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Phylogenetic analysis of RT-PCR detected infectious bronchitis virus locally infected broiler farms in Diyala Governorate-IRAQ

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Abstract--- Background and Aim: One of the primary pathogenic causes linked to economic losses in the chicken business is the infectious bronchitis virus. This virus exploited its S (spike) gene-encoded protein to hook into the host receptor. The biological diversity of the viral S gene may be linked to the chicken industry's vaccination status. Recently, the virus was detected in broilers of six farms located in different districts of Diyala Governorate by the use of RT-PCR. The current study aimed to explore the pattern of biological diversity of infectious bronchitis viruses based on the possibility of genetic variations of the S gene. Materials and Methods: Ten tissue samples were collected from broilers of six farms located in different districts of Diyala Governorate and named R1 to R10. The chickens were recently vaccinated, but naturally infected with infectious bronchitis virus; a particular RT-PCR fragment partially encompassing the coding domains of the S gene was recently obtained. The amplified fragments were immediately submitted to direct sequencing tests to examine the genetic variation pattern in the samples acquired from various chicken sources. Then unique comprehensive trees were generated to validate the correct genotyping of the identified variations and their

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phylogenetic distribution. Results: Our findings revealed the existence of four nucleic acid variations in the samples studied, namely C>T94, C>T99, G>A200, and G>A207, in poultry infected with infectious bronchitis viruses. Only one of them (C>T99) was discovered in seven samples, whereas three of them (C>T94, G>A200, and G>A207) were present in all samples (R1, R3, R4, R5, R7, R8, and R9). The direct nucleic acid translation revealed that both C>T94 and G>A200 had a silent variation (p.Thr268= and p.Gln302=), whereas the other two C>T99 and G>A200 had two missense mutations (p.Thr>Met269 and p.Cys>Tyr305) on the S-encoded protein. Our analyzed samples were found to be suitable in the local area of Iranian and Iraqi strains of identical viral sequences, according to the tree. Furthermore, large evolutionary distances were discovered between the clade of our samples and the clade of vaccination strains. According to our analysis, the currently under investigation poultry-infecting infectious bronchitis virus isolates were found to be of Iranian / Iraqi origins. Conclusions: It was concluded that vaccination had no evolutionary influence on creating any detectable nucleic acid mutation in the virus strains studied. For these reasons, our research implies that S-based amplicons might be a useful tool for delving further into the genotypes of Infectious Bronchitis Virus sequences that have been found.

***Keywords*---**Infectious bronchitis viruses, S (spike) gene, RT-PCR.

Introduction

One of the most important respiratory diseases of poultry is infectious bronchitis (IB). It is a contagious disease that can infect all ages. The causative viral agent is a very significant pathogen. The infectious bronchitis virus belongs to the Coronavirinae subfamily with the family Coronaviridae. The IBV genome was Single-stranded, positive-sense RNA virus belonging to the genus gammacoronavirus (Cavanagh and Gelb, 2008).

In North Dakota, USA, the first Infectious bronchitis virus caused by the Mass serotype was recorded and later become widespread worldwide with an almost 90-100% occurrence rate regardless of the vaccination status of the flock by using live attenuated and inactivated vaccines program for the control of the infection (Schalk, 1931; Jordan, 2017). Later more than hundreds of new variants of IBV genomes have been continually emerging due to genetic instability throughout the virus transmission or evolution, leading to mainly changes with S1 glycoprotein as a hypervariable region of IBV that carry most of the neutralizing epitopes of the virus, which has significant biological properties.

Chickens are the most significant natural hosts for infection with IBV, whereas other species like pigeons, geese, peafowl, pheasants, and ducks can also play a role in the spread and infection with IBV but clinical signs seem to be minimal (Liu *et al.,* 2005; Awad *et al.,* 2014). The incubation period of IBV in poultry is relatively short (24-48) hours and the causative agent spreading horizontally by aerosol (coughing and sneezing) and, through contaminated poultry litter, feed,

drinking water, equipment, or other fomites leading to the entire infected flock within one or two days (Chhabra *et al.,* 2015). Transmission of IBV by vertical route within the embryo was never reported, but it is possible to get the IB virus from infected hens by contaminating the shell of hatching eggs through shedding from the alimentary tract or the oviduct (Boltz *et al.,* 2004; Saif *et al.,* 2008). Chickens of all ages are susceptible to and affected by the IB virus and are mostly associated with economic losses, but the very young chicks showed more pronounced respiratory distress associated with increased morbidity and high mortality rate compared with older chickens (Britton and Cavanagh, 2007; Cavanagh and Gelb, 2008). Initially, it was believed that all isolates of the IB virus strains belonged to a single prototype called Massachusetts (Mass) which was detected from commercial poultry (Cavanagh and Naqi, 2003). Then, a different variant of serotypes and prototypes continues to emerge worldwide (Bochkov *et al.,* 2006). In the Middle East, a considerable number of IBV as variant genotypes were circulating including (Iraq/ Sul/01/09, Iran/793B/19/08, Egypt/ Beni-Seuf/01, and CK/CH/SCYA/10I) (Mahmood *et al.,* 2011; Kahya *et al.,* 2013). These strains were detected and isolated from layer and broiler farms and considered a dominant strain in these countries (Liu *et al.,* 2006). It was noticed that many outbreaks of IBV infections occurred in poultry farms of Diyala governorate regardless of the vaccination programs followed by private sectors to control the infection. These outbreaks caused economic losses in the poultry industry of Diyala Governorate. The failure of vaccination programs against the disease was widely reported in the governorate because of no-cross- protection between all serotypes of IBV and random doses of the imported vaccines that did not match the local antigenicity isolates that circulating in the field, therefore, continuous production of new vaccines from local strain are important to controlling the IBV (Casais *et al.,* 2003). The present study was designed to point out more data on IBV infections in poultry farms associated with circulating IBV or might be attributed to new strains or variants of the virus. Little information is known on molecular evidence of IBV in the Diyala Governorate, located in the eastern part of Iraq. Therefore, the purpose of this is to investigate the phylogenetic analysis of detected viruses to point out the possibility of variations or emerging strains among the circulating viruses in comparison to the reference strain of NCBI.

Materials & Methods

Ethical approval

The Scientific Ethical Committee of College of Veterinary Medicine, Uinversity of Diyala, Iraq approved this study (Approval no: Vet Medicine (134); September 2020, K, A, T and K

Background

Infectious Bronchitis Virus (IBV) was detected by the use of specific Oligonucleotide primers (Integrated DNA Technologies Company, Canada) as previously described by (Jones *et al.,* 2005) for partial amplification of S1 gene of IBV RNA genome. Out of 30 tissue samples collected from broilers of naturally infected but IBV vaccinated farms, 25 samples were resulted in a DNA amplicon of 393 bp by the use of RT-PCR (Karar *et al.,* 2022, manuscript under

publication). Clear and clean band resulted from the amplification was subjected to sequencing. The partial fragment sequence of the S1 gene of the IBV has been sequenced. Following the instruction manuals of the commercial kit (Macrogen Inc. Geumchen, Seoul, South Korea Sequence Company), the resolved PCR amplicons from both directions, forward and reverse directions were sequencing. By comparing the observed genomic sequences of local samples with the returned nucleic acid sequences from GenBank, the virtual positions and other information of the acquired PCR fragments were evaluated. With BioEdit Sequence Alignment Editor Software in Version 7.1, the current sequencing of the PCR products of the targeted samples was reviewed, aligned, and modified, and compared to the respective sequences in the reference database (DNASTAR, Madison, WI, USA). Each sequenced sample's detected differences were numbered in PCR amplicons and their matching positions within the reference genome. SnapGene Viewer ver. 4.0.4 (https://www.snapg ene.com) was used to annotate each discovered variation within the viral sequences. For each studied sample, the identified changes were stored in the NCBI-bankit database under a unique accession number.

Translation of nucleic acid variations into amino acid residues

The amino acid sequences of the targeted S-encoded spike protein were obtained using the ID number AXT92415.1 from the protein data bank (http://www.ncbi.nlm.nih.gov). There were 1156 amino acid residues in this protein. Using the Expasy online software, the detected nucleic acid variations in the coding regions were translated into a reading frame matching to the relevant amino acid residues in the generated protein. Using the BioEdit server's "align" script, multiple amino acid sequence alignment was performed between the reference amino acid sequences and their observed mutant counterpart.

Comprehensive phylogenetic tree construction

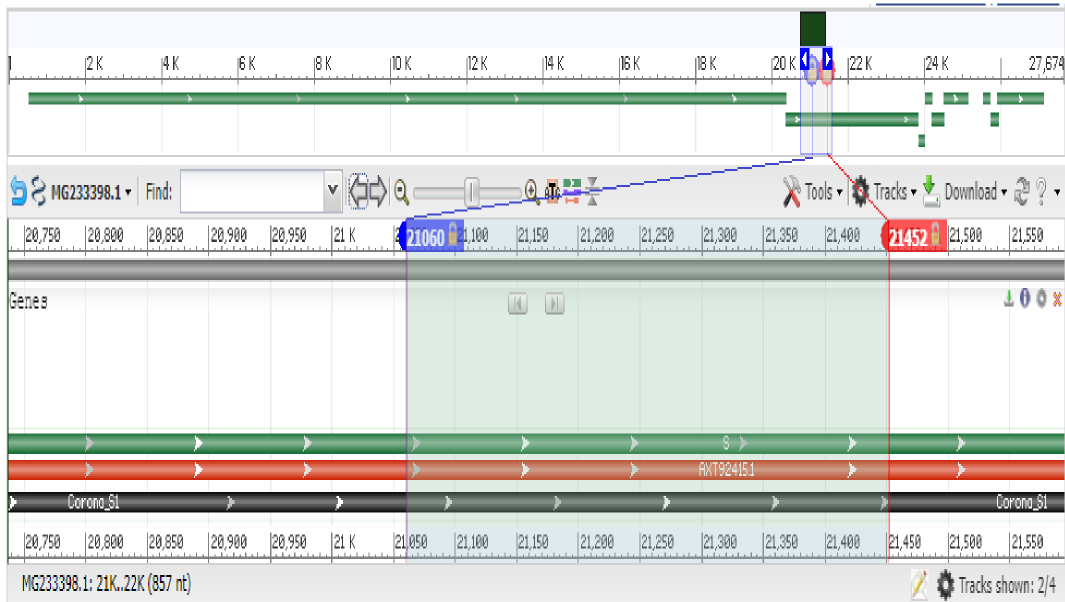
In this work, a particular comprehensive tree was built using the neighbor-joining procedure provided by Al-Dabbagh *et al* (2019). Using the NCBI-BLASTn service, the detected variations were matched to their homologous reference sequence neighbors (Zhang *et al.* 2000). Then, using the iTOL suit, a complete inclusive tree was generated, including the observed variation, using the neighbour-joining approach and displayed as a circular cladogram (Letunic and Bork, 2019).

Results

Sequence and Phylogenetic Analysis

Ten (spike) gene sequences of (IBV) were chosen from 25 RT-PCR positive samples from this location. The NCBI BLASTn engine discovered 99 percent sequence similarity between the sequenced local samples and the intended NCBI reference target sequences for the 393 bp amplicons from these ten samples. Through represent R1 to R10 samples, the observed 10 local sample variants were placed in the NCBI-bankit database under the accession numbers (OL321799, OL321800, OL321801, OL321802, OL321803, OL321804, OL321805, OL321806, OL321807, and OL321808). By comparing the observed nucleic acid sequences of these 10 studied local samples with the obtained nucleic acid sequences

(GenBank acc. MG233398.1) as a reference sequence, the precise positions of the retrieved PCR fragments were identified. As seen in the diagram (1)



393 bp PCR amplicon length

Figure (1) The exact site of the produced 393 bp amplicon partially contained a section (start and end point) of the S gene within Infectious bronchitis virus genomic sequences from reference sequences (Accession NO. MG233398.1). The blue arrow points to the starting location of this amplicon, whereas the red arrow points to the terminus.

The alignment findings of the 393 bp of local samples indicated the existence of four nucleic acid changes represented by four nucleic acid substitutions in the analyzed samples (GenBank acc. no. MG233398.1) when compared to the most comparable referring reference nucleic acid sequences, as shown in (Figure 2).

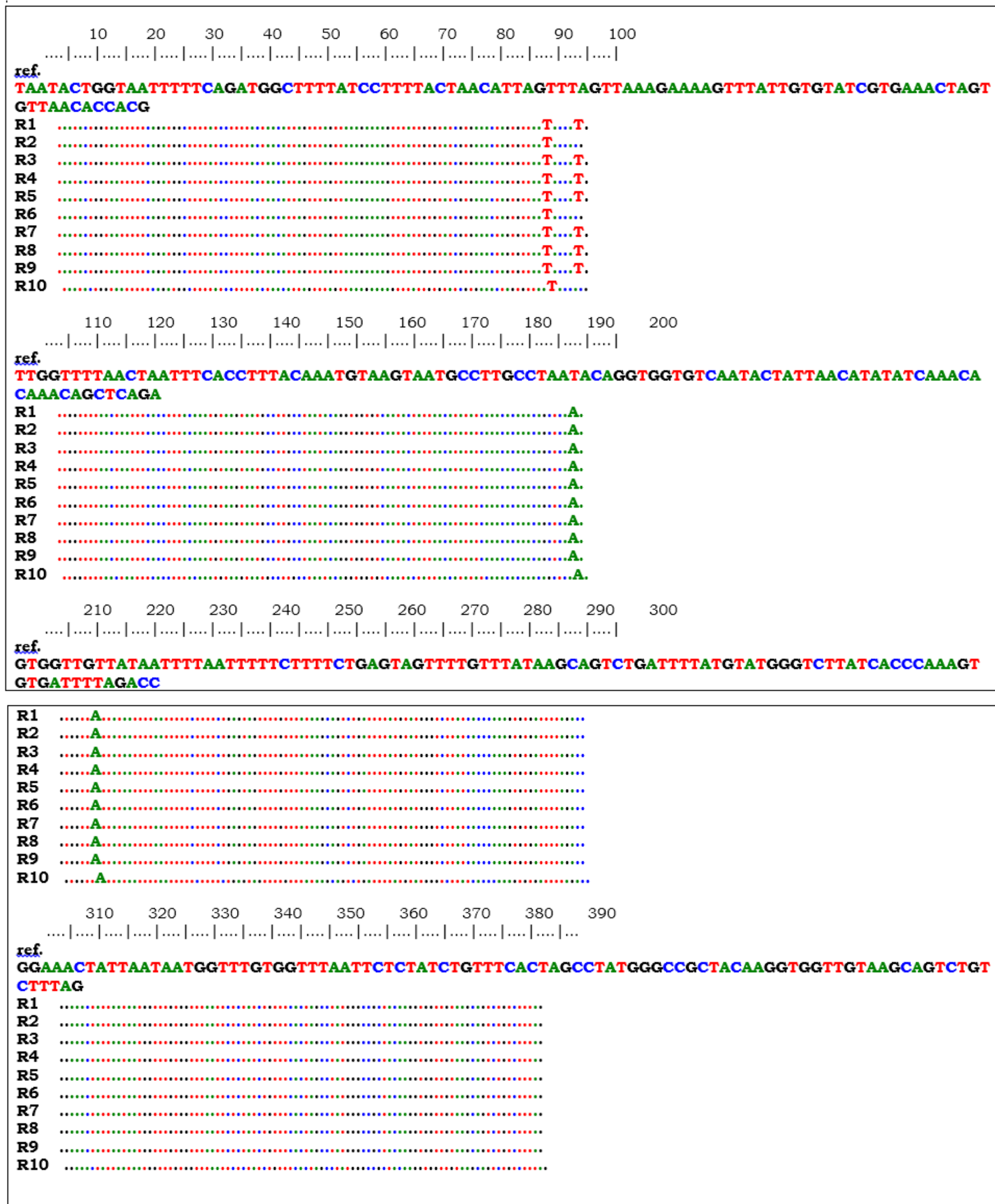


Fig. 2: Nucleic acid sequences of 10 local samples and their associated reference sequences of the S genetic sequences' 393 bp amplicons. The letters "R" followed by a number, which varies from R1 to R10, signify for the NCBI reference sequence, whereas the sign "ref" stands for the NCBI referencing sequence.

Our findings revealed the occurrence of four nucleic acid variations in the studied 10 local samples, namely C>T94, C>T99, G>A199, and G>A207. Three of these variations, namely (C>T94, G>A199, and G>A207), were detected in all isolated

samples (R1-R10), but just one (C>T99) was observed in seven isolated samples (R1-R10) (R1, R3, R4, R5, R7, R8, and R9). To confirm these extremely high numbers of variations, the sequencing chromatograms of the investigated local samples, as well as their detailed annotations as a reference from NCBI, were verified and documented, and the chromatograms of their sequences were shown according to their positions in the PCR amplicons (Fig.3). The presence of each of these variations, as well as the absence of any possible technical mistake, was confirmed in the original chromatogram.

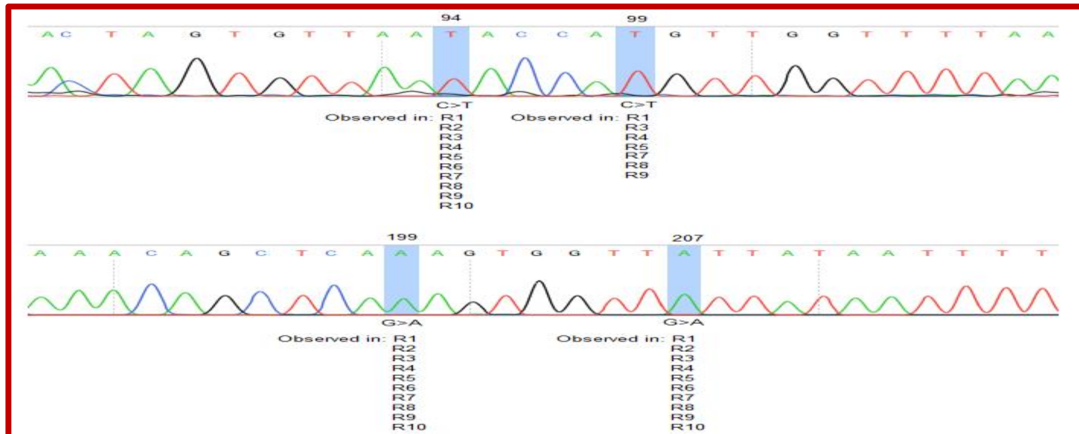


Fig. 3: Chromatogram of IBV-infecting samples discovered in chicken. The contamination-free technical parameters used to assess each variant in the present samples are referred to as the clarity of the identified peaks. The letter "R" denotes the code allocated to the local isolated samples investigated in this study.

The amino acid sequences of all ten isolated samples from the immediate area were translated using the (Expasy translate suite). Two mutations had a little influence on the spike polypeptide, according on amino acid alignment of these samples and their NCBI references (Fig 4. A). These synonymous variations, specifically p.T268= (p.Thr268=) and p.Q302= (p.Gln302=), were found across the spike protein sequences (Fig4 B). The other two variants, p.T>M269 (p.Thr>Met269) and p.C>Y305 (p.Cys>Tyr305), were shown to cause direct missense mutations of two amino acid changes.

A) sequences of amino acid residues within PCR amplicons

```
          10        20        30        40        50        60        70        80        90
100
    ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
ref.
NTGNFSDGFYPFTNISLVKEKFIVYRETSVNTTLVLTNFTFTNVSNALPNTGGVNTI
NIYQTQTAQSGCYNFNFSFLSSFVYKQSDFMYGSYHPKCDFRP
R1   ................................M...................................Y...............................
R2   ...................................................................Y...............................
R3   ................................M...................................Y...............................
R4   ................................M...................................Y...............................
R5   ................................M...................................Y...............................
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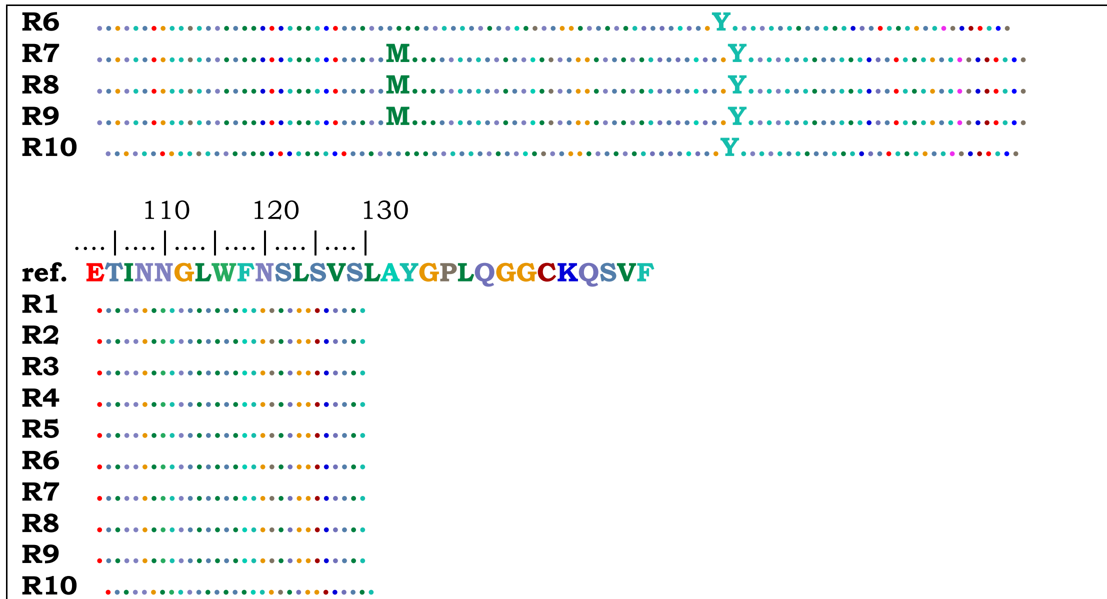

B) sequences of amino acid residues within the entire protein

The entire structure of the spike protein (protein id= AXT92415.1)

MLVKSLFIVTLLFALCSAALFDNNQAVYYYQSAFRPSSGWHKHGGAYAVANVSLEYAN
AGSSTHCTAGAIYWSKNFTASSVAMTAPGTGMSWSTAQFCTAHCNFSDFTVFVTHCY
KSGDVCPLTGLIPSGYIRISAMTKGTTSLFYNLTVPVTKYPKFKSLQCVDNFTSVYLNGD
LVFTSNETKDVSAAGVHFKAGGPITYKVMEKVDVLAYFVNGTAQDVILCDNSPRGLLA
CQYNTGNFSDGFYPFTNISLVKEKFIVYRETSVNT**T**LVLTNFTFTNVSNALPNTGGVNTI
NIYQTQTAQSG**C**YNFNFSFLSSFVYKQSDFMYGSYHPKCDFRPETINNGLWFNSLSVS
LAYGPLQGGCKQSVFSNRATCCYAYSYNGPRLCKGVYIGELQQYFECGLLVYVTKSD
GSRIQTRNEPLVLTHHNYNNITLDRCVEYNIYGRSGQGFITNVTAAAANYNYLADGGLA
ILDTSGAIDIFVVQGEYGPNYYKVNPCEDVNQQFVVSGGGIVGVLTSHNETGSQQLEN
LFYVKLTNSTRRTRRSTIANVTTCPYVSYGRFCIKPDGLVSEIVPQELDYFVAPLLNVTE
HVLIPNSFNLTVTDEYIQTRMEKVQINCLQYVCGNSIECRNLFQQYGPVCDNILSIVNSV
GQREDMESLTFYSSTKPKGYNTPIFSNISTGDFNISLMLTPPSSPSGRSFIEDLLFTSVE
TVGLPTDAEYKKCTAGPLGTLKDLICAREYNGLLVLPPIITADMQTMYTASLVGAMAFG
GITSAAAIPFATQIQARINHLGITQSLLMKNQEKIAASFNKAIGHMQEGFRSTSLALQQI
QDVVNKQSAILTETMNSLNKNFGAITSVIQDIYAQLDAIQADAQVDRLITGRLSSLSVLA
SAKQSEYIRVSQQRELATQKINECVKSQSNRYGFCGSGRHVLSIPQNAPNGIVFIHFTY
TPESFVNVTAIVGFCVSPANASQYAIVPANGRGIFIQVNGTYYITARDMYMPRDITAGDI
VTLTSCQANYVNVNKTVITTFVEDDDFDFDDELSKWWNETKHEIPDFDEFNYTVPILN
ISSEIDRIQGVIQGLNDSLINLEELSIIKTYIKWPWYVWLAIGFAIIIFILILGWVFFMTGCC
GCCCGCFGIIPLMSKCGKKSSYYTTFDNDVVT

Figure (4). Amino acid residues alignment of the observed variants of the S-encoded spike protein. A) The amino acid alterations are marked in the amplified 393 bp locus according to their respective locations. B) The amino acid substitutions are marked in the protein according to their respective locations.

The increased area of the S-encoded glycoprotein is shown by the grey highlights. The blue hues in the alignment map relate to the amino acid replacement

Comparison between infection due to IBV and vaccine strain IB4/91

To determine the distance of nucleic acid differences with these strains, we must compare our viral infection of local isolates with strains often utilized in the vaccination of chicken flocks in Diyala Governorate. This type of comparison is valuable for determining the potential impact of a vaccination campaign on the samples we studied. Seventy-two (72) nucleic acid changes were discovered when local samples infected with IBV were compared to strain IBV (4/91), a prototype of vaccine strain (Fig. 5). As a result, there are several variances between our tested samples and this strain. These findings might imply that immunization with IB4/91 had no effect on producing any noticeable nucleic acid changes in the virus strains studied.

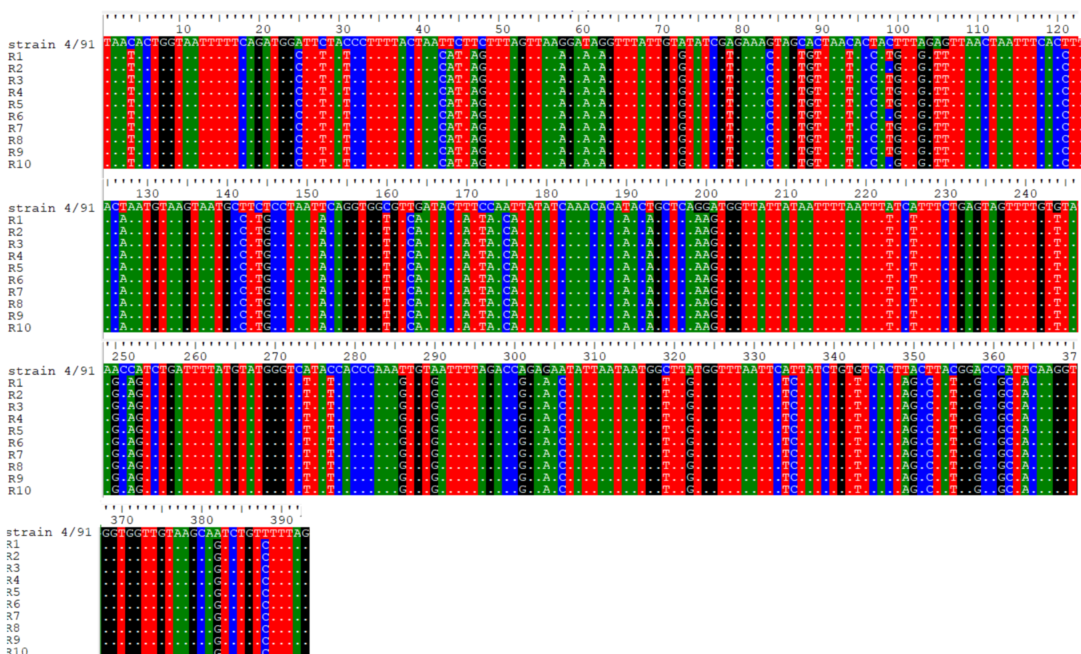

Fig. 5 Comparative nucleic acid alignments between the examined poultry-infecting samples with IBV and the vaccination (strain IB 4/91) utilizing multiple nucleotide sequence (Bioedit version 7.0.9). The colorful A, G, C, and T letters showed the existence of diversity in nucleic acid sequences, whereas the dots indicated sequence identity. The letter "R" represents the code for the samples that were studied in this study.

Phylogenetic analysis

A thorough phylogenetic tree was built in the current study based on nucleic acid changes detected in the amplified 393 bp of the S gene amplicons to offer a phylogenetic comprehension of the real distances between our studied samples and the vaccine IBV 4/91 strain. R1 to R10 samples were included in this phylogenetic tree, as well as additional related nucleic acid sequences of

infectious bronchitis virus sequences from NCBI. Our investigated samples were to make up the majority of the cladogram's incorporated sequences within this tree. In addition, the vaccination strain IBV 4/91 and its associated genomes were included in the same tree. In this complete tree, there were a total of 100 aligned nucleic acid sequences. Within the infectious bronchitis virus sequences, the studied samples were grouped into two phylogenetic clades in the cladogram. Our samples showed two unique sites within this large lineage. The first cluster was made up of R1, R, 3, R4, R5, R7, R8, and R9 viral samples, while the second cluster was made up of the remaining three (R2, R6, and R10) viral samples (Fig 6).

This location was made possible by the lack of one genetic variant, C>T99, in the R2, R6, and R10 samples. The other three nucleic acid changes (C>T94, G>A199, and G>A207) were found in all of the viral samples examined. This type of placement suggested that the detected C>T99 variation played a minor impact in causing a notable divergence from the initial location of these viral samples inside the primary clade of this cladogram. Despite the fact that two cluster positionings were discovered in the R1–R10 samples, it is conceivable to assume a close positioning of these samples within close phylogenetic locations.

Furthermore, the clustering of all viral samples under consideration might imply that these genomes have just two closely linked evolutionary distribution patterns. Because it indicated the real neighbour-joining-based placement in such reported alterations, the present observation of this tree has validated sequencing reactions. The investigated samples (R2, R6, and R10), as well as the other seven viral samples (R1 to R7), were all within a few miles of the MG233398.1 entry number. Other four Iranian strains are also closely related (GenBank acc. no. of MF322831.1, MF322829.1, MF322846.1, and KT583600.1) Surprisingly, three Iraqi strains that belonged to the same poultry-infecting viral sequences were found near the local Iraqi samples (GenBank acc. no. KU143889.1, KU143887.1, KU143885.1). The findings revealed that the viral sequences of these strains within the primary clade had a high level of genetic similarity and were also closely connected to one another. The vaccination 4/91 strain (GenBank accession number KF377577.1) and its associated sequencesoccupy huge phylogenetic locations apart from main clade sequences in the minor clade.

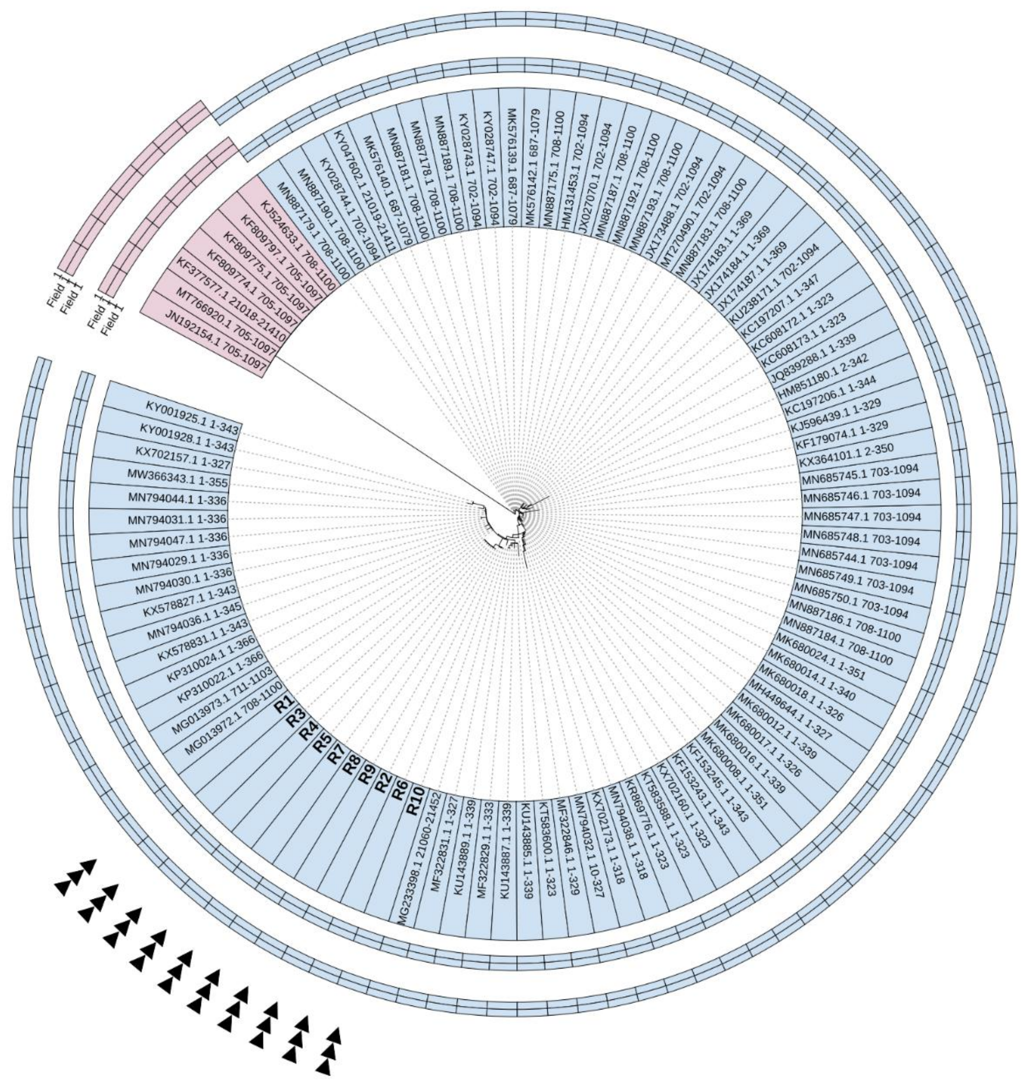

Fig. 6 cladogram depicting the evolutionary tree of genetic variations of the S gene section of 10 poultry-infecting Infectious bronchitis virus samples. The black-colored triangle represents the viral variants that were investigated. The numbers all related to the GenBank entry number for each referring species. The number "0.1" at the top of the tree represents the scale range of the creatures categorized by the comprehensive tree. The letter "R#" stands for the code of the examined local samples.

Discussion

This IBV disease is economically very important as it causes tremendous economic important losses with high mortality and poor weight gain in broilers as well as decreasing egg production and quality in layers (Cavanagh and Naqi, 2003). In addition to the respiratory and reproductive system, the virus has been associated with nephritis. The viral genome is a single-stranded, non-segmented RNA molecule of positive polarity. The virus has a worldwide distribution and many variants with changes in the genome have been identified (Cavanagh, 2007).

Sequence and Phylogenetic Analysis

This is the first research in the Diyala Governorate to use molecular and phylogenetic techniques to assess genotyping and the rate of IBV infection in broiler flocks. In this investigation, a single PCR fragment partially encompassing the coding domains of the S gene in IBV-infected broiler chickens was generated. To examine the pattern of genetic variation in the samples acquired from various chicken sources, the amplified fragments were immediately submitted to direct sequencing tests. The four nucleic acid variants discovered in the current investigation were just a slight tilt within the same viral genotype, with no discernible evolutionary influence on the current placement of the R1 – R10 samples.

Ten samples of IBV local isolates (OL321799 to OL 321808) were grouped into two phylogenetic clades within the infectious bronchitis virus sequences after partial S-gene sequence alignment. The first clade consists of seven local samples (R1, R3, R4, R5, R7, R8, and R9), whereas the second clade consists of three viral samples (R2, R6, and R10). This result clearly suggests that the R1–R10 sequences are known genetic variants of Infectious Bronchitis Virus sequences. Our findings revealed that there were four nucleic acid variations, three of which (C>T94, G>A199, and G>A207) were discovered in all local samples and just one (C>T99) in seven local samples (R1, R3, R4, R5, R7, R8, and R9). As these viral samples inhabited the major branch of this cladogram, this type of location suggested that the discovered C>T99 variation played only a modest effect in producing a significant divergence from the original position.

Previous investigations by Zanaty *et al.* (2016) shown that Egyptian IBV isolates are grouped into two different genotypes based on phylogenetic analysis. Notably, the use of S gene sequences in the current investigation has provided additional evidence for the presence of a precise genotype identification of this viral organism.

As a result, these pieces of evidence are consistent with one another, indicating that the divergence of this poultry-infecting pathogenic viral sequences from nearby Iraqi – Iranian origins is plausible. When the NCBI BLASTn engine evaluated the S-gene partial sequence alignment of 10 IBV local isolates (R1-R10) to the published sequence from GenBank, the homology sequences among them were around (99 percent) and their identities dropped. A comparison of the S region of different IBV strains from across the world revealed that this gene segment formed a hypervariable region (HVR) (Cavanagh, 2007). According to a previous research from Sulaymaniyah (Iraq), the initial IBV isolate from

Sulaymaniyah (Sul/01/09) only differed by 6% from Egypt's Benisuef/01 isolate and 5% from Israel's (IS/885) and (Isr/720/99) isolates. While the nucleotide sequences of these four isolates are almost identical, with a similarity of 96–97%, (Mahmood *et al.,* 2011). The investigated samples (R2, R6, and R10), as well as the other seven viral samples (R1 to R7), were all within a few miles of the MG233398.1 entry number.

This IBV reference strain was very recently discovered in Iran (Mousavi et al., 2018). This IBV variant may have emerged as a result of genetic modifications that conferred changes in pathogenicity and antigenicity, leading to both mutation and recombination being involved in the emergence of IBV variants in the field and despite intensive vaccinations; this strain is considered the dominant genotype in Iran and has spread to other countries.

The Iraqi locally detected IBVs were divided into two genetic groupings, which were assigned accession numbers in the NCBI-bankit database (OL321799 to OL321808). They had a lot of nucleotide sequence in common with IBV isolates from Iran, which had whole genome strains published under accession numbers (GenBank acc. no. of MF322831.1, MF322829.1, MF322846.1, and KT583600.1). The country's long border and considerable economic ties with Iran, notably the import of chicken and poultry products since 2003, can be connected to the significant degree of similarity between Iraqi strains and Iranian isolates. Surprisingly, the Iraqi provenance of the investigated local samples cannot be overlooked. This was owing to their grouping into two groups surrounding three strains recovered from IBV-infected broiler chickens in southern Iraq (GenBank accession numbers KU143889.1, KU143887.1, and KU143885.1) (Seger *et al.,* 2016).

The results of alignment of the 10 local samples were checked against the original chromatogram to see if the modifications caused any changes in the spike protein's corresponding locations. In comparison to the most similar referring reference nucleic acid sequences, two variants exhibited two silent effects on the spike protein S1, namely p.T268= (p.Thr268=) and p.Q302= (p.Gln302=), as well as two missense mutations of two amino acid substitutions, namely p.T>M269 (p.Thr>Met269) and p.C>Y305 (p.Cys>Tyr305). These changes, on the other hand, might be created by the invading organism as a response to medications or vaccinations directed at its targeted spike protein (Legnardi *et al.,* 2020).

Comparison between infection due to IBV and vaccine strain IB4/91

The tree demonstrated that our ten local samples were compatible with Iranian and Iraqi strains of similar viral sequences in the nearby area for the examined S-encoded protein. Furthermore, considerable evolutionary gaps between the clade of our samples and the clade of vaccination strains have been identified.

Seventy-two (72) nucleic acid changes were discovered in the studied local samples that were infected with IBV when compared to strain IBV (4/91), a prototype of vaccinated strain. The sources of the recently researched poultry-infecting infectious bronchitis virus isolates were proven to be Iranian / Iraqi, according to the current study inquiry. Furthermore, it was concluded that

vaccination had no evolutionary influence on creating any detectable nucleic acid mutation in the virus strains studied. For these reasons, our research implies that S-based amplicons might be a useful tool for delving further into the genotypes of Infectious Bronchitis Virus sequences that have been found.

Conclusion

In a final conclusion, differences in the genetic buildup of our locally detected IBV and the vaccine strain were pointed up and might be related to vaccine failure to protect the broilers in our province against IBV infection, accordingly isolation of IBV from locally infected broilers and preparation of IBV vaccine from local isolates was strongly recommended to be under wide study for evaluation in comparison to commercial vaccines.

Authors' Contributions

KAJ: Designed the study, **KAJ**, **AKA, TJK and KSA**: collected the samples and processing in the lab and editing of the manuscript and analysis. All authors read and approved the final manuscript for submission.

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Competing Interests

The authors declare that they have no competing interests

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