

# First complete genome sequence of canine bocavirus 2 in mainland China

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## Abstract

We obtained the first full-length genome sequence of canine bocavirus 2 (CBoV2) from the faeces of a healthy dog in Guangzhou city, Guangdong province, mainland China. The genome of GZHD15 consisted of 5059 nucleotides. Sequence analysis suggested that GZHD15 was close to a previously circulated Hong Kong isolate.

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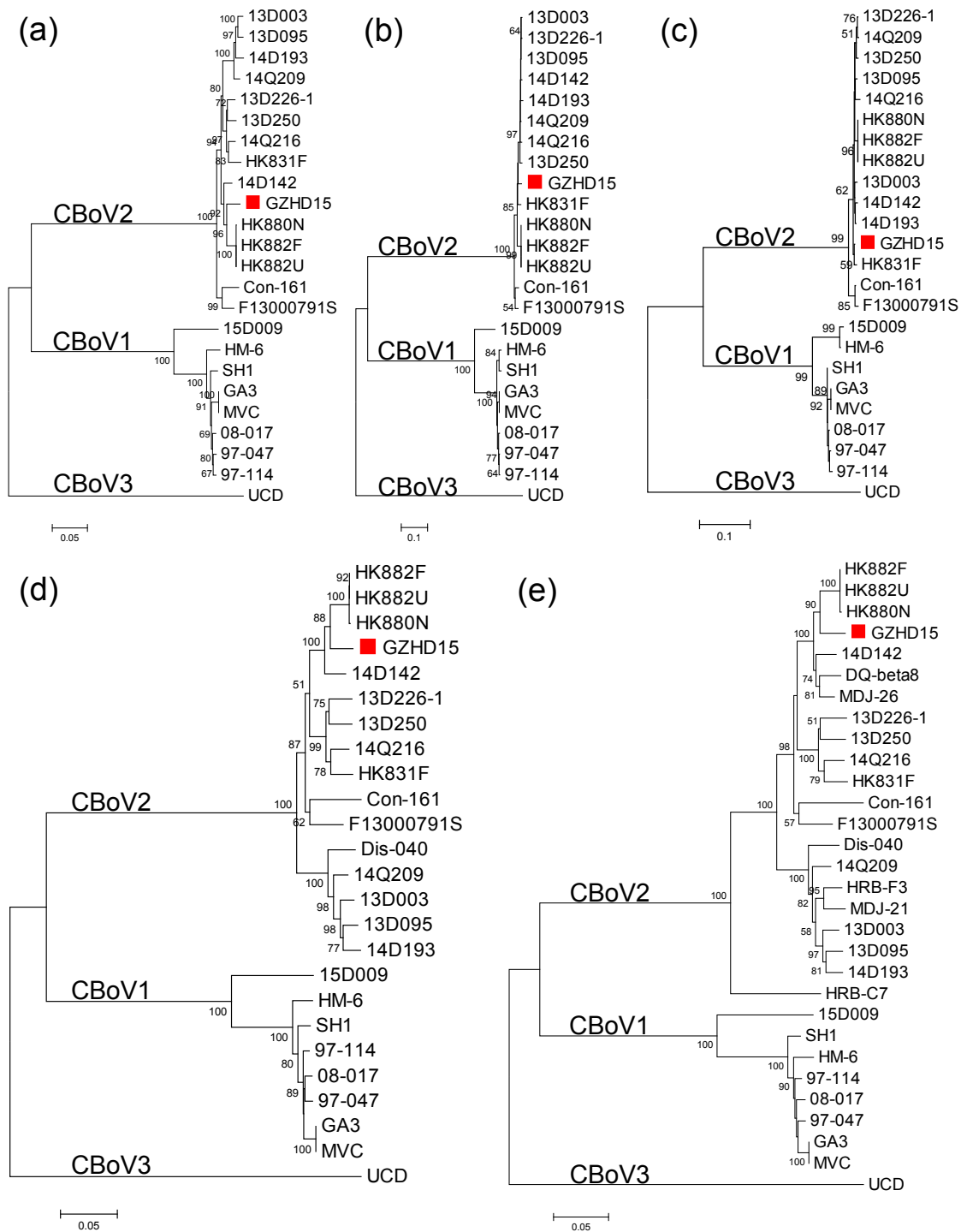
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Canine bocavirus (CBoV) belongs to *Bocavirus* genus, *Parvovirinae* subfamily, in the *Parvoviridae* family [1]. Its genome comprises about 5200 nucleotides (nt) with three open reading frames (ORFs) encoding NS1, NP1 and VP1/2 respectively. To date, three CBoV genotypes (CBoV1, CBoV2 and CBoV3) have been identified. CBoV1 was first reported in German shepherd dogs [2]. It was later found to be prevalent in Japan, United States, China, Italy and South Korea [3–6]. CBoV2 and CBoV3 were discovered in 2012 and 2013 respectively [7,8]. CBoV2 was also circulating in Germany, South Korea, mainland China and Hong Kong [9–12]. However, CBoV3 only emerged in the United States [8].

Although CBoV1 and CBoV2 have been reported in Shanghai, Heilongjiang (China) and Hong Kong [5,9,10], the existing epidemiologic data of CBoV are still limited. Therefore, in this study, a universal PCR detection method was developed to investigate the epidemic status of CBoV1, CBoV2 and CBoV3 in 425 dog faeces samples collected from Guangzhou city, Guangdong province, mainland China. Among them, 391 samples were from healthy dogs, while the remaining 34 samples were from dogs with diarrhoea. PCR primers were CBoV-F (5'-AAR AGR AAR CTY TAT TTT GC-3') and CBoV-R (5'-TGC CAG TCT TGW GGH GAR AA-3') targeting at all three CBoV genotypes. The corresponding PCR fragment size of CBoV1, CBoV2 and CBoV3 was 377 bp, 386 bp or 404 bp, and 386 bp respectively. A 25 µL PCR system contained 0.5 µL CBoV-F primer (10 µM), 0.5 µL CBoV-R primer (10 µM), 12.5 µL PCR Mixture (Tiangen, Beijing), 8.5 µL of ddH<sub>2</sub>O and 3 µL of sample DNA or controls. DNA amplification was initiated by preheating for 5 minutes at 94°C, followed by 40 cycles at 94°C for 30 seconds, 55°C for 30 seconds and 72°C for 30 seconds, and a final extension for 10 minutes at 72°C. Moreover, to obtain genome information of CBoV, PCR primers were used to amplify complete nucleotide sequences of CBoV from those positive samples [11].

After initial testing, only one CBoV isolate (named GZHD15) was identified from a healthy poodle dog. Following



**FIG. 1.** Phylogenetic analysis of different canine bocaviruses (CBoVs). (a) Phylogenetic tree based on complete genome sequences of CBoVs. (b) Phylogenetic tree based on complete NSI sequences of CBoVs. (c) Phylogenetic tree based on complete NP1 sequences of CBoVs. (d) Phylogenetic tree based on complete VP1 sequences of CBoVs. (e) Phylogenetic tree based on complete VP2 sequences of CBoVs. Phylogenetic trees were constructed by neighbour-joining method in MEGA 5.1 software. One thousand bootstrap replicates were used. CBoV obtained in this study indicated by box.

conventional Sanger sequencing, we found that the full-length genome of GZHD15 was 5059 nt (50.37% G+C). Its ORF1 (2.382 nt) encoded a 793 aa NS1 protein, while its ORF2 (2.139 nt) encoded a 712 aa overlapping capsid protein (VP1/VP2). ORF3 (588 nt) encoded a 195 aa NPI protein. Sequence alignment analysis demonstrated that GZHD15 had the highest nucleotide similarity (97.2%) with a Hong Kong CBoV2 strain (HK880U) [9] and the lowest nucleotide similarity (58.4%) with a US CBoV3 strain (UCD) [8]. Phylogenetic analysis based on different gene sequences all showed that GZHD15 was clustered together with other CBoV2 strains (Fig. 1).

In summary, this study for the first time reported low prevalence of CBoV2 in southern China. The complete genome sequence of this recent CBoV2 isolate as described here will be critical to study the genetic evolution and biology of CBoV.

### Nucleotide sequence accession number

The genome sequences of GZHD15 have been deposited in GenBank under accession number KY038922.

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### Conflict of Interest

None declared.

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