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Short Communication

Genotyping of Infectious Bronchitis Viruses in the East of Iran, 2015

Ghalyanchi-Langeroudi A[1*], Karimi V[2], Jannat A[2], Hashemzadeh M[3], Fallah MH[3], Gholami F[2], Zabihi MT[2], Heidarzadeh M[4]

1. Department of Microbiology and Immunology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
2.Department of Avian Medicine, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
3.Department of Research and Production of Poultry Viral Vaccine, Razi Vaccine and Serum Research Institute, Karaj, Iran.
4.Iranian Veterinary Organization, Zahedan , Iran.

Abstract

Background and Aims: Infectious bronchitis virus (IBV) is the causative agent of avian infectious bronchitis, which is characterized by respiratory, reproductive, and renal signs. IBV is a highly variable virus with a large number of genotypes. S1 gene sequencing has been used for molecular epidemiological studies and genotypic characterization of IBV. To better understand the molecular epidemiology of IBV in Iran, we sequenced the S1 gene of IBV field isolates, a total of 40 tracheal and kidney tissue specimens from different commercial broiler flocks in the East of Iran were collected from 2015.

Materials and Methods: 15 IBV-positive samples were selected from among the total and were further characterized by sequencing the spike glycoprotein gene. The isolates were confirmed by real-time polymerase chain reaction (PCR) and characterized by sequencing the spike glycoprotein gene.

Results: Three genotypes were detected. The percentage of Variant 2 (IS/1494 like), 793/B, and QX genotypes was 66.7%, 26.7%, and 6.6% respectively. We reported the QX as the first time and Variant2 was the dominant genotype in this area.

Conclusion: It is an updated and comprehensive study of genotyping of IBV and completes IBV puzzle in the East of Iran.

Keywords: Avian Infectious Bronchitis, Iran, Genotyping, Spike.

Introduction

Avian infectious bronchitis virus (IBV), the causative agent of infectious bronchitis (IB), is a gammacoronavirus in the family Coronaviridae. IBV was first recognized as the avian respiratory pathogen in 1930 after that many IBV vaccines were introduced to tackle this problem (1 4/91(793/B) and other strains) (2).Coronaviruses have a single-stranded positive-senseRNA genome ranging in size from 27 to 30 kb, with a 50cap and a 30 poly-A tail. Transcription occurs through aleader-primed RNA synthesis mechanism that results forIBV in six 30 co-terminal sub genomic mRNA molecules. Four structural proteins—spike (S), envelope (E), membrane(M), and nucleocapsid (N)—along with the viral RNA

[*] **Corresponding author**: Arash Ghalyanchi-Langeroudi. Department of Microbiology and Immunology,Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
Email: arashghalyanchi@gmail.com
Tel: (+98) 912-8117714

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make up the enveloped virion (3). IBV readily undergoes mutation in chickens resulting in the emergence of new variant serotypes and genotypes. Mutations occur frequently in hyper variable regions (HVR) of the S1 subunit of the envelope spike (S) glycoprotein gene. New variant strains may differ as much as 55% in their S1 amino acid sequence compared with vaccine strains (4). The continuous emergence of variant strains of IBV has been reported, and at least 30 serotypes have been identified worldwide (5). In Iran, several serotypes and genotypes of IBV have been reported from different regions; the first isolation of IBV in Iranian chicken flocks was reported in 1994. Later, several Iranian researchers identified the 793/B serotype, which turned out to be one of the most predominant types of IBV circulating in the country (6). Genotyping of IBV strains isolated in Iran were classified into seven distinct phylogenetic groups (Mass, /B like, IS/1494 like, IS/720-like, QX-like, IR-1, and IR-2) based on analysis of mainly HVRs of the S1 glycoprotein gene (7, 8). The aim this study was determination prevalence rate of IBV in the respiratory complex in the East of Iran and genotyping of positive samples.

Methods

Study area, Sampling, and virus Isolation. In this study, tissue (tracheal and kidney) samples were collected from 40 broiler farms, selecting farms from two provinces in the East of Iran from two provinces: 1- Khorasan-e-Jonobi 2- Sistan-o-Balochestan (As shown in figure 1), 2015.

RNA Extraction. RNA was extracted using CinnaPure RNA (Sinaclon Co.) according to manufacturer's instructions. The purity of the extracted RNA was determined by taking the ratio of the readings at 260 and 280 nm.

cDNA synthesis. The extracted RNA was used in reverse transcription (RT) reaction to generate cDNA through cDNA synthesis kit (Thermo scientific). The cDNA was stored at -20°C until use.

Real- time PCR for IBV detection. Real – time PCR for IBV detection based on 5′ UTR was used in this study. The amplification was performed by using the amplification kit (Bioneer, South Korea), with the forward primer 5′GCTTTTGAGCCTAGCGTT3′, reverse primer 5′GCCATGTTGTCACTGTCTATTG3′ and Taqman® dual-labeled probe FAM-CACCACCAGAACCTGTCACCTC-BHQ1, as described by Callison et al (9).

Nested PCR and Sequencing for detection of genotype. A nested PCR assay was used for genotyping (10). Initial PCR was performed in 20 µl volumes in a mixture containing 2µl of distilled water, 2 µl of primers from SX1 (5′-CACCTAGAGGTTTGYTWGCATG-3′) and SX2 (5′-TCCACCTCTATAAACACCYTTAC-3′), 3 µl of cDNA, and 13 µl of Sinaclon 2x PCR master mix (Sinaclon, Iran). The amplification reaction was performed in an Eppendorf Master Cycler gradient thermocycler (Eppendorf, Hamburg, Germany) for an initial denaturation period at 94℃ for 2 min and 35 cycles with denaturation at 94℃ for 15 s, annealing at 58°C for 30s, and polymerization at 72℃ for 30s. Final extension was performed at 72℃ for 10 min. For nested PCR, a 1 µl aliquot of 1:100 dilution of the first amplicon was subjected to a second round of amplification using SX3 (5′-TAATACTGGYAATTTTTCAGATGG-3′) and SX4 (5′-AATACAGATTGCTTACAACCACC-3′) primers and the same cycling procedures. The reaction products were analyzed by electrophoresis in 1.5% agarose gels in Tris/Borate/EDTA (TBE) buffer, stained with GelRed™ (Biotium, USA) and visualized under UV light. The AccuPrep® PCR Purification Kit (Bioneer Co., Korea) was used for purification of the PCR products. Sequencing was performed with the primers (both directions) used in the PCR (Bioneer Co., Korea). We did nucleotide BLAST (NCBI) the results of edited obtained sequences for detection of IBV genotypes.

Results

Real-time PCR for IBV detection based on the 5 'UTR was used in this study. From the 40

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Fig. 1. The samples collection areas for genotyping of infectious bronchitis viruses from broiler farms in the East of Iran, 2015.

Table 1: Distribution of genotypes of Infectious Bronchitis Virus (IBV) in the East of Iran.

IBV Genotype	Detected Positive/Total Positive
Variant 2 (IS-1494 like)	10/15 (66.66%)
793/B	4/15 (26.7%)
QX	1/15 (6.6%)

samples during this study, 15 samples were positive for IBV with real-time PCR. The total rate of infection was 37.5% . To IBV genotyping, all 15 positive samples were sequenced based S1 partial. A total of three genotypes were detected. The total percentage of Variant 2 IS/1494, 793/B, and QX genotypes in five was 66.7%, 26.7%, and 6.6% respectively (Table 1).

Discussion

IB has occurred frequently in recent years in Iran. It is one of the major poultry pathogens and causes heavy economic losses. Currently, Ma5, H120, and attenuated 4/91 IBV-based vaccination strategies have been applied for IB control in poultry farms in Iran (11, 12). In our study, the prevalence rate was 37.5% and Variant-2 like being the dominant genotype in this region. In addition, we detected the QX-like IBV for the first time in the East of Iran.

.Variant 2 like IBV (IS/223/96), which was described by Callison et al. in 2001. Later other viruses, which are highly related to Variant 2 (IS/378/97, IS/572/98, IS/585/98, IS/589/98), were recovered from the respiratory or renal disease in Israel (13). The presences of variant 2 viruses (IS/1494/06 like) in Iranian commercial flocks have been demonstrated (8). Hosseini et al (2015) reported Variant-2 like with frequency 17.2% during their molecular surveillance of IBV genotypes involved in outbreaks between 2010 –2014 (14) . The Variant-2 like IBV is still a major IBV variant involved in Jordan, Egypt, Turkey, Israel and other countries in the Middle East (8, 15-17). In September 1997, an outbreak of the disease, characterized mainly by swelling of the stomach (proventriculitis), diarrhea and loss of body weight in 25- to 70-day-old chickens occurred in chicken flocks in Qingdao, China (18). However, it was not recognized as a novel type of IBV at that time. From 1999 to 2004, we investigated five chicken flocks in four provinces in China and found that the QX-like variant (taking the LX4 strain as representative) is a novel genotype of nephropathogenic (19).

The first isolation of IBV in Iranian chicken flocks was reported by Aghakhan *et al* (1994) (20) which was Massachusetts serotype. Later, several Iranian researchers identified the 793/B serotype (21). Vasfi *et al* (2001) did serotyping on some Iranian IBV isolates between 1997-2000.They found the strain may antigenically different from H120 Massachusetts strain and predict the presence of new IBV genotype (22). The 793/B serotype is regarded as one of the dominant genotypes of IBVs circulating in Iran (11). Akbari Azad et al (2004) worked on Iranian IBV isolated trough RFLP analysis. Base on RFLPs patterns and comparison with RFLP references patterns (793/B, D274, M41, H120), 8 of 12 strains showed the 793/B

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pattern and the rests (4 of 12) showed Mass pattern in RFLPs (23).Vasfi et al (2007) did the molecular analysis of three Iranian isolates belonged to 793/B serotype of IBVs. Three Iranian strains belonged to 793/B genotype with nucleotide differences of 5.64-6.07 % to UK/793/B as a prototype of 793/B and 26.02-26.16 % to H120 as a vaccinal strain (21). Shoushtari *et al* (2008), showed the phylogenetic study based on S1 gene revealed the presence of 793/B and Massachusetts strains in Iran (24). Recently, six distinct phylogenetic groups (IS/1494/06 [Var2] like, 4/91-like, IS /720-like, QX-like, IR-1 and Mass-like) based on phylogenetic analysis of S1 glycoprotein gene were described (25). Jahantigh *et al* (2013) were conducted to identify the IBV with group-specific primers in Zabol, Southeast of Iran. The results of this study showed that 36.36% of the sampled flocks were positive to IBV by RT-PCR. Moreover, the Mass type was the identified serotype of IBV (26).This job is the first research in this area of Iran. There is limited evidence in the literature describing the prevalence of poultry disease in Pakistan. One report describes the analysis of 900 serum samples from 23 flocks in Pakistan and reported an incidence of 3strains of IBV (M-41, Arkansas, Connecticut) in Pakistan (27). Ahmed *et al* did detection and seroprevalence of IBV strains in commercial poultry in Pakistan. Overall, 88% of the flocks were seropositive for M-41 antibodies, whereas40, 52, and 8% of the flocks were positive for D-274, D-1466, and 4-91 IBV strains, respectively (28). Molecular Identification of Agents Causing Respiratory Infections in Chickens from Southern Region of Pakistan from October 2007 to February 2008, revealed out of 50 samples, 20 samples were positive for NDV, 28 for AIV and 2 were negative for both (29). In addition, we do not have any data from Afghanistan. It would be necessary for doing IBV genotyping in the Afghanistan.

According to the border with Pakistan and Afghanistan, permanent monitoring is recommended to check the entry and circulating of new IBV genotypes. It is an updated and comprehensive study of genotyping of IBV and completes IBV puzzle in the East of Iran. The phylogenetic study shows that the detected genotypes have high homology with IBV strains, which are infected broiler, pullet, and layers in the region.

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