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Plague as a Mortality Factor in Canada Lynx (*Lynx canadensis*) Reintroduced to Colorado

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ABSTRACT: As part of a species recovery program, 129 Canada lynx (*Lynx canadensis*) originating from British Columbia, the Yukon, Manitoba, and Quebec, Canada, and Alaska, USA, were reintroduced to southwestern Colorado, USA, from 1999 to 2003. Of 52 lynx mortalities documented by October 2003, six lynx, including a female and her 5-mo-old kitten, had evidence of *Yersinia pestis* infection as determined by fluorescent antibody test and/or culture. Postmortem findings in these lynx were characterized by pneumonia, ranging from acute suppurative pneumonia, to multifocal necrotizing pneumonia, to fibrinous bronchopneumonia. Histopathologic examination of lung revealed multiple areas of inflammation and consolidation, areas of edema and hemorrhage, and bacteria surrounded by extensive inflammation. Spleens had severe lymphoid depletion and hypocellular red pulp. Lymphadenomegaly was observed in only one plague-affected lynx. We hypothesize that these Canada lynx were exposed to *Y. pestis* by infected prey, and these are the first reports of plague in this species.

Key words: Canada lynx, *Lynx canadensis*, mortality factors, plague, *Yersinia pestis*.

The Canada lynx (*Lynx canadensis*) in the contiguous United States is listed as a threatened species under the Endangered Species Act (US Fish and Wildlife Service, 2000). Reintroduction of lynx to the southern portions of their historic range is one approach being used to recover the species. However, evaluation of the current suitability of the southern portions of the historic range is critical because habitat conditions within these areas are markedly different from what they were in the last century. Habitat changes have resulted from changing land use patterns, human encroachment, and the introduction of exotic diseases.

Plague, a flea-borne disease caused by

the bacterium *Yersinia pestis*, was introduced into the United States near the turn of the twentieth century (Biggins and Kosoy, 2001). Sylvatic plague is maintained by wild rodent populations in semiarid areas of North America. The bacterium spreads to resistant nonrodent hosts, including some carnivores, and occasionally to susceptible nonrodent hosts, such as humans and felids (Gasper and Watson, 2001). Domestic cats exhibit high mortality in response to experimental plague challenges (Gasper et al., 1993), and a single fatal case of plague has been reported in a bobcat (*Lynx rufus*) in Texas (Tabor and Thomas, 1986). Antibodies to *Y. pestis* have been reported in other wild felids; an antibody prevalence of 40% is reported for mountain lions (*Felis concolor*) sampled in California (Paul-Murphy et al., 1994). Cases of plague have not been previously reported in lynx; however, a low antibody prevalence (2/39 lynx sampled) to *Y. pestis* has been reported from lynx sampled in Montana (Biek et al., 2002).

In an effort to restore the species to its historic southern range, free-ranging lynx were trapped in British Columbia, the Yukon, Manitoba, and Quebec, Canada, and Alaska, USA, then transported and released in southwestern Colorado. A total of 129 lynx were released in 1999, 2000, and 2003 (Shenk, 2004). Satellite and very high frequency (VHF) collars were placed on lynx to monitor movements and detect mortalities.

During the period February 1999 through October 2003, 52 mortalities were detected. These included 31 carcasses that

TABLE 1. Vital information and diagnostic test results for six plague-affected Canada lynx from Colorado. *Yersinia pestis* tests performed by the Centers for Disease Control and Prevention, Fort Collins, Colorado, included fluorescent antibody test on lynx tissues (FA), fluorescent antibody test following mouse inoculation (mouse FA), and culture.

Date sample collected	Lynx ID	Sex	Estimated age (mo)	Plague confirmatory test
24 May 2000	BC00F03	F	12	FA; mouse FA; culture lung & spleen
17 August 2000	YK00F06 ^a	F	27	FA; mouse FA; culture lung
14 September 2000	AK00F01	F	16	FA; mouse FA
2 February 2001	YK00F12	F	21	Mouse FA; culture lung
10 October 2003	YK00F16	F	65	Mouse FA; culture lung
10 October 2003	CO03F07 ^b	F	5	Mouse FA; culture lung

^a Death due to trauma from vehicle collision.

^b Offspring of YK00F16.

were suitable for complete postmortem diagnostic examination, and of these, cause of death was determined on 28. *Yersinia pestis* was detected in six of these carcasses using the fluorescent antibody test (FA) and/or culture (Centers for Disease Control and Prevention, Fort Collins, Colorado, USA; Table 1). Plague appeared to be the direct cause of death in five of the six affected lynx, including a female and her 5-mo-old kitten. Each of these lynx exhibited pneumonia, ranging from acute suppurative pneumonia, to multifocal necrotizing pneumonia, to fibrinous bronchopneumonia. Histopathologic examination of lung revealed multiple areas of inflammation and consolidation. Areas of consolidation were characterized by alveolar septal capillaries filled with bacteria surrounded by extensive infiltration of neutrophils. In some cases these areas were surrounded by large zones of edema and hemorrhage. Fibrin was apparent in two of the cases. Splenomegaly was not noted grossly in any of the cases; however, spleens that were examined histologically from four lynx had severe lymphoid depletion and hypocellular red pulp.

Lymphadenomegaly was observed in only one plague-affected lynx (YK00F12). The face of this lynx appeared swollen, and a moderate amount of edema was present within the subcutaneous tissue of the throat and anterior aspects of the

trachea extending dorsally to the base of the ears. The associated mandibular lymph nodes were enlarged and reddened. On histopathologic examination, these nodes were characterized by edema accompanied by infiltration of neutrophils and severe depletion of lymphocytes. Interestingly, on the tongue of this lynx, small focal areas of necrosis were apparent grossly; these were confirmed on histopathologic examination. This finding suggests that oral inoculation of *Y. pestis* may have occurred in this lynx.

The remaining plague-affected lynx (YK00F06) died from trauma after being struck by a car. Gross lesions suggestive of plague were not observed on postmortem examination; however, the carcass had undergone moderate to severe postmortem autolysis, and plague was not initially included in the differential diagnoses. On histopathologic examination, sections of lung had mild congestion with mild alveolar collapse and multifocal to coalescing areas of edema. Presence of *Y. pestis* was detected in lung using FA and culture. We can only speculate as to whether plague would have led to death in this lynx had it not succumbed to fatal trauma. However, clinical signs of illness, such as lethargy and stupor, may have increased the susceptibility of this lynx being struck by a vehicle, and further, had it not died of trauma, it may have died from pneumonic plague. A review by

Eidson et al. (1991) reported that only one of nine domestic cats survived pneumonic plague in the absence of antibiotic therapy.

Pneumonic plague is generally an acute disease. In domestic cats, plague is characterized clinically by a triad of lethargy, anorexia, and fever (Eidson et al., 1991). Lethargy and anorexia are serious features of disease in wild carnivores where supportive care is not available and where unsuccessful hunting or anorexia can lead into a spiral of starvation or increased susceptibility to other mortality factors. Cause of death of reintroduced lynx was undetermined in 24 cases, and it is possible that plague was a direct or indirect cause of death in some of these. We attempted to test this possibility by testing marrow samples recovered from six highly decomposed carcasses; however, all tested negative on FA for *Y. pestis*.

Based on observed lesions, lynx were most likely exposed during consumption of *Y. pestis*-infected rodents or lagomorphs. Peripheral lymphadenopathy, as would be expected if exposure occurred via flea bite, was not observed. This is consistent with findings in domestic cats in New Mexico, where ingestion was considered a more important route of transmission than flea bites. In those domestic cats, observations of bilateral involvement of submandibular or cervical lymph nodes led to the conclusion that oral inoculation of bacteria through ingestion of infected rodents occurred (Eidson et al., 1991). Cervical lymphadenopathy was not a consistent finding in lynx with plague. Blood cultures were not performed on any of the cases, so bacteremic status cannot be confirmed. If lynx were bacteremic, pneumonic plague may have been secondary to the bacteremia. However, pneumonic lesions could also have been due to inhalation of aerosolized droplets containing *Y. pestis* while lynx were hunting or consuming infected carcasses. In a laboratory study in which black-footed ferrets (*Mustela nigripes*) were challenged with mice infected with

Y. pestis, one ferret died from plague without ingesting the mouse. It was speculated that the ferret was infected when it sniffed or licked the infected carcass (Rocke et al., 2006).

Survival time of plague-susceptible species has been shown to vary with route of exposure and dose (Poland and Barnes, 1979). Gasper and Watson (2001) reported that *Y. pestis* isolates coming directly from a mammalian host produce virulence factors that assist in evading the immune response. Therefore, the virulence is higher in exposure via ingestion or inhalation than from a flea bite.

Antibodies to *Y. pestis* were not detected in these lynx prior to release in Colorado (Biek et al., 2002). However, a healthy adult male lynx from the Colorado reintroduction that was recaptured in Albany County, Wyoming, USA, in March 2001 had a positive antibody titer of 512 using a passive hemagglutination/passive hemagglutination inhibition test (T. M. Shenk, unpubl. data). This lynx remained in good health throughout a 3-wk rehabilitation period prior to rerelease in Colorado. This finding suggests that exposure to *Y. pestis* is not invariably fatal in lynx. A small number of Siberian polecats (*Mustela ermine*) that survived initial *Y. pestis* challenge were protected in a subsequent challenge of ingestion of an infected mouse (Castle et al., 2001). In domestic cats, survival rates were higher in bubonic than pneumonic plague presentations (Eidson et al., 1991). Lynx that are found with antibody titers to *Y. pestis* may have been exposed to low doses of organisms, perhaps via a flea bite.

Limited plague surveillance in Montezuma and LaPlata counties of southwestern Colorado during 2000–04 detected *Y. pestis* infection in domestic cats, Gunnison's prairie dogs (*Cynomys gunnisoni*), and flea pools, as well as these lynx. *Yersinia pestis* infection has also previously been reported in humans within 100 km of the detection in lynx (Colorado De-

partment of Public Health and Environment, 2005; J. Pape, pers. comm.). Plague activity in this area appears most prevalent at elevations of about 1,800–2,700 m but has also been detected >3,000 m (L. Carter, pers. comm.). In Colorado, mean elevation of lynx locations ($n=5,635$ over 5 yr) was 3,170 m (range 1,232–4,310 m) (Shenk, unpub. data). Diet of the reintroduced lynx is composed primarily of snowshoe hare (*Lepus americanus*) and red squirrel (*Tamiasciurus hudsonicus*), species that typically occur at elevations >2,400 m (Shenk, 2004). Although plague was not documented in either of these species in this area from 2000 to 2004, tree squirrels and rabbits are susceptible hosts (Gasper and Watson, 2001). Plague has been detected in cottontail rabbits (*Sylvilagus nuttalli*) in New Mexico, and rabbits have been associated with human plague cases in Colorado (J. Pape, pers. comm.) and other areas in the American West (von Reyn et al., 1976). Thus, lynx may have been exposed via their primary prey. Alternatively, because lynx opportunistically consume a variety of rodents as a minor part of their diet, exposure may have occurred from any of a number of species over a broader elevation gradient that lynx may range.

Lack of initial antibody titers to *Y. pestis* (Biek et al., 2002) and the apparent rarity of plague north of the 50th parallel (Gibbons and Humphreys, 1940; Leighton, 2001; Centers for Disease Control, 2005) indicate these lynx from Canada and Alaska were naïve to plague prior to arrival in Colorado. Additional serologic surveillance for *Y. pestis* in lynx is necessary to determine if lynx commonly mount an immune response following exposure or if *Y. pestis* infections are generally fatal. Should the latter be the case, development of a vaccine would likely be necessary to protect individual lynx. Given the incidence of plague detected in this population, plague may be a significant mortality factor that warrants investigation to determine the

potential for impact on the recovery of lynx in their southern range. Further, these findings provide support for concern regarding the disruption that plague, as an invasive species, is exerting on North American ecosystems (Biggins and Kosoy, 2001).

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