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Research paper

Influence of vaccine strains on the evolution of canine distemper virus

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ABSTRACT

Canine distemper virus (CDV) is a major dog pathogen belonging to the genus *Morbillivirus* of the family *Paramyxoviridae*. CDV causes disease and high mortality in dogs and wild carnivores. Although homologous recombination has been demonstrated in many members of *Paramyxoviridae*, these events have rarely been reported for CDV. To detect potential recombination events, the complete CDV genomes available in GenBank up to June 2015 were screened using distinct algorithms to detect genetic conversions and incongruent phylogenies. Eight putative recombinant viruses derived from different CDV genotypes and different hosts were detected. The breakpoints of the recombinant strains were primarily located on fusion and hemagglutinin glycoproteins. These results suggest that homologous recombination is a frequent phenomenon in morbillivirus populations under natural replication, and CDV vaccine strains might play an important role in shaping the evolution of this virus.

1. Introduction

Canine distemper virus (CDV) affects a great number of animal species, primarily terrestrial carnivores. In dogs, CDV causes a systemic disease with respiratory, nervous and digestive signs. CDV is a small, enveloped virus belonging to the genus *Morbillivirus* within the family *Paramyxoviridae* of the Mononegavirales order, possessing a non-segmented single-stranded negative RNA genome (15,690 kb long) encoding six structural proteins: hemagglutinin (H), fusion (F), envelope-associated matrix (M), phospho- (P), large polymerase (L) and nucleocapsid (N) (Lamb and Parks, 2013). Among these, the F and H glycoproteins are protective antigens that induce humoral antibodies (Hirama et al., 2003; Wild and Buckland, 1997).

Based on the H gene, which has the highest genetic variability, CDV strains segregate into at least ten major geographically related genetic lineages: America-I (including the vaccine strains), America-II, Asia-I, Asia-II, Europe Wildlife, Arctic, South Africa, South America-I/Europe, South America-II and Rockborn-like (Budaszewski et al., 2014; Calderón et al., 2007; Martella et al., 2006; Panzera et al., 2012; Woma et al., 2010). Moreover, in Mexico and Asia, some CDV strains substantially diverge and might represent novel geographically related groups (Gámiz et al., 2011; Radtanakatikanon et al., 2013).

The introduction and extensive use of live-attenuated CDV vaccines in the 1950s has drastically reduced the incidence of canine distemper in dogs. However, CDV infections and diseases in immunized dogs have been observed on several occasions (Martella et al., 2011). Wild-type CDV strains are genetically divergent from the vaccine strains, and a number of studies have described antigenic differences between these variants, especially considering the influence of N-linked glycans at the H protein (Sawatsky and von Messling, 2010). However, the influence of vaccine immunity on CDV evolution remains unknown.

Copy choice recombination plays an important role in the evolution of positive sense RNA viruses (Lai, 1992; Nagy and Simon, 1997; Weber et al., 2015), whereas negative sense RNA viruses were characterized by low rates of recombination (Chare et al., 2003; Schierup et al., 2005). For the *Paramyxoviridae* family, recombination events have previously been documented in some species, primarily in measles virus (Schierup et al., 2005), respiratory syncytial virus (Spann et al., 2003) and Newcastle disease virus (Chare et al., 2003; Miller et al., 2009), but there are limited studies concerning CDV (Han et al., 2008; Ke et al., 2015; McCarthy et al., 2007). Thus, the aim of the present study was to perform extensive phylogenetic and recombination analyses using the available complete CDV genomes of different host species to detect potential recombination events that might shape viral genetic diversity.

2. Material and methods

2.1. Datasets

All CDV complete genome sequences deposited in GenBank (up to June 2015) were retrieved from the National Center for Biotechnology

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Table 1
GenBank accession numbers of the complete CDV genome sequences used in the recombination analysis.

Accession no.	Strain	Host[a]	Country[a]	Genotype[b]	Year[a]	Reference
AY443350.1	00-2601	Raccoon	USA	America-II	2000	Lednicky et al., 2004
AY649446.1	01-2689	Raccoon	USA	America-II	2001	Lednicky et al., 2004
EU716337.1	164071	Dog	USA	America-II	2004	Unpublished
KJ123771.1	171391-513	Dog	USA	America-II	2004	Unpublished
AF164967.1	A75/17 (Cornell)	Dog	USA	America-II	–	Unpublished
AY386316.1	5804	Dog	Germany	South America-I/Europe	–	von Messling et al., 2003
KJ466106.1	CDV SY	Raccoon	China	Asia-I	2012	Cheng et al., 2015
KC427278.1	Hebei	Mink	China	Asia-I	2008	Unpublished
HM852904.1	MKY-KM08	Rhesus monkey	China	Asia-I	2008	Qiu et al., 2011
AB687720.2	CYN07-dV	Cynomolgus monkey	Japan	Asia-I	2008	Sakai et al., 2013
AB687721.2	CYN07-hV	Cynomolgus monkey	Japan	Asia-I	2008	Sakai et al., 2013
JN896331.1	PS	Dog	China	Asia-I	2010	Yi et al., 2013
JX681125.1	HLJ1-06	Fox	China	Asia-I	2006	Jiang et al., 2013
AB474397.1	007Lm	Dog	Japan	Asia-II	–	Unpublished
AB476401.1	011C	Dog	Japan	Asia-II	–	Sultan et al., 2009
AB476402.1	50Con	Dog	Japan	Asia-II	–	Sultan et al., 2009
AB475099.1	55L	Dog	Japan	Asia-II	–	Sultan et al., 2009
AB475097.1	M25CR	Dog	Japan	Asia-II	–	Sultan et al., 2009
KF914669.1	CDV2784/2013	Dog	Italy	Artic	2013	Marcacci et al., 2014
AY445077.2	98-2645	Raccoon	USA	America-I	1998	Lednicky et al., 2004
AY542312.2	98-2646	Raccoon	USA	America-I	1998	Lednicky et al., 2004
AY466011.2	98-2654	Raccoon	USA	America-I	1998	Lednicky et al., 2004
AF305419.1	Onderstepoort	Dog (attenuated)	–	America-I	–	Gassen et al., 2000
EU726268.1	CDV3	Mink (attenuated)	China	America-I	–	Unpublished
HM046486.1	Phoca/Caspian/2007	Seal	Kazakhstan	America-I	2007	Unpublished
HM063009.1	Shuskiy	Mink	Kazakhstan	America-I	1989	Unpublished
JN896987.1	Snyder_Hill	Dog (attenuated)	USA	America-I	–	Ludlow et al., 2012

[a] Data retrieved from GenBank or corresponding reference, when available.
[b] Genotype according to the phylogenetic analysis of the complete H gene.

Information (NCBI; http://www.ncbi.nlm.nih.gov/nucleotide) using 'canine distemper virus complete genome' as key words. Fifty-seven complete CDV genomic sequences were retrieved, edited using BioEdit version 7.2.5 and aligned with ClustalW using MEGA 6 software (Tamura et al., 2013). To avoid evolutionary discrepancies, identical sequences and attenuated isolates other than the vaccine strains were removed from the dataset, resulting in twenty-seven complete genomic sequences (Table 1).

Table 2
Putative recombination events detected using the software RDP4 in canine distemper virus.

Recombinant		00-2601 (AY443350.1)	00-2601 (AY443350.1)	01-2689 (AY649446.1)	01-2689 (AY649446.1)	171391-513 (KJ123771.1)	171391-513 (KJ123771.1)
Major parent		01-2689 (AY649446.1) (99%)	01-2689 (AY649446.1) (99.8%)	5804 (AY386316.1) (96.4%)	Unknown strain (00-2601[a])	5804 (AY386316.1) (96%)	164071 (EU716337.1) (99.3%)
Minor parent		Unknown strain (164071[a])	Unknown strain (171391-513[a])	00-2601 (AY443350.1) (100%)	164071 (EU716337.1) (99.6%)	00-2601 (AY443350.1) (99.7%)	Unknown strain (01-2689[a])
P-values determined by							
RDP4	RDP	9.743×10^{-21}	2.773×10^{-10}	5.921×10^{-07}	3.601×10^{-06}	5.921×10^{-07}	1.317×10^{-07}
	GENECONV	1.562×10^{-19}	5.923×10^{-12}	3.561×10^{-07}	4.543×10^{-05}	3.561×10^{-07}	ND
	BootScan	2.692×10^{-20}	1.136×10^{-12}	7.452×10^{-10}	ND	7.452×10^{-10}	2.990×10^{-06}
	MaxChi	2.287×10^{-10}	1.138×10^{-05}	1.212×10^{-06}	ND	1.212×10^{-06}	ND
	Chimaera	2.204×10^{-09}	4.069×10^{-05}	1.722×10^{-05}	ND	1.722×10^{-05}	ND
	Siscan	3.844×10^{-40}	1.200×10^{-28}	8.551×10^{-14}	3.636×10^{-05}	8.551×10^{-14}	1.975×10^{-06}
	3Seq	5.148×10^{-18}	ND	ND	4.679×10^{-03}	ND	2.963×10^{-03}
Fragment							
SH test	A	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	B	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	C	0.0665	0.0000	0.0831	0.0701	0.0598	0.0000
	Concatened tree	0.8415	0.7934	0.8139	0.8477	0.8022	0.7738
ELW test	A	0.0067	0.0000	0.0000	0.0000	0.0000	0.0000
	B	0.0000	0.0277	0.0000	0.0000	0.0000	0.0000
	C	0.7598	0.0000	0.9662	0.9746	0.9668	0.9538
	Concatened tree	0.2335	0.9723	0.0338	0.0254	0.0332	0.0462
Beginning breakpoint (position in alignment)		4936 (99% CI: 4897–4966)	9095 (99% CI: 8090–10642)	Undetermined (2525; 99% CI: 2485–2686)	Undetermined (5741; 99% CI: 2470–5821)	Undetermined (2475; 99% CI: 2485–2686)	Undetermined (5800; 99% CI: 2470–5821)
Ending breakpoint (position in alignment)		6956 (99% CI: 6888–7010)	2223 (99% CI: 1787–2234)	Undetermined (4529; 99% CI: 7033–7095)	7065 (99% CI: 6979–7151)	Undetermined (4529; 99% CI: 7033–7095)	7073 (99% CI: 6979–7151)
Recombination rate		0.033003	0.018414	0.003564	0.026	0.007321	0.013130

ND: Recombination not detected with this algorithm.
[a]Strain considered as parent to perform bootscan analysis.