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GENOME ANNOUNCEMENT

Complete Genome Sequence of an Infectious Bronchitis Virus Chimera between Cocirculating Heterotypic Strains

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To date, multiple serotypes and genotypes of infectious bronchitis virus (IBV) have been isolated and identified. In order to provide more information on the viral evolution of IBVs, a new virulent strain named GX-NN09032, isolated from Guangxi, China, in 2009, was sequenced, and phylogenetic and recombination analyses were conducted. Furthermore, potential recombination events associated with GX-NN09032 were found in four IBV strains, including GX-YL5, DY07, CK/CH/SD09/005, TC07-2. The present study suggested that GX-NN09032 might contribute to the emergence of modern IBV variants through recombination.

Coronavirus infectious bronchitis virus (IBV), belonging to group III of genus *Coronavirus*, family *Coronaviridae*, is a positive-sense, single-stranded RNA virus known to infect fowls, including turkey and pheasant (2, 3, 11). Recently, different serotypes and genotypes of IBV have been reported and identified to be generated by RNA recombination, insertions, and point mutations due to replication and evolution (4, 5, 7). Characterization of the isolates for natural outbreaks of IB has been reported in our lab between 1985 and 2008 (8, 10); however, recombination and chimera have been submitted only for a small part of the genome (1, 13). In this report, the full-length genome sequence of an isolate, GX-NN09032, was analyzed and compared with recently reported strains and other typical IBVs by phylogenetic and recombination analysis.

The 5′ and 3′ terminals of the genome were obtained by using a rapid amplification of cDNA ends (RACE) kit (TaKaRa, Japan). All of the cloned products were sequenced with an Applied Biosystems 3730 DNA analyzer, and the assembly of contiguous sequences, multiple sequence alignments, and phylogenetic analysis were conducted using the vector NTI 11.5.1 software (Invitrogen) and MEGA4 (12) program. Recombination analysis was performed by employing the SimPlot 3.5.1 (6) software program.

The complete genome sequence of isolate GX-NN09032 is 27,684 nucleotides (nt) in length, excluding the poly(A) tail, and includes 10 open reading frames (ORFs). Its genome organization was identified to be 5′-Pol-S-3a-3b-E-M-5a-5b-N-3′. The sequence of GX-NN09032 (located at nt 20374 to 22011) showed the highest nucleotide acid sequence identities (99%, 98%, and 98%) to those of Chinese strains CK/CH/SD09/005 (GenBank accession no. HM230749.1), TC07-2 (GenBank accession no. GQ265948.1), and CK/CH/GD/KP10 (GenBank accession no. HQ018919.1), respectively, which confirmed the serotype of the virus as a different serotype (9), and the two region sequences of GX-NN09032 (located at 24533 to 25213 and 25959 to 27189) have 99% and 96% homology, respectively, with CK/CH/SD09/005 at the nucleotide level. Phylogenetic analysis based on S1, M, and N genes indicated that GX-NN09032 was closer to CK/CH/SD09/005, although GX-NN09032 was the closest to TC07-2 and CK/CH/GD/KP10.

All the employed recombination detection methods supported that four isolates of IBV, including GX-YL5 (GenBank accession no. HQ848267), DY07 (GenBank accession no. HM245923.1), CK/CH/SD09/005, and TC07-2, were identified to be virus chimera, with GX-NN09032 as one of putative parents. The first and second recombination regions were located at positions 21124 to 22012 and 22058 to 23578 in the S gene, and the rest of recombination regions were located in the M and N genes (24533 to 25213 and 25959 to 27189), respectively. The results showed that recombination has probably happened within the S, M, and N structural proteins and that GX-NN09032 is a possible virus chimera of CK/CH/SD09/005 and TC07-2, which were circulated during 2007 to 2009 and evolved independently by recombination and mutations among field strains.

Nucleotide sequence accession number. The full-length genome sequence of GX-NN09032 has been deposited in NCBI GenBank under accession no. JX897900.

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REFERENCES

1. **Ammayappan A, Upadhyay C, Gelb J, Vakharia VN.** 2008. Complete genomic sequence analysis of infectious bronchitis virus Ark DPI strain and its evolution by recombination. Virol. J. **5**:157.
2. **Cavanagh D.** 2007. Coronavirus avian infectious bronchitis virus. Vet. Res. **38**:281–297.
3. **Cavanagh D, Elus MM, Cook JKA.** 1997. Relationship between sequence variation in the S1 spike protein of infectious bronchitis virus and the extent of cross protection in vivo. Avian Pathol. **26**:63–74.
4. **Cavanagh D.** 2001. A nomenclature for avian coronavirus isolates and the question of species status. Avian Pathol. **30**:109–115.
5. **Kottier SA, Britton CDP.** 1995. Experimental evidence of recombination in coronavirus infectious bronchitis virus. Virology **213**:569–580.
6. **Lole KS, et al.** 1999. Full-length human immunodeficiency virus type 1 genomes from subtype C-infected seroconverters in India, with evidence of intersubtype recombination. J. Virol. **73**:152–160.

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7. **Ladman BS, Loupos AB, Gelb J.** 2006. Infectious bronchitis virus S1 gene sequence comparison is a better predictor of challenge of immunity in chickens than serotyping by virus neutralization. Avian Pathol. **35**:127–133.
8. **Li M, et al.** 2012. Serotype and genotype diversity of infectious bronchitis viruses isolated during 1985–2008 in Guangxi, China. Arch. Virol. **157**: 467–474.
9. **Luo H, et al.** 2011. Phylogenetic analysis of the S1 glycoprotein gene of infectious bronchitis viruses isolated in China during 2009–2010. Virus Genes **44**:19–23.
10. **Mo ML, et al.** 2012. Complete genome sequences of two Chinese virulent avian coronavirus infectious bronchitis virus variants. J. Virol. **86**:10903–10904.
11. **Stern D, Sefton B.** 1982. Coronavirus proteins: biogenesis of avian infectious bronchitis virus virion proteins. J. Virol. **44**:794–803.
12. **Tamura K, Dudley J, Nei M, Kumar S.** 2007. MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol. Biol. Evol. **24**:1596–1599.
13. **Zhang Y, et al.** 2010. Complete genome sequence and recombination analysis of infectious bronchitis virus attenuated vaccine strain H120. Virus Genes **41**:377–388.