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EXPERIMENTAL INFECTION OF DOMESTIC FERRETS (*MUSTELA PUTORIUS FURO*) AND SIBERIAN POLECATS (*MUSTELA EVERSMANNI*) WITH *YERSINIA PESTIS*

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ABSTRACT: Eight domestic ferrets (*Mustela putorius furo*) and two Siberian polecats (*M. eversmanni*) were inoculated subcutaneously with 12 to 1.2 × 10^7 *Yersinia pestis* originally isolated during an epizootic of plague in white-tailed prairie dogs (*Cynomys leucurus*) near Meeteetse, Park County, Wyoming (USA) in 1985. None of the ferrets or polecats developed clinical signs of disease which suggested that black-footed ferrets (*M. nigripes*), a congener, also would be resistant to plague. All animals receiving ≥ 1.2 × 10^3 organisms produced serum antibodies detected by the passive hemagglutination test with titers peaking at 1:1,024 and remaining positive until at least 219 days postinoculation. Sera collected from 12 free-ranging black-footed ferrets near Meeteetse in 1984 and 1985 were negative for antibodies against *Y. pestis*. Prevalence of antibodies against *Y. pestis* was high in other carnivores collected from the same area in 1986.

Key words: *Mustela putorius furo*, *Mustela eversmanni*, *Mustela nigripes*, black-footed ferrets, Siberian polecats, plague, *Yersinia pestis*, experimental infection, serology.

INTRODUCTION

Sylvatic plague was diagnosed in June 1985 in white-tailed prairie dogs (*Cynomys leucurus*) in colonies near Meeteetse (Park County, Wyoming, USA) (Ubico et al., 1988). These prairie dog colonies were also inhabited by the last known population of free-ranging black-footed ferrets (*Mustela nigripes*) (Thorne and Williams, 1988). Prairie dogs are the most important component of the diet of black-footed ferrets (Sheets et al., 1972; Campbell et al., 1987). Therefore, diagnosis of plague was of major concern to management agencies responsible for the black-footed ferrets for two reasons; it was known that prairie dogs are highly susceptible to plague (Barnes, 1982; E. S. Williams, unpub.), thus potentially endangering the prey base for ferrets (Quan, 1982), and the susceptibility of black-footed ferrets to plague was unknown, leading to allegations that black-footed ferrets were dying of plague. The experimental portion of this study was undertaken to determine the susceptibility to plague of domestic ferrets (*Mustela putorius furo*) and Siberian polecats (*Mustela eversmanni*), as surrogates for black-footed ferrets.

Serologic testing of some carnivores, because of their relative resistance to plague, is a useful technique for epidemiologic investigation of plague activity (Barnes, 1982). Therefore, sera from free-ranging black-footed ferrets were tested for antibodies against *Y. pestis* in order to learn about the prevalence and effects of exposure to plague experienced by black-footed ferrets. A survey for antibodies to *Y. pestis* in other carnivores from the area inhabited by black-footed ferrets also was conducted.

MATERIALS AND METHODS

Eight juvenile castrated male domestic ferrets were obtained from a commercial breeder (Marshall Farms, North Rose, New York 14516, USA). Two captive-born adult male and female Siberian polecats, which had been housed at the Wyoming Game and Fish Department's Sybille Wildlife Research Unit (Bosler Route, Wheatland, Wyoming 82201, USA) for several years prior to initiation of the study, were also used. Ferrets and polecats were housed at the Wyoming State Veterinary Laboratory (1174 Snowy

Range Road, Laramie, Wyoming 82070, USA) in stainless steel wire bottom cages in a P2 biocontainment room (Centers for Disease Control-National Institutes of Health, 1984) and fed cat food (Ralston-Purina, St. Louis, Missouri 63164, USA) and fresh water. The room was maintained at 22 C on a 12 hr light-12 hr dark cycle.

Ferrets and polecats were inoculated subcutaneously in the right inguinal region with 0.1 ml physiologic saline suspension of *Y. pestis*, originally isolated from a prairie dog that died of plague in the Meeteetse colony in 1985. The inoculum was prepared according to Quan et al. (1985). The dose of bacteria inoculated was calculated by plating 0.1 ml of bacterial suspension on blood agar, incubating at 28 C for 48–72 hr, and averaging colony counts. Two ferrets each were inoculated with 12, 1.2×10^3, 1.2×10^5, and 1.2×10^7 organisms; polecats were inoculated with 12 and 120 organisms. Virulence of the inoculum was established by concurrent inoculation of 6-wk-old laboratory mice (NIH general purpose strain, Centers for Disease Control, Fort Collins, Colorado 80522, USA) in groups given ascending doses from approximately 1 to 1.2×10^7 organisms. Mortality data were used to calculate the mean lethal dose (LD_{50}) for the mice (Reed and Muench, 1938).

Ferrets and polecats were observed twice daily for evidence of disease. Blood was obtained periodically by venipuncture from animals anesthetized with ketamine hydrochloride (Ketaset, Bristol Laboratories, Syracuse, New York 13201, USA) and diazepam (Valium, Hoffman-La Roche Inc., Nutley, New Jersey 07110, USA). Sera were collected and frozen at −20 C until tested by the passive hemagglutination (PHA) test for antibodies to *Y. pestis* Fraction 1 (World Health Organization, 1970). Serum titers of ≥1:8 were considered positive.

Three weeks postinoculation, two domestic ferrets that had been inoculated with 1.2×10^5 and 1.2×10^7 organisms were anesthetized, killed by intracardiac injection (T-61 Euthanasia Solution, Hoechst-Roussel Agri-Vet Company, Sommerville, New Jersey 08876, USA), and necropsied. Spleen, liver, and inguinal lymph nodes were examined by fluorescent antibody (FA) technique (Moody and Winter, 1959; Winter and Moody, 1959) and cultured for *Y. pestis* (Thorne et al., 1987). Sections of most organs were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 6 μm and stained with hematoxylin and eosin for histopathology.

Blood from 12 free-ranging black-footed ferrets was collected during research trapping operations at the Meeteetse site in late summer and fall 1984 and 1985. Black-footed ferrets were anesthetized and blood collected by jugular venipuncture (Thorne et al., 1985). Sera were collected within 48 hr and held at −20 C until tested. Blood was collected from 13 badgers (*Taxidea taxus*), eight coyotes (*Canis latrans*), four raccoons (*Procyon lotor*), and two skunks (*Mephitis mephitis*) shot or trapped in the Meeteetse area from May to September 1986 (Williams et al., 1988). Sera were collected from the blood within approximately 24 hr and frozen at −20 C until tested. Carcasses were chilled on ice, transported to the Wyoming State Veterinary Laboratory usually within 24 hr, and necropsies were conducted on most carcasses. Sections of most organs were collected for histopathology.

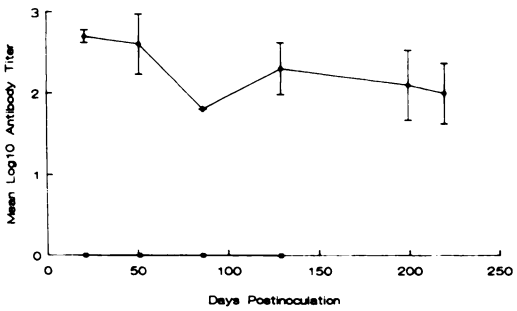

FIGURE 1. Mean (±SE) passive hemagglutination antibody titers (log_{10}) of domestic ferrets and Siberian polecats to *Y. pestis* exposure. ● response of ferrets inoculated with ≥1.2×10^3 *Y. pestis*; ■ = response of ferrets and Siberian polecats inoculated with approximately 12 and 120 *Y. pestis*.

RESULTS

None of the ferrets or polecats developed clinical signs of disease following inoculation with virulent *Y. pestis*. The virulence of the inoculum was confirmed by a calculated LD_{50} of approximately one organism for the laboratory mice. The six ferrets which received ≥1.2×10^3 organisms developed PHA serum antibodies by day 21 (Fig. 1). No antibodies were detected in two ferrets receiving approximately 12 organisms or in the polecats. Serum antibody titers peaked at 1:1,024 in two ferrets on days 21 and 51 and in two other ferrets at 1:512 on day 21 postinoculation. Antibody titers then declined erratically, but were maintained at ≥1:32 to at least day 219 postinoculation.

Yersinia pestis was not isolated and FA tests were negative on tissues collected from ferrets killed 21 days postinoculation.

Moderate lymphoid hyperplasia was present in spleen, prefemoral and mesenteric lymph nodes, and small aggregates of lymphocytes and plasma cells were found in portal zones of livers from these animals.

Six black-footed ferret sera collected in 1984 and six sera collected in 1985 did not contain detectable PHA antibodies to *Y. pestis*. Eleven of 13 (85%) badgers, seven of eight (88%) coyotes, one of two (50%) skunks, and none of four raccoons were seropositive and titers ranged from 1:8 to 1:128. Both juvenile and adult animals had positive titers. There was no gross or microscopic evidence of plague in these animals.

DISCUSSION

Absence of clinical disease in domestic ferrets and Siberian polecats when challenged with *Y. pestis* strongly suggests they are resistant to plague. In addition, inability to detect *Y. pestis* in tissues of domestic ferrets killed 21 days postinoculation also indicates that these animals are resistant. Moderate lymphoid hyperplasia suggested active antibody production; these animals had high serum antibody titers at the time they were killed. The serologic response of ferrets to *Y. pestis* was similar to a small number of orally infected coyotes, skunks and raccoons which remained seropositive for 6 to 8 mo (Barnes, 1982); our ferrets maintained positive titers for at least 7 mo.

Ferrets and polecats receiving the lower doses of bacteria did not develop measurable PHA antibody titers indicating infection did not develop in these animals. Carnivores may be infected with *Y. pestis* via consumption of rodents with plague (Poland and Barnes, 1979) thus receiving a massive dose of *Y. pestis* or via bite of infected fleas where exposure probably would be considerably less ($\leq 1 \times 10^4$ organisms) (Burroughs, 1947). Based on our experimental data, exposure to $\leq 1 \times 10^2$ organisms, which might occur via bite of an infected flea, probably would not result in infection of ferrets and therefore the animals would not develop antibodies to *Y. pestis*.

The response of domestic ferrets and Siberian polecats to *Y. pestis* suggests black-footed ferrets are also resistant to plague. We believe domestic ferrets and Siberian polecats were reasonable surrogates for black-footed ferrets because of the very close taxonomic relationship between these species (Anderson, 1989; O'Brien et al., 1989). Also, most mustelids appear resistant to plague based on field observations and serosurveys of badgers (Hetlet, 1968; Fitzgerald, 1970; Poland et al., 1973; Barnes, 1982; Hopkins and Gresbrink, 1982; Messick et al., 1983), striped and spotted skunks (*Spilogale gracilus* (Wolff and Hudson, 1974; Barnes, 1982; Hopkins and Gresbrink, 1982), pine marten (*Martes americana* (Barnes, 1982; Zielinski, 1984); and long-tailed weasels (*Mustela frenata*) (Barnes, 1982). Skunks seroconverted following ingestion of a *Y. pestis* infected meal, but did not become ill, bacteremic or shed *Y. pestis* in their feces (Poland and Barnes, 1979; Barnes, 1982). However, a long-tailed weasel apparently died of plague following subcutaneous inoculation with a large dose of *Y.* (*Bacillus*) *pestis* (McCoy, 1911).

Absence of antibody titers against *Y. pestis* in sera of black-footed ferrets collected in 1984 and 1985 may be interpreted in several ways. A likely explanation is that these animals were never exposed to *Y. pestis*. Plague may not have been present in the Meeteetse colonies in 1984; and in 1985, plague was patchy in its distribution (Ubico et al., 1988). Also, the number of black-footed ferrets sampled was small and seropositive animals could have been missed if the prevalence was low. If black-footed ferrets had been exposed to $\leq 1 \times 10^2$ organisms, they might not have become infected and developed detectable serum antibodies to *Y. pestis*. Ingestion of a prairie dog with plague would likely expose black-footed ferrets to enough bacteria to cause infection, but it is possible that ferrets avoid eating sick or

dead prairie dogs. It seems extremely unlikely, based on the results of this study, that lack of seropositive black-footed ferrets was due to death of exposed individuals.

The prevalence of antibodies against *Y. pestis* in skunks, badgers, and coyotes in 1986 was high. Antibody titers in juvenile as well as adult carnivores reflected ongoing plague in the prairie dogs during the summer. Coyotes and badgers range more widely than black-footed ferrets (Bekoff, 1982; Lindzey, 1982; Biggins et al., 1985) and would be more likely to encounter and consume prairie dogs from colonies experiencing plague epizootics. It is not known if plague was present in 1984 or if it was more widespread in 1986 than in 1985, but this serologic information strengthens the observations, based on culture of fleas and dead prairie dogs, that plague was widespread across the colonies in 1986 (Menkens and Anderson, 1987).

The challenge of domestic ferrets and Siberian polecats reported here and the known resistance of other mustelids to plague suggests black-footed ferrets are resistant to *Y. pestis*. Therefore, concern about black-footed ferret mortality directly due to *Y. pestis* infection is probably not warrented in the event of a plague epizootic in a black-footed ferret occupied prairie dog colony.

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