

EPIZOOTIOLOGIC INVESTIGATIONS OF SELECTED INFECTIOUS DISEASE AGENTS IN FREE-RANGING EURASIAN LYNX FROM SWEDEN

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ABSTRACT: Serum samples from 106 Eurasian lynx (*Lynx lynx*) from across Sweden, found dead or shot by hunters in 1993–99, were investigated for presence of antibodies to feline parvovirus (FPV), feline coronavirus, feline calicivirus, feline herpesvirus, feline immunodeficiency virus, *Francisella tularensis*, and *Anaplasma phagocytophila*, and for feline leukemia virus antigen. In addition, tissue samples from 22 lynx submitted in 1999 were analyzed by real-time polymerase chain reaction (PCR) to detect nucleic acids specific for viral agents and *A. phagocytophila*. Except for FPV antibodies in one lynx and *A. phagocytophila* in four lynx, all serology was negative. All PCR results also were negative. It was concluded that free-ranging Swedish lynx do not have frequent contact with the infectious agents considered in this study.

Key words: *Anaplasma phagocytophila*, Eurasian lynx, feline viruses, *Francisella tularensis*, *Lynx lynx*, serologic survey, serology.

INTRODUCTION

The Eurasian lynx (*Lynx lynx*) is locally endangered and strictly protected in many European regions. In Sweden, the species is now found in most parts of the country north of 60° latitude, and in 2001, the population number was estimated at about 1,500 individuals.

Although Eurasian lynx have been studied intensively in different countries during the past decade, little is known about infectious diseases in this species. Except for sarcoptic mange, which commonly affects lynx in areas of endemic disease in red fox (*Vulpes vulpes*; Holt and Berg, 1990; Mörner, 1992a; Ryser-Degiorgis et al., 2002), few isolated cases of infectious diseases have been reported in free-ranging Eurasian lynx. Notable disease reports include rabies (e.g., Fernex, 1976; Schneider et al., 1989; Stahl and Vandell, 1999; Tschirch, 2001), feline parvovirus (FPV) infections (Stahl and Vandell, 1999; Schmidt-Posthaus et al., 2002), Borna disease (Degiorgis et al., 2000), and notoedric mange (Ryser-Degiorgis et al., 2002). In captive individuals, feline infectious peri-

tonitis (Hyslop, 1955) and a cowpox virus infection (M. Bennett, pers. comm., cited in Tryland et al., 1998) have been reported. Antibodies to orthopoxvirus were demonstrated in free-ranging Eurasian lynx from Fennoscandia (Tryland et al., 1998).

A study performed in Sweden that included more than 500 lynx collected over 20 yr did not reveal clinical cases of infection with common feline pathogens (M.-P. Ryser-Degiorgis et al., unpubl. data). To our knowledge, there is no available information about the prevalence of exposure to common feline viruses and other infectious agents in free-ranging Eurasian lynx, although several viruses of domestic cats also are known to exist in nondomestic felid populations.

Feline parvovirus infections occur in all feline species in zoos (Gass, 1987). Feline coronavirus (FCoV) infection/feline infectious peritonitis (FIP) has been described in several wild feline species (e.g., Hyslop, 1955; Heeney et al., 1990; Roelke et al., 1993; Hofmann-Lehmann et al., 1996). Feline calicivirus (FCV) occurs worldwide in domestic cats, and it has been suggested that all members of the Felidae are sus-

ceptible. Feline calicivirus has been demonstrated in several large felids in captivity and in the wild (Roelke et al., 1993; Paul-Murphy et al., 1994; Hofmann-Lehmann et al., 1996). Feline herpesvirus (FHV) is considered pathogenic to most wild felids (Fowler, 1986).

Antibodies to FPV, FCV, and FHV were found in free-ranging Canada lynx (*Lynx canadensis*; Biek et al., 2002), bobcats (*Lynx rufus*; Fox, 1983), European wildcats (*Felis sylvestris sylvestris*; Artois and Remond, 1994; Daniels et al., 1999; Leutenegger et al., 1999a), and other feline species. Antibodies to FCoV have been reported in free-ranging Canada lynx (Biek et al., 2002).

Antibodies reacting with feline immunodeficiency virus (FIV) antigens were found in at least half of the species in the family Felidae (Carpenter and O'Brien, 1995). In free-ranging populations, exposure to FIV has been documented in several large felids from North America (Barr et al., 1989; Olmsted et al., 1992; Roelke et al., 1993) and Africa (e.g., Olmsted et al., 1992; Brown et al., 1993; Hofmann-Lehmann et al., 1996).

Infections with feline leukemia virus (FeLV) and resulting disease are rare in nondomestic felids (e.g., Rasheed and Gardner, 1981; Jessup et al., 1993; Sleenman et al., 2001). Serologic studies in free-ranging populations of wild feline species from North America, Japan, and Africa demonstrated that FeLV infections are rare, if present at all, in these populations (Mochizuki et al., 1990; Roelke et al., 1993; Hofmann-Lehmann et al., 1996). However, FeLV infection has been detected in several free-ranging populations of European wildcats (McOrist, 1992; Artois and Remond, 1994; Daniels et al., 1999; Leutenegger et al., 1999a), suggesting that the situation could be different depending on the study area, the species, or both.

Francisella tularensis is a highly infectious bacterium causing tularemia. It is primarily a disease of lagomorphs and rodents (Mörner et al., 1988) but has been

reported from 190 mammalian species, including humans (Hopla, 1974; Pfahler-Jung, 1989). Tularemia occurs frequently among varying hares (*Lepus timidus*) and small rodents in Fennoscandia (Mörner, 1992b). Lynx often feed on hares (Haglund, 1966; Nowicki, 1997) and sometimes harbor ectoparasites including ticks (M.-P. Ryser-Degiorgis et al., unpubl. data). Because infection can occur by contact with blood or tissues of infected animals, ingestion of contaminated meat, inhalation of infected aerosols or particles, or bites of blood-feeding arthropods (Hopla, 1974; Jellison, 1974), lynx could also become infected with *F. tularensis*. Antibodies to *F. tularensis* were detected in free-ranging bobcats and Canada lynx (Heidt et al., 1988; Biek et al., 2002).

Anaplasma phagocytophila (previously known as *Ehrlichia phagocytophila*; Dummer et al., 2001) is the cause of tickborne fever in domestic ruminants in several countries in Europe (Woldehiwet, 1983). Compared with domestic animals and humans, little is known about anaplasma and ehrlichial infections among wild mammals. In Switzerland, the presence of *A. phagocytophila* was reported in red foxes (Pusterla et al., 1999b) and wild ungulates (Liz et al., 2002). In Scandinavia, infections with *A. phagocytophila* have been documented in dogs, cattle, and horses from Sweden (Olsson Engvall et al., 1996) and in several wild ungulate species from Norway (Jenkins et al., 2001; Stuen et al., 2002).

The main goal of this study was to determine the prevalence of exposure to selected infectious agents known to occur in other feline species in the free-ranging Swedish lynx population. Furthermore, we aimed to compare the results in healthy and diseased lynx to determine whether diseases like sarcoptic mange might be associated with latent viral infections; sarcoptic mange is not considered to be a typical disease of feline species but is the most common infectious cause of death of free-ranging lynx in Sweden (M.-P. Ryser-

Degiorgis et al., unpubl. data). Lesions of mange in lynx are suggestive of an anergic state (Pence and Ueckermann, 2002). Therefore, it has been hypothesized that cases of mange in Eurasian lynx might be linked to immunosuppression.

MATERIALS AND METHODS

Animals and sampling

Since 1995, there has been regulated hunting of the Swedish lynx population, and submission of all lynx carcasses for analysis is compulsory. Lynx shot or found dead are to be submitted within 2 days for complete necropsy to the National Veterinary Institute in Uppsala.

One hundred six animals from most parts of the lynx range in Sweden, including areas with low and high human density, were sampled for this study (Fig. 1). Eighty-seven lynx were sampled in 1999. To enlarge the sample size of diseased lynx, an additional 19 animals found dead during 1993–98 were included in the study.

Eighty-one lynx died by trauma (hunting, traffic accident) and were considered to be healthy. Twenty-five were considered diseased: 17 lynx were severely affected with sarcoptic mange and one with Borna disease (Degiorgis et al., 2000). Five died of trauma but were seropositive for *Sarcoptes scabiei* ($n=1$) or were in poor body condition and had lymphadenopathy ($n=4$). One died during anesthesia and was in a poor body condition. Two juvenile lynx died of starvation.

Sex and age were determined. Age classification was based on sexual maturity and therefore social behavior: 29 were juvenile (first year of life), 37 were subadults (second year of life for females and second and third year for males), and 40 were adults (≥ 2 -yr-old for females and ≥ 3 -yr-old for males).

Blood samples were taken from the thoracic cavity or from the heart and immediately centrifuged. The serum was then stored at -20 C (1993–98) or -70 C (1999). In addition, for the lynx submitted in 1999, specimens of spleen were collected and frozen at -70 C. Not all sera were tested for evidence of exposure to all agents because of insufficient quantities of serum.

Serology

Serum samples were analyzed for the presence of antibodies to FPV, FCoV, FCV, and FHV as described previously (Hofmann-Lehmann et al., 1996). Antibodies to the transmembrane protein of domestic cat FIV were analyzed by enzyme-linked immunosorbent as-

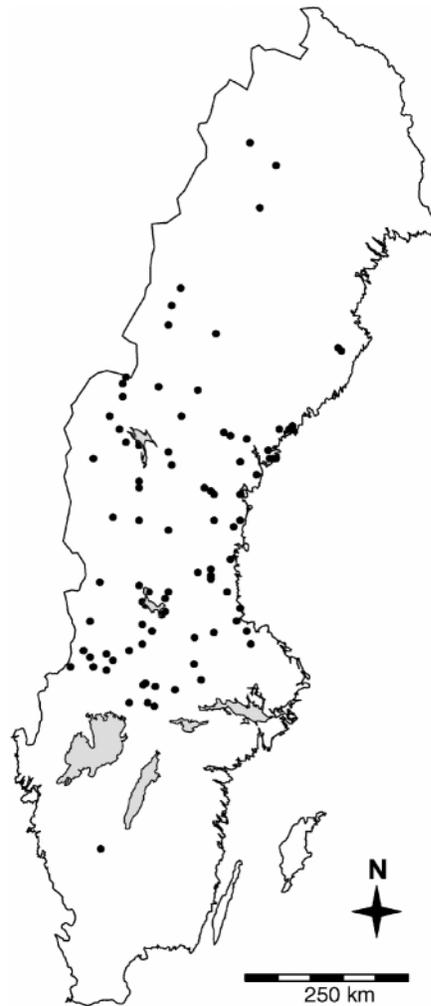


FIGURE 1. Map of the Swedish mainland (grey areas are large lakes), showing the origin of lynx (black dots) examined for infections with selected infectious disease agents.

say (ELISA; Calzolari et al., 1995) and to Gag proteins of FIV by western blot analysis (Lutz et al., 1988). Antibodies to the p45 recombinant *env* gene product (the unglycosylated form of gp70) were measured by ELISA as described with 0.2 μ g of p45 per well for coating (Lutz et al., 1980; Lehmann et al., 1991). Feline leukemia virus p27 antigen was assayed by double-antibody sandwich ELISA (Lutz et al., 1983). Feline leukemia virus western blot analysis was performed as described (Lutz et al., 1988). Detection of antibodies to *F. tularensis* was by tube agglutination test (Anonymous, 1996). Sera were tested for presence of antibodies to *A. phagocytophila* as described by Pusterla et al. (1999a).

In all assays, positive and negative control sera were included. The positive control sera had been collected from domestic cats either vaccinated with the respective antigen or characterized for presence of specific antibodies with the use of well-characterized antigens. As negative controls, sera from nonvaccinated specified pathogen-free (SPF) cats were used.

Polymerase chain reaction

DNA for detection of FHV, FIV, FeLV, and *A. phagocytophila* was extracted from 22 randomly selected spleen tissue samples (QIAamp DNA tissue kit, Qiagen AG, Basel, Switzerland). For detection of viral (FHV), proviral (FIV and FeLV), or rickettsial DNA (*A. phagocytophila*), we used fluorogenic probe-based (TaqMan) real-time polymerase chain reaction (PCR) assays as described (Leutenegger et al., 1999b; Pusterla et al., 1999a; Hofmann-Lehmann et al., 2001; Vöglin et al., 2002).

RESULTS

One of 102 lynx sampled (1%) was seropositive for FPV. It was a subadult male affected by sarcoptic mange. The animal had been shot close to houses in 1994 because of its diseased condition.

Four serum samples were positive for antibodies to FIV transmembrane antigen, and 28 and 58 samples were positive by ELISA for antibodies to FeLV recombinant gp70 or FeLV p27 antigen, respectively. All ELISA-positive samples with reactivity to FIV and 20 FeLV-positive samples were tested by western blot analysis. All were negative. Therefore, all 104 samples were considered to be negative for FIV and FeLV.

Four of 102 serum samples (4%) were positive for antibodies to *A. phagocytophila*. These lynx were two males and two females (two adults and two juveniles) from four different counties. Three were healthy animals killed by hunters in 1999, and one was a juvenile lynx that had died of starvation in 1994.

Antibodies to FCoV, FCV, FHV (102 serum samples tested), and *F. tularensis* (91 serum samples tested) were not be detected. Tissue samples from 22 lynx tested by PCR were negative. Because of the very low prevalence of antibodies to the

selected infectious agents, no difference in prevalence could be detected in healthy versus diseased lynx.

DISCUSSION

Samples originated from healthy and diseased lynx of different sex and age classes from across Sweden and had been collected over a period of 7 yr. Thus, our results should be representative of the infection status of the Swedish lynx population.

The true seroprevalence in this study could have been underestimated because of the quality of the samples examined. Many samples were collected from lynx in various stages of decomposition, and most serum samples were hemolytic. Leutenegger et al. (1999a) investigated whether hemolysis leads to false positive or negative results of serologic tests for FPV, FCoV, FCV, FHV, and FeLV. In comparing hemolytic and fresh blood from domestic cats and wildcats, they found a loss or gain of sensitivity of only 7–10%. Furthermore, detection of antibodies in wildcats found dead and frozen prior to sample collection were an additional proof for adequate quality of samples collected in this manner. Thus, decreased sensitivity of serologic tests associated with sample quality in this study likely does not exceed 10%.

Our results of low seroprevalence to FCoV, FHV, FCV, FIV, and FPV were similar to those found in free-ranging European wildcat populations (McOrist et al., 1991; McOrist, 1992; Artois and Remond, 1994; Daniels et al., 1999; Leutenegger et al., 1999a) and Canada lynx (Biek et al., 2002). The findings in wildcats and Eurasian lynx suggest that infections with FCoV, FHV, FCV, FPV, and FIV are not readily maintained or reach only very low viral loads in free-ranging populations of wild felids in Europe, in contrast to the situation in domestic cats. It has been postulated that the solitary social system of free-ranging wildcats decreases the risk of spread of infectious diseases (Leutenegger et al., 1999a). In agreement with Biek et al. (2002), we suggest that it is also true

for these viral infections in lynx populations because lynx are solitary living animals.

Feline leukemia virus infections were not demonstrated in Swedish lynx in our study, and they have not been found in other larger felid populations (Roelke et al., 1993; Hofmann-Lehmann et al., 1996). In contrast, high prevalences were reported in free-ranging European wildcats (Artois and Remond, 1994; Daniels et al., 1999; Leutenegger et al., 1999a), suggesting that the virus does not represent a major health problem for this species. If intraspecific contacts are rare, an infectious agent can only persist within the population if it causes latent infection, it is non-pathogenic, or the disease development is very slow.

Feline leukemia virus-related diseases have been reported in a mountain lion (*Puma concolor*; Jessup et al., 1993) and a bobcat (Sleeman et al., 2001) in captivity. In both cases, a domestic cat was suspected as source of infection. These observations indicate that wild feline species are susceptible to FeLV infection. However, it appears that the virus is not maintained in these populations, and an interspecific contact is required (e.g., with an infected domestic cat) for an individual to acquire the infection (Jessup et al., 1993; Sleeman et al., 2001).

This assumption is probably true for other infectious diseases as well, as postulated by Gass (1987). In the case of the Eurasian lynx, an external source of infection has been speculated for infections with rabies virus (e.g., Fernex, 1976; Schneider et al., 1989; Stahl and Vandell, 1999; Tschirch, 2001), orthopoxvirus (Tryland et al., 1998), Borna virus (Degiorgis et al., 2000), *S. scabiei* (Jeu and Xiang, 1982; Holt and Berg, 1990; Mörner, 1992a; Ryser-Degiorgis et al., 2002), and *Notoedres cati* (Ryser-Degiorgis et al., 2002). This is likely for the reported case of FIP in captive lynx (Hyslop, 1955) and seems to be probable for FPV infections in wild populations as well.

The true prevalence of the viral infections studied in this paper also could have been underestimated because of a decline of antibody titers below the level of detection within a few months. Antibodies to FPV, FHV, and FCV induced by vaccination persist for up to 48 mo in domestic cats (Mouzin et al., 2004). These studies were done in cats that had regular contact with other cats and therefore could have received an occasional booster from these contacts. In free-ranging lynx, frequent contacts are likely not to occur. Therefore, we postulate that antibodies will persist for many months in lynx but might decline before the 48 mo found in domestic cats.

Several lynx were tested seropositive for FIV and FeLV in ELISA tests. However, western blot analyses did not indicate infection, and the results of the ELISAs were considered false positive. Antigens for the FIV and FeLV ELISAs were made in *Escherichia coli* (Lehmann et al., 1991; Calzolari et al., 1995). Therefore, cross-reactions of antibodies against *E. coli* antigens still present in the antigen preparation might occasionally lead to false-positive results in the antibody ELISA. Additionally, detection of antibodies to recombinant FeLV gp70 could be explained by exposure of the lynx to murine leukemia viruses, which are closely related to FeLV, by eating mice. False positive FeLV p27 results can be explained by the presence of antibodies to mouse IgG in the serum of the lynx tested. Anti-mouse IgG antibodies have been observed in domestic cats vaccinated with rabies vaccines produced in mouse brains (Jacobson and Lopez, 1991). We postulate that anti-mouse IgG antibodies can be induced in lynx after consumption of mice.

None of the lynx sampled was seropositive for *F. tularensis*. In Canada lynx, Biek et al. (2002) found very low seroprevalence as well (1/195). *Francisella tularensis* infections in Scandinavian hare populations peak in late summer and early fall (Mörner et al. 1988); thus, lynx might be less likely to encounter the agent in winter

and spring, when most of our samples were taken (hunting season). However, although the longevity of antibody titers following *F. tularensis* infection in lynx is unknown, antibodies can be detected several years postinfection in naturally infected humans (Ericsson et al., 1994). Tularemia is an acute fatal disease in hares, and the varying hare, in which the disease is predominantly found, is considered an accidental host and not a reservoir (Mörner et al., 1988). This could explain why lynx do not come into frequent contact with the agent.

The seroprevalence to *A. phagocytophila* of about 4% corresponds to that observed in red foxes in Switzerland (Pusterla et al., 1999b). The duration of seropositivity following infection with granulocytic ehrlichiae is not known for nondomestic carnivores, but experimental studies in dogs and cats reveal that animals can be seropositive for up to 12 mo (Foley et al., 1999). Thus, lynx infected more than 1 yr before death might be undetected. The analysis of sera collected after thawing of frozen carcasses of mountain lions from California revealed a seroprevalence of 17% for granulocytic ehrlichiae, with rates ranging from 0% in winter up to 29% in summer and fall (Foley et al., 1999). Because many of the lynx sampled in our study died in winter and early spring, the overall prevalence might be underestimated as the result of a seasonal bias.

Absence of antibodies to FIV and other viruses suspected to cause immunodeficiency in the lynx we studied suggests that sarcoptic mange and other infectious diseases like Borna disease are not a result of concomitant viral infection.

The free-ranging Swedish lynx population does not seem to be in frequent contact with the infectious agents considered in this study. Sporadic undetected fatal infections might occur, but subclinical or recovered cases are surely not widespread. As a superpredator, the lynx is exposed to diseases through its prey (red fox, hares, rodents, and domestic cats), but because

of its solitary behavior, this felid has only rare opportunities to transmit the infectious agent before a fatal outcome of (or recovery from) the disease. However, similar studies in other lynx populations are needed to corroborate these observations.

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