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Brief Report

In search of virus carriers of the 1988 and 2002 phocine distemper virus outbreaks in European harbour seals

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Summary

European harbour seal (*Phoca vitulina*) populations decreased substantially during the phocine distemper virus (PDV) outbreaks of 1988 and 2002. Different hypotheses have stated that various seals and terrestrial carnivore species might be the source of infection. To further analyse these hypotheses, grey (*Halichoerus grypus*) and ringed (*Phoca hispida*) seals, polar bears (*Ursus maritimus*) and minks (*Mustela lutreola*) were sampled from the North Sea and East Greenland coasts between 1988 and 2004 and investigated by RT-PCR using a panmorbillivirus primer pair. However, all samples were negative for morbillivirus nucleic acid.

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Mass mortalities of the European harbour seal (*Phoca vitulina*) populations were reported in 1988 and 2002 [8, 16, 17]. Phocine distemper virus (PDV), a member of family *Paramyxoviridae*, subfamily *Paramyxovirinae*, genus *Morbillivirus*, was identified as the causative agent [20, 25]. Different hypotheses concerning the origin of PDV and its geographical and chronological dissemination pattern have been presented. These include virus spread from less susceptible marine mammals, infection from diseased terrestrial animals and promotion by immunosuppressive environmental factors [16].

Thus, searching for possible PDV vectors, other infected migratory seal species that are in contact with European harbour seals were studied. Morbillivirus antibodies were detected in Canadian harp seals (*Phoca groenlandica*) even before their massive migration from Greenland to the North Sea coasts, in winter 1987 [6, 8, 11, 18, 22]. Whether these seals could have served as a virus reservoir for the 1988 mass die-off by introducing a virus that was highly virulent for harbour seals in a naive

seal population remains an intriguing hypothesis [8, 15, 17]. Although no harp seal migration was noted before the 2002 epidemic, experiments with tagged seals showed that during 2001–2002 some harp seals moved from Paulatuk (Canada) westwards to the Dutch North Sea coast (www.beaufortseals.com), indicating that individual animals could represent the source of infection. Furthermore, between the two epizootics, harp, hooded (*Cystophora cristata*), grey (*Halichoerus grypus*), and ringed (*Phoca hispida*) seals, and also harbour seals from the Western North Atlantic and Canadian Arctic were found to be seropositive for PDV [9–11]. The North Atlantic coasts represent a unique area where the habitats of these seal species overlap [26]. Therefore, a potential transmission of PDV between different seal species living in this area and thereby triggering both epidemics by migrating animals seems possible.

In addition, between 1990 and 1991, grey seals from the coasts of Estonia showed signs of PDV infection. It was also observed that grey seals acted as carriers of the PDV infection among different seal populations during the 2002 epidemic [14]. Thus, due to their high migratory behaviour, Baltic grey seals might have served as PDV carriers between the North Sea and the Baltic seal populations in 2002 [12].

In 1988 and 2002, harbour seals were infected with an almost identical PDV strain [20, 23]. This raises the question whether PDV was continuously circulating among the European seals between the two epizootics, or if it was re-introduced. Between 1988 and 1993, grey and harbour seals from the Dutch Wadden Sea were found to be seropositive for PDV [30]. Similar results were obtained for the grey seals from the British Farne Island in 1992 [1]. Whether these findings indicate a persistent infection or are residuals from the 1988 epidemic remained undetermined. Contrary to this, others found no evidence of a PDV infection in harbour and grey seals in the Wash and other British sites of the North Sea after the 1988 outbreak [19]. Similarly, a screening for virus RNA in seals after 1997 revealed consistently negative results [20]. Likewise, the majority of seals from German waters investigated before the 2002 outbreak lacked morbillivirus-specific antibodies [23].

In addition, it was suggested that, similar to the mass death of Baikal (*Phoca sibirica*) and Caspian (*Phoca caspica*) seals [21], infected terrestrial carnivores could represent the primary source of infection in harbour seals. However, in those cases, CDV was identified as the cause of the epizootics and not PDV. It still remains a possibility that PDV, with a reduced virulence for terrestrial mammals, is circulating in these species and causes mass die-offs in pinnipeds after crossing the species barrier [4]. Before 1988, cross-species infection between minks and seals had been reported [4, 24], but little is known about the role of terrestrial carnivores such as minks and polar bears during the second PDV outbreak. Virus-infected minks from coastal areas around the North Sea, as well as Canadian Arctic polar bears preying on arctic seals could be a potential source of infection or transmission of PDV to seals. Serological investigation between 1990 and 1998 showed that polar bears from the Norwegian coasts and the pack ice of the Barents Sea were exposed to CDV [29]. In addition, polar bears captured between 1984 and 1996 in the Canadian Arctic were positive for antibodies to PDV [5, 27].

Environmental factors such as high pollution burdens in seals from the Baltic and North Seas were also mentioned as contributing factors to the high mortality during the epizootics. It has been shown that accumulations of polychlorinated biphenyls (PCBs) and heavy metals in the food chain could impair reproduction and immune functions in harbour, grey and ringed seals as well as in harbour porpoises [2, 3, 7, 28]. However, it is difficult to establish a direct causal link between the susceptibility for infection and the exposure to environmental factors.

The aim of this study was to investigate the presence of morbillivirus transcripts in grey and ringed seals, polar bears and minks that could serve as a PDV vector or reservoir for the 1988 and 2002 outbreaks.

The investigated tissues (lung, spleen, liver and kidney) were collected from 207 dead adult terrestrial and marine mammals, geographically (Fig. 1) and annually distributed around the two PDV outbreaks in Europe (Table 1). Thus, 41 grey seals were sampled from the German and Dutch North

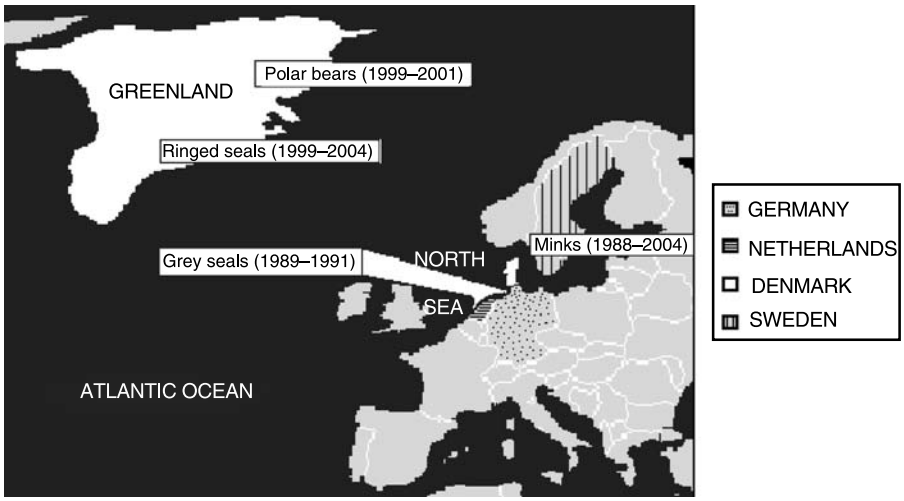

Fig. 1. Geographical distribution of the regions from which the investigated animals were collected

Table 1. Species, number and source of animals tested for morbillivirus infection

Species	Number of animals	Year	Organs	Source	Total number of animals per species
Grey seals (*Halichoerus grypus*)	36	1989	lung and spleen	Germany	41 grey seals
	2	1989	liver	Netherlands	
	2	1990	liver		
	1	1991	liver		
Ringed seals (*Phoca hispida*)	15	1999	liver/kidney	East Greenland, Denmark	76 ringed seals
	16	2000	liver/kidney		
	16	2002	liver/kidney		
	14	2003	liver/kidney		
	15	2004	liver/kidney		
Polar bears (*Ursus maritimus*)	female 5 male 7	1999	kidney	East Greenland, Denmark	63 polar bears
	female 16 male 13	2000	kidney		
	female 11 male 11	2001	kidney		
European minks (*Mustela lutreola*)	male 1	1988	lung and spleen	SW Sweden	27 European minks
	female 1	1989	lung and spleen		
	male 7	1990	lung and spleen		
	female 5 male 2	2002	lung and spleen		
	female 5 male 6	2004	lung and spleen		

Sea coasts between 1989 and 1991, 76 ringed seals from East Greenland, aboriginal hunted between 1999 and 2004, 27 European wild minks (*Mustela lutreola*) from the southwestern Swedish North Sea coast, and 63 free-ranging polar bears (*Ursus maritimus*) from the coast of East Greenland hunted between 1999 and 2001. Tissues were stored at −20 °C until used. For RNA isolation, the RN easy Maxi Kit (Qiagen, Hilden, Germany) followed by a DNase treatment according to the manufacturer's instructions was applied. Subsequently, RT-PCR was performed using 2 µg total RNA, random pri-

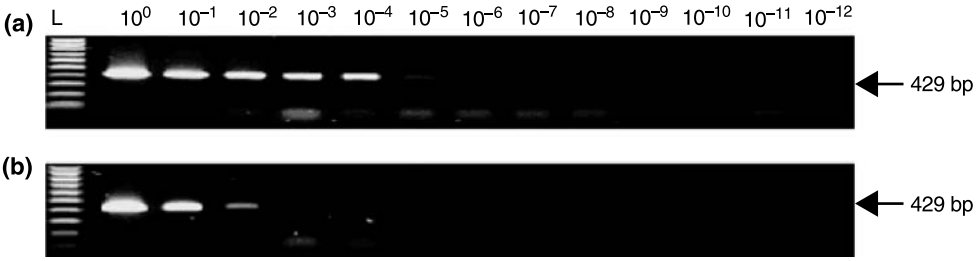

Fig. 2. RT-PCR reactions of serial 10-fold dilutions of viral RNA extracted from Vero cells infected with PDV (**a**) and Onderstepoort-CDV (**b**) using universal morbillivirus primers. The starting RNA concentrations were 1 µg/µl for PDV and 1 ng/µl for Onderstepoort-CDV. L-100 bp DNA ladder

mers (Promega) and the Omniscript RT-PCR kit (Qiagen, Hilden, Germany). The cycling temperature profile for the PCRs was: 94 °C for 5 min, 40 cycles consisting of denaturation at 95 °C for 0.30 min, annealing at 60 °C for 1 min, extension at 72 °C for 2.50 min, and a final extension step at 72 °C for 10 min.

As a control for the integrity of RNA, PCR with specific primers for canine glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were used [13]. The length of the PCR product corresponded to a 364-bp band. For identification of morbillivirus RNA, a set of universal morbillivirus primers was used [1]. The expected amplicon length is 429 bp. Negative controls included RNA extracted from non-infected Madin-Darby canine kidney (MDCK) cells, lung from a healthy dog and two seals previously identified as negative for morbillivirus antigen, and viral RNA [23]. RNA extracted from DH82 cells infected with Onderstepoort-CDV and Vero cells infected with PDV, Onderstepoort-CDV, porpoise morbillivirus or the Edmonston strain of measles virus were used as positive controls [12]. In addition, RNA extracted from two seals previously tested positive for morbillivirus antigen and RNA were included [23].

To determine the sensitivity of the PCR reactions, PDV and Onderstepoort-CDV RNA was isolated by using the NucleoSpin RNA virus kit (Macherey-Nagel, Düren, Germany) from 300 µl viral suspensions containing $10^{4.75}$ and $10^{7.75}$ $TCID_{50}$/ml, respectively. Viral RNA was eluted in 50 µl distilled H_2O and subsequently, serial dilutions were prepared (from 10^{0} to 10^{-10}) and the presence of viral nucleic acid was analyzed by RT-PCR using the universal morbillivirus primers (Fig. 2). Using this procedure, at least 10 $TCID_{50}$ units of morbillivirus could be detected.

Morbillivirus nucleic acid was detected in all investigated morbillivirus strains including PDV, CDV, the Edmonston strain of measles virus and the porpoise morbillivirus and, as expected, not in the negative controls. All investigated samples from grey and ringed seals, polar bears (*Ursus maritimus*) and minks were positive for GAPDH RNA. However, no morbillivirus transcripts were present in the samples. These negative findings provide further indirect support for the hypothesis that the PDV source for the outbreaks were migrating animals originating from the northwestern or eastern sides of the North Sea, the North Atlantic and the Baltic Sea. Therefore, a circulating PDV strain among the autochthonous seal populations seems less likely; however, due to the limited number of investigated animals, this still remains a possibility. On the other hand, the investigated animals were collected before, during and after the two epizootics from geographical areas relevant for both PDV outbreaks. In addition, the RNA was often isolated from organs including lung and spleen, which are primarily infected in phocine distemper. RNA integrity was also confirmed by demonstrating intact house-keeping gene RNAs.

Like grey seals, infected ringed seals could also play a role in the spread of the second epidemic. Ringed seals are the most widespread seals in the arctic and they are the main prey of polar bears. Thus, they could contribute to the outbreak by virus transmission to (i) other seal species due to their migratory behaviour and/or (ii) to their predators.

Between 1984 and 1987, harp and ringed seals from Greenland were positive for PDV antibodies [8], and based on this observation it was suspected that Greenlandic seals represent a PDV reservoir for the first epidemic. However, in the present study, 76 ringed seals and 63 polar bears from Greenland, collected between 1999 and 2001, lacked morbillivirus nucleic acid. Thus, it seems possible that, due to the limited number of investigated animals, individuals acting as potential morbillivirus carriers were not detected.

A direct contact between infected minks and seals around the Isle of Anholt during the second epizootic could not be excluded. Consequently, we investigated 27 European minks found dead on the southwestern Swedish North Sea coast near the area where both epizootics started, but none of the minks were positive for PDV or a related morbillivirus.

To summarise, despite investigating a variety of marine and terrestrial mammal species, the source of the two PDV outbreaks in European harbour seal and the origin of the virus remains undetermined.

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References

1. Barrett T, Crowther J, Osterhaus ADME, Subbarao SM, Groen J, Haas L, Mamaev LV, Titenko AM, Visser IKG, Bostock CJ (1992) Molecular and serological studies on the recent seal virus epizootics in Europe and Siberia. Sci Total Environ 115(1–2): 117–132
2. Beineke A, Siebert U, McLachlan M, Bruhn R, Thron K, Failing K, Müller G, Baumgärtner W (2005) Investigations of the potential influence of environmental contaminants on the thymus and spleen of harbour porpoises (Phocoena phocoena). Environ Sci Technol 39: 3933–3938
3. Bernt KE, Hammill MO, LeBoeuf M, Kovacs KM (1999) Levels and patterns of PCBs and OC pesticides in harbour and grey seals from the St Lawrence Estuary, Canada. Sci Total Environ 243/244: 243–262
4. Blixenkrone-Møller M, Svansson V, Have P, Botner A, Nielsen J (1989) Infection studies in mink with seal-derived morbillivirus. Arch Virol 106: 165–170
5. Cattet MRL, Duignan PJ, House CA, St Aubin DJ (2004) Antibodies to canine distemper and phocine distemper viruses in polar bears from Canadian Arctic. J Wildl Dis 40(2): 338–342
6. Daoust PY, Haines DM, Thorsen J, Duignan PJ, Geraci JR (1993) Phocine distemper in harp seal (*Phoca groenlandica*) from the Gulf of St. Lawrence, Canada. J Wildl Dis 29: 112–117
7. de Swart RL (1995) Impaired immunity in seals exposed to bioaccumulated environmental contaminants. Dr. Thesis, Erasmus University, Rotterdam, Netherlands, 140 pp
8. Dietz R, Hansen CT, Have P, Heide-Jørgensen MP (1989) Clue to seal epizootic. Nature 338: 627
9. Duignan PJ, Sadove S, Saliki JT, Geraci JR (1993) Phocine distemper in harbour seals (*Phoca vitulina*) from Long Island, New York. J Wildl Dis 29: 465–469
10. Duignan PJ, Saliki JT, St. Aubin DJ, Early G, Sadove S, House JA, Kovacs K, Geraci JR (1995) Epizootiology of morbillivirus infection in North American harbour seals (Phoca vitulina) and grey seals (Halichoerus grypus). J Wildl Dis 31(4): 491–501
11. Duignan PJ, Nielsen O, House C, Kovacs KM, Duffy N, Early G, Sadove S, St Aubin DJ, Rima BK, Geraci JR (1997) Epizootiology of morbillivirus infection in harp, hooded, and ringed seals from the Canadian Arctic and Western Atlantic. J Wildl Dis 33: 7–19
12. Frisk AL, König M, Moritz A, Baumgärtner W (1999) Detection of canine distemper virus nucleoprotein RNA by reverse transcription-PCR using serum, whole blood, and cerebrospinal fluid from dogs with distemper. J Clin Microbiol 37(11): 3634–3643
13. Gröne A, Frisk AL, Baumgärtner W (1998) Cytokine mRNA expression in whole blood samples from dogs with natural canine distemper virus infection. Vet Immunol Immunopathol 65: 11–27
14. Hammond JA, Pomeroy PP, Hall JA, Smith JA (2005) Identification and real-time PCR quantification of *Phocine distemper virus* from two colonies of Scottish grey seals in 2002. J Gen Virol 86: 2563–2567
15. Have P, Nielsen J, Botner A (1991) The seal death in Danish waters 1988. 2. Virological studies. Acta Vet Scand 32(2): 211–219
16. Härkönen T, Dietz R, Reijnders P, Teilmann J, Harding K, Hall A, Brasseur S, Siebert U, Goodman SJ, Jepson PD, Rasmussen TD, Thompson P (2006) A review of the

1988 and 2002 phocine distemper virus epidemic in European harbour seals. Dis Aquat Organ 68: 115–130
17. Heide-Jørgensen MP, Härkönen T, Dietz R, Thompson PM (1992) Retrospective of the 1988 European seal epizootic. Dis Aquat Organ 13: 37–62
18. Henderson G, Trudgett A, Lyons C, Ronald K (1992) Demonstration of antibodies in archival sera from Canadian seals reactive with the European isolate of phocine distemper virus. Sci Total Environ 115: 93–98
19. Hughes DE, Carter SD, Robinson I, Clarke DD, Clarke CJ (1992) Anti-canine distemper virus antibodies in common and grey seals. Vet Rec 130: 449–450
20. Jensen T, van de Bildt M, Dietz HH, Andersen TH, Hammer AS, Kuiken T, Osterhaus ADME (2002) Another phocine distemper outbreak in Europe. Science 297: 209
21. Kennedy S, Kuiken T, Jepson PD, Deaville R, Forsyth M, Barrett T, van de Bildt MWG, Osterhaus ADME, Eybatov T, Duck C, Kydyrmanov A, Mitrofanov I, Wilson S (2000) Mass die-off of Caspian seals caused by canine distemper virus. Emerg Infect Dis 6: 637–639
22. Markussen NH, Have P (1992) Phocine distemper virus infection in harp seals (Phoca groelandica). Mar Mamm Sci 8: 19–26
23. Müller G, Wohlsein P, Beineke A, Haas L, Greiser-Wilke I, Siebert U, Fonfara S, Harder T, Stede M, Gruber AD, Baumgärtner W (2004) Phocine distemper in German seals, 2002. Emerg Infect Dis 10: 723–725
24. Orvell C, Blixenkrone-Møller M, Svansson V, Have P (1990) Immunological relationships between phocid and canine distemper virus studied with monoclonal antibodies. J Gen Virol 71: 2085–2092
25. Osterhaus ADME, Vedder AEJ (1988) Identification of a virus causing recent seal deaths. Nature 335: 20
26. Perrin WF, Würsig B, Thewissen JGM (2002) Encyclopedia of marine mammals, 1st edn. Academic Press, San Diego, 1414 pp
27. Philippa JD, Leighton FA, Daoust PY, Nielsen O, Pagliarulo M, Schwantje H, Van Herwijnen R, Martina BEE, Kuiken T, Van De Bilt MWG, Osterhaus ADME (2004) Antibodies to selected pathogens in free-ranging terrestrial carnivores and marine mammals in Canada. Vet Rec 155: 135–140
28. Reijnders PJH (1986) Reproductive failure in common seals feeding on fish from polluted coastal waters. Nature 324: 456–457
29. Tryland M, Neuvonen E, Huovilainen A, Tapiovaara A, Osterhaus ADME, Wiig Ø, Derocher AE (2005) Serologic survey for selected virus infections in polar bears at Svalbard. J Wildl Dis 41: 310–316
30. Visser IKG, Vedder EJ, Vos HW, van de Bildt MWG, Osterhaus ADME (1993) Continued presence of phocine distemper virus in the Dutch Wadden Sea seal population. Vet Rec 133: 320–322