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ANTIBODIES AGAINST ORTHOPOXVIRUSES IN WILD CARNIVORES FROM FENNOSCANDIA

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ABSTRACT: Two hundred and three sera obtained in 1993–96 from red foxes (*Vulpes vulpes*), lynx (*Lynx lynx*), brown bears (*Ursus arctos*) and wolverines (*Gulo gulo*) in Fennoscandia (Norway, Sweden, and Finland) were examined for the presence of anti-orthopoxvirus antibodies by a competition enzyme linked immunosorbent assay (ELISA). High prevalences were found for the red foxes in Norway (7/62, 11%) and Finland (7/14, 50%). While only one of 73 (1%) lynx from Finland had anti-orthopoxvirus antibodies, a high prevalence was found in sera from the Sarek National Park in Sweden (5/17, 29%). In addition, anti-orthopoxvirus antibodies were found in one brown bear from the same area (1/45, 2%), whereas none of the 14 wolverines were seropositive. This is the first report of anti-orthopoxvirus antibodies in the brown bear and the lynx, and the first screening for such antibodies in Sweden and Finland. These results indicate that orthopoxviruses are distributed in Sweden and Finland as well as in Norway, and that the red fox and the European lynx may serve as indicator species for the presence of orthopoxviruses in the local populations of small mammals.

Key words: Brown bear, carnivores, competition enzyme linked immunosorbent assay, cowpox virus, ELISA, lynx, *Lynx lynx*, red fox, rodents, serosurvey, *Ursus arctos*, *Vulpes vulpes*.

INTRODUCTION

Vaccinia virus, a poxvirus in the genus *Orthopoxvirus* used in the world-wide vaccination campaign against smallpox, also has been used in another application during the last decades as a vaccine vector in recombinant vaccines designed for use in human, domestic animals, and wildlife (Perkus et al., 1995; Yilma, 1994). Recombinant vaccinia-based vaccines, with an insert of a glycoprotein-coding gene from rabies virus, have been spread with baits in Belgium and France since 1988. They are directed toward the main reservoir for rabies in Europe, the red fox (*Vulpes vulpes*) (Pastoret and Brochier, 1996). Bait trials for wildlife species like the red fox, skunk (*Mephitis mephitis*), raccoon (*Procyon lotor*) and wild boar (*Sus scrofa*) in the USA and Canada also have been conducted (Artois et al., 1990; Fletcher et al., 1990; Hable et al., 1992; Winkler and Bögel, 1992). Cowpox virus, which is a close relative of vaccinia virus, has emerged during this pe-

riod as a pathogen in several species of wild Felidae from zoos in Russia and the UK (Marennikova et al., 1977; Baxby et al., 1982), and in the domestic cat in several European countries (Thomsett et al., 1978; Bennett et al., 1990; Bomhard et al., 1992; Tryland et al., 1996). It has been assumed that both zoo animals and domestic cats become infected from small rodents, lately implicated as a reservoir for cowpox virus based on the isolations of virus from big gerbils (*Rhombomys opimus*) and susliks (*Citellus fulvus*) in Turkmenia (Marennikova et al., 1978) and from a red-tailed Libyan jird (*Meriones libicus*) in Georgia, and a root vole (*Microtus oeconomus*) on the Kola Peninsula in Russia as referred to by Pilaski and Jacoby (1993). Serological screenings of rodents in several European countries have revealed that orthopoxviruses are widely distributed in small wild rodents in Europe (Kaplan et al., 1980; Pilaski and Jacoby, 1993; Crouch et al., 1995; Boulanger et al., 1996). On

several occasions cowpox virus has been transmitted from zoo animals and cats to humans. Two human cases of cowpox virus infections appeared in southern Sweden in 1990 (Cronqvist et al., 1991), and in 1994 one human (Myrmel et al., 1997) and one feline case (Tryland et al., 1996) appeared in Norway. No clinical cases of orthopoxvirus infection have been reported from Finland. It has recently been demonstrated that small wild rodents and the common shrew (*Sorex araneus*) from different parts of Norway carry both orthopoxvirus specific DNA (T. Sandvik, unpublished data) and antibodies (Tryland et al., 1998). Anti-orthopoxvirus antibodies also are present in domestic cats in Norway (M. Tryland, unpublished data).

The potential of orthopoxviruses to evoke disease in wildlife species is unknown, although a captive lynx (*Lynx lynx*) died from cowpox virus infection in a zoo in the UK (M. Bennett, pers. comm.). Recent serological surveys of red foxes in Germany have revealed anti-orthopoxvirus antibody prevalences of 7% ($n = 703$), 16% ($n = 1,040$), and 19% ($n = 830$), respectively, (Henning et al., 1995; Mayr et al., 1995; Müller et al., 1996), whereas no such antibodies were found in 70 adult and 55 juvenile red foxes from areas in Belgium and France where recombinant rabies vaccines have been used (Boulanger et al., 1996). Anti-orthopoxvirus antibodies also have been detected in 8 of 215 sera (4%) from wild boars in Germany (Mayr et al., 1995).

Rodents are the main prey for the red fox, and thus have considerable influence on the population dynamics for the latter. A study of the fecal contents of red foxes in a mountainous region in Norway (Finse), showed that 11% and 90% of the scats contained remnants of Norway lemmings (*Lemmus lemmus*) and voles, respectively (Frafjord, 1995), whereas examination of stomach contents from 146 lynx from Norway demonstrated remnants of small rodents in 8% of the individuals (Birkeland and Myrberget, 1980).

The 76 lynx from Finland included in

this study were part of an investigation to determine their choice of prey by registration of the remains in the gastrointestinal tracts, as an indication of ingesta during the previous 24 hr. Five hundred eighty five animals were examined between 1967–90 (Pulliainen et al., 1995). Vole species and lemmings were found in 2% to 3% in eastern Finland (the animals in this study), and 12% in southwestern Finland, showing that the lynx has a broad contact with rodents and that rodents constitute a part of their prey during the winter. In addition, remnants from domestic cats were found in 4% of the animals.

The wolverine (*Gulo gulo*) is a generalist and an opportunist in its feeding behaviour. Although it may kill large prey like reindeer (*Rangifer tarandus*), it is a poor hunter, a real carrion-feeder, and it feeds on rodents as well. The wolverine is a mobile and non-territorial hunter, and visits all kinds of habitats (Pulliainen, 1988). Hence, it may be in contact with many rodent species and populations. Rodents also are regarded as supplementary diets for brown bears (Craighead et al., 1995).

The bait vaccination of red foxes against rabies in western Europe has been successful, both in areas where attenuated rabies vaccines and recombinant vaccinia virus vectored vaccines have been used (Müller et al., 1994). However, non-target species like rodents, mustelids and wild boars (Brochier et al., 1989) and probably also mammals like badgers (*Meles meles*), pine martens (*Martes martes*), lynx, brown bears, and wolverines may consume baits as well and become infected by recombinant vaccinia virus. The role of these wildlife species as hosts for orthopoxviruses is unknown.

The aim of this study was to investigate whether large carnivores in Fennoscandia have antibodies directed against orthopoxvirus. Their feeding habits and territorial range imply a broad contact with different species and populations of rodents and shrews, and the carnivores may serve as indicator species of whether or not ortho-

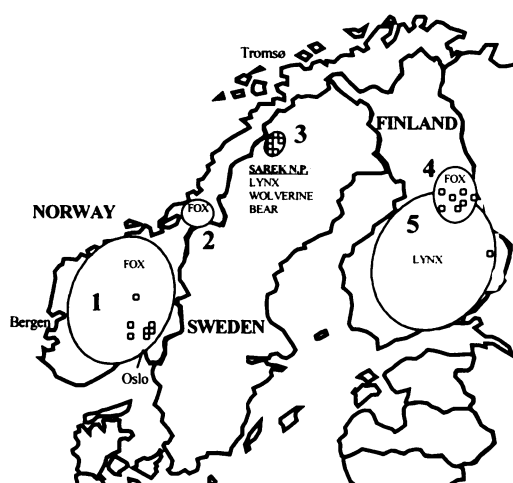


FIGURE 1. Geographical locations in Fennoscandia where blood samples from red foxes, lynx, brown bears, and wolverines were obtained. Areas marked by circles include (1) at 58°30' to 63°15'N, 07°00' to 11°30'E; (2) 63°45' to 64°30'N, 11°00' to 12°00'E; (3) vicinity of Kvikkjokk Mountain at 67°00'N, 17°40'E; (4) 65°15' to 66°00'N, 27°10' to 30°00'E; and (5) 60°45' to 65°45'N, 22°00' to 31°15'E. Seropositive individuals (red foxes, lynx and brown bear) from the respective locations are marked by boxes.

poxviruses are present in the populations of small mammals. Recombinant poxvirus-based vaccines might be used more extensively in the future, and information about the role of the carnivores in the ecology of orthopoxviruses is important in that context.

MATERIAL AND METHODS

Animals

Blood or tissue fluid from 225 wild carnivores were collected from hunter-killed animals and from animals that were chemically immobilized for radio-telemetry studies in different parts of Norway, Sweden and Finland (Fig. 1). Among the 62 red foxes from Norway that were tested, 17 were killed in November and December 1994, and 45 were killed in January ($n = 13$), February ($n = 11$), March ($n = 20$) and April ($n = 1$) 1995. Sarcoptic mange was diagnosed in 18 animals. From the Sarek National Park in northern Sweden and surrounding areas, blood from 14 wolverines, 45 brown bears, and 17 lynx were sampled during radio tagging (Linden et al., 1995). The lynx blood were sampled from February to May 1996, whereas samples from brown bears and red

foxes were obtained from May to July 1996. In addition, 73 hunter-killed lynx from the eastern Finland were examined and blood collected at the Department of Biology (University of Oulu, Oulu, Finland) from December to March 1993–95. From northeastern Finland, 14 red foxes were sampled from November to March 1992–95. Blood from chemically immobilized animals was taken from the jugular or femoral vein, serum was separated in the field and stored at -20 C until testing. From the hunter-killed animals, blood was collected whenever available; in some instances this was several days after death and some of the samples were heavily hemolyzed. In the red foxes of Norway tissue fluids from the abdominal cavity were collected and prepared in the same way as the blood samples.

Control sera

Propagation, purification and inactivation of vaccinia virus [strain Western Reserve, American Type Culture Collection (ATCC), Rockville, Maryland, USA, number VR119], preparation of polyclonal hyperimmune serum against inactivated vaccinia virus in rabbit, and preparation of biotinylated immunoglobulin G (IgG) are described elsewhere (Tryland et al., 1998). Polyclonal hyperimmune serum from the rabbit was used as a positive control and a reference serum on each enzyme linked immunosorbent assay (ELISA) plate. In addition, polyclonal hyperimmune serum from a domestic cat immunized with live cowpox virus, kindly provided by M. Bennett (Liverpool, England) was used as positive control.

Serological analysis

The competition ELISA is described in detail elsewhere (Tryland et al., 1998). Briefly, flat-bottomed 96-well plates (Nunc-Immuno[®] PolySorp, NUNC A/S, Roskilde, Denmark) were coated overnight with live vaccinia virus (VR 119) at a final concentration of $4.5\ \mu\text{g}$ protein/ml, measured by a BCA protein assay reagent kit (Pierce, Rockford, Illinois, USA). As cowpox virus is the only orthopoxvirus expected to be responsible for anti-orthopoxvirus antibody production in wild animals in Europe, this assay is based on the immunological cross-reactivity between the species within the genus *Orthopoxvirus* (Fenner et al., 1989a). Coated plates were blocked for 2 hr with 3% Tween 20 in phosphate buffered saline (PBS). Serum samples (test sera and controls) were diluted 1:10 and 1:100 in PBS with 0.05% Tween 20 and biotinylated IgG ($150\ \mu\text{g}/\text{ml}$, 1:100). Preimmune rabbit serum (1:100), obtained from the rabbit prior to immunization with vaccinia vi-

rus, and Vero cell protein (1 mg/ml, 1:100) obtained from vero cells (ATCC number CCL81) cultivated in Eagles Minimum Essential Medium (Gibco-BRL, Life Technologies Inc., Gaithersburg, Maryland, USA) supplemented with 5% bovine calf serum, L-glutamine (2 mmol/litre) and antibiotics consisting of benzylpenicillin 1.04×10^5 units/litre and streptomycin sulphate 7.87×10^4 units/litre (Sigma Chemical Co., St. Louis, Missouri, USA) were added, and the serum dilutions were incubated for 2 hr. After application of sera, the coated plates were incubated for one hour. Streptavidin-Peroxidase conjugate (Streptavidin-POD, Böhringer Mannheim, GmbH, Mannheim, Germany) was added in a 1:10,000 dilution in PBS with 0.05% Tween 20 and incubated for 30 min, followed by 10 min incubation with orthophenylenediamine (OPD, DAKO, Glostrup, Denmark), 1.5 mg/ml in citric acid phosphate buffer as substrate. The plates were read in an eight channel vertical light path filter photometer (Multiskan Bichromatic type 348, Labsystems, Helsinki, Finland) at 492 nm. Percent reduction of the photometer extinction of the biotinylated rabbit IgG by the competing test sera in 1:10 dilution compared to the rabbit hyperimmune serum (1:10 dilution defined as 100%) was calculated by the formula: % inhibition = $[\text{OD}_{492} \text{ rabbit preimmune serum} - \text{OD}_{492} \text{ test serum}] / [\text{OD}_{492} \text{ rabbit preimmune serum} - \text{OD}_{492} \text{ rabbit hyperimmune serum}] \times 100$. The hyperimmune serum from the domestic cat, immunized with infectious cowpox virus, gave an inhibition of 112% compared to the rabbit hyperimmune serum.

To demonstrate that the ability of the sera to evoke inhibition in the competition ELISA was IgG-specific, the IgG fraction of sera from lynx, red fox, and brown bear was purified by affinity chromatography on a 5 ml protein-A column (HiTrap[®] affinity column, Pharmacia Biotech, Uppsala, Sweden) and a FPLC-system (LCC-501 Plus Core System, Pharmacia), according to the manufacturers instructions. The separations gave varying amounts of IgG from the different animal species, due to differences in the ability of the IgG to bind to protein A (Lindmark et al., 1983). IgG from brown bear, lynx, and especially from the red fox, showed high affinity to protein A. The IgG fractions were dialyzed against PBS and tested in the competition ELISA.

RESULTS

Screening results for the sera from wolverine, brown bear, red fox and lynx are summarized in Table 1, and seropositive animals are indicated in Figure 1. The

TABLE 1. Prevalence of anti-orthopoxvirus antibodies in wolverines, brown bears, red foxes and lynx. Inhibition levels of >80% and >90% in the competition ELISA are shown.

Species	Competition-ELISA: % Inhibition	
	>80%	>90%
Wolverine		
Sweden	0/14 (0) ^a	0/14 (0)
Brown bear		
Sweden	13/45 (29)	1/45 (2)
Red fox		
Finland (east)	10/14 (71)	7/14 (50)
Norway (south)	17/62 (27)	7/62 (11)
Total	27/76 (36)	14/76 (18)
Lynx		
Finland (southeast)	16/73 (22)	1/73 (1)
Sweden	12/17 (71)	5/17 (29)
Total	28/90 (31)	6/90 (7)

^a Number of seropositive individuals/number tested (% seropositive).

competition ELISA was evaluated by comparing different inhibition levels of sera from bank voles (*Clethrionomys glareolus*) and parallel immunofluorescence results (Tryland et al., 1998). A serum giving an inhibition level of 90% or more compared to the rabbit hyperimmune serum (calculated from the formula above) was defined as antibody-containing, and this level was used in the further discussion of the results, although the >80% level is shown in Table 1 for comparison.

Among the red foxes from Norway that were tested, 7 of 62 individuals (11%) had inhibition levels >90%. All the seropositive red foxes were caught in Oslo, Akershus, Oppland and Buskerud counties in southern Norway, where 28 of the 62 animals tested had been caught (Table 1), giving a collective prevalence of anti-orthopoxvirus antibodies of 25% in all these counties. Of the 62 red foxes from Norway, 18 (29%) had sarcoptic mange, and in three of these animals anti-orthopoxvirus antibodies were detected as well.

In sera from the Sarek National Park in Sweden, antibodies were found in one of 45 brown bears (a 2-yr-old female), and in 5 of 17 (29%) lynx (one male, four fe-

males). Three of the female lynx were immobilized in the same area on the same day. Anti-orthopoxvirus antibodies were not detected in any of the 14 wolverines tested, inhibition levels varying from 40 to 70%.

One of 73 lynx (1%) (a 9.5 kg 1-yr-old male) and 7 of 14 red foxes (50%) from Finland had anti-orthopoxvirus antibodies.

Testing the IgG fractions from sera of brown bear, lynx and red fox in the competition ELISA demonstrated a high ability of inhibition, from 60% to 100% compared to rabbit hyperimmune serum.

DISCUSSION

This is the first report of anti-orthopoxvirus antibodies in the brown bear and lynx. It also is the first report of anti-orthopoxvirus antibodies in wildlife from Sweden and Finland. The sera were tested by a competition ELISA, designed to test sera from different species without the need for species specific antibodies. The competition ELISA has given rational results on sera from bank voles when compared with an immunofluorescence assay (Tryland et al., 1998). Testing the IgG preparations in the ELISA demonstrated that the ability of the test sera to compete for the antigen sites in the coat was linked to the IgG fraction. Due to differences in the affinity to protein A, both between species and between different subclasses of IgG (Lindmark et al., 1983), and several dilution steps during the preparation, these inhibition levels are not directly comparable to the original sera or between species.

For the red foxes from Norway included in this investigation, a total anti-orthopoxvirus antibody prevalence of 11% was found. For the 28 serum samples obtained from the counties where seropositive animals were detected (Oslo, Akershus, Oppland and Buskerud), there was a prevalence of 25%. Collective data from the surveys of rodents and shrews and red foxes, cover major parts of southern Norway, and demonstrate that orthopoxviruses are

widely distributed in this part of the country.

The samples from Sweden included in this investigation are restricted to the Sarek National Park and surrounding areas in the northern part of the country. Wolverines, brown bears and lynx are present in wide areas of the western parts of the country along the Norwegian border, and some individuals range on both Swedish and Norwegian territories. In fact, one lynx during a period of 6 mo moved into Norway, about 250 km from where it was originally radio-collared. Samples from a higher number of individuals and from different geographical areas should be investigated in order to estimate the prevalences of anti-orthopoxvirus antibodies in carnivores from Sweden. However, one of 45 brown bears and 5 of 17 lynx were seropositive, which demonstrates that orthopoxviruses are present in the study area, and that these species are susceptible to orthopoxvirus infection. No firm conclusions can be drawn with regard to presence or absence of anti-orthopoxvirus antibodies in wolverines, since only 14 individuals were tested.

The number of red foxes tested from Finland was small, and the geographical distribution was restricted to the Kainuu district in Oulu county (Fig. 1). Nevertheless, 7 of 14 animals were seropositive, indicating a very high prevalence of anti-orthopoxvirus antibodies in this area. Of the 73 lynx sera tested, sampled mainly from more southern areas (Fig. 1), anti-orthopoxvirus antibodies was detected in only one individual. This is in contrast to the results obtained from lynx in Sweden. Considering the relatively high number of serum samples representing lynx from most of the eastern and southern parts of Finland, and the fact that remnants from voles and Norwegian lemmings were found in the stomach of 3% of the individuals, orthopoxviruses may not be as widely distributed in rodents and shrews in this part of Finland as in southern Norway.

Little is known about the pathogenicity of orthopoxviruses for wildlife species, although cowpox virus has a very wide host range, causing lesions in almost every species that have been tested (Fenner et al., 1989b). Cowpox virus was isolated from apparently healthy wild rodents in Turkmenia (Marennikova et al., 1978), and cowpox virus infections with clinical signs in wildlife have not been reported. Cowpox virus seems more pathogenic to captive wild felids than to the domestic cat, and an additional pulmonary form of the infection without dermal lesions have been observed in felids from zoos (Marennikova et al., 1977; Baxby et al., 1982). In the Moscow zoo, the outbreak involved all the Felidae and Edentata housed in the building, whereas three bears (two *Melursus ursinus* and one *Tremarctos ornatus*) and a hyena (*Crocuta crocuta*) appeared to be resistant to infection (Marennikova et al., 1977). Whether this is the case for wild brown bears is unknown.

The actual route of orthopoxvirus infection for the tested carnivore species are unknown. Orthopoxvirus DNA have been detected in several organs, and especially in the lungs, of small rodents and common shrews in Norway. Carnivores might conceivably become infected by the respiratory and/or alimentary routes during feeding, or the virus entry might be through the skin as suggested for domestic cats (Bennett et al., 1990). In addition to contracting infections from rodents and shrews, lynx may be infected by close contact with foxes and, occasionally, domestic cats as prey. Of the 63 red foxes from Norway three animals had both clinical evidence of sarcoptic mange and orthopoxvirus antibodies. Due to few individuals tested, no conclusions can be drawn as to whether individuals with *Sarcoptes scabiei* infections are predisposed to contract orthopoxvirus infections. Sarcoptic mange also is occasionally found in lynx in Scandinavia (Mörner, 1992).

Although the species and numbers of individuals examined from Sweden and

Finland are limited, and no screenings have been performed on small mammals in these countries, we have demonstrated that anti-orthopoxvirus antibodies are present in carnivore species in Norway, Sweden, and Finland. This is important information for the evaluation of possible non-target effects connected to the use of poxvirus-vectored recombinant vaccines. Although the pathogenicity of orthopoxviruses in wild carnivores is unknown, they are susceptible to the virus and they respond to an infection by producing antibodies. In areas where carnivores like the red fox and lynx are hunted or chemically immobilized and blood samples are available, serologic surveys for anti-orthopoxvirus antibodies may be a rapid easy way to obtain information as to whether or not orthopoxvirus(es) are circulating in the regional populations of small wild mammals.

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