Virus Genes (2010) 40:421–422
DOI 10.1007/s11262-010-0461-z

Genomic characterization of a proventriculitis-associated infectious bronchitis coronavirus

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Received: 14 March 2009 / Accepted: 11 February 2010 / Published online: 27 February 2010

Abstract Transmissible proventriculitis associated with infectious bronchitis virus (IBV) was at first seen in eastern China in mid-1995, and is now endemic in China. Herein, the complete genome sequence of a proventiculitis-associated infectious bronchitis coronavirus (ZJ971) was sequenced and analyzed. Compared with the genome of the vaccine strain H120, ZJ971 had 54 nucleotide substitutions and a deletion in the 3′-UTR. The substitutions were in the regions of nsp2–nsp5, nsp7, nsp12, nsp13, nsp15, S and N genes, and the untranslating region. The results indicated that ZJ971 could be a variant of IBV strain H120.

Keywords Infectious bronchitis virus · Proventriculitis · Complete genome

Proventriculitis has been seen in chickens throughout the world since the early 1970s [1]. However, the causative agent has not yet been definitively established. Both infectious and dietary agents have been implicated as contributing causes [1]. Some studies have reported infectious bronchitis virus (IBV) to be a candidate etiological agent [2, 3]. In mid-1995, there was an outbreak of disease in the eastern China characterized by a swollen proventriculus and since then this disease has become endemic in China. Several proventriculitis-associated IBV strains have been isolated and analyzed [4–7], but the full genomes of these viruses have not yet been determined. Here, we report the complete genome sequence of the proventriculitis-associated IBV isolate ZJ971 [4, 8].

The entire genome of ZJ971 is 27,627 nucleotides (nt) in length (GenBank accession No. EU714028), with an A + T content of 61.8%, excluding the polyA tail, and has the same genomic organization as the classical IBV strain Beaudette [9]. Its 5′-UTR is 528 (1–528) nt in length. Gene 1 (polymerase gene) consists of 19,836 nt, encompassing ORF 1a and ORF 1b. ORF 1a is 11,802 nt in length (529–12,330) and encodes a polyprotein of 3933 amino acids (aa; polyprotein 1a, pp1a), while ORF 1b is 7,959 nt in length (12,405–20,363), and encodes a protein of 2652 aa (pp1b). Gene 2 (spike, S) of ZJ971 is 3,489 nt in length (20,314–23,802), and encodes a protein of 1162 aa. There is a 50 nt overlap between the 3′-end of ORF 1b and the 5′-end of the S gene. Gene 3 is 678 nt in length, and contains three ORF: 3a (23,802–23,975), 3b (23,975–24,169), and the envelope (E) protein (3c, 24,150–24,479). The length of gene 4 (membrane protein, M) is 678 nt (24,451–25,128), with a 29 nt overlap between the 3′-end of the E gene and the 5′-end of the M gene. There is a non-coding region of 359 nt between the 3′-end of the M gene and gene 5. Gene 5 contains ORF 5a (25,488–25,685) and 5b (25,682–25,930). Gene 6 (25,873–27,102) encodes the N protein (409 aa). There is a 58 nt overlap between the 3′-end of 5b and the 5′-end of the nucleocapsid (N) gene. The 3′-UTR of ZJ971 is 525 nt long, 205 nt and 20 nt longer than those of strains M41 (AY851295) and Beaudette (NC_001451), respectively.

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The complete genomic sequence of ZJ971 is very similar to that of the vaccine strain H120 (FJ888351), which is widely used in China, with 99.8% sequence identity, and only 54 nucleotide substitutions and a deletion of three bases in the 3′-UTR detected in ZJ971. The 54 nucleotide substitutions were distributed throughout the genome, two in 5′-UTR, 35 in gene 1, 14 in the S gene, two in the N gene, and one in the 3′-UTR. The 35 nucleotide substitutions in gene 1 resulted in 19 amino acids changes, with six in non-structural protein (nsp) 2 (198T → A, 322G → D, 331S → P, 358A → T, 475A → V, and 501 V → I), three in nsp3 (1126 N → D, 1489 N → D, and 1910R → K), three in nsp4 (2348I → M, 2498 N → T, and 2562R → K), two in nsp5 (2772G → V and 2879A → T), two in nsp7 (3368E → K and 3418I → V), one in nsp12 (4488P → Q), one in nsp13 (4919P → S), and one in nsp15 (6129I → M). The 14 nucleotide substitutions in S gene resulted in nine aa substitutions, including seven in the S1 subunit (19A → V, 52 I → V, 118G → V, 122I → L, 130S → F, 355E → Q, and 440T → I) and two in the S2 subunit (1047S → F and 1136R → K). The two nucleotide substituions in the N gene resulted in one amino acid change (22S → P). There are eight epitope clusters within the S glycoprotein of IBV strain D207, located between amino acid residues 24–61 (S1D), 132–149 (S1E), 291–398 (S1C/A/B), 497–543 (S1F), 548-574(S2G), and 1095-1118(S2H) [10, 11]. Three of the nine aa difference between the S proteins of H120 and ZJ971 were located in these epitope clusters (52 I → V, 130S → F, and 355E → Q).

These findings suggest ZJ971 could be a variant of IBV strain H120, with mutations distributed throughout the genome.

Acknowledgments This study was supported by grants from the Ministry of Science and Technology of the People's Republic of China (Grant No. 2006BAD06A03), from the earmarked fund for Modern Agro-industry Technology Research System (Grant No. nycytx-41), and from the National Science Foundation of China (Grant No. 30625030).

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