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Phylogenetic analysis of the infectious bronchitis virus in Iraqi farms

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Abstract

IBV is one of the viral illnesses that occurs most often in the poultry sector, and it is caused by the Gamma Coronavirus genus and the Coronaviridae family. In this experiment, fifty specimens of trachea and lung were taken from various locations inside Iraq (Baghdad, Wasit, Diyala, Salahuddin, and Babylon). The chickens' ages ranged from 12 to 26 days, and they displayed respiratory symptoms such as gasping and tracheal rales. The majority of samples were taken from broiler chickens in the range of morbidity and postmortem examination, such as pus in the trachea. Real-time RT-PCR was used to diagnose IBV, which amplified the S1 gene. The result of the RT-PCR test was positive for eight of the specimens. In order to sequence the S1 gene, the Anicon laboratory in Germany, using the Sanger method, was provided with three samples to analyze. The results of the sequencing of the S1 gene indicated that the isolated copies were related to those of the Iranian strain IS/1494/06 that were placed at the National Center for Biotechnology Information website in the Genbank database.

Keywords
IBV, chicken, real-time RT-PCR, and sequencing for the S1 gene

One of the most dangerous viruses that can infect chickens is called avian infectious bronchitis virus (IBV), and this virus represents a challenge to the chicken industry around the world (Flageul, Allйe, Courtillon et al., 2022; Ulaiwi 2019). Since 1931, when it was first seen in the United States, it has been widespread (Lin and Chen 2017). Iraq has reported diseases since 1989 (Al-Zuhairy 2017). The disease caused by a virus from the Gamma coronavirus genus and Coronaviridae family belongs to the order Nidovirales (Franzo, Massi, and Tucciarone 2017). This virus causes a very contagious, severe form of the illness. Death rates range between 20% and 30%, while morbidity may exceed 100%. Secondary infections may increase flock mortality (Isa, Abdo, and Al-Barzinji 2022). The virus enters via inhalation and mostly multiplies in the respiratory organs in the ciliated epithelium and mucus secretion cells, which leads to breathing signs such as tracheal rales and respiratory symptoms, including wheezing, coughing, sneezing, and nasal secretions (Benyeda, Szeredi, and Mato 2010). Infected laying hens have been discovered to be suffering from respiratory problems and a drop in egg production as a result of the infection (Najimudeen, Hasan, and Goldsmith 2021). Along with the implementation of a vaccination plan for IBV, a significant number of the flock that was impacted shows respiratory signs of late-onset respiratory disease and an increase in the airsaculitis condemnation that was induced by IBV (Khamas 2008). Vaccines are available; nonetheless, infection control is challenging due to the fact that there is no cross-protection across different virus genotypes and the fact that genes might vary (Buharideen, Hasan, and Niu 2021). Hence, the genetic detection of circulating IBV strains is essential for the direction of national or regional plans to control IB through the establishment of appropriate diagnostic and

immunization procedures (Quinteros, Lee, and Noormohammadi 2022). IBV is a gammacoronavirus that is part of the third group of the Coronavirus genus, which is part of the Coronaviridae family, which is part of the order Nidovirales. It has a positive-sense, single-stranded RNA genome that is around 27.6 kilobases in length and encodes four component proteins. These proteins are the spike glycoprotein (S), membrane glycoprotein (M), envelope protein (E), and nucleocapsid protein (N) (Cabal and Wu 2022). Protein S is the primary structure of the infectious bronchitis virus and is also found on the exterior of the virus envelope. It is the primary effect in stimulating the production of antibodies capable of neutralizing the virus, as well as being responsible for linking to and entering host cells (Kathiravan, Radhakrishnan, and Namasivayam 2021).Furin-like host cell proteases are responsible for its dissection into the S1 and S2 subunits (Jackwood and De wit 2013). It is believed that mutations, insertions, deletions, or deletions of S1 genes, as well as RNA recombination, are responsible for S1 variety (Ghorbiani, Boroomand, and Mayahi 2020). This diversity may result in a significant level of biological heterogeneity between strains, and the S1 protein may undergo partial amino acid alterations, which may allow for the emergence of new serotypes (Ren, Han, Zhao, and Liu 2020). There are around 20 different IBV species that have been discovered across the world (Seger, Karimi, and Madadger 2016). One of the biggest challenges for Iraq's poultry industry is still IB. Nevertheless, RT-PCR remains the gold standard for diagnosing IBV because of its dependability and speed (Promkuntod 2016). Despite the fact that collective vaccination against infectious bronchitis has been practiced throughout the whole of the commercial chicken sector, outbreaks of this illness have continued to occur up until the present day (Abdulwahid, Zahid, and Kadhum 2016). The purpose of this research was to sequence IB virus isolates and identify currently circulating IB virus isolates in Iraqi fields.

Methodology

Selection of samples

From October 2021 to January 2022, 50 specimens (trachea and lung) were collected from various regions of Iraq (Baghdad, Wasit, Babylon, Salahuddin, and Diyala) from broiler and layer farms with respiratory signs and abnormalities in the shape of eggs. After that, the specimens were transferred into test tubes and kept at a temperature of -20 degrees Celsius until they were needed.

Extraction of RNA and RT-PCR protocol

The extraction of RNA was carried out by using the QI gene kit. The forward primer sequence was 5'-GCTTTTGAGCCTAGCGTT-3`, and the reverse primer's nucleotide sequence was 5`-GCCATGTTGTCACTGTCTATTG- 3`.The twenty µl PCR reaction master mix was prepared according to the producer's instrument. Display Tables 1 and 2.

Table.1; RT-PCR reagent was shown.

NO.	Reagent	Company	Storage
1	Probe:FAM IBV 5 G BHQ1 5 FAM-CACCACCAGAACCTGTCACCTC-BHQ3	αDNA	-20 °C (+2-10)°C
2	Primer: IBV 5 GU391 5`- GCTTTTGAGCCTAGCGTT- 3`	αDNA	-20 °C (+2-10)°C
3	Primer: IBV 5 GL 533 5`- GCCATGTTGTCACTGTCTATTG- 3`	αDNA	-20 °C (+2-10)°C
4	Bidistilled water	αDNA	RT/-20°C(+2-10)°C

Table.2; RT-PCR protocol.

Stage	NO. of cycles	Temperature	Time (sec.)
Reverse transcription	1	42 °C	900 sec.
RT inactivation/Hot start activation	1	95 °C	30 sec.
3 step qPCR -Denaturation	40	95 °C	30 sec.
Annealing -Extension		60 °C	60 sec.

Sequencing of the S1 gene of the infectious bronchitis virus

The RT-PCR results were sequenced in Germany by AniCon Laboratories GmbH by Sanger sequencing. Accession numbers were assigned to the sequences of the Iraqi IBV-strain S1 gene that were placed in the GenBank database at the National Center for Biotechnology Information (NCBI) website.

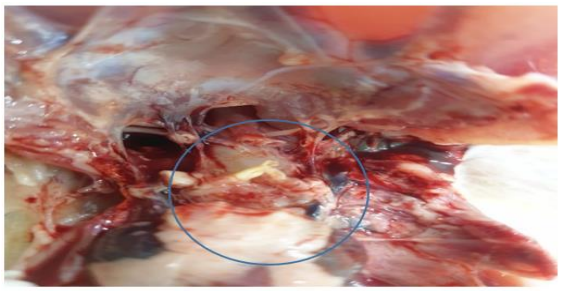

Figure.1; Caseous exudate in the tracheal bifurcation

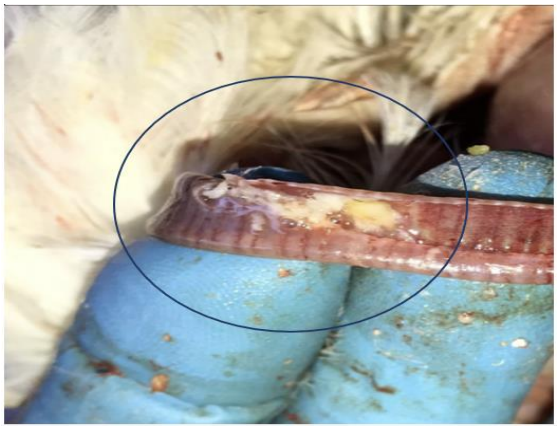

Figure.2; Caseous exudate in the trachea

Results

Clinical manifestations and examinations after death

Broiler chicken farms exhibited respiratory symptoms of infectious bronchitis (IB) disease; death rates from infectious bronchitis (IB) disease reached 25% within five days. The following symptoms were observed in birds: gasping, ruffled feathers, sneezing, and the full range of morbidity. Caseous exudate was noticed in the trachea and tracheal bifurcation, as shown in Figures 1 and 2.

Real-time RT-PCR

The RT-PCR outcome showed that out of fifty specimens, eight (8) were positive. Show the outcome in Table 3.

Table 3; shows the number of samples, region, and Ct of infectious bronchitis virus using RT-PCR, as well as bird type.

NO. of samples	Regions	Ct. of IBV	Birds type
1	Baghdad	35.4	Layers
4	Babylon/ Al-Hilla	33.1	Broilers
6	Wasit/ Al Hayy	26.2	Broilers
7	Babylon/ Al-Mahaweel	31	Broilers
8	Wasit/ Numaniyah	23.2	Broilers
9	Wasit/Al-Kut	29.3	Broilers
10	Salahuddin/ Samarra	29.4	Broilers
14	Diyala/ Baqubah	27.1	Broilers

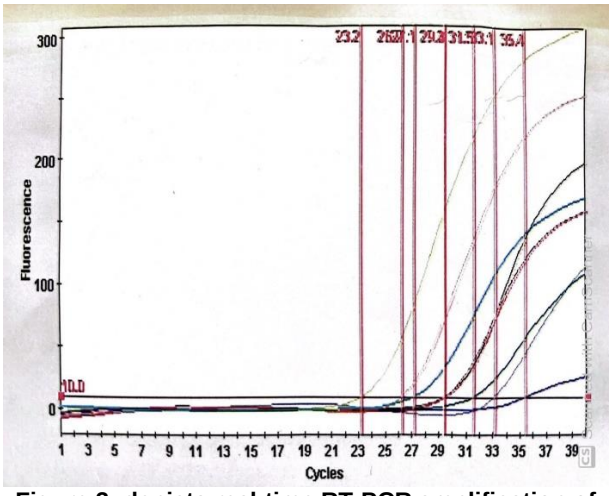

Figure 3; depicts real-time RT-PCR amplification of the S1 gene as a method for detecting the Avian IB virus.

The sequencing of the S1 gene in the IBV virus

AniCon Labor GmbH in Germany was responsible for sequencing the S1 gene. The sequences were submitted to the GenBank database of the NCBI, so they were assigned accession numbers OQ162300, Bankit 2659945, and also S1, respectively. Figure 4 depicts the results for isolation related to Iranian strain IS1494/06.

Phylogenetic tree

The tree contains many clusters; each cluster has a group of clusters that trace back to a common ancestor; all clusters in the diagram refer to one father from which they all emerged, as shown in figure 4.

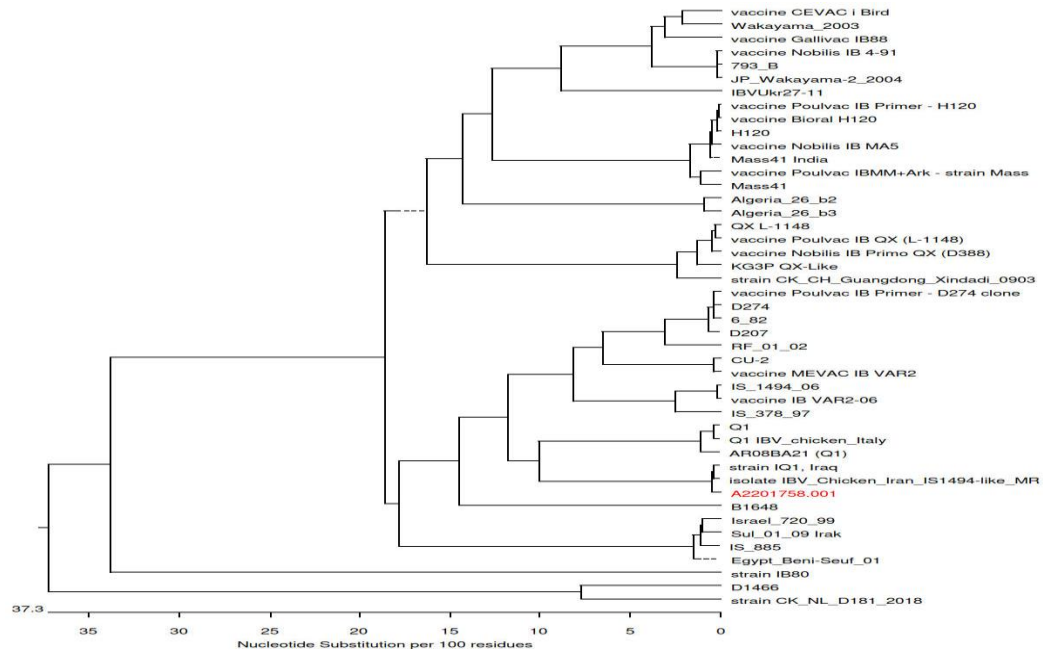

Figure 4: Phylogenetic analysis of infectious bronchitis virus for the gene S1. This analysis used the nucleotide sequence of S1 from the A2201758.001 strain and 15 previously published IBV strains.

Discussion

The coronavirus causes avian infectious bronchitis (IB), a severe and highly contagious disease of poultry that can cause complex respiratory problems and is among the most widespread poultry diseases (Mohammed 2013). Since the afflicted flocks were both vaccinated and unvaccinated, the occurrence of this illness resulted in enormous economic losses in Iraq. The trachea and lung were collected because IBV initially infected the respiratory tract, and 3 days after virus replication inoculation, the highest virus titers were found in the trachea. Virus strains play a role, which may persist for up to 5 days and gradually decrease and maintain a low level for up to 28 days. The presence of IBV in the lungs was also high after infection; high levels of virus were detected in the lungs 4 days after the inoculation, 11 days after the illness (Tran and Nguyen 2018). The infectious bronchitis virus strain IS-1494 affects the respiratory system (Najafi, Ghalyanchi, and Hashemzadeh 2016). Samples were taken from various farms and different ages of chicken and were aggregated in the first stages of the disease as soon as the clinical symptoms of the illness (tracheal rales, dyspnea, coughing, and gasping) appeared (Najimudeen, Barboza, Ali, and Isham 2022). The golden test, RT-PCR, was used to diagnose IBV, and this assay was sensitive, accurate, and useful for detecting the virus (Chacon, Astolfi-Ferreira, and David 2019). The sequencing of the S1 gene is important because most mutations in IBV appear in this gene. The S1 gene of the infectious bronchitis virus was sequenced, and the whole specimens were sent to Germany for Sanger sequencing. The phylogenetic analysis revealed that the isolated strain was related to Iranian strain IS 1494/06, a result that was agreed upon (Mousavi, Hosseini, Sadri, and Ghafouri 2018). Sequencing is critical for detecting any mutation in the genome or protein; by sequencing, the divergence between the vaccine in Iraqi marketing and the isolated virus can be determined, and the genetic sequence of the virus can be determined. As a result, the majority of mutations occurred within the S gene, which is consistent with (Xu, Cheng, Zhao and Zhang 2021).

Conclusion

Infectious bronchitis virus (IBV) was found in several areas of Iraq in this study, including the capital Baghdad, Wasit, Babylon, Diyala, and Salahuddin. IBV is a difficult problem on Iraqi poultry farms, causing a recent economic downturn. The S gene has the most infectious

bronchitis virus mutations. The sequencing of the S1 gene refers to the IBV isolate related to Iranian strain IS/1494. To get the best results, it's recommended to use RT-PCR and sequencing.

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To my father, my mother, my wife, my brothers, and all my friends, To My College and to all of the doctors in the microbiology department:

Conflict of Interest

The authors of this study say they have no conflicts of interest.

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