

Identification of the avian infectious bronchitis coronaviruses with mutations in gene 3

Shengwang Liu¹, Qingxia Zhang¹, Jianfei Chen, Zongxi Han, Yuhao Shao, Xiangang Kong*, Guangzhi Tong

Division of Avian Infectious Diseases, National Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute, The Chinese Academy of Agricultural Sciences, Harbin 150001, People's Republic of China

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Abstract

The sequence of a 6.0-kb fragment was compared in the 3'-encoding region of the genome in 27 infectious bronchitis virus (IBV) strains. All these strains have the same S-3-M-5-N gene order, as is the case for other IBVs. However, the sizes of the corresponding open reading frames (ORFs) of some genes varied among the virus strains. Phylogenetic analysis and sequence alignments demonstrated that recombination events had occurred in the origin and evolution of the strains CK/CH/LSD/03I and CK/CH/LLN/98I and the possible recombinant junction sites might be located at the 3c and M genes, respectively. The normal product of ORF 3a is 57 amino acids long, whereas a 43-bp deletion at the 3'-end of the CK/CH/LSD/03I 3a gene was detected, resulting in a frameshift event and C-terminally truncated protein with 47 amino acids. Comparison of the growth ability in embryos and replication and pathogenicity in chickens with IBV carrying the normal 3a gene indicated that this deleted sequence in the 3a gene of CK/CH/LSD/03I was not necessary for viral pathogenesis and replication either *in vitro* or *in vivo*. Occurrence of a mutation at the corresponding position of the CK/CH/LLN/98I start codon in the 3a gene led to the absence of ORF 3a in this virus, resulting in a novel genomic organization at the 3'-encoding regions: S-3b, 3c-M-5a, 5b-N. Comparison with other viruses carrying the normal 3a gene revealed that CK/CH/LLN/98I had replication and pathogenicity abilities *in vivo* similar to those of other IBVs; however, its growth ability in embryos was lower, although the relationship between the lower growth ability and the ORF 3a defect requires further confirmation.

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Keywords: Infectious bronchitis coronavirus; Gene order; Mutation; Replication; Pathogenicity

1. Introduction

Coronaviruses belong to the family *Coronaviridae*, a member of the order *Nidovirales*, and are classified into 3 groups based on the lack of genetic and antigenic relationships

Abbreviations: IB, infectious bronchitis; IBV, infectious bronchitis virus; ORF, open reading frame; SARS-CoV, severe acute respiratory syndrome-associated coronavirus; ns, nonstructural; S, spike; M, membrane; E, envelop; N, nucleocapsid; RT, Reverse transcription; PCR, polymerase chain reaction; EID₅₀, 50%; (median) embryo infectious doses; TRS, transcription regulatory sequence; TOC, tracheal organ cultures.

* Corresponding author. Tel.: +86 451 85935000; fax: +86 451 82733132.

E-mail address: xgkong@hvri.ac.cn (X. Kong).

¹ These two authors contributed equally to the work.

between the species of different groups (González et al., 2003; Masters, 2006). They have been known to cause upper and lower respiratory diseases, gastroenteritis, and central nervous system infection in a number of avian and mammalian hosts, including humans (Weiss and Navas-Martin, 2005). The etiological importance of coronaviruses has received much attention since the discovery of the newly emerged severe acute respiratory syndrome-associated coronavirus (SARS-CoV) in 2003. In particular, how coronaviruses break the host species barrier, cause interspecies infection, and become zoonotic are questions of interest to the public. The infectious bronchitis virus (IBV), an avian coronavirus, together with the genetically closely related turkey coronavirus (Cavanagh et al., 2003; Guy, 2000), pheasant coronavirus (Cavanagh et al., 2002), and

viruses recently detected in 3 species of wild birds (Cavanagh et al., 2001), forms group 3 coronaviruses. IBV primarily causes respiratory disease in domestic fowl, although it also replicates at many epithelial surfaces of the alimentary tract, oviduct, and kidney (Cavanagh, 2003), and it is one of the most economically important pathogens in the poultry industry (Cavanagh et al., 2003).

Coronaviruses have the largest RNA viral genomes ranging from 26 to 32 kilobases (kb) in length (Masters, 2006). Genetic diversity among coronaviruses is generated by point mutations, insertions, and deletions introduced into the viral genome by viral RNA-dependent RNA polymerase, which lacks proofreading capabilities, and by genetic recombination, which occurs by a genomic template-switching mechanism (Masters, 2006). Two-

thirds of the viral genome encodes replicase activity and the remaining one-third, structural proteins and small nonstructural (ns) accessory proteins (Masters, 2006). IBV has 4 essential structural proteins, the 3 membrane proteins being the spike (S), integral membrane (M), and small envelope (E) proteins, and a phosphorylated nucleocapsid (N) protein. Although the S1 subunit of the S protein carries virus-neutralizing and serotype-specific determinants, the S2 subunit may also induce neutralizing antibodies, and the IBV serotypes can be grouped based on the S2 gene sequence (Bosch et al., 2003). The N gene and N-terminus of the IBV M protein also vary between strains (Cavanagh, 2007). Furthermore, mutations and recombination events have been observed in multiple structural genes of IBV recovered from naturally occurring infections.

pF/CH/LKQ3/03	ATGTGGTAA <u>CTGAACAATA</u> CAGACCTAAA AAGTCTGTTT <u>AA</u> <u>ATG</u> ATCCAA AGTCCCACGT
CK/CH/LJL/04I	ATGTGGTAA <u>CTGAACAATA</u> CAGACCTAAG AAGTCTGTTT <u>AA</u> <u>ATG</u> ATTCAG AATCCAACAT
CK/CH/LSD/03I	ATGTGGTAA <u>CTGAACAATA</u> CAGACCTAAA AAGTCTGTTT <u>AA</u> <u>ATG</u> ATTCAG AAGCCAACAT
CK/CH/LLN/98I	ATGTGGTAA <u>CTGAACAATA</u> CAGACCTAAA AAGTCTGTTT <u>AA</u> TTGTTCAA ACTCCCGTAT
pF/CH/LKQ3/03	CCTTCTTAAT AGTATTAATT TTGCTTTGGT GTAAACTTGT ACTAAGTTGT TTTAGAGAGT
CK/CH/LJL/04I	CTTTTCTAAT AGTGTTAATT CTTCCTTGGT TTAAACTTGT GCTAAGTTGT TTTAGAGAGT
CK/CH/LSD/03I	CTTTTCTAAT AGTGTTAATT CTTCCTTGGT TTAAACTTGT GCTAAGTTGT TTTAGAGAGT
CK/CH/LLN/98I	CTTTTGTAAT ACTATTAATT TTTCCTTGGT TTAAACTTGC ATTAAGTTGT TTCAGTGAGT
pF/CH/LKQ3/03	TTATTATAGC GCTCCAACAA CTAATACAAG TTTTACTCCA AATTATCAAT AGTAACTTAC
CK/CH/LJL/04I	GTGTGTTAGC ACTCCTACAA CTAATACAAG TTCTACACCA AATTATTAAT AGTAACTTAC
CK/CH/LSD/03I	GTGTGTTAGC ACTCCTACAA CTAATACGAG TTCTACTCCA AATTATTAAT A-----
CK/CH/LLN/98I	GCATTGTAGC ACTTCAACAG CTAATACAAG TTCTACTCCA AATTATTAAT AATAATTTAC
pF/CH/LKQ3/03	AGCCTAGACT GACCCTTTGT CACAGCTAG AC <u>TAATG</u> TTA AACTTAGAAG CAATTATTGA
CK/CH/LJL/04I	AGTCTAGGCT GTCCTTTGG CACAGCCTAG AC <u>TAATG</u> TTA GATTTTGAGA AAATAATTGC
CK/CH/LSD/03I	----- -ATGTTA GATTTTGA)GA AAACAATTGA
CK/CH/LLN/98I	AATCTAGGCT GTCCTTTGG CACAGCCTAG AC <u>TAATG</u> TTA GATTTTGC)GA AAATAATTGA
pF/CH/LKQ3/03	AACTGGTGAG CAAGT-GATT CAAAAATCA GTTTC AATTT ACAGCATATT TCAAGTGAT
CK/CH/LJL/04I	AACTGGTGAA GTAGT-AGTA CAACAAATCA GTTTC AATTT ACAACATATT TCAAGTGTTT
CK/CH/LSD/03I	AACAGGTGAA GTAGT-AGTA CAACAAATCA GTTTC AATTT ACAACATATT TCAAGTGTTT
CK/CH/LLN/98I	AACAGGTGGA ACAGTTAGTA CAACAAATCA GTTTC AATTT ACAACATATT TCAAGTGTTT
pF/CH/LKQ3/03	TAAACACAGA AGTATTTGAC CCCTTTGACT ATTGTTATTA CAGAGGAGGT AATTTTGGG
CK/CH/LJL/04I	TAGAAACACA AATTTTGGAC CCATTTGAGT GCTGCTATTA TTCAGTGGT AGTTTTATG
CK/CH/LSD/03I	TAGAAACACA AGTTTTGGAC CCATTTGAGT GTTGTACTA TTCAGTGGT AGTTTTATG
CK/CH/LLN/98I	TAGAAACACA GATTTTGGAC CCATTTGAGT GTTGCTATTA TTCAGTGGT AGTTTTATG
pF/CH/LKQ3/03	AAATAGAGTC AGCTGAAGAT TGTT CAGGTG <u>ATG</u> ATGAATT TATTGAA <u>TAA</u> GTCGCTAGAG
CK/CH/LJL/04I	AAATAGAGTC AGCTGACGAT TGTT CAGGTG <u>ATGA</u> ---ATC TTAT <u>TAA</u> TAA ATCCTAGAA
CK/CH/LSD/03I	AAATAGAGTC AGCTGACGAT TTTTCAGATG <u>ATGA</u> ---GTT TACTGAA <u>TAA</u> ATCCTAGAA
CK/CH/LLN/98I	AAATAGAGTC AGCTGACGAT TGTT CAGGTG <u>ATGA</u> ---ATC TTAT <u>TAA</u> TAA GTCGCTAGAG

Fig. 1. Sequence alignment of ORFs 3a and 3b of 4 IBV strains. Deletion in gene 3 of IBV CK/CH/LSD/03I results in a 3'-end truncated 3a gene and the mutation in gene 3 of CK/CH/LLN/98I results in the absence of ORF 3a in this virus. Sequences of the pF/CH/LKQ3/03 and CK/CH/LJL/04I strains as representatives of the normal 3a gene were compared. The putative transcription regulatory sequence (TRS), CTGAACAA, of gene 3 is indicated in gray. The ATGs with a single underline in boldface are the start codons of genes 3a, 3b, and 3c. The single mutation at the first site of the start codon of gene 3 in the CK/CH/LLN/98I strain (ATG→ATT) results in the absence of ORF 3a in this virus. The TAAs/TGAs in boxes are the termination codons of genes S, 3a, and 3b, respectively. A 43-bp nucleotide sequence is deleted in the CK/CH/LSD/03I strain (represented as -).

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