Plague Prevalence in Lower Brule Indian Reservation Black-Tailed Prairie Dog Colonies

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INTRODUCTION

South Dakota has been very successful in reintroducing and maintaining black-footed ferret (*Mustela nigripes*) populations. This success is important to the very survival of the black-footed ferret and is due in part to the lack of plague in the state until recently. Given that the ferret depends exclusively on black-tailed prairie dog (*Cynomys ludovicianus*) colonies in South Dakota, it is important that these colonies be protected against plague epizootics to the extent possible. By testing for *Yersinia pestis* (the causative agent of plague) on the Lower Brule Indian Reservation, in conjunction with testing in other areas of the state (e.g., Badlands and Wind Cave National Parks), we can provide managers with a tool to assess current plague prevalence on the study colonies and potentially predict epizootics before they occur. It should also be noted that plague can present a significant risk to the public. Early detection of even low plague prevalence allows appropriate actions such as restricting access to affected colonies and dusting with deltamethrin.

This project expanded the geographic scope of an ongoing study funded by the National Park Service in Wind Cave and Badlands National Parks on to the Lower Brule Indian Reservation which lies near the eastern edge of the distribution of the black-tailed prairie dog in South Dakota. The ultimate goal of this research is to understand how *Y. pestis* spreads across the prairie landscape. We used a sensitive nested polymerase chain reaction (PCR)-based technique for detecting plague bacteria in fleas collected from prairie dog burrows that was developed at USD (Hanson et al. 2007). This work has revealed that black-tailed prairie dog colonies in Montana and Colorado can remain apparently unaffected by plague with about 20% of the burrows yielding plague-positive fleas (Hanson et al. 2007, Jones et al. 2009). The recent occurrence of sylvatic plague in South Dakota provides a unique opportunity to apply this method to naïve prairie dog colonies and to potentially track the progress of plague over time.

We collected fleas from black-tailed prairie dog burrows on the Fort Hale Bottom prairie dog complex on the Lower Brule Indian Reservation in June and July, 2009 (Figure 1). The approximately 15 prairie dog colonies on this complex support a small population of the Federally Endangered black-footed ferret. We typed sampled fleas to species, extracted DNA from the fleas, and tested them for the presence of *Y. pestis* using our PCR-based assay. The results will inform management on the Lower Brule Reservation about the risk of plague epizootics in prairie dog colonies that support black-footed ferrets and other prairie-obligate mammals.

The project objectives were to:

1) Work with the wildlife staff at the Lower Brule Indian Reservation to sample fleas from black-tailed prairie dog burrows on colonies that support black-footed ferrets.

2) Type the sampled fleas to species in order to estimate the prevalence of prairie dog fleas (genus *Oropsylla*) in the Lower Brule flea fauna.

3) Assay the sampled fleas for the presence of *Y. pestis* using a sensitive PCR-based detection protocol (Hanson et al. 2007). Plague prevalence will be estimated from the results of the assays.

4) Make recommendations regarding the management of potential plague epizootics and future monitoring efforts based on the results.

METHODS

Fleas were sampled from black-tailed prairie dog colonies during June and July, 2009, using the burrow-swabbing method described in Ubico et al. (1988). Fleas were also collected in June 2010 as part of ongoing studies in South Dakota. Fleas were sampled directly from prairie dog burrows using 30 cm squares of flannel fabric affixed to the end of a plumbers' snake. Each flannel collecting cloth was run down the burrow being sampled until it hit bottom or was stopped at a bend or other obstruction in the burrow. The cloth was left in the burrow for 1 min, then reeled up and immediately sealed in a large ziplock bag with a unique identifying label. About 10 ml of ethanol was added to the bag to kill and preserve the fleas collected on the cloth. Each sampled burrow was given a unique number and its GPS location was recorded. Only active prairie dog burrows were sampled; active burrows were defined as those with fresh scat or a prairie dog was observed in the entrance. Presence of prairie dogs nearby was also noted.

Standard CDC safety procedures were used during flea collection. These included the following: long-sleeved, light colored shirt and latex or other protective gloves were worn; long pants and close-toed shoes/boots were recommended, and DEET – containing insect repellent was applied to sleeves, pants cuffs, boots, and gloves.

Additional fleas were collected by combing prairie dogs that were captured by Lower Brule Sioux Tribe Department of Wildlife, Fish and Recreation personnel. To incapaciate fleas and allow collection, prairie dogs received a dose of isoflurane (Isoflo, Abbott Laboratories, Chicago, IL) to induce anesthesia and breathing was monitered continuously. Once anesthetized, fleas were removed using forceps or by brushing the prairie dog with a flea comb over a white pan. Collected fleas were placed in vials filled with ethanol, labeled, and sent to Britten's lab at USD for plague assays. Swabbed and combed fleas were subject to the same analytical protocols.

Individual fleas were identified to species under a dissecting microscope following Lewis (2002) and Furman and Catts (1982). Genomic DNA from the sampled fleas was extracted using the Easy-DNA kit (Invitrogen) following a modified mouse tail extraction procedure (Protocol 8, using $1/10^{th}$ volumes of reagents; Invitrogen Life Technologies). All fleas from collected from each burrow were assayed for *Y. pestis*. A nested PCR procedure (Hanson 2005, Hanson et al. 2007) modified from Hinnebusch and Schwan's (1993) PCR-based protocol was used to assay each flea for *Y. pestis* (i.e., plague) DNA. DNA extracted from these fleas was stored at -80ºC for potential future analysis. Plague prevalence by colony was estimated as: (number of plague-positive burrows/total number of burrows from which fleas were collected)*100.

RESULTS

Nine black-tailed prairie dog colonies yielded fleas via burrow-swabbing (Table 1). Of the 250 fleas identified from these colonies for 2009 73% (N = 182) were *Oropsylla hirsuta* (Table 2). Table 2 contains the species counts by colony for all the swab-collected fleas in 2009. A total of 171 *Y. pestis* assays have been completed at this time. Table 1 provides the results from the burrow-swabbing effort and the *Y. pestis* assays. Overall, five burrows from three colonies contained *Y. pestis* – positive fleas for a total prevalence of 5% (Table 1).

Seventeen black-tailed prairie dogs from five colonies yielded a total of 153 fleas for 2009; all were identified as *O. hirsuta* (Table 3). At this time, 1 of 4 of the prairie dog fleas have tested positive for presence of *Y. pestis* on Junior's colony (Figure 1).

Fleas were collected from burrows as well as prairie dogs in 2010 as part of an ongoing study. Seven of nine colonies swabbed for fleas during June 2010 yielded fleas (Table 4). Sixty-seven fleas were recovered by swabbing in 2010, 86.6% (N = 58) of which were *O. hirsuta* (Table 4). Sixty-six of 114 prairie dogs trapped in 2010 yielded fleas, though they are awaiting identification (Table 5). At this time, no assays for presence of *Y. pestis* have been conducted from fleas collected from Lower Brule in 2010.

DISCUSSION

While sylvatic plague has been in South Dakota since 2005, our results indicating the presence of *Y. pestis*, even at low prevalence, on Lower Brule black-tailed prairie dog colonies was somewhat surprising given its location near the eastern edge of the range of the black-tailed prairie dog. Once notified of these results, Lower Brule wildlife personnel carefully monitored the *Y. pestis* – positive colonies for signs of die-off, but none have been noted to date. We detected similar prevalences on prairie dog colonies in Wind Cave National Park during the same time period as this study with no apparent effect on the prairie dog colonies there (Mize and Britten, unpubl data). In contrast, we documented the presence of *Y. pestis* in burrow-collected fleas on the Kosher Flat prairie dog colony in Badlands National Park in 2009 (Mize and Britten, unpubl data). The Kosher Flat colony and others near it were decimated by a plague epizootic beginning in 2009.

Y. pestis is extremely virulent in black-tailed prairie dogs, but evidence is mounting that suggests that the pathogen can exist in prairie dog colonies in an apparent enzootic state (Hanson et al. 2007, Biggins et al. 2010, and Griffin et al. 2010). In other words, the pathogen may be present at low prevalence in prairie dog colonies without triggering an epizootic. While the mechanisms that trigger epizootics are little understood, they probably involve a combination of ecological factors that impinge on the fleas and/or the pathogen, as well as, the condition of the prairie dogs themselves. In addition, our nested PCR-based assay is extremely sensitive and is capable of detecting *Y. pestis* at very low prevalence prior to any indication of an epizootic (Hanson et al. 2007).

MANAGEMENT IMPLICATIONS

Information gathered from this research was directly influential in management actions taken at Lower Brule. Biologists at Lower Brule began closely monitoring the health of colonies where black-footed ferrets were known to live, looking for evidence of plague epizootics including a noticeable reduction in prairie dog densities and/or prairie dogs mortalities. In addition to increased monitoring, biologists also began dusting burrows on several colonies at Lower Brule using the insecticide deltamethrin (Delta dust) which has been shown to effectively reduce flea loads in black-tailed prairie dog colonies (Biggins et al. 2010). In an effort to protect black- footed ferrets directly, managers have also begun vaccinating ferrets.

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Table 1: Fleas collected by burrow swabbing from Lower Brule Indian Reservation from 2009. The total number of burrows surveyed for fleas by colony, number of burrows with fleas per colony, number of fleas collected from burrows per colony and the number of fleas testing positive for *Yersinia pestis* with the prevalence of *Y. pestis* by burrow.

Colony name	Total burrows sampled	No. of burrows with fleas	No. of fleas assayed	No. of *Yersinia pestis* positive fleas (*Y. pestis* prevalence)
Badgerhead	119	10	15	1 (10%)
Cattleguard	108	45	150	2 (4.4%)
Moe's	51	4	12	NA
Sheldon's East	13	1	1	NA
Sheldon's South	6	3	6	NA
Charley's North	63	12	19	0
Charley's South	45	14	19	2 (14.3%)
Junior's	79	9	13	0
Unknown	unknown	2	15	NA
Totals	**484**	**100**	**250**	**5 (5%)**

Table 2: Number of each flea species collected by burrow swabbing by colony in 2009.

Colony name	No. *Oropsylla hirsuta*	No. *Oropsylla tuberculata*	No. *Oropsylla* spp	No. *Pulex* spp	No. *Hoplopsyllus cenomalus*	Unknown
Badgerhead	12*	3	0	0	0	0
Cattleguard	100*	45*	4	0	0	1
Moe's	10	0	0	1	0	1
Sheldon's East	1	0	0	0	0	0
Sheldon's South	5	0	0	0	0	1
Charley's North	10	8	1	0	0	0
Charley's South	16*	2	0	0	1	0
Junior's	13	0	0	0	0	0
Unknown	15	0	0	0	0	0
Totals	**182**	**58**	**5**	**1**	**1**	**3**

*Species that tested positive for *Yersinia pestis* by colony.

Table 3: Fleas collected from prairie dogs at Lower Brule Indian Reservation in 2009. The number of prairie dogs with fleas by colony, number of *Oropsylla hirsuta* collected by colony and the number testing positive for *Yersinia pestis* including the total number of fleas tested to date.

Colony name	No. of prairie dogs with fleas	No. of *Oropsylla hirsuta*	No. of *Yersinia pestis* positive fleas (No. tested)
Badgerhead	2	8	NA
Cattleguard	5	20	NA
Charley's South	3	28	NA
Charley's	4	16	NA
Junior's	3	81	1 (4)
Totals	**17**	**153**	

Table 4: Fleas collected from burrow swabbing at the Lower Brule Indian Reservation in 2010. The total number of burrows surveyed for fleas by colony, number of burrows with fleas per colony, number of fleas collected from burrows per colony and the number of each flea species collected from burrow swabbing by colony.

Colony name	Total burrows sampled	No. of burrows with fleas	No. of fleas	No. *Oropsylla hirsuta*	No. *Oropsylla tuberculata*
Badgerhead	51	5	10	8	2
Cattleguard	20	10	27	27	0
Moe's	61	0	0	0	0
Sheldon's East	2	0	0	0	0
Sheldon's South	16	4	6	4	2
Charley's North	24	7	9	8	1
Charley's South	36	4	4	3	1
Charley's	40	5	7	4	3
Junior's	65	3	4	4	0
Totals	**315**	**38**	**67**	**58**	**9**

Table 5: Fleas collected from prairie dogs at Lower Brule Indian Reservation in 2010. The number of prairie dogs examined for fleas and the number of prairie dogs with fleas by colony.

Colony name	No. prairie dogs examined	No. of prairie dogs with fleas
Badgerhead	14	11
Cattleguard	6	6
Sheldon's South	10	7
Junior's	20	10
Moe's	25	12
Charlie's	18	10
Charlie's South	21	10
Totals	**114**	**66**

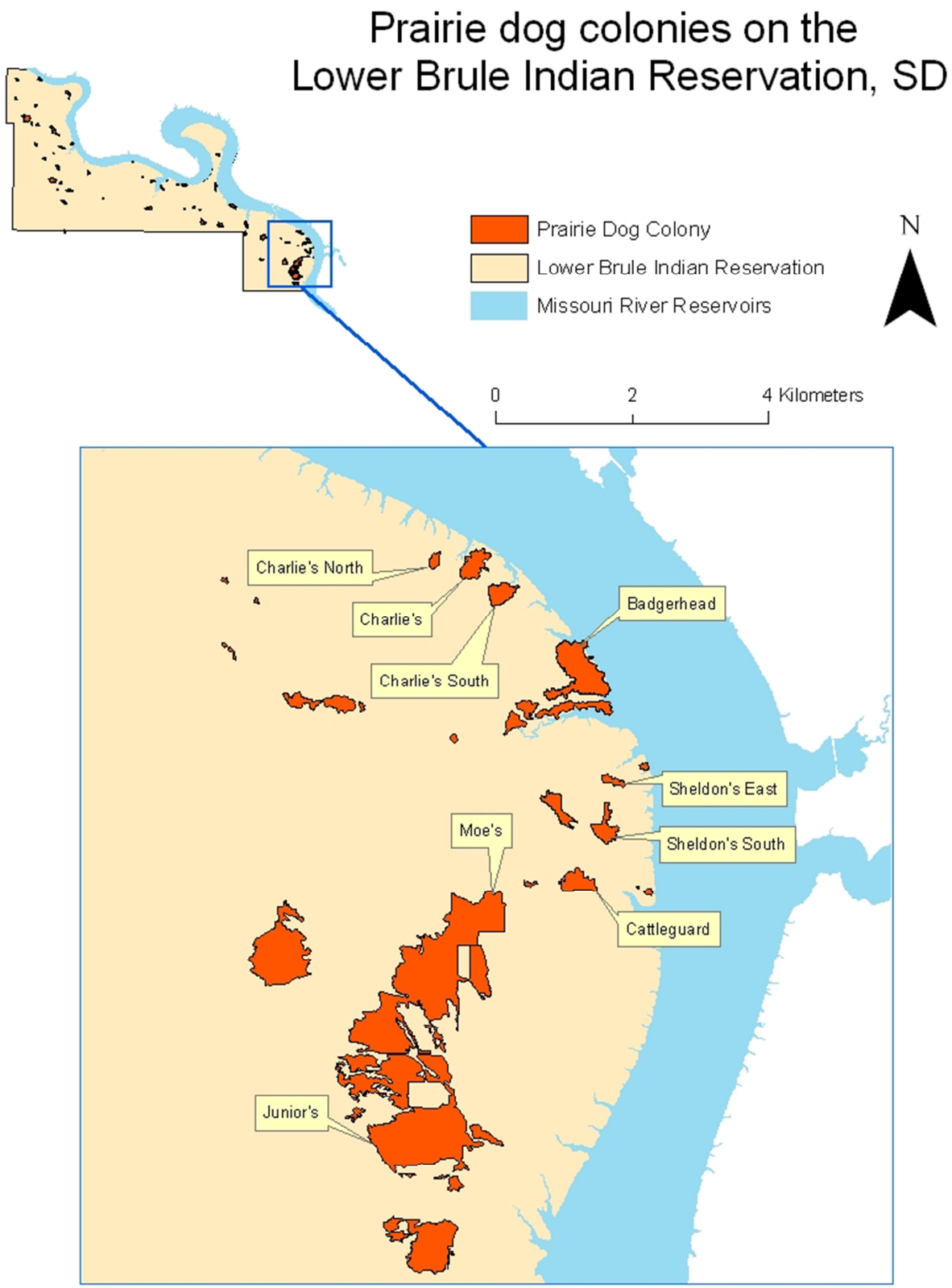

Figure 1. Locations of black-tailed prairie dog colonies sampled by burrow-swabbing on the Lower Brule Indian Reservation in summers 2009 and 2010.