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# SUSCEPTIBILITY OF THE SIBERIAN POLECAT TO SUBCUTANEOUS AND ORAL *YERSINIA PESTIS* EXPOSURE

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ABSTRACT: To determine if the Siberian polecat (*Mustela eversmannii*) represents a suitable model for the study of plague pathogenesis and prevention in the black-footed ferret (*Mustela nigripes*), polecats were exposed to $10^3$, $10^7$, or $10^{10}$ *Yersinia pestis* organisms by subcutaneous injection; an additional group was exposed to *Y. pestis* via ingestion of a plague-killed mouse. Plague killed 88% of polecats exposed to *Y. pestis* (71% mortality in the $10^3$ group, 100% mortality in the $10^7$ and $10^{10}$ groups, and 83% mortality in the mouse-fed group). Within the challenged group, mean day of death post-challenge ranged from 3.6 to 7.6 days; all polecats died on or before day 12 post-challenge. Animals receiving the lowest parenteral dose survived significantly longer than those receiving higher parenteral doses. Within challenged animals, mean survival time was lower in those presenting with significant weight loss by day 3, lethargy, and low fecal output; time to onset of lethargy and other signs was also related to risk of dying and/or plague dose. Six polecats developed serum antibody titers to the *Y. pestis* F1 protein. Three seropositive polecats survived the initial challenge and a subsequent exposure to a plague-killed mouse, while two seropositive animals later died. This study confirms that the Siberian polecat is susceptible to plague and suggests that this species will offer an appropriate surrogate for black-footed ferrets in future plague studies and related vaccine trials.

*Key words:* Black-footed ferret, experimental infection, ferret, *Mustela eversmannii, Mustela nigripes,* plague, Siberian polecat, *Yersinia pestis.*

## INTRODUCTION

The black-footed ferret (*Mustela nigripes*) recovery program has successfully employed a captive breeding strategy to produce surplus animals for reintroduction into native habitat. Unfortunately, plague (caused by *Yersinia pestis*) is common throughout many of the proposed black-footed ferret release sites (Ubico et al., 1988). Plague causes periodic and sometimes dramatic die-offs of prairie dogs (*Cynomys* spp.) (Menkens and Anderson, 1991), and may adversely affect reintroduction efforts by reducing the ferret prey base and through direct mortality of black-footed ferrets.

The susceptibility of some *Mustela* spp. and *Mustela* spp. hybrids to plague has been recently investigated. Williams et al. (1991) challenged groups of domestic ferrets (*M. putorius furo*) with doses ranging from 12 to $1.2 \times 10^7$ *Y. pestis* organisms and challenged two Siberian polecats (*M. eversmannii*) with 12 or 120 *Y. pestis* organisms. None of the animals developed clinical signs of plague, but domestic ferrets exposed to $1.2 \times 10^3$ or more *Y. pestis* organisms developed serum antibody titers. Based on information available at that time, ferrets and polecats were thought to be resistant to *Y. pestis*, so the authors suggested that black-footed ferrets are unlikely to succumb to plague.

The death of a black-footed ferret due to a *Y. pestis* infection (Williams et al., 1994) demonstrated the susceptibility of the species to plague. Recent multiple deaths of black-footed ferrets at the Pueblo Chemical Depot (Colorado, USA; D. Biggins, unpubl. data) due to ingestion of prairie dog meat contaminated with *Y. pestis* have underscored the potential hazard of plague to the species. Two Siberian



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polecats also died from plague at Pueblo, illustrating that both species can suffer mortality from plague, and that species differences in plague susceptibility, if present, are relative. Two black-footed ferrets and two polecats developed serum antibody titers to *Y. pestis* at Pueblo and survived, demonstrating that both species can survive some level of *Y. pestis* exposure (D. Biggins, unpubl. data). However, the minimum lethal dose, lethal routes of exposure, and clinical signs of illness have not been characterized for pure-bred Siberian polecats or for black-footed ferrets exposed to a *Y. pestis* strain with well-defined virulence factors.

The Siberian polecat has been used as an investigational model for a number of studies applicable to the black-footed ferret (Hill and Carpenter, 1982; Powell et al., 1985; Williams et al., 1991). The two animals are similar behaviorally and morphologically, and conspecificity has been debated (Anderson et al., 1986; O'Brien et al., 1989). If, however, the Siberian polecat has developed some resistance to *Y. pestis* due to its association with the bacillus over evolutionary time, then it may be an unsuitable surrogate for black-footed ferrets in plague-challenge studies. *Yersinia pestis* is thought to have originated in the Old World (Poland and Barnes, 1979), and it was isolated from dead polecats in central Asia as early as 1911 and 1929 (Peshkov, 1954). The polecat may be a secondary carrier of plague over its natural range, and may therefore have a role in the natural epidemiology of plague (Kydyrbaev, 1988).

To study the suitability of Siberian polecats as surrogates for black-footed ferrets in studies of plague, we exposed Siberian polecats (*M. eversmannii dauricus*) to a well-characterized, virulent strain of *Y. pestis* via subcutaneous injection or ingestion of a plague-killed mouse. Our objectives were to determine (1) the effect of dose and route of exposure on mortality, (2) whether eating *Y. pestis*-infected dead rodents provides a similar level of exposure as a simulated flea bite, (3) whether animals that survive exposure to *Y. pestis* develop sufficient immunity to reduce their risk of dying from a second exposure, (4) the dynamics of clinical *Y. pestis* infection, and (5) the optimal challenge dose in polecats to use during plague-vaccine evaluations.

## MATERIALS AND METHODS

### General

All procedures were approved by the Animal Care and Use and Biosafety Committees at Colorado State University (CSU; Fort Collins, Colorado, USA). The experiment was conducted between 21 October and 21 December 1997.

### Animals

Siberian polecats used in this study were captive-bred descendants (six generations in captivity) of animals wild-caught in Eastern Inner Mongolia, China. Thirty-five animals (20 females and 15 males) from 1 to 3 yr-old were used. Polecats were stratified based upon founder female and breeding pedigree, and randomly assigned to five treatment groups of seven animals.

Polecats were moved from the Pueblo Chemical Depot breeding facility to a biosafety level-3 containment facility at Colorado State University (Fort Collins) 1 wk prior to the start of the experiment. Polecats were housed individually in stainless steel rabbit cages (0.5 m × 0.5 m × 0.5 m); pans of wood shavings were placed under each cage to collect feces and urine. Each cage contained a hiding tube constructed of a 35 cm long × 10 cm diameter piece of plastic pipe fitted with an endcap. Animals were provided with 60 g of commercial mink chow each day, and had continual access to water. Constant ambient temperature (21–23 C) and photoperiod (12L:12D) were maintained.

### Body temperature

Body temperature ($T_B$) of each animal (unanesthetized) was determined by placing the animal in an 8 cm diameter cylindrical wire-mesh tube and inserting a K-J thermocouple (Fluke Company, Inc., Everritt, WA, USA) 2.5 cm into the rectum and allowing it to equilibrate for approximately 15 to 20 sec. Temperature was read on a Fluke telethermometer (Fluke Company, Inc., Everritt, WA, USA). The thermocouple was decontaminated with a

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10% bleach solution and thoroughly rinsed between animals.

### Blood collection and physical examination

On the day of plague exposure (day 0), and on days 3, 9, 15, and 21 post-exposure, all animals were anesthetized with isoflurane (AErrane, Fort Dodge, Iowa, USA) prior to blood collection and physical examination. Anesthesia was induced with 5% isoflurane and 4 l $O_2$/min while the polecat was in its plastic hiding tube, and was maintained by mask with 2 to 3% isoflurane in oxygen at 1.5 l/min using a bain nonrebreathing circuit. While the animals were anesthetized, 1 to 3 ml of blood were collected by jugular venipuncture. Blood was also collected from animals just prior to euthanasia when possible. One ml of blood was placed into a tube containing sodium EDTA, and a complete blood cell count (CBC) and differential white blood cell count were performed. The remaining sample was placed into a glass serum tube, allowed to clot for approximately 2 hr, then centrifuged to recover the serum.

Aliquots of serum were analyzed immediately or quick-frozen and stored at −70 C until analyzed. Hematologic parameters were determined by the Clinical Pathology Section of the Veterinary Teaching Hospital at CSU. Parameters measured included red blood cell count (RBC), white blood cell count (WBC), platelet count, differential count, hemoglobin concentration (g/dl), and packed cell volume (PCV, %). Standard red cell indices were calculated, including mean corpuscular volume (MCV, fl), mean corpuscular hemoglobin (MCH, pg), and mean corpuscular hemoglobin concentration (MCHC, g/dl).

Physical examinations were performed on anesthetized animals, and included observation of the oral cavity and eyes for lesions, swelling, mucosal injection, and icterus; palpation of the spleen, liver, retropharyngeal, axillary, inguinal, and popiliteal lymph nodes, and inspection of the subcutaneous inoculation site for swelling and necrosis.

### Dosing regime

After blood collection and physical examination on day 0, anesthetized polecats were dosed with *Y. pestis.* Group 1 animals received no inoculation, and served as controls. Groups 2, 3, and 4 were inoculated subcutaneously (s.c.) in the right inguinal region with $10^3$, $10^7$, and $10^{10}$ *Y. pestis* (strain CO963188, cat isolate) organisms, respectively, and will be referred to as the "$10^3$-," "$10^7$-," and "$10^{10}$-dose" groups. The $10^3$ dose was given to simulate a flea bite (Burroughs, 1947). Group 5 animals were each fed a 5-wk-old male Swiss-Webster outbred strain mouse (Centers for Disease Control and Prevention; CDC) that had died of *Y. pestis* (strain CO963188, cat isolate) infection under standard CDC protocols, and will be referred to as the "mouse-fed" group. The *Y. pestis* isolate was chosen because its virulence factors have been well-characterized by CDC and it has the same virulence features of an isolate used in a previous study conducted by CDC researchers (Gasper et al., 1993).

### Daily observations

Animals were observed and scored twice daily for the first week after inoculation, and once daily thereafter. Each day, the following criteria were subjectively scored: behavior (alert, aggressive, lethargic, non-responsive); physical status (coat groomed or not, molting, abnormal vocalizations, ataxic or not); amount and appearance of feces (normal, diarrheic, bloody) and respiration (rate, presence of cough, wheezing, or dyspnea); and the presence of oral or nasal discharge or other abnormalities. Food intake was subjectively assessed, but was not quantitated because many polecats dispersed their food throughout their cage and onto the floor, making individual animal measurements difficult.

Polecats were anesthetized with isoflurane as described above and euthanized with an intracardiac dose of 3 ml sodium pentobarbital (Beuthanasia D, Shering-Plough Animal Health Corporation, Union, New Jersey, USA) if the animal exhibited any of the following: body temperature higher than 41.7 C; no response to stimuli; blood from any orifice; cyanotic mucous membranes; ataxia or other severe neurological signs.

### Necropsy

Polecats that died or were euthanized were frozen at −70 C until necropsy. All necropsies were conducted at the conclusion of the experiment at the Bacterial Zoonose Branch diagnostic laboratory of the CDC (Fort Collins, Colorado, USA). At necropsy, we noted and subjectively scored external and internal lesions, hemorrhage, swelling, lymphadenopathy, and changes in spleen and liver appearance and size, relative to a polecat that had died of other causes. Spleen and liver samples were smeared onto two-well microscope slides for direct fluorescent antibody (DFA) analysis (see below). Unfortunately, samples were not collected for histopathology prior to freezing of polecat carcasses.

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### Recovery of *Yersinia pestis*

To isolate *Y. pestis* from dead polecats, an asceptically collected section of liver was streaked onto 6% sheep blood agar (SBA). Plates were incubated at 37 C for 24 to 48 hr, and examined for *Y. pestis* colonies, which were 1 to 2 mm in diameter, gray-white to yellow in color, with raised irregular "hammered" shiny surface and edge. Cultures were confirmed by bacteriophage lysis and supported by direct fluorescent antibody (DFA) testing, following standard CDC procedures (Chu, 2000).

### *Yersinia pestis* virulence

The virulence of the inoculating strain of *Y. pestis* CO963188 was determined by eight × eight titration in ICR outbred mice. A 24-hr culture of CO963188 was diluted in normal saline to McFarland's turbidity standard #1 and then serially ten-fold diluted. Samples of each dilution were spread on agar plates to determine colony forming units. Each of eight diluted inoculum, $10^0$ to $10^7$ cfu/ml, was injected s.c. at 0.1 ml volumes into eight mice/dilution. The $LD_{50}$ was determined by the method of Reed and Meunch (1938) to be between 1 to 100 cfu. CO963188 was also determined to contain the full complement of *Y. pestis* plasmids by electrophoresis, and was positive by Congo red agar (Chu, 2000).

### Serology

Antibodies in serum of plague-exposed polecats were detected by passive hemagglutination (PHA) test, following standard CDC methods (Chu, 2000). A titer of >1:10 was considered positive. All positive sera were verified by repeat titration by PHA and by inhibition for specificity.

### Second plague challenge

Twenty-eight days after the initial *Y. pestis* challenge, five controls and three animals which survived the initial challenge were each fed a plague-killed mouse (as above). Polecats were sampled, monitored and scored on a daily basis for 21 d as described above.

### Statistical analyses

All data were analyzed using the SAS statistical package (SAS Institute, Inc., Cary, NC, USA) or SPSS for the Macintosh (SPSS Inc., Chicago, Illinois, USA). Kaplan-Meier survival analysis (log rank test) was performed to determine the unadjusted effects of *Y. pestis* dose and route of administration on survival time. In our univariate analyses, we used the Chi-square test in evaluating the effects of categorical variables on dichotomous variables, and the ANOVA test to examine the effects of categorical variables on continuous outcomes. Multiple Cox regression was employed to determine the independent effects of plague dose and route, and to assess the influence of potential confounders, including gender and founder female, on survival time. We also assessed the effect of *Y. pestis* dose and route on the development of 11 clinical signs, including significant weight loss (defined as >10% from baseline), fever (defined as maximum temperature increase from baseline), lethargy, ataxia, unkempt fur, low fecal output, bloody diarrhea, green diarrhea, wheezing, coughing, and dyspnea. In the text, results are presented as the mean ± one standard error (in parentheses).

## RESULTS

Because all 12 polecats initially challenged with a plague-killed mouse on day 0 ($n = 7$) or day 28 ($n = 5$) exhibited similar responses, in terms of day of death (ANOVA; $P = 1.0$), maximum body temperature, (ANOVA; $P = 0.70$) and subjectively scored behavioral, physical, and fecal changes, results for those twelve animals were pooled for all subsequent analyses.

None of the control animals died during the first 21 days of the experiment. One control animal and one mouse-fed survivor died between days 21 and 28. The absence of clinical signs, gross lesions, and isolation of *Y. pestis* indicated that plague was not the cause of death in either case. All infected animals that died during the experiment were *Y. pestis* culture positive and phage lysis positive, providing strong evidence that plague was the cause of death.

Mortality was significantly higher in each experimental group than in the controls (Chi-square test, $P < 0.016$ for all individual comparisons), suggesting that even a relatively low *Y. pestis* does ($10^3$ organisms) increased the risk of dying. Overall, 29 of 33 (88%) polecats exposed to *Y. pestis* died (Table 1). All polecats that died, regardless of dose, died on or before day 12 post-challenge. Considering only animals that died from *Y. pestis* infection, the average day of death was significantly

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TABLE 1. Numbers of Siberian polecats that died after exposure to *Yersinia pestis*, and mean ± SE days to death.

| Route and dose | Died/total exposed | Day of death[a] |
|---|---|---|
| Subcutaneous | | |
| $10^3$ | 5/7 | $7.6^1 \pm 1.7$ |
| $10^7$ | 7/7 | $3.6^2 \pm 0.3$ |
| $10^{10}$ | 7/7 | $4.5^2 \pm 0.9$ |
| Mouse-fed | 10/12 | $4.0^2 \pm 0.5$ |
| Total | 29/33 | Overall $4.6 \pm 0.5$ |

[a] Means within a column followed by different numerical superscripts are significantly different (Fisher's protected least significant difference, $P < 0.05$).

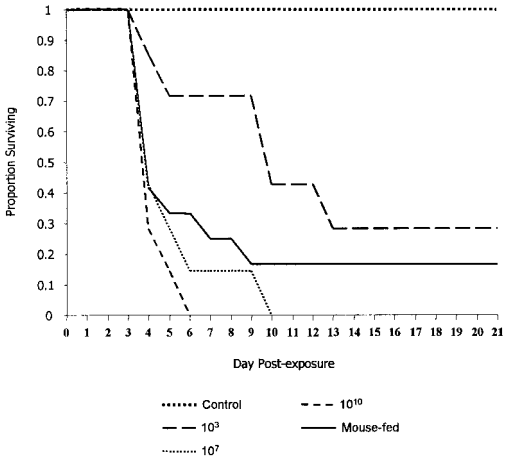



FIGURE 1. Comparison of the proportion survivorship of Siberian polecats exposed to *Yersinia pestis* via subcutaneous injection of $10^3$, $10^7$, $10^{10}$ organisms, or via ingestion of a plague-killed mouse. The proportion alive at the end of each day is presented. See text for statistical comparisons among groups.

higher in the $10^3$ group than all other groups (Table 1).

Within the subcutaneously challenged group, survival time fell progressively and significantly with increasing *Y. pestis* does (Fig. 1). Animals receiving the lowest dose ($10^3$) had significantly longer survival times than those receiving the two higher doses ($10^7$ and $10^{10}$) and those ingesting plague-killed mice (Kaplan Meier log rank test, $P < 0.001$). Gender and founder lines were not significantly related to survival times of Siberian polecats (log rank test, $P > 0.05$).

Clinical signs of illness that were significantly associated with shorter survival times over all subcutaneous *Y. pestis* doses were greater than 10% weight loss by day 3 post-challenge (log rank test, $P = 0.012$), low fecal output (log rank test, $P = 0.015$) and lethargy (log rank test, $P < 0.001$).

Maximum body temperature ($T_B$) attained by experimental animals was significantly higher than that attained by the control group (ANOVA, $P < 0.001$), however there were no significant differences in maximum $T_B$ among the experimental groups (ANOVA, $P = 0.42$). Two animals attained $T_B$ of over 41.7 C before they were euthanized. Of the 29 polecats that died, 27 had $T_B$ over 40.6 C.

The average increase in $T_B$ above baseline (day 0) of the experimental groups was significantly higher than that of the control group (ANOVA, $P < 0.001$). Animals that survived the *Y. pestis* challenge exhibited a lower maximum $T_B$ than those which died (ANOVA, $P = 0.034$). Similarly, the maximum increase in body temperature above baseline of animals that survived was less than that of animals which died ($P = 0.008$). Body temperature of three survivors of the initial challenge increased by about 1.4 C after the second challenge, whereas $T_B$ of the five polecats exposed to *Y. pestis* for the first time on day 28 increased by about 2.8 C; that difference in $T_B$ increase approached significance (ANOVA, $P = 0.06$).

Among the challenged animals, *Y. pestis* dose significantly affected the onset of lethargy (log rank test, $P = 0.026$), occurrence of low fecal output (log rank test, $P = 0.001$), and green diarrhea (log rank test, $P = 0.017$), with onset of signs in animals receiving the $10^3$ dose occurring later than in those receiving $10^7$ or $10^{10}$ doses of *Y. pestis*. Other signs, including wheezing, coughing, dyspnea, bloody diarrhea, and ataxia, did not appear strongly related to either survival time or *Y. pestis* dose. At no time during the trial period did any controls present with lethargy, ataxia, or significant weight loss, or show any respiratory or gastrointestinal signs.

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Four of 33 *Y. pestis*-exposed polecats exhibited nasal discharge prior to death; two were in the $10^3$-dose group and two were in the $10^{10}$-dose group. Four of seven $10^3$-dose animals and three of seven $10^7$-dose animals developed red, swollen areas around the inoculation site by day 3. All $10^{10}$-dose animals developed similar lesions by day 3, and one $10^{10}$-dose polecat developed open sores around the injection site.

Our assessment of blood parameters was hindered because most animals became moribund rapidly and died. Data suggest the development of anemia and thrombocytopenia, with lymphocytosis, neutrophilia and a left shift in *Y. pestis*-challenged animals. Those signs are consistent with a response to an acute bacterial infection.

*Yersinia pestis* organisms were clearly visible on blood smears of three polecats (one each from the $10^7$, $10^{10}$, and mouse-fed groups); all three of animals died on day 3 post-challenge, after blood collection.

All pre-infection serum samples were negative. Six of 33 polecats exposed to *Y. pestis* developed antibody titers, including four animals exposed to $10^3$ organisms and two animals that ingested infected mice. Two of the seropositive $10^3$-dose animals subsequently died before the next scheduled bleeding; blood collected at the time of death was seronegative. Titers were first detected 9 days after exposure (range: 1:64 to 1:2048, $n = 6$), and peaked 21 days after exposure (range: 1024 to 2048, $n = 4$). Titers initially decreased after the surviving polecats were re-exposed to *Y. pestis* on day 28. On days 9 and 15 after the second exposure, all three polecats exhibited titers of 1:256. Titers again peaked on day 21 post-exposure (range 1:1024 to 1:2048, $n = 3$). Titers for those three animals ranged from 1:256 to 1:512 at the final blood draw, 53 days after the second exposure.

The most common gross lesions observed at necropsy were an enlarged, rounded spleen (23 of 29 animals) and petechial hemorrhages on the skin (21 of 29 animals). Petechial hemorrhage occurred in both subcutaneously injected animals and those fed plague-killed mice. Other noteworthy abnormalities included hemorrhage in the intestinal mesenteries (15 of 29 animals) and yellow-tinged liver (9 of 29 animals). The inoculation site of most polecats was marked by hemorrhage and necrosis (15 of 19 inoculated animals).

## DISCUSSION

Plague killed a high percentage (88%) of Siberian polecats in this study, regardless of dose or route of exposure. In previous studies, all ($n = 8$) domestic ferrets survived subcutaneous challenge with up to $10^7$ *Y. pestis* organisms, as did two Siberian polecats exposed to 12 or 120 organisms (Williams et al., 1991). More recently, E. S. Williams (pers. comm.) exposed black-footed ferret × Siberian polecat hybrids to *Y. pestis* by subcutaneous injection or by feeding plague-killed mice. All hybrids dosed with $10^3$ *Y. pestis* survived, as did three of four challenged with $10^4$ organisms; nine of twelve orally-dosed hybrids became infected and died, however. The mortality rate of orally-challenged hybrids was therefore very similar to that seen in our pure Siberian polecats. Subsequent work by E. S. Williams (pers. comm.) resulted in 100% mortality of four black-footed ferrets exposed to about 800 organisms of *Y. pestis* by subcutaneous injection.

Survival time of species susceptible to plague has been shown to vary with route of exposure and dose (Poland and Barnes, 1979). Mean survival time (4.5 days) of Siberian polecats in this study varied with dose, but was comparable to that observed in a variety of susceptible species, including: black footed ferrets, black-footed ferret × Siberian polecat hybrids (E. S. Williams, pers. comm.), domestic cats (Gasper et al., 1993) rock squirrels (*Spermophilus variegatus*) (Quan et al., 1985), and grasshopper mice (*Onychomys leucogaster*)

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(Thomas et al., 1988, 1989). Mortality in humans typically occurs in 5 to 7 days in untreated cases of bubonic plague, and in 1 to 2 days following respiratory exposure (Poland and Barnes, 1979).

It has been suggested that a subcutaneous injection of *Y. pestis* may not truly mimic a flea bite (Perry and Fetherstone, 1997) independent of the possible difference in the number of organisms injected. At the typical body temperatures of a flea, which roughly correspond to ambient temperature less than 37 C, the bacterium does not produce the F1 virulence factor, and needs first to be phagocytized by the host's WBCs. After reproducing in the host's cells at approximately 37 C, the bacterium possesses the F1 protein, and becomes fully virulent. Our *Y. pestis* inoculum was incubated at 37 C in the lab, so therefore may have caused a greater (or at least faster) mortality than may be seen after a true flea bite. Other *Y. pestis* virulence factors exist, and their production may also differ between lab-reared and flea-incubated *Y. pestis.*

In our study, polecats with prior plague exposure appeared to be protected against a second challenge; because of the small sample size these results must be viewed cautiously. All three animals that survived the first challenge with *Y. pestis* survived a subsequent exposure via ingestion of a plague-killed mouse. A small and transient increase in $T_B$ was the only measurable response seen in animals after their second exposure; there were no changes in behavioral, physical, or fecal attributes. Antibody titers in the pre-exposed animals were high before and after the second challenge, so it is likely that at least part of the protection was conferred by adequate humoral immunity. Few experiments (outside of vaccine trials) have looked at the responses of previously plague-challenged animals to re-exposure. Two rock squirrels that were seropositive at capture survived consecutive exposures to approximately $10^4$ and $10^6$ organisms; one of the squirrels maintained detectable antibodies at least 50 mo after the second challenge (Quan et al., 1985).

The time frame of a second *Y. pestis* exposure may influence survival also. It is possible that if enough time elapses between exposures, antibody titers could drop below the level at which they are protective. Our animals were re-challenged within 30 days of their initial exposure, so their antibody titers may have been unnaturally high. The minimum protective level is undetermined for most species, but titers of 1:128 appear to be protective in humans (Poland and Barnes, 1979). Domestic ferrets maintained detectable antibodies for at least 219 d post-exposure (Williams et al., 1991), and our surviving polecats maintained detectable titers for at least 96 days post-exposure.

Generally, seroconversion after *Y. pestis* exposure is associated with a greater chance of survival (Rust et al., 1971; Gasper et al., 1993); however, serological responses are variable within and between species. Siberian polecats that survived exposure to *Y. pestis* in our study developed antibodies, and two animals that died also seroconverted; seroconversion was not detected in the other 27 polecats that died. Three black-footed ferret × Siberian polecat hybrids that survived subcutaneous challenge with $10^3$ *Y. pestis* seroconverted, as did three of four hybrids dosed with $10^4$ organisms; one orally-challenged hybrid that died seroconverted, but the three orally-challenged survivors failed to seroconvert (E. S. Williams, pers. comm.). All dogs exposed to *Y. pestis* seroconverted by day 8 post-exposure, and all survived (Rust et al., 1971). Two of five cats exposed by Rust et al. (1971) seroconverted and survived, one cat seroconverted and died, and two cats died without seroconverting. Ten of ten cats that survived an oral challenge seroconverted, while four of six animals that died had seroconverted (Gasper et al., 1993). Two cats died in that study without seroconverting. Rodents (grasshopper mice and rock squirrels) that survived *Y. pestis* challenges were also more likely to

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have antibodies than those that died, but some animals that survived did not seroconvert (Quan et al., 1985; Thomas et al., 1988), a phenomenon also seen in black-footed ferret × Siberian polecat hybrids (E. S. Williams, pers. comm.) but not observed in the present study.

Two polecats dosed with $10^3$ *Y. pestis* were seropositive early on day 9, but subsequently died on days 9 and 12. Serum taken on the day of death, however, revealed no detectable PHA titer. This suggests that in the presence of circulating antigen due to overwhelming disseminated bacterial infection, the antibody present may not have been able to effectively protect the animal. Furthermore, the antibody could have complexed with the massive amounts of free antigen, thus effectively acting as an inhibitor to the PHA test. Unfortunately, the final serum samples from those animals were not of sufficient volume to test for the presence of free F1 antigen. High levels of circulating antigen could also account for the initial decline in antibody titers observed after the three survivors were re-exposed to *Y. pestis*.

Few of the clinical signs of plague we noted were significant predictors of impending death, and none were unique to *Y. pestis* infection. Animals that lost >10% of their body mass in the first 3 d after challenge, animals that had low fecal output, and those that became lethargic had significantly shorter survival times. Those three signs are probably closely related, and may reflect anorexia after polecats were infected. Food intake was not quantified during this study, but it was apparent, based upon rate of refilling feed dishes, that *Y. pestis*-challenged animals usually decreased their food intake considerably.

Fever was an excellent, albeit nonspecific, indicator of plague infection, but did not predict death. Gasper et al. (1993) reported that domestic cats dying from plague attained a $T_B$ of 40.7 to 41.3 C, while those that became ill but survived had $T_B$ of 40.2 to 41.2 C, supporting this view. Results of the present and previous studies suggest that body temperature alone should not be used as a criterion for euthanasia in laboratory plague-challenge trials. Animals that survived plague exposure often exhibited body temperatures similar to animals that died. Animals exhibiting high $T_B$ in combination with abnormal behavioral and/or physical signs (e.g., lethargy and low fecal output) are not likely to survive, however, and interventive treatment or euthanasia should be considered in those cases.

Because high mortality rates were observed in all the plague-challenged groups, we were not able to calculate a minimum lethal dose or an $LD_{50}$. However, the $LD_{50}$ is likely less than $10^3$ with this defined strain, and future plague-challenge experiments should include much lower doses if they are to determine an $LD_{50}$. Our results further indicate that a subcutaneous exposure to $10^4$ to $10^5$ *Y. pestis* organisms of the strain used in our study would be a reasonable challenge dose in plague vaccine trials, because exposure to $10^7$ *Y. pestis* was fatal to all polecats exposed to that dose, and a challenge dose should not kill all exposed animals.

Because Siberian polecats are highly susceptible to infection with the virulent strain of *Y. pestis* administered subcutaneously or via a plague-killed rodent, the Siberian polecat is a useful surrogate for the black-footed ferret in plague-challenge trials. The purebred polecats used in our study did not appear to exhibit resistance to *Y. pestis* postulated at the outset, but succumbed to infection rapidly. Unfortunately, no published reports compare Siberian polecats and black-footed ferrets exposed to the same doses of virulent *Y. pestis* at the same time. However, in light of the similar responses of black-footed ferrets and Siberian polecats to accidental ingestion of plague-infected prairie dog meat in Pueblo, the 100% mortality seen in parenterally challenged black-footed ferrets (E. S. Williams, pers. comm.), and

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the high mortality seen in the present study, we expect black-footed ferrets to be at least as susceptible to plague as Siberian polecats. Hence, it is likely that black-footed ferrets are at risk for plague infection in the field via flea bites as well as by ingestion of plague-infected prey and carrion.

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