# *Yersinia pestis* prevalence in fleas collected from South Dakota swift fox and black-footed ferrets

Erica L. Mize, M.S. and Hugh B. Britten, Ph.D.

Department of Biology, The University of South Dakota, Vermillion SD, 57069

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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 sylvatic 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.

The proposed project expands the scope of a study funded by the National Park Service in Wind Cave and Badlands National Parks as well as a previous study funded by South Dakota Department of Game Fish and Parks on the Lower Brule Sioux Tribe (Britten and Mize 2010). The ultimate goal of this research is to understand how *Y. pestis* spreads across the prairie landscape by examining the role of two prairie mesopredators swift fox (*Vulpes velox*) and black-footed ferrets as potential reservoir hosts and vectors of infected fleas. We identified fleas to species, extracted DNA, and tested for the presence of *Y. pestis* using a PCR-based assay. Tested fleas were collected from black-footed ferrets recovered from the Fort Hale Bottom prairie dog complex on the Lower Brule Sioux Tribe in 2009 – 2012 by Shaun Grassel (Lower Brule Sioux Tribe) and fleas collected from swift foxes surveyed in the Conata Basin area of the Buffalo Gap National Grassland in 2010 by Greg Schroeder and Josh Delger (National Park Service). In addition, we obtained fleas combed from black-footed ferrets during 2006 – 2011 in the Buffalo Gap National Grassland and 2010 in Wind Cave National Park by Travis Livieri (Prairie Wildlife Research).

The results will inform management about the role of mesopredators in plague epizootics in prairie dog colonies and provide a framework to make management decisions regarding the black-footed ferret and the swift fox which are both animals of conservation concern. Better informed management may also reduce the prevalence of this zoonotic disease in South Dakota prairie dog colonies. Furthermore, increased knowledge of how this exotic pathogen spreads improves the likelihood that black-tailed prairie dogs and their ecological associates will be sustained in South Dakota for the enjoyment of residence and visitors into the future.

The project objectives were to:

Objective 1: Determine species of fleas and their abundance on swift foxes and black-footed ferrets.

Objective 2: Determine the prevalence of *Yersinia pestis* in fleas collected from swift foxes and black-footed ferrets.

Objective 3: Determine if swift foxes and black-footed ferrets act as a reservoir for sylvatic plague in South Dakota.

Objective 4: Provide wildlife managers with data and recommendations to make decisions that benefit swift fox and black-footed ferret management objectives as well as sylvatic plague management objectives.

## METHODS

In October 2010, fleas were collected from 17 swift foxes in the Conata Basin area of the Buffalo Gap National Grassland and 31 black-footed ferrets were examined for fleas from 2009 – 2012 at the Fort Hale Bottom prairie dog complex on the Lower Brule Sioux Tribal lands. In addition, fleas sampled from black-footed ferrets by Travis Livieri from Wind Cave National Park in 2011 and in the Conata Basin from 2006 – 2011 were available for *Y. pestis* assays (note that 2006 fleas were stored in saline and DNA cannot be successfully extracted from them; the rest were stored in ethanol). These fleas were identified to species using the keys of Lewis (2002), Furman and Catts (1982) and Stark (1958). DNA was extracted from fleas using PrepGem (Zygem, New Zealand) extraction kits and tested for *Y. pestis* using a nested polymerase chain reaction (PCR) technique used by Jones et al. (2010) and Hanson et al. (2007) and modified from Hinnebusch and Schwan (1998). In total, 350 fleas were tested for the presence of *Y. pestis*; 100 fleas from swift fox captured in the Conata Basin, 250 fleas from ferrets captured at Lower Brule (93 fleas from 2011), Conata Basin (135 fleas), and Wind Cave National Park (22 fleas). This report also includes preliminary data mentioned in our proposal for 176 fleas collected from black-footed ferrets at Lower Brule in 2009.

## RESULTS

*Swift Fox* – Conata Basin

Three hundred sixty fleas were collected from 17 swift fox captured by Badlands National Park personnel in the Conata Basin in October 2010. Of the 360 fleas collected, 98% (N=353) were *Pulex irritans*. Of the 100 *Pulex irritans* fleas tested for the presence of *Y. pestis*, two fleas from two foxes (male pup and adult female) tested positive for the presence of *Y. pestis* for a total prevalence of 2% (Table 1).

*Black-footed Ferret* – Conata Basin

One hundred thirty six *Oropsylla hirsuta* fleas were examined from nine black-footed ferrets captured at least twice by Prairie Wildlife Research personnel in the Conata Basin. Of 136 fleas examined, two fleas collected from an adult female in 2007 tested positive for the presence of *Y. pestis* for a total prevalence of 1.5% (4.1% prevalence for the individual ferret) (Table 2).

*Black-footed Ferret* – Lower Brule

Two hundred sixty nine fleas were examined from 31 black-footed ferrets captured by Lower Brule personnel at Lower Brule Sioux Reservation in 2009 and 2011. Of the 269 collected, 98% (N=263) were *Oropsylla hirsuta* (Table 3). Of 176 fleas examined for the presence of *Y. pestis* in 2009, four fleas from black-footed ferrets (2 male kits and 1 adult male)

tested positive for the presence of *Y. pestis* for a prevalence of 2.3% in 2009 (Table 3). Of the 93 fleas examined for the presence of *Y. pestis* in 2011, one flea from a male kit black-footed ferret tested positive for the presence of *Y. pestis* for a prevalence of 1.1% in 2011 (Table 3). Total *Y. pestis* prevalence among fleas collected from black-footed ferrets at Lower Brule from 2009 and 2011 was 1.9% (Table 3).

*Black-footed Ferret* – Wind Cave National Park

Twenty two fleas were examined from six black-footed ferrets captured by Prairie Wildlife Research personnel in Wind Cave National Park. Of the 22 fleas collected 95% (N=21) were *Oropsylla hirsuta* (Table 4). All 22 fleas tested negative for the presence of *Y. pestis* (Table 4).

## DISCUSSION

*Swift Fox*

The few studies that have examined the role of swift fox in the plague system in the United States present mixed results (McGee et al. 2006, Salkeld et al. 2007). McGee (2006) found all six foxes examined tested seropositive for plague antibodies indicating exposure to the pathogen, yet none of the fleas sampled from the foxes were positive for plague using a mouse inoculation test. Salkeld et al. (2007) found 24% of swift foxes examined were seropositive though none of the fleas tested positive for the presence of *Y. pestis* using a single-reaction PCR method similar to Hinnebusch (1998). While these results are enigmatic, our nested PCR method is not only a very specific, but a highly sensitive method of detecting the presence of *Y. pestis*, far more so than Hinnebusch's (1998) single-reaction assay (Hanson et al. 2007). Single-reaction PCR assays for plague like Hinnebusch (1998) have been shown to be more sensitive than mouse inoculation (Engelthaler et al. 1999). This may explain why previous studies were unable to detect *Y. pestis* in fleas even though the hosts were seropositive for exposure to *Y. pestis*.

*Pulex irritans* was the most common flea recovered from swift foxes in the Conata Basin. This flea is commonly referred to as the "human flea" and is often found on swift fox and a wide range of hosts including other predators such as badgers (*Taxidea taxus*) and coyotes (*Canis latrans*)(Table 1, Stark 1958). Hubbard (1947) notes prairie dogs, including black-tailed prairie dogs, are often parasitized by two fleas, *O. hirsuta* and *P. irritans* across their range. While we did not recover *O. hirsuta* from swift fox living on black-tailed prairie dog colonies in the Conata Basin, it is possible that swift fox are a potential source of *P. irritans* for prairie dog colonies. This study, in conjunction with the work of McGee et al. (2006) and Salkeld et al. (2007), suggests that swift fox may serve as a reservoir host for *Y. pestis*.

*Black-footed Ferret*

To the best of our knowledge, this is the first study to examine plague prevalence in fleas collected from black-footed ferrets. This may be in part due to the fact that ferrets are highly susceptible to plague and suffer high mortality within a few days of exposure to *Y. pestis* (Godbey et al. 2006, Matchett et al. 2010). However, implementation of a vaccination program to protect ferrets from plague has increased their survival when plague exists in an enzootic state (no noticeable die-offs of prairie dogs) (Matchett et al. 2010). This protection against contracting plague may alter the role ferrets play in the plague system; changing them from incidental hosts to a potential transporter of *Y. pestis*-infected fleas within and between prairie

dog colonies. In addition, the primary flea species collected from ferrets was *O. hirsuta*. Prairie dogs are considered a "true host" for this flea (Hubbard 1947) and it is almost exclusively found parasitizing prairie dogs (Brinkerhoff 2008 and Brinkerhoff et al. 2011). Indeed, *O. hirsuta* is a major component of the flea fauna collected from prairie dogs and their burrows on Lower Brule (Britten and Mize 2010). Little is known about the parasite species of black-footed ferrets since their population recovery; it is unclear if there were any parasites specific to ferrets before their population decline in the wild (Gompper and Williams, 1998). Holland (1985) lists *Rhadinopsylla fraterna* as a flea parasite of black-footed ferrets from a single record by Jordan in 1937 from Montana; otherwise this flea was recorded from a suite of rodent hosts as well as long-tailed weasels (*Mustela fraternal*). Trapping of ferrets in Philip's County, Montana, from 1996 – 2002 yielded *O. hirsuta*, *O. tuberculata*, and *Pulex* spp (R. Matchett personal communication).

*Comparison to burrow swabbing*

While this study did not examine individual hosts for exposure to the plague pathogen *Y. pestis*, it does provide evidence that both swift fox and black footed ferrets are capable of carrying *Y. pestis*-positive fleas. The flea species collected from ferrets strongly resembles the flea species found on black-tailed prairie dogs and in their burrows in South Dakota (Britten and Mize 2010, Mize and Britten unpublished data). Whereas flea species commonly found on swift fox were not found on black-tailed prairie dogs or in their burrows in South Dakota. However, other studies have documented collections of *P. irritans* from prairie dogs in other areas.

Prevalence of *Y. pestis*-positive fleas collected from swift fox (2%) was similar to fleas collected from burrow swabs at Buffalo Gap National Grasslands (BUGA) in 2009 (2.2%). Fleas collected from BUGA in 2010 were pooled by burrow and *Y. pestis* prevalence in burrows was 24% (number of burrows with *Y. pestis-positive* fleas/total number of burrows examined). Average prevalence of *Y. pestis* from fleas collected from swift fox pooled by fox is 11.7% (number of foxes with *Y. pestis*-positive fleas/total number of foxes with fleas examined). Ferrets at Lower Brule showed *Y. pestis* prevalences of 2.3% and 1.1% in their fleas. *Y. pestis* prevalences in fleas collected from burrow swabbing at Lower Brule was also similar to that of ferret-collected fleas (3% in 2009 and 2010). Previous studies have shown collecting fleas directly from hosts is a more sensitive method for parasite collection and surveillance of vector-borne pathogens than direct collection from the environment (Hamer et al. 2010, Mize et al. 2011).

## MANAGEMENT IMPLICATIONS

Successful reintroduction and establishment of self-sustaining populations of the black-footed ferrets is not possible without consideration of the effects of sylvatic plague on ferrets and prairie dogs, their primary food source. Examination of fleas from ferrets at Lower Brule and the Conata Basin yielded *Y. pestis*-positive fleas before plague outbreaks occurred on prairie dog colonies at both locations. Ferret populations are actively monitored through spotlighting and routine trapping, we recommend that fleas should be collected from ferrets during these monitoring activities and tested for the presence of *Y. pestis* as a monitoring tool for the presence of plague on active prairie dog colonies. If positive fleas are identified, this should trigger management activities such as dusting prairie dog burrows with Deltamethrin to kill fleas on colonies. Using ferrets as early sentinels for the presence of plague will help to target dusting

efforts to the preservation of ferrets on the landscape. In addition, vaccinated ferrets may serve as transporters of infected fleas within and between colonies. A greater understanding of the spread of plague across the landscape will better inform managers of potential risks and aid in enacting proactive versus reactive management strategies.

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Table 1: Flea species abundances and plague prevalence in fleas collected from swift fox captured in the Conata Basin in October 2010.

| Animal | Age | Sex | No. Fleas | No. *Pulex irritans* | No. *Pulex* spp | Other | No. tested | No. + *Y. pestis* | Prevalence |
|---|---|---|---|---|---|---|---|---|---|
| 1017 | Pup | Male | 14 | 13 | 1 | 0 | 12 | 0 | 0.0% |
| 967 | Adult | Male | 7 | 6 | 0 | 1 unknown | 5 | 0 | 0.0% |
| 972 | Adult | Male | 5 | 5 | 0 | 0 | 5 | 0 | 0.0% |
| 1028 | Pup | Female | 5 | 5 | 0 | 0 | 5 | 0 | 0.0% |
| 1030 | Pup | Male | 11 | 10 | 0 | 1 lost | 6 | 1 | 16.7% |
| 828 | Adult | Male | 16 | 16 | 0 | 0 | 5 | 0 | 0.0% |
| 920 | Adult | Female | 25 | 25 | 0 | 0 | 6 | 1 | 16.7% |
| 970 | Adult | Female | 24 | 24 | 0 | 0 | 6 | 0 | 0.0% |
| 1022 | Pup | Female | 58 | 56 | 1 | 1 *Aetheca wagneri* | 6 | 0 | 0.0% |
| 927 | Adult | Male | 16 | 16 | 0 | 0 | 5 | 0 | 0.0% |
| 1024 | Pup | Female | 44 | 44 | 0 | 0 | 6 | 0 | 0.0% |
| 1026 | Pup | Male | 17 | 17 | 0 | 0 | 7 | 0 | 0.0% |
| 974 | Adult | Male | 15 | 15 | 0 | 0 | 6 | 0 | 0.0% |
| 726 | Adult | Male | 38 | 37 | 0 | 1 *Epitedia* spp | 6 | 0 | 0.0% |
| 861 | Adult | Male | 12 | 12 | 0 | 0 | 6 | 0 | 0.0% |
| 976 | Adult | Male | 37 | 37 | 0 | 0 | 4 | 0 | 0.0% |
| 978 | Adult | Male | 13 | 13 | 0 | 0 | 4 | 0 | 0.0% |
| **Total** | 17 Foxes | | 357 | 351 | 2 | 4 | 100 | 2 | 2.0% |

Table 2: Abundance and plague prevalence of *Oropsylla hirsuta* fleas collected from black-footed ferrets with multiple captures in the Conata Basin between 2006 and 2010. Year references the year of capture when fleas were collected. For number of captures "C" means fleas were collected during the first capture and "R1" means fleas were collected during the first recapture.

| Year | Animal | No. captures | Age | Sex | No. Fleas | No. *Oropsylla hirsuta* | No. tested | No. + *Y. pestis* | Prevalence |
|---|---|---|---|---|---|---|---|---|---|
| 2007 | 05-161 | 2 (R1) | Adult | Male | 6 | 6 | 6 | 0 | 0.0% |
| 2007 | 06-021 | 2 (R1) | Adult | Female | 33 | 33 | 33 | 0 | 0.0% |
| 2007 | 06-042 | 2 (R1) | Adult | Female | 49 | 49 | 49 | 2 | 4.1% |
| 2007 | 07-142 | 2 (C) | Kit | Male | 1 | 1 | 1 | 0 | 0.0% |
| 2007 | 06-090 | 3 (R1) | Adult | Female | 7 | 7 | 7 | 0 | 0.0% |
| 2007 | 05-046 | 3 (R1) | Adult | Female | 5 | 5 | 5 | 0 | 0.0% |
| 2007 | 05-110 | 3 (R1) | Adult | Male | 12 | 12 | 12 | 0 | 0.0% |
| 2007 | 07-069 | 2 (C) | Kit | Male | 22 | 22 | 22 | 0 | 0.0% |
| 2009 | 06-093 | 2 (R1) | Adult | Female | 1 | 1 | 1 | 0 | 0.0% |
| **Total** | 9 Ferrets | | | | 136 | 136 | 136 | 2 | 1.5% |

Table 3: Flea species abundances and plague prevalence in fleas collected from black-footed ferrets captured at Lower Brule Sioux Reservation in 2009 and 2011.

| Year | Animal | Age | Sex | No. Fleas | No. *Oropsylla hirsuta* | No. *Oropsylla* spp | Other | No. tested | No. + *Y. pestis* | Prevalence |
|---|---|---|---|---|---|---|---|---|---|---|
| 2009 | 104-294-820 | Kit | Male | 12 | 12 | 0 | 0 | 12 | 1 | 8.3% |
| 2009 | 104-335-632 | Kit | Female | 1 | 1 | 0 | 0 | 1 | 0 | 0.0% |
| 2009 | 104-262-512 | Adult | Female | 18 | 18 | 0 | 0 | 18 | 0 | 0.0% |
| 2009 | 104-123-881 | Adult | Female | 4 | 3 | 0 | 1 *Epitedia wenmanni* | 4 | 0 | 0.0% |
| 2009 | 104-124-637 | Adult | Male | 32 | 31 | 0 | 1 F: Ceratophyllidae | 32 | 0 | 0.0% |
| 2009 | 104-292-012 | Kit | Male | 3 | 3 | 0 | 0 | 3 | 0 | 0.0% |
| 2009 | 104-111-080 | Kit | Male | 2 | 1 | 0 | 1 *Peromyscopsylla selenis* | 2 | 0 | 0.0% |
| 2009 | 104-295-874 | Kit | Male | 6 | 6 | 0 | 0 | 6 | 0 | 0.0% |
| 2009 | 104-299-060 | Kit | Female | 1 | 1 | 0 | 0 | 1 | 0 | 0.0% |
| 2009 | 104-277-818 | Kit | Female | 2 | 2 | 0 | 0 | 2 | 0 | 0.0% |
| 2009 | 104-345-846 | Kit | Male | 19 | 17 | 0 | 1 F: Ceratophyllidae, 1 unknown | 19 | 2 | 10.5% |
| 2009 | 104-256-336 | Adult | Male | 33 | 32 | 1 | 0 | 33 | 1 | 3.0% |
| 2009 | 104-298-558 | Kit | Female | 10 | 10 | 0 | 0 | 10 | 0 | 0.0% |
| 2009 | 104-122-582 | Adult | Female | 2 | 2 | 0 | 0 | 2 | 0 | 0.0% |
| 2009 | 104-285-627 | Kit | Male | 22 | 22 | 0 | 0 | 22 | 0 | 0.0% |
| 2009 | 104-273-052 | Kit | Female | 9 | 9 | 0 | 0 | 9 | 0 | 0.0% |
| **Total 2009** | 16 Ferrets | | | 176 | 170 | 1 | 5 | 176 | 4 | 2.3% |
| 2011 | 045 053 075 | Kit | Female | 6 | 6 | 0 | 0 | 6 | 0 | 0.0% |
| 2011 | 045 054 791 | Adult | Female | 9 | 9 | 0 | 0 | 9 | 0 | 0.0% |
| 2011 | 045 055 598 | Kit | Female | 14 | 14 | 0 | 0 | 14 | 0 | 0.0% |
| 2011 | 045 084 821 | Adult | Male | 2 | 2 | 0 | 0 | 2 | 0 | 0.0% |
| 2011 | 045 095 801 | Kit | Female | 12 | 12 | 0 | 0 | 12 | 0 | 0.0% |
| 2011 | 045 094 333 | Kit | Male | 50 | 50 | 0 | 0 | 50 | 1 | 2.0% |
| **Total 2011** | 6 Ferrets | | | 93 | 93 | 0 | 0 | 93 | 1 | 1.1% |
| **Total** | **31 Ferrets** | | | **269** | **263** | **1** | **5** | **269** | **5** | **1.9%** |

Table 4: Flea species abundances and plague prevalence in fleas collected from black-footed ferrets captured in Wind Cave National Park in 2011.

| Animal | Age | Sex | No. Fleas | No. *Oropsylla hirsuta* | No. *Oropsylla* spp | Other | No. tested | No. + *Y. pestis* | Prevalence |
|---|---|---|---|---|---|---|---|---|---|
| 09-011 | Adult | Female | 14 | 14 | 0 | 0 | 14 | 0 | 0.0% |
| 09-010 | Adult | Female | 2 | 2 | 0 | 0 | 2 | 0 | 0.0% |
| 09-009 | Adult | Male | 1 | 1 | 0 | 0 | 1 | 0 | 0.0% |
| 10-009 | Adult | Female | 3 | 2 | 1 | 0 | 3 | 0 | 0.0% |
| 09-007 | Adult | Female | 1 | 1 | 0 | 0 | 1 | 0 | 0.0% |
| 10-010 | Adult | Male | 1 | 1 | 0 | 0 | 1 | 0 | 0.0% |
| **Total** | 6 Ferrets | | 22 | 21 | 1 | 0 | 22 | 0 | 0.0% |