Research Article

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Molecular and Pathological Investigation of Infectious Bronchitis in Broiler Flocks in Sulaimani Province, Iraq

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Abstract

This research's goal was to examine the molecular analysis and pathological lesions linked to IBV infection in flocks of naturally infected broiler chickens from non-vaccinated and vaccinated (H120, MA5+IB 4-91) flocks. One hundred sixteen birds were collected in Sulaimani provinces between November 2021 and July 2022 from various broiler chicken flocks of vaccinated (H120, MA5+IB 4-91) and non-vaccinated birds who suffered from respiratory illness. Viral isolation and PCR were used to confirm the infection. The majority of the necropsied birds showed congested trachea and the presence of mucus with caseated debris at the bifurcation. There was a white precipitate in the ureters, also the kidneys were enlarged and congested. Sequencing of the S1 glycoprotein gene revealed that four isolates found and after phylogenetic analysis of the strains' deduced amino acid sequences indicated that they had a close relationship with the Iranian strains MG013972 and MG013973 as well as the Iraqi strain MH747093. The non-vaccinated chickens' histopathological evaluation of all organs was more severe than that of the vaccinated chicks, with catarrhal exudate obstructing the tracheal lumen and tracheal epithelium undergoing hyperplasia to squamous metaplasia. In lung sections, catarrhal exudate partially to completely blocked the parabronchial lumen and the intrabronchial septa thickened due to alveolar capillary congestion, and an inflammatory reaction. Mild to severe glomerulonephritis with considerable collecting epithelial cell degeneration was seen in the kidney segment. Finally, it can be said that the infectious bronchitis virus is a significant contributor to the respiratory and/or renal issues affecting the flocks of broiler chickens in Sulaimani Province.

Keywards: H120, MA5+IB 4-91, Nephrotropic, SULZNS1-2022

الفحص الجزيئي والمرضي لالتهاب الشعب الهوائية المعدي في حقول فروج اللحم فى محافظة السليمانية

الخلاصة

يهدف هذا البحث لفحص التحليل الجزيئي والآفات المرضية المرتبطة بعدوى التهاب الشعب الهوائية المعدي في **حقول فروج اللحم** المصاب طبيعياً في الحقول غير الملقحة والملقحة. جمعت مائة وستة عشر طائرا من حقول مختلفة في محافظة السليمانية في الفترة ما بين تشرين الثاني **2021** و تموز **2022** من الطيور الملقحة ب **(H120, MA5+ IB 4/91)** وغير الملقحة التي كانت تعاني من علامات تنفسية. تم استخدام العزل الفيروسي و **تقنية النسخ العكسي لتفاعل البلمرة المتسلسل ل**لتأكد من الإصابة. **ظهرت** الطيور التي تم تشريحها حتقانا في القصبة الهوائية ووجود مخاط مع تنخر متجبن عند التشعب الرغامي. كان هناك ترسب أبيض في الحالبين مع وجود تضخم في الكلى واحتقانهما. كشف التسلسل الجيني للبروتين السكري **S1** عن وجود أربع عزلات وبعد تحليل النشوء والتطور لتسلسل الأحماض الأمينية المستخلصة للسلالات التي أشارت إلى وجود علاقة وثيقة مع السلالات الإيرانية **MG013972** و **MG013973** وكذلك مع السلالة العراقية **MH747093**. التقييم النسجي المرضي لجميع أعضاء الافراخ غير الملقحة أكثر شدة من تلك الموجودة في الافراخ الملقحة مع وجود نضحة نزلية مؤدية الى انسداد تجويف القصبة الهوائية فضلا عن خضوع ظهارة القصبة الهوائية للتضخم والى الحؤول الحرشفية. اما في مقاطع الرئة لوحظ بان النضحة النزلية تغلق التجويف شبه القصبي جزئيًا الى كليا وكذلك تتخن الحاجز داخل القصبة بسبب احتقان الشعيرات السنخية ورد فعل التهابي. لوحظ التهاب خفيف الى شديد في كبيبات الكلى مع ملاحظة وجود تنكس كبير فيالنبيبات الكلوية . أخيرًا يمكن القول بأن فيروس التهاب الشعب الهوائية المعدي يساهم بشكل كبير في مشاكل الجهاز التنفسي و / أو الكلوي التي تؤثر على فروج اللحم فى حقول التربية فى محافظة السليمانية.

Introduction

The avian gammacoronavirus, which causes infectious bronchitis virus (IBV), is an acute and highly contagious upper respiratory tract disease that affects chickens and other poultry. IBV causes severe economic losses in the poultry industry, particularly in young birds, and it also causes loss of appetite, growth retardation, and mortality (1-3).

IBV is a member of the Coronaviridae family and is made up of a single positive-sense RNA that is around 27 Kb in length and encodes the four structural proteins nucleocapsid (N), membrane (M), envelope (E), and spike (S) (4). The SI gene is one of the effective genomic markers for distinguishing between IBV strains (5, 6). The virus seems to have its natural host in chickens and other bird species (7). Infectious bronchitis (IB) is an airborne illness spread through both direct and indirect ways (8).

Infectious Bronchitis may cause dyspnea, coughing, sneezing, depression, nasal discharge, and mortality, among other clinical symptoms. Some strains of IBV, on the other hand, can reproduce in the ciliated epithelial cells of organs such as the kidney, reproductive organs, and enteric tracts, causing severe nephritis caused by enteropathogenic IBV strains (9), and in broiler breeders, reproductive problems (10).
The virus also damages the kidneys and is thought to be a predisposing factor for respiratory bacterial infection, particularly air vasculitis, as well as other pathological effects on crucial organs that result in decreased egg production and quality (11).

Vaccination programs mainly depend on IBV strains such as H120, MA5, Massachusetts, and 4/91, also the most commonly utilized IBV vaccine strains in Iraq (12). Despite the use of these vaccinations, the prevalence of IBV in vaccinated chicks continues to have a considerable economic impact, and a new strain (Sul5/17) has been discovered in Sulaimani City in the Kurdistan region (13). Vaccination resistance upon IBV commonly occurs as a result of the virus's genetic material evolution (14). Although both inactivated and live attenuated vaccinations are being used to combat IBV in poultry farms in Iraq with the (H120 and 4/91) strains, outbreaks of IB have nevertheless been noted on broiler farms (15). The first report of the detection and genotyping of IBV isolates in Iraq came from Kurdistan-Iraq and showed the appearance of 793/B (with a prevalence rate of 25%) and a novel IBV variant (Sul/01/09) in vaccinated (Ma5, or 4/91) broiler farms (16).

According to prior research, the inadequacy of the vaccinations to protect against the various genotypes of IB means that it continues to create major problems for the Iraqi/Kurdistan region poultry industry. The differential diagnosis of poultry in Iraq is only based on clinical indicators and gross lesions due to the limited amount of poultry diagnostic laboratories in the nation of Iraq. The purpose of the research was to determine and describe the pathologic lesions formed by naturally produced neuropathogenic strains of IBV in vaccinated and nonvaccinated broiler farms in Sulaimani province. The characterization of IBV has raised further issues in terms of both epidemiology and control. Furthermore, the samples were identified using PCR based on partial S1 sequencing and histopathology methods, which allowed us to compare the groups of vaccination strain(s) that have already been used in our area with the non-vaccinated strain.

Materials and Methods

Location of the study and collection of samples:

The current investigation was conducted between November 2021 and July 2022. One hundred sixteen (116) suspected chicken samples in the Sulaimani Province, have been taken from multiple industrial broiler farms at various times and from five distinct aged groups (group 1; n=1st week, group 2; n=2nd week, group 3; n=3rd week, group 4; n=4th week, and group 5; n=5th week) were gathered non-vaccinated and vaccinated (H120 and MA5+IB4-91), the infection was frequently observed in the second and third weeks. Some chicks in the infected groups had typical IB clinical indications, such as tracheal rales, coughing, and gasping at 21 days. The chicks were killed through cervical dislocation before being exposed to gross and microscopic examination. Tissue samples (1 cm) from the trachea, lung, and kidneys were obtained from each chick after being inspected grossly and taken a capture from each of them and deposited in two different containers, the first containing 10% neutral buffered formalin for histological investigation and kept at room temperature in the dark area. The second container for molecular analysis is kept at -20 °C.

Molecular approaches were used to identify chicken IBV S1:

Preparation of the Sample:

The receiving organs, which included trachea, lung, and kidney tissue samples (0.5-1 gm), were collected and mixed in a five-volume RNA/later solution in a sterile tube before being stored at -20°C. Phosphate-buffered saline (PBS) (10% wt/vol) was used to homogenize each tissue sample. By centrifuging the homogenates samples at 4 °C for 30 minutes at 13000 rpm, the samples were made clear 200µl of the supernatant was taken and used for the RNA isolation. According to the manufacturer's instructions, viral RNA was isolated using a commercial small kit (Addprep Total RNA extraction kit, Korea).

RT-PCR:

An Addscript RT-PCR kit was used to perform one-step RT-PCR for the virus's detection (5UTR, 143 bp) and typing (Spike gene, 448 bp) as seen in (Table 1). Briefly, The RNA was reverse transcribed and amplified in 20 µl reaction mix by using 8 µl of 2.5X reaction buffer, 1 µl (10 µM) of each primer, 1 µl (20X) add script-enzyme mix, 1 to 3 µl RNA, and the volume was finished by adding diethyl pyrocarbonate-treated H_2O. At first, the PCR reaction was set at 52°C for 30 min to reverse transcriptase RNA to cDNA, then it was followed by an initial denaturing step starting at 95 °C for 10 min, followed by 35 cycles of denaturation at 95°C for 30 sec, annealing at 55°C for 30 sec for primer (5UTR, 143 bp) at 55°C and for (Spike gene, 448 bp) at 52°C, extension at 72°C for 1 min, final extension step was been at 72°C for 5 min to amplify the sequence and finally the reaction was held at 4°C.

Table (1): Target genes primers for detection and typing of the virus.

GENE	Name	Primer	Purpose	Size	Annealing
5UTR	5UTRF 143	5′-GCTTTTGAGCCTAGCGTT-3′	Detection	143 bp	55
	5UTRR 143	5′-GCCATGTTGTCACTGTCTATTG-3′			
SPIKE GENE	SGF 448	5′-GTTTACTACTACCAAAGTGCCTT -3′	Sequencing	448 bp	52
	SGR 448	5′-GTGTAAACAAGGTCACCATTTA-3′			

Gel Electrophoresis:

The amplified bands were found by running the amplicon across a 1% agarose gel. The PCR products were separated using electrophoresis on a 1% agarose gel (Cleaver Scientific LTD, UK). The preparation of agarose gel involved mixing 0.75 g of agarose with 50 ml of TBE buffer. The gel was cast onto the tray after being dyed with safe dye (EURx, Banino, Poland), combs were added, and the gel was allowed to harden for 20 minutes. The 10 µl PCR products were set into the wells. The central well of the gel was loaded with 10 µl of a 100 bp DNA ladder (Add Bio in the Republic of Korea). After 45 minutes of electrophoresis on the gel at 100 volts, DNA was viewed under UV light, and the picture was analyzed and captured using a gel documentation device (SYNGENE, Ingenius3, UK).

Sequencing analysis:

The GenBank database was used to gather partial nucleotide sequences of the spike glycoprotein S1 gene from all around the world. MEGA 7 used the ClustalW technique to construct multiple alignments of these sequences (17,18). MEGA 7 was used to conduct phylogenetic analysis with Neighbor-Joining (NJ). The bootstrap values were calculated using 1000 replicates of the original data.

Examinations of Histopathology:

Tissue samples about 1 cm were collected from the trachea, lung, and kidney tissues after taking gross examination of each them, the tissues were fixed for at least 48 hours in 10% neutral buffered formalin, then processing the samples through a graded series of ethanol solutions for sample dehydration. After being dipped with xylene baths, the tissues were preserved in paraffin wax and from each paraffinized block samples 4µm sections were placed on glass slides and stained with hematoxylin and eosin dye (H&E) and the slides were inspected using an eyepiece grid under a microscope at 10-400 magnification by a pathologist who was blind to the study, the lesions were estimated by conventional light microscope (Leica, Germany) through computer-assisted image investigation software to study slices (Am Scope^{TM}, Japan). All this techniques procédures was held in the Histopathology Lab of Anwar Shexa Medical City/Sulaimani Governorate .

Statistical Analysis:

Statistical analysis were performed with SPSS statistics version 24 (IBM, SPSS Inc, Chicago, USA). A p-value < 0.05 was considered statistically significant. The Shapiro-Wilk test was used to test the normality of the data. Data were analyzed by Duncan test evaluating the IB infection in both vaccinated and non-vaccinated groups and different ages groups of chicken.

Results and Discussion

Molecular evaluation

The RT-PCR finding results of the five groups of age were presented in Tables 2 and 3. Among the 116 tissue samples, there were 101 positives (87.06%) for IB and 19 positives (18.81%) for H120 vaccinated, 26 positives (25.74%) for IB MA5+IB4-91 vaccinated, while 21 positive (20.79%) for IB non-vaccinated. There are no significant differences between the vaccinated and non-vaccinated groups, whereas multiple differences exist between different age groups which are highly significant difference ($P< 0.05$) as seen in table 2 by different letters. The viral genome was amplified using RT-PCR, which amplified the 143 bps 5UTR and the 448 bps Spike Glycoprotein S1gene region (Figure 1). Al-Jameel and Al-Mahmood concluded that the

findings demonstrated that the virus may harm birds at all ages (19).

Table (2): For each age group of broiler chickens, a molecular analysis of IBV-positive test findings was performed.

Groups	Numbers	Positive result		
		accinated		Non vaccinated
		H120	MA5+IB4-91	
1st week	4	1 (%0.9)	1 (%0.9) a,b,c,d	1(%0.9) a
2nd week	24	4 (%3.9)	8 (%7.9) a	6(%5.9) a
3rd week	41	10 (%9.9)	12 (%11.8) b	11(%10.8) a
4th week	23	4 (%3.9)	4 (%3.9) c	3(%2.9) a
5th week	9	0 (%0)	1 (%0.9) d	0(%0) b
Total	101	19	26	21

Various letters are used to indicate the variations between the age groups (P< 0.05).

Table (3): For each age group of broiler chickens, molecular analysis was performed on the negative IBV sample results.

Groups	Numbers	Negative result		
		Vaccinated		Non vaccinated
		H120	MA5+IB4-91	
1st week	4	/	/	1(%0.9)
2nd week	24	2 (%1.9)	2 (%1.9)	2(%1.9)
3rd week	41	2 (%1.9)	2 (%1.9)	4 (%3.9)
4th week	23	3 (%2.9)	4 (%3.9)	5 (%4.9)
5th week	9	2 (%1.9)	3 (%2.9)	3 (%2.9)
Total	101	9	11	15

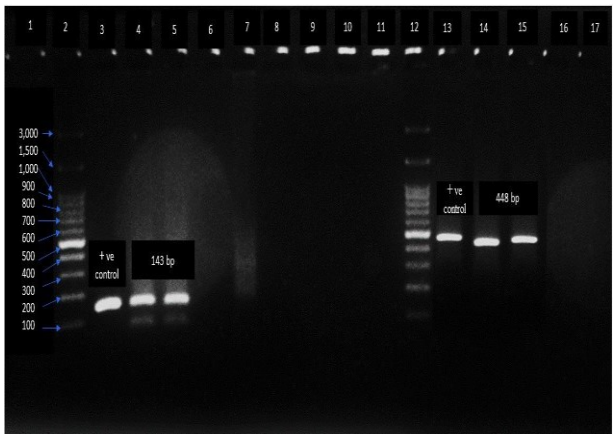

Figure (1): IBV-positive infection is indicated by an amplicon with the proper size (448 bp for the spike gene and 143 bp for the 5UTR) on an agarose gel electrophoresis.

Sequence analysis

The virus's Spike Gene S1 region was amplified by PCR and sequenced in 25 ml (Macrogen sequencing service, Korea) in both directions (forward and reverse). Sequences were aligned using Mega X software, and then they were uploaded to GenBank with the accession number (OP038915), identifying the isolate as SULZNS1-2022. Field-isolated viruses (SULZNS1-2022) showed a relationship to other Iranian strains in the partial S1 gene nucleotide sequences of the chosen strains, which were around 362 nucleotides long. As shown in Figure 2, a phylogenetic comparison of the infectious bronchitis genomes shows that individual isolates cluster primarily depending on the host to which they corresponded to that of the Iranian IBV strains (MG013972 and MG013973) were closely related to the current strain's SGS1gene sequence (SULZNS1-2022), with the highest identities (89-90%). Phylogenetic analysis confirmed the appearance of novel variations (OP038915) in Sulaimani Governorate and revealed the greatest identity of the new isolate with an Iraqi strain (MH747093) by 99% similarity (20), while the two Iranian strains (MG013972 and MG013973) demonstrated 89-90% sequence similarity with Glade I based on the complete genome and capsid protein versus our strain genome relied on a partial S1 gene sequence (21). This discovery indicates that the Iranian strain is at least one of the primary causes of IBV infection in our nation Kurdistan/Iraq.

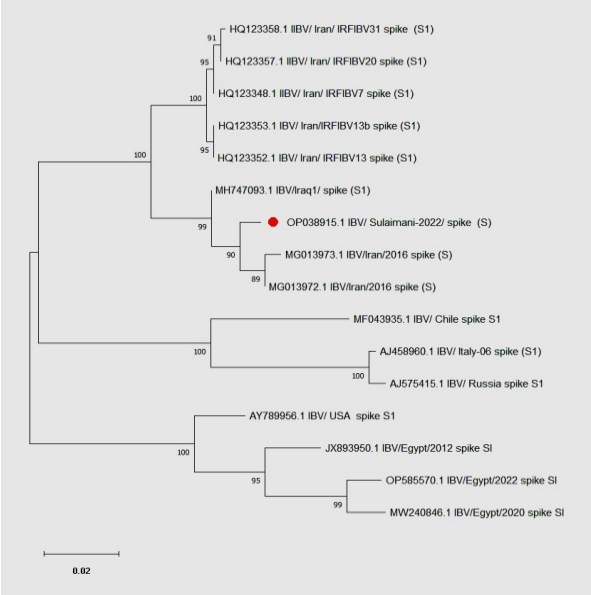

Figure (2): Based on the partial Spike glycoprotein S1 gene nucleotide sequences, a phylogenetic tree of IBV relationships has been constructed. Using the neighbor-joining approach and 1000 bootstrap repetitions, the tree was created using the MEGA X program.
Red cubic highlights the viruses that have been given genogroup designations.

Alignment of the sequence

Several sequences of the field virus's partial spike glycoprotein S1 gene were aligned with the amino acid residues of other reference strains, revealing that certain locations were different among all references and were replaced by new amino acids, such as the amino acid residues at positions (Asparagine -N31) instead of (Therionine-T31) and (Asparagine -N56) instead of (Histidine-H56), as shown in (Figure 3).

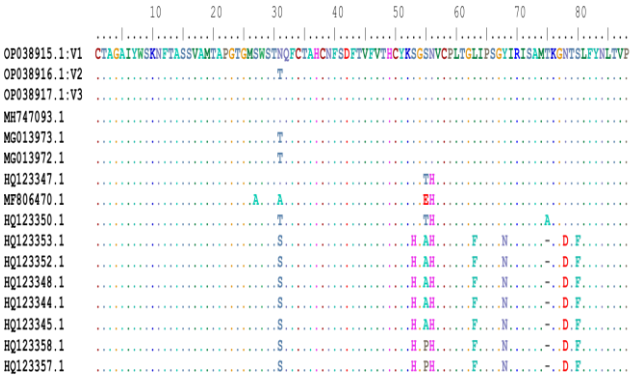

Figure (3): Numerous sequence alignments of the amino acid sequences of the field isolate's partial spike glycoprotein S1gene region (SULZNS1, S2, and S3 -2022) with other reference strains.

Gross observations and histopathologic alterations

Gross necropsy findings

At necropsy, abnormalities in the respiratory system, particularly the trachea and kidney of chicks were detected. The trachea was markedly congested in the non-vaccinated group (Figure 4a) in comparison to the infected chicken from the H120-vaccinated group displayed significant tracheal congestion, especially in the proximal and distal portions (Figure 4c) vs. to the chick groups that received the MA5 and 491 vaccinations had moderate to severe tracheal edema and congestion (Figure 4e). In non-vaccinated group, the kidney was significantly congested and displayed a characteristic lobulation pattern, as well as a swollen kidney with a distended ureter containing urate were detected (Figure 4b). While, in the H120-vaccinated group, the kidneys revealed severe edema and congestion with enlarged form, and distended ureters with urates (Figure 4d) in comparison to the MA5 and 491 vaccine group that showed, swelling-pale kidney, vascular congestion with noticeable enlargement, and dilated ureter with urate (Figure 4f). In accordance with the previous studies that revealed abnormalities in various organs mostly nephropathogenicity (22).

Infected bird in non-vaccinated group, histopathologic analysis of the tissues demonstrated severe hyperplasia of the tracheal epithelium with total loss of goblet cells and blockage of the tracheal lumen by catarrhal exudate mixed with necrotic debris (Figure 5a, b). The findings of the present investigation were in agreement with those of previous research teams that demonstrated IBV replication inside tracheal cells resulted in cellular necrosis and damage with epithelial hyperplasia (23). In addition, Seifi and Boroomand (2015) found that the tracheal epithelium indicated complete deciliation, rupture, the production of abundant mucus, and goblet cell hypertrophy (24). While, chicks that had received the H120 vaccine and had IB infection were examined histopathologically. The tracheal sections showed marked hyperplasia with sloughing of the lining epithelium and loss of goblet cells, severe vascular congestion, and infiltration of inflammatory cells, particularly macrophages, and heterophils from the lamina propria to the adventitia (Figure 6 a,b). Grgić et al. (25) suggest that IBV has a significant association with various phases of tracheal hyperplasia in accordance with our findings received the H120 vaccine. In comparison to the non-vaccinated and H120-vaccinated groups, the IB 491+MA5-vaccinated group showed marked sloughing of lining epithelium with loss of goblet cells and obstruction of the tracheal lumen by catarrhal exudate, severe edema, and congestion, especially in the mucosal layer, with mucous inflammatory exudate diffusely via all layers (Figure 7 a,b).

The lung section in the non-vaccinated group displayed inflammatory reaction, particularly heterophil infiltration, and marked congestion of pulmonary vasculature and alveolar capillaries. The parabronchial lumen was partial to completely blocked by catarrhal exudate, sloughing of parabronchial lining

epithelium, and the intrabronchial septa was thickened due to alveolar capillaries congestion (Figure 5c, d). Following the previous research, which revealed that the trachea and lungs demonstrated hyperplasia and degenerative alterations in their epithelium, the tertiary bronchi and atria were filled by homogeneous eosinophilic material with mononuclear cell infiltrations (26). The additional documentation showed distinct histological lesion findings in all IBV-infected birds, including the primary and secondary bronchi epithelial hyperplasia, and mononuclear inflammatory cell infiltration of the lamina propria (27). The lung in H120 vaccinated group section showed sloughing of parabronchial lining epithelium with the presence of necrotic debris in their lumen, severe pulmonary vascular congestion, and alveolar capillaries congestion is also seen, thickening of inter-bronchial septa due to hyperplasia of interatrial tissue and alveolar cells with necrotic debris inside the atrium, the inflammatory reaction within atrium (Figure 6 c,d). While the lung portions in the IB 491+MA5-vaccinated group revealed partial and complete obstruction of the parabrachial lumen by necrotic debris with mucous exudate, sloughing of the parabronchial lining epithelium, thickening of the intrebronchial septa due to interatrial cell hyperplasia, severe congestion of the pulmonary vasculature and air capillaries, and an inflammatory response throughout the parenchyma, especially infiltration of heterophil and macrophages (Figure 7 c,d). In disagreement with our study, the previous report indicated that Nobilis Ma5 and IB 4/91 vaccines administered at 1 day and 14 days, correspondingly, keep chickens from IB infection and decrease the severity of infection (28).

In non-vaccinated chicken group, the kidney sections showed moderate proliferative glomerulonephritis along with severe mesangial cell proliferation was seen in the renal section, marked degeneration of collecting tubules, and moderate interstitial hemorrhage (Figure 5e, f). The general results in the renal tubules concurred with earlier investigations, as did those noted by (29,30). While in H120 vaccinated group, the kidney section showed focal atrophy of glomeruli, marked degeneration of collecting tubules, and interstitial hemorrhage with infiltration of few inflammatory cells including heterophil (Figure 6 e, f). In accordance with the study's findings, dilated renal tubules were blocked by intra-luminal casts comprising a combination of necrosed epithelia, mucus, disintegrating heterophils,andproliferativebglomerulonephrit is (31). In comparison to the IB 491+MA5-vaccinated group, kidney segments showed mild mesangial cell and matrix proliferation with enlargement of Bowman's space, marked degeneration of collecting tubules, interstitial hemorrhage, and infiltration of heterophil and macrophage inflammatory cells, also showed moderate proliferative glomerulitis with swelling of glomeruli (Figure 7 e, f). Moreover, the present research's histological investigation of renal tissue concurred with the previous studies' findings of renal tubular degeneration and necrosis with the inflammatory response (32,33).

The type of vaccine used and the technique of vaccination impact how the clinical challenge turns out (34). The severity of the lesions might be impacted by poor care. The severity of certain instances may have been exacerbated by an additional illness present at the same time (35).

The vaccination program in use in our région was unable to protect broiler farms from IBV illness and was not able to reduce the economic harm brought on by infectious bronchitis disease as a result of the sequence analysis provided in this study. The failure of

vaccination programs to defend poultry against IBV is related to the chickens' inability to produce an adequate immune response after infection, décause the vaccination prépare from non-local strains of IB (36)

The selection of the most virulent serotypes, choosing the applications based on flocks that need to be revaccinated, and the potential for long-term protection are all related to using appropriate IBV vaccines. Farm owners criticize vaccines for failing to immunize their flocks. Poor vaccine administration was shown to be responsible for more than half of immunization failures in flocks that had received vaccinations. Moreover, the quality of vaccine administration and storage as well as the increased risk of vaccine delivery are crucial reasons for vaccination rejection that result in IBV outbreaks in vaccinated farms (37).

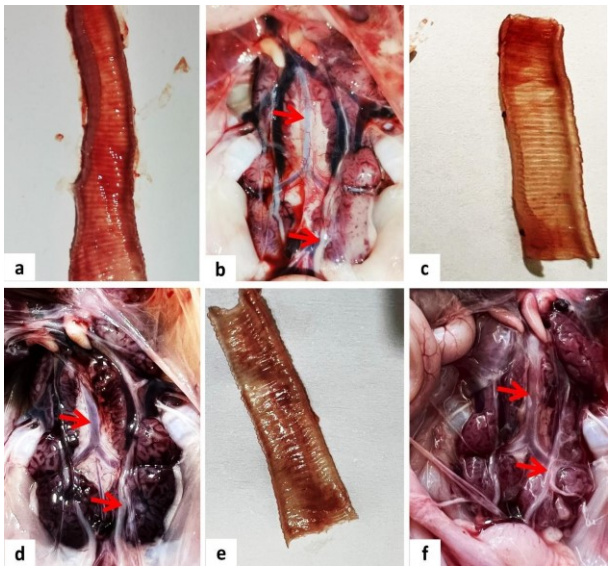

Figure (4): Gross lesions of IB-infected chicks revealed abnormalities in the trachea and kidney in images: a and b; the trachea displayed edema and severe congestion, a palecongested kidney showed swelling with pus exudate (red arrows) and lobulation, and the nonvaccinated group's ureters were swollen and filled with urates, c, and d: In the H120vaccinated group, e, and f, there were moderate tracheal congestions, severe tracheal congestions, swollen kidneys, noticeable enlargements and distended ureter with urate (red arrows), also in the MA5 and 491 vaccine group, and there was moderate to severe edema and congestion of the trachea. The kidneys were palely swollen and had vascular congestion with obvious enlargement, The ureters were filled with urates (red arrows).

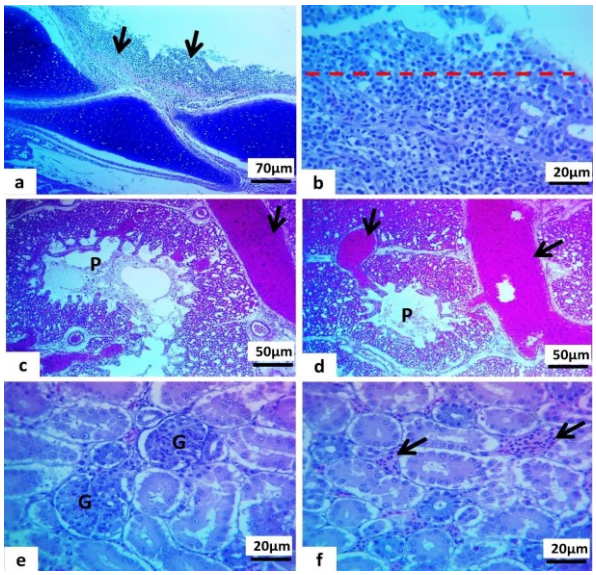

Figure (5): The following characteristics of non-vaccinated chicks with IB infection were seen in microscopic sections: a: Marked hyperplasia (red dashed line) of the lining epithelium with total loss of goblet cells, and occlusion of the tracheal lumen by catarrhal exudate. c, and d: Interatrial septa hyperplasia, inflammation throughout the parenchyma, sloughing of the parabronchial lining epithelium, thickening of the interbronchial septa, and partial to complete obstruction of the parabrachial (P) lumen by mucous exudate are all symptoms of this condition. e, and f: Moderate proliferative glomerulonephritis (G) with significant mesangial cell and matrix proliferation, noticeable collecting tubule degradation, and interstitial bleeding, as shown by black arrows, (H&E stain).

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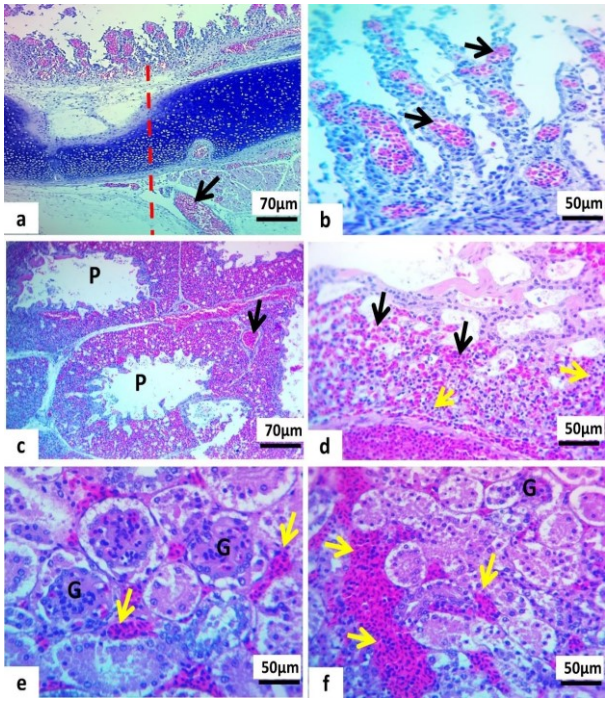

Figure (6): Microscopic analysis of IB-infected (H120-vaccinated) chicks showed: a and b: significant hyperplasia with sloughing of lining epithelium and loss of goblet cells, severe congestion (red arrows), and infiltration of inflammatory cells across all layers. c and d: Necrotic debris within the atrium with hyperplasia of interatrial septa, the inflammatory response inside the atrium and interatrial septa (yellow arrows), sloughing of the parabronchial lining (P) epithelium with necrotic debris in the lumen, thickening of the interbroncial septa, severe vasculature (black arrows) and air capillaries congestion, e and f: The glomeruli (G) show localized atrophy, the collecting tubules show clear degeneration, and there is interstitial bleeding as seen by the yellow arrows, (H&E stain).

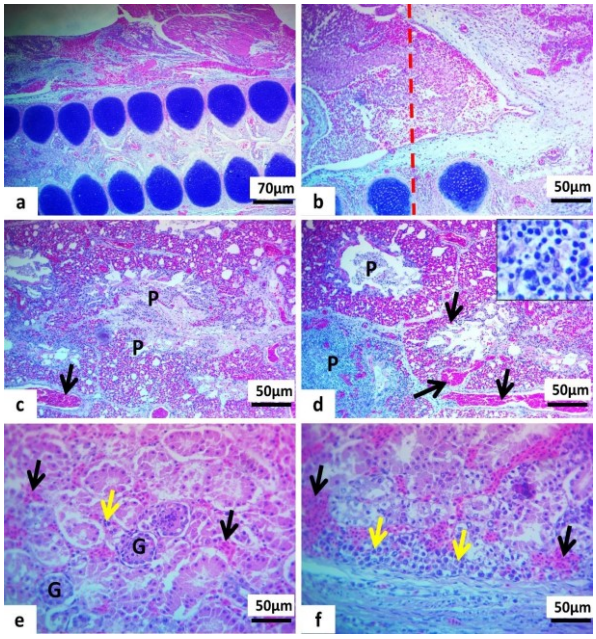

Figure **(7):** The subsequent characteristics can be seen in a microscopic portion of IB-infected chicks given the IB 491+MA5 vaccine: a: marked lining epithelium sloughing with loss of goblet cells, obstruction of the tracheal lumen by catarrhal exudate, severe edema and congestion, and marked inflammatory response diffusely across all layers (red dash line). c and d: The parabronchial (P) lumen is blocked by necrotic debris and mucous exudate, the parabronchial lining epithelium sloughs off, the interbronchial septa thickens, the air capillaries and vasculature are severely congested (black arrows), the interatrial septa are hyperplastic, and the parenchyma is inflamed overall (inset). e and f: Moderate glomerular swell (G), mild mesangial cell and matrix proliferation with enlargement of the bowman's space, significant collecting tubule degeneration (black arrows), interstitial bleeding, and infiltration of inflammatory cells are all seen in the H&E stain.

Conclusions

The analyses of phylogeny reported the formation of new variations in the Governorate of

Sulaimani. The IBV OP038915 isolate is the most comparable to the Iraqi (MH747093) and Iranian (MG013972 and MG013973) strains. It is most likely among the most prevalent IBV

strain in Kurdish broiler farms. According to the sequence analysis results given in this study, the vaccinations supplied were ineffective in protecting broiler farms from IBV infection and in reducing economic losses caused by infectious bronchitis illness. As a result, additional research into the most effective vaccine strain with strong protection against IBV respiratory illness is required.

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Conflict of Interests

All authors declare no conflict of interest.

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