Neutralizing antibodies to Phocine Distemper Virus in Atlantic Walruses (Odobenus rosmarus rosmarus) from Arctic Canada

Authors: Duignan, Pádraig J., Saliki, Jeremiah T., St. Aubin, David J., House, James A., and Geraci, Joseph R.

Source: Journal of Wildlife Diseases, 30(1) : 90-94

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-30.1.90

Journal of Wildlife Diseases, 30(1), 1994, pp. 90–94

Neutralizing antibodies to Phocine Distemper Virus in Atlantic Walruses (*Odobenus rosmarus rosmarus*) from Arctic Canada

Pádraig J. Duignan,[1,3] Jeremiah T. Saliki,[2] David J. St. Aubin,[1] James A. House,[2] and Joseph R. Geraci,[1] [1] Department of Pathology, Ontario Veterinary College, University of Guelph, Guelph, Ontario N1G 2W1, Canada; [2] National Veterinary Services Laboratories, Foreign Animal Diseases Diagnostic Laboratory, Greenport, New York 11944, USA; [3] Author for reprint requests.

ABSTRACT: The first evidence of phocine distemper virus (PDV) infection in Atlantic walruses (*Odobenus rosmarus rosmarus*) from Nottingham Island, Northwest Territories, Canada, is reported. Blood samples were collected from three male walruses killed by Inuit hunters in the fall of 1990. Differential virus neutralization test for each animal yielded higher titers against PDV than against other members of the *Morbillivirus* genus including canine distemper, peste des petits ruminants, rinderpest and measles viruses. Thus, PDV infection may be enzootic in walruses of the eastern Canadian Arctic.

Key words: Walrus, *Odobenus rosmarus*, morbillivirus, phocine distemper virus, antibodies, virus neutralizing titer, epizootic.

We report the first serological evidence of phocine distemper virus (PDV) infection in Atlantic walruses (*Odobenus rosmarus rosmarus*) from the eastern Canadian Arctic. Since 1987, morbilliviruses have emerged as the most potent agents of mass mortality in both pinnipeds and odontocete cetaceans worldwide. The cluster of epizootics began in 1987 when a virus similar to canine distemper virus (CDV) killed thousands of Baikal seals (*Phoca sibirica*) in the Soviet Union (Grachev et al., 1989). A similar, but unrelated, epizootic caused by PDV was equally devastating to European harbor seals (*Phoca vitulina*) during the 1988 breeding season (Kennedy, 1990). In contrast to harbor seals, morbidity in gray seals (*Halichoerus grypus*) was high but mortality was negligible (Kennedy et al., 1989; Harwood, 1989). Related morbilliviruses also have caused a distemper-like disease in European harbor porpoises (*Phocoena phocoena*) and Mediterranean striped dolphins (*Stenella coeruleoalba*) (Kennedy et al., 1988; Duignan et al., 1992).

More recently, PDV, or a closely related morbillivirus, has emerged as a cause of disease in North American harp seals (*Phoca groenlandica*) and harbor seals (Daoust et al., 1993; Duignan et al., 1993). Based on retrospective serology, PDV infection may be enzootic in some populations of pinnipeds in Atlantic Canada and along the northeast coast of the United States (Henderson et al., 1992; Ross et al., 1992; Duignan, Saliki and Geraci, unpubl.). Based on the high prevalence of morbillivirus antibodies in harp seals from Greenland (Dietz et al., 1989), the Barents Sea and Jan Mayen (Markussen and Have, 1992), and eastern Canada (Henderson et al., 1992), they may be the reservoir host for PDV. Increasing harp seal populations and unusual distribution patterns in recent years may have been responsible for introducing PDV into susceptible pinniped populations in both Europe and North America (Roff and Bowen, 1986; Harwood, 1989; Odell, 1991). Here we provide the first evidence that the Atlantic walrus, which seasonally shares part of its range with harp seals in Baffin Bay, Davis Strait, Foxe Basin, Hudson Bay and Hudson Strait has been exposed to PDV.

As part of ongoing studies by the Canadian Department of Fisheries and Oceans, specimens are routinely collected from marine mammals taken by Inuit hunters throughout the Arctic. Three male Atlantic walruses were sampled in Hudson Strait, south of Nottingham Island (63°20′N, 77°55′W), one on 26 September 1990, and two others on 1 October 1990. One of the animals (OrN90-16) was of mature body size (370 cm total length), and

a second (OrN90-15) was a subadult (280 cm); no measurements were obtained on the third (OrN90-13). Blood was collected from the heart 30 to 40 min after death, and kept on ice for 4 to 5 hr before centrifugation to collect serum. Serum samples were held on ice for 1 to 2 days, then stored at −20 C until tested for virus neutralizing activity against a panel of morbilliviruses. The following viruses were used in the virus neutralizing (VN) test; CDV, Onderstepoort strain, and measles virus (MV), Edmonston strain (kindly provided by Dr. Max Appel, Cornell University); peste des petits ruminants (PPRV) Nigeria 75/1 attenuated strain (Diallo et al., 1989); phocine distemper virus (PDV) provided by Dr. A. D. M. E. Osterhaus, National Institute of Public Health, The Netherlands; and rinderpest virus (RPV) RBOK vaccine strain from the Foreign Animal Disease Diagnostic Laboratory repository. All viruses were adapted to grow in Vero cells (Culture # CCL 81, American Type Culture Collection, Rockville, Maryland, USA). For preparation of virus stocks for VN test, Vero cells grown in minimum essential medium supplemented with 5% bovine fetal serum (Rossiter et al., 1985) were infected in suspension at a multiplicity of infection (MOI) of 0.01 tissue culture infective doses ($TCID_{50}$/cell) and allowed to form monolayers. Virus was harvested when cytopathic effects (CPE) were seen in over 80% of the cell monolayer, clarified by centrifugation at 1000 × *g* for 10 min, and stored in 1.8 ml aliquots at −70 C. Virus infectivity titer was determined by the Reed and Muench method (Reed and Muench, 1937).

The VN test was performed using the following modification of the micromethod described for RPV and PPRV (Rossiter et al., 1985). Briefly, two-fold dilutions of sera were made in triplicate in wells of 96-well microtiter plates using minimum essential medium with Earle's salts (EMEM) supplemented with 5% fetal bovine serum (Rossiter et al., 1985). An equal volume (50 µl) of virus containing approximately

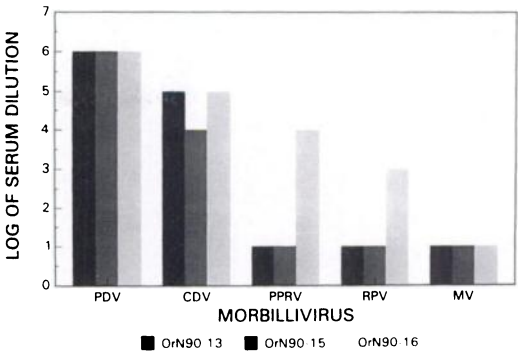

FIGURE 1. Distribution of serum virus neutralizing titers in three Atlantic walruses (OrN90-13, OrN90-15, OrN90-16) against PDV, CDV (Onderstepoort strain), PPRV (Nigeria 75/1, attenuated strain), RPV (RBOK vaccine), MV (Edmonston strain). Titers are expressed as $\log_2$ of the reciprocal of the highest dilution of serum that completely neutralized CPE in two of two wells. Titers ≤3 are regarded as negative.

100 $TCID_{50}$ was added to two wells of each triplicate while the third well received an equal volume of EMEM as serum control. Plates were incubated at 20 C for 2 hr; Vero cells (100 µl, 10^4 cells) then were added to each well and the plates incubated at 37 C in 5% carbon dioxide. The test was read on day 4 (for CDV), day 7 (for MV, PPRV and RPV) or day 8 (for PDV) by examining cell monolayers for virus-specific cytopathic effects (CPE). Titers were expressed as $\log_2$ of the reciprocal of the highest dilution of serum that completely neutralized CPE in duplicate wells. Virus neutralizing titers against PDV were 6 for three walruses, at least one log dilution less against CDV while titers against PPRV, RPV and MV were either low (one animal) or negative (Fig. 1).

Titration of the sera against all five morbilliviruses was warranted by the fact that the viruses cross-react serologically, titers being highest against the homologous virus (Imagawa, 1968; Visser et al., 1990). Based on differential virus neutralization, these walruses were infected by a morbillivirus similar, if not identical, to PDV. The variations in VN titers between the walruses most likely reflects the stage of the im-

mune response during which the sera were collected rather than a difference in viral strains. Following infection, morbillivirus antibody titers rise, stabilize, and decline steadily over a period of years (Appel et al., 1981). Furthermore, as all morbilliviruses consist of only one serotype (Appel et al., 1981), the static VN test used here may not be used to differentiate between strains.

We cannot ascertain when or how phocine distemper virus was introduced into this population. Atlantic walruses seasonally share the same range as harp, ringed (*Phoca hispida*) and bearded seals (*Erignathus barbatus*), and occasionally harbor seals (Mansfield, 1958). Opportunities for casual contact between walruses and these other species of pinnipeds are considered rare, since under most circumstances seals avoid walruses because of their large size, aggressiveness, and infrequent habit of preying on smaller seals (Mansfield, 1958). Nevertheless, we propose three alternative interpretations of the data. First, PDV may be an enzootic infection in Atlantic walruses with a high level of immunity in the population and low mortality. Second, a PDV epizootic may have spread through this population at some time in the past causing little mortality but inducing persistent virus neutralizing titers in affected animals. Third, walruses are dead-end hosts, supporting sufficient virus replication to elicit neutralizing antibody following exposure from an undetermined source, but without sufficient virus shedding to permit transmission. Further sampling of walruses in the Canadian Arctic will be required to determine which is the more likely senario.

The risk of an epizootic in any pinniped species depends on introduction of the virus, social organization, probability of contact with infective individuals, susceptibility and, possibly, environmental factors (Harwood and Grenfell, 1990). Walruses are highly gregarious and tend to form large, dense aggregations at haul-out sites on ice or land, a behavior that would facilitate virus transmission (Fay, 1981; Harwood and Grenfell, 1990). In a late-maturing, low-fertility species such as the walrus a phocine distemper epizootic causing high mortality could be devastating (McLaren, 1990), not only for the species but also to the economy of the Inuit (Mansfield, 1958).

Sporadic mass mortality has occurred in Pacific walruses (*Odobenus rosmarus divergens*) and the most recent event, in 1978, resulted in the death of approximately 1,000 animals on St. Lawrence Island in the Bering Sea (Fay and Kelly, 1980). On initial investigation, the cause of death was identified as trauma due to overcrowding at haul-out sites; however, the authors noted that 15% of the carcasses were aborted fetuses and did not rule out the possibility of an infectious agent (Fay and Kelly, 1980). In a recent survey of 158 Pacific walruses sampled prior to 1988 in the east and west Bering Sea, Osterhaus et al. (1988) found no CDV neutralizing antibodies. Virus neutralizing antibodies against PDV were not measured in that study. Thus, the Pacific walrus population, estimated at 160,000 animals (Estes and Gol'tsev, 1984) appears to be immunologically-naive with respect to morbilliviruses. Consequently, the potential for a phocine distemper epizootic in the western Arctic would appear to be much greater than in the east.

The significance of PDV infection as a cause of natural mortality in Atlantic walruses is not known. Currently the walrus population in the Canadian Arctic is estimated to be at least 10,000 animals (Richard and Campbell, 1988) and an increase in natural mortality has not been noted in recent years. Perhaps the epizootiology of phocine distemper infection in this species is similar to that in North American harbor and harp seals in which sporadic mortality occurs in juvenile animals (Daoust et al., 1993; Duignan et al., 1993). More extensive serological surveys and continuous monitoring of natural mortality in Arctic pinnipeds will be required to determine

the prevalence, and significance, of phocine distemper infection in this ecosystem.

We thank Marilyn Patterson, Marge Llewellyn and Michael LaRocco for technical assistance. We are grateful to Dr. Charles Mebus for permitting laboratory testing in the USDA Foreign Animal Disease Diagnostic Laboratory. Heather Copland, Pathology Department, University of Guelph, assisted with production of the manuscript. We acknowledge the interesting comments of the anonymous reviewers. The work was funded in part by the United States Office of Naval Research (Grant #N00014-87-J-114 to DJS and JRG) and the Department of Fisheries and Oceans (Canada) Science Subvention Program (to JRG).

LITERATURE CITED

APPEL, M. J. G., E. P. J. GIBBS, S. J. MARTIN, V. TER MEULEN, B. K. RIMA, J. R. STEPHENSON, AND W. P. TAYLOR. 1981. Morbillivirus diseases in animals and man. *In* Comparative diagnosis of viral disease IV, E. Kurstak and C. Kurstak (eds.). Academic Press, New York, New York, pp. 235–297.

DAOUST, P.-Y., D. M. HAINES, J. THORSEN, P. J. DUIGNAN, AND J. R. GERACI. 1993. Phocine distemper in a harp seal (*Phoca groenlandica*) from the Gulf of St. Lawrence, Canada. Journal of Wildlife Diseases 29: 114–117.

DIALLO, A., W. P. TAYLOR, P.-C. LEFEVRE, AND A. PROVOST. 1989. Atténuation d'une souche de virus de la peste des petits ruminants: Candidat pour un vaccin homologue vivant. Revue d'Elevage et de Médecine Véterinaire des Pays Tropicaux 42: 311–319.

DIETZ, R., C. T. ANSEN, P. HAVE, AND M. P. HEIDE-JORGENSEN. 1989. Clue to seal epizootic? Nature 338: 627.

DUIGNAN, P. J., J. R. GERACI, J. A. RAGA, AND N. CALZADA. 1992. Pathology of morbillivirus infection in striped dolphins (*Stenella coeruleoalba*) from Valencia and Murcia, Spain. Canadian Journal of Veterinary Research 56: 242–248.

———, S. SADOVE, J. T. SALIKI, AND J. R. GERACI. 1993. Phocine distemper in harbor seals (*Phoca vitulina*) from Long Island, New York. Journal of Wildlife Diseases 29: 465–469.

ESTES, J. A., V. N. GOL'TSEV. 1984. Abundance and distribution of the Pacific walrus (*Odobenus rosmarus divergens*): Results of the first Soviet-American joint aerial survey, Autumn 1975. National Oceanographic and Atmospheric Administration Technical Report Number 12, National Marine Fisheries Service, U.S. Department of Commerce, Springfield, Virginia, pp. 67–76.

FAY, F. H. 1981. Walrus, *Odobenus rosmarus* (Linnaeus, 1758). *In* Handbook of marine mammals, Vol. 1, S. H. Ridgway and R. J. Harrison (eds.). Academic Press, London, England, pp. 1–23.

———, AND B. P. KELLY. 1980. Mass natural mortality of walruses (*Odobenus rosmarus*) at St. Lawrence Island, Bering Sea, Autumn 1978. Arctic 33: 226–245.

GRACHEV, M. A., V. P. KUMAREV, L. V. MAMEV, V. L. ZORIN, L. V. BARANOVA, N. N. DENEKINA, S. I. BELIKOVA, E. A. PETROV, V. S. KOLESNIK, V. N. DOROFEEV, A. M. BEIM, V. N. KUDELIN, F. G. NAGIEVA, AND V. N. SIDOROV. 1989. Distemper virus in Baikal seals. Nature 338: 209.

HARWOOD, J. 1989. Lessons from the seal epidemic. New Scientist 121: 38–42.

———, AND B. GRENFELL. 1990. Long term risks of recurrent seal plagues. Marine Pollution Bulletin 21: 284–287.

HENDERSON, G., A. TRUDGETT, C. LYONS, AND K. RONALD. 1992. Demonstration of antibodies in archival sera from Canadian seals reactive with a European isolate of phocine distemper virus. The Science of the Total Environment 115: 93–98.

IMAGAWA, D. T. 1968. Relationships among measles, canine distemper and rinderpest viruses. Progress in Medical Virology 10: 160–193.

KENNEDY, S. 1990. A review of the 1988 European seal morbillivirus epizootic. The Veterinary Record 127: 563–567.

———, J. A. SMYTH, P. F. CUSH, S. J. MCCULLOUGH, G. M. ALLEN, AND S. MCQUAID. 1988. Viral distemper now found in porpoises. Nature 336: 21.

———, J. A. SMYTH, P. CUSH, P. J. DUIGNAN, M. PLATTEN, S. J. MCCULLOUGH, AND G. M. ALLEN. 1989. Histopathologic and immunocytochemical studies of distemper in seals. Veterinary Pathology 26: 97–103.

MCLAREN, I. A. 1990. Pinnipeds and oil: Ecological perspectives. *In* Sea mammals and oil: Confronting the risks, J. R. Geraci and D. J. St. Aubin (eds.). Academic Press, New York, New York, pp. 55–101.

MANSFIELD, A. W. 1958. The biology of the Atlantic walrus (*Odobenus rosmarus rosmarus*) in the eastern Canadian Arctic. Manuscript Report 653, Fisheries Research Board of Canada, Ottawa, Canada, pp. 1–46.

MARKUSSEN, N. H., AND P. HAVE. 1992. Phocine distemper virus infection in harp seals (*Phoca groenlandica*). Marine Mammal Science 8: 19–26.

ODELL, D. K. 1991. A review of the Southeastern United States Marine Mammal Stranding Network, 1978–1987. *In* Marine mammal strandings in the United States, J. E. Reynolds and D. K.

Odell (eds.). National Oceanic and Atmospheric Administration Technical Report 98, National Marine Fisheries Service; U.S. Department of Commerce, Springfield, Virginia, pp. 19–24.

OSTERHAUS, A. D. M. E., J. GROEN, P. DEVRIES, F. G. C. M. UYTDEHAAG, B. KLINGEBORN, AND R. ZARNKE. 1988. Canine distemper virus in seals. Nature 335: 403–404.

REED, L. J., AND H. MUENCH. 1937. A simple method of estimating fifty percent endpoints. American Journal of Hygiene 27: 493–497.

RICHARD, P. R., AND R. R. CAMPBELL. 1988. Status of the Atlantic walrus, *Odobenus rosmarus rosmarus*, in Canada. The Canadian Field-Naturalist 102: 337–350.

ROFF, D. A., AND W. D. BOWEN. 1986. Further analysis of population trends in the northwest Atlantic harp seal (*Phoca groenlandica*) from 1967–1985. Canadian Journal of Fisheries and Aquatic Science 42: 553–564.

ROSS, P. S., I. K. G. VISSER, H. W. J. BROEDERS, M. W. G. VAN DE BILT, W. D. BOWEN, AND A. D. M. E. OSTERHAUS. 1992. Antibodies to phocine distemper virus in Canadian seals. The Veterinary Record 130: 514–516.

ROSSITER, P. B., D. M. JESSETT, AND W. P. TAYLOR. 1985. Microneutralization system for use with different strains of peste des petits ruminants and rinderpest virus. Tropical Animal Health and Production 17: 75–81.

VISSER, I. K. G., V. P. KUMAREV, C. ORVELL, P. DE VRIES, H. W. J. BROEDERS, M. W. G. VAN DE BILT, J. GROEN, J. S. TEPPEMA, M. C. BURGER, F. G. C. M. UYTDEHAAG, AND A. D. M. E. OSTERHAUS. 1990. Comparison of two morbilliviruses isolated from seals during outbreaks of distemper in northwest Europe and Siberia. Archives of Virology 111: 148–164.

Received for publication 8 March 1993.