Short Communication

Novel sequence variants of viral hexon and fibre genes in two dogs with canine adenovirus type 1-associated disease

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Abstract

There is little information on sequence variation of canine adenovirus type 1 (CAdV-1), the aetiological agent of infectious canine hepatitis (ICH). This study reports hexon and fibre gene sequence variants of CAdV-1 in a dog with systemic ICH and a dog with the ocular form of the disease (‘blue eye’) in Northern Italy in 2013. One of the sequence variants matched a CAdV-1 fox sequence previously detected in Italy.

Keywords: Canine adenovirus type 1; Infectious canine hepatitis; Anterior uveitis; Sequence variants.

Canine adenovirus type 1 (CAdV-1) is the aetiological agent of infectious canine hepatitis (ICH). Although ICH is relatively uncommon in veterinary practice, there is evidence that CAdV-1 continues to circulate in carnivores (Decaro et al., 2007; Balboni et al., 2013, 2014; Walker et al., 2016). This study reports variant CAdV-1 hexon and fibre gene sequences in two domestic dogs with naturally occurring CAdV-1 infection in Northern Italy in 2013.

Case 1 (417-2013) was an unvaccinated 14-month-old male mixed breed dog from a rural area that died after three days of hospitalisation with petechial haemorrhages, jaundice, haemorrhagic diarrhoea, seizures, acute liver failure and disseminated intravascular coagulation (platelet count 18 x 10^3/µL, reference range 160-350/µL; prothrombin time 17.6 s, reference range 5.0-7.5 s; activated partial thromboplastin time 48.1 s, reference range 8.0-16.5 s), acute liver failure (elevated hepatic enzymes and bilirubin [Editor's note: Please specify values for specific hepatic enzymes and bilirubin]), acute kidney injury, haemorrhagic enteritis and encephalitis. Case 2 (574-2013) was a 3-month-old male mixed breed dog from a shelter that had anterior uveitis/corneal oedema ('blue eye'), from which it recovered in 2-3 weeks, with no evidence of systemic disease.

Infection with CAdV-1 was confirmed using PCR by amplifying a fragment of the E3 gene (Hu et al., 2001) from liver, kidney, brain and intestine from case 1, and a rectal swab from case 2. Both dogs were negative for the CAdV-2 E3 gene by PCR, for antibodies against *Leishmania* spp., *Ehrlichia canis*, *Anaplasma phagocytophilum* and *Leptospira* spp., and for antigens of *Dirofilaria immitis* and *Giardia* spp.

At post-mortem examination, case 1 had an enlarged liver; in histological sections of liver stained with haematoxylin and eosin, intranuclear eosinophilic inclusions were observed in hepatocytes, along with occasional Küpffer cells and endothelial cells (see Appendix:

Supplementary Fig. 1A). In the kidney, there were deposits of periodic acid-Schiff (PAS) positive material in the glomeruli, consistent with membranoproliferative glomerulonephritis, but no evidence of intranuclear inclusions. Using a goat polyclonal antibody with a broad range of cross reactivity against numerous adenoviruses (0151-9004, AbD Serotech; 1:1600 dilution; see Appendix: Supplementary material), ~70% of hepatocyte nuclei were strongly positive for adenoviral antigen by immunohistochemistry (see Appendix: Supplementary Fig. 1B), whereas there was no positive immunohistochemical staining in the kidney.

Virus was isolated from the liver of case 1 and the rectal swab of case 2 using Madin Darby canine kidney (MDCK) cells (see Appendix: Supplementary material), producing cytopathic effects at the third and seventh passages, respectively. CAdV-1 infection was confirmed by quantitative real-time PCR of cell suspensions (Balboni et al., 2015).

The complete CAdV-1 hexon and fibre genes were amplified from the liver (L) of case 1 and the rectal swab (RS) of case 2 (see Appendix: Supplementary material). The amplicons from the E3, hexon and fibre genes were sequenced, and the nucleotide sequences were assembled and aligned with reference sequences of canine and bat adenoviruses from GenBank[1] using ClustalW (see Appendix: Supplementary material). Nucleotide sequences were translated into amino acid sequences using BioEdit 7.2.5[2].

The CAdV-1 E3 gene sequences (dog 1: KP670423, KP840546, KP840547; dog 2: KP670424, KP840548, KP840549) were identical with six CAdV-1 reference sequences (Y07760, M60937, JX416838, JX416839, JX416840 and KF676977). The hexon gene sequences had >99% identity with the reference strains at both the nucleotide and the amino acid levels. The nucleotide sequences from cases 1 and 2 had 99.9% nucleotide identity with CAdV-1 sequence 113-5L from a

[1] See: http://www.ncbi.nlm.nih.gov/genbank (accessed 10 January 2017).
[2] See: http://www.mbio.ncsu.edu/BioEdit/bioedit.html (accessed 10 January 2017).

red fox in Italy in 2011 (GenBank KP840545). Amino acid alignment showed complete identity between 417-2013-L (case 1), 574-2013-RS (case 2) and 113-5L; position 388 of the Italian sequences differed from the other reference sequences by having serine instead of asparagine (Table 1). Nucleotide alignment showed an identity of 99.6% between 417-2013-L and 574-2013-RS fibre gene sequences, and an identity of 99.8% between these two viruses and 113-5L (KP840544). There was 99.6% identity between amino acid sequences of 417-2013-L and 574-2013-RS, as well as between 574-2013-RS and 113-5L (Table 1). The predicted CAdV-1 fibre amino acid sequences of 417-2013-L and 113-5L were identical. Phylogenetic relationships among the hexon and the fibre gene sequences were evaluated using MEGA 6.0.6[3] and the viruses grouped in the CAdV-1 cluster (Fig. 1).

In this study, distinctive nucleotide and predicted amino acid sequences were found in the hexon and fibre genes of one dog with systemic ICH and one dog with 'blue eye', demonstrating that genetic variants of CAdV-1 circulate in Italy. In particular, amino acid position 388 in the hexon protein may differentiate Italian CAdV-1 sequences from those in other regions. Additional studies are needed to confirm these genetic differences and to determine whether they have any effect on the pathogenesis of CAdV-1 infection.

Conflict of interest statement

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

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[3] See: http://www.megasoftware.net/ (accessed 10 January 2017).

Appendix: Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi: …

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Table 1

Amino acid mutation allowing differentiation of the hexon and fibre sequences obtained between both themselves and worldwide reference sequences.

Hexon protein								
CAdV-1 sequences	**Amino acid residues**							
	8	**12**	**225**	**231**	**245**	**388**	**783**	**790**
RI261_Y07760	P	Y	R	T	D	N	A	R
CLL_U55001	P	Y	R	A	D	N	P	R
IN2007_EF206692	A	C	K	T	G	N	A	G
CCC-V6_EF559262	P	Y	K	A	D	N	A	R
Italian sequences:								
113-5L_ KP840545	P	Y	R	T	D	S	A	R
417-2013-L_ KP840547	P	Y	R	T	D	S	A	R
574-2013-RS_ KP840549	P	Y	R	T	D	S	A	R

Fibre protein						
CAdV-1 sequences	**Amino acid residues**					
	6	**7**	**23**	**110**	**376**	**506**
RI261_Y07760	S	A	P	E	T	A
CLL_U55001	R	S	P	E	A	R
GLAXO_M60937	S	A	P	E	A	R
Italian sequences:						
113-5L_ KP840544	S	A	T	E	A	A
417-2013-L_ KP840546	S	A	T	E	A	A
574-2013-RS_ KP840548	S	A	P	D	A	A

CAdV-1, Canine adenovirus type 1.

Framed: Amino acid mutations which differentiate the Italian sequences from the other sequences.

In grey: Amino acid mutations which differentiate the Italian sequences between themselves.

Figure legend

Fig. 1. Phylogenetic trees constructed with nucleotide sequences of the canine adenovirus type 1 (CAdV-1) hexon and the fibre genes determined in this study, and with canine and bat adenovirus reference sequences retrieved from GenBank. The best-fit model of nucleotide substitution was determined for each sequence alignment using the Find Best DNA/Protein Model function implemented in MEGA version 5.05 [Editor's note: Previously in the manuscript, it is stated that you used MEGA version 6.06; please check and correct if necessary]. The hexon gene phylogenetic tree was constructed using the maximum likelihood method and the Hasegawa-Kishino-Yano (HKY) model with a γ distribution was used for nucleotide substitution. The fibre gene phylogenetic tree was constructed using the maximum likelihood method, and the HKY model with invariant sites was used for nucleotide substitution. Bootstrap values were determined by 1000 replicates to assess the confidence level of each branch pattern and values > 80% are indicated on the respective branches. Italian nucleotide reference sequences are in bold. Nucleotide sequences generated in this study are underlined.