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Short Communication

Emergence of canine distemper virus strains with two amino acid substitutions in the haemagglutinin protein, detected from vaccinated carnivores in North-Eastern China in 2012–2013

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ABSTRACT

A total of 16 strains of canine distemper virus (CDV) were detected from vaccinated minks, foxes, and raccoon dogs in four provinces in North-Eastern China between the end of 2011 and 2013. Upon sequence analysis of the haemagglutinin gene and comparison with wild-type CDV from different species in the same geographical areas, two non-synonymous single nucleotide polymorphisms were identified in 10 CDV strains, which led to amino acid changes at positions 542 (isoleucine to asparagine) and 549 (tyrosine to histidine) of the haemagglutinin protein coding sequence. The change at residue 542 generated a potentially novel *N*-glycosylation site. Masking of antigenic epitopes by sugar moieties might represent a mechanism for evasion of virus neutralising antibodies and reduced protection by vaccination.

Canine distemper virus (CDV) is a *Morbillivirus* of the family *Paramyxoviridae* and is a highly contagious pathogen for several carnivore species. CDV is an enveloped virus with a single-stranded negative-sense RNA genome, encoding six structural and two non-structural proteins. Two surface glycoproteins, namely haemagglutinin (H) and fusion protein (F), play a key role in virus attachment and entry into host cells and are the main targets for the immune response. The glycosylated H protein mediates receptor binding and is much more variable than other CDV proteins; thus representing a suitable gene for investigating CDV genetic/antigenic diversity (Zhao et al., 2010). Analysis of CDV strains has revealed pronounced genetic diversity in the H gene, with eleven main geographically-distinct lineages (genotypes) described (Zhao et al., 2010; Nikolin et al., 2012b).

Phylogenetic and molecular evolutionary analyses of CDV have revealed that the emergence of CDV infection in novel host species might be associated with mutations that affect the receptor (CD150/SLAM or Nectin-4) binding site (amino acid 549) of the H protein. CDV strains in domestic dogs usually carry tyrosine at residue 549, whereas histidine is encoded at this location for most strains identified from non-canine hosts (McCarthy et al., 2007; Sekulin et al., 2011). However, recent investigations have shown that there are exceptions to this apparent variability in host-specificity, determined by residue 549 (Nikolin et al., 2012b).

Between 2011 and 2013, canine distemper-like disease was observed in vaccinated breeding minks (*Mustela lutreola*), foxes (*Vulpes vulpes*) and raccoon dogs (*Nyctereutes procyonoides*) from Shandong, Liaoning, Hebei and Heilongjiang provinces in the North-East of China, where the main fur industry is located. All the animals had been vaccinated with either CDV3 or Onderstepoort CDV vaccine strains. At necropsy, CDV infection was confirmed in 16 animals, on the basis of the histopathological lesions, detection of CDV antigen using a commercial immunochromatographic test (Shanghai Quicking) and demonstration of CDV RNA in tissue samples by reverse-transcription (RT)-PCR.

Full-length H genes were sequenced in 16 CDV strains detected from minks, foxes and raccoon dogs between 2011 and 2013 in four provinces of China (Table 1). In order to characterize the phylogenetic and molecular evolution of the H genes, these sequences were compared with wild-type CDV, identified from

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Table 1
Origin of the 16 CDV strains investigated.

Number	Strain	Province	Date	Host	Source	GenBank Accession number
1	LN(11)1	Liaoning	08/2011	Fox	Lung	JX844218
2	LN(12)1	Liaoning	08/2012	Fox	Lung	JX844219
3	SD(12)1	Shandong	07/2012	Mink	Brain	JX844220
4	SD(12)2	Shandong	08/2012	Fox	Lung	JX844221
5	SD(12)3	Shandong	09/2012	Mink	Lung	KC494688
6	HeB(13)1	Hebei	01/2013	Fox	Brain	KF006819
7	LN(13)1	Liaoning	01/2013	Raccoon dog	Lung	KF006820
8	HLJ(13)1	Heilongjiang	08/2013	Fox	Lung	KF767692
9	LN(13)2	Liaoning	10/2013	Mink	Lung	KF880677
10	SD(13)1	Shandong	10/2013	Raccoon dog	Lung	KF880678
11	SD(13)2	Shandong	10/2013	Raccoon dog	Liver	KF880679
12	SD(13)3	Shandong	10/2013	Fox	Lung	KF880680
13	SD(13)4	Shandong	10/2013	Raccoon dog	Liver	KF880681
14	SD(13)5	Shandong	10/2013	Mink	Liver	KF880682
15	SD(13)6	Shandong	10/2013	Mink	Liver	KF880683
16	SD(13)7	Shandong	10/2013	Mink	Lung	KF880684

different species in the same region before 2013. RNA was extracted from tissue samples using the RNeasy Mini Kit (Qiagen). RT-PCR was carried out as described previously (Zhao et al., 2010). Sequences were determined using an ABI Prism 310 genetic analyser (Applied Biosystems) and aligned with other CDV H protein sequences retrieved from GenBank[1] using Clustal*W* program within the MEGA5.0 software package.[2] The potential *N*-linked glycosylation sites of the H protein were determined using NetNGlyc1.0 software.[3] Phylogenetic analysis was performed using the Neighbour-Joining method and setting the *p* distance algorithm of correction with the MEGA5.0 software package.

Amino acid sequence identity in the full-length H protein, comparing the 16 Chinese CDV strains, ranged from 98.4% to 100%. Upon phylogenetic analysis, all 16 strains segregated into the Asia-1 genotype, along with other Chinese wild-type CDV strains. All the Shandong CDV strains and strain LN(13)2, detected from a mink in Liaoning province, were grouped in a unique branch (Fig. 1).

Upon inspection of the H protein sequence alignment, the 10 CDV strains from Shandong province possessed a tyrosine to histidine substitution at residue 549, unlike the majority of CDV strains collected from the other provinces. Interestingly, with the exception of one Shandong strain, SD(12)2, which was detected in a fox, most of the Shandong strains (9/10) also exhibited a novel potential *N*-glycosylation site (542–544), due to substitution of asparagine with isoleucine at amino acid residue 542 (Fig. 2).

It has been suggested that amino acid residues 530 and 549 in the H protein are associated with host specificity (McCarthy et al., 2007). Substitutions in these two key positions are related to cross-species transmission, based on CDV-H specificity for CD150/SLAM binding (Nikolin et al., 2012a). When the dog-derived CDV strain 5804 was passaged in ferrets (*Mustela putorius*), the H protein acquired a mutation leading to substitution of tyrosine to histidine at amino acid position 549 (von Messling et al., 2003). CDV isolates with histidine at residue 549 were highly virulent for raccoons, compared to those strains lacking this substitution (Lednicky et al., 2004).

All the CDV strains identified in the present study were characterized as Asia-1 genotype. This particular genotype seems to be predominant throughout China, while it is less common in Japan and Korea, where the Asia-2 genotype predominates (Zhao et al., 2010). All the CDV strains detected from Shandong province and a strain identified in a mink from Liaoning province, displayed the specific amino acid substitution tyrosine to histidine at residue 549. Before the year 2012 in China, only two Shandong CDV strains, SD(07)1 and SD(09)1, identified from a mink and fox, respectively, possessed this specific mutation (Fig. 2). There is a large population of potential non-canine hosts (chiefly mink) for CDV in Shandong province, but the limited geographical coverage of the sampling is a limitation for our study.

A potentially novel *N*-glycosylation site (amino acids 542–544) was predicted in the H protein of nine Shandong CDV strains. The Liaoning CDV strain, LN(13)2, was phylogenetically related to these Shandong strains, suggesting spread of the virus between regions. This potential *N*-glycosylation site was also described in two Brazilian CDV stains, BR2 (EU098103) and BR3 (EU098104) (Fig. 2), of the South America-1 genotype. Changes in *N*-glycosylation have been associated with increased virulence in CDV strains (Sawatsky and von Messling, 2010). Moreover, the three-dimensional structure of morbillivirus H protein suggests that extensive masking of antigenic epitopes by sugar moieties can hinder binding of neutralising antibodies to the H protein (Hashiguchi et al., 2007). Accordingly, the novel *N*-glycosylation site, identified in the H protein of the Shandong CDV strains, might be one explanation for the observed CDV vaccine failures. The isoleucine to phenylalanine amino acid change at residue 542 was also observed in two simian CDV isolates (CYN07-dV and BJ-01) suggesting a possible role for this residue in adaptation of CDV to new hosts (Fig. 2).

CDV is capable of undergoing mutation, whereby relatively few amino acid changes at key positions of surface antigens potentially result in substantial changes to virus species specificity or tissue tropism (Nikolin et al., 2012a; Sakai et al., 2013). Surveillance for CDV infection in wildlife and domesticated animals is important for identifying novel CDV variants. Moreover, molecular and antigenic characterisation of CDV strains is pivotal for our understanding of the mechanisms that are involved in CDV vaccine failure.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

[1] See: http://www.ncbi.nlm.nih.gov/genbank/.
[2] See: http://www.megasoftware.net/.
[3] See: http://www.cbs.dtu.dk/services/NetNGlyc/.