

Exposure of Captive Black-footed Ferrets to Plague and Implications for Species Recovery

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Abstract

Plague, a disease caused by the bacterium *Yersinia pestis*, was introduced into North America ca. 1900 and is now common within the ranges of three species of prairie dogs (*Cynomys* spp.) that collectively composed the former range of the highly endangered black-footed ferret (*Mustela nigripes*). An experimental population of black-footed ferrets living in quasi-natural outdoor pens suffered 90 percent mortality after they ate prairie dogs infected with *Y. pestis*. Lethal and sublethal exposure of Siberian polecats (*Mustela eversmannii*) subsequently released into those pens suggested that live *Y. pestis* can be maintained in animal tissues within burrow systems for at least 2 months. A combination of low levels of prairie dog mortality and persistence of *Y. pestis* in dead hosts may pose a chronic hazard for free-ranging black-footed ferrets in areas where plague is enzootic.

Keywords: black-footed ferret, disease, introduced disease, invasive species, *Mustela eversmannii*, *Mustela nigripes*, plague, Siberian polecat, *Yersinia pestis*.

Background

Plague was once believed to be millions of years old, but recent genetic evidence suggests that the causative bacterium, *Yersinia pestis*, may have evolved from *Y. pseudotuberculosis* only 1,500–20,000 years ago (Achtman and others, 1999). The disease has caused devastating epidemics in humans. Plague-like symptoms were recorded in human populations of Asia and Africa as early as 541 A.D. Most scientists believe that plague was introduced into North America from Asia in the late 19th century via rats (*Rattus* spp.) transported by ships

(Biggins and Kosoy, 2001). There is now evidence of plague infection in wild mammals or fleas (Insecta: Siphonaptera) from 17 western States in the United States (Gage and Kosoy, this volume).

There are multiple transmission modes for plague, including vector transport (flea bites), aerosol, and consumption of contaminated food items (Gage and Kosoy, this volume). Early cases of plague were linked with rodent infestations and assumed to be from rodent bites, but it was soon recognized that fleas could spread the disease among hosts (Gage, 1998). Aerosol transmission involves expulsion of contaminated droplets of fluid from the lungs of infected animals as they cough; the droplets containing *Y. pestis* may be ingested or inhaled by another potential host. Transmission has also been documented through consumption of infected animals (Gage and Kosoy, this volume). Although some carnivores become infected and do not survive, other species seem quite resistant (Barnes, 1982; Gage and others, 1995).

Plague is common within the ranges of three species of prairie dogs (*Cynomys* spp.) that collectively composed the former range of the highly endangered black-footed ferret (*Mustela nigripes*). The black-footed ferret is extremely dependent on prairie dogs and their colonies (Biggins and Godbey, 2003). Plague causes periodic and sometimes dramatic die-off of prairie dogs, indirectly affecting ferret survival through reduction of prey biomass (Oldemeyer and others, 1993). In 1985, discovery of plague in the white-tailed prairie dogs (*C. leucurus*) supporting the last known population of wild ferrets in Meeteetse, Wyo. (Ubico and others, 1988), caused great concern about the future of ferret habitat. White-tailed prairie dogs were found to be highly susceptible to the disease, but susceptibility of the black-footed ferret was unknown (Williams, 1986). The fears of habitat loss and an unstable prey base proved well founded. A 10-year decline in prairie dogs at Meeteetse left only a remnant population. The initial steep decline of prairie dogs at Meeteetse (fig. 1) was accompanied by a decline in ferrets, which may have been exacerbated by a second disease, canine distemper (Forrest and others, 1988). The dramatic ferret population decline

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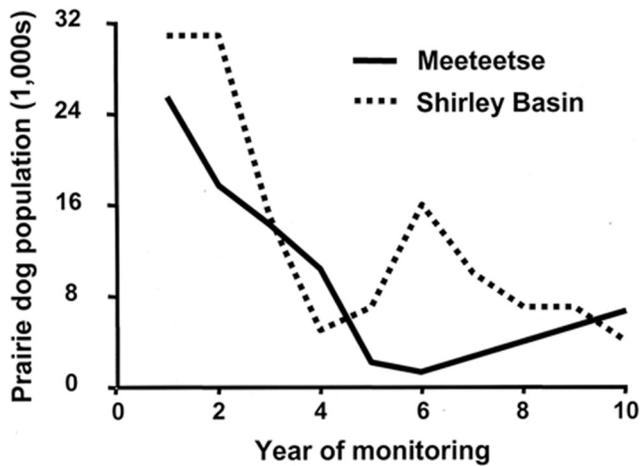


Figure 1. Changes in Wyoming white-tailed prairie dog (*Cynomys leucurus*) populations in areas with known plague. (Adapted from Biggins and Kosoy, 2001. Reprinted with permission of the *Journal of the Idaho Academy of Science*, Pocatello, Idaho.)

prompted the capture of remaining ferrets for captive breeding (Biggins and others, 1997).

The captive breeding program to produce animals for reintroduction into native habitat (i.e., complexes of prairie dog colonies) was ultimately successful (Biggins and Godbey, 2003). Reintroductions of ferrets were begun in 1991 into Wyoming white-tailed prairie dog colonies at Shirley Basin where plague was known to be established. The Shirley Basin population of prairie dogs also declined (fig. 1), but more recently the population has shown some signs of recovery. In 1994, releases of ferrets began in a Montana black-tailed prairie dog (*C. ludovicianus*) complex also known to have plague. Plague has been documented at most reintroduction or potential reintroduction sites, with the exception of those in South Dakota, throughout the ferret range.

Plague was not believed to be a direct hazard to ferrets at the time of the first reintroductions. Williams and others (1991) initially reported that domestic ferrets (*Mustela putorius furo*) and Siberian polecats (*Mustela eversmannii*) were resistant to plague and suggested that “concern about black-footed ferret mortality directly due to *Y. pestis* infection is probably not warranted.” It was therefore surprising to hear of the death of a black-footed ferret due to plague infection (Williams and others, 1994). Williams’s further work with black-footed ferret × Siberian polecat hybrids provided additional evidence on the direct hazard of plague. Nine of 12 hybrids tested became infected and died from ingestion of plague-killed mice; the three survivors failed to show an antibody response (E. Williams, oral commun., 1996). A subsequent trial resulted in 100 percent mortality of four black-footed ferrets exposed to about 800 organisms (equivalent to one flea bite dose) of *Y. pestis* by subcutaneous injection (E. Williams, oral commun., 1999).

Plague Exposure of Captive Black-footed Ferrets at Pueblo

On November 19, 1995, an experimental colony of black-footed ferrets was inadvertently exposed to plague at a research facility housed at the U.S. Army’s Pueblo Chemical Depot, Pueblo, Colo. The facility consisted of modified buildings and enclosures that provided quasi-natural environments for rearing and conditioning black-footed ferrets prior to release. Indoor cages and outdoor pens of various sizes were also used. Outdoor pens consisted of earth-filled structures (fig. 2) with combinations of natural burrows dug by prairie dogs, seminatural burrows constructed of 10.2-cm corrugated plastic drain pipe buried to a depth of about 1 m, and nest boxes. Studies on ferret behaviors were being conducted by using Siberian polecats, black-footed ferrets, and domestic ferrets reared in various environments. There were 64 resident black-footed ferrets in three categories at the time of the exposure. Twenty-three ferrets were assigned to the behavioral studies. Twenty-six ferrets had just been received and were being conditioned as experimental groups for release in Arizona and Montana. Fifteen ferrets 4–7 years old were being held awaiting transfer to zoos as display animals. Most of the black-footed ferrets were provided a diet of prairie dog portions on alternating days; Siberian polecats and domestic ferrets were fed commercial mink chow. The prairie dogs were live-trapped from various sources, quarantined for 10 days, sacrificed, and then frozen until used.

Thirty ferrets were fed on November 19, 1995. The food included portions from five quarantined black-tailed prairie dogs originating in Montana that were removed from one freezer and two nonquarantined Gunnison’s prairie dogs (*C. gunnisoni*) captured from a site near Cortez, Colo., in August



Figure 2. Black-footed ferrets (*Mustela nigripes*) occupied complex burrow systems dug by prairie dogs (*Cynomys* spp.), making them difficult or impossible to locate during and after the outbreak of plague.

1994 and stored in a second freezer. Only the Montana prairie dogs were to be fed, but new animal care personnel were unaware of the distinction. All seven prairie dogs were cut into large pieces on a common cutting board and placed into a bowl for transport to the pens.

Two days after feeding (November 21, 1995), the crew discovered the first obviously ill black-footed ferret in an outdoor pen. The ferret died soon after it was captured. Food-borne disease or poisoning was immediately suspected, so the remaining food was removed, the facilities were quarantined for 10 days, and vitamin K was administered to counteract possible rodenticide poisoning. Ten uneaten or partially eaten pieces of prairie dog were found. Black-tailed and Gunnison's prairie dog parts could not be distinguished because the skin had been removed. The recovered food and the bowl were sent to the Centers for Disease Control (CDC) in Fort Collins, Colo., for testing. Several whole prairie dogs from the Montana shipment and two Gunnison's prairie dogs remaining in the second freezer were also sent to the CDC. Repeated searches of the pens over the next 2 days disclosed other sick and dead ferrets. Clinical signs included lethargy and bloody stools. Of the 30 animals possibly exposed, 19 died and 8 were missing and presumed dead in underground burrows. Black-footed ferret remains were sent to Colorado State University for necropsy, and tissue samples were forwarded to the CDC for plague testing. The three surviving animals were quarantined, and blood was drawn and sent to the CDC.

Three of the 10 recovered prairie dog pieces, the two remaining Gunnison's prairie dogs, and a swab taken from the inner surface of the transport bowl tested positive for plague. There was no evidence of plague in the tested Montana black-tailed prairie dogs. All dead ferrets were positive for plague in one or more tissue samples. Internal organs showed various stages of infection, but all included intestinal hemorrhaging and congested lungs. Clinical signs were consistent with advanced stages of plague.

Labels on recovered freezer bags indicated that the two Gunnison's prairie dogs fed to the ferrets had died during capture or shipment. During 1994 and 1995, former technicians working at the Pueblo facility received several shipments of Gunnison's prairie dogs from Mr. Gay Balfour of Dog Gone, Inc., Cortez, Colo. Mr. Balfour used a modified industrial street cleaning machine with a large vacuum to extract live prairie dogs from their burrows. A small percentage of his catch was injured or killed during capture, and a few prairie dogs may have been dead in the burrow when extracted by the vacuum. These nonquarantined prairie dogs were to be tested later for plague and stored separately from quarantined prairie dogs. One or both of the Gunnison's prairie dogs fed to the ferrets was likely infected with *Y. pestis*. It is unlikely that all 30 black-footed ferrets received Gunnison's prairie dog pieces. We believe the infected portions of Gunnison's prairie dog cross-contaminated the rest of the prairie dog pieces during processing on the cutting board and/or while being carried in the transport bowl.

Surviving ferret #1148 shared a pen with another black-footed ferret (#268) that died from plague. Initial serum samples from #1148 (December 14, 1995) showed no evidence of plague exposure as judged by passive hemagglutination assay; however, surviving ferrets #565 (titer 1:128) and #1508 (titer 1:256) did show evidence of exposure (fig. 3). Ferrets #1508 and #1148 were transferred to reintroduction sites (Montana and Arizona, respectively) before additional blood samples could be taken. Ferret #565 remained at Pueblo, and blood samples were taken at 2-week intervals to follow the immune response. The titer level for ferret #565 increased to 1:2,048 and then diminished to 1:64 over the next 5 months (fig. 3).

Questions arose regarding the persistence of plague underground, and we elected to move some of the resident Siberian polecats from cages to the outdoor pens for exposure testing. On January 23, 1996, 11 male-female pairs of polecats were transferred into pens that had held ferrets that either died or disappeared. We radio tagged the polecats and took baseline serum samples prior to the transfer. Polecats were located each day visually or via radio telemetry. Additional blood samples were taken approximately monthly for 5 months, and irregularly thereafter.

On January 28, 1996, polecat #889 was found dead underground via radio telemetry. Necropsy and tests of tissues indicated plague as the cause of death. The pen had previously housed a black-footed ferret (#1410) whose body was not recovered. On February 13, 1996, polecat #800 carried the partially mummified remains of a formerly missing black-footed ferret (#1471) into a nest box. Subsequent tests of the polecat's blood indicated no evidence of exposure to plague; however, the remains of ferret #1471 were positive for plague.

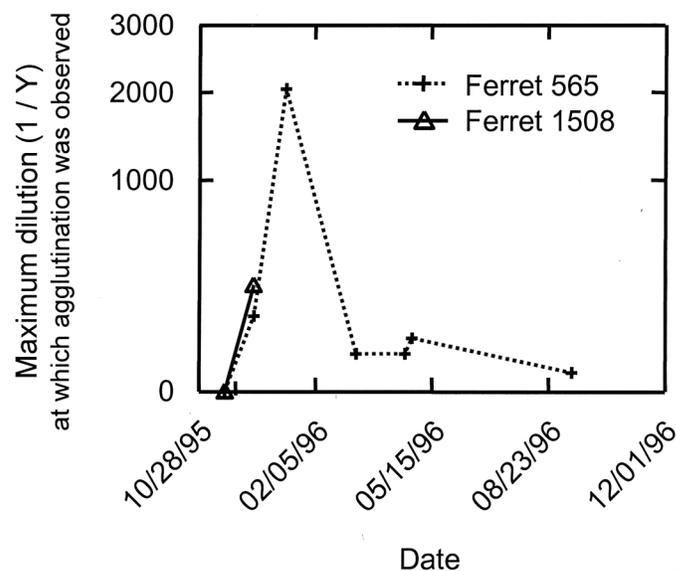


Figure 3. Antibody responses (as determined by passive hemagglutination) of black-footed ferrets (*Mustela nigripes*) #1508 and #565. Estimated date of exposure was 11/19/1995.

On February 23, 1996, polecat #094 recovered the remains of black-footed ferret #636. Serum samples indicated that polecat #094 was positive for plague and remained so for more than 3 months without clinical symptoms (fig. 4). The recovered body of black-footed ferret #636 also tested positive for plague. Siberian polecat #293, housed in a pen where ferret #526 had disappeared, also tested positive for plague. Thus, of the 22 polecats moved to the black-footed ferret pens, 3 tested positive for plague, 1 of which died. One of the seropositive surviving polecats was likely exposed when it recovered the remains of a plague-positive ferret. However, an additional polecat that recovered a plague-positive ferret tested negative. The remaining 18 polecats, including the pen mates of the three that were seropositive for plague, tested negative.

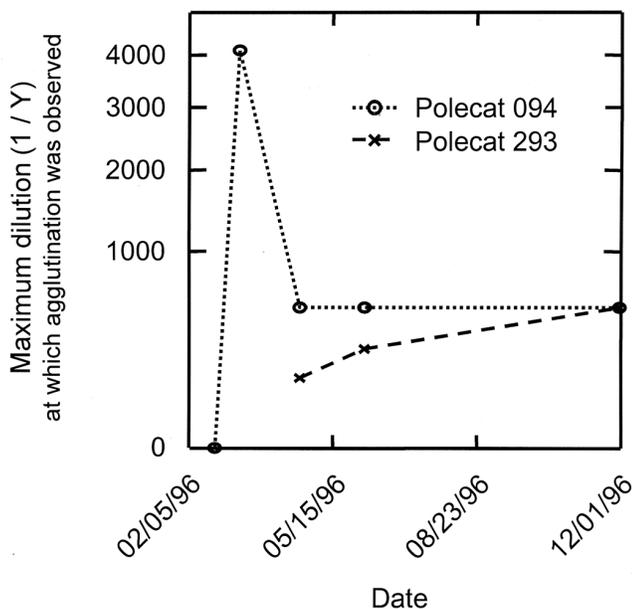


Figure 4. Antibody responses (as determined by passive hemagglutination) of two Siberian polecats (*Mustela eversmannii*) exposed to plague. Earliest potential date of exposure was 1/23/1996, when polecats were moved into pens.

Discussion

Black-footed ferrets may die within 48 hours of consuming plague-infected meat. Of the 30 animals in the group potentially exposed, 27 likely died (some were missing), and 3 survived (2 with antibody responses and 1 with no seroconversion even though its pen mate died of plague). This high rate of mortality was surprising given the circumstances of exposure. Some ferrets apparently ate prairie dog pieces that were surface-contaminated (by mixing with other pieces from infected prairie dogs) and probably received a fairly low dose of *Y. pestis*. Perhaps the two ferrets that survived exposure and showed antibody response consumed very low numbers of bacteria.

Black-footed ferrets are known to scavenge opportunistically; that habit, combined with the fact that plague has been repeatedly detected at most of the black-footed ferret reintroduction sites, suggests that plague-killed rodents constitute a real and eminent hazard for free-ranging black-footed ferrets. Because of the persistence of live *Y. pestis* in carcasses for more than 2 months in relatively cool and humid prairie dog burrows, the hazard may linger long after an epizootic has killed the rodents. If *Y. pestis* resides in prairie dog colonies, occasionally causing disease in individual prairie dogs or other rodents, the risk posed by even widely spaced carcasses could be serious for the relatively mobile foraging ferrets.

Although titers of the Siberian polecats declined, they remained sufficiently high during the course of monitoring (ca. 1 year) to suggest immunity to plague (fig. 4). Because the native habitats of Siberian polecats are centered on Asian foci of plague, these polecats were hypothesized to be more resistant than black-footed ferrets to the disease. Nevertheless, plague killed 88 percent of 33 polecats exposed to *Y. pestis* through subcutaneous injections and consumption of plague-killed mice (Castle and others, 2001), a loss rate similar to the suspected mortality rate for black-footed ferrets (90 percent) in the Pueblo incident.

The initial plague exposure of ferret #565 was more than 3 weeks before the first blood sample was taken on December 14, 1995. The greatest measured antibody response (1:2,048) was on January 11, 1996 (fig. 3), followed by a decline. The relatively low titers after just 3 months may have been insufficient to confer protection against subsequent exposure to plague (fig. 3). Consequently, long-term protection against plague via vaccination may be problematic in black-footed ferrets. More research is clearly needed.

It is unlikely that plague can be eliminated from the wild in North America. Protection of the black-footed ferret from this disease may depend in part on the ability to reduce its spread among and within prairie dog colonies and complexes. The use of pesticides to reduce flea populations provides some hope of reducing plague outbreaks and stabilizing treated areas (Durbian and others, 1997; Karhu and Anderson, 2000; Seery and others, 2003). Repeated dusting of burrows with pesticides, however, is labor intensive and perhaps not practical for large colonies and complexes. If a management tool (e.g., insecticide) can eliminate plague from a prairie dog colony, both ferrets and prey will be afforded some protection. Initial results suggest that flea control may reduce or eliminate epizootics of plague on prairie dog colonies but may not eliminate *Y. pestis* completely. If low levels of enzootic plague remain on such colonies, the threat to ferrets may be substantial, and additional management intervention (e.g., vaccination of ferrets) may be necessary.

Plague is currently common throughout the majority of the black-footed ferret's historical range. Remaining plague-free areas have become vital to reestablishment of the ferret. Why some prairie dog complexes are plague free and how long they will remain so are unknowns. The few remaining

plague-free areas provide a unique opportunity to learn about black-footed ferret habitat before plague becomes endemic. As experimental reintroductions and plague research continue, special consideration should be given to existing plague-free areas for recovering the black-footed ferret and for increasing our knowledge of plague dynamics through comparisons of areas with and without the disease.

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