*Journal of Wildlife Diseases*, 47(4), 2011, pp. 888–892


# ASSESSMENT OF A RECOMBINANT F1-V FUSION PROTEIN VACCINE INTENDED TO PROTECT CANADA LYNX (*LYNX CANADENSIS*) FROM PLAGUE

**Lisa L. Wolfe,[1,4] Tanya M. Shenk,[1] Bradford Powell,[2] and Tonie E. Rocke[3]**

[1] Colorado Division of Wildlife, Wildlife Research Center, 317 West Prospect Road, Fort Collins, Colorado 80526-2097, USA

[2] Bacteriology Division, United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland 21702, USA

[3] United States Geological Survey, Biological Resources Division, National Wildlife Health Laboratory, 6006 Schroeder Road, Madison, Wisconsin 53711, USA

[4] Corresponding author (email: lisa.wolfe@state.co.us)

ABSTRACT: As part of an ongoing restoration program in Colorado, USA, we evaluated adverse reactions and seroconversion in captive Canada lynx (*Lynx canadensis*) after vaccination with a recombinant F1-V fusion protein vaccine against *Yersinia pestis*, the bacterium that causes plague. Ten adult female lynx received the F1-V vaccine; 10 source- and age-matched lynx remained unvaccinated as controls. All of the vaccinated and control lynx remained apparently healthy throughout the confinement period. We observed no evidence of injection site or systemic reactions to the F1-V vaccine. Among vaccinated lynx, differences in $\log_{10}$ reciprocal antibody titers measured in sera collected before and after vaccination (two doses) ranged from 1.2 to 5.2 for anti-F1 antibodies and from 0.6 to 5.2 for anti-V antibodies; titers in unvaccinated lynx did not change appreciably over the course of confinement prior to release, and thus differences in anti-F1 ($P=0.003$) and anti-V ($P=0.0005$) titers were greater among vaccinated lynx than among controls. Although our findings suggest that the F1-V fusion protein vaccine evaluated here is likely to stimulate antibody responses that may help protect Canada lynx from plague, we observed no apparent differences in survival between vaccinated and unvaccinated subject animals. Retrospectively, 22 of 50 (44%; 95% confidence interval 29–59%) unvaccinated lynx captured or recaptured in Colorado during 2000–08 had passive hemagglutination antibody titers >1:16, consistent with exposure to *Y. pestis*; paired pre- and postrelease titers available for eight of these animals showed titer increases similar in magnitude to those seen in response to vaccination, suggesting at least some lynx may naturally acquire immunity to plague in Colorado habitats.

*Key words:* Antibody, Canada lynx, *Lynx canadensis*, plague, titer, vaccine, *Yersinia pestis*.

## INTRODUCTION

Plague, caused by *Yersinia pestis*, was introduced into North America in the early 1900s, and its impacts on some native wildlife species since that time have been substantial (Cully, 1993; Wuerthner, 1997; Biggins and Kosoy, 2001; Gasper and Watson, 2001; Biggins et al., 2010). Epizootics in prairie ecosystems have been well documented and probably contributed to the marked declines observed in both prairie dogs (*Cynomys* spp.) and black-footed ferrets (*Mustela nigripes*) over the last century (Cully, 1993; Biggins et al., 2010). Although less extensively studied, it seems likely that plague has impacted other wildlife species as well (Biggins and Kosoy, 2001; Gasper and Watson, 2001; Biggins et al., 2010).

Canada lynx (*Lynx canadensis*) historically resided in Colorado, USA (Fitzgerald et al., 1994), but their numbers were reduced below sustainable levels by the late 1970s. Whether plague played any role in the near disappearance of lynx from Colorado is not known. Regardless of plague's role in the historical decline, this disease emerged as a potentially important source of mortality in the course of efforts to reestablish Canada lynx in southwestern Colorado. Within the first 4 yr after lynx were reintroduced into southern Colorado, six cases of *Y. pestis* infection were confirmed among 52 documented mortalities (12%) both in released and native-born individuals (Wild et al., 2006). Our interest in potentially incorporating plague vaccination into the Colorado Division of Wildlife's lynx reintroduction protocol was

motivated by these findings. Effective vaccines for preventing plague in mammalian species, including felids, have been developed only recently (Heath et al., 1998, Gasper and Watson, 2001; Rocke et al., 2004; Powell et al., 2005). Of these, a recombinant capsular F1-V fusion protein vaccine (Powell et al., 2005) has shown a promising combination of safety and efficacy in black-footed ferret recovery (Rocke et al., 2004, 2008) and appeared potentially useful in lynx restoration as well. We report on assessment of responses to a recombinant F1-V fusion protein vaccine (US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland, USA) in captive lynx being held in southwestern Colorado prior to release as part of a reintroduction program.

## MATERIALS AND METHODS

Our study was conducted during December 2003–April 2004. Subject lynx had been captured in British Colombia or Québec, Canada, and transported to the Frisco Creek Wildlife Rehabilitation Center, Del Norte, Colorado, USA, where they were held in covered pens prior to the study, throughout the study, and until release (Devineau et al., 2010). Lynx were fed primarily domestic rabbits, with their diet occasionally supplemented with a commercial carnivore diet (Dallas Crown, Inc., Kaufman, Texas, USA).

We observed lynx for adverse reactions and tested for seroconversion after vaccination with the F1-V fusion protein. Vaccine study methods were approved by the Colorado Division of Wildlife Animal Care and Use Committee (file 01-2004). Ten adult females received the F1-V vaccine; 10 source- and age-matched lynx (five females and five males) remained unvaccinated as controls. Vaccine was diluted and combined with 0.2% Alhydrogel adjuvant (United Vaccines, Madison, Wisconsin, USA) as described by Heath et al. (1998). The vaccine-adjuvant mixture was rocked gently overnight at 4 C. We administered vaccine (2 ml) via subcutaneous injection in the hindquarter on day 0, and a second (booster) dose was given 27–28 days later. For examination and blood collection, we restrained lynx in a squeeze cage and anesthetized them with medetomidine (0.07 mg/kg) and ketamine (3.5 mg/kg) intramuscularly (IM) by hand injection. After examination and sampling, we antagonized medetomidine effects with atipamezole (5 mg/mg medetomidine) injected IM. We observed vaccinated lynx immediately after vaccination, upon recovery from anesthesia, and daily thereafter for evidence of adverse effects (e.g., localized swelling, depression, or changes in behavior or appetite).

To evaluate serologic responses of vaccinated lynx, we collected blood 28–49 days prior to initial vaccination (in conjunction with entry health examinations), 27–28 days after initial vaccination (in conjunction with booster vaccination), and 46 days after initial vaccination (27–28 days after the booster vaccination, in conjunction with prerelease health examinations). We collected blood samples from controls in conjunction with entry health examinations and again in conjunction with prerelease health examinations 69–104 days later. Serum was separated and stored frozen until assayed. We submitted serum to the Centers for Disease Control and Prevention (CDC, Fort Collins, Colorado, USA) for measurement of antibody titers to *Yersinia pestis* using passive hemagglutination (PHA). Serum was also submitted to the US Geological Survey, National Wildlife Health Center (Madison, Wisconsin, USA) for measurement of antibody titers against *Y. pestis* F1 and V antigens (US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland, USA) using an enzyme-linked immunosorbent assay (Rocke et al., 2004), modified to analyze lynx sera by using horseradish peroxidase–labeled goat anti-cat IgG (1:2,000; Kirkegaard and Perry, Inc., Gaithersburg, Maryland, USA) as the secondary antibody.

For the 10 principal vaccinates and controls, we compared changes in $\log_{10}$ anti-F1 and anti-V reciprocal antibody titers ($\log_{10}$ reciprocal titer hereafter) using a paired Wilcoxon rank sum. For each lynx, the change in titer was calculated by subtracting the $\log_{10}$ reciprocal titer at entry (=prevaccination) from the $\log_{10}$ reciprocal titer measured from samples collected $\geq$69 days later at prerelease (=postvaccination for individuals receiving vaccine). Survival of vaccinated and unvaccinated lynx also was monitored postrelease.

In addition to assessing vaccine reactions and responses, we collected blood from all lynx that were recaptured in Colorado at various times during 2000–08 as part of the reintroduction monitoring program. This sample included both lynx that were part of the experiment and those that were not. Sera from these animals were submitted to the CDC for PHA antibody titers to evaluate

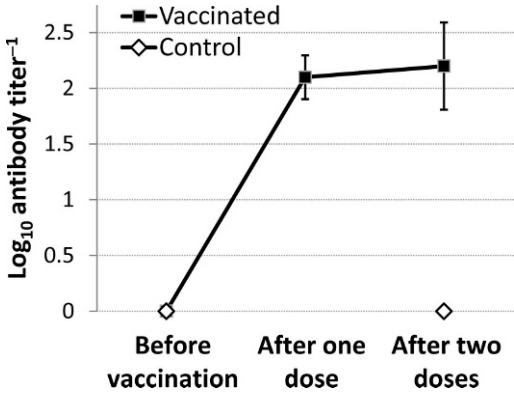


FIGURE 1. Serum antibody response to *Yersinia pestis* in Canada lynx (*Lynx canadensis*) vaccinated with a F1-V fusion protein vaccine. Lines connect mean (±95% confidence interval [CI]) $\log_{10}$ reciprocal hemagglutinating serum antibody titers from lynx measured prior to or after receiving one or two vaccine doses (vaccinated) as compared to lynx not vaccinated (control). Titers to *Y. pestis* were measured by passive hemagglutination. Vertical lines are ±95% CI of mean observations. See Methods for details of vaccination and sampling schedule.

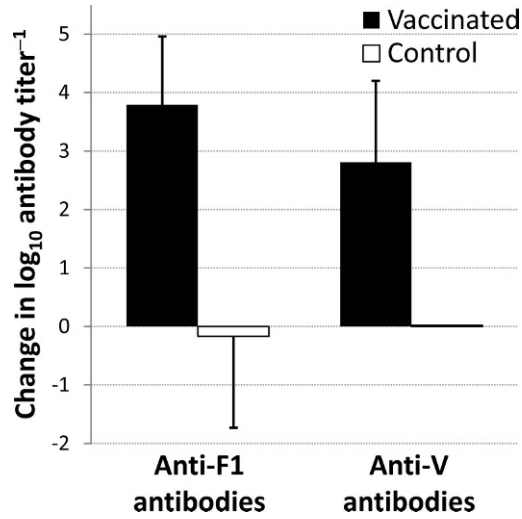


FIGURE 2. Changes in serum antibody titers to *Yersinia pestis* F1 and V antigens in Canada lynx (*Lynx canadensis*) vaccinated with a F1-V fusion protein vaccine. Bars are the mean difference in $\log_{10}$ reciprocal antibody titers (titer after two doses minus titer before vaccination) for lynx receiving two vaccine doses or not vaccinated (control). Titers to *Y. pestis* F1 and V antigens were measured by enzyme-linked immunosorbent assay. Vertical lines are ±95% confidence interval of mean observations.

seroconversion arising from natural exposure to plague. Tissues from recovered carcasses were tested for plague using methods described previously (Wild et al., 2006).

## RESULTS

All of the vaccinated and control lynx remained apparently healthy throughout the confinement period. We observed no evidence of injection site or systemic reactions to the F1-V vaccine.

Serum antibody titers to *Y. pestis* increased in response to vaccination (Fig. 1). Among vaccinated lynx, differences in $\log_{10}$ reciprocal antibody titers measured in sera collected before and after administration of two vaccine doses ranged from 1.2 to 5.2 for anti-F1 antibody titers (mean=3.8; Fig. 2) and from 0.6 to 5.2 for anti-V antibody titers (mean=2.8; Fig. 2). In contrast, titers in unvaccinated lynx did not change appreciably over the course of confinement prior to release (Figs. 1, 2). Differences in both anti-F1 ($P$=0.003) and anti-V ($P$=0.0005) titers were greater in vaccinated animals as compared to controls (i.e., the control and treatment groups were significantly not symmetric around zero difference).

All experimental animals were released within 2 mo after the booster vaccine dose was administered. Survival of vaccinated and control animals was similar 1 yr and 6 yr after release. Of the 10 vaccinated individuals, seven were still alive and three had died by 1 yr postrelease; by 6 yr postrelease three were still alive, five had died, and the status of two was unknown. Of the known causes of death among vaccinated lynx, one was hit by a vehicle and two were killed by other predators. Of the 10 control individuals, seven were still alive 1 yr postrelease, two had died and the status of one was unknown; by 6 yr postrelease, five were still alive, four were dead and the status of one was unknown. The known causes of death among control lynx included gunshot (two) and predation (one). Of eight lynx carcasses (four

vaccinated individuals, four controls) where sufficient tissues were available for testing, *Y. pestis* was detected in none.

Among the 50 unvaccinated lynx that were sampled when captured in Colorado, 22 individuals (44%; 95% confidence interval 29–59%) had positive PHA antibody titers (>1:16) and the remainder had no antibody titers to *Y. pestis*. Of the 22 lynx with positive PHA titers, paired pre- and postrelease sera from eight individuals showed titer increases similar in magnitude to those seen with seroconversion following vaccination; paired titers were not available for the remainder, which included six Colorado-born individuals.

## DISCUSSION

The F1-V fusion protein vaccine stimulated seroconversion in translocated lynx prior to their release in southwestern Colorado (Fig. 1). The magnitude of changes in antibody titers specific to the F1 and V antigens appeared similar to or greater than changes reported in black-footed ferrets vaccinated with the same F1-V fusion protein vaccine (Rocke et al., 2004, 2008). Although our study did not include challenging lynx with *Y. pestis* to explicitly assess the protective efficacy of this vaccine, the anti-F1 and anti-V $\log_{10}$ reciprocal titer responses for all 10 vaccinated lynx resembled those seen in captive black-footed ferrets that survived challenged with *Y. pestis* under laboratory conditions (Rocke et al., 2008). Consequently, it appears likely that vaccination with this F1-V fusion protein vaccine would confer some amount of protection to lynx naturally exposed to plague.

Plague-related mortalities in lynx clearly occur, but our field data also suggest that some lynx may survive natural exposure to *Y. pestis*. Nearly half of the free-ranging lynx reintroduced to Colorado and opportunistically sampled since their release showed serologic evidence of exposure (and seroconversion in eight cases) based on PHA titers. The mechanism (or mechanisms) for natural resistance to or protection from plague in lynx are not clear. The rate of lynx exposure to *Y. pestis* suggested from our field data appears to be considerably higher than would be expected in more northern ranges (Biek et al., 2002; Wobeser et al., 2009), but is comparable to rates estimated for both bobcats (*Lynx rufus*) and mountain lions (*Puma concolor*) in Colorado (Bevins et al., 2009). The apparent difference in exposure compared to lynx elsewhere could be the result of greater plague activity in Colorado, greater use of atypical prey by reintroduced lynx, or some combination of these (Wild et al., 2006).

Based on our findings, we regard the F1-V fusion protein vaccine as likely to stimulate antibody responses that may help protect lynx from plague and relatively unlikely to cause adverse reactions. Although some protection from plague may have been conferred by vaccination, the similarity of short- and long-term survival of vaccinated and unvaccinated lynx and lack of additional cases may indicate that plague was not a particularly important source of mortality during the period when these data were collected. Considering the potential benefits and apparently small risks, however, we believe that this or an antigenically similar vaccine could be useful in Canada lynx reintroduction attempts in other areas where plague is endemic.

## ACKNOWLEDGMENTS

This study was funded by the Colorado Division of Wildlife. We thank S. and H. Deiterich for assistance with handling and care of captive lynx; K. Griffin, L. Baeten and I. Levan for laboratory assistance; T. Spraker for conducting the necropsies; L. Carter and J. Young for laboratory analyses; P. Lukacs for analyzing serology data; and M. W. Miller and anonymous reviewers for providing helpful comments on earlier drafts of our manuscript.

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*Submitted for publication 16 July 2010.*
*Accepted 25 May 2011.*