VIROLOGICA SINICA

## Electronic Supplementary Material

# Genetic variation of multiple serotypes of enteroviruses associated with hand, foot and mouth disease in Southern China

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Supporting information to DOI: 10.1007/s12250-020-00266-7

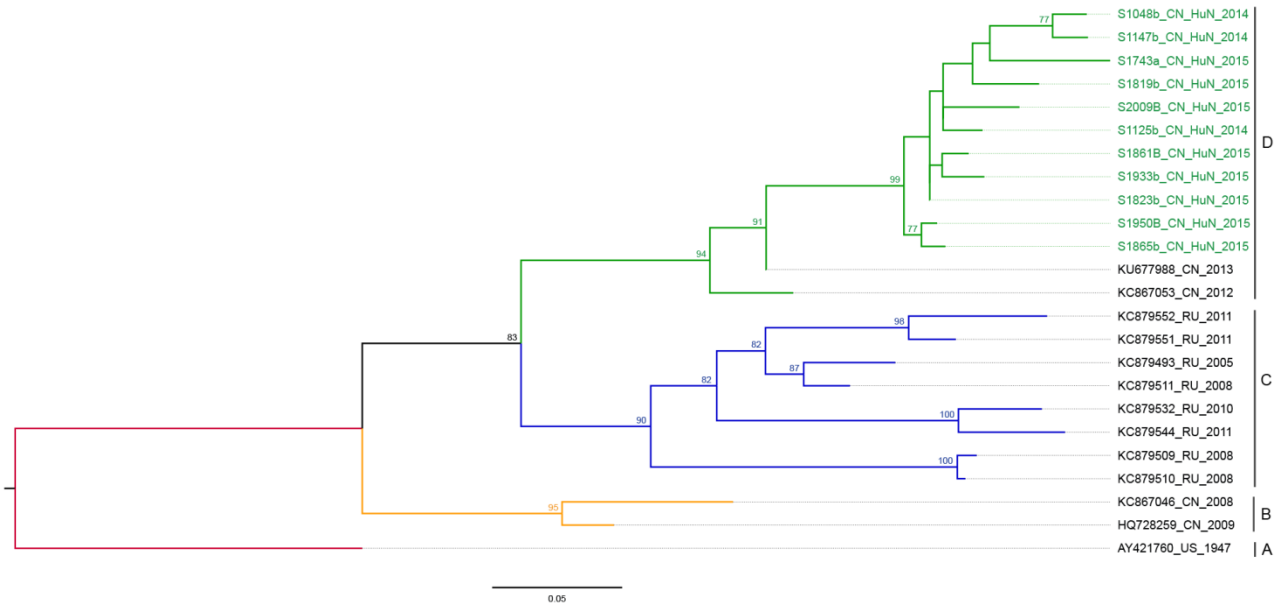


**Fig. S1**. Phylogenetic analyses of the VP1 sequences (342 bp) of CVA2 from Anhua County based on ML methods. The tree was rooted on genotype A of the CVA2 strain. The branches of sequences are color-coded according to the CVA2 lineage. The names of the study strains are colored in green, the other reference strains are uncolored. Bootstrap values >70% were shown on the branches. This phylogenetic tree indicates that evolutionary branch D was responsible for infections in Anhua County during 2013-2016.

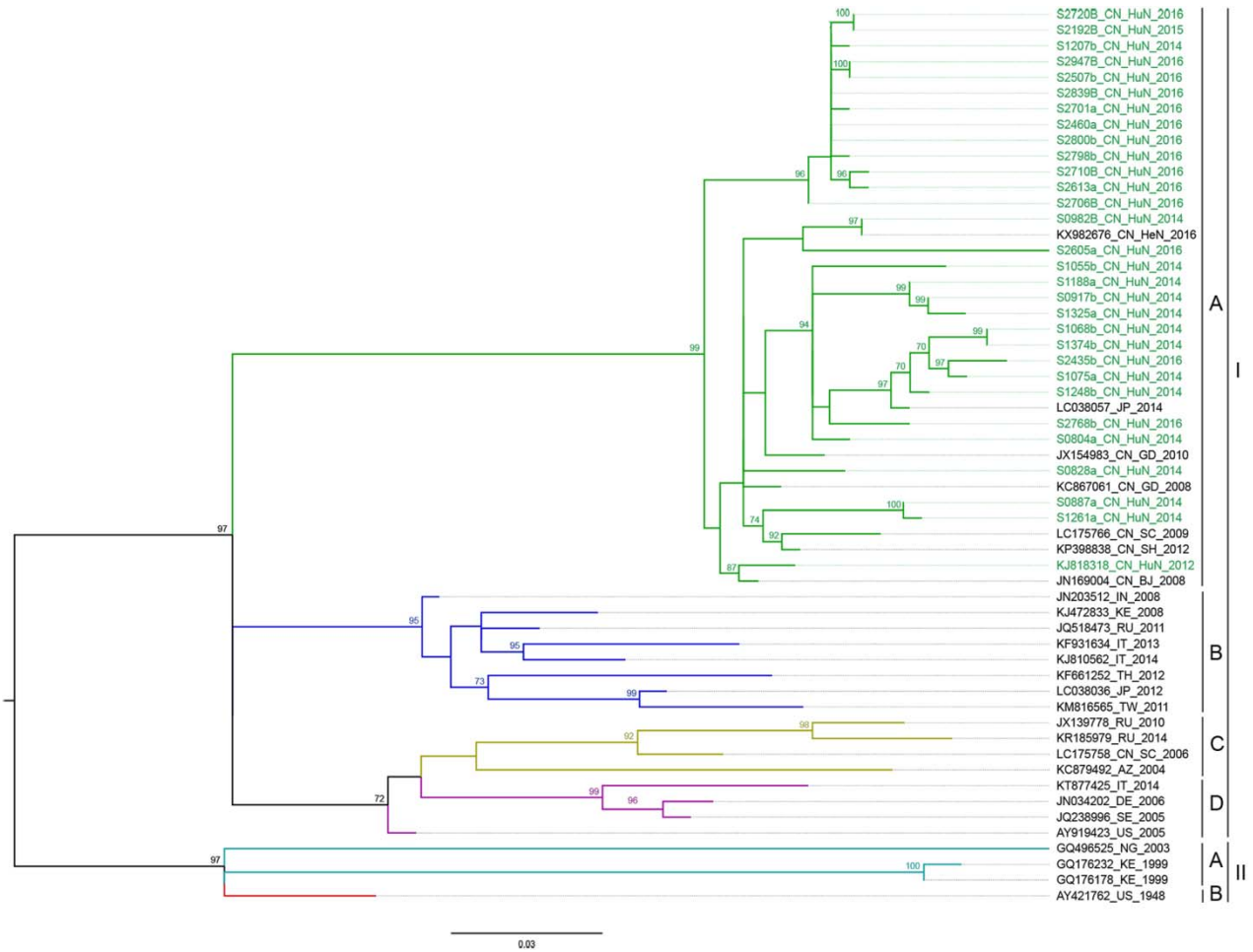


**Fig. S2**. Phylogenetic analyses of the VP1 sequences (234 bp) of CVA4 from Anhua County based on ML methods. The tree was rooted at the midpoint. The branches of sequences are color-coded according to the CVA4 lineage. The names of the study strains are colored in green, the other reference strains are uncolored. Bootstrap values >70% were shown on the branches. This phylogenetic tree indicates that evolutionary branch I-A was responsible for infections in Anhua County during 2013-2016.

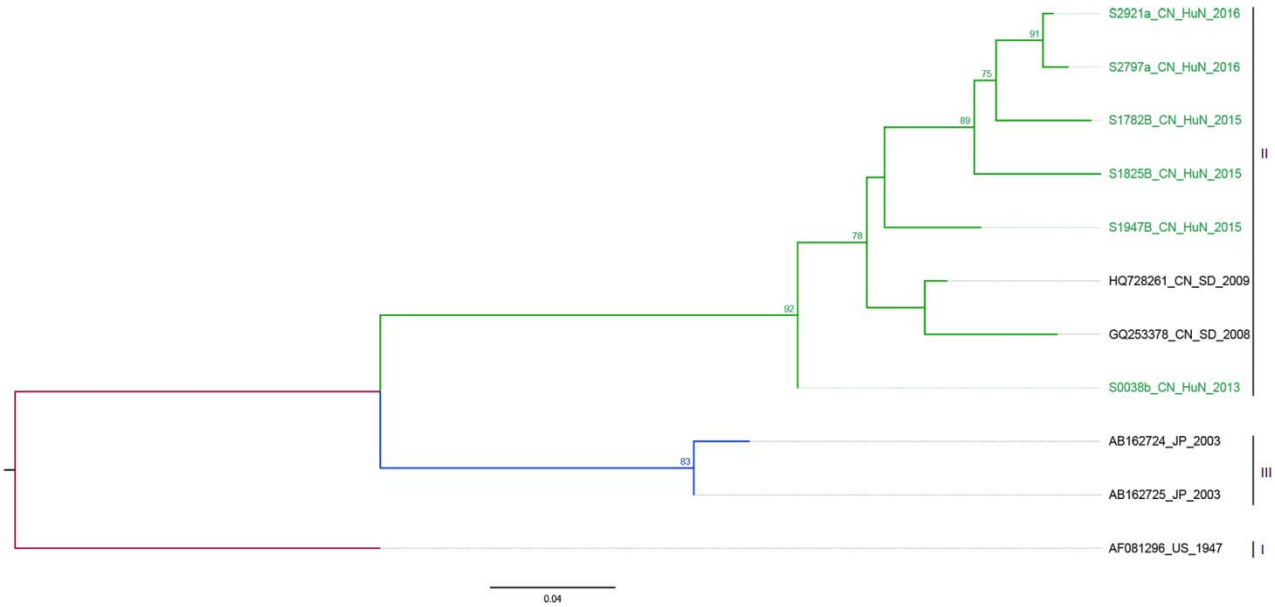


**Fig. S3.** Phylogenetic analyses of the VP1 sequences (295 bp) of CVA5 from Anhua County based on ML methods. The tree was rooted on genotype I of the CVA5 strain. The branches of sequences are color-coded according to the CVA5 lineage. The names of the study strains are colored in green, the other reference strains are uncolored. Bootstrap values >70% were shown on the branches. This phylogenetic tree indicates that evolutionary branch II was responsible for infections in Anhua County during 2013-2016.

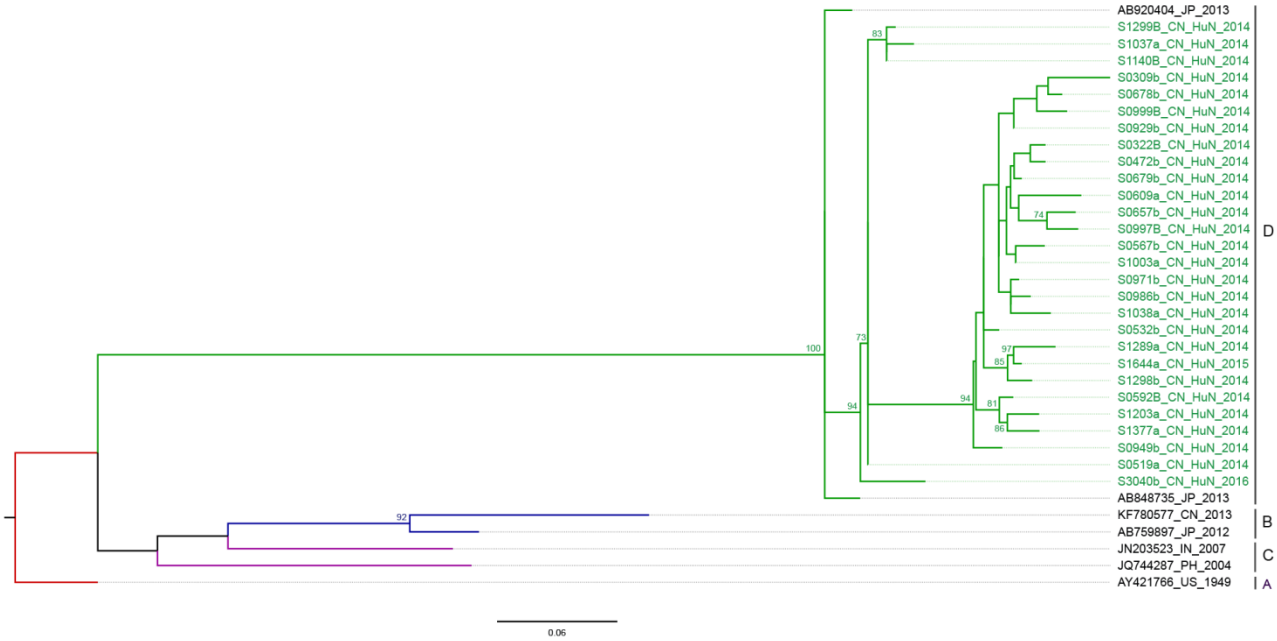


**Fig. S4**. Phylogenetic analyses of the VP1 sequences (329 bp) of CVA8 from Anhua County based on ML methods. The tree was rooted on genotype A of the CVA8 strain. The branches of sequences are color-coded according to the CVA8 lineage. The names of the study strains are colored in green, the other reference strains are uncolored. Bootstrap values >70% were shown on the branches. The phylogenetic tree indicates that evolutionary branch D was responsible for infections in Anhua County during 2013-2016.


**Fig. S5** Phylogenetic analyses of the VP1 sequences (308 bp) of CVA10 from Anhua County based on ML methods. The tree was rooted on genotype A of the CVA10 strain. The branches of sequences are color-coded according to the CVA10 lineage. The names of the study strains are colored in green, the other reference strains are uncolored. Bootstrap values >70% were shown on the branches. The phylogenetic tree indicates that evolutionary branch C was responsible for infections in Anhua County during 2013-2016.

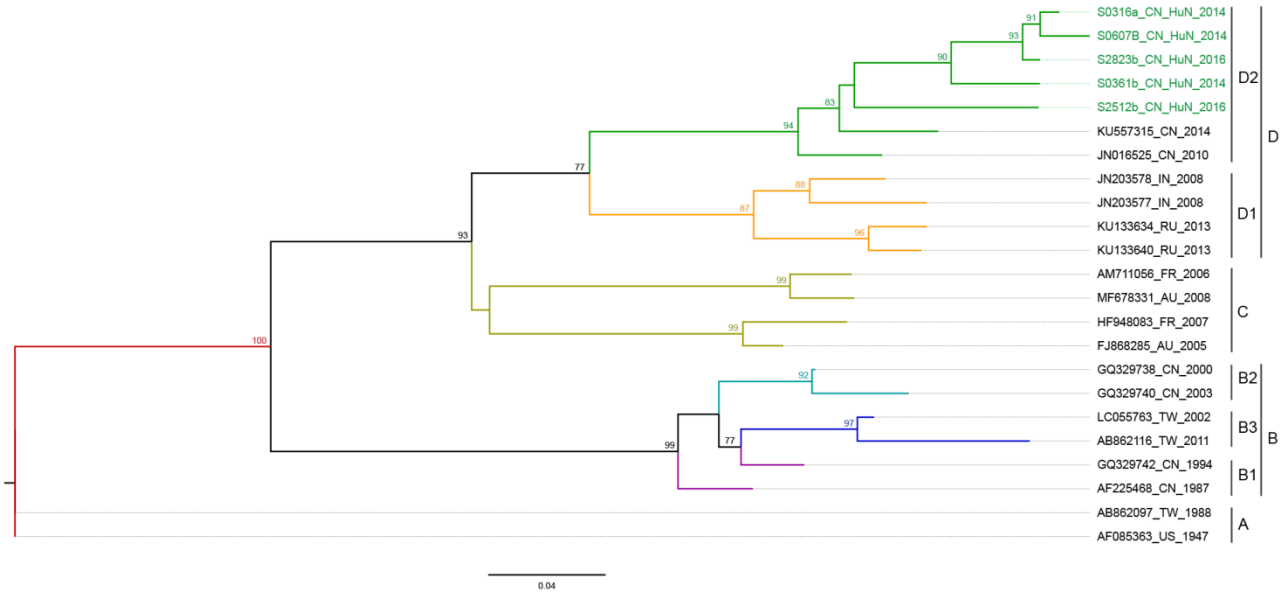


**Fig. S6.** Phylogenetic analyses of the VP1 sequences (389 bp) of CVB2 from Anhua County based on ML methods. The tree was rooted on genotype A of the CVB2 strain. The branches of sequences are color-coded according to the CVB2 lineage. The names of the study strains are colored in green, the other reference strains are uncolored. Bootstrap values >70% were shown on the branches. The phylogenetic tree indicates that evolutionary branch D2 was responsible for infections in Anhua County during 2013-2016.

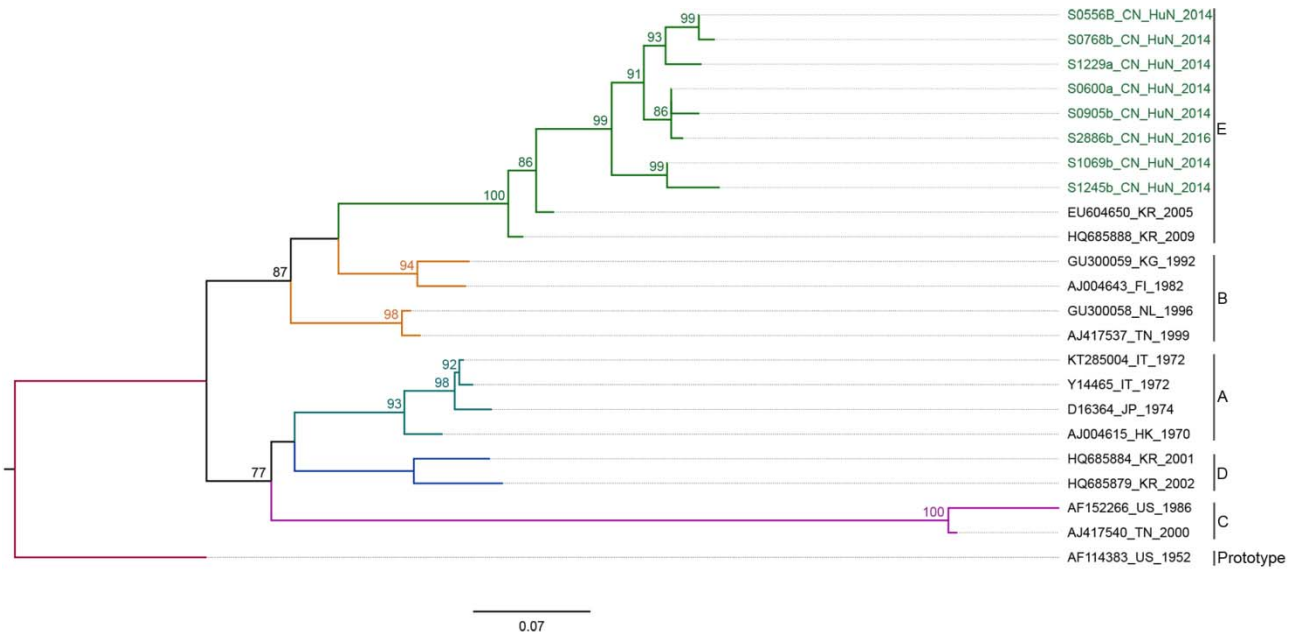


**Fig. S7.** Phylogenetic analyses of the VP1 sequences (381 bp) of CVB5 from Anhua County based on ML methods. The tree was rooted on Prototype of the CVB5 strain. The branches of sequences are color-coded according to the CVB5 lineage. The names of the study strains are colored in green, the other reference strains are uncolored. Bootstrap values >70% were shown on the branches. The phylogenetic tree indicates that evolutionary branch E was responsible for infections in Anhua County during 2013-2016.

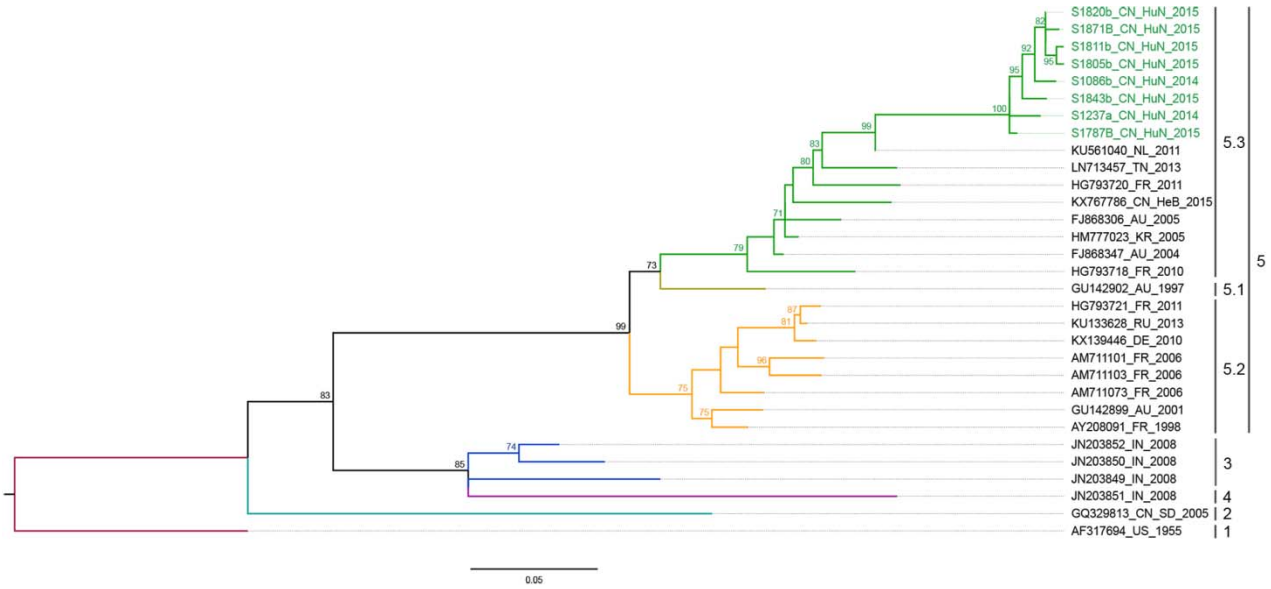


**Fig. S8**. Phylogenetic analyses of the VP1 sequences (344 bp) of E18 from Anhua County based on ML methods. The tree was rooted on genotype 1 of the E18 strain. The branches of sequences are color-coded according to the E18 lineage. The names of the study strains are colored in green, the other reference strains are uncolored. Bootstrap values >70% were shown on the branches. The phylogenetic tree indicates that evolutionary branch 5.3 was responsible for infections in Anhua County during 2013-2016.

**Table S1**. The Primers Sequences of Complete VP1 Sequencing Used in this Study.

| Primer/Probe | Sequence (5'→3') | Position | Region | Methods |
|---|---|---|---|---|
| EV-A71-VP1 | GCAGCCCAAAAGAACTTCAC | 2372-2391 | VP3 | RT-PCR (EV-A71) |
| EV-A71-VP1 | AAGTCGCGAGAGCTGTCTTC | 3454-3435 | 2A | |
| CVA6-OUTER | GARGCTAACATYATAGCTCTTGGAGC | 2347-2372 | VP3 | |
| CVA6-OUTER | CCYTCATARTCHGTGGTGGTTATGCT | 3326-3301 | VP1 | Nested RT-PCR (CVA6) |
| CVA6-INTER | GACACYGAYGARATYCAACAAACAGC | 2407-2432 | VP3 | |
| CVA6-INTER | CGRTCRGTTGCAGTGTTWGTTATTGT | 3296-3271 | VP1 | |
| CVA16-OUTER | GTCGTGCCATGGATCAGTAA | 2215-2234 | VP3 | |
| CVA16-OUTER | ACAATTGCACCTAGCGATGG | 3504-3485 | 2A | Nested RT-PCR (CVA16) |
| CVA16-INTER | TSAARYTGTGCAARGACAC | 2429–2448 | VP3 | |
| CVA16-INTER | GCICCIGAYTGITGICCRAA | 3408–3389 | 2A | |

**Table S2**. Serotype homology based on the genome VP1 region

| Serotype | Identity of nucleotides | Identity of amino acids |
|---|---|---|
| CVA16 | 92%-99% | 99%-100% |
| CVA6 | 94%-99% | 97%-99% |
| EV-A71 | 93%–99% | 97%-100% |
| CVA10 | 85%-99% | 95%-100% |
| CVA4 | 89-99% | 96%-100% |
| CVA8 | 96%-98% | 92%-100% |
| CVA2 | 93%-100% | 97%-100% |
| E18 | 96%-99% | 98%-100% |
| CVA5 | 93%-97% | 98%-100% |
| CVB5 | 92%-99% | 98%-100% |
| CVB2 | 94%-99% | 98%-100% |

**Table S3**. Numbers of VP1 deduced amino sequences of EV-A71, CVA16 and CVA6

***Note to Table S3:*** Red - Numbers of variation sites; Green - BC loop regions; Orange - EF loop regions; Blue - GH loop region.