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Plague in a Black-footed Ferret (*Mustela nigripes*)

Elizabeth S. Williams,[1] Kenneth Mills,[1] Donald R. Kwiatkowski,[2] E. Tom Thorne,[3] and Amy Boerger-Fields,[1] [1] Department of Veterinary Sciences, University of Wyoming, Laramie, Wyoming 82070, USA; [2] Wyoming Game and Fish Department, Sybille Wildlife Research and Conservation Education Unit, Wheatland, Wyoming 82201, USA; [3] Wyoming Game and Fish Department, Research Laboratory, Laramie, Wyoming 82071, USA

ABSTRACT: Diagnosis of sylvatic plague in a captive black-footed ferret (*Mustela nigripes*) was based on gross and microscopic lesions, fluorescent antibody tests, culture of *Yersinia pestis*, and immunohistochemistry. Gross lesions consisted of acute hemorrhage and necrosis associated with cervical and mesenteric lymph nodes, subcutaneous hemorrhages, and pulmonary edema. Acute edema, hemorrhage, and necrosis with numerous bacteria in blood vessels and sinusoids characterized microscopic lesions. Occurrence of fatal plague in a black-footed ferret potentially has significant implications for recovery of this endangered species due to the widespread distribution of plague in prairie dog colonies throughout historic black-footed ferret range.

Key words: Black-footed ferret, *Mustela nigripes*, plague, *Yersinia pestis*, endangered species, conservation biology, case report.

Sylvatic plague, caused by *Yersinia pestis*, is an important widespread zoonotic disease in the western United States (Barnes, 1982). It occurs in prairie dogs (*Cynomys* spp.) (Barnes, 1982) throughout most of the historic range of the endangered black-footed ferret (*Mustela nigripes*) (Nowak, 1991). Prairie dog populations may be severely affected by plague and mortality may reach 100% in Gunnison's prairie dogs (*C. gunnisoni*) (Rayor, 1985); however, in white-tailed prairie dog (*C. leucurus*) colonies mortality may be less and recovery of individual colonies within a complex may be more rapid than in other species (Heller, 1991; Menkens and Anderson, 1991). Prairie dogs are the primary prey of black-footed ferrets; thus the presence and distribution of plague in colonies of prairie dogs is an important consideration in planning reintroduction of black-footed ferrets to the wild (Hnilicka and Luce, 1992).

Black-footed ferrets have been assumed to be resistant to plague based on the apparent resistance of carnivores, with the exception of cats, to plague (Barnes, 1982; Gasper et al., 1993); resistance of striped skunks (*Mephitis mephitis*) to be experimental oral infection (Barnes, 1982); and field observations and serological surveys of mustelids. Badgers (*Taxidea taxus*) (Barnes, 1982; Hopkins and Gresbrink, 1982; Messick et al., 1983), pine martens (*Martes americana*) (Barnes, 1982; Zielinski, 1984), striped and spotted skunks (*Spilogale gracilus*) (Barnes, 1982; Hopkins and Gresbrink, 1982), and long-tailed weasels (*Mustela frenata*) (Barnes, 1982) have been found to carry antibodies against *Y. pestis*. In addition, experimental infection of eight domestic ferrets (*M. putorius furo*) and two Siberian polecats (*M. eversmanni*) via subcutaneous injection of 12 to 1.2×10^7 *Y. pestis* resulted in seroconversion in animals given $\geq 10^3$ organisms; no clinical signs of plague were observed in artificially infected animals (Williams et al., 1991). A single report of plague-induced mortality in a mustelid was an experimentally infected long-tailed weasel that died following subcutaneous injection with *Y.* (*Bacillus*) *pestis* (McCoy, 1911). None of 12 free-ranging black-footed ferrets from Meeteetse, Wyoming (USA) had serum antibodies against *Y. pestis*; it was not known if these animals had been exposed to plague (Williams et al., 1991).

In February 1993, a 1-yr-old male black-footed ferret housed in a large outdoor enclosure at the Wyoming Game and Fish Department's Sybille Wildlife Conservation and Education Unit (Wheatland, Wyoming) escaped via a small opening into an adjoining enclosure. These adjacent enclosures (6.7 × 6.1 m) were filled 2.4 m with soil, contained white-tailed prairie dogs and their burrows, and were used for

research and acclimation of black-footed ferrets prior to release into the wild (Thorne et al., 1993). The black-footed ferret was trapped 2 days later and placed in indoor quarantine facilities as is routine when bringing animals housed outside back into the main indoor facility (Williams et al., 1992b). The animal was anesthetized the next morning with a mixture of ketamine hydrochloride (Ketaset, Bristol Laboratories, Syracuse, New York, USA) and diazepam (Valium, Hoffman-LaRoche, Inc., Nutley, New Jersey, USA) at 17 mg/kg and 0.07 mg/kg, respectively, given by intramuscular injection for physical examination and positive identification. Physical examination and immediate recovery from anesthesia were normal. The black-footed ferret was clinically normal that afternoon but did not eat during the night. It was found dead in it's cage approximately 32 hr after anesthesia and refrigerated. Necropsy was conducted at the Wyoming State Veterinary Laboratory (Department of Veterinary Sciences, University of Wyoming, Laramie, Wyoming) within 24 hr.

On gross examination, the black-footed ferret was in excellent body condition (1,060 g). Red foam was present in the external nares. Acute subcutaneous hemorrhage was found over the lower back; around the pharynx, especially on the right side; and along fascial planes in the musculature of the neck. The lungs failed to collapse normally and were severely edematous; white froth was in the trachea and large bronchi. Numerous small black foci were on the gastric mucosa and stomach contents were red-black and mucoid. Hemorrhage was present in the area of the mesenteric lymph nodes. A small amount of hemorrhage was in the meninges surrounding the cervical spinal cord. Other organs appeared grossly normal. Pieces of skin, eye, tongue, skeletal muscle, brain, spinal cord, lymph nodes, liver, lung, trachea, esophagus, thyroid gland, parathyroid gland, kidney, body fat, heart, aorta, adrenal gland, thymus, testicle, epididymis, preputial gland, stomach, small intestine, colon, mesenteric lymph node, spleen, pancreas, salivary gland, and urinary bladder were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 5 to 6 μm and stained with hematoxylin and eosin (Luna, 1968). Selected tissue sections were stained by the Brown-Hopps Gram stain method for bacteria (Luna, 1968).

On microscopic examination of the tissues, large areas of hemorrhage, necrosis, and massive numbers of Gram-negative coccobacilli in sinuses and blood vessels of mandibular, pharyngeal, and mesenteric lymph nodes were observed. Similar bacteria were numerous in vessels of the lung. Multiple foci of acute hemorrhage and marked perivascular and alveolar edema occurred in the lung. Blood vessels in many organs also contained bacteria, but other microscopic lesions were minimal.

Imprints of lung were positive for *Y. pestis* antigen by fluorescent antibody test (Moody and Winter, 1959; Winter and Moody, 1959). Lung, intestine, and feces were cultured for bacteria on Columbia blood agar with 5% sheep blood at 25 C (Carter and Cole, 1990). *Yersinia pestis* was identified in lung, but not other samples, by typical morphology and the following biochemical reactions: catalase positive, oxidase negative, indole negative, sorbitol negative, rhaminose negative, L-arabinose positive, urease negative, esculin positive, Voges-Proskauer negative, citrate negative, and raffinose negative.

Sections of paraffin-embedded lung and lymph node were predigested with 0.05% protease (Sigma Chemical Co., St. Louis, Missouri, USA) for 15 min. Tissues were incubated with murine hybridoma supernatant monoclonal antibodies, diluted 1:200, against *Y. pestis* capsular fraction 1 (Dr. Kelly Davis, Fort Detrick, Maryland, USA), with detection using an avidin-biotin complex reaction kit (Vector Laboratories, Inc., Burlingame, California, USA) and 3,3′ diaminobenzadine tetrachloride/nickel chloride (Zymed Laboratories, Inc.,

San Francisco, California) according to Haines and Chelack (1991) for immunohistochemical identification of *Y. pestis* antigen. Sections were weakly positive.

Based on gross and microscopic lesions, fluorescent antibody tests, bacterial cultures, and immunohistochemistry, plague was diagnosed as the cause of death of this black-footed ferret. No other black-footed ferrets or animal caretakers developed plague. We conclude that isolation and quarantine protocols prevented exposure of other black-footed ferrets to the affected animal and that safety measures developed early in the captive breeding program to protect black-footed ferrets against pathogens carried by humans (Williams et al., 1992b) also served to protect caretakers against a disease of ferrets.

The source of plague for the black-footed ferret is not known. Plague is common in wild rodents in Wyoming and has been identified in many parts of the state since 1936 (Quan, 1982; Ubico et al., 1988; Williams et al., 1992a). On two occasions, plague has been diagnosed in free-ranging wild rodents at the Sybille Unit, Wheatland, Wyoming. A bushy-tailed woodrat (*Neotoma cinerea*) was found dead and *Y. pestis* isolated from its tissues in 1980 (Thorne and Walthall, 1981) and a yellow-bellied marmot (*Marmota flaviventris*) found dead near the black-footed ferret building was determined to have died of plague in 1988 (Anderson et al., 1988). The black-footed ferret complex is surrounded by a 5.5 cm mesh chain-link fence and the outdoor prairie dog cages are enclosed in 2.5 cm mesh chain-link. These barriers exclude most mammals, but not small rodents such as chipmunks (*Eutamias* sp.), woodrats, mice (*Mus musculus, Peromyscus maniculatus*) or voles (*Microtus* spp.) that could carry plague or infected fleas. Another possible source of plague was prairie dogs trapped from colonies in Carbon County, Wyoming and held in quarantine for 7 to 10 days before being used to feed ferrets or placed in the outdoor enclosures. Maximum incubation period in experimentally infected white-tailed prairie dogs was 10 days (E. S. Williams and K. Mills, unpubl.). Possibly an infected but clinically normal prairie dog was introduced into the outside pens. Black-footed ferrets were not housed in this cage for approximately a year.

The black-footed ferret appeared to have been orally exposed based on the presence of bubos in the neck and mesentery. Domestic ferrets were resistant to plague when exposed to *Y. pestis* via subcutaneous injection, mimicking flea bite exposure. The usual route of natural infection in carnivores is probably by consumption of infected prey, and perhaps the oral route increases the likelihood of disease in ferrets. Dose is important and consumption of a rodent that died of plague would probably provide much greater exposure to *Y. pestis* than a flea bite.

Yersinia pestis may have been introduced into North America in the early 1900's (Olsen, 1981) and then spread from California across the west in resident rodents. Black-footed ferrets probably were present in North America for at least 100,000 years (Anderson, 1989). It is possible that they are more susceptible due to lack of exposure and failure to develop resistance to this organism over time than the closely related domestic ferret and Siberian polecat that occur in areas where plague historically has been present. Increased resistance to experimental plague was demonstrated in voles (*Microtus californicus*) (Hubbert and Goldenberg, 1970) and grasshopper mice (*Onychomys leucogaster*) (Thomas et al., 1988) from areas of enzootic plague in comparison to animals from areas free of the disease. However, most other North American carnivores which generally have a historic relationship with plague similar to black-footed ferrets appear to be resistant (Barnes, 1982). It also is possible that black-footed ferrets, due to their low genetic variability (O'Brien et al., 1989), are more susceptible to this pathogen than other closely related species. Low genetic vari-

ability has been shown to be associated with increased susceptibility to feline infectious peritonitis of the endangered cheetah (*Acinonyx jubatus*) (O'Brien et al., 1985), and similar mechanisms could influence susceptibility of black-footed ferrets to a variety of pathogens, including *Y. pestis* and canine distemper (Williams et al., 1988). Additional research, including experimental infections and field study, are currently being conducted to establish the relative susceptibility of black-footed ferrets to plague.

Because plague is very widespread in prairie dogs throughout the historic range of black-footed ferrets, recovery of this endangered species could be jeopardized if they prove to be highly susceptible to plague induced mortality. Some black-footed ferrets have persisted and reproduced in areas of high plague prevalence in Wyoming (Thorne and Williams, 1988); thus some black-footed ferrets may be relatively resistant to plague or are not exposed under free-ranging conditions.

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