SHORT COMMUNICATION

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Detection and molecular characterization of infectious bronchitis virus from recent outbreaks in broiler flocks in Sulaimani Governorate

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Abstract

Infectious Bronchitis (IB) is one of the most important viral diseases of poultry. It causes major financial losses in the poultry industry. It is caused by the infectious bronchitis virus (IBV). This study investigated the genetic origin and diversity of IBV by analyzing the partial S1 sequence derived from local broiler farms in different regions of Sulaimani Governorate. Detection was performed using a newly designed primer for the partial S1 gene. The present was undertaken to understand the molecular relationship between the strains across the world compared with field virus isolates by phylogenetic tree and sequences-analysis. Phylogenetic tree demonstrated that field isolate made a novel group (sul-5/17-like strain), indicating new variants. Also, field virus sequences were closely matched to Iranian strain HQ123347/RFIBV6 (97.00%). Further sequences analysis exhibited that field isolate was not homologous but high divergences with reverence vaccines strains. These results indicated that differences in S1 protein of the locally circulated IBV could be a reason for currents vaccination failure.

Introduction

Infectious bronchitis (IB) is an acute and highly contagious respiratory disease of chicken.[1] The disease is caused by the infectious bronchitis virus (IBV), which belongs to the genus Coronavirus of the Coronaviridae family,[2] that replicates in the cell cytoplasm and contains an unsegmented, single-stranded, positive-sense, enveloped RNA genome.[3] The viral genome encodes for four structural proteins, namely the membrane protein (M), the envelope protein (E), the nucleocapsid protein (N), and the spike protein (S).[4] The spike protein is the major protein of the virus that is cleaved into two smaller proteins, namely SP1 and SP2. SP1 gene contains two hypervariable regions responsible for the induction of neutralizing and serotype-specific antibodies.[5] The virus mainly affects the respiratory tract in broiler chicken. It results in respiratory signs such as gasping, tracheal rales, sneezing, coughing, reduced weight gain, and mortality.[6] The infected bird appears lethargic and dislikes to move, and have foamy conjunctivitis with abundant lacrimation.[7] The IBV can associate with renal and reproductive infections.[8] Nephropathogenic IBV strains cause clinical signs, including wet dropping and excessive water intake.[9] In layers flocks IB virus causes severe damage to the oviduct leading to decreased egg production and low egg quality.[3,8] Collectively, the pathological effects of IBV make it one of the essential single causes of infectious disease-related economic loss in the poultry industry.[3] All breed types and ages are susceptible to IBV infection; however, the disease is more severe in younger chicks than adults. Similarly, resistance to infection was mentioned to increase with aging.[10] The disease is transmitted through the respiratory route, direct chicken to chicken contact, and indirectly by the mechanical spread.[11] The necessity of IB prevention in chicken regarding the nature of the virus with a high mutation rate in the Spike 1 gene instructs the need to improve effective vaccines. The first step is to study the virus strains spread in the geographical region and define their antigenicity and pathogenicity to choose a suitable virus strain for vaccination.[12] However, amplification and sequencing of the S1 gene supply a reasonable means for genotypic classification of new IBV strains.[13] The PCR on reverse-transcribed RNA is a sensitive and fast technique

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for detection of IBV.[14] Genome sequencing have almost changed traditional serology and virus cultivation methods in IBV diagnosis.[15]

Materials and Methods

Sampling. Research proposal was accepted and registered under (No. 1 on 13/12/2017) by the ethics committee of veterinary medicine college. The tissue samples, trachea, and lung were obtained from 3-5 sick chicks from 10 clinically suspected IB broiler farms and submitted to the laboratory for investigations. The RT-PCR examined a total number of 10 IBV suspected farms in the study during the six-months. The results of five of them were positive.

RNA extraction. Total RNA extracted from the tracheas and lung tissue as directed by whole RNA mini kit tissue from (GeNet Bio, Daejeon, South Korea).

Oligonucleotides. A pair of primers specific for partial amplification sequence of S1 spike glycoprotein were used for the PCR analysis, forward primer f-IBV-S1 (5'-GTTTACTACTACCAAAGTGCCTT-3') it is a position in S1 gene is (20446-20468) and reverse primer r-IBV-S1 (5'-GTGTAAACAAGGTCACCATTTA-3') it is a position in S1 gene is (20873-20894). These primers were provided by (Macrogen, Republic Korea) in lyophilized forms. To prepare a working solution, they were dissolved in sterile distilled water to a final concentration of 10.00 pmol μL^{-1}.

RT and polymerase chain reaction (PCR). A partial sequence of S1 IBV was amplified by using SuprimeScript RT-PCR Premix (2X). This kit provides a complete system for fast, high yield, and reliable single tube one-step RT-PCR (GeNet Bio). The reactions were carried out in a 0.20 mL PCR tube based on the following specifications: 10.00 µL supreme script RT-PCR premix, 5.00 µL RNA, 1.00 µL forward (10 pmol), 1.00 µL reverse primer (10.00 pmol), and 3.00 µL ultra-pure water to make up the final volume of 20.00 µL. The conventional PCR machine (Hercuvan Lab Systems, Cambridge, UK) was programmed as followed: cDNA synthesis 50.00 ˚C for 30 min, initial denaturation at 95.00 ˚C for 10 min followed by 35 cycles of 95.00 ˚C for 30 sec; annealing at 52.00 ˚C for 30 sec, and extension at 72.00 ˚C for 30 sec, and a final extension at 72.00 ˚C for 10 min.

Agarose gel electrophoresis. The polymerase chain reaction (PCR) product was analyzed by electrophoresis on 1.00% agarose gel and stained with SimplySafe™ dye (EURx, Gdańsk, Poland) in Tris-acetate-EDTA (TAE) buffer (1X). The PCR product was run via gel electrophoresis and visualized under a UV transilluminator.

Nucleotide sequence accession numbers. The partial sequences of IBV S1 were deposited in the Genbank under accession numbers (MF806472, MF806473, MF806474, MF806475, and MF806475).

Phylogenic and sequence analysis. Nucleotide sequences from the S1 gene was produced and translated by Expasy translate tool software to deduce the S1 glycoprotein amino acids. A Basic Local Alignment Search Tool (BLAST®) analysis was initially performed using the S1 sequence of our genotypes to establish its identity to Genbank accessions available from the National Center for Biotechnology Information (NCBI) Infectious bronchitis Viruses Resource. A comparative analysis of S1 sequences was performed using the CLUSTAL W Multiple Sequence Alignment Program (UCD, Dublin, Ireland). The phylogenetic tree, based on partial S1 sequences, was constructed using MEGA (version 6.0; Biodesign Institute, Tempe, USA) employing the Maximum Likelihood method with Kimura 2-parameter nucleotide substitution model.[16]

Results

The primer pair used in this study amplify the hypervariable region within the partial S1 gene of IBV given a product size of 448 bp (Fig. 1). It was a sensitive and newly designed primer for the identification of the IB virus by PCR. The reverse transcriptase PCR (RT-PCR) method is extensively used in the rapid, sensitive and reliable diagnosis of IBV in advanced laboratories.[17]

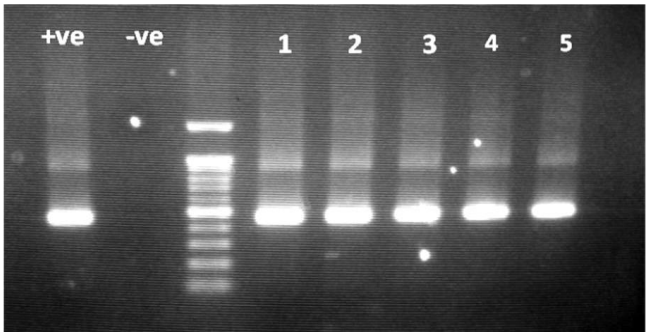

Fig. 1. Agarose gel showing amplification of the partial S1 gene (448 bp). Lanes 1 to 5: (448) PCR product of Sulaimani sequences virus. Lane +ve: positive control (H120 vaccine strain), and Lane –ve: Negative control (distilled water).

Phylogenetic analysis was conducted based on the nucleotide sequence, and the samples were classified as new IBV variants (Fig. 2).

Phylogenetic analysis was based on the nucleotide sequences of the five isolates' S1 partial gene, and other published results in the GeneBank database. The five IBV isolates were aligned with additional references and related IBV strains. It revealed that all five IBV isolates were making Novel grouped with (MF806470/sul/13, HQ123347/RFIBV6, KU238176/IR and KF153244/IBV/Erbil/12) in one cluster named Sul5/17-likes strain, the nucleotide homology of these isolates with grouped (98.00%, 97.00%, 94.00%, and 95.00% respectively). In contrast, field virus sequences' identities ranged (97.50 - 99.80%), and amino acid sequences ranged (97.00% - 100%) among them. Further analysis of phylogenetic tree indicated that at least four distinct genetic groups of IBV

were present in chicken flocks in Iraq, named S/149-LIKE, S885-LIKE & OXIBV- like strain, and variant 1. Interestingly phylogenetic tree showed that the field virus sequences were heterologous with all commercial vaccine strains currently used in vaccination programs in Iraq, such as H120, H52, MA5, Mass41, Massachusetts, and 4/91 strain. The nucleotide sequences identities of these vaccines strain with field virus sequences ranged 70.00%, 69.50%, 70.00%, 69.50%, 71.00% and 72.50%) respectively, and amino acid identities (67.00%, 63.00%, 67.00, 63.00%, 67.00%, and 70.00%), respectively.

Amino acid sequence alignments used the multiline tool and revealed many point mutations, insertion, and deletion in the S1 gene. The S glycoprotein is the primary functional protein for IBV; thus, the putative differences between the IBV/SULI strains and IBV reference vaccine strains were investigated (Supplementary files).

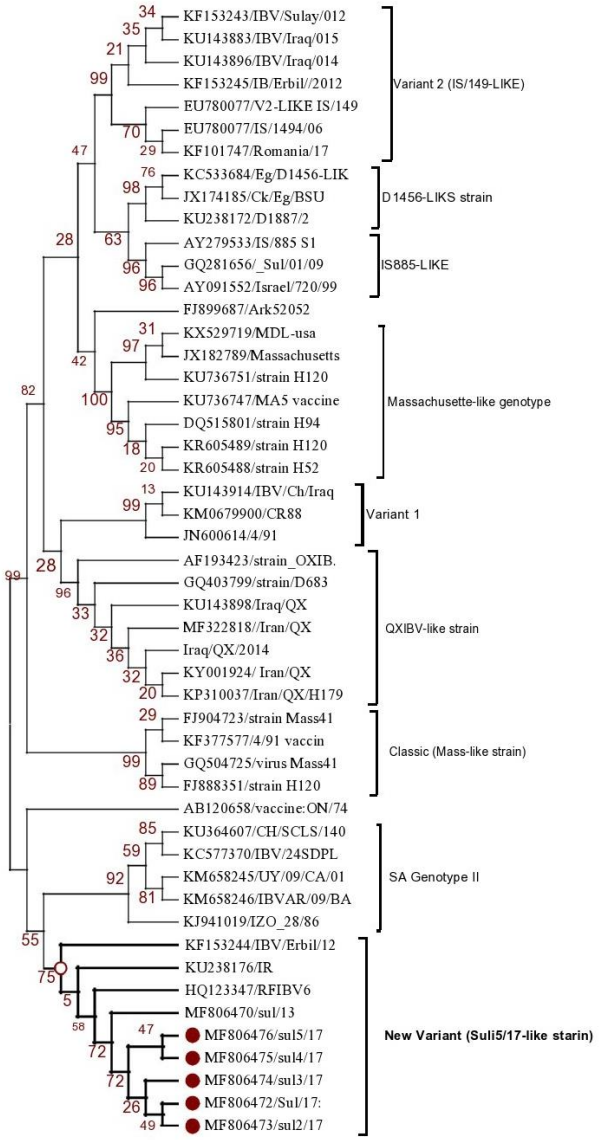

Fig. 2. Maximum likelihood tree phylogenetic relationships of the obtained Sulaimani isolates and selected reference strains based on partial S1 nucleotide sequences determined using MEGA 6.0 with the Clustal W method. Numbers along the branches refer to bootstrap values.

Discussion

The avian infectious bronchitis virus (IBV) is recognized as an essential global infectious disease because new variants are a constant threat to the poultry industry worldwide. The primer pair used in this study amplified the hypervariable region within the partial S1 gene of IBV given a product size of 448bp (Fig. 1), and it was a sensitive and newly designed primer for identification of the IB virus. Phylogenetic analysis was conducted based on the nucleotide sequence, and the samples were classified as new IBV variants (Fig. 2).

The finding of the present study revealed new branches within the IBV genotype. It indicated the emergence of new strains (Sul5/17- likes strains. The identity of Sul5/17-likes strains with Iranian isolates of HQ1233447/RFIBV6, and KU238176/IR was (97.00% and 94.00%, respectively, and this was due to the long border and huge trade transactions in the import of chicken and chicken products from Iran.

Genotype group A (MF806470/sul/13) was found in Sulaimani province in 2014 and was positively related to Iranian isolates (IRFIBV6).[18] These findings showed close similarity with this study results, and these variants are still detected in broiler flocks of Sulaimani province.

The main site for the multiplication of the IBV is the respiratory tract, especially during the first 3 to 5 days after infection.[19] Furthermore, in the present study, new variant genotypes were detected in the trachea of the infected chickens with respiratory signs of infectious bronchitis. The continuous variation of IBV makes it very difficult to control infectious bronchitis using live attenuated vaccines for immunization.[7]

Vaccination programs mostly rely on using the IBV strains such as H120, MA5, Massachusetts, and 4/91, which are also the most commonly used IBV vaccine strains in Iraq. However, despite using these vaccines, the familiar presences of IBV in vaccinated chickens continue to have a significant adverse economic impact. In the present study, IB variants were detected from chickens vaccinated with live attenuated Massachusetts types H120, MA5, vaccines, and nucleotide sequences identities of these vaccines strains with field virus sequences ranged (70.00%). These divergences between them may lead to increased pathogenicity of the virus and inadequate immune response induced by the hosts' vaccines. The inadequate protection caused by the vaccines containing heterologous strains against the IBV isolates present in the field in Iraq indicated the necessity of developing vaccines from local strains. Others reported the identification and

genotyping of IBV isolate in the Kurdistan region of Iraq in symptomatic vaccinated broiler and found low efficacy of vaccines used in this region since vaccinated broilers were symptomatic and found positive by PCR.[20]

In summary, different types of primers have been used for the detection of the IB virus in other researches. In this study, the new sensitive pair of the oligonucleotide for the S1 gene was used to detect IB virus by RT- PCR. The phylogenetic analyses revealed the emergence of five new variants in Sulaimani Governorate. Based on the sequence analysis presented in this study, the vaccines administered could not protect the broiler farms from IBV infection and could not reduce the economic losses caused by infectious bronchitis disease.

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Conflict of interest

The authors declare no conflict of interest.

References

1. Butcher GD, Shapiro DP, Miles RD. Infectious bronchitis virus: Classical and variant strains. One of a series of the Veterinary medicine-large animal clinical sciences department, Florida cooperative extension service. UF/IFAS Extension 2011; VM127.
2. Hafez HM. Avian adenoviruses infections with special attention to inclusion body hepatitis/hydropericardium syndrome and egg drop syndrome. Pak Vet J 2011; 31(2): 85-92.
3. Cavanagh D. Coronavirus avian infectious bronchitis virus. Vet Res 2007; 38(2):281-297.
4. Su JL, Zhou ZT. Guo ZS, et al. Identification of five bronchitis virus (IBV) strains isolated in China and phylogenetic analysis of the S1 gene. Afri J Microbiol Res 2012; 6(9):2194-2201.
5. Haqshenas G, Assasi K, Akrami H. Isolation and molecular characterization of infectious bronchitis virus, isolate Shiraz3. IBV, by RT-PCR and restriction enzyme analysis. Iran J Vet Res 2005; 6(2):9-15.
6. Poorbaghi SL, Mohammadi A, Asasi K. Molecular detection of avian infectious bronchitis virus serotypes from clinically suspected broiler chicken flocks in Fars province of Iran. Pak Vet J 2012; 32(1):93-97.
7. Terregino C, Toffan A, Serena Beato M, et al. Pathogenicity of a QX strain of infectious bronchitis virus in specific pathogen free and commercial broiler chickens, and evaluation of protection induced by a vaccination programme based on the Ma5 and 4/91 serotypes. Avian Pathol 2008; 37(5):487-493.
8. Balestrin E, Fraga AP, Ikuta N, et al. Infectious bronchitis virus in different avian physiological systems- a field study in Brazilian poultry flocks. Poult Sci 2014; 93(8):1922-1929.
9. Boltz DA, Nakai M, Bahra JM. Avian infectious bronchitis virus: a possible cause of reduced fertility in the rooster. Avian Dis 2004; 48(4):909-915.
10. Crinion RA, Hofstad MS. Pathogenicity of four serotypes of avian infectious bronchitis virus for the oviduct of young chickens of various ages. Avian Dis 1972; 16(2):351-363.
11. Pohuang T, Chansiripornchai N, Tawatsin A, et al. Detection and molecular characterization of infectious bronchitis virus isolated from recent outbreaks in broiler flocks in Thailand. J Vet Sci 2009; 10(3):219-223.
12. Boroomand Z, Asasi K, Mohammadi A. Pathogenesis and tissue distribution of avian infectious bronchitis virus isolate IRFIBV32 (793/B serotype) in experimentally infected broiler chickens. Sci World J 2012;403537 doi:10.1100/2012/402537
13. Zhu JG, Qian HD, Zhang YL, Hua XG, Wu ZL. Analysis of similarity of the SI gene in infectious bronchitis virus (IBV) isolates in Shanghai, China. Arch Med Vet 2007;39(3):223-8.
14. Zwaagstra, KA, van der Zeijst, BA, Kusters, JG. Rapid detection and identification of avian infectious bronchitis virus. J Clin Microbiol 1992; 30(1):79-84.
15. Cavanagh D. Severe acute respiratory syndrome vaccine development: experiences of vaccination against avian infectious bronchitis coronavirus. Avian Pathol 2003: 32(6):567-582.
16. Tamura K, Stecher G, Peterson D, et al. MEGA6: Molecular evolutionary genetics analysis version 6.0. Mol Bio Evol 2013; 30(12):2725-2729
17. Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 1994;22(22):4673-80.
18. Rauf HS. Investigation and molecular characterization of avian infectious bronchitis virus in suspected broiler farms in Slemani Governorate. MS Thesis, University of Sulaimani. Sulaymaniyah, Iraq: 2014.
19. de Wit JJ, Nieuwenhuisen-van Wilgen J, Hoogkamer A, et al. Induction of cystic oviducts and protection against early challenge with infectious bronchitis virus serotype D388 (genotype QX) by maternally derived antibodies and by early vaccination. Avian Pathol 2011; 40(5):463-471.
20. Mahmood ZH, Sleman RR, Uthman AU. Isolation and molecular characterization of Sul/01/09 avian infectious bronchitis virus, indicates the emergence of a new genotype in the Middle East. Vet Microbiol 2011; 150(1):21-7.