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Emergence of a genotype I variant of avian infectious bronchitis virus from Northern part of India

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ABSTRACT

Infectious bronchitis virus (IBV) is one of the foremost causes of a persistent economic burden to poultry industries worldwide. IBV belongs to the genus *Gammacoronavirus* within the family *Coronaviridae*. The IBV infection leads to respiratory and nephrogenic symptoms in broiler chickens. In addition, its infection leads to reduced fertility and hatchability in layer birds. We determined the first complete genome sequence of a variant IBV from an outbreak in Haryana state of the Northern part of India using next generation sequencing. On phylogenetic analysis of the IBV isolate, it clustered with genotype I lineage 1 (GI-1). The deduced amino acid sequence of S gene of IBV isolates showed a high level of identity with strains isolated from Tamil Nadu and the reference vaccine strains. Our result suggests that the IBV virus isolated from unvaccinated chicken flocks in North India might be a revertant strain originally evolved from the live attenuated vaccine strains used in the region. Determination of the complete genome sequence of additional IBV isolates from India is necessary to understand the epidemiology of IBV in India.

Infectious bronchitis (IB) is a highly contagious disease of poultry and is the reason for a constant monetary load with commercial poultry enterprises worldwide. It is caused by infectious bronchitis virus (IBV), which belongs to the genus *Gammacoronavirus* within the family *Coronaviridae*, order *Nidovirales* (Bande et al., 2017; Cavanagh and Naqi, 2003). The natural host of IBV is the domestic fowls of *Gallus gallus* species, however, IBV-like avian coronaviruses are also reported from quail, penguin, partridge, geese, pigeon, guinea fowl, teal, duck and peafowl (Bande et al., 2017; Cavanagh, 2005b, 2007). IBV can be classified into two major pathotypes based on its tissue tropism viz. respiratory and nephropathic (Bande et al., 2017). The respiratory pathotype leads to respiratory symptoms, which varies in severity depending on the strains of the virus (Cavanagh and Naqi, 2003). The virus leads to deciliation of respiratory epithelium followed by secondary bacterial infection (Cavanagh, 2005a; Matthijs et al., 2003). Apart from respiratory symptoms, the virus can also replicate in kidney, gonads and bursa of Fabricius leading to the emergence of nephropathic strains of IBV (Liu and Kong, 2004). The virus drastically affects poultry industry by causing retardation in sexual maturity, irreparable damage to reproductive organs, reduced egg quality, fertility and hatchability leading to “false layers syndrome” and high mortality (Cavanagh and Naqi, 2003; de Wit et al., 2011). IBV is an enveloped, pleomorphic virus with a mean diameter of 120 nm enclosing approximately 27.6 kb single-stranded RNA genome with a positive polarity (Cavanagh, 2005b). The genome codes for both structural (spike protein [S], envelope protein [E], membrane glycoprotein [M] and nucleoprotein [N]) and nonstructural (product of gene1, 3 and 5) proteins (Lai and Cavanagh, 1997; Spaan et al., 1988; Sutou et al., 1988). The S protein, located on the surface of the viral membrane is involved in viral attachment with the host cell receptor and fusion of the virion with the cell membrane (Cavanagh, 2007; Cavanagh and Davis, 1986; Koch et al., 1990; Niesters et al., 1987). Besides this, the S protein is also supposed to play a major role in inducing virus neutralizing antibody and protection against IBV infection (Bochkov et al., 2007; Cavanagh et al., 1988; Johnson et al., 2003; Winter et al., 2008). The S protein is post-translationally cleaved into the amino-terminal S1 and the carboxyl-terminal S2 subunits at a multi-basic cleavage site (Cavanagh and Davis, 1986; Cavanagh et al., 1986; Valastro et al., 2016). The S1 protein is highly variable and nucleotide heterogeneity is mostly contained within three different hypervariable regions (HVRs) (aa 38–67, 91–141 and 274–387) of the S1 protein (Cavanagh et al., 1988; Moore et al., 1997). One of the most recent and comprehensive classification followed S1 gene-based phylogeny of infectious bronchitis virus and identified six main genotypes (I–VI), 32 lineages and a number of inter-

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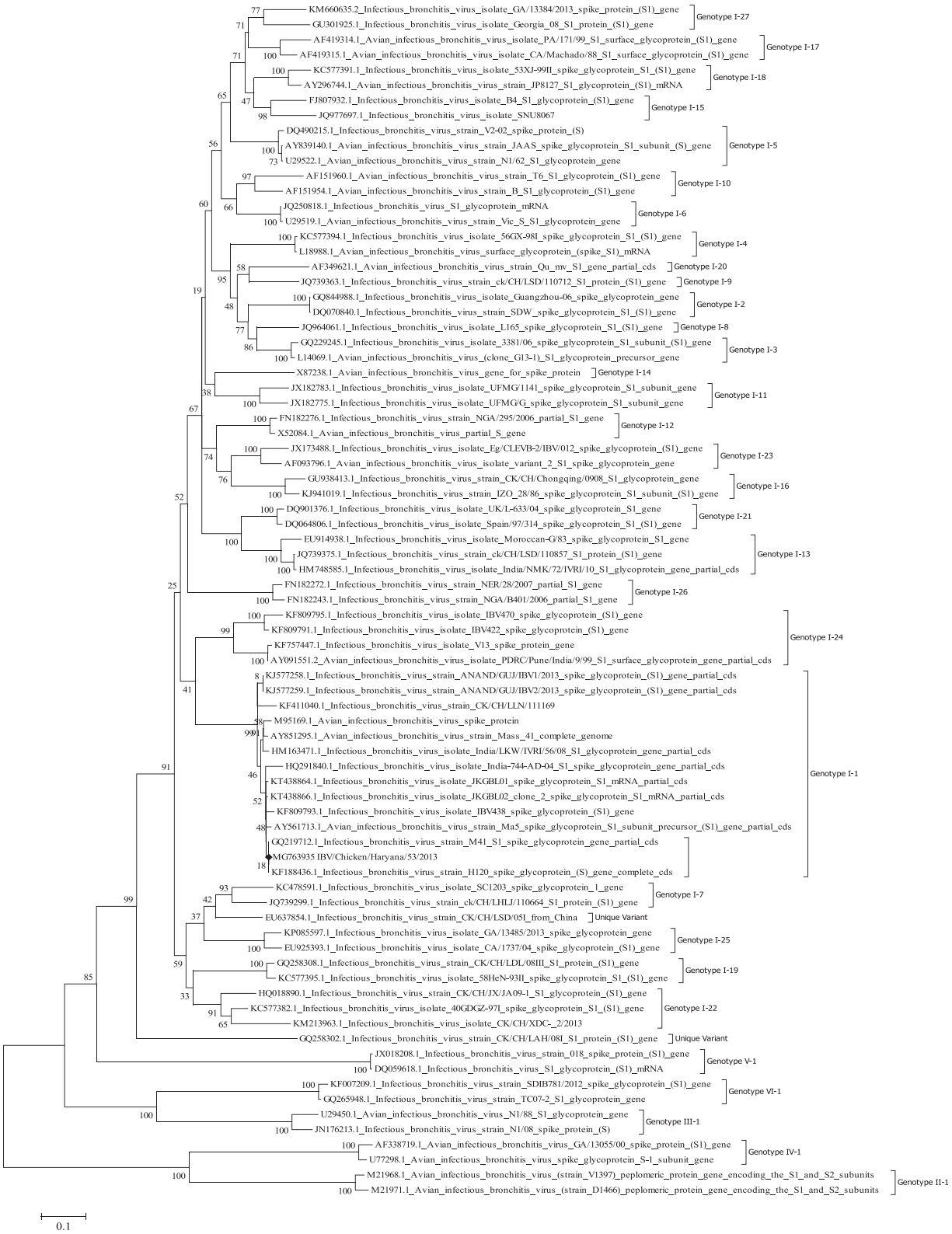

Fig. 1. Molecular Phylogenetic analysis of complete S1 nucleotide sequence of IBV isolate from North India by Maximum Likelihood method. The analysis involved 79 nucleotide sequences. The evolutionary history was inferred by using the Maximum Likelihood method based on the General Time Reversible model. The tree with the highest log likelihood (−41992.7846) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree (s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.9051)). The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 11.7116% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Codon positions included were 1st + 2nd + 3rd + Noncoding. There were a total of 2033 positions in the final dataset. Evolutionary analyses were conducted in MEGA6.

lineage recombinants in global strains of IBV (Valastro et al., 2016). In case of IBV, vaccination is partially successful due to continual emergence of antigenic variants and requires the application of genotype-matched vaccines for effective protection (Bijlenga et al., 2004). IBV strains within a geographic region are unique and distinct in different parts of the world. The impact of IB infection is higher in developing countries like India and other Asian subcontinents (Bayry et al., 2005). In India, IBV isolates have been reported to be circulating in Maharashtra, Uttar Pradesh, Tamil Nadu, Andhra Pradesh, Orissa and Assam (Patel et al., 2015; Sumi et al., 2012). More recently, IBV have been reported from broiler chickens in Kashmir and nephropathic strains were isolated from chickens in Anand, Gujarat (Bayry et al., 2005; Parveen et al., 2018; Patel et al., 2015; Sumi et al., 2012). IBV strains from India mostly belong to genotype-I, lineages 1 and 24 and serotype Massachusetts (Valastro et al., 2016). However, very little is known about the complete genome sequences of IBV isolated from different parts of India.

This study deals with the analysis of complete genome sequence of a variant IBV isolated from an outbreak in a poultry farm in Haryana state, India. The infected birds showed clinical signs of respiratory distress and oedema of the head. Postmortem examination revealed necrosis in the caecal tonsils with varying degrees of tracheitis. The virus isolated from infected tissue samples (kidney and trachea) was propagated more than three passages in the allantoic cavity of 9-day-old specific pathogen free embryonated chicken eggs. The allantoic fluid was collected 48 h post-inoculation and RNA was extracted using TRIZOL reagent following manufactures instruction (Sigma, USA). Viruses were identified as IBV by observing dead embryos (curling and dwarfing) and further by doing RT-PCR for the N gene. Furthermore, cDNA library was prepared by the standard procedure using RNA-Seq Kit v2 (Invitrogen, USA). Each cDNA library was subjected to emulsion PCR and DNA-positive beads were recovered. The recovered positive beads were enriched and subjected to sequencing using a 316 chip on an Ion Torrent PGM sequencer (Life Technologies) following the standard protocol. The raw sequencing output was first mapped with the genome of chicken in order to remove the contaminating sequences. The remaining sequences were further filtered using PRINSEQ with a minimum read length of 45 bases and a mean base quality of 25 for the entire read (Schmieder and Edwards, 2011). Finally, the sequences were assembled by GS denovo assembler (Roche Diagnostics) and MIRA 4 (http://sourceforge.net/projects/mira-assembler/) using the default parameters for ion torrent platform. The largest contig assembled by both the assembler was mapped using BWA aligner (Li and Durbin, 2010). The complete genome sequence of the IBV was submitted to GenBank and its phylogenetic analysis was performed using MEGA6 software (Nei and Kumar, 2000; Tamura et al., 2013). A total of 79 different S gene sequences of prototype IBV strains belonging to several countries around the globe were taken for the analysis.

The complete genome sequence of IBV isolate IBV/Chicken/Haryana/53/2013 was submitted to GenBank (Accession number MG763935). The complete genome of IBV isolate Haryana consisted of 27,631 nucleotides (nt), and the genome consisted of six genes and ten open reading frames. The order was as follows: 5′ untranslated region (5′UTR)-1a-1b-spike-3a-3b-envelope (3c/sM)-membrane-5a-5b-nucleocapsid-3′UTR. The molecular signatures of the S gene were analyzed for the IBV strain Haryana. The spike (S) protein gene of IBV isolate Haryana was 3489 nucleotides long (1163 amino acids [aa]) with a cleavage site of ^{533}RRFRR^{537}. The S1 glycoprotein of IBV isolate Haryana contains the amino acids Aspartic acid (D), Histidine (H), Serine (S) and Isoleucine (I) at the critical positions 38, 43, 66 and 69 which contribute to the binding of the spike. On phylogenetic analysis, the IBV isolate Haryana clustered with prototype sequence belonging to Beaudette (accession number M95169) and the reference Mass 41 (AY851295) representing genotype I lineage 1 (GI-1) (Fig. 1). Among the IBV strains reported from India and the reference vaccine strains Ma5, H120, and Mass41, the nucleotide sequence of S gene of IBV strain Haryana showed 97.5–99.8% identity with the reference vaccine strains and the highest identity of 99.8% with a strain reported from Andhra Pradesh in 2009. The amino acid sequence of the S gene showed maximum identity with the vaccine strain H120 and the strain reported from Andhra Pradesh in 2009 while minimum identity was observed with PDRC/Pune/India/9/99 S1strain.

This is the first report of the complete genome sequence of a field strain of IBV from the Northern part of India. The whole genome sequencing was performed using ion torrent PGM. *Coronaviruses* have one of the longest RNA genomes and hence, traditional sequencing methodology using overlapping PCR fragments followed by Sanger sequencing can be quite tedious and inefficient (Abro et al., 2011; Mondal and Cardona, 2007). Here, the next generation sequencing approach has significantly increased the efficiency of the whole sequencing process. In the S1 glycoprotein of IBV isolate Haryana, the amino acids critical for the binding of the spike, D38, H43, S66, and I69 are similar to the S1 glycoprotein of H120 vaccine strain (Promkuntod et al., 2013). The IBV isolate Haryana belongs to GI-1 genotype. GI-1 is the most widely distributed genotype all over the world since the most extensively used vaccine strains are homologous to the GI-1 lineage (Parveen et al., 2018).As the IBV vaccines used in India are live-attenuated in nature, there is a high chance of pathogenic reversion of the vaccine strains (Kamble et al., 2014). Although our strain was isolated from a non-vaccinated broiler flock, it shows a high percentage of nucleotide and amino acid identity with the reference vaccine strains of GI-1. Presumably, it might be a revertant strain originally evolved from the live attenuated vaccine strains used in the region. It also shows a high percentage of identity 99.4–99.8% with IBV strains reported from Andhra Pradesh and Kashmir suggesting its dissemination across the region by the trade of domestic birds or wild migratory birds (Parveen et al., 2018; Sumi et al., 2012). It will be interesting to sequence the other Indian IBV isolates to analyze the level of divergence among the isolates.

Conflict of interest

The authors declare no conflict of interest.

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