

Genetic and evolutionary analysis of a new Asia-4 lineage and naturally recombinant canine distemper virus strains from Thailand

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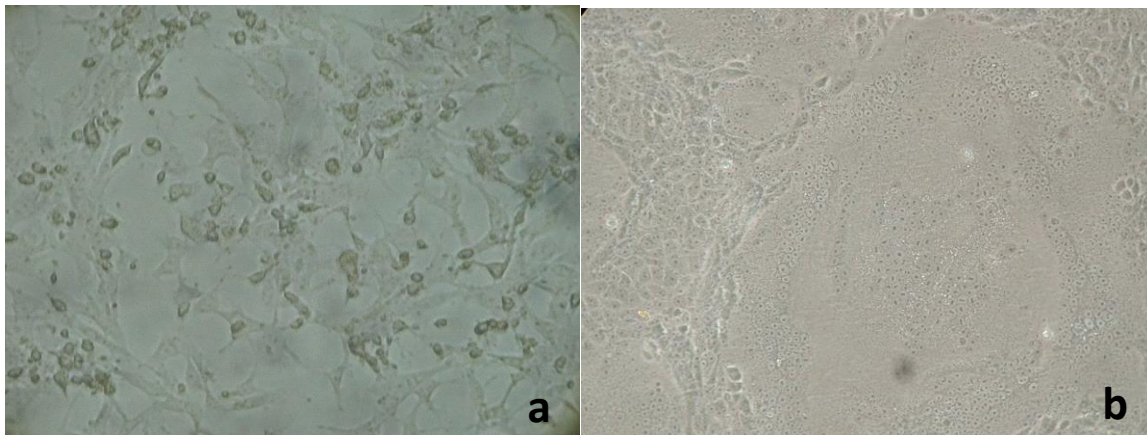


Fig. S4. Cytopathic effect (CPE) after CDV Thai strain inoculation in Vero-DST cell line. Stellate cell formation after 24 hours inoculation Vero-DST with CDV6 TH/2014 (a). and syncytial cell formation (b) after CDV2 TH/2014 inoculation.

Table S1. Sixteen CDV primer sets used for whole genome sequencing

Direction	Primer	Position
Forward	5'-ATGGCTAGCCTTCTCAAGAGC-3'	1-21
Reverse	5'-CAAATCATTTCAGCAATTCTAGGC-3'	764-741
Forward	5'-GAATGAACAAAATATGGCTTGATAT-3'	736-760
Reverse	5'-GATTCTCTCTGAGGGCTTTGAG-3'	1867-1846
Forward	5'-CTTAGGACCCAGGTCCAACAA-3'	1739-1759)
Reverse	5'-GAATCAGTCTCTCCTTTAAGCAG-3'	2843-2821
Forward	5'-GCAAAGATGACCCAGAAATGCAA-3'	2635-2657
Reverse	5'-CCAGTTGTTCTTGACACCTG-3'	3765-3745
Forward	5'-GGGAGAGATTCAGGAAGAGCA-3'	2995-3015
Reverse	5'-GAGCGGGTAACACAGGATTTTC-3'	4283-4262
Forward	5'-GGGATTAGTGTTTGCTCTAGGA-3'	4163-4184
Reverse	5'-CTCACCTAATTCTGCTTTGGTAC-3'	5495-5473
Forward	5'-GGTTAGTCCTATGGTGCATTGG-3'	5287-5308
Reverse	5'-CCAGTGGGCAGGTATCGGAG-3'	6537-6518
Forward	5'-CAGCCATTTGTAGCCAGAACTC-3'	6289-6310
Reverse	5'-AGGTGGATTTATGCACCAATGG-3'	7507-7486
Forward	5'-GAAAACCTTAGGGCTCAGGTAGT-3'	7051-7072
Reverse	5'-AACCGCCCATAAGATGGCAAC-3'	8304-8284
Forward	5'-GGTAAACTTATTTGCTAGTTCCT-3'	7763-7787
Reverse	5'-GGGATAAGATAACAAGGTCTGATT-3'	9270-9246
Forward	5'-GTCCATCTAGATAGCCCAATTG-3'	9069-9090
Reverse	5'-CCATGTCTATCCCGATAACCG-3'	10243-10223

Forward	5'-GAAATTCAGGAGATTTTACAGGAC-3'	9960-9983
Reverse	5'-GTTTCATTTGCTTTCAAATGATGTC-3'	11497-11473
Forward	5'-CCCCTAACTCTCAAATATTCATC -3'	11203-11225
Reverse	5'-GTGGTTCCTTAATGCTCTCGC-3'	12458-12438
Forward	5'-GGATAGGAAAATTATTATCCCAAG-3'	12179-12202
Reverse	5'-TTTCAAAGCCCCAGTTGATTGC-3'	13524-13503
Forward	5'-CAGATTGTTGCGTAATACCCATG-3'	13129-13151
Reverse	5'-GTAAGGTGAAATCTCTCGTTGTC-3'	14511-14488
Forward	5'-GATTAGGAGTCCGGA ACTATGAG-3'	14257-14279
Reverse	5'-ACCAGACAAAGCTGGGTATGATA-3'	15690-15668

Table S2. Complete CDV sequences retrieving from GenBank used in genetic alignment for phylogenetic analysis.

Accession no.	Strain	Host	Country	Genotype^a	Year
AY443350	00-2601	Raccoon	USA	America-2	2000
AY649446	01-2689	Raccoon	USA	America-2	2001
KJ123771	171391-513	Dog	USA	America-2	2004
AF164967	A75/17	Dog	USA	America-2	N/A
KJ466106	CDV SY	Raccoon	China	Asia-1	2012
KC427278	Hebei	Mink	China	Asia-1	2008
HM852904	MKY-KM08	Monkey	China	Asia-1	2008
JN896331	PS	Dog	China	Asia-1	2010
JX681125	HLJ1-06	Fox	China	Asia-1	2006
AB474397	007LM	Dog	Japan	Asia-2	N/A
AB476401	011C	Dog	Japan	Asia-2	N/A
AB476402	50Con	Dog	Japan	Asia-2	N/A
AB475097	M25CR	Dog	Japan	Asia-2	N/A
AY445077	98-2645	Raccoon	USA	America-1	1998
AY542312	98-2646	Raccoon	USA	America-1	1998
AY446011	98-2654	Raccoon	USA	America-1	1998
MF041963	CDV-06	Wolf	Ethiopia	Europe/wildlife	2016
KP677502	Louguan thai	Panda	China	Asia-1	2015
KM280689	Uy251	Dog	Uruguay	Europe/South America-1	2012
KY971532	WT02SA	Hyena	Africa	South Africa	2017

^a Genotype classification based on complete H gene of the CDV.

N/A: No available data

Table S4. Evidence for positive and negative selection using various detection methods

CDV genome	Selection pressure analysis	SLAC	FEL	FUBAR
H gene	Positive selection	0	4	3
	Negative selection	47	125	134
	Overall dN/dS	0.240	0.228	0.177
N gene	Positive selection	0	0	0
	Negative selection	30	90	143
	Overall dN/dS	0.094	0.087	0.102
F gene	Positive selection	0	8	8
	Negative selection	26	95	100
	Overall dN/dS	0.296	0.280	0.203
P gene	Positive selection	0	2	10
	Negative selection	16	56	42
	Overall dN/dS	0.362	0.336	0.524
M gene	Positive selection	0	0	0
	Negative selection	13	55	73
	Overall dN/dS	0.047	0.039	0.051
L gene	Positive selection	0	1	1
	Negative selection	139	398	278
	Overall dN/dS	0.097	0.086	0.102

Table S5. Viral titer of individual CDV's TH/2014 strain.

CDV strain	Log 10 TCID₅₀ /ml
CDV1 TH/2014*	10 ^{2.75}
CDV2 TH/2014*	10 ^{3.5}
CDV3 TH/2014*	10 ⁵
CDV4 TH/2014	10 ^{2.167}
CDV5 TH/2014*	10 ^{2.875}
CDV6 TH/2014	10 ³
CDV7 TH/2014	10 ^{2.5}
CDV8 TH/2014*	10 ^{2.625}

* The strains were classified as CDV Asia-4.