

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

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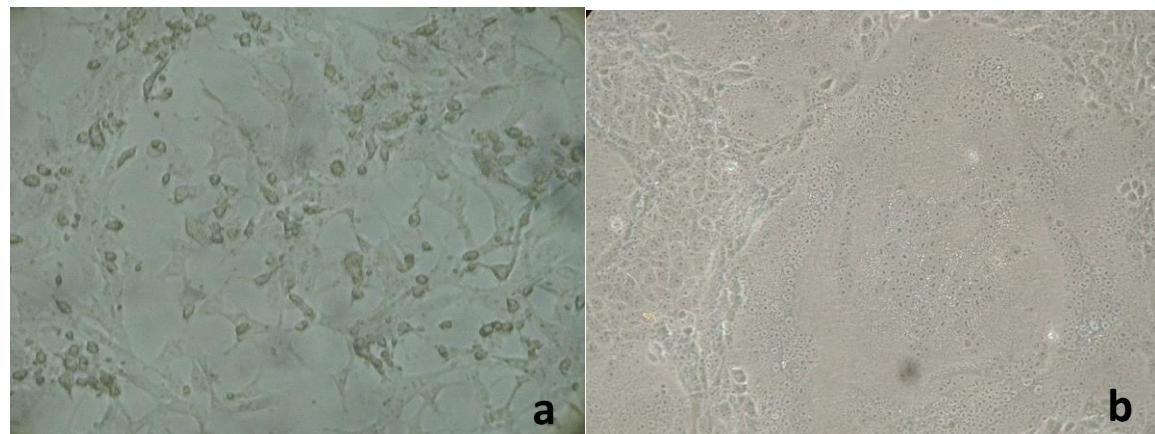


Fig. S4. Cytopathic effect (CPE) after CDV Thai strain inoculation in Vero-DST cell line. Stellate cell formation after 24 hours inoculation Vero-DST with CDV6 TH/2014 (a). and syncytial cell formation (b) after CDV2 TH/2014 inoculation.

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

| Direction | Primer | Position |
|----------------|---------------------------------|-------------|
| Forward | 5'-ATGGCTAGCCTCTCAAGAGC-3' | 1-21 |
| Reverse | 5'-CAAATCATTTCAGCAATTCTAGGC-3' | 764-741 |
| Forward | 5'-GAATGAACAAAATATGGCTTGATAT-3' | 736-760 |
| Reverse | 5'-GATTCTCTCTGAGGGCTTGAG-3' | 1867-1846 |
| Forward | 5'-CTTAGGACCCAGGTCCAACAA-3' | 1739-1759) |
| Reverse | 5'-GAATCAGTCTCTCCTTAAGCAG-3' | 2843-2821 |
| Forward | 5'-GCAAAGATGACCCAGAAATGCAA-3' | 2635-2657 |
| Reverse | 5'-CCAGTTGTTCCCTTGACACCTG-3' | 3765-3745 |
| Forward | 5'-GGGAGAGATTCAAGGAAGAGCA-3' | 2995-3015 |
| Reverse | 5'-GAGCGGGTAACACACAGGATTTC-3' | 4283-4262 |
| Forward | 5'-GGGATTAGTGTGCTCTAGGA-3' | 4163-4184 |
| Reverse | 5'-CTCACCTAATTCTGCTTGGTAC-3' | 5495-5473 |
| Forward | 5'-GGTTAGTCCTATGGTGCATTGG-3' | 5287-5308 |
| Reverse | 5'-CCAGTGGGCAGGTATCGGAG-3' | 6537-6518 |
| Forward | 5'-CAGCCATTGTAGCCAGAACTC-3' | 6289-6310 |
| Reverse | 5'-AGGTGGATTATGCACCAATGG-3' | 7507-7486 |
| Forward | 5'-GAAAACCTAGGGCTCAGGTAGT-3' | 7051-7072 |
| Reverse | 5'-AACCGCCCATAAGATGGCAC-3' | 8304-8284 |
| Forward | 5'-GGTAAAACCTATTGCTAGTCCT-3' | 7763-7787 |
| Reverse | 5'-GGGATAAGATAACAAGGTCTGATT-3' | 9270-9246 |
| Forward | 5'-GTCCATCTAGATAGCCCCATTG-3' | 9069-9090 |
| Reverse | 5'-CCATGTCTATCCCGATAACCG-3' | 10243-10223 |

| | | |
|----------------|--------------------------------|-------------|
| Forward | 5'-GAAATTCAAGGAGATTTACAGGAC-3' | 9960-9983 |
| Reverse | 5'-GTTTCATTCGCTTCAAATGATGTC-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'-CAGATTGTTGCGTAATAACCCATG-3' | 13129-13151 |
| Reverse | 5'-GTAAGGTGAAATCTCTCGTTGTC-3' | 14511-14488 |
| Forward | 5'-GATTAGGAGTCCGGAACTATGAG-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 | Louguanthai | 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 S3. Pairwise nucleotide identity of complete CDV sequences.

| | |
|--|---|
| AY466011_CDV_Raccoon_isolate_98-2654_America-1 | 0.000 |
| AY445077.2_CDV_Raccoon_isolate_98-2645_America-1 | 0.001 0.001 |
| AY542312.2_CDV_Raccoon_isolate_98-2646_America-1 | 0.029 0.029 0.028 |
| AF164967.1_CDV_Dog_isolate_A75/17_America-2 | 0.029 0.030 0.030 0.004 |
| EU716337.1_CDV_Dog_isolate_164071_America-2 | 0.036 0.037 0.036 0.012 0.009 |
| KJ123771.1_CDV_Dog_isolate_171391-513_America-2 | 0.028 0.028 0.028 0.013 0.013 0.021 |
| AY649446.1_CDV_Raccoon_isolate_01-2689_America-2 | 0.026 0.026 0.026 0.016 0.017 0.024 0.014 |
| MF041963.1_CDV_Wolf_isolate_CDV06_Europe_wildlife | 0.057 0.057 0.057 0.042 0.043 0.049 0.049 0.049 |
| MH496775_CDV_Dog_isolate_CDV4_TH/2014 | 0.052 0.052 0.052 0.034 0.036 0.042 0.043 0.043 0.014 |
| KJ466106.1_CDV_Raccoon_isolate_SY_Asia-1 | 0.055 0.055 0.055 0.040 0.041 0.048 0.047 0.047 0.013 0.009 |
| MH496779_CDV_Dog_isolate_CDV6_TH/2014 | 0.055 0.055 0.054 0.038 0.040 0.046 0.045 0.045 0.012 0.011 0.010 |
| KC427278.1_CDV_Mink_isolate_Hebei_Asia-1 | 0.054 0.054 0.054 0.039 0.040 0.046 0.045 0.046 0.013 0.012 0.011 0.009 |
| JN896331.1_CDV_Dog_isolate_PS_Asia-1 | 0.047 0.047 0.047 0.031 0.033 0.039 0.038 0.038 0.012 0.012 0.011 0.009 0.009 |
| MH496776_CDV_Dog_isolate_CDV7_TH/2014 | 0.054 0.054 0.054 0.038 0.040 0.046 0.045 0.045 0.014 0.014 0.013 0.011 0.011 |
| JX681125.1_CDV_Fox_isolate_HLJ1-06_Asia-1 | 0.055 0.055 0.055 0.043 0.044 0.050 0.048 0.049 0.018 0.018 0.017 0.016 0.015 0.012 0.017 |
| KP677502.1_CDV_Panda_isolate_Louguantai_1_Asia-1 | 0.058 0.058 0.058 0.042 0.044 0.049 0.048 0.049 0.021 0.020 0.020 0.018 0.018 0.012 0.019 0.023 |
| HM852904.1_CDVMonkey_isolate_MKY-KM08_Asia-1 | 0.055 0.055 0.055 0.040 0.042 0.048 0.046 0.047 0.029 0.026 0.026 0.025 0.025 0.020 0.026 0.031 0.028 |
| MH496772_CDV_Dog_isolate_CDV1_TH/2014 | 0.055 0.055 0.055 0.038 0.041 0.046 0.045 0.045 0.057 0.052 0.054 0.054 0.053 0.047 0.054 0.057 0.057 0.056 |
| MH496773_CDV_Dog_isolate_CDV2_TH/2014 | 0.055 0.055 0.055 0.039 0.041 0.047 0.046 0.046 0.058 0.052 0.055 0.054 0.054 0.048 0.055 0.057 0.058 0.057 0.003 |
| MH496777_CDV_Dog_isolate_CDV8_TH/2014 | 0.055 0.055 0.055 0.038 0.040 0.046 0.046 0.046 0.057 0.052 0.055 0.054 0.054 0.048 0.054 0.057 0.058 0.056 0.003 0.002 |
| MH496778_CDV_Dog_isolate_CDV5_TH/2014 | 0.055 0.055 0.055 0.040 0.041 0.047 0.046 0.047 0.058 0.053 0.056 0.054 0.054 0.048 0.055 0.058 0.059 0.057 0.009 0.008 0.009 |
| MH496774_CDV_Dog_isolate_CDV3_TH/2014 | 0.054 0.054 0.054 0.038 0.040 0.046 0.045 0.045 0.057 0.051 0.054 0.053 0.053 0.047 0.054 0.057 0.058 0.056 0.012 0.011 0.012 0.012 |
| KM280689.1_CDV_Dog_isolate_Uy251_Europe/SouthAmerica-1 | 0.052 0.052 0.052 0.039 0.040 0.045 0.043 0.043 0.056 0.052 0.055 0.054 0.053 0.047 0.054 0.057 0.056 0.055 0.054 0.055 0.055 0.055 |
| AB474397.1_CDV_Dog_isolate_007Lm_Asia-2 | 0.052 0.052 0.052 0.039 0.040 0.046 0.042 0.044 0.059 0.054 0.057 0.056 0.056 0.049 0.056 0.059 0.060 0.057 0.056 0.057 0.056 0.056 |
| AB476402.1_CDV_Dog_isolate_50Con_Asia-2 | 0.053 0.053 0.052 0.040 0.042 0.047 0.046 0.046 0.059 0.054 0.057 0.056 0.055 0.049 0.056 0.058 0.059 0.056 0.055 0.056 0.056 0.055 0.009 |
| AB476401.1_CDV_Dog_isolate_011C_Asia-2 | 0.054 0.054 0.054 0.041 0.043 0.048 0.047 0.047 0.060 0.055 0.058 0.057 0.057 0.050 0.057 0.060 0.060 0.057 0.057 0.057 0.057 0.003 |
| AB475097.1_CDV_Dog_isolate_M25CR_Asia-2 | 0.054 0.054 0.054 0.041 0.043 0.048 0.047 0.047 0.060 0.055 0.058 0.057 0.057 0.050 0.057 0.060 0.060 0.058 0.056 0.057 0.057 0.003 0.000 |
| KY971532.1_CDV_Hyena_isolate_WT02SA_South_Africa | 0.057 0.057 0.057 0.044 0.046 0.052 0.050 0.050 0.061 0.058 0.060 0.059 0.059 0.052 0.059 0.062 0.062 0.062 0.061 0.061 0.061 0.060 0.059 0.056 0.054 0.055 0.055 |

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

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