Virus Research 144 (2009) 323–328

Contents lists available at ScienceDirect

Virus Research

journal homepage: www.elsevier.com/locate/virusres

Short communication

Genetic diversity and phylogenetic analysis of the attachment glycoprotein of phocine distemper viruses of the 2002 and 1988 epizootics

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ARTICLE INFO

Article history:
Received 1 March 2009
Received in revised form 23 April 2009
Accepted 26 April 2009
Available online 3 May 2009

Keywords:
Phocine distemper virus
Morbillivirus
Canine distemper virus
Hemagglutinin
Phylogeny
Molecular epidemiology

ABSTRACT

To investigate the possible origin and spread of the dramatic re-emergent 2002 distemper epizootic observed among seals in Danish Waters, we have sequenced wild-type genes of the attachment (H) glycoproteins of viruses from both the 2002 and 1988 epizootics. Phylogenetic analysis of the H genes of phocine distemper virus (PDV) together with other morbilliviruses, suggests that the re-emergent 2002 PDV is more closely related to a putative recent ancestral PDV than the 1988 PDV isolates. Moreover, upsurges of distemper disease in land-living carnivores linked in time and locality to the 2002 seal epizootic in Danish Waters was investigated and determined to be caused by canine distemper virus, the closest relative of PDV, revealing no direct epidemiological link to the seal epizootics.

1. Introduction

The origin of the phocine distemper virus (PDV) infection which started the devastating seal plague in the spring 2002 in Danish Waters remains an enigma. The epizootic spread quickly among seals throughout Northern Europe decimating the harbour seal (*Phoca vitulina*) populations (Jensen et al., 2002; Härkönen et al., 2006).

During the 9-month epizootic it has been estimated that more than 22,000 harbour seals and grey seals (*Halichoerus grypus*) succumbed to PDV infection (Barrett et al., 2003; Härkönen et al., 2006). Affected animals died with clinical signs characteristic of distemper disease such as respiratory and nervous system manifestations. This re-emergence of the seal plague occurred 14 years after the disease was first observed among harbour seals along the coasts of Anholt in the Kattegat area of Denmark in 1988. The progression of distemper through the seal population in 2002 was comparable to the situation seen in the 1988 epizootic (Härkönen et al., 2006). During the first epizootic, it was suggested that PDV jumped the species barrier into terrestrial animals causing distemper disease outbreaks in 1989 among farmed mink in Denmark (Blixenkrone-Møller et al., 1990, 1992).

The distemper viruses, *phocine distemper virus* (PDV) and *canine distemper virus* (CDV), are closely related and belong to the Order *Mononegavirales*, Family *Paramyxoviridae*, sub-family *Paramyxovirinae*, genus *Morbillivirus*, a group of enveloped, non-segmented negative strand RNA viruses (Faquet et al., 2005). The molecular properties of PDV isolates from the 1988 epizootics have previously been characterized using cell culture adapted viruses (Kovamees et al., 1991; Curran et al., 1992; Blixenkrone-Møller, 1993). It is widely recognised that the viral hemagglutinin (H) glycoprotein contains essential determinants for the host range of the different morbilliviruses and is involved in interactions with the cellular receptors including the signal lymphocyte activating molecule (SLAM) (Tatsuo et al., 2000, 2001). Sequence variation among the H genes of wild-type morbilliviruses makes this a highly relevant focus for molecular epidemiological analysis (Blixenkrone-Møller et al., 1996; Mochizuki et al., 1999; WHO, 2001; Lednicky et al., 2004; Bailey et al., 2005). In the search for further clues to the origin of the re-emergent distemper disease, we have amplified and compared genetic material for wild-type H glycoproteins derived from tissues taken from seals infected with distemper viruses from the beginning of the 2002 and 1988 epizootics in Danish Waters. The data generated for full length PDV H genes were compared

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doi:10.1016/j.virusres.2009.04.023

with available PDV H genes originating from other parts of North-Western Europe.

Lung tissue samples stored at −80 °C from a total of eight affected harbour seal carcasses stranded at Danish coasts were examined (Kovamees et al., 1991). Total RNAs were extracted from lung tissue using RNA Now (Ozyme, Biogentex, St Quentin Yvelines, France) as described previously (Blixenkrone-Møller et al., 1998). PDV wild-type H genes were amplified directly from the RNA extractions as six overlapping fragments by reverse transcriptase polymerase chain reaction (RT-PCR) (One-step RT-PCR, Invitrogen,) using primers described previously (Kovamees et al., 1991). The following conditions were used: 94 °C 30 s, 50 °C 30 min, 94 °C 2 min, 40 cycles of (94 °C 1 min, 50 °C 1 min, 72 °C 1 min) and 72 °C 8 min. Cycle sequencing was carried out on purified PCR

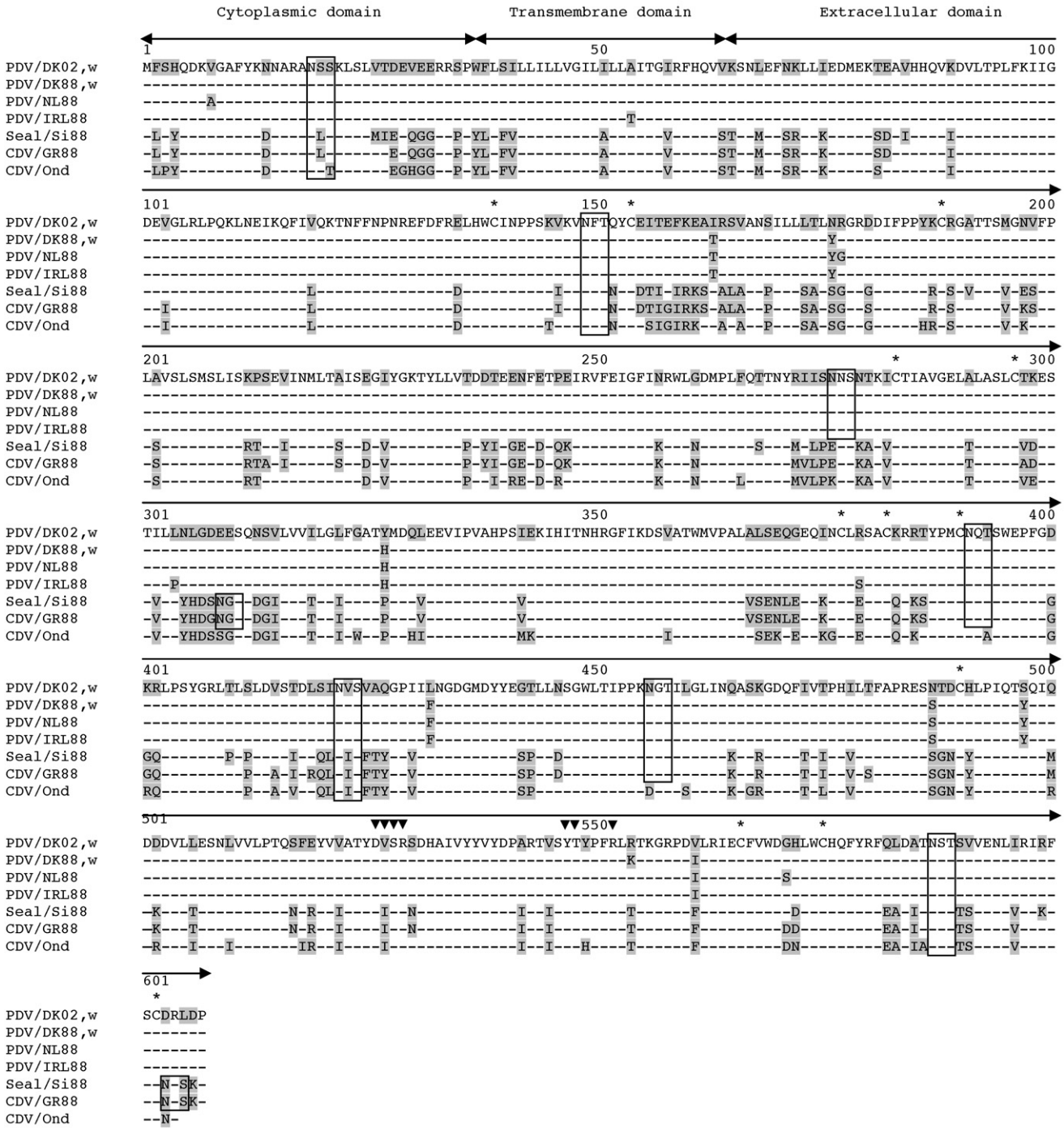

Fig. 1. Alignment of H protein sequences of wild-type PDVs from the 2002 and 1988 seal epizootic in Danish Waters with those of Irish and Dutch 1988 PDVs and Arctic 1988 CDVs. Stippled lines indicate identity and overlined areas indicate hydrophobic regions. Potential N-linked glycosylation sites are boxed and arrowheads mark amino acid residues which for CDV is known to have strong effect on SLAM-dependent fusion. The asterisks mark conserved cysteine residues. GenBank accession numbers are: PDV/DK02 w (FJ648456), PDV/DK88 w (AF479274), PDV/NL88 (AJ224707), PDV/IRL88 (D10371), Seal/Si88 from Siberian seal (X84998), CDV/GR88 from Greenlandic dog (Z47760), and reference strain CDV/Ond (AF305419).