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GENOME ANNOUNCEMENT

Complete Genome Sequences of Two Chinese Virulent Avian Coronavirus Infectious Bronchitis Virus Variants

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Avian coronavirus infectious bronchitis virus (IBV) is variable, which causes many serotypes. Here we reported the complete genome sequences of two virulent IBV variants from China, GX-YL5 and GX-YL9, belonging to different serotypes. Differences between GX-YL5 and GX-YL9 were found mainly in stem-loop structure I in the predicted RNA secondary structure of open reading frame (ORF) 1b and the S protein gene fusion region, which will help us understand the molecular evolutionary mechanism of IBV and the disconcordance between the genotypes and serotypes of coronavirus.

Infectious bronchitis virus (IBV), a member of the genus *Gammacoronavirus* of the family *Coronaviridae* in the order *Nidovirales*, is an enveloped, nonsegmented, single-stranded, positive-sense RNA virus. The coronavirus employs a discontinuous RNA synthesis mechanism for its transcription, and multiple subgenomic mRNAs contain a short 5′ leader sequence. The leader and mRNA body sequences are joined within a short conserved sequence motif named the transcription-regulating sequence, which precedes each transcription unit (4, 8, 10, 12). In this study, two IBV field strains from China, named GX-YL5 and GX-YL9, showed strong pathogenicity and belonged to the same genotype but different serotypes (5). The complete genomes of GX-YL5 and GX-YL9 were sequenced and analyzed to further understand the molecular mechanism of IBV.

The 5′ and 3′ terminals of the GX-YL5 and GX-YL9 genomes were confirmed by using the 5′-Full random amplification of cDNA ends (RACE) kit and 3′-Full RACE core set, version 2.0 (TaKaRa, Japan), and the other parts were generated by 25 and 24 overlapping cDNA fragments, respectively. All sequencing was done using an ABI Prism 3730 sequencer (Applied Biosystems) and assembled using the SeqMan software program (DNASTAR Inc.). Sequence alignment was conducted and a phylogenetic tree was constructed using the software program MEGA5 (3). RNA secondary structure prediction was conducted using the program Mfold (15). Recombination analysis was performed using the RDP 4.14 (7) and SimPlot 3.5.1 (6) software programs.

The complete genomes of GX-YL5 and GX-YL9 are 27,706 and 27,582 nucleotides (nt), respectively, in length, after excluding the polyadenylated tract, which shared 95.9% nucleotide identity. Compared with the most popularly used vaccine strain, H120, they had lower nucleotide identities, 86.0% and 85.9%, respectively. Their genome organizations are classical IBV genomes with the characteristic gene order 5′-Pol-S-3a-3b-E-M-5a-5b-N-3′. The differences between GX-YL5 and GX-YL9 were found in the S1 subunit within nt 20429 to 20830, a membrane gene within nt 24559 to 25579, and the 3′ untranslated region (UTR) within nt 27193 to 27405. The recombination sites of genes S2, membrane, nucleocapsid, and 3′UTR in GX-YL5 and the recombination sites of genes 3a, membrane, 5a, nucleocapsid, and 3′UTR in GX-YL9 had been predicted.

The S1 subunit of the IBV genome is the major determiner of serotype (1, 2, 9, 13). In our study, the predicted RNA secondary structure of the ORF 1b and the S protein gene fusion region (nt 20375 to 20430), based on GX-YL5, showed that the nucleotide mutations were found mostly in stem-loop structure I, and multiple nucleotide differences were also found in the fusion region between GX-YL5 and GX-YL9. Interestingly, GX-YL5 and GX-YL9 belonged to the same genotype but different serotypes (5). During synthesis of the cDNA strand of coronavirus, a process known as copy choice can lead to genetic recombination (14). The high frequency of recombination in IBV likely plays a major role in the generation of new serotypes of the virus (11).

The results will help us understand the molecular evolutionary mechanisms of IBV and disconcordance between the genotypes and serotypes of coronaviruses.

Nucleotide sequences accession numbers. The full genomic sequences of GX-YL5 and GX-YL9 are available in GenBank under accession numbers HQ848267 and HQ850618, respectively.

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