

# Complete Genome Sequence Analysis of a Natural Reassortant Infectious Bursal Disease Virus in China

Feng Chen,<sup>a</sup> Jiajia Liu,<sup>a</sup> Zhuanqiang Yan,<sup>a</sup> Di Liu,<sup>a</sup> Jun Ji,<sup>a</sup> Jianping Qin,<sup>b</sup> Haiyan Li,<sup>b</sup> Jingyun Ma,<sup>a</sup> Yingzuo Bi,<sup>a</sup> and Qingmei Xie<sup>a</sup>

College of Animal Science, South China Agricultural University, Tianhe District, Wushan Road, Guangzhou, Guangdong, People's Republic of China,<sup>a</sup> and Guangdong Wen's Foodstuffs Group Co. Ltd., Yunfu, Guangdong, People's Republic of China<sup>b</sup>

**A novel isolate of infectious bursal disease virus (IBDV) was designated GX-NN-L. The GX-NN-L IBDV was a very virulent infectious bursal disease virus (vvIBDV) isolated from broiler flocks in Guangxi province, China, in 2011. The GX-NN-L IBDV caused high mortality, immunosuppression, low weight gain, and bursal atrophy in commercial broilers. Here, we report the complete genome sequence of the GX-NN-L IBDV, a reassortment strain with segments A and B derived from very virulent strains and attenuated IBDV, respectively. These findings from this study provide additional insights into the genetic exchange between attenuated and very virulent strains of IBDV and continuous monitoring of the spread of the virus in chicken.**

Infectious bursal disease virus (IBDV) belongs to the family *Birnaviridae* and the genus *Avibirnavirus*, which is the causative agent of a highly contagious immunosuppressive disease in young chickens (7). The genome of IBDV is a bisegmented double-stranded RNA consisting of 2 segments (A and B); segment A encodes VP5 and polyprotein (VP2-VP4-VP3), while segment B encodes VP1 protein (5). The coinfection with very virulent IBDV (vvIBDV) and attenuated IBDV strains led to exchange of the double-stranded genomic RNA segments to generate new reassortant viruses (2, 3, 11).

The GX-NN-L IBDV was isolated from 21-day-old commercial broiler flocks vaccinated with a classical IBDV vaccine in Guangxi province, China, in 2011. The GX-NN-L IBDV caused immunosuppression, low weight gain, bursal atrophy, about 18.6% mortality, and 75% morbidity in clinic. The whole genome of the GX-NN-L isolate was amplified and cloned into the pMD19-T vector (TaKaRa Bio Inc., Japan), followed by sequencing three times using an ABI 3730 Sanger-based genetic analyzer (Carlsbad, CA). The DNA sequences were assembled using DNASTar (version 7). Multiple-sequence alignment was performed with Clustal X (BioEdit version 7). A phylogenetic tree was constructed for genome sequences using the MEGA 5.1 program (10).

Comparative analyses showed that the nucleotide sequence identities of segment A between the GX-NN-L IBDV and other known vvIBDV isolates from GenBank ranged from 96.8 to 97.6% and that segment A was highly homologous to that of the YS07 strain (99.1%). Segment B of GX-NN-L shared homology ranging from 88% to 89% with the other known vvIBDV isolates and shared only 88.3% homology with YS07. The phylogenetic analyses indicated that segment A of GX-NN-L formed a cluster with all vvIBDV strains while segment B of GX-NN-L formed a cluster with attenuated and classical virulent strains. The results further suggested that segment A of GX-NN-L was derived from a vvIBDV strain and segment B was derived from an attenuated strain (12). Alignment analysis results indicated that VP5 of GX-NN-L had a four-amino-acid (MLSL) deletion in the N-terminal extension which met vvIBDV characteristics (11). There were four unique nucleotide substitutions (E18K, A51T, T135I, and W137R) in VP5 of GX-NN-L compared to that of other vvIBDV isolates, differences which might be responsible for the variability

of the GX-NN-L isolate in virulence and adaptability (1, 8). A single amino acid mutation (D212N) occurred in the first large hydrophilic region of VP2 of GX-NN-L IBDV, a change which might result in the antigenic change of GX-NN-L IBDV (4, 9). The VP1 protein of vvIBDV strains contains 15 characteristic amino acid residues (4V, 13K, 61I, 145T, 146D, 147N, 242E, 287A, 390M, 393D, 508K, 511S, 562P, 687P, and 695R) (8). In contrast, the GX-NN-L strain differs in seven characteristic amino acid residues (D146E, N147G, E242D, M390L, D393E, P562S, and R695K), changes which make it identical to most of the classical, variant, and attenuated IBDV strains. These data are useful for analyses of epidemiology and evolutionary characteristics of IBDV in China.

**Nucleotide sequence accession numbers.** The complete genome sequences for segments A and B of GX-NN-L are available from GenBank under accession numbers [JX134483](#) and [JX134484](#), respectively.

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Address correspondence to Qingmei Xie, [qmx@scau.edu.cn](mailto:qmx@scau.edu.cn).

F.C. and J.L. contributed equally to this study.

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