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FULL PAPER

Seroepidemiological survey of morbillivirus infection in Kuril harbor seals (*Phoca vitulina stejnegeri*) of Hokkaido, Japan

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Abstract

Serological analysis was performed to detect morbillivirus infection in Kuril harbor seals in Hokkaido, Japan. Serum samples were collected from the seals at Nosappu (231 sera), Akkeshi (16), and Erimo (75) between 1998 and 2005. Antibodies to phocine distemper virus (PDV) were detected by ELISA in seals from Nosappu and Erimo. Antibodies to PDV were found in 56% (5/9) of the sampled seals from Nosappu in 1998, versus only 5% (3/66) for 2003, 1% (1/79) for 2004, and 1% (1/77) for 2005. These suggest epidemic caused by the virus in or before 1998. As antibody-positive seals included juvenile seals in 2003 and 2005, sporadic infections of the virus are thought to have occurred in recent years. In Erimo, antibodies to PDV were found in 50% (14/28) of sampled seals in 2004, versus only 13% (1/8) for 1999, 7% (1/15) for 2003, and 0% (0/24) for 2005. These suggest sporadic infection

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by the virus before 2003 and the epizootic between after autumn in 2003, when samples of 2003 were collected, and 2004. Since antibodies to canine distemper virus (CDV) were detected in one adult seal from Nosappu in each year from 2003 to 2005, sporadic infections of the virus were suggested. There were no difference in incidence of seals with antibodies to the viruses between males and females and between juveniles and adults.

Key Words : Canine distemper virus, Kuril harbor seal, Marine mammal, Morbillivirus, Phocine distemper virus

Introduction

Canine distemper virus (CDV) and phocine distemper virus (PDV), two closely related viruses of the genus *Morbillivirus*, family *Paramyxoviridae*, are single-stranded negative RNA viruses. CDV is highly contagious pathogen that has a worldwide distribution[3,12] and wide host range that includes terrestrial carnivores[5] and phocids[35].

Epizootics in Baikal seals (*Phoca sibirica*) in 1987[13] and Caspian seals (*P. caspica*) in 2000[23] were attributed to CDV. PDV was first recognized in 1988 after being isolated from an epizootic in which more than 17,000 Eastern Atlantic harbor seals (*P. vitulina vitulina*) and grey seals (*Halichoerus grypus*) died in the North Sea[34]. In 2002, more than 22,000 dead Eastern Atlantic harbor seals were again found in the North Sea[15,37]. The cause of death was a PDV infection that had infected the same populations in 1988[21]. Infections of CDV and PDV in seals resulted in polysynthetic disorders such as bronchopneumonia, lymphoid depletion, encephalitis, and dermatitis. Widespread screenings suggest that many populations of pinnipeds in the North Atlantic were exposed to these viruses before and after the 1988 PDV outbreak[7-10,41]. Serological investigation of CDV and PDV infection in seals of other areas including the Antarctic Ocean[1] and the Northwestern Pacific[31] revealed that infections occurred in the animals.

The Kuril harbor seal (*P. v. stejnegeri*) is one of five harbor seal subspecies[2,25]. Kuril harbor seals are distributed in the Northwestern Pacific from the coast of Hokkaido northward through the Kuril Islands and eastern Kamchatka as far north as the Commander Islands. The population of Kuril harbor seals in Hokkaido dramatically declined in the 1960s and 1970s from hunting[20]. The number of hauling-out sites also decreased during that period. Although the population has been rebounding for the past twenty years, exceeding 900 individuals in 2002, the number of hauling-out sites has not recovered[40]. Approximately 60% of the Kuril harbor seals in Hokkaido are concentrated at two hauling-out sites (Daikoku Island, Akkeshi and Erimo) of the seven[4,22,40]. Overpopulation at hauling-out sites may make the seals susceptible to infectious disease. Mass mortalities of marine mammals caused by viral infection have not been reported in Hokkaido, but morbillivirus infection in pinnipeds in Hokkaido, including Kuril harbor seals, was reported before 1998[31]. In this study, we surveyed morbillivirus infection in Kuril harbor seals on the coast of Hokkaido in recent years.

Materials and Methods

Samples

Serum samples were collected from Kuril harbor seals at Nosappu (231 sera), Akkeshi

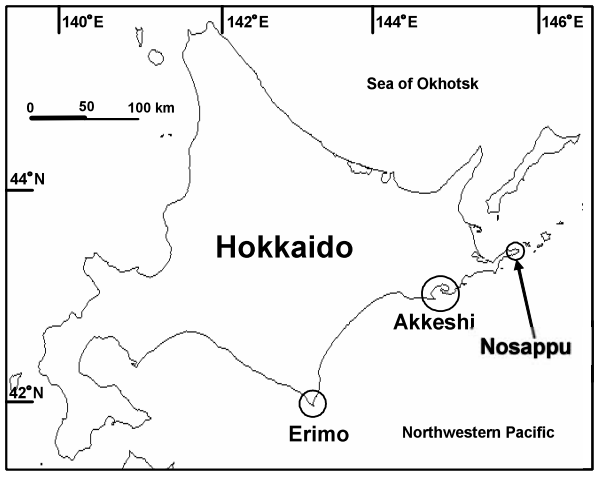

Fig. 1 Map of the sampling area
Serum samples were collected from Kuril harbor seals at Nosappu (231 sera), Akkeshi (16), and Erimo (75) between 1998 and 2005.

(16), and Erimo (75) between 1998 and 2005 (Fig. 1). All samples from Nosappu and Akkeshi were obtained from seals by-caught in fixed salmon nets from late August to November. Of the samples from Erimo, 46 were collected from seals captured under investigative capture from late June to early July and 29 samples were collected from seals by-caught in fixed salmon nets from late August to November. Body length (nose to tip of tail) was measured as an index of age. Seals measuring 125 cm or less were judged to be juveniles (age < 2 years)[11,29,42]. Of the juvenile seals at Erimo, those captured from late June to early July and having glossy hair were judged to be newborns younger than 2 months, because birthing season is mid to late May[29,30] and hair of seals that are 1 year old before molting season (July-August) is not glossy[11]. The newborn seals are considered to contain maternal antibodies[39,46].

Cells and viruses

Vero cells were cultivated in Eagle's MEM supplemented with 5% calf serum. PDV and the Onderstepoort strain of CDV from the repository of the Laboratory Animal Research Center, The Institute of Medical Science, The University of Tokyo were propagated in Vero cells. Antigens of Vero cells infected with PDV or CDV were dissolved in lysis buffer (1% Triton X-100, 1 mM iodoacetamide, 0.2 U/ml of the trypsin inhibitor aprotinin, 1 mM phenylmethylsulfonyl fluoride, 1% sodium deoxycholate, 0.14 M NaCl, and 10mM Tris-HCl at pH 8.0) and used as antigens in enzyme-linked immunosorbent assay (ELISA). Uninfected cell lysate was used as antigen for negative controls.

Enzyme-linked immunosorbent assay (ELISA)

Antibodies to PDV or CDV were detected by ELISA according to modified procedure described previously[24,32]. The lysate of Vero cells infected with PDV or CDV and uninfected cells was used as antigen to precoat the 96-well microtiter plates at 4℃ for at least 2 hr. After the wells were blocked with 1% bovine serum albumin (BSA) in PBS for 1 h at room temperature (RT), they were washed with PBST (PBS that contains 0.05% concentration of Tween 20). Fifty μl of serum diluted at 1 : 100 in PBS containing 0.5% BSA and 0.05% Tween 20 (BSA-PBST) was added to each well. After incubation at RT for 1 hr, the plates were washed with PBST. Fifty μl of Peroxidase-conjugated Protein G (Sigma) diluted at 1 : 200 in BSA-PBST was added to each well, and the plate was incubated for 1 hr at RT. The plates were washed with PBST, and 100 μl of substrate solution (0.05 M citrate buffer pH4.0, 0.008% hydrogen peroxide; 40m M2.2'-azino-di-3-ethyl-benzothiazobine-6-sulfuric acid) was added to each well. After RT incubation for 30 min, the optical density (OD) of each well was read by spectrophotometer using a 405 nm filter. Samples with OD values at least twice as great as the negative control were regarded as positive[32].

Table 1 Number of anti-PDV antibodies positive sera collected from Kuril harbor seals in Nosappu, Akkeshi, and Erimo, Hokkaido, Japan.

	Nosappu					Akkeshi					Erimo				
	Juvenile[a]		Adult		Total	Juvenile		Adult		Total	Juvenile (Juvenile excluding newborn[b])		Adult		Total
	Male	Female	Male	Female		Male	Female	Male	Female		Male	Female	Male	Female	
1998	0/0[c]	1/3	2/2	2/4	5/9	–	–	–	–	–	–	–	–	–	–
1999	–	–	–	–	–	–	–	–	–	–	0/3 (0/1)	1/2 (0/0)	0/3	–	1/8
2003	2/13	1/16	0/18	0/19	3/66	–	–	–	–	–	0/6 (0/4)	0/2 (0/1)	1/3	0/4	1/15
2004	0/12	0/6	0/35	1/26	1/79	–	–	0/2	0/2	0/4	8/13 (4/6)	4/10 (2/7)	1/3	1/2	14/28
2005	0/11	1/9	0/32	0/25	1/77	0/5	0/4	0/2	0/1	0/12	0/13 (0/3)	0/9 (0/2)	0/2	–	0/24

a) Seals measuring 125 cm or less, were judged to be juvenile (age< 2 years). Juvenile seals of Nosappu and Akkeshi did not contain newborn seals.
b) Of the juvenile seals at Erimo, those captured from late June to early July and having glossy hair were judged to be newborn younger than 2 months.
c) Number of positive / number of sample.

Table 2 Number of anti-CDV antibodies positive sera collected from Kuril harbor seals in Nosappu, Akkeshi, and Erimo, Hokkaido, Japan.

	Nosappu					Akkeshi					Erimo				
	Juvenile[a]		Adult		Total	Juvenile		Adult		Total	Juvenile (Juvenile excluding newborn[b])		Adult		Total
	Male	Female	Male	Female		Male	Female	Male	Female		Male	Female	Male	Female	
1998	0/0[c]	0/3	0/2	0/4	0/9	–	–	–	–	–	–	–	–	–	–
1999	–	–	–	–	–	–	–	–	–	–	0/3 (0/1)	0/2 (0/0)	0/3	–	0/8
2003	0/13	0/16	1/18	0/19	1/66	–	–	–	–	–	0/6 (0/4)	0/2 (0/1)	0/3	0/4	0/15
2004	0/12	0/6	0/35	1/26	1/79	–	–	0/2	0/2	0/4	0/13 (0/6)	0/10 (0/7)	0/3	0/2	0/28
2005	0/11	0/9	0/32	1/25	1/77	0/5	0/4	0/2	0/1	0/12	0/13 (0/3)	0/9 (0/2)	0/2	–	0/24

a) Seals measuring 125 cm or less, were judged to be juvenile (age< 2 years). Juvenile seals of Nosappu and Akkeshi did not contain newborn seals.
b) Of the juvenile seals at Erimo, those captured from late June to early July and having glossy hair were judged to be newborn younger than 2 months.
c) Number of positive / number of sample.

ELISA was performed in duplicate.

Results

Antibodies to morbillivirus were detected from serum of Kuril harbor seals from Nosappu and Erimo. Antibodies to PDV were found in 55.6% (5/9) of seals from Nosappu in 1998, versus 4.5% (3/66) for 2003, 1.3% (1/79) for 2004, and 1.3% (1/77) for 2005 (Table 1). There were statistical differences in the incidence between 1998 and other years (Fisher's exact test, $P<0.05$). Antibodies to PDV were found in 50% (14/28) of the seals from Erimo in 2004, versus 12.5% (1/8) for 1999, 6.7% (1/15) for 2003, and 0% (0/24) for 2005. There were statistical difference in the incidence between 2004 and 2003, and between 2004 and 2005 (Fisher's exact test, $P<0.05$). Incidence of seals with antibodies to PDV did not differ between males and females and between juveniles and adults at either areas in each year (Fisher's exact test, $P>0.05$). One out of 1 antibodies-positive juvenile seal in 1998 and 6 out of 12 antibodies-positive juvenile seals in 2004 from Erimo were considered to be newborn with maternal antibodies. There were no differences in incidence between juvenile males and juvenile females (excluding newborns), and there were no differences in incidence between juveniles excluding newborns and adults at Erimo in each year (Fisher's exact test, $P>0.05$). The range of OD for positive sera in PDV-ELISA was 0.22-0.56.

Antibodies to CDV were detected in the serum of 1 adult seal from Nosappu in each year from 2003 to 2005 (Table 2). There were no differences in incidence of antibodies to CDV between male and female and between juvenile and adult in each year (Fisher's exact test, $P > 0.05$). The range of OD for positive sera in CDV-ELISA was 0.27-0.47. No seals had antibodies to both PDV and CDV.

Discussion

We detected anti-PDV antibodies from the sera collected from Kuril harbor seals at Nosappu and Erimo. Ohashi and Kai[31] reported antibodies to PDV in 83% (19/23) of Kuril harbor seals of Hokkaido in 1996 and 100% (2/2) in 1997. In addition, >50% incidence was reported in other pinnipeds, such as steller sea lions (*Eumetopias jubatus*) and larga seals (*P. largha*), around Hokkaido from 1994 to 1998. Those authors suggested that PDV infection in pinnipeds in Hokkaido had occurred. In our study, though the incidence of antibodies to PDV in seals at Nosappu in 1998 was as high as the incidence reported for pinnipeds of Hokkaido from 1994 to 1998, the incidences in 2003, 2004, and 2005 were low. These results suggested that the viral infection in the seals from Nosappu may have decreased during the last few years. However, because antibodies to PDV have been detected in juvenile seals in recent years, it is considered that sporadic infections have occurred in recent years. The seals of the southern Kuril Islands also may be exposed to the virus. This is suspected because most of the seals by-caught in fixed salmon nets at Nosappu are considered to be from Habomai Islands[17,44]. In Erimo also, antibodies to PDV were detected from Kuril harbor seals. Sporadic infection was suggested by the samples of 1999 and 2003. The incidence of antibodies to PDV in 2004 was high, especially for juveniles. However, because 6 of 12 antibodies-positive juvenile seals in 2004 were newborn and may have had maternal antibodies, it should not be concluded that the antibody-positive rates translate directly into morbidity rates. The incidence of the antibodies in juvenile seals excluding newborns in 2004 was 46% (6/13), which was also high. The antibodies to PDV were not detected in sera in 2005. These results suggest that the epizootic of PDV occurred between after autumn 2003, when the sera of 2003 were collected, and 2004 in the population of Erimo. Antibodies to CDV were detected in 1 adult seal from Nosappu in each year from 2003 to 2004. These suggest CDV sporadic infection in the Kuril harbor seals from Nosappu. Kuril harbor seals in Hokkaido may be exposed to PDV more frequently than to CDV. For definitive diagnosis, detection of the viruses from the tissue or mucus of seals is required.

Kuril harbor seals haul out in dense concentrations[30]. The hauling-out periods should be considered opportunities for transmission of the virus. Behavioral differences between male and female Kuril harbor seals have been reported, including frequency of observation at hauling-out sites[30], however, no difference in incidence of antibodies to PDV and CDV between males and females was confirmed in this study.

Whereas incidence of seals from Nosappu with antibodies to PDV tended to decrease in 2000s, the incidence of seals from Erimo tended to increase in 2004. The distance from Erimo to Akkeshi is about 170 km, there are no hauling-out sites between the two areas[40]. Analysis of mtDNA has shown that there is little movement of Kuril harbor seals between Erimo and eastern Hokkaido (from Akkeshi to Nosappu)[45]. The difference in the incidence trends between Nosappu and Erimo may relate to the exiguity of contact between the

Kuril harbor seals inhabiting eastern Hokkaido and the Kuril harbor seals inhabiting Erimo.

In North Sea, PDV caused mass mortality of Eastern Atlantic harbor seals[15,34,37]. In Hokkaido, it was suggested that PDV infection has occurred in Kuril harbor seals, however, mass mortality associated with the viral infection in this area has not been reported. The high mortality rate of harbor seals in the North Sea may be attributed in part to environmental pollutants that suppress their immunity[6,14,33,38 43]. The level of contamination may be one of the factors in the differing mortality of seals between the North Sea and Hokkaido.

Grey seals are probably asymptomatic carriers of PDV in North Sea[14,36]. These seals are thought to be carriers for PDV between Arctic seals and harbor seals in the 1988 and 2002 outbreak in the North Sea. In Hokkaido, there are five species of seal : Kuril harbor seal, larga seal, ringed seal (*P. hispida*), ribbon seal (*P. fasciata*) and bearded seal (*Erignathus barbatus*). However Kuril harbor seals are found on Hokkaido coasts year round[30], and the four other species visit in winter and spring, drifting south to Hokkaido with pack ice[19,28]. Seasonally migrating seals may be able to carry the virus to Kuril harbor seals, which are known for their sedentary behavior. Larga seals are special note for this, because they often share hauling-out sites with Kuril harbor seals[18,27] and have great range of movement[26].

Mass mortalities in animal populations lead to dramatic changes in abundance and community structure, and it has been suggested that these events play an important role in shaping long-term population dynamics and, thereby, evolutionary processes[16]. Continual epidemiological study, including sympatric animals, is required for management and conservation of the marine mammals.

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