawk ® live traps (Size #70, Tomahawk Live Trap Company, Tomahawk,Wisconsin, USA) were set around each burrow for a total of 76-104 traps per colony. Traps were baited with a combination of rolled oats and sweet feed and wired open for at least 4 days prior to trapping to acclimatize prairie dogs to traps.

At a processing station removed from the trapping site, each prairie dog was weighed to the nearest gram with a Pesola ® scale (Baar, Switzerland), sexed, and given a uniquely numbered ear tag (Gey Band and Tag Co, Norristown, PA, USA). Animals were processed with the aid of canvas cones described in Hoogland (2005). Blood samples were collected by clipping a toenail on a rear foot just distal to the quick and blotting the blood onto a Nobuto filter strip (Toyo Roshi Kaisha, Ltd., Tokyo, Japan). Toenails were thoroughly cleaned with alcohol pads before clipping and treated with a sulfur compound (styptic) after clipping to prevent infection. Nobuto strips were air dried and put into an envelope for short term storage. Fleas were collected by holding each animal over a plastic basin containing a 20 x 20 cm felt cloth and thoroughly brushing the fur with a flea comb and/or toothbrush until all observed fleas had fallen into the basin. Fleas were then collected from the felt cloth, placed into a labeled cryovial and flash frozen in liquid nitrogen (-70˚ C). After processing, prairie dogs were returned to their trap and released at the site of capture. Prairie dogs were processed only during the first capture of each trapping period.

Flea Identification- Fleas were examined under a dissecting microscope and identified to genus, species or subspecies according to Furman and Catts (1982), Hubbard (1947) and Lewis (2002). Voucher specimens of each flea species were deposited in the Division of

Vector-borne Infectious Diseases of the Center for Disease Control and Prevention in Fort Collins, Colorado.

Bartonella methods

DNA extraction for Whole Blood Samples- Whole blood samples were processed with DNeasy Blood and Tissue Kits (DNeasy 250 or 96, Qiagen, Hilden, Germany) using manufacturer's instructions with minor modifications for tissue samples. For each sample, 25 µl of blood was combined with 125 µl of Bovine brain serum and the entire mixture was used in the extraction process. Final products were gathered from a single elution of 50 µl buffer AE.

*DNA extraction procedures for Nobuto filter strip*s- We used the Qiagen DNeasy® Blood and Tissue Kit and the dried blood protocol from the QIAamp® 96 DNA mini Kit. The following modifications were made to the published protocol: The pK lyses step was extended to 2 hours; an additional spin step was added after the addition of Buffer AW2. Final products were gathered in a single elution of 150 µl Buffer AE.

PCR amplification of Bartonella DNA

Blood Samples- A nested PCR procedure using Promega Taqman or Promega GoTaq (Promega Corp, Madison Wisconsin, USA) and *Bartonella*-specific primers for the citrate synthase (*gltA*) gene were used to amplify target from whole blood samples. The first set of primers correspond to a 500 bp target of the gltA gene (Sheff et al., unpublished protocol): Forward primer 600F (5'-TAT GTG TTT TTC TGT TCC TTG TGA-3'), and reverse 1243R(5'-AGA GTT GGC GTG GTC GGC TAA T-3'); The second set of primers amplified a 329 bp product of the same gene as described before

(Bai et al., 2008). Forward and reverse primers were BhCS781.*P* (5'-GGG GAC CAG CTC ATG GTG G-3') and BhCS137.n (5'-AAT GCA AAA AGA ACA GTA AAC A-3'), respectively. Distilled water and DNA of *B. doshiae* were used as negative and positive controls, respectively, in all PCR runs. All PCR amplifications were conducted in 96 well plates on BioRad or Applied Biosystems Thermocyclers.

For each reaction, 2.5 µl template DNA was added to a 50 µl reaction mixture containing 2.5 µl Taq, 5 µl 10X Buffer with MgCl (or 10 µl 10X Buffer with MgCl), 0.5 µl dNTP's (40µM solution of Promega's deoxynucleoside triphosphate mixture dATP, dCTP, dGTP, and dTTP), and 0.5 µl of each primer 600F, 1243R (10µM). This reaction was then run in the following thermocycler program: Initial 5 minute denaturization at 95˚ C , 4 cycles of 95˚ C for 1 minute, 56.3˚ C for 1 minute, and 72˚C for 1 minute, followed by 34 cycles of 95˚ C for 55 seconds, 56.3˚ C for 55 seconds, 72˚ C degrees for 1 minute, and finally 95˚C for 1 minute, 56.2˚C for 1 minute and a final annealing stage of 72˚ C for 10 minutes. The second reaction used 2.5 µl of the first reaction and an identical reaction mixture but substituted the inner primer set (BhCS781.p, BhCS137.n). The thermocycler program for the second step was: 95˚ C for 5 minutes, followed by 34 cycles of 95˚C for 1 minute, 58.3˚C for 1 minute, and 72˚C for 1 minute, followed by a final annealing step of 72 ˚C for 10 minutes.

Initial runs of the nested protocol on dried blood samples gave poor results with many positive samples showing weak and hard to interpret bands as compared to whole blood samples in side by side experiments. After modifying DNA extraction steps as described above for Nobuto strips, results improved somewhat. However, results were improved considerably by using the inner primer set (BhCS781.p, BhCS137.n) in a

reoptimized single step PCR procedure. Thus, the great majority of Nobuto derived DNA samples were amplified using this single step PCR procedure and the inner primer pair. Specifically, a 25 µl PCR reaction mixture containing 2.5 µl DNA template (DNA=1/10 reaction volume), 5 µl 5X buffer, 0.25 µl dNTPs (40mM), 0.25 µl each primer (10µM), 0.25 Taq DNA polymerase (GoTaq Green, Promega Co), 3 µl of $MgCl_2$ (for a final concentration of 4.5mM of $MgCl_2$) and water to volume was run in the following thermocycler program: 5 minute denaturization at 95˚C , 34 cycles of 95˚C for 1 minute, 52.3˚C for 1 minute, and 72˚C for 1 minute, followed by a final annealing stage of 72˚C for 10 minutes.

The high concentration of $MgCl_2$ led to nonspecific banding in certain instances (most commonly manifested as a rodent amplicon measuring 250 bp), which was remedied by processing samples on ice or using a hotstart method. We also found the 5' HotMaster Mix (Fischer) with variable Mg2+ to work very well with this protocol.

Flea samples: Fleas were assessed for presence of *Bartonella* DNA using a multiplex reaction as described elsewhere (Stevenson et al., 2003). Briefly, primers OF-G2 and OR-G2 described above were used to amplify a 328 kb length of the citrate synthase (gltA) gene and as described elsewhere (Stevenson et al., 2003). Each reaction used 2.5 µl of Flea DNA or homogenate in a 50 µl a solution containing the following reagents: 0.5 µl dNTP's, 0.5 µl each primer, and 2.5 µl of Taq (Promega Taqman) and was run in the following thermocycler program: 95˚ C for 5 minutes, followed by 34˚ C cycles of 95˚ C for 1 minute, 56˚ C for 1 minute, 72˚ C for 1 minute followed by a final annealing step of 72˚ C for 10 minutes.

All amplified products were visualized on a 1.5% Agar gel for run for 10-20 minutes followed by a five minute soak in an Ethidium Bromide solution. A 50bp ladder (Invitrogen) was used as a standard. We also used E-gel precast agarose gels (E-Gel 96®, Invitrogen) for high throughput processing of samples.

Statistical Analysis

Bartonella prevalence was calculated as the number of infected samples/total samples tested for each rodent species and all rodent species combined at each site. *Bartonella* prevalence was calculated for each trapping period and web or transect at each site. Because a large number of transects had no data due to lack of captures, we also calculated prevalence for all transects combined within a trapping period and used these values in generalized linear models to assess the relationship between prevalence and season and site. Rodent densities were calculated for each web in each site and each trapping period (typically twice per year) using a uniform cosine model in DISTANCE (Thomas et al., 2010). The trapping scheme used for prairie dogs did not allow us to estimate prairie dog density and this specie is not included in the density analyses. Relationships between *Bartonella* prevalence and density used web capture data, whereas seasonal patterns of *Bartonella* infection were analyzed using the monthly transect data. To account for the effect of sampling effort in analyses, which did not include density (tests for effect of season), we calculated the average number of captures/100 trap-nights and used this as a covariate in linear model analysis (offset variable).

Spearman correlations were used to assess the relationships between prevalence of *Bartonella* in small mammal populations and sampling effort, capture numbers, and prevalence of *Bartonella* in fleas (PROC CORR, SAS 9.2). We used a generalized linear

model with binomial distribution and logit link (PROC GLIMMIX SAS 9.2.) for analysis of the site level relationships between prevalence and rodent density. We also used this model to examine the seasonal trends in the prevalence of *Bartonella*. For web density analyses, elevation and sampling period were included as fixed effects. We treated web (nested within elevation) as a random effect and, to account for the repeated sampling at each site, we included sampling period (Year-Season) as a random effect with web-within-elevation as a subject. We fit our model by a backwards stepwise process where we first fit the fully crossed model and then sequentially dropped factors with the highest *P* value until only significant factors remained.

For transect data, the full model with season and site fixed effects and a random sampling period with transect as a subject would not converge. Therefore, we ran a model that used the average of all transects and sampling periods condensed into seasons as fixed effects. December, January, February were classified as winter, March, April, and May were classified as spring, Summer was June, July, and August, and Fall was September, October and November. Our final model used site (Sandia Mountains, Sevilleta NWR, VCNP) and season as fixed effects and included a season nested within years and as a random effect and defined the subject as “site”. Differences among significant variables were assessed using means tests with Tukey adjusted *P* values to account for multiple comparisons (PROC GLIMMIX. SAS 9.2). All tests were considered significant at the 0.05 alpha level.

Results

Bartonella Prevalence- We sampled 3,515 rodents for *Bartonella* over the course of this study (Table 4.1). Overall prevalence was lowest at the Sevilleta NWR (24.6%) and highest at the VCNP (53%). Of those species with greater than 10 samples, *Onychomys arenicola* and *Perognathus flavus* both from the Sevilleta NWR had the highest and lowest overall prevalence (64.8 and 9%, respectively). Prevalence was similar among species (with greater than 10 captures) at different sites with the following exceptions: *Bartonella* was 3 times as prevalent in *Dipodomys ordii* captured from the Sandia Mountains (28 vs. 6%) than animals captured on the Sevilleta NWR. Similarly, *Perognathus flavus* and *Peromyscus leucopus* from the Sandia Mountains had twice the prevalence of *Bartonella* over those captured at the Sevilleta NWR (21 vs 9 % and 40 vs 22%, respectively).

Of the 827 fleas that were tested for *Bartonella*, 478 (60%) were positive for *Bartonella*. Overall, 24 flea species were positive for *Bartonella* (Table 4.2; see Appendix 5 for a complete list of flea species by each rodent host and site). Thirteen percent of the fleas from the Sandia Mountains and 20% of the fleas from the Sevilleta NWR and VCNP were positive for *Bartonella*.

Bartonella prevalence was correlated with captures/100 trap-nights across all years and sites for both transect (r=0.38, P=0.0001, n=66) and web (r=0.225, P=0.0077, n=139) data. When analyzed by year, only 2005 showed a significant correlation for transects (r=0.595, P=0.053, n=11).

Infection Cycle- Numerous individual rodents (*n*=381) were captured and tested for *Bartonella* more than once over the course of this study (Fig. 4.1). The majority of animals (72%) were captured twice, 17% were captured 3 times, 4.7% were capture 4 times, 3.7 % 5 times and just under 1% were captured 6 or more times. The greatest number of recaptures was recorded for one *P. boylii* from the Sandia Mountains that was caught 8 times over the span of 15 months. The longest record for an individual rodent was seen for another *P. boylii* from the Sandia Mountains that was captured 6 times over a 24 month period.

Rodents in the Sandia Mountain site had the highest recapture rate (13.7%) and VCNP the lowest (7.5%). Of those species with at least 10 individuals captured more than once, *Peromyscus leucopus* (19.6%) from the Sevilleta NWR, *P. boylii* from the Sandia Mountains (18.3%) followed by *Onychomys arenicola* (17.9%) had the three highest recapture rates, whereas *P. maniculatus* from the VCNP (8%), and *P. truei (*(9%) and *Perognathus flavus* (8%) from the Sevilleta NWR had the lowest. Recaptured *P. boylii* had a much lower prevalence of *Bartonella* at the Sevilleta NWR than the Sandia Mountains, but otherwise all other species were similar (<3% difference) between sites. Of those animals captured only 2 times, 50.4% were captured on consecutive months (Figure 4.1a), 27.5% were captured over a 3-4 month span, 12% over 5-6 months and the remaining 10% were captured in periods ranging from 7-18 months.

Consecutive captures- One hundred and sixty two animals were captured on 2 or more consecutive months (Figure 4.1a-c). The majority (139/162 or 85%) were captured on two consecutive months. Sixty three percent (102/162) of the overall captures (93 of 139 of 2 month captures) showed no change in infection. The Sandia Mountains and

Sevilleta were mostly negative for *Bartonella* infections and remained so when captured 1 month later (Figure 4.1a). Most animals sampled for 2 months in the VCNP were *Bartonella* positive for both samples. About equal proportions gained or lost infections from the first to second sampling at each site.

Animals tested for 3 consecutive months tended to show a change in infection status though 1 animal from the Sevilleta was found to be positive for all 3 months (Figure 4.1b). The Sandia Mountains and Sevilleta had 1 and 2 animals, respectively, which were never positive for *Bartonella,* whereas all other captures showed some change in infection status over the course of the study. One *Onychomys arenicola* from the Sevilleta was captured 5 times in five months, gained *Bartonella* infection, which lasted for 2 months, was negative at the next sample and positive during a final survey.

Bimonthly captures- One hundred and twenty animals were captured on a bimonthly basis, 49 of which showed some change infection status over the course of the study. Animals sampled over a three month period were largely negative for *Bartonella* for both samples (Figure 4.1d). There was a slight tendency to lose infections from first to second sampling. Over a four-month period, six animals from the Sandia Mountains and three from VCNP appeared to have chronic infections with *Bartonella*, whereas 11 from the Sandia Mountains, four from Sevilleta, and one from the VCNP were negative for the same period (Figure 4.1e).

Duration of infections- Fifty-three animals tested positive for two or more consecutive months. The longest consecutive infection recorded in this study was three months (1 *Onychomys* from Sevilleta, and 2 *Peromyscus maniculatus* from the VCNP). Within

those animals captured bimonthly, we found two *P. boylii* and two *P. leucopus* from the Sandia Mountains positive in 3 out of 4 months (potential 4 month infections). One *P. boylii* from the Sandia Mountains was positive in surveys conducted every other month for 6 months. *Peromyscus* species, in particular *P. boylii* from the Sandia Mountains and *P. maniculatus* from the VCNP, typically had infections lasting 2 months. *Peromyscus boylii* tended to have 2 month infections (9 vs 5 instances), whereas *P. leucopus* tended to have a single month infection (25 vs 15) when tested two or 3 times. *Onychomys* were also often found with 2 month infections.

Bartonella Prevalence-Host Density relationships (Fig. 4.2)- Prevalence of *Bartonella* in the Sandia Mountains rodents was influenced by density and sampling period (X^2=1.13; F=4.32, *P*=0.046 and F= 4.49, *P*=0.042, respectively). Prevalence was significantly higher in spring of 2007 than fall of 2006. *Bartonella* prevalence was influence by a density*sampling period interaction in Sevilleta rodents (X^2=1.28, F=2.22, *P*=0.048). Density and prevalence followed similar trends in the spring but not fall seasons (Fig. 4.2). For the VCNP, density (X^2=0.89; F=3.19, *P*=0.021), elevation (F =3.66, *P*=0.091), density*sampling period (F =3.19, *P*=0.0214) and elevation*sampling period (F =2.64, *P*=0.008) were significant effects. Prevalence of *Bartonella* was significantly lower in the fall of 2006 than in Spring 2004 and 2005.

Bartonella Seasonal Trends (Fig 4.3, 4.4) - Rodents were captured over 31 trapping periods. Not all sites were trapped all 31 periods. Fluctuations in prevalence of *Bartonella* were evident at each of the sites (Fig 4.3). In analyses of all sites combined, we found significant correlations between *Bartonella* prevalence in rodents and capture effort (r=0.432, *P*<0.0001), total fleas collected (r=0.28, *P*=0.0066), and prevalence of

Bartonella within fleas (r=0.48, *P*<0.0001). These correlations held true for analysis of just the Sandia Mountains (r=0.49, 0.46, and 0.36 with *P*=<0.001, 0.0012 and 0.014, respectively), but not Sevilleta where no significant correlations were found. Within the VCNP only the prevalence of *Bartonella* in rodents and fleas was significantly correlated (r=0.55, *P*=0.02).

Within the Sandia Mountains, species level correlations were found for *Peromyscus boylii* and *P. leucopus* for rodent prevalence and capture effort (r=0.46, *P*=0.02 and r=0.44, *P*=0.021) (Fig 4.4.). Rodents captured from the Sevilleta and the VCNP showed no significant correlations between these variables except for *Neotoma mexicana* in the VCNP which had a perfect relationship between flea and rodent prevalence (though n= 3).

Model analysis showed no significance for season or season*site effects (F=0.87, *P*=0.47 and F=1.07, *P*=0.41, respectively). Site was significant (F=4.43, *P*=0.03) with the Sandia Mountains having a significantly lower prevalence of *Bartonella* than Sevilleta and the VCNP.

Discussion

This is the first comprehensive survey of *Bartonella* in rodent and flea communities of the southwestern United States. *Bartonella* infected on average 20-50% of the animals surveyed in this study, which is comparable to rates reported elsewhere (Kosoy et al., 1997; Holmberg et al., 2003; Bai et al., 2002; Birtles et al., 1994). Three species from the Sevilleta, *Dipodomys merriami, D. ordii* and *Perognathus flavus,* had unusually low parasetemia (<10% infected). These levels are similar to those reported for

rodents from Thailand (9%) where it was suggested that a lack of flea vectors might be the cause (Castle et al, 2004). We did collect fewer fleas from rodents captured on the Sevilleta (Table 4.1), but a greater proportion of those fleas carried *Bartonella* (19% versus 13%). In addition, though fewer fleas were collected on *D. merriami, D. ordii* and *P. flavus* as compared to other rodent species on the Sevilleta, *Bartonella* was found in those fleas (Table 4.1 and 4.2). Conversely, *Bartonella* was not always found in fleas from rodents, which had high prevalence of *Bartonella* (e.g. *Neotoma albigula* and *P. flavus* from The Sandia Mountains). Therefore, the presence of infected fleas does not always correspond to an infected host.

Differences in the immune response of different hosts, alternative non-flea vectors, or a decrease host interactions that influences transmission might also cause a low prevalence of *Bartonella*. Immunity is probably not a factor for *P. flavus*, which shows 21% infected at the Sandia Mountains though only 9% of animals at the Sevilleta were positive for *Bartonella*. It may be that there are different *Bartonella* with different immunological profiles infesting *P. flavus* at each site. Alternatively, there may be an alternative flea or tick vector present within the Sandia Mountains but not the Sevilleta. Fleas were rarely collected from this species at either site, though of these, only a flea from the Sevilleta was found to contain *Bartonella*. Interestingly, temporal patterns of capture success show that the Sevilleta populations were much more stable (Fig. 4.4) compared to the Sandia Mountains population. Though the Sandia Mountains showed a near absence of *P. flavus* for many trapping sessions, peaks in capture numbers were an order of magnitude greater at the Sandia Mountains than Sevilleta. It may be that animals at the Sandia Mountain site are reaching a critical density threshold beyond which

transmission of *Bartonella* increases substantially resulting in high prevalence. This pattern is also evident for *P. leucopus* trapped from both sites (Fig. 4.2). Cross-site comparisons cannot be made for *D. merriami* and *D. ordii*, but their congener *D. spectabilis* had a relative high prevalence of *Bartonella* and *Dipodomys*-specific fleas positive for *Bartonella* were pulled from *D. ordii* and *D. spectabilis*. Presumably, *Dipodomys* species would be infected by the same or a closely related suite of *Bartonella* species (Kosoy et al., 1997, 2000), though this does not necessarily mean these hosts would respond similarly to the infections.

We report a number of potential flea vectors of *Bartonella* (Table 4.2 and Appendix 5). Most of these flea species have not previously been surveyed for *Bartonella* and, as a result, this paper adds 16 new species to the list of fleas already reported to carry *Bartonella* in the U.S. (Stevenson et al., 2003; Reeves et al., 2004, 2007; Morway et al., 2008). It is clear from this survey that *Bartonella* is widespread in the rodent flea community. An average of 20% of the fleas analyzed carried *Bartonella* and the prevalence of *Bartonella* in rodents was strongly correlated to the prevalence of *Bartonella* in fleas collected at the same site. When analyzed by site, these trends were strongest for the Sandia Mountains. However, fewer fleas were tested from the Sevilleta, which reduced our capacity to examine these relationships at this site.

It is generally accepted that *Bartonella* can be transmitted by fleas (Krampitz, 1962; Lucey et al., 1992; Chomel et al, 1996; Pappalardo et al., 1997; Parola et al., 1999; Stevenson et al., 2003; Bown et al., 2004). However, other vectors, including ticks are also known to carry and transmit *Bartonella* (Chang et al., 200; Pappalardo et al., 1997; Kim et al., 2005) and it is unclear how important fleas might be to the persistence of

Bartonella within the environment. Though the presence of *Bartonella* in fleas is not enough in and of itself to determine whether fleas are transmitting *Bartonella,* the large number of *Bartonella* positive flea species found in this study points to their potential as vectors in these systems.

Characteristics of *Bartonella* Infections- Rodents in this study were transiently infected with *Bartonella*. Most species appear to maintain infections for about two months and experience frequent reinfections with *Bartonella*. Thus, in support of the findings of Kosoy et al. (1997), it appears unlikely that these rodents are acquiring immunity to *Bartonella*. Arguments for the development of acquired immunity in these hosts come from studies, which report a significantly higher prevalence of *Bartonella* in juvenile versus adult animals (Kosoy et al., 2004; Jardine et al., 2006; Tefler et al., 2007a). We did not examine the demographics of the infected rodent population and it may be that young rodents are infected more frequently by *Bartonella*. However, the consistent nature of the infections observed at times on animals captured over the span of several months (Fig 4.1) indicates that, in general, these rodents remain susceptible to *Bartonella* infections. Interestingly, Kosoy et al. (2004a) and Birtles et al., (2001) found *Sigmodon hispidus* sequentially infected by novel *Bartonella* variants, which may indicate immunity at the species level, though not against *Bartonella* in general. However, Birtles et al. (2001) report repeated infections within a single animal by the same variants though these infections were always separated in time. Acute infections have also been reported in black-tailed prairie dogs (*Cynomys ludovicianus*) in the Western U.S. (Bai et al., 2008) and bank voles (*Clethrionomys glareous)* and wood mice (*Apodemus sylvaticus*) populations in England (Birtles et al., 2001). We found species level differences in the

duration of infection in rodents (2 months in *P. boylii* and 1 month in *P. leucopus*), but did not detect site level differences in length of infection of different rodent species. Therefore, if rodents are exhibiting differential immunity to *Bartonella* species then similar *Bartonella* are infesting rodents at both sites.

Seasonal patterns of *Bartonella* prevalence- *Bartonella* prevalence changed significantly over time (when analyzed across individual trapping periods) but was not significantly different among seasons. Prevalence is expected to increase during warmer months, which corresponds to the activity of the ectoparasite that transmits these organisms. We did find a tendency at all three sites for a peak in prevalence during the spring-summer months. However, we also saw peaks during fall collections in the VCNP and in winter months for the Sevilleta (Fig 4.3, 4.4). Three studies have found that *Bartonella* infections peak in late summer and autumn (Jardine et al., 2006; Calvet et al., 2000; Kosoy et al., 2004) though these studies sampled only part of the year. Jardine et al. (2006) identified late summer as the period of greatest transmission where prevalence of both *Bartonella* and fleas was highest. Significant seasonal effects have also been observed in studies conducted year-round on two rodent species in England (Telfer et al., 2007a). This study found similar patterns for three species of *Bartonella*. Specifically, prevalence of *Bartonella* peaked in the late summer/fall followed by a drop in the winter and spring seasons. However, as observed in our data, Telfer et al., (2007a) also found a peak in Bartonella prevalence in the winter/early spring that they attributed to a single *Bartonella* species. Though they suggest that a non-flea or non-ecto mode of transmission may be responsible for this pattern, it is also possible that these patterns are due to nest or other fleas, which may not be dormant during winter months.

Seasonal patterns are particularly interesting to examine in light of our observations of *Bartonella*. It is likely that different *Bartonella* species are driving the patterns seen in our study (Figs. 4.1, 4.3, 4.4). These species-specific trends in timing of peak prevalence indicate some reliance on different flea species with unique ecological and microclimate requirements. Through this mechanism, climate is expected to play a significant role in influencing overall trends in *Bartonella* prevalence. Bai et al. (2008) found geographic variation in the timing of peak prevalence in their study of black tailed prairie dogs that may be due to the impact of climatic variations on flea activity patterns. Similarly, Jardine et al. (2006) note that cool and wet weather conditions may have negatively influenced the presence of *Bartonella* by reducing flea vector reproduction. The sites surveyed in this study each showed unique patterns of prevalence rise and fall (Figs. 4.1, 4.3, and 4.4) that may be due to climate variations among the sites. Sevilleta, the warmest and driest site, shows a consistent drop off in prevalence during the hottest months, which may be due to reductions in flea activity. This pattern is not seen at the other two sites. Conversely, all three sites showed an increase in prevalence during the fall of 2006 that may have resulted from regional wide climate patterns, which favored *Bartonella* transmission.

Flea vs. Rodent mediated transmission dynamics- Previous studies in the U. S. failed to find density-prevalence relationships in black-tailed prairie dogs and Richardson's' ground squirrels (Jardine et al., 2006; Bai et al., 2008). However, the longitudinal study conducted in England (Tefler et al., 2007a) found both delayed and current density dependence for two rodent species. In this study, the prevalence of *Bartonella* was influenced by rodent density though this relationship was differently affected by season

and sampling location within the three sites surveyed in this study. *Bartonella* prevalence was related to host density in spring seasons on the Sevilleta but appear inversely related for fall seasons. Similarly, within the VCNP, density and prevalence corresponded during spring but not fall seasons (for low elevation sites). These patterns may reflect a shift from rodent mediated transmission dynamics to flea mediated processes. Specifically, transmission of *Bartonella* during spring months, when flea populations are small is determined primarily by host contact rates, which corresponds to host density. As summer progresses, flea populations become much larger (see Chapter 3) and transmission becomes a function not only of host contact rate but also of the increased likelihood of begin bitten by a flea vector. In particular, transmission likely becomes a function of the presence and abundance of certain flea species. Therefore, fall seasons may vary from year to year depending on the characteristics of flea communities, which in turn are influenced by seasonal and annual patterns of weather. Elevational trends in density were evident at the VCNP where density was lowest in upper elevation webs and highest in lower elevations. Despite these differences in density, prevalence of *Bartonella* was similar across all three elevations. However, unlike the low elevation populations, prevalence of *Bartonella* at the highest sites tended to follow trends in density despite the season in which samples were collected. At mid elevations, density and prevalence were inversely related during the first three sampling periods, but corresponded during the last three sampling periods (Fig 4.2). Again, this may reflect a change in the mechanism driving *Bartonella* cycles. The lower elevations sites on the VCNP contain prairie dog towns, which tend to carry high flea loads relative to other species (Table 4.1). At higher elevations, prairie dogs are absent and flea communities may be more restricted by cold

weather. Seasonal patterns in the abundance of some flea communities have been attributed to differential tolerances to cold temperatures (Krasnov et al. 2001). Therefore, at higher elevation sites, transmission of pathogens between individuals is dependent on host contact rates as influenced by host density. Conversely, low elevation sites exhibit large build-ups in the flea populations (see Chapter 3), which presumably increases the probability of inter-host contacts, thereby increasing the likelihood of disease exchange. In addition, host contact may be more limiting at upper elevation sites, which show a lower rodent density than lower elevation sites (Fig. 4.2).

Bartonella is a dynamic parasite that appears to maintain a steady cycle of infection in wild rodent species. Prevalence of *Bartonella* appears to be influenced not only by annual variations in temperature, but also by latitudinal and elevational gradients ,which are characterized by climate gradients. These seasonal and environmental changes in prevalence point to the importance of flea-mediated mechanisms of *Bartonella* transmission. However, host rodent species also play significant roles in determining overall prevalence in these rodent communities.

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Table 4.1. Prevalence of *Bartonella* species in rodents and their fleas collected from 3 sites in New Mexico. Only those rodent species with more than 10 captures are listed here.

Species	Total	% positive	No. fleas collected (tested)	No. flea species	No. positive species	% (fleas)
Placitas						
Dipodomys ordii	46	28%	0	--	--	--
Neotoma albigula	52	52%	6	2	0	--
Neotoma micropus	67	27%	5	3	2	67
Perognathus flavus	87	21%	1	1	0	--
Peromyscus boylii	301	42%	136	7	3	18
P. leucopus	513	40%	117	13	5	19
P. truei	309	20%	58	6	2	7
Reithrodontomys megalotis	54	20%	1	1	0	--
Placitas Total	1450	34%	335 (330)*	16*	7*	13*
Sevilleta						
Cynomys gunnisoni	14	36%	20	1	1	5
Dipodomys merriami	27	4%	0	--	--	--
D. ordii	17	6%	6	2	1	50
D. spectabilis	113	30%	49	2	1	29
Neotoma albigula	93	47%	51	6	4	12
Onychomys arenicola	56	64%	14	6	2	21
Perognathus flavus	198	9%	3	3	1	33
Peromyscus boylii	49	43%	14	6	1	7
P. leucopus	92	22%	13	6	0	--
P. truei	235	18%	23	5	1	4
Sevilleta Total	920	25%	134 (133)*	20*	8*	19*
Valles Caldera						

Species	Total	% positive	No. fleas collected (tested)	No. flea species	No. positive species	% (fleas)
Cynomys gunnisoni	128	42%	513	4	4	11
Microtus longicaudus	10	60%	6 (2)	6	1	50
Neotoma cinerea	10	30%	13	5	2	38
N. mexicana	42	31%	66 (40)	3	2	38
Peromyscus maniculatus	1002	56%	308 (243)	18	6	19
Spermophilus lateralis	16	19%	4	1	0	--
Tamias minimus	52	56%	6	2	2	50
Valles Caldera Total	1145	54%	404 (309)*	23*	13*	19*
Overall	3515	38%	930 (870)*			

*Numbers include fleas collected from rare rodent species not listed here.

Table 4.2. List of *Bartonella* positive fleas collected from rodents captured at three sites in New Mexico.

Site	Species	Flea Species	No. Tested	Prevalence
Placitas	*Neotoma micropus*	*Orchopeas s. agilis*	1	1
		Orchopeas s. neotomae	1	1
	Peromyscus boylii	*Malaraeus sinomus*	116	0.20
		Orchopeas leucopus	4	0.25
		Peromyscopsylla hesperomys	11	0.09
	P. leucopus	*Malaraeus sinomus*	45	0.09
		Orchopeas leucopus	30	0.32
		Peromyscopsylla adelpha	2	1
		Peromyscopsylla hemispherium	3	0.33
		Peromyscopsylla hesperomys	24	0.17
	P. truei	*Malaraeus sinomus*	41	0.07
		Orchopeas leucopus	7	0.14
Sevilleta	*Dipodomys ordii*	*Meringis arachis*	4	0.75
	D. spectabilis	*Meringis arachis*	44	0.32
	Neotoma albigula	*Echidnophaga gallinacea*	22	0.05
		Orchopeas s. agilis	10	0.3
		Orchopeas s. schisintus	11	0.09
		Orchopeas s.agilis	2	0.5
	Onychomys arenicola	*Malaraeus telchinus*	7	0.14
		Pleochaetis e. exilis	2	0.5
	O. leucopus	*Pleochaetis e. triptus*	1	1
	Perognathus flavus	*Meringis shannoni*	1	1

Site	Species	Flea Species	No. Tested	Prevalence
	Peromyscus boylii	*Pleochaetis e. exilis*	1	1
	P. truei	*Malaraeus sinomus*	13	0.08
Valles Caldera	*Microtus longicatudus*	*Megabothris abantis*	1	1
	N. cinera	*Orchopeas s. agilis*	4	0.5
		Orchopeas s. sexdentatus	5	0.6
	N. mexicana	*Orchopeas s. neotomae*	37	0.38
		Stenoponia alpina	1	1
	P. maniculatus	*Aetheca w. ophidius*	72	0.13
		Aetheca w. wagneri	50	0.28
		Hystrichopsylla g. dippei	2	0.5
		Malaraeus sinomus	17	0.06
		Malaraeus telchinus	29	0.14
		Stenoponia americana	54	0.3
	Tamias minimus	*Eumolpianus e. cyrturus*	5	0.4
		Eumolpianus e. eumolpi	1	1

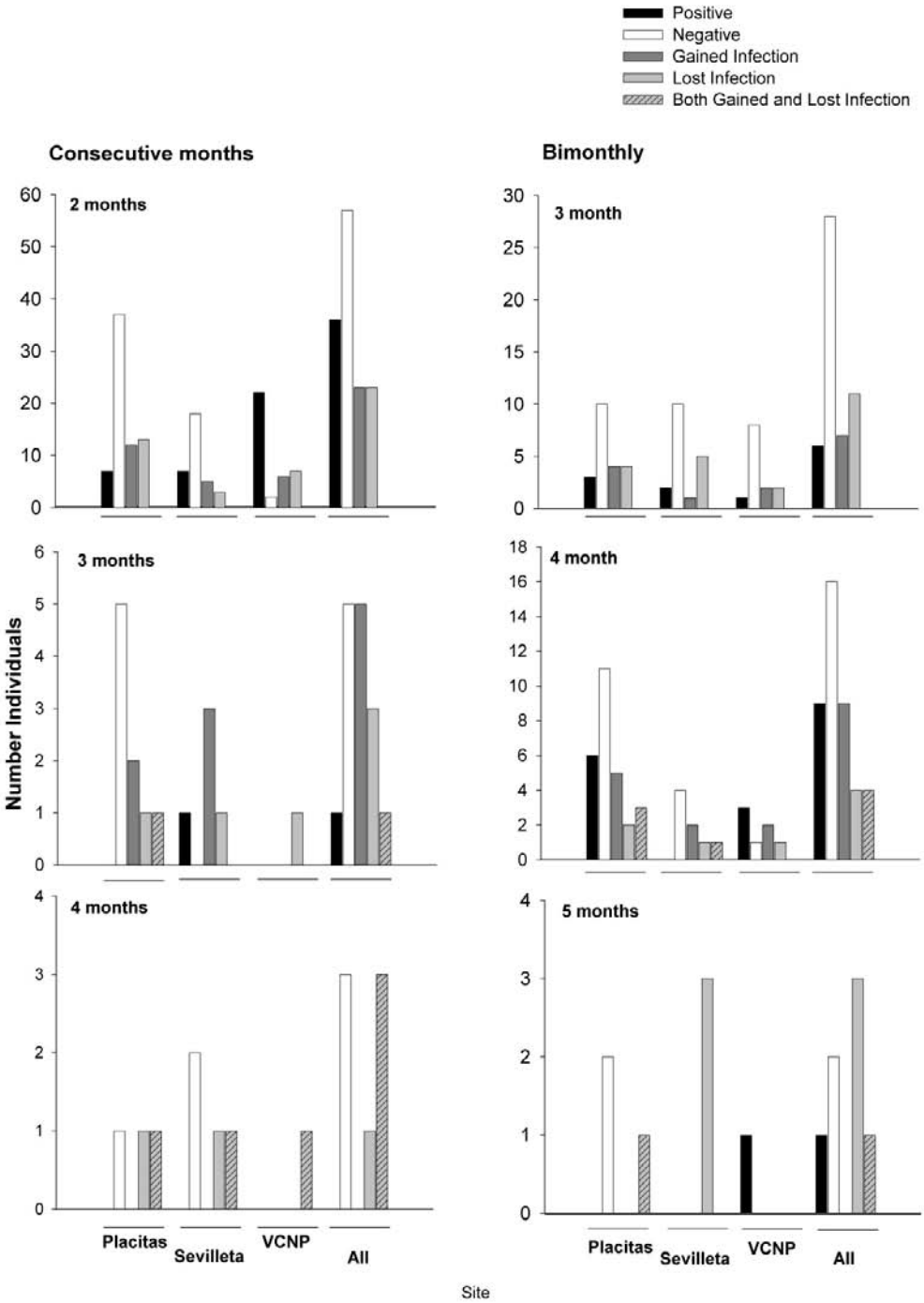

Figure 4.1. Number of recaptured animals that were *Bartonella* positive, negative, or with a loss or gain of infection. Left hand figures represent the infection status of animals caught for 2, 3 and 4 sequential months and right hand figures represent the infection status of animals caught every other month over 3, 4 and 5 month periods.

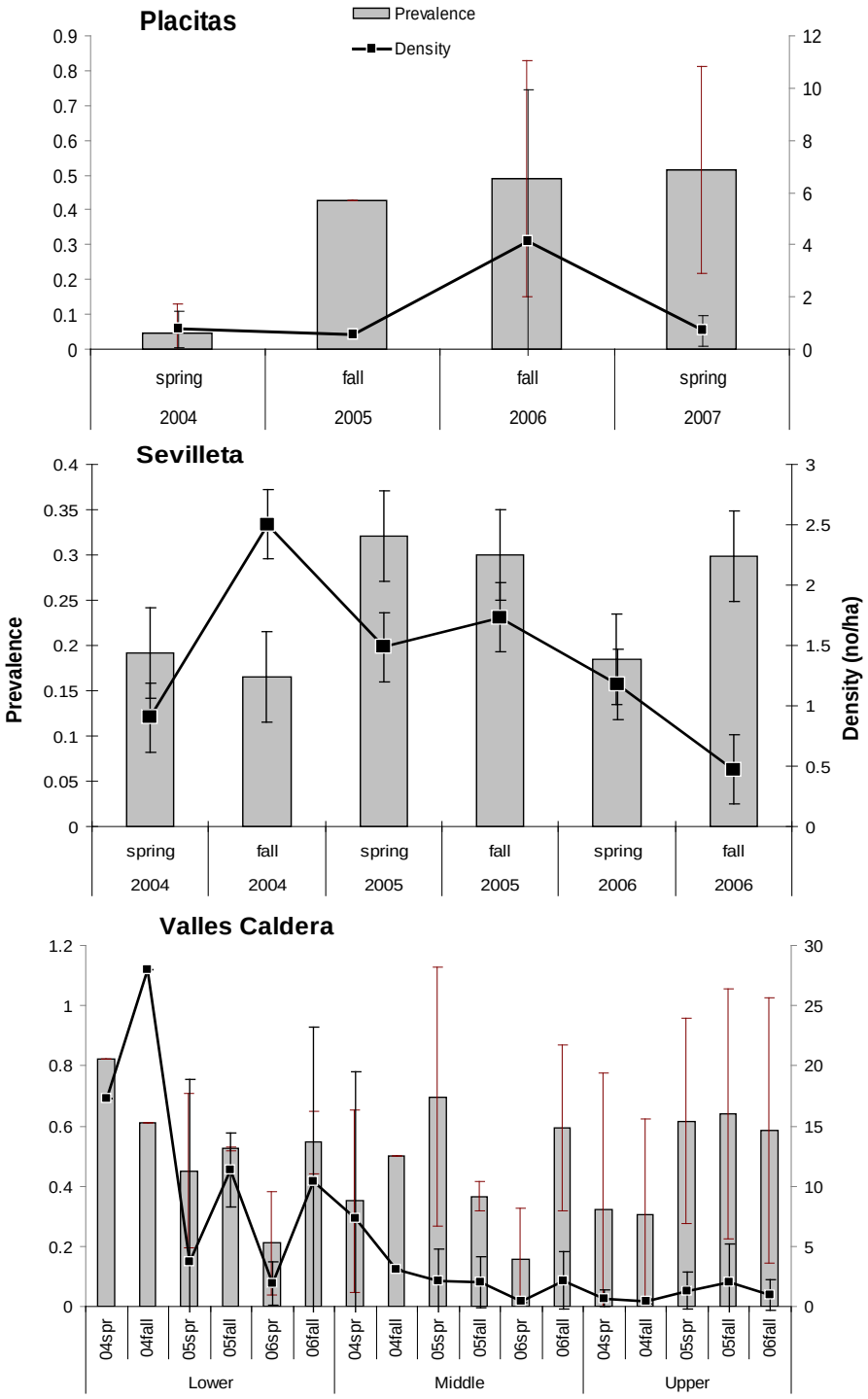

Figure 4.2. Density of rodents and prevalence of *Bartonella* caught on webs trapped twice each year from May 2004 through May 2007 at three sites in New Mexico. Figures display results of a generalized linear model analysis of density-prevalence-trapping period relationships. Placitas had significant season and density effects. Trapping period*Density was significant for Sevilleta rodents. Prevalence of *Bartonella* in Valles Caldera was influenced Density, Elevation Density*Elevation and Density*sampling period effects. Not all sites were trapped at all time periods and is reflected in these figures. Bars represent standard deviation.

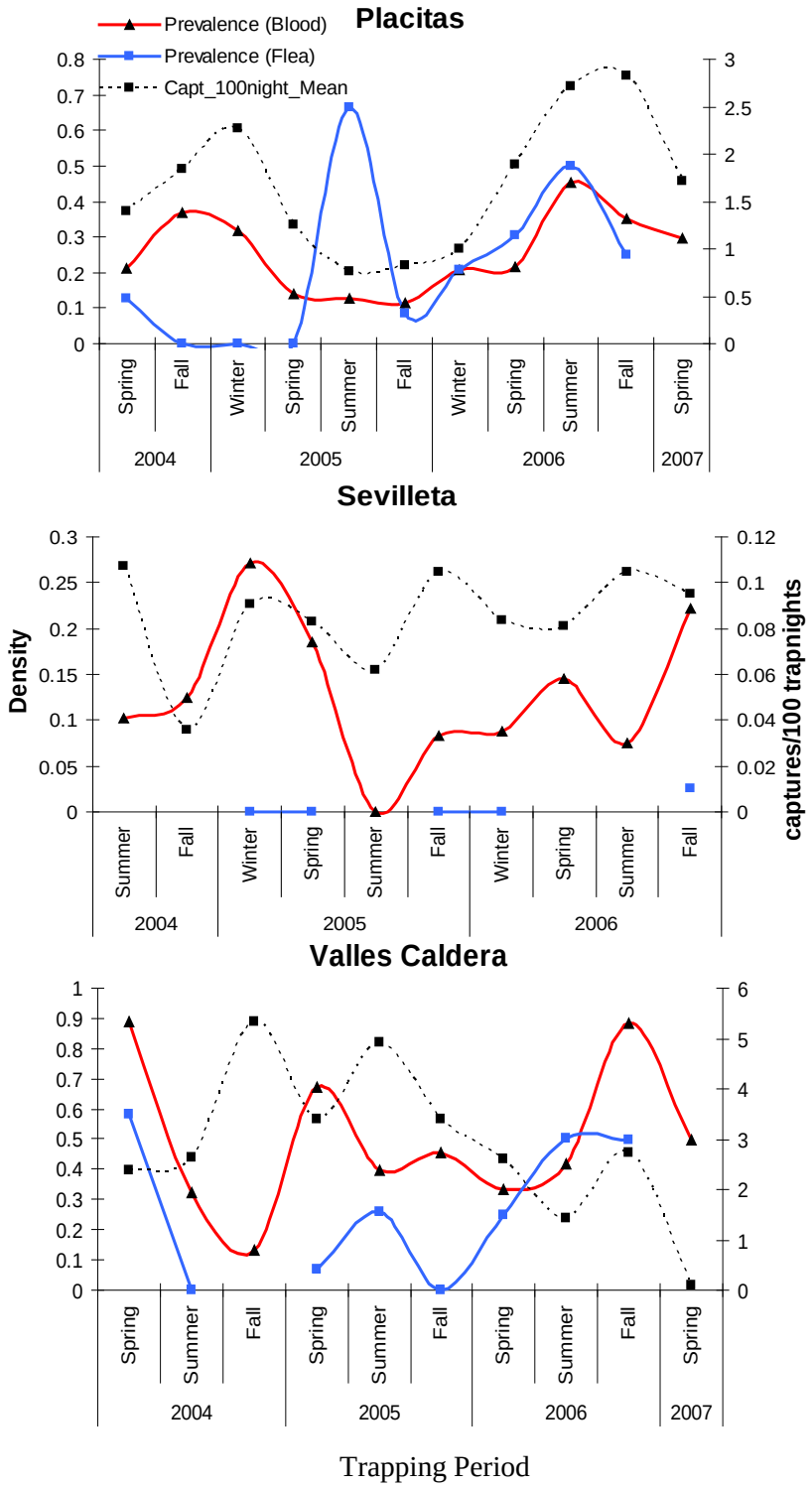

Figure 4.3. Seasonal patterns of rodent capture (standardized to animals/100 trap nights), and *Bartonella* prevalence in rodent blood and flea samples. Months were divided into seasons according to their climatic similarities, where winter is December, January, February (three coldest months), Spring is March, April, and May, Summer is June, July, and August, and Fall is September, October and November.

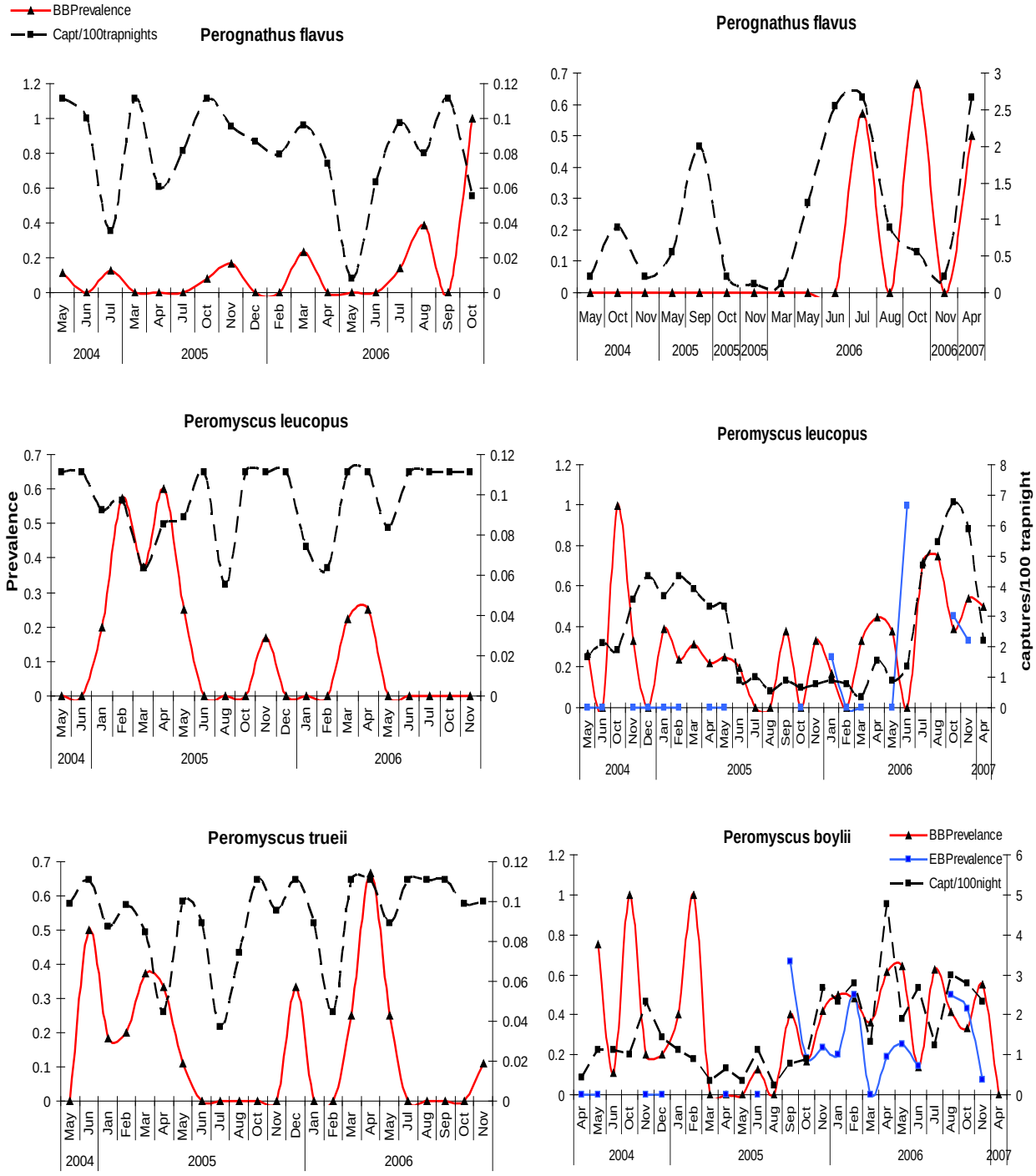

Figure 4.4. Monthly prevalence of *Bartonella* in rodents and their fleas capture from 2 sites. *Perognathus flavus* and *Peromyscus leucopus* were capture at both sites, whereas *P. truei* and *P. boylii* were not.

CHAPTER 5: DISCUSSION AND CONCLUSIONS

Fleas are important for the maintenance of plague over time and are the primary mechanism by which plague is transmitted among hosts (Gage and Kosoy 2005). Flea lifecycles, which include both on host and off host stages, are influenced not only by their physical surroundings but also by changes in host populations. These influences are dynamic and interact to determine the final composition of flea communities at a given site and time. The analysis presented in Chapter 2 demonstrates a relationship between flea community composition and habitat disturbance. The impact of human land use and change on flea communities is mediated through changes in host populations and the physical environment of their habitat. Loss of diversity favors generalist species, both in host and flea communities. Generalist rodents tend to carry high flea burdens and diverse flea communities and are commonly reservoirs to zoonotic diseases (Keesing et al., 2006; Escogue 1976; Wilcox and Gubler, 2006). *Peromyscus maniculatus*, a reservoir of Hanta virus and potentially of plague, is an excellent example of such a species captured during this study. This species harbored the most diverse flea communities and was among the most heavily infested rodents (Table 4.2). Generalist flea species, which infest a diversity of hosts, are often important vectors for diseases (Molynuex et al., 2003; Hawlena et al., 2007; Neito et al., 2007). Not only do these species facilitate transmission among different wildlife species, but they also tend to be numerically dominate on their hosts. Two flea species found in this study, *Aetheca sinomus* and *Malareus telchinus*, infest a diversity of hosts and are known to transmit *Y. pestis* (Appendix 5, Table 4.2).

High flea burdens and a high prevalence of fleas are also associated with increased disease transmission in rodent communities. In Chapter 2, both intensity of

infestation and prevalence increased with increasing disturbance, which indicates high disturbance sites may be more prone to disease outbreak. An increase in flea abundance leads to greater transmission of flea borne diseases by increasing the likelihood that fleas will be transfer between host species. This effect may also be achieved when host density increases and leads to greater contact between individuals and a greater likelihood of flea and disease exchange. Thus, the conclusions of Chapter 4, which found a relationship between current rodent density and prevalence of *Bartonella*, indicate a role of fleas in transferring this pathogen. In Chapter 3, increases in flea abundance observed just prior to a prairie dog plague epizootic and it may be that plague arose because the ideal conditions were present in the flea communities.

The prairie dogs inhabiting the Valles Caldera underwent a plague epizootic during this study, which allowed us to examine the characteristics of the flea communities associated with plague affected and non-affected towns. We found the number of fleas per host and per infested host and burrow were higher in plague-affected than non-affected colonies. Though some of the increase was due to the sudden loss of host species, which left a large number of fleas remaining in burrows and concentrated on surviving animals, we do not have an explanation for the pre-plague build-up in flea populations. One likely source of variation, not examined in this study, is the influence of seasonal and annual weather patterns on flea reproduction and survival. *Oropsylla hirsuta* and *O. t tuberculata* were the primary fleas involved in prairie dog plague epizootics. *Oropsylla hirsuta* is the most common flea implicated in the spread of plague in prairie dog towns (Cully and Williams, 2001) particularly with respect to supporting the fast moving epizootics commonly reported to occur in prairie dog colonies (Ubico et al.,

1988; Cully et al., 1997). However, this was the first report of plague infected *O. t. tuberculata* from prairie dog burrows. One of the most important conclusions to come from these observations regards the important role of the prairie dog burrow for the exchange of fleas and flea-borne diseases.

P. maniculatus and *O. leucopus* have been proposed as potential enzootic hosts. Both species show variable resistance and exhibit the population characteristics (e.g. high reproductive rates) that are characteristic of enzoonotic hosts (Gratz, 1999). In this study, *Peromyscus maniculatus* were abundant around prairie dog towns that became infected with plague (Chapter 3) and *P. maniculatus* carried a diversity of fleas that could potentially carry plague (Table 4.2). However, plague was not found in *P. maniculatus* nor any of rodents of the Valles Caldera (other than prairie dogs) surveyed in this study. Plague was detected in a number of non-prairie dog fleas however. Plague positive fleas were pulled from *Peromyscus maniculatus* (2), *Neotoma mexicana* (1) and *Spermophilus spilosoma* (1) in the VCNP and from *Dipodomys spectabilis* (2), *N. albigula* (1), and *Onychomys arenicola* (1) on the Sevilleta. Thus, there is only indirect evidence that these species may be reservoirs of plague. However, the results of these surveys point to the potentially greater sensitivity of flea over rodent surveys for the detection of plague in the environment.

Ground squirrels, *Spermophilus lateralis*, may play a role in transferring infected fleas between reservoir host species (Lechleitner et al., 1968; Anderson and Williams, 1997). Ground squirrels and prairie dogs often share flea species and exchange between these hosts is particularly evident during plague outbreaks (Ecke and Johnson 1950; Anderson and Williams, 1997; Cully and Williams, 2001). In the Valles Caldera, ground

squirrels were abundant on prairie dog towns, readily used prairie dog burrows, and ground squirrel associated fleas were positive for plague. It seems likely that *S. lateralis* are important to the plague cycle in the Valles Caldera and should be included in future investigations of potential enzootic hosts.

In conclusion, environmental factors can influence the realized role of fleas as disease vectors in a number of ways. In this dissertation, I show that anthropogenic disturbance can increase the risk of flea borne disease spread through changes in flea community composition. Specifically, fleas infested a larger proportion of hosts with a greater number of individuals in high disturbance sites. These characteristics appear important in prairie dog epizootics where a buildup in flea populations in burrows were associated with prairie dog plague outbreaks in the Valles Caldera. In addition, rodents in the Valles Caldera carried more fleas and a greater diversity of flea species, which may explain why we see plague in the Valles Caldera but not the Sevilleta. *Bartonella* cycles within rodent populations most likely reflect a relationship with flea vector species. In addition, it is likely that pathogen-vector interactions are species-specific where seasonal variations in the prevalence of *Bartonella* match variations seen for their favored flea species. Thus, it is likely that the emergence of *Bartonella* as a pathogen of global importance will be prone to the same influences as plague and other flea-borne diseases. *Bartonella* may prove valuable for future research that aims to identify species-specific interactions within flea-borne pathogen systems. Through these individual analyses, I found evidence for anthropogenic mediated and seasonally related changes in flea communities that are associated with an increased risk for disease transmission and perhaps initiated an outbreak in a Gunnison's prairie dog colony. This supports

predictions and observations made by others that warn of the potential for increase in the range and frequency of vector borne diseases (Githeko et al. 2000; Epstein, 2001; Harvell et al. 2002). Arthropod vectors are pivotal components in the disease cycles. At this time, it appears that many of the conditions projected for the future will benefit those species and conditions that promote disease transmission.

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APPENDICES

Appendix 1- Papers used in comparative analysis of anthropogenic disturbance on flea communities and flea-host associations. 135

Appendix 2. Scatter plot diagrams with loess (locally weighted scatterplot smoothing) lines for variables relating to mammal and flea community characteristics compiled from 63 studies reporting the fleas of small mammal communities at a variety of locations across the world. All values are log transformed. 142

Appendix 3. List of small mammal species reported in 70 studies used for a comparative analysis of anthropogenic disturbance on flea communities and flea- host associations. Parentheses indicate original reporting name 143

Appendix 4-List of flea species reported in 70 studies used for a comparative analysis of anthropogenic disturbance on flea communities and flea- host associations. Parentheses indicate original reporting name 150

Appendix 5. List of flea species, number and prevalence with *Bartonella* that were collected from rodent species trapped at three sites in New Mexico from May 2004 through May 2007. Questionable flea identifications are not listed here. 156

Appendix 1- Papers used in comparative analysis of anthropogenic disturbance on flea communities and flea-host associations.

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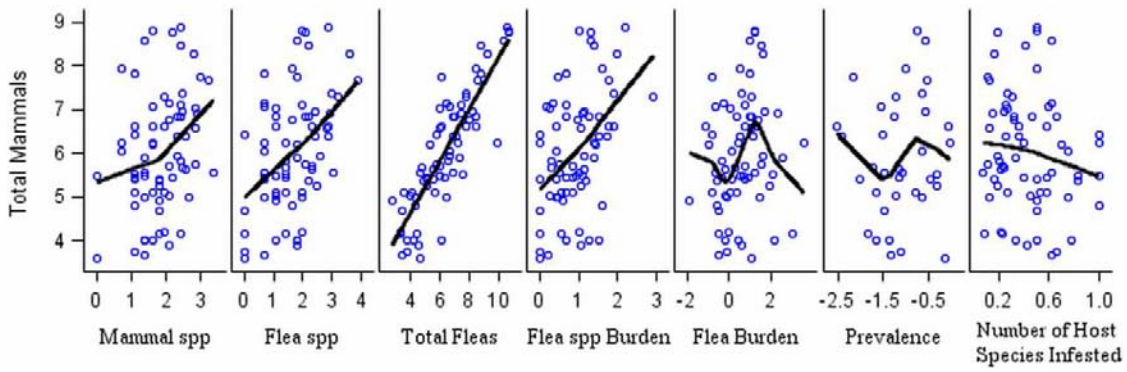
Appendix 2. Scatter plot diagrams with loess (locally weighted scatterplot smoothing) lines for variables relating to mammal and flea community characteristics compiled from 63 studies reporting the fleas of small mammal communities at a variety of locations across the world. All values are log transformed.



Appendix 3. List of small mammal species reported in 70 studies used for a comparative analysis of anthropogenic disturbance on flea communities and flea- host associations. Parentheses indicate original reporting name

Aethomys kaiseri

Akodon montensis

Akodon olivaceus

Akodon serrensis

Apodemus agrarius

Apodemus agrarius corea

Apodemus flaviocollis

Arvicola terrestris

Bandicota bengalensis

Bolomys lasiurus

Calomys tener

Calomyscus bailwardi

Caluromys philander

Abrothix longipilis

Acomys cahirinus cahirinus

Acomys cahirinus dimidiatus

Acomys russatus

Acomys spinosissimus

Aethomys namaquensis

Akodon azarae

Akodon cursor

Apodemus peninsulae

Apodemus sylvaticus

Apodemus sylvaticus

Arvicanthis niloticus

Arvicanthis niloticus niloticus

Arvicantis abyssinicus

Bandicota indica

Bandicota savilei

Berylmys berdmorei

Berylmys bowersi

Berylmys dermorei

Blarina blarina manitobensis

Blarina brevicauda

Agouti paca

Apodemus microps

Blarina blarina carolinensis

Cavia a. aperea

Chaetodipus (Perognathus) formosus

Chaetodipus (Perognathus) hispidus

Chaetodipus (Perognathus) intermedius

Chaetodipus (Perognathus) penicillatus

Chaetodipus californicus

Chiropodomys gliroides (trees)

Chromyscus chiropus

Citellus spilosoma

Citellus t. tridecemlineatus

Citellus variegatus

Clethrionomys gapperi

Clethrionomys gapperi loringi

Clethrionomys glareolus

Clethrionomys rufocanus

Clethrionomys rutilus

Coendou prehensilis

Cratogeomys castanops

Cricetulus migratorius

Cricetus cricetus

Crocidura lasiura

Crocidura renticola

Crocidura suaveolens

Cryptotis parva

Cynomys gunnisoni

Cynomys leucurus

Cynomys ludovicianus

Dasyprocta leporina

Delomys sublineatus

Deltamys kempi

Dendrogale murina

Dendromus sp.

Didelphis albiventris

Didelphis aurita

Didelphis marsupialis

Didelphis virgininia

Dipodillus dasyurus

Dipodomys agilis

Dipodomys heermanni

Dipodomys merriami

Dipodomys mesomelas

Dipodomys ordii

Dipodomys spectabilis

Dremomys rufigenis

Echimys chrysurus

Eliomys malanurus

Eliomys quircinus

Eothenomys regulus

Eutamias minimus

Eutamias minimus borealis

Eutamias quadrivittatus

Euxerus sp.

Gerbillus dasyurus

Gerbillus gerbillus

Gerbillus henleyi

Gerbillus nanus

Gerbillus pyramidum

Glaucomys sabrinus canescens

Grammomys dlichurus

Hermpestes spp

Holochilus brasiliensis

Hylomys suillus

Isothrix sinnamariensis

Jaculus jaculus

Lagurus curtatus

Leggada

Lemniscomys striatus

Leopoldamy sabanus

Leopoldamys edwarsi

Lepus californicus

Lepus townsendii

Liomys irroratus

Lophouromys aquilus

Lophuromys flavopunctatus

Makalata armata

Marmosops incanus

Marmota flaviventris

Marmota marmota canadensis

Mastomys (Praomys) natalensis

Mastomys coucha

Maxomys moi

Maxomys surifer

Menetes berdmorei

Meriones crassus

Meriones persicus

Meriones sacramenti

Mesomys hispidus

Metachirus nudicaudatus

Micoureus demerarae

Micromys minutus

Microsorex h. hoyi

Microtus agrestis

Microtus arvalis

Microtus californicus

Microtus drummondii

Microtus fortis

Microtus longicaudus

Microtus mexicanus

Microtus montanus

Microtus ochrogaster

Microtus pennsylvanicus

Microtus pinetorum

Microtus socialis

Millardia meltada

Mus caroli

Mus cervicolor

Mus minutoides

Mus mus castaneus

Mus musculus

Mus musculus brevirostris

Mus musculus praetextus

Mus norvegicus

Mus pahari

Mus triton

Myoprocta acouchy

Nectomys squamipes

Neomys anomalus

Neotoma albigula

Rodent species (cont).

Neotoma cinerea

Neotoma floridana

Neotoma fuscipes

Neotoma goldmani

Neotoma mexicana

Neotoma micropus

Niviventer cremoriventer

Niviventer fulvescens

Niviventer langbianis

Niviventer niniventer

Ochontona princeps

Ochrotomys nuttalli

Oenomys sp.

Oligoryzomys flavescens

Oligoryzomys microtis

Oligoryzomys nigripes

Oligorzoyms delticola

Oligorzoyms longicaudatus

Onlychomys torridus

Onychomys leucogaster

Oryzomys angoya

Oryzomys flavescens

Oryzomys nigriges

Oryzomys palustris

Oryzomys russatus

Oryzomys subflavus

Otomys angoniensis

Otomys denti

Otomys irroratus

Otomys unisulcatus

Oxymycterus judex

Oxymycterus roberti

Oxymycterus rufus

Pearsonomys annectus

Perognathus baileyi

Perognathus californicus

Perognathus flavus

Perognathus parvus

Peromuscus maniculatus

Peromyscus boylii

Peromyscus californicus

Peromyscus crinitus

Peromyscus difficilis

Peromyscus difficillus

Peromyscus eremicus

Peromyscus gossypinus

Peromyscus leucopus

Peromyscus maniculatus

Peromyscus maniculatus bairdii

Peromyscus maniculatus nubiterrae

Peromyscus n. aureolus

Peromyscus pectoralis

Peromyscus polionotus

Peromyscus truei

Phenacomys intermdius

Phenacomys u. soperi

Philander opossum

Pitomys subterraneus

Pitymus p. pinetorum

Proechimys iheringi

Psammomys obesus

Rattu rattus alexandrinus

Rattus (koratensis) sikkimensis

Rattus argentiventer

Rattus bartelsii

Rattus blanfordi

Rattus bukit

Rattus everetti

Rattus exulans

Rattus hawaiiensis

Rattus losea

Rattus megalotus

Rattus nitidus

Rattus norvegiucs

Rattus rattus diardii

Rattus rattus frugivorus

Rattus rattus kijabius

Rattus rattus palelae

Rattus rattus rattus

Rattus rattus rufescens

Rattus surifer

Rattus tanezumi

Rattus tiomanicus

Reithrodontomys fulvescens

Reithrodontomys humulis

Reithrodontomys megalotis

Reithrodontomys montanus

Rhabdomys pumilio

Rhipidomys sp.

Saccostomus capestri

Scapteromys aquaticus

Sciurillus pusillus

Sciuris aestuans

Sciuris arizonensis

Sciurus aberti

Sciurus aestuans

Sciurus carolinensis

Sekeetamys calurus

Sigmodon hispidus hispidus

Sigmodon minimus

Sigmodon orchrognathus

Sorex minutus

Sorex araneus

Sorex articus laricorum

Sorex caecutiens

Sorex cinereus cinereus

Sorex merriami

Sorex ornatus

Sorex palustris

Sorex palustris palustris

Sorex vagrans

Spermophilus beecheyi

Spermophilus lateralis

Spermophilus townsendii

Spermophilus variegatus

Spermphilus spilosoma

Speromphilus armatus

Speromphilus beecheyi

Speromphilus lateralis

Sphiggurus insidiosus

Suneus murinus

Sylvilagus aquaticus

Sylvilagus audubonii

ASylvilagus bachmani

Sylvilagus f. mallurus

Sylvilagus floridanus

Sylvilagus idahoensis

Sylvilagus nuttallli

Sylvilagus palustris

Synaptomys cooperi

Tachyoryctes splendens

Tamias merriami

Tamias s. griseus

Tamias striatus

Tamiasciurus (Tamias) hudsonicus

Tamiops macclellandi

Tatera indica

Thaptomys nigrita

Thomomys talpoides

Thomomys umbrinus

Tupaia glis

Vandeleuria oleracea

Zapus h. hudsonius

Appendix 4-List of flea species reported in 70 studies used for a comparative analysis of anthropogenic disturbance on flea communities and flea- host associations. Parentheses indicate original reporting name

Acropsylla girshami

Adoratopsylla (T) i. intermedia

Adoratopsylla antiquorum

Aetheca (E.) fornacis

Aetheca (Monopsylla) e. americanus

Aetheca (Monopsylla) e. cyrturus

Aetheca (Monopsylla) tripus

Aetheca (Monopsylla) vison

Aetheca (Monopsylla) wagneri systalius

Aetheca (Monopsyllus) anisus

Aetheca (Monopsyllus) e. eumolpi

Aetheca (Monopsyllus) e. kansensis

Aetheca (Monopsyllus) exilis

Aetheca (Monopsyllus) thambus

Aetheca (Monopsyllus) wagneri

Aetheca wagneri

Amalaraeus p. pedias

Amophalius necopinus

Amphipsylla siberica

Anomiopsylla novomexicanensis

Anomiopsylla nudatus

Anomiopsyllus amphibous

Anomiopsyllus f. congruens

Anomiopsyllus falsicialifornicus

Anomiopsyllus nudatus nudatus

Atyphloceras echis echis

Atyphloceras longipalpus

Atyphloceras m. multidentatus

Atyphlooeras multidentatus

Barreropsylla excelsa

Callistopsyllus campetris

Callistopsyllus terinus

Callistopsyllus terinus

Carteretta carteri

Catallagia calsheri

Catallagia decipiens

Catallagia luski

Cediopsylla inaequalis

Cediopsylla interrupta interrupta

Cediopsylla simplex

Ceratophyllus acutus

Ceratophyllus fasciatus

Chaetopsylla lotoris

Chiastopsylla rossi

Chiliopsylla alloophyla allophyla

Conorhinopsylla nidocola

Coptopsylla africana

Corrodopsylla birulai

Corrodopsylla c. curvata

Craneopsylla m. wolffheugeli

Craneopsylla minerva minerva

Ctenocephalides canis

Ctenocephalides felis

Ctenoparia inopinata

Ctenophthalmus (Ethioctenaphthalmus) machadoi

Ctenophthalmus agyrtes

Ctenophthalmus assimilis

Ctenophthalmus calceatus

Ctenophthalmus caviae

Ctenophthalmus crataepus

Ctenophthalmus felis strongylus

Ctenophthalmus solutus

Ctenophthalmus topali

Ctenophthalmus vulceatus

Ctenophthalums pseudogyrtes

Ctenophthalumus cabirus

Ctenophyllus erribilis

Ctenopthalmus congeneroides congeneroides

Dactylopsylla rara

Delotelis telegoni

Diamanus montanus

Dinopsyllus ellobius

Dinopsyllus lypusus

Dinopsyllus smiti

Doratopsylla blarinae

Doratopsylla c. curvata

Echindophaga gallicacea

Echinocephalus (C.) u. unicinatus

Epitedia stanfordi

Epitedia wenmanni

Eumolpi e. eumolpi

Foxella ignota

Gryphopsylla jacobsoni

Hechtiella lakoi

Hoplopsyllus affinis

Hoplopsyllus anomalus

Hoplopsyllus g. foxi

Hystrichopsylla dippiei

Hystrichopsylla dippiei neotomae

Hystrichopsylla dippiei truncata

Hystrichopsylla linsdalei

Hystrichopsylla microti

Hystrichopsylla occidentalis

Hystrichopsylla orientalis orientalis

Hystrick lindalei

Lentistivalius insolli

Lentistivalius klossi

Lentistivalius occidentayunnanus

Leptopsylla algira costai

Leptopsylla musculi

Leptopsylla nuttalli

Leptopsylla segnis

Letopsylla aethiopica

Letopsylla musculi

Listropsylla agrippinae

Macrostylophora pilata

Malaraeus bitterrootensis

Malaraeus euphorbi

Malareaus telchinum

Malareus euphorbi

Malareus sinomus

Malareus vonfintelis

Megaborthris clantoni

Megabothris a. megacoplus

Megabothris abantis

Megabothris d. divisus

Megabothris quirini

Megabothris rectangulatus

Megabothrisobscurus

Megarthroglossus cavernicolus

Megarthroglossus d. bisetis

Megarthroglossus weaveri

Megarthroglossus wilsoni

Megarthroglussus d.(aff.) divisus

Megarthroglussus pygaerus

Merengis dipodomys

Merengis jamesoni

Merengis parkeri

Meringis bilsingi

Meringis cummingi

Meringis hubbardi

Meringis nidi

Meringis parkeri

Meringis rectus

Meringis rectus

Meringis shannoni

Micropsylla sectilis

Micropsylla sectilis sectilis

Myoxopsylla laverani traubi

Nearctopsylla spp.

Neopsylla avida

Neopsylla bidentiatiformis

Neopsylla inopina

Neopsylla specialis

Neopsylla tricata

Nosopsyllus fasciatus

Nosopsyllus iranus

Nosopsyllus theodori

Odontopsyllus dentatus

Opisodasys k. nesiotus

Opisodasys keeni

Opisodasys pseudoarctomys

Opisodasys robustus

Orchopeas c. caedens

Orchopeas howardii

Orchopeas leucopus

Orchopeas pennsylvanicus

Orchopeas s. agilis

Orchopeas s. pennsylvanicus

Orchopeas sexdentatus

Orchopeas sexdentatus neotomae

Orchopeas wickhami

Oropsylla (Opisocrostis tuberculatus) tuberculata tuberculata

Oropsylla (Opisocrostis) bruneri

Oropsylla (Opisocrostis) labis

Oropsylla (Opisocrostis) washingtonensis

Oropsylla arctomys

Oropsylla hirsuta (Opisocrotis hirsutus)

Oropsylla idahoensis

Oropsylla labis

Oropsylla montana

Oropsylla pandorae

Oropsylla rupestris

Oropsylla t. cynomuris

Palaeopsylla s. soricis

Palaeopsylla soricis starki

Parapulex chephrenis

Peromyscopsylla b. bidentata

Peromyscopsylla catitina

Peromyscopsylla draco

Peromyscopsylla h. adelpha

Peromyscopsylla h. cuneata

Peromyscopsylla h. vigens

Peromyscopsylla scotti

Peromyscopsylla selenis

Peromyscopsylla sylvatica

Peromysopsylla hesperomys

Phalacropsylla allos

Pleocheatis exilis

Plusaetis sibynus

Polygenis a. axius

Polygenis atopus

Polygenis axius pessoai

Polygenis b. bohlsi

Polygenis frustratus

Polygenis gwyni

Polygenis k. klagesi

Polygenis massoiai

Polygenis occidentalis

Polygenis pradoi

Polygenis puelche

Polygenis pygaeurus

Polygenis r. beebei

Polygenis rimatus

Polygenis tripus

Polygenus occidentalis occidentalis

Polygenus roberti roberti

Polyplax spinulosa

Pulex irritans

Pulex simulans

Rectofrontia fraterna

Rhadinopsylla multidenticulata

Rhadinopsylla concava

Rhadinopsylla fraterna

Rhadinopsylla insolata

Rhadinopsylla masculana

Rhadinopsylla sectilis

Rhopalopsylllus garbei

Rhopalopsylllus gwyni

Rhopalopsyllus australis australis

Rhopalopsyllus l. lugbris

Sphinctopsyllaa ares

Stenistomera macrodactlya

Stenoponia alpina

Stenoponia americana

Stenoponia macrodactyla

Stenoponia montanta

Stenoponia sidimi

Stenoponia tripectinata medialis

Stivalius cognatus

Tetrapsyllus rhombus

Thrassis a. capestris

Thrassis aridis

Thrassis b. johnsoni

Thrassis bacchi

Thrassis campestris

Thrassis fotus

Thrassis francisi

Thrassis howelli

Thrassis o. coloradensis

Thrassis pandorae

Thrassis petiolatus

Thrassis standfordi

Thrips sp.

Xenopslla cheopis

Xenopsylla astia

Xenopsylla bantoum

Xenopsylla baxtoni

Xenopsylla braziliensis

Xenopsylla cheopis

Xenopsylla conformis mycerini

Xenopsylla dipodilli

Xenopsylla hawaiiensis

Xenopsylla nubicus

Xenopsylla ramesis

Xenopsylla robertsi

Xenopsylla scopulifer

Xenopsylla vexabilis

Xiphiopsylla hyparetes

Appendix 5. List of flea species, number and prevalence with *Bartonella* that were collected from rodent species trapped at three sites in New Mexico from May 2004 through May 2007. Questionable flea identifications are not listed here.

Site	Rodent Species	Flea Species	Number collected	Number Tested	Prevalence
Placitas	*Neotoma albigula*				
		Malaraeus sinomus	4	4	0
		Orchopeas s. sexdentatus	2	2	0
	Neotoma micropus				
		Orchopeas s. agilis	1	1	1
		Orchopeas s. neotomae	1	1	1
		Orchopeas s. schisintus	3	3	0
	Perognathus flavus				
		Malaraeus sinomus	1	1	0
	Peromyscus boylii				
		Aetheca w. ophidius	1	1	0
		Malaraeus sinomus	117	116	0.198
		Malaraeus telchinus	1	0	
		Orchopeas leucopus	4	4	0.25
		Peromyscopsylla hesperomys	11	11	0.090
		Peromyscopsylla selenis	2	2	0
	P. leucopus				
		Epitedia wemmani	1	1	0
		Malaraeus bitterootensis	1	1	0
		Malaraeus sinomus	47	45	0.0888
		Opisodaysis keeni	3	3	0
		Orchopeas leucopus	30	30	0.321
		Orchopeas s. agilis	1	1	0
		Orchopeas s. neotomae	1	1	0
		Orchopeas s. schisintus	1	1	0
		Orchopeas sp.	4	4	0.25
		Peromyscopsylla adelpha	2	2	1
		Peromyscopsylla hemispherium	3	3	0.333
		Peromyscopsylla hesperomys	24	24	0.166
		Peromyscopsylla selenis	1	1	0
	P. maniculatus				
		Malaraeus sinomus	1	1	0
		Peromyscopsylla hesperomys	1	1	0

	P. nasutus				
		Malaraeus sinomus	2	2	0
	P. truei				
		Malaraeus sinomus	41	41	0.073
		Opisodaysis keeni	2	2	0
		Orchopeas leucopus	7	7	0.14
		Orchopeas nepos	1	1	0
		Orchopeas s. agilis	1	1	0
		Peromyscopsylla hesperomys	4	3	0
	Reithrodontomys megalotis				
		Orchopeas leucopus	1	1	0
	Spermophilus lateralis				
		Hoplopsyllus anomalus	2	2	0
Sevilleta	*Ammospermophilus interpres*				
		Oropsylla idahoensis	1	1	0
	Dipodomys ordii				
		Echidnophaga gallinacea	2	2	0
		Meringis arachis	4	4	0.75
	D. spectabilis				
		Echidnophaga gallinacea	7	5	0
		Meringis arachis	44	44	0.318
	Neotoma albigula				
		Echidnophaga gallinacea	22	22	0.045
		Orchopeas s. agilis	10	10	0.3
		Orchopeas s. neotomae	1	1	0
		Orchopeas s. schisintus	11	11	0.091
		Orchopeas s. schisintus/intermedius	1	1	0
		Orchopeas s. sexdentatus	4	4	0
		Orchopeas s. sexdentatus/s.agilis	2	2	0.5
	N. microtus				
		Echidnophaga gallinacea	3	3	0
		Malaraeus sinomus	1	0	
		Orchopeas s. sexdentatus	3	3	0
	Onychomys arenicola				
		Malaraeus sinomus	1	1	0
		Malaraeus telchinus	7	7	0.143
		Meringis dipodomys	1	1	0
		Peromyscopsylla hesperomys	1	1	0
		Pleochaetis e. exilis	2	2	0.5
		Pleochaetis e. triptus	1	1	0
	Onychomys leucopus				
		Malaraeus telchinus	3	3	0

	Oropsylla hirsuta	1	1	0
	Pleochaetis e. triptus	1	1	1
Perognathus flavus				
	Meringis dipodomys	1	1	0
	Meringis shannoni	1	1	1
	Orchopeas leucopus	1	1	0
P. boylii				
	Atyphloceras echis	1	1	0
	Malaraeus sinomus	5	5	0
	Orchopeas leucopus	4	4	0
	Peromyscopsylla hesperomys	1	1	0
	Pleochaetis e. exilis	1	1	1
	Thrassis bacchi	1	1	0
P. leucopus				
	Malaraeus sinomus	2	2	0
	Malaraeus telchinus	1	1	0
	Opisodaysis keeni	1	1	0
	Orchopeas leucopus	5	5	0
	Peromyscopsylla adelpha	1	1	0
	Peromyscopsylla hesperomys	3	3	0
P. truei				
	Malaraeus sinomus	13	13	0.077
	Megabothris d. divisus	1	1	0
	Orchopeas leucopus	7	7	0
	Orchopeas s. neotomae	1	1	0
	Peromyscopsylla hesperomys	1	1	0
Spermophilus spilosoma				
	Thrassis a. desertorum	1	1	0
	Thrassis pansus	2	2	0
Valles Caldera				
Microtus longicatudus				
	Malaraeus sinomus	1	0	
	Malaraeus telchinus	1	0	
	Megabothris abantis	1	1	1
	Megabothris quirini	1	1	0
	Peromyscopsylla selenis	2	0	
N. cinera				
	Orchopeas s. agilis	4	4	0.5
	Orchopeas s. neotomae	2	2	0
	Orchopeas s. schisintus	1	1	0
	Orchopeas s. sexdentatus	5	5	0.6
N. mexicana				
	Malaraeus sinomus	3	2	0

	Orchopeas s. neotomae	62	37	0.378
	Stenoponia alpina	1	1	1
Peromyscus maniculatus				
	Aetheca w. ophidius	72	72	0.125
	Aetheca w. wagneri	98	50	0.28
	Anomiopsyllus nudatus	1	1	0
	Catallagia decipiens	5	3	0
	Hystrichopsylla g. dippei	2	2	0.5
	Malaraeus bitterootensis	2	1	0
	Malaraeus euphorbi	1	1	0
	Malaraeus sinomus	20	17	0.059
	Malaraeus telchinus	36	29	0.138
	Megabothris quirini	1	1	0
	Opisodaysis keeni	2	1	0
	Orchopeas s. neotomae	1	1	0
	Orchopeas s. schisintus	1	1	0
	Orchopeas s. sexdentatus	1	1	0
	Peromyscopsylla hesperomys	3	3	0
	Peromyscopsylla ravalliensis	2	1	0
	Peromyscopsylla selenis	3	3	0
	Stenoponia americana	55	54	0.296
Spermophilus lateralis				
	Oropsylla idahoensis	4	4	0
Tamias minimus				
	Eumolpianus e. cyrturus	5	5	0.4
	Eumolpianus e. eumolpi	1	1	1
T. quadivatticus				
	Eumolpianus e. cyrturus	1	1	0