

Complete Genome Sequence of Phocine Distemper Virus Isolated from a Harbor Seal (*Phoca vitulina*) during the 1988 North Sea Epidemic

Rory D. de Vries,^a R. Joyce Verburgh,^a Marco W. G. van de Bildt,^a Albert D. M. E. Osterhaus,^{a,b} Rik L. de Swart^a

Department of Viroscience, Erasmus MC, Rotterdam, The Netherlands^a; Artemis, Research Institute for Wildlife Health in Europe, Utrecht, The Netherlands^b

Phocine distemper virus (PDV) was identified as the cause of a large morbillivirus outbreak among harbor seals in the North Sea in 1988. PDV is a member of the family *Paramyxoviridae*, genus *Morbillivirus*. Until now, no full-genome sequence of PDV has been available.

Received 10 April 2013 Accepted 14 May 2013 Published 27 June 2013

Citation de Vries RD, Verburgh RJ, van de Bildt MWG, Osterhaus ADME, de Swart RL. 2013. Complete genome sequence of phocine distemper virus isolated from a harbor seal (*Phoca vitulina*) during the 1988 North Sea epidemic. *Genome Announc.* 1(3):e00291-13. doi:10.1128/genomeA.00291-13.

Copyright © 2013 de Vries et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 3.0 Unported license](http://creativecommons.org/licenses/by/3.0/).

Address correspondence to Rik L. de Swart, r.deswart@erasmusmc.nl.

Morbilliviruses are highly infectious, are spread via the respiratory route, and cause profound immunosuppression. Moreover, these viruses cause large outbreaks with high morbidity and mortality rates in previously unexposed populations. The prototype morbillivirus is measles virus (MV), an important pathogen of humans. Other members of the genus include canine distemper virus (CDV), rinderpest virus (RPV), dolphin morbillivirus (DMV), porpoise morbillivirus (PMV), peste des petits ruminants virus (PPRV), and feline morbillivirus (FmPV) (1, 2). Phocine distemper virus (PDV) was identified as a member of the *Morbillivirus* genus in 1988 (3, 4), infecting seals as a natural host and causing clinical signs and lesions similar to those of CDV in dogs and other carnivores (5). Since then, outbreaks and individual cases of PDV have been reported repeatedly (6–8). The morbillivirus genome consists of negative-sense single-stranded RNA, typically 15,500 to 16,000 nucleotides (nt) in length, comprising six genes that encode 8 proteins. Every 6-nt section of the viral genome is covered by one nucleocapsid (N) protein; therefore, the genome lengths of morbilliviruses always obey the “rule of six” (9).

We isolated PDV (previously referred to as PDV-1) from an organ suspension obtained from harbor seals that died during the 1988 North Sea outbreak (3). The organ suspension was previously used as challenge material in a PDV vaccination study (10); the isolated virus was passaged twice in Vero-dogSLAM cells (11). The RNA was isolated, and a sequence of the complete viral genome was obtained using 28 primer sets generating overlapping fragments, based on previously published partial PDV sequences (GenBank accession no. D10371, Y09630, and X75717). The 3′ and 5′ ends of the genome were sequenced following rapid amplification of cDNA ends (RACE). The genome was found to be 15,696 nt in length, consistent with the “rule of six,” and contained 6 nonoverlapping genes in the order N–P/V/C–M–F–H–L, which is typical for morbilliviruses. The amino acid lengths of the eight proteins encoded by the genome were N, 523 amino acids (aa); P, V, and C, 507 aa, 299 aa, and 174 aa, respectively; M, 335 aa; F, 631 aa; H, 607 aa; and L, 2,184 aa. Genes were flanked on either

side by highly conserved transcription start and stop signals. It has been noted previously that the starting points of the six transcripts in morbilliviruses have a conserved phase (12). The phase of the PDV gene starts proved to be fully conserved compared to CDV. The genome contained a 55-nt leader region at the 3′ end, which was conserved when compared to other morbilliviruses (13) (84% homologous to CDV, 72% to MV). The trailer at the 5′ end was found to be 38 nt in length and was also highly conserved (14) (79% homologous to CDV, 82% to MV). This full-genome sequence can be used for comparative morbillivirus studies and may allow for the generation of an infectious molecular clone.

Nucleotide sequence accession number. The complete genome sequence of the strain PDV/Wadden_Sea.NLD/1988 is available at GenBank under the accession no. **KC802221**.

ACKNOWLEDGMENTS

We acknowledge Martin Ludlow, Linda Rennick, Paul Duprex, and Bert Rima for helpful suggestions and critical comments on the manuscript.

This research received financial support from the EU (Antigone, FP7 278976).

REFERENCES

- Barrett T. 1999. Morbillivirus infections, with special emphasis on morbilliviruses of carnivores. *Vet. Microbiol.* 69:3–13.
- Woo PC, Lau SK, Wong BH, Fan RY, Wong AY, Zhang AJ, Wu Y, Choi GK, Li KS, Hui J, Wang M, Zheng BJ, Chan KH, Yuen KY. 2012. Feline morbillivirus, a previously undescribed paramyxovirus associated with tubulointerstitial nephritis in domestic cats. *Proc. Natl. Acad. Sci. U. S. A.* 109:5435–5440.
- Osterhaus AD, Vedder EJ. 1988. Identification of virus causing recent seal deaths. *Nature* 335:20.
- Visser IK, Kumarev VP, Örvell C, De Vries P, Broeders HWJ, Van de Bildt MWG, Groen J, Teppema JS, Burger MC, UytdeHaag FGCM, Osterhaus ADME. 1990. Comparison of two morbilliviruses isolated from seals during outbreaks of distemper in North West Europe and Siberia. *Arch. Virol.* 111:149–164.
- Kennedy S. 1998. Morbillivirus infections in aquatic mammals. *J. Comp. Pathol.* 119:201–225.
- Jensen T, Van de Bildt MWG, Dietz HH, Andersen TH, Hammer AS, Kuiken T, Osterhaus ADME. 2002. Another phocine distemper outbreak in Europe. *Science* 297:209.

7. Rijks JM, Van de Bildt MWG, Jensen T, Philippa JDW, Osterhaus ADME, Kuiken T. 2005. Phocine distemper outbreak, The Netherlands, 2002. *Emerg. Infect. Dis.* 11:1945–1948.
8. Earle JAP, Melia MM, Doherty NV, Nielsen O, Cosby SL. 2011. Phocine distemper virus in seals, east coast, United States, 2006. *Emerg. Infect. Dis.* 17:215–220.
9. Sidhu MS, Chan J, Kaelin K, Spielhofer P, Radecke F, Schneider H, Masurekar M, Dowling PC, Billeter MA, Udem SA. 1995. Rescue of synthetic measles virus minireplicons: measles genomic termini direct efficient expression and propagation of a reporter gene. *Virology* 208:800–807.
10. Visser IKG, Van de Bildt MWG, Brugge HN, Reijnders PJH, Vedder EJ, de Vries P, Groen J, Walvoort HC, UytdeHaag FGCM, Osterhaus ADME. 1989. Vaccination of harbour seals (*Phoca vitulina*) against phocid distemper with two different inactivated canine distemper virus (CDV) vaccines. *Vaccine* 7:521–526.
11. Seki F, Ono N, Yamaguchi R, Yanagi Y. 2003. Efficient isolation of wild strains of canine distemper virus in Vero cells expressing canine SLAM (CD150) and their adaptability to marmoset B95a cells. *J. Virol.* 77: 9943–9950.
12. Rima BK, Collin AMJ, Earle JAP. 2013. Completion of the sequence of a cetacean morbillivirus and comparative analysis of the complete genome sequences of four morbilliviruses. *Virus Genes* 30:113–119.
13. Banyard AC, Grant RJ, Romero CH, Barrett T. 2008. Sequence of the nucleocapsid gene and genome and antigenome promoters for an isolate of porpoise morbillivirus. *Virus Res.* 132:213–219.
14. Minet C, Yami M, Egzabhier B, Gil P, Tangy F, Brémont M, Libeau G, Diallo A, Albina E. 2009. Sequence analysis of the large (L) polymerase gene and trailer of the peste des petits ruminants virus vaccine strain Nigeria 75/1: expression and use of the L protein in reverse genetics. *Virus Res.* 145:9–17.