Serologic Survey for Viral and Bacterial Infections in Western Populations of Canada Lynx (*Lynx canadensis*)

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ABSTRACT: A serologic survey for exposure to pathogens in Canada lynx (*Lynx canadensis*) in western North America was conducted. Samples from 215 lynx from six study areas were tested for antibodies to feline parvovirus (FPV), feline coronavirus, canine distemper virus, feline calicivirus, feline herpesvirus, *Yersinia pestis,* and *Francisella tularensis.* A subset of samples was tested for feline immunodeficiency virus; all were negative. For all other pathogens, evidence for exposure was found in at least one location. Serologic evidence for FPV was found in all six areas but was more common in southern populations. Also, more males than females showed evidence of exposure to FPV. Overall, prevalences were low and did not exceed 8% for any of the pathogens tested. This suggests that free-ranging lynx rarely encounter common feline pathogens.

Key words: Canada lynx, canine distemper virus, feline calicivirus, feline coronavirus, feline herpesvirus, feline immunodeficiency virus, feline parvovirus, FIV, *Francisella tularensis, Lynx canadensis,* serologic survey, *Yersinia pestis.*

The Canada lynx (*Lynx canadensis*) is a mid-sized carnivore inhabiting the boreal forests of North America. Lynx are highly specialized predators that feed primarily on snowshoe hare (*Lepus americanus*). Both lynx and hare populations undergo cycles with peak numbers reached at approximately 10 yr intervals (Elton and Nicholson, 1942). Lynx are still considered abundant across much of their range; however, populations at the southern extent of the distribution are smaller, more scattered, and may not display population cycles (Ruggiero et al., 2000). In 2000, lynx in the contiguous United States were listed as "threatened" under the Endangered Species Act because agencies lacked management plans that offered adequate protection (US Fish and Wildlife Service, 2000). In addition to these conservation concerns, very little is known about the occurrence of infectious diseases in free-ranging populations of lynx. Therefore, the objective of our study was to determine the prevalence of common feline pathogens from different parts of lynx range.

Between 1993 and 2001, 215 lynx were captured from six areas of western North America (Fig. 1, Table 1). Serum samples were collected during capture for ongoing management and research projects. Study areas were characterized by different types of coniferous forest and have been described elsewhere (Bailey et al., 1986; Poole, 1994; Poole et al., 1996; Reynolds, 1999; Squires and Laurion, 2000). All trapping occurred between October and May. Sex and age information was collected for 208 and 150 individuals, respectively. Sex was determined based on external physical characteristics. Kittens (<1 yr) were distinguished from adults (>1 yr) based on dentition and morphological features (Slough, 1996). Because individuals sampled from the same area may be related, our sample is not completely random.

Sera were tested for evidence of exposure to the following agents using standard diagnostic tests (Table 2): feline parvovirus (feline panleukopenia; FPV), feline infectious peritonitis virus/feline enteric corona virus (FIP/FECV), canine distemper virus (CDV), feline calicivirus (FCV), feline herpesvirus (FHV), *Francisella tularensis,* and

FIGURE 1. Sampling areas used for survey of Canada lynx. Area 1: Interior Alaska (USA; 64°05′–64°15′N, 147°27′–147°50′W); Area 2: Kenai Peninsula, Alaska (USA; 60°25′–60°50′N, 150°00′–151°15′W); Area 3: Yukon (Canada; 60°06′–61°52′N, 133°00′–137°06′W); Area 4: Northwest Territories (Canada; 61°30′–61°35′N, 116°45′–117° 30′W); Area 5: British Columbia (Canada; 50°00′–52°09′N, 120°00′–121°52′W); Area 6: Montana (USA; 112°40′–113°50′N, 46°55′–47°50′W). Potential distribution of lynx based on habitat relationships according to Schwartz et al., (2002) is shown in dark.

Yersinia pestis. Tests for feline leukemia virus were not conducted because this viral pathogen is rarely detected in wild felids (Murray et al., 1999). In addition, a subset of samples from Areas 1,3, 5, and 6 were evaluated for infection with feline immunodeficiency virus (FIV) using three serologic techniques. First, sera (n=16) from Area 6 were tested at the Washington State Disease Diagnostic Laboratory (Pullman, Washington, USA) with an enzyme-linked immunosorbent assay (ELISA) designed for detection of domestic cat FIV antibodies. Secondly, lynx sera were tested for antibody recognition of cougar-derived FIV (*Puma concolor*) using an immunoblot assay (n=92) and a flow cytometric assay (n=64) at the University of Montana (Missoula, Montana, USA). Both tests reliably detect FIV antibodies in cougars (Poss, unpubl. data).

Not all sera were tested for evidence of exposure to all agents due to insufficient quantities of sample. Disease prevalence data based on serologic detection is sum-

TABLE 1. Serum antibody prevalence for feline parvovirus (feline panleukopenia; FPV), feline infectious peritonitis virus/feline enteric corona virus (FIP/FECV), canine distemper virus (CDV), feline calicivirus (FCV), feline herpesvirus (FHV), Francisella tularensis, and Yersinia pestis in lynx from six areas of western North America (see Fig. 1 for site information and Table 2 for diagnostic methods used).

Area	Time period	n	FPV	FIP/FECV	CDV	Antibody prevalence			
						FCV	FHV	F. tularensis	Y. pestis
1	1999–2000	30	1/30 (3)[a]	1/30 (3)	0/28	0/23	0/23	1/30 (3)	0/30
2	1993–1999	56	1/56 (2)	1/56	1/53 (2)	0/51	0/53	0/44	0/44
3	1999–2000	38	2/38 (5)	5/38 (13)	0/29	0/35	0/27	0/38	0/38
4	1993–1996	16	2/16 (13)	0/16	3/16 (16)	0/16	0/16	0/14	0/14
5	1999–2000	30	6/30 (20)	3/30 (10)	1/27 (4)	0/30	0/27	0/30	0/30
6	1998–2001	45	5/45 (11)	2/45 (4)	0/43	2/43 (5)	1/42 (2)	0/39	2/39 (5)
Total		215	17/215 (8)	12/215 (6)	5/196 (3)	2/198 (1)	1/188 (1)	1/195 (1)	2/195 (1)

[a] Number of lynx positive/number of lynx tested (%).

TABLE 2.   Diagnostic methods used for determining exposure of Canada lynx to selected viral and bacterial pathogens.

Agent	Test method	Positive threshold	Reference
Feline parvovirus[a]	IFA[b]	25	modified after Helfer-Baker et al. (1980)
Feline coronavirus[a]	IFA[b]	25	Heeney et al. (1990)
Canine distemper virus[a]	VNT[c]	4	Appel and Robson (1973), modified according to Guo et al. (1986)
Feline calicivirus[a]	VNT[c]	4	Scott (1977)
Feline herpesvirus[a]	VNT[c]	4	Scott (1977)
Francisella tularensis[d]	MA[e]	128	Brown et al. (1980)
Yersinia pestis[d]	cELISA[f]	4	Chu (2000)
	PH/PHI[g]	10	Chu (2000)

[a] Conducted at Washington State Disease Diagnostic Laboratory, Pullman, Washington.
[b] Immunofluorescence assay.
[c] Virus neutralization test.
[d] Test conducted at Wyoming State Veterinary Laboratory, Laramie, Wyoming.
[e] Microagglutination test.
[f] Competitive enzyme-linked immunoabsorbent assay.
[g] Passive hemagglutination/passive hemagglutination inhibition test (if cELISA positive at 1:4).

marized in Table 1. All antibody titers in the text are reported as reciprocals of the respective serum dilutions (i.e., if the last positive reaction in a specific test was at a serum dilution of 1:128, the titer is reported as 128).

Prevalence of exposure to disease agents tested was low in lynx from all areas (Table 1). All samples were negative for FIV antibodies. For each of the other pathogens, there was evidence for exposure in at least one area. However, prevalence within a study area was less than 20% for any agent. Also, the cumulative prevalence for a pathogen across study areas did not exceed 8%. The only agent for which evidence was detected in all six populations was FPV. Together, these results suggest that lynx in the sampled areas have a low level of exposure to common infectious diseases of felids.

The effects of regional distribution, sex, and age group on antibody prevalence for FPV and FIP/FECV were tested with Fisher's exact test (two sided). For other pathogens, prevalences were not high enough for such an analysis. To determine if prevalence differed regionally, results from Areas 1–4 and Areas 5–6 were pooled.

Overall, FPV prevalence in the two southern populations was higher than further north (11 of 75 in the south vs. six of 140 in the north, $P=0.014$). This regional difference may be related to the distribution of other wild felid species or occurrence of common sources of infection (e.g., domestic cats). Previous serologic surveys have revealed widespread and frequent exposure of cougars to FPV (Roelke et al., 1993; Paul-Murphy et al., 1994). Feline parvovirus infections have also been described in bobcats (*Lynx rufus*; Wassmer et al., 1988). It is possible that southern lynx are more frequently exposed to parvoviruses from cougars or bobcats, species that do not occur in the northern lynx range. On the other hand, parvovirus infections occur in many carnivores and some virus genotypes may be able to infect multiple species. It cannot be determined serologically whether lynx with antibodies against FPV have all encountered the same viruses because they are antigenically very similar (Steinel et al., 2001).

Antibody prevalence to FPV was significantly higher in males than females (12/107 vs. 3/101, $P=0.030$). As in most felids, the home ranges of male lynx tend to be larger than those of females (Slough and

Mowat, 1996; Squires and Laurion, 2000), which may increase the probability of encountering disease agents, including FPV. A higher degree of territory-marking behavior could also predispose males to FPV infection because virus transmission occurs mainly through the fecal-oral route (Reif, 1976). None of 29 kittens tested had evidence of exposure to FPV compared to 14 of 129 adults. However, this difference was not statistically significant ($P=0.220$).

The fact that evidence of FPV exposure was detected in all six study areas suggested that this virus is enzootic in wild lynx populations. Antibody titers of lynx in Alaska (Areas 1–2) were low (25). However, some lynx in the other areas (Areas 4–6) had high titers ($\geq 3,125$) indicative of recent FPV infection or re-exposure.

Exposure to FIP/FECV was confirmed in all areas except Area 4. The three positive lynx from Areas 5 had high antibody titers (125–625) suggesting they were recently exposed. In contrast, antibody titers were low (25) in the other nine positive animals. The three positive lynx were 1–2 yr old and were captured in 2000. All lynx sampled in the same area in 1999 were negative. Together these data suggest a recent increase of feline coronavirus exposure in Area 5. None of the seropositive animals exhibited clinical signs. Thus, the significance of this increase in feline coronavirus exposure in Area 5 cannot be determined. Prevalence of FIP/FECV did not vary with sex (seven of 107 males vs. five of 101 females, $P=0.769$), age group (nine of 129 adults vs. two of 21 kittens, $P=0.653$), or region (five of 75 in the south vs. seven of 140 in the north, $P=0.756$).

Evidence for exposure to FHV and FCV was found only in one and two individuals, respectively. In all three cases, antibody titers were low (6–48). Sensitivity and specificity of the employed tests for lynx has not been determined, so it is possible that the actual prevalence of infection with these agents may be somewhat higher or lower than reported. However, it is noteworthy that the viruses that are absent or at low prevalence in lynx populations (CDV, FHV, FCV, and FIV) are those that require close contact for transmission and do not persist well outside a host. In contrast, infections with FPV and FIP/FECV, which can persist temporarily in the environment, were more commonly detected. Thus, the solitary life style of the lynx may explain the observed low prevalence of some common feline pathogens.

Only two lynx, both from interior Alaska, had antibodies against *F. tularensis.* This bacterium is distributed throughout North America, including Canada and Alaska (Mörner and Addison, 2001). Serologic prevalences of 10–25% have been reported for wolves (*Canis lupus*) from Alaska and coyotes (*Canis latrans*) from Wyoming (Zarnke and Ballard, 1987; Gese et al., 1997). Moreover, snowshoe hares, the primary prey of the Canada lynx, are considered a common host for *F. tularensis* (Jellison, 1974; Morton, 1981). Thus it seems likely that lynx would come into frequent contact with the agent. *Francisella tularensis* infections in Scandinavian hare populations peak in late summer and early fall (Mörner et al., 1988). If this seasonal pattern holds for North American hare, lynx may be less likely to encounter the agent in the winter when our sampling was conducted. However, this explanation would also require that *F. tularensis* infection in lynx generate a short-lived humoral response. Although the longevity of tularemia antibody titers following *F. tularensis* infection in lynx is unknown, antibodies can be detected in naturally infected humans several years postinfection (Ericsson et al., 1994). Thus, the low prevalence of serologic evidence for *F. tularensis* in lynx is difficult to explain.

The two lynx with evidence of exposure to *Y. pestis* originated in Montana, the southernmost area examined. This result is concordant with the known distribution of plague, which has only occasionally been observed north of the Canadian border (Centers for Disease Control, 1998). In contrast, carnivore populations further

south frequently show evidence of exposure (Paul-Murphy et al., 1994; Gese et al., 1997).

The low prevalence of antibodies against several common pathogens across a large geographic area suggests that infectious diseases rarely occur in lynx populations. However, we have to caution that lack of serologic evidence of disease does not necessarily equate with a lack of exposure. Specific data on longevity of antibody responses or on the sensitivity of the tests used are unavailable for lynx. Also, a low serologic prevalence for a pathogen may be related to exposed lynx developing clinical disease and dying. It is therefore possible that the current survey underestimated the true degree of exposure to the pathogens tested. However, the most parsimonious explanation for the low prevalences observed remains that free-ranging lynx have only minimal exposure to the agents included in this study.

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