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Phylogenetic analysis and full-length characterization of S1 gene of IS-1494 (Variant 2) like infectious bronchitis virus isolates, Iran, 2015

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ABSTRACT

Infectious bronchitis virus (IBV), a major pathogen of commercial poultry flocks, circulates in the form of different genotypes. Three IB viruses were isolated from broiler chickens showing respiratory and renal lesions. The isolates were characterized by reverse transcriptase polymerase chain reaction and sequence analysis of the Full-length of the S1. Three isolates were belonged to Variant 2 like (IS/1494 like) IBV genotype. Phylogenetic analysis showed that Variant 2 like isolates formed two clusters and the Iranian and Iraqi isolates were included in the cluster II. Cluster I composed of Israeli, Egyptian and Turkish Variant 2 like IBV Isolates. Three hyper variable regions (HVR) of S1 were determined. The Most variation was seen in HVR2. The findings emphasize the importance of continuous monitoring of IBV, in addition to adjust diagnostic methods, molecular epidemiological studies, development and use of vaccines which are adapted to the changing disease scenario.

1. Introduction

Infectious bronchitis (IB), caused by infectious bronchitis virus (IBV) is one of the most economically important viral diseases of poultry and can result in respiratory disease, drops in egg production and hatchability, nephritis and enteric problems. The IBV belongs to the genus *Gammacoronavirus* within the *Coronaviridae* family. IBV positive-sense single-stranded RNA genome (27.6 kb) encodes four structural proteins: the spike glycoprotein, the membrane glycoprotein, the envelope protein, and the phosphorylated nucleocapsid protein. The spike (S) proteins are the major structure proteins of IBV proteins, which are responsible for the induction of neutralizing and serotype-specific antibodies. Diversity in S1 probably results from mutation, insertions, deletions, or RNA recombination of the S1 genes. Moreover, three hypervariable regions (HVRs) comprising amino acid residues 38 to 51, 99 to 115 and 274 to 387, respectively, have been located within the S1 subunit and have been associated with haemagglutination-inhibiting and virus-neutralizing epitopes. Numerous genotypes have been detected, some of which have quickly disappeared while others have caused major worldwide disease with economic relevance. Genotyping of IBV strains isolated in Iran showed seven distinct phylogenetic groups (Mass, 793/B like, IS/1494 like, IS/720-like, QX-like, IR-1, and IR-2) based

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on analysis of mainly HVRs of the S1 gene (Najafi et al., 2014–2015; Hosseini et al., 2015). In spite of the use of different vaccines (Mass & 793/B type) in poultry farms in Iran, outbreaks have been occurred with high mortality. The presence of Variant 2 viruses (IS/1494/06 like) in Iranian commercial flocks have been demonstrated (Hosseini et al., 2015). The isolation of IS/1494/06, one of the Israeli Variant 2 isolates, and information on its S1 gene sequence in GenBank (Accession number: EU780077) has first been reported by Meier & Maher from Israel (Meir et al., 2004). The IS/1494/06 is still a major IBV variant involved in Israel chickens' farm and in Jordan, Egypt, Turkey and other countries in the Middle East (Hosseini et al., 2015; Kahya et al., 2013; Hussein et al., 2014). IS/1494/06 is known to be nephropathogenic and it also affects the respiratory system (Susan et al., 2010).The aim of this study was to determine the complete nucleotide sequences and phylogenetic analysis of spike gene of Iranian IS-1494 (Variant 2) like IBVs.

2. Materials and methods

2.1. Sample collection & virus isolations

In this study, samples were collected from Iranian broiler chicken farms in 2015. The samples (Trachea and kidney) were taken from chickens showed IB suspected clinical signs (respiratory problems such as gasping, sneezing and bronchial rales, and nephritis lesions such as enlargement, and congestion in kidneys). The samples were collected aseptically and frozen at −70 °C. The details of positive samples are available in Table 1. Samples from each bird were homogenized, and a 10% (w/v) suspension was made in PBS. Subsequently, samples were centrifuged at 1500 ×*g* for 20 min at 4 °C. The supernatant content was used to inoculate fertile specific pathogen-free (SPF) eggs. Homogenized tissue samples supplemented with 10,000 IU penicillin, 10,000 IU streptomycin, and 250 IU amphotericin B ml^{-1} were used for this isolation. After a period of 1 h at room temperature, 200 μL aliquots of the homogenates were inoculated into the allantoic cavity of 9–11-day-old SPF embryonated chicken eggs. Five eggs were used for each sample. The inoculated eggs were incubated at 37 °C and candled daily to check for embryonic viability. After 2–3 days of incubation, the allantoic fluid was harvested and used for subsequent passages. In addition, 3 uninoculated SPF eggs considered as a control in each isolation process.

2.2. RNA extraction

RNA was extracted from samples using Cinna Pure RNA Extraction Kit (Sinaclone, Iran) according to manufacture instructions.

2.3. cDNA synthesis and PCR reaction for S1 amplification

For cDNA synthesis, 1 μL (0.2 μg) of random hexamer primer (SinaClon, Iran) was added to 5 μL of extracted RNA and the mixture was heated at 65 °C for 5 min. Fourteen microliters of cDNA master mix containing 7.25 μL DEPC-treated water (SinaClon, Iran), 2 μL dNTP mix (SinaClon, Iran), 0.25 μL RiboLock RNase Inhibitor (Thermo Fisher Scientific, USA), 0.5 μL Revert Aid Reverse Transcriptase (Thermo Fisher Scientific, USA), and 4 μL 5× RT Reaction *Buffer* was added to each tube, resulting in a final volume of 20 μL. Then, the mixture was incubated at 25 °C for 5 min, 42 °C for 60 min, 95 °C for 5 min, and 4 °C for 1 min, respectively. RT-PCR was carried out using primer sets New oligo 5–5′TGAAACTGAACAAAAGACA 3′- and New oligo 3-5′ CCATAAGTAACATAAGGRCRA 3′-targeting the S1 subunit of spike glycoprotein of IBV. The PCR condition for amplification was 94 °C for 5 min, 30 cycles of 94 °C for 40 s, 60 °C for 40 s, and 72 °C for 2 min, followed by 72 °C for 10 min. The products were analyzed on 1.0% agarose gel. The PCR products were cloned into pTZ57R/T vector (InsTA clone PCR Cloning Kit, Cat No: K1213) for later sequencing.

2.4. Phylogenetic analysis

Sequencing was performed with the universal primers (both directions) (Bioneer Co., Korea). Chromatograms were evaluated with CromasPro (CromasPro Version 1.5). Multiple amino acid alignments were performed on S1 genes of representative viruses using Clustal W (MEGA5) and CLC sequence viewer. The phylogenetic tree was constructed by using of the MEGA 5.1 software with neighbor-joining method and each tree was produced using a consensus of 1000 bootstrap replicates (Tamura et al., 2011).The nucleotide sequences of a full length of the S1 gene were compared with several S1 sequences from gene bank. The mentioned sequences of the IBV was submitted to the GenBank database with the following accession numbers: XXXXX-YYYY.

Table 1
The sample data of Iranian IBV–IS-1494 like strains used for S1 gene charechterization.

Strain name	Flock type	Year	Vaccines used in flock	Organ of isolation	Accession numbers
GKHWNF-16	Broiler	2015	H120, 4/91	Kidney	
GKHWNF-4	Broiler	2015	H120, 4/91	Trachea	
GKHWNF-5	Broiler	2015	Ma5, 4/91	Kidney	