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SEROLOGIC SURVEY FOR SELECTED VIRUS INFECTIONS IN POLAR BEARS AT SVALBARD

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ABSTRACT: Polar bears (*Ursus maritimus*) were chemically immobilized and sampled at Svalbard, Norway, and on the pack ice in the Barents Sea from late March to mid-May between 1990 and 1998. Plasma samples were tested for the presence of antibodies to canine distemper virus (CDV), calicivirus, phocid herpesvirus type 1 (PhHV-1), and rabies virus. A seroprevalence of 8% to CDV and 2% to calicivirus were found, whereas no antibodies were detected against PhHV-1 or rabies virus. This serologic survey indicates that polar bears in this region are exposed to morbillivirus and calicivirus, although the nature of these viruses and infections are unknown. Morbillivirus and calicivirus are potential pathogens in seals, but it is unknown whether they may cause health problems in polar bears.

Key words: Arctic rabies, calicivirus, herpesvirus, morbillivirus, serology, *Ursus maritimus*.

INTRODUCTION

Morbillivirus antibodies have been detected in polar bears from Alaska and Russia with prevalences ranging from 26% to 46% between different years (Follmann et al., 1996). Morbillivirus infections are well known in seals, especially after two recent epidemics among harbor seals (*Phoca vitulina*) in Europe. In 1989–90, more than 18,000 harbor seals and a number of grey seals (*Halichoerus grypus*) died (Heide-Jørgensen et al., 1992; de Swart et al., 1995), and in 2002 over 21,000 harbor seals may have died as a result of morbillivirus infection (Thompson et al., 2002; Jensen et al., 2002). Antibodies to morbillivirus were detected in four of 90 ringed seals (*Phoca hispida*) from northwestern and eastern Greenland (Dietz et al., 1989). Morbillivirus antibodies have also been detected in harp seals (*Phoca groenlandica*) from western Greenland, eastern Greenland, and from the Barents Sea (Dietz et al., 1989; Stuen et al., 1994). High seroprevalences, without reports of related mortality, suggest that morbillivirus may

be endemic in these populations of harp and ringed seals.

Two caliciviruses, San Miguel sea lion virus (SMSV) and vesicular exanthema of swine virus (VESV), are indistinguishable and are regarded as a single virus that was transferred from the marine environment to swine, causing the now-extinct disease, vesicular exanthema of swine (Smith et al., 1973a). SMSV was first isolated in 1972 from California sea lions (*Zalophus californianus*) that had aborted and that had vesicular lesions of the flippers (Smith et al., 1973a). Virus and/or calicivirus antibodies have been detected in a wide range of whale and seal species, including walrus from the Chukchi Sea (Lenghaus et al., 2001; Ganova-Raeva et al., 2004). No information has been reported on calicivirus infections or antibodies in bears.

Phocid herpesvirus type 1 (PhHV-1) infections represent a major cause of death in seals in rehabilitation centers and cause respiratory disease in adults and generalized infections in neonates and pups (Martina et al., 2003). Also phocid herpesvirus

type 2 (PhHV-2), a tentatively classified gammaherpesvirus, has been isolated from harbor seals in Europe and North America (Harder et al., 1996). Antibodies against PhHV-1 and PhHV-2 were reported in several marine mammal species from Alaska and Russia (Osterhaus et al., 1985; Zarnke et al., 1997), including the ringed seal and the bearded seal. Antibodies against phocid herpesvirus have been detected in harp seals from the Barents Sea and in harp and hooded seals (*Cystophora cristata*) from the Greenland Sea north of Jan Mayen (Stuen et al., 1994). A genetic characterization of PhHV-1 has revealed a close relationship between PhHV-1 and alphaherpesviruses of other carnivores (canid herpesvirus and felid herpesvirus) as compared with herpesviruses from other host species (Martina et al., 2003).

Rabies was diagnosed at Svalbard for the first time in 1980 in 12 arctic foxes (*Alopex lagopus*), three reindeer (*Rangifer tarandus platyrhynchus*), and in one ringed seal (Ødegård and Krogsrud, 1981). No rabies virus was found when brain tissue from 23 polar bears, 846 arctic foxes, 19 reindeer, and 5 ringed seals were tested by direct fluorescent antibody test (Prestrud et al., 1992). However, rabies was recently diagnosed in a few arctic foxes at Svalbard, and the disease is regarded as endemic in this population (Mørk and Prestrud, 2004). The arctic fox is regarded as the main reservoir for the virus, and the arctic rabies virus variant (P 41) seems to be represented throughout the area in which the arctic fox is distributed (Johnston and Fong, 1992). Species other than the arctic fox seem to be infected only incidentally. Only one case of clinical polar bear rabies has been reported (Loewen et al., 1990; Taylor et al., 1991). In 1989, a lame polar bear was shot in Canada by Inuit hunters, and the rabies diagnosis was based upon a strongly positive immunoperoxidase reaction against rabies virus antigen in the lumbar spinal cord and the Gasserian ganglion sections (Taylor et al., 1991).

Little is known about the health status of the polar bears at Svalbard. High levels of environmental pollutants have been detected (Bernhoft et al., 1997; Norstrom et al., 1998), and recent studies have shown that organochlorines may impair the immune function of the bears and thus make them more susceptible to infectious agents and diseases (Bernhoft et al., 2000; Lie et al., 2004). We have previously found antibodies against *Brucella* sp. in polar bears of this population (Tryland et al., 2001). The aim of this study was to investigate whether polar bears of the Svalbard population were exposed to selected viruses known to cause disease in seals.

MATERIALS AND METHODS

Blood samples were collected from late March to mid-May in 1990–98 at Svalbard, Norway (76°–81°N, 15°–25°E), and on the pack ice in the Barents Sea (74°N–77°N, 37°E–43°E) (Fig. 1). The bears were chemically immobilized from helicopter by remote injection of a drug-filled dart (Palmer Cap-Chur Equipment, Douglasville, Georgia, USA). The drug Zoletil vet.[®] (a 1:1 mixture by weight of the dissociative anesthetic tiletamine and the tranquilizer zolazepam; Virbac International, Carros Cedex, France) was used (200 mg/ml) at a dosage of 5–10 mg/kg of body mass (Stirling et al., 1989). Blood was drawn from the femoral vein into heparinized, evacuated blood-collecting tubes. The samples were transported to the laboratory within 8 hr after sampling, where plasma was prepared by centrifugation and stored at –20 C until analysis. The samples were not hemolyzed as assessed by visual inspection. Age was determined by removing a vestigial first premolar tooth and counting cementum growth layer groups (Calvert and Ramsay, 1998). Of the 275 polar bears sampled, 52% were males and 48% were females; age varied from 3 mo to 28 yr (mean 11 yr, SD 6.3).

Plasma samples ($n=242$) were tested in virus neutralization tests for antibodies against canine distemper virus (CDV) (Osterhaus et al., 1990) and PhHV-1 (isolate PB84; Osterhaus et al., 1985). Two-fold plasma dilutions were tested for their ability to neutralize 100 median tissue culture infective doses (TCID₅₀) of CDV and PhHV-1. After incubation for four days, the virus-neutralizing antibody titer was determined microscopically on the basis of observed cytopathic changes. A plasma dilution was con-

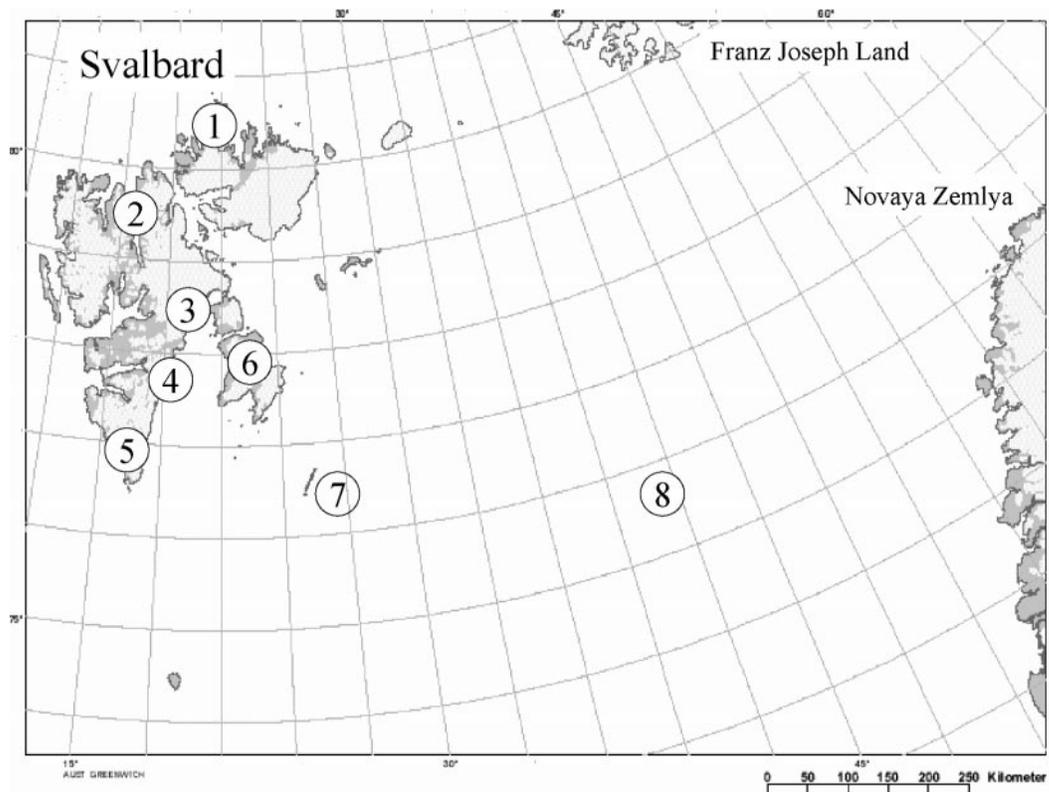


FIGURE 1. Map of Svalbard and the Barents Sea showing sampling locations for polar bears investigated for antibodies against morbillivirus (CDV), calicivirus (FCV), phocid herpesvirus type 1 (PhHV-1), and rabies virus (numbers corresponds with locations in Table 1).

sidered positive if cytopathic effect (CPE) was not observed.

A modified microplate version of the rapid fluorescence focus inhibition test (RFFIT), originally developed by Smith and coworkers (1973b), was used to test 266 polar bear plasma samples for rabies antibodies. Serial, twofold, 1:10 to 1:640 dilutions of the test plasma and of the human-positive control serum (WHO, Statens Seruminstitut, Copenhagen, Denmark) were prepared in 96-well microplates. Diluted sera were mixed with a constant dose of challenge virus standard 11 (CVS11; Federal Research Centre for Virus Research, Tübingen, Germany) forming 30–50 fluorescent foci per microplate well. The mixture of plasma and virus were incubated at 37 C for 90 min. After incubation, baby hamster kidney (BHK; Federal Research Centre for Virus Research, Tübingen, Germany) cells were added to the plasma/virus mixtures, and after a further 24 hr of incubation, the cell monolayer was acetone fixed and stained with FITC-labeled antibodies

immunoglobulin to detect the presence of non-neutralized virus (fluorescent foci). Fluorescent foci were counted in each dilution and compared with the corresponding dilutions of the positive control serum. The positive threshold for the test was the dilution in which the amount of the fluorescent foci were reduced by 50%.

Samples from 262 polar bears were tested for antibodies against calicivirus in a virus neutralization test as described (OIE, 1996) with feline calicivirus (American Type Culture Collection, strain VR529) as antigen. Plasma samples were inactivated at 56 C for 30 min. Virus (100 TCID₅₀) was incubated with the sample at 37 C for 60 min and added to feline lung cell monolayers with Eagles Minimum Essential Medium (GibcoBRL, Life Technologies Inc., Gaithersburg, Maryland, USA) and 10% fetal bovine serum (FBS; Gibco). A reduction of CPE of 100% by a serum sample diluted 1:4 or higher was considered as serologic evidence of exposure.

TABLE 1. Polar bears with antibodies against morbillivirus (CDV) and calicivirus (feline calicivirus; FCV) (number positive/number tested).

Locality	Morbillivirus	Calicivirus
Nordautlandet (1)	1/1	0/1
Wijdefjord (2)	0/2	0/2
Sabineland (3)	2/7	0/7
Heerland (4)	0/4	0/4
Sørkappland (5)	0/9	1/9
Edgeøya (6)	2/58	0/66
Hopen (7)	14/117	2/127 ^a
Barents Sea (8)	1/44	1/46 ^a
Total	20/242 (8%)	4/252 (2%)

^a No animals tested positive for antibodies to both viruses.

RESULTS

Morbillivirus neutralizing antibodies were detected in 20 individuals (8%), 7 females and 13 males, with ages varying from 3 mo to 26 yr (mean 9.4, SD 6.9) (Table 1). Calicivirus neutralizing antibodies were detected in samples from 4 animals (2%) (Table 1), with titers ranging from 1:4 to 1:16. Two of these animals were adult males, and two were adult females. Antibodies against PhHV-1 and rabies virus were not detected.

DISCUSSION

Morbilliviruses, including CDV, phocine distemper virus (PDV-1 and PDV-2), dolphin morbillivirus (DMV), and porpoise morbillivirus (PMV), have caused disease outbreaks in various marine mammal species and populations. Although disease associated with these viruses has not been reported in polar bears, a high seroprevalence (36%) was found against CDV in polar bears from Alaska and Russia (Follmann et al., 1996). Higher neutralizing antibody titers to CDV than to PDV, DMV, and PMV suggest that these bears were exposed to a morbillivirus of terrestrial origin. The morbillivirus responsible for the antibodies in polar bears has not been identified and may be unique to this species (Garner et al., 2000). A much lower seroprevalence (8%) against CDV was found in the Svalbard polar bear population compared with animals from Alaska

and Russia. Previous studies suggest that morbillivirus is endemic in the North Atlantic and Barents Sea harp seal populations where seroprevalences ranging from 67% to 100% ($n=183$) have been reported. Serologic results in these studies were similar when using either CDV or PDV as antigen (Stuen et al., 1994), indicating serological cross-reactivity between these viruses. Although no information exists on morbillivirus exposure of ringed seals from the Svalbard region, neutralizing antibodies have been detected in ringed seals from Canada and Greenland (Dietz et al., 1989; Duiganan et al., 1997). Polar bears may also be exposed to morbillivirus through harp seals, which in addition to ringed and bearded seals, may constitute an important food item for these animals at Svalbard (Derocher et al., 2002). Whether the morbillivirus infection in polar bears at Svalbard is a result of an interspecies transmission from seals or a unique polar bear morbillivirus (Garner et al., 2000) is unknown, and virus isolation and characterization would be required to answer this question.

A comparison of the hypervariable sequences of the capsid protein gene of caliciviruses indicates a close relationship between the members of the genus *Vesivirus*, which includes both SMSV and feline calicivirus (FCV) (Fenner and Fantini, 1999). However, a high degree of antigenic heterogeneity has been demonstrated between these viruses, and different antigenic types may be isolated even during the same outbreak (Radford et al., 2003). The calicivirus antibodies found in polar bears only indicate that these animals were exposed to a calicivirus; it is unknown if the calicivirus originated from terrestrial or marine sources. Because interspecies transmission of calicivirus between marine and terrestrial mammals has been demonstrated and because SMSV seems to have a zoonotic potential (Smith et al., 1998), further investigations on calicivirus in marine mammals in this region seem relevant.

Rabies antibodies were not detected in polar bears in this study, which is in concordance with a previous investigation among polar bears in Alaska (E. Follmann, pers. comm.). Rabies virus seems to be distributed throughout the circumpolar region, and many epizootics have been reported over the past several decades (Mørk and Prestrud, 2004). A common opinion has been that rabies kills the great majority of infected individuals and that antibodies would only be found in animals in the incubation phase of the disease. However, clinically healthy arctic foxes with rabies antibodies have been reported, and some animals may survive infection (Ballard et al., 2001). In another carnivore, the spotted hyena (*Crocuta crocuta*) in Tanzania, rabies antibodies and rabies virus RNA were found in 37% and 13% of the population, respectively, despite lack of symptomatic rabies or decreased survival recorded in the population over 9 to 13 years (East et al., 2001). Reports of rabies in other bear species are scarce. Experimental infections of brown (grizzly) bears (*Ursus arctos*) and black bears (*U. americanus*) have shown that both species are susceptible, but compared with canine species, a larger infective dose was needed to cause disease. This suggests that rabid foxes may not excrete sufficient quantities of virus in the saliva to infect bears (Rausch, 1975). Based on the negative serologic results from this study and previous reports of negative results from brain tissues from polar bears from Svalbard (Prestrud et al., 1992), this species should be regarded only as an occasional or rare host for rabies virus.

Polar bears at Svalbard are exposed to both morbillivirus and calicivirus, both of which are potential pathogens of seals. Further investigations may reveal whether these viruses cause health problems in this population of polar bears.

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