

indoors and out. It has been the practice on the farm to spread the bedding from the lambing pens, after six months storage, on to the pasture. Pastures were grazed simultaneously by sheep, dry cows and in-calf heifers, and some weeks later by milking cows alone.

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## Serological investigation of virus infections in harp seals (*Phoca groenlandica*) and hooded seals (*Cystophora cristata*)

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HERPES, influenza A and morbillivirus have caused mortality in seals, especially in the harbour seal (*Phoca vitulina*) (Geraci and others 1982, Osterhaus and others 1985, Osterhaus and Vedder 1988). More than 18,000 harbour seals died of a newly discovered phocine distemper virus (PDV) infection during the seal epizootic in European waters in 1988 and, according to earlier assumptions, the harp seal (*Phoca groenlandica*), may have been a vector for the infection in that epizootic (Goodhart 1988, McGourty 1988, Heide-Jørgensen and others 1992, Visser and others 1993a).

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Antibodies to herpesviruses have been detected in the harbour seal (Osterhaus and others 1985, Osterhaus 1988), California sea lion (*Zalophus californianus*) (Kennedy-Stoskopf and others 1986), Weddell seal (*Leptonychotes weddelli*) and crabeater seal (*Lobodon carcinophagus*) (Harder and others 1991). While antibodies to influenza A virus have been detected in the harbour seal and grey seal (*Halichoerus grypus*) (Geraci and others 1982, 1984). In addition, antibodies to canine distemper virus (CDV) and PDV have been detected in eight different seal species, including the hooded seal (*Cystophora cristata*) from Canadian waters (Heide-Jørgensen and others 1992) and the harp seal (Markussen and Have 1992). The present investigation was performed to examine the presence of serum antibodies against seal herpesvirus, influenza A virus, CDV and PDV in the harp and hooded seal.

Serum samples from harp and hooded seals were collected in the Barents Sea ('East Ice') and north of Jan Mayen ('West Ice') in April/May 1991 and 1992. Sera were collected from harp seals in both areas and from hooded seals north of Jan Mayen. One hundred samples (30 in 1991 and 70 in 1992) from hooded seals and 183 samples (43 in 1991 and 140 in 1992) from harp seals were analysed. Age distribution among the seals was not recorded, but most of the hooded seals were between one and four years old and most of the harp seals were adults (four years and older).

Sera were heat-inactivated for 30 minutes at 56°C before analysis. A virus neutralisation assay using seal kidney monolayer cell cultures with seal herpesvirus was performed as described by Osterhaus and others (1985). Serum antibody titres  $\geq 10$  were considered positive. An ELISA was used to investigate the presence of antibodies against influenza A viruses according to methods described by de Boer and others (1990). Serum antibody titres  $\geq 2$  were considered positive. Neutralising antibodies in serum against the Onderstepoort strain of CDV and a Danish isolate of PDV were analysed according to Markussen and Have (1992). Serum antibody titres  $\geq 10$  were considered positive.

Neither clinical signs nor pathological changes were recorded during sampling, although the lungs and hearts from 26 per cent of hooded seals but from only 1.5 per cent of harp seals were infected with the parasite *Dipetalonema spirocauda*. The results of the serum analyses are shown in Tables 1 and 2. These show that both the harp and hooded seals had antibodies against herpesvirus, influenza A virus and morbillivirus. Major differences in seroprevalence were not observed between harp seal populations from the Barents Sea and from north of Jan Mayen.

Antibodies against herpesvirus and influenza A virus have not previously been observed in harp and hooded seals, although sera from 102 harp and seven hooded seals from eastern Canada were examined for antibodies against influenza A virus (Geraci and others 1984).

The prevalence of CDV and PDV antibodies in the harp seal in the present study is similar to that observed in 1987 and 1989 by Markussen and Have (1992). As found in these previous studies, the mean neutralising serum titre in the harp seal against PDV (mean, 303; titre range, 10 to >1280) was higher than the mean neutralising titre against CDV (mean, 116; titre range 10 to >1280) (data not shown). The same trend was found in sera from hooded seals and indicated that these seal species had been infected with a virus more closely related to PDV and CDV.

**TABLE 1: Examination of serum samples for antibodies against seal herpesvirus, influenza A virus, canine distemper virus (CDV) and phocine distemper virus (PDV) in the harp seal (*Phoca groenlandica*)**

Year	Area	Number	Seal herpesvirus		Influenza A virus		CDV		PDV	
			+	-	+	-	+	-	+	-
1991	W	43	16	27	7	36	29	14	29	14
			% seropositive		37.2		16.2		67.4	
1992	E	70	29	41	7	63	61	9	70	0
			% seropositive		41.4		10.0		87.1	
	W	70	20	50	19	51	65	5	65	5
			% seropositive		28.6		27.1		92.9	

W 'West Ice', E 'East Ice'

+ Number seropositive, - Number seronegative



**TABLE 2: Examination of serum samples for antibodies against seal herpesvirus, influenza A virus, canine distemper virus (CDV) and phocine distemper virus (PDV) in the hooded seal (*Cystophora cristata*)**

Year	Area	Number	Seal herpesvirus		Influenza A virus		CDV		PDV	
			+	-	+	-	+	-	+	-
1991	W	30	2	28	0	30	1	29	1	29
% seropositive			6.7		0.0		3.3		3.3	
1992	W	70	3	67	8	62	2	68	2	68
% seropositive			4.3		11.4		2.9		2.9	

W 'West Ice'  
+ Number seropositive, - Number seronegative

No morbillivirus has yet been isolated and characterised from either the harp or hooded seals, although the first case of clinical phocine distemper has recently been reported in a two-month-old harp seal from Canadian waters (Daoust and others 1993). The antibodies detected in the present study could come from a PDV infection or infection from closely related morbilliviruses. These do not include the viruses isolated from porpoises (*Phocoena phocoena*) (Welsh and others 1992) and from striped dolphins (*Stenella coeruleoalba*) (Osterhaus and others 1992), since these were shown to be more closely related to the ruminant morbilliviruses than to PDV and CDV (for review see Visser and others 1993a).

In the present investigation, the seroprevalence of PDV antibodies in harp and hooded seals ranged from 6.7 to 100 per cent and 2.9 to 3.3 per cent, respectively. The present results also indicate a difference in seroprevalence of seal herpesvirus and influenza A virus specific antibodies between these two species. This difference in prevalence between harp and hooded seals could partly be a result of infection with different subtypes of viruses. Different morbilliviruses can give rise to different numbers of seropositive animals when tested against PDV and CDV (Cornwell and others 1992, Ross and others 1992), and depending on the herpesvirus isolates used in serological tests a significant difference has been observed in the frequency of seropositive seals (Frey and others 1989). Although different subtypes of influenza A virus have been isolated from harbour seals (Hinshaw and others 1984), the ELISA used detected antibodies to a conserved epitope on the N protein, and thus to all subtypes of influenza A virus.

Another explanation for the difference in prevalence of seropositive animals could be the result of different epidemiological situations, like differences in social contact and aggregation behaviour between individuals within each seal species. While very little is known about the behaviour and distribution of both harp and hooded seals during the major part of the year (Folkow and Blix 1992), it is known that the hooded seal does not aggregate in large social groups and is predominantly a solitary animal. The hooded seal remains scattered during pupping and moulting, typically forming small groups of only two or three animals (King 1983).

In contrast, the harp seal is known to stay in groups both in water and on land, and can form large groups at parturition and moulting (King 1983). Thus aerosol transmission of infection may easily occur between animals during this aggregation on land. Spread of virus infections through sea water may have less importance, since virus particles seem to lose infectivity rapidly in seawater (Suttle and Chen 1992).

A further explanation for the difference in prevalence of seropositive animals could be that hooded seals are less susceptible to infection or mount a weaker immune response than harp seals. The hooded seals sampled were younger than the corresponding harp seals and earlier investigations indicate that the ability of juvenile seals (less than 18 months) to mount an effective immune response is not as good as the ability of adults (Carter and others 1990). It should be noted that the three hooded seals seropositive for PDV were adults.

In conclusion, the present investigation confirms that harp seals in the Barents Sea and north of Jan Mayen are normally exposed

to morbillivirus and to a lesser extent herpesvirus and influenza A virus. Hooded seals north of Jan Mayen are also infected with these viruses. The difference in prevalence of seropositive animals between these two species could be due to differences in social contact and thereby the possibility of virus transmission within each population.

The present study, together with the studies of Dietz and others (1989) and Markussen and Have (1992), indicate that morbillivirus infections are endemic in the harp seal populations in the North Atlantic waters. This raises questions about the persistence of PDV in seals and about the duration of the virus excretion by infected animals (Visser and others 1993b).

Further studies of PDV infection in the harp seal should concentrate on the pathogenesis and virus persistence in this species which may also indicate whether the harp seal has indeed served as a vector for this virus and may be likely to do so in the future.

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