Supplementary Information

**MATERIALS AND METHODS**

**Cells**

Vero.Dog SLAMtag (VDS) cells were grown in Dulbecco’s modified Eagle’s medium (Invitrogen, California, USA) supplemented with 5% fetal bovine serum. Tissues were collected between 1993 and 1994 from 4 different species, a lion, 2 hyenas, a bat eared fox and a domestic dog which died of CDV infection (Table 1). Samples were obtained from wild animals in the Serengeti Park in Africa which died due to natural infection. Tissues were taken at post mortem. Further details of these samples are given in reference 7. Viruses were isolated by inoculating homogenised tissue onto VDS cells which gave rise to syncytia formation.

**Reverse Transcription-PCR and DNA sequencing**

Total RNA was extracted from infected cells using TRIzol reagent (Invitrogen, California, USA). cDNA synthesis was carried out using oligo-dT primers and the Superscript first strand synthesis kit (Invitrogen, California, USA). PCR was performed using the High Fidelity Taq kit (Invitrogen, California, USA). RT-PCR was carried out on the first passage with a range of primers designed to conserved regions of the H and P genes.
H, P and F gene primer sequences for RT-PCR and sequencing were either previously published (1, 15, 16, 17) or designed for this study. Primer sequences for successful primer sets are given in Table 2. DNA sequencing was performed using a Bigdye 3.1 Terminator Cycle sequencing kit (Applied Biosystems, California USA). Completed PCR products were sent to the Genomics Core Facility, Queens University Belfast for chromatographic preparation.

Despite repeated RNA preparations and RT-PCR reactions with additional primer sets we were unable to obtain the total sequence for viruses except for the earliest isolate Hyena 2. This suggests primer mismatch to sequences in later isolates. For the H gene, contigs corresponding to 2,008 nt for the Hyena 2 starting at the end of the F gene and covering the entire H gene; 939 nt for Hyena 1, 948 nt for the lion, 936 nt for the bat eared fox and 1,704 nt for Duramine vaccine were obtained. For the P gene 419 nt for Hyena1, 1613 nt for Hyena 2, 415 nt for the dog, 378 nt for the bat eared fox and 680 nt for Duramine vaccine were obtained. The PCR products were sequenced (submitted to GenBank) and aligned with all CDV sequences previously published in GenBank.
Phylogenetic Analysis

Nucleotide alignments were made using the NCBI BLAST programme. Unrooted neighbour joining phylogenetic and fast minimum evolution trees were constructed using DNASTAR software (Version 7.1) Megalign package with the CLUSTAL W method.
Supplementary Figure 1

Alignment of CDV P/V amino acid sequences 1 to 250 for selected viruses. Highlighted residues: Yellow- synapomorphies shared by the Serengeti strains; Magenta- differences between Nikolin et al (ref 30) and isolates reported in the current study; Red consensus; Turquoise- residues associated with disease in wildlife: turquoise; Green-residues associated with vaccine/attenuated strains
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---
Domestic dog (KJ415358)  
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Bat eared fox (MN335913)  
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Bat eared fox (KJ415357)  
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Lion1 (MN335916)  
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African Lion (KJ415362)  
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Hyena2 (MN335915)  
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Spotted hyena (KU578258)  
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Hyena2 (MN335915)

Spotted hyena (KU578258)

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Monkey CYN07-dV (AB687720.2) GIKKRDGKTLQFPHNPEGKTEDPECGSIKGTGERSASHGMIVASTNG250
Supplementary Figure 2

Alignment of CDV C amino acid sequences. Highlighted residues: yellow, synapomorphies shared by the Serengeti strains; red, consensus sequence; green, differences from the consensus.
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Bat eared fox (MN335913)

hyena_2 (MN335915)

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Supplementary Figure 3  H Sequence Alignment

Alignment of CDV H amino acid sequences. Highlighted residues: yellow, synapomorphies shared by the Serengeti strains; red, consensus sequence; magenta, differences from the consensus; turquoise specific to monkey virus. Substitutions in PDV have not been highlighted. Boxes indicate: red, residues critical for SLAM binding; blue, residue critical for Nectin-4 binding; green, site suggested by McCarthy et al.[56] to be associated with spread to non-canine host.
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Vacc-P_gi|224579344|gb\|FJ70523 TVLLYH SNGSQDGILVTVTLGIFGAT MDQVEEVIPVHAHPSEKEHIITNH 350
Dog_argentina_gi|134801370|emb ----YH SNGSQDGILVTVTLGIFGAT MDQVEEVIPVHAHPSEKEHIITNH 350
German_dog_gi|693948|emb\|X8500 TVLLYH SNGSQDGILVTVTLGIFGAT MDQVEEVIPVHAHPSEKEHIITNH 350
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Hyena 2 (MN335910)      DKDVLSNLVVLPTQNYFVYIATYHSHGDHAIVYYVYDYPTISYTP 550
Spotted Hyena (KJ415364)      DKDVLSNLVVLPTQNYFVYIATYHSHGDHAIVYYVYDYPTISYTP 550
Bat_Eared_Fox (MN335908)      DKDVLSNLVVLPTQNYFVYIATYHSHGDHAIVYYVYDYPTISYTP 550
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DOG_H.2_1 (MN335909)      DKDVLSNLVVLPTQNYFVYIATYHSHGDHAIVYYVYDYPTISYTP 550
Domestic dog (JN812976)      DKDVLSNLVVLPTQNYFVYIATYHSHGDHAIVYYVYDYPTISYTP 550
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