SPATIAL PATTERNS OF HISTORICAL PLAGUE INCIDENCE ON BLACK-TAILED PRAIRIE DOG (*CYNOMYS LUDOVICIANUS*) COLONIES

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Abstract

During successive plague outbreaks over the past twenty years in Boulder County, CO, some black-tailed prairie dog (*Cynomys ludovicianus*) colonies have been regularly affected by plague while others have repeatedly escaped plague. Previous research by S. Collinge and others indicates that this spatial pattern of historical plague occurrence is correlated with landscape context. We tested the hypothesis that landscape-level characteristics are correlated with colony-level characteristics that play a direct role in plague epidemiology. To elucidate the causal mechanism driving the observed historical plague pattern, we examined the following colony-level characteristics on 14 prairie dog colonies of known positive or negative plague history: microclimate, vegetation, vector (flea) and host (prairie dog and small mammal) populations, and disease prevalence. From February through September, 2004, we trapped prairie dogs and small mammals, and tested the animals and fleas for plague and bartonellosis.

Preliminary results indicate that the relative abundance of the two dominant flea species collected from prairie dogs in spring 2004 differed between historically plague positive and negative sites. Additionally, bartonellosis prevalence was greater in the flea species of relatively greater abundance on historically plague positive sites. Plague prevalence in prairie dogs and their fleas was 0% on all sites in spring 2004. Additional data on microclimate, vegetation cover, and are currently being analyzed. Fleas from the remainder of the trapping season are being identified, and tested for disease. Analysis of all results will be complete by May 2005.

Introduction

Understanding spatial patterns of plague incidence is crucial to manage prairie dog populations effectively. Plague, habitat loss, and poisoning (Cully and Williams 2001) are primary causes of the 98% decline in prairie dog colony coverage since the early 1900's (Miller et al. 1994). Although plague transmission via animal bite is possible, flea bites are considered the primary mode of transmission between individual prairie dogs (Gage 1999). Social behaviors such as allelogrooming—grooming between individuals (Hoogland 1995)—provide ample

opportunity for flea exchange. Because black-tailed prairie dogs (*Cynomys ludovicianus*) are highly social and have uniformly low resistance to plague, they are at high risk for infection (Biggins and Kosoy, 2001). As a result of these vulnerabilities, plague epizootics (outbreaks) frequently kill greater than 99% of individuals in colonies (Biggins and Kosoy 2001, Cully and Williams 2001).

Intermittent to epizootics in prairie dogs, *Yersinia pestis,* the bacterial agent of plague, is maintained by mammals and flea vectors in a poorly characterized enzootic cycle (Cully and Williams 2001). Although deer mice (*Peromyscus maniculatus*), among others, have been suggested as potential reservoir hosts, no definitive evidence exists to support this contention. What is still unclear is the number of species involved in disease maintenance, as well as the identity of these species. The mechanism for transmission from the enzootic cycle to the epizootic cycle is also unclear. Because of gaps in our knowledge of both epizootic and enzootic dynamics, we are unable to make predictions concerning the spatial pattern of epizootics that could aid prairie dog management.

Due to the infrequent and unpredictable nature of plague outbreaks in prairie dogs in Boulder County, CO, we decided to utilize historical plague records to study the spatial pattern of outbreaks. Collinge et al. (in review) collected data on historical plague incidence from 1981 through 2003 for Boulder County, CO. Major plague epizootics affecting most colonies in the area occurred in 1986 and 1994; much less extensive epizootics occurred in 1991, 1999 and 2000. Using historical plague data and current GIS layers of landscape cover, Collinge et al. predicted that plague incidence is negatively affected by cover of roads, streams, and lakes in the surrounding landscape.

The authors (Collinge et al. in review) suggested three potential mechanisms for the observed correlation between landscape context and plague history: 1) Cover of roads, lakes and streams in the surrounding landscape could directly impede movement of infectious propagules into prairie dog colonies. 2) Habitat on colonies could vary spatially as a result of landscape context and affect disease dynamics directly and/or indirectly. 3) Roads, streams and lakes could be associated with other features affecting disease dynamics. In this study, we conducted further exploration of the second proposed mechanism. The objective of this research was to understand how putative disease related factors vary spatially in relation to landscape context and plague history. Furthermore, we examined how these disease correlates might directly or indirectly

influence spatial disease incidence in vectors and mammalian hosts. Specifically, we tested the hypothesis that abiotic and/or biotic conditions that play an important role in plague epidemiology differ between historically plague positive and historically plague negative colonies.

Methods

Prairie Dog Trapping:

We trapped during early spring and summer in 2004 (see Tables 1 & 2). In spring (February through March) we trapped prairie dogs on five plague positive and five negative sites. In summer (June through July) we trapped on nine plague positive and five negative sites. All subsequent data collection occurring on the same colonies was conducted in the same general area. We trapped each colony for four consecutive days, approximately three hours per day. We used 49 Tomahawk live-traps placed 25 meters apart and arranged in a grid. Traps were pre-baited with corn, oats and barley, and locked open for five days prior to trapping, and during hours intermittent to trapping. We will use mark-recapture data obtained from the summer trapping session to estimate relative population sizes for each colony.

Exceptions to the above trapping regimen include spring trapping on plague positive colonies three and six. We utilized targeted trapping rather than a grid design, placing approximately 50 traps per site at burrow entrances. Trapping was conducted for three days instead of four. In addition, traps on site three were placed in a different area of the colony than the area where all other data collection occurred.

Table 1. Plague history of study colonies. Properties in bold are owned by County Open Space, with the exception of Beech, which is jointly owned by City and County Open Space.

Site	Plague History	Plague History Details	Area	Property Name
1	**positive**	**plague 94**	**130.9**	**Dowe Flats**
3	**positive**	**plague 94**	**150.3**	**RCF**
6	**positive**	**plague 94**	**17.3**	**Kaharias**
8	positive	plague 94 & 86	243.6	Axelson
10	positive	plague 94	10.7	Gallucci
11	positive	plague 94 & 99	29.1	Flatirons
17	positive	plague 94	17.5	Belgrove
19	**positive**	**plague 94 & 86**	**35.8**	**Beech**
20	positive	plague 94	24.7	Waneka
18	negative	survived '86, '91, & '94 plague	24.1	Andrus
30	negative	survived 94 plague	25.2	Johnson/Dawson
47	negative	survived '86, '91, & '94 plague	41.3	S. Dam Boulder Res.
60	negative	survived '86, '91, & '94 plague	6.2	Klein
106	negative	survived 94 plague	29.1	Ute

Table 2. Data collection timeline followed by a key to sites upon which data collection occurred during various time periods.

Activity	Early Spring	Spring	Summer	Fall
Prairie dog trapping				
Small mammal trapping				
Prairie dog visual counts				
Vegetation surveys				
Microclimate data collection				

Sites	# Plague Pos. Sites	# Plague Neg. Sites
1,3,6,11,18,19,30,47,60,106	5	5
1,3,6,8,10,11,17,18,19,20,30,47,60,106	9	5

Small Rodent Trapping

We trapped small rodents on nine plague positive colonies and five negative colonies twice in 2004, in May through June and again in August through September. 49 Sherman live traps were placed 20 m apart and arranged in a grid. This grid was placed in the same general location as the larger prairie dog grid, and the prairie dog grid encompassed between 83 – 100 % of the small mammal grid. We trapped each site for four consecutive nights. Traps were opened and baited with rolled oats in the evening, then checked and closed in the morning between 6 and 11 am.

Animal Processing

Prior to handling, we anesthetized animals with gaseous isoflurane. We then identified, sexed, weighed and measured each animal. Additionally, fleas and blood were collected from each animal. We removed all fleas with tweezers; this was facilitated by the effects of the anesthetic on the fleas. All fleas were stored frozen in 2 % saline solution until identification. Prairie dogs were permanently marked with a PIT (Passive Integrated Transponder) tag, to allow identification of recaptured individuals. A small patch of fur was shaved from the hind quarter of the small mammals; this allowed us to distinguish processed from unprocessed recaptures, but did not allow us recognize unique individuals. A blood sample was taken from each animal. For prairie dogs, approximately 0.5 – 0.7 ml of blood was collected from the femoral vein using 1 ml

heparinized syringes. For the small mammals, capillary tubes were used to collect 0.3 – 0.4 ml of blood from the retro-orbital sinus.

Sample Analysis

Fleas: All fleas were identified to the level of species using a dissecting microscope. During spring prairie dog trapping, only two dominant flea species were collected, therefore we used a chi-squared analysis to test for differences in relative abundance of the two species on plague positive and plague negative sites.

Following identification, fleas were tested for presence of *Bartonella* and *Y. pestis* at the Centers for Disease Control using a multiplex Polymerase Chain Reaction (PCR) (Stevenson et al. 2003). Prior to PCR, DNA was extracted from fleas using Qiagen DNeasy Tissue extraction. One of two methods was used to break open cells prior to extraction: fleas were either hand-ground and subjected to 20 minutes of lysozyme treatment or mechanically ground using a sand-blasted glass pestle.

To determine the effects of flea species identity and site history on *Bartonella* prevalence in prairie dog fleas collected in the spring, we used the CATMOD procedure in SAS (Statistical Analysis Software 8.02, SAS Institute Inc., Cary, NC, USA). This procedure fits a linear model to categorical data using maximum likelihood techniques.

Blood: Blood samples were tested for the presence of *Y. pestis* using antibody tests. Additionally, the samples were tested for the presence of *Bartonella* using culturing techniques and PCR.

Prairie Dog Visual Counts

In order to estimate prairie dog density on colonies, visual counts were performed concurrently with prairie dog trapping, mid-June through the beginning of August (Menkens et al. 1990, Johnson and Collinge 2004). On nine plague positive and five negative sites, three 50 by 50 m grids per colony were established. Using hand-held binoculars, the number of prairie dogs in each grid was counted for three consecutive days at twenty minute intervals from 8 – 11 am. We arrived at least 20 minutes before 8 am to allow prairie dogs to become accustomed to our presence, and remained in the same position throughout the morning. When weather conditions were rainy, we waited until conditions improved. Because maximum counts better correlate with mark-recapture data than average counts (Severson and Plumb 1998), we used the maximum average count to estimate prairie dog density. To calculate this measure, we added the

maximum numbers of animals counted in each of three grids over all three days, and divided that number by three (the number of grids). We then converted this value to number of animals per hectare (ha). These values represent a relative index of density, not an absolute value.

Vegetation Sampling.

During July 2004, vegetation cover was measured on nine plague positive site and five plague negative sites. We used point-intercept techniques to estimate cover based on physiognomic properties of the vegetation (Kent and Coker 1994). Specifically, we used a systematic subsample of 25 of the 49 trap locations on each small mammal grid. At each sample point, we placed a 1 x 1 m quadrat over the vegetation and scored 25 points for vegetation type. Physiognomic classifications of the vegetation included grasses, forbs, shrubs, and succulents, as well as bare ground and litter (Zavala-Hurtado et al. 1996).

Microclimate Data Collection.

From March through November 2004, we used data loggers to collect microclimate data (temperature and relative humidity) at four hour intervals from prairie dog burrows. We placed one data logger per colony, approximately one meter from the burrow entrance, on five plague positive and five negative colonies. We modified the loggers to withstand potential chewing by animals and fixed each one to a metal coil to allow retrieval.

Preliminary Data and Analysis:

In the following section, I will present data collected thus far, and summarize what remains to be done. Most of the data are in summary form and have not yet been analyzed. All subsequent data analysis will be completed by May 2005.

Prairie Dog Trapping:

Table 3. Summary of the number of unique prairie dogs captured, recaptured animals, and animals released unprocessed and unmarked during the spring.

Site	Unique Individuals	Recaptured	Released Unprocessed
1	24	3	0
3	30	2	0
6	24	6	0
11	8	2	0
19	27	6	6
18	11	2	0
30	3	0	0
47	8	0	0
60	8	5	0
106	23	4	1

Prairie dog trapping data from summer has not yet been summarized.

Small Mammal Trapping

Table 4. Total captures over four nights of all small mammals trapped in the spring. Species captured include: *Chaetodipus hispidus* (CHHI), *Microtus ochrogaster* (MIOC), *M. pennsylvanicus* (MIPE), *Peromyscus maniculatus* (PEMA), *Reithrodontomys megalotis* (REMA), and *Spermophilus tridecemlineatus* (SPTR). Species richness is also calculated for each site.

Site	CHHI	MIOC	MIPE	PEMA	REMA	SPTR	Species Richness
1	4	0	0	54	0	0	2
3	0	0	0	83	0	0	1
6	0	0	0	69	0	0	1
8	2	0	0	30	0	2	3
10	6	0	0	133	0	0	2
11	2	0	0	55	1	0	3
17	0	0	0	90	0	0	1
19	1	1	0	107	0	0	3
20	0	0	0	71	1	0	2
18	0	0	0	42	0	0	1
30	0	0	0	50	0	0	1
47	0	0	0	139	0	0	1
60	0	0	5	75	0	0	2
106	0	0	0	56	0	0	1

Small mammal trapping data from fall has not yet been summarized.

Flea Samples

Flea Identification:

Thus far, all fleas collected from prairie dogs in the spring have been identified. Fleas collected during spring and fall small mammal trapping and summer prairie dog trapping are not yet identified. 1722 prairie dog fleas were collected in the spring. Two dominant flea species were identified: 733 fleas were *Oropsylla hirsuta*, and 983 fleas were *Oropsylla tuberculata cynomuris*. Four of the fleas belong to the genus *Pulex*. Two of the fleas were crushed beyond our ability to identify them. *O. hirsuta* was significantly more abundant relative to *O. t. cynomuris* on historically plague positive sites ($\chi^2 = 400.9$, n = 1716, $p < 0.0001$) (Fig. 1).

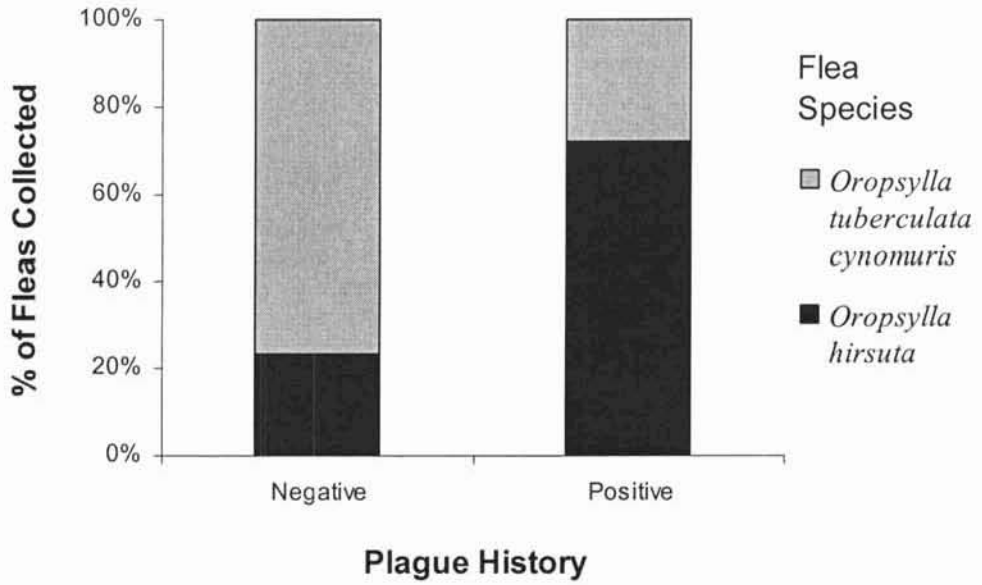

Fig. 1. Relative abundance of the two dominant flea species collected from prairie dogs in the spring from historically plague positive and negative sites.

Disease Prevalence in Fleas:

All prairie dog fleas collected in the spring were tested for *Y. pestis*, and prevalence was 0%. The remainder of the fleas are currently untested for *Y. pestis* and *Bartonella*.

Of the 1722 fleas collected in the spring from prairie dogs, 409 fleas were assayed for bartonellosis. This subset was composed of fleas collected from 2-6 animals per site from all ten sites. The number of fleas per animal ranged from 1-49 fleas. We found that *Bartonella* prevalence was significantly greater in *O. hirsuta* (24.0%) than in *O. t. cynomuris* (1.3%) (χ^2 = 16.0, n = 409, $p<0.0001$), but did not differ significantly between historically plague positive and negative sites ($p>0.05$) (Fig. 2). There was no significant interaction between site history and flea species identity ($p>0.05$).

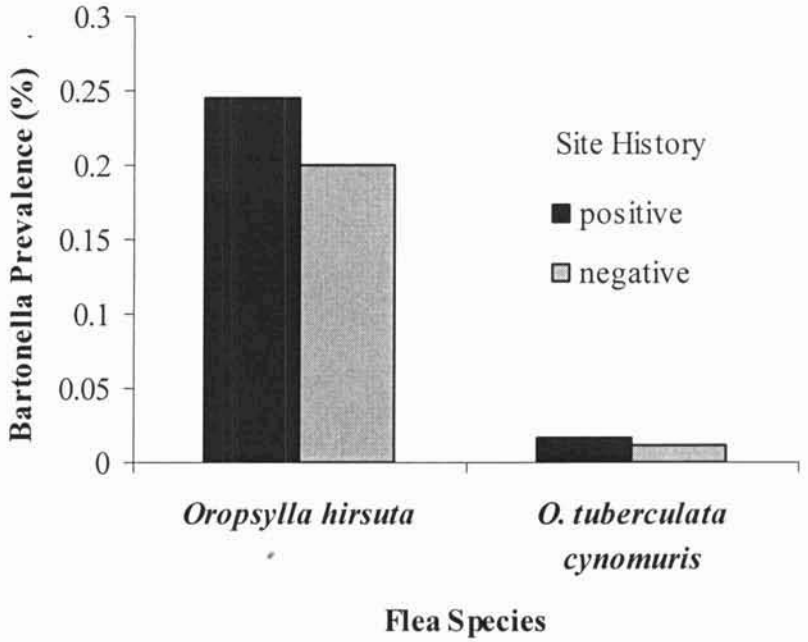

Figure 2. Bartonella prevalence in a subset of fleas collected from prairie dogs in the spring.

Blood Samples

We tested 148 blood samples collected from prairie dogs in the spring for *Y. pestis*; prevalence was 0%. We tested 126 prairie dog blood samples collected in the spring for the presence of *Bartonella* using culturing techniques. Three animals tested positive for *Bartonella*; these animals were from sites 6, 11, and 60. The prevalence of *Bartonella* in all blood samples collected was 2.4%. All of these samples will be retested for the presence of *Bartonella* using PCR based techniques. Blood samples from spring and fall small mammal trapping, and summer prairie dog trapping will be tested for *Bartonella* and *Y. pestis* using only PCR, but are not yet tested.

Visual Counts

Table 5. Number of active burrow entrances per hectare (ha) for each site. Number of prairie dogs per ha estimated using visual counts.

Site	Burrow Density (burrows/ha)	Prairie Dog Density (animals/ha)
1	346.7	90.7
3	86.7	58.7
6	153.3	26.7
8	193.3	61.3
10	93.3	49.2
11	193.3	33.3
17	246.7	57.2
19	206.7	45.3
20	146.7	53.2
18	233.3	24.0
30	193.3	38.7
47	280.0	44.0
60	326.7	65.2
106	293.3	29.3

Vegetation

Table 6. Summary of count data from all vegetation quadrats conducted on each site. I will use ordination methods to look for differences in vegetation community composition between plague positive and negative sites.

Site	Grasses	Forbs	Shrubs	Cacti	Yucca	Litter	Bare
1	142	157	0	0	0	145	181
3	238	181	1	15	0	110	80
6	294	137	0	0	0	93	101
8	3	361	0	0	0	105	156
10	6	421	0	0	11	34	153
11	100	351	0	0	0	92	82
17	210	263	0	0	0	52	100
19	204	235	0	0	0	98	88
20	67	317	0	0	0	106	135
18	1	313	0	0	14	142	155
30	92	389	0	0	0	98	46
47	8	340	0	0	0	164	113
60	168	326	0	0	0	79	52
106	37	358	0	0	0	71	159

Microclimate

Data loggers have been removed from all burrows, and the data are currently being analyzed.

Summary

Preliminary results indicate black-tailed prairie dogs had two dominant flea species in spring 2004, and the relative abundance of these two species differed between historically plague positive and negative prairie dog colonies. Additionally, bartonellosis prevalence was greater in the flea species of relatively greater abundance on historically plague positive sites. Plague prevalence in prairie dogs and their fleas was 0% on all sites in spring 2004. Additional data on microclimate, vegetation cover, and are currently being analyzed. Fleas from the remainder of

the trapping season are being identified, and tested for disease along with the remainder of the blood samples. Analysis of all results will be complete by May 2005.

Citations

Biggins, D. E., and M. Y. Kosoy. 2001. Influences of introduced plague on North American mammals: Implications from ecology of plague in Asia. Journal of Mammalogy **82**:906-916.

Collinge, S. K., W. C. Johnson, C. Ray, R. Matchett, J. Grensten, J. F. J. Cully, K. L. Gage, M. Y. Kosoy, J. E. Loye, and A. P. Martin. in review. Landscape structure and plague occurrence in black-tailed prairie dogs. Ecological Applications.

Cully, J. F., and E. S. Williams. 2001. Interspecific comparisons of sylvatic plague in prairie dogs. Journal of Mammalogy **82**:894-905.

Gage, K. L. 1999. Plague Surveillance. Pages 135-165 *in* Plague Manual: Epidemiology, Distribution, Surveillance and Control. WHO/CDS/ECD/EDC.

Hoogland, J. L. 1995. The black-tailed prairie dog : social life of a burrowing mammal. University of Chicago Press, Chicago.

Johnson, W. C., and S. K. Collinge. 2004. Landscape effects on black-tailed prairie dog colonies. Biological Conservation **115**:487-497.

Kent, M., and P. Coker. 1994. Vegetation description and analysis: a practical approach. Wiley, Chichester; New York.

Menkens, G. E., D. E. Biggins, and S. H. Anderson. 1990. Visual Counts as an Index of White-Tailed Prairie Dog Density. Wildlife Society Bulletin **18**:290-296.

Miller, B., G. Ceballos, and R. Reading. 1994. The Prairie Dog and Biotic Diversity. Conservation Biology **8**:677-681.

Severson, K. E., and G. E. Plumb. 1998. Comparison of methods to estimate population densities of black-tailed prairie dogs. Wildlife Society Bulletin **26**:859-866.

Stevenson, H. L., Y. Bai, M. Y. Kosoy, J. A. Montenieri, J. L. Lowell, M. C. Chu, and K. L. Gage. 2003. Detection of novel Bartonella strains and Yersinia pestis in prairie dogs and their fleas (Siphonaptera : ceratophyllidae and pulicidae) using multiplex polymerase chain reaction. Journal of Medical Entomology **40**:329-337.

Zavala-Hurtado, J. A., P. L. Valverde, A. Diaz-Solis, F. Vite, and E. Portilla. 1996. Vegetation-environment relationships based on a life-forms classification in a semiarid region of tropical Mexico. Revista De Biologia Tropical **44**:581-590.