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Genotypic Analysis and Pathological Findings of Infectious Bronchitis Virus in some Poultry Farms in Ismailia, Egypt

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ABSTRACT

Infectious bronchitis virus (IBV) variants constantly emerge and causes significant economic losses by affecting both vaccinated and unvaccinated chickens. This study, conducted in 2022-2023, investigated frequent outbreaks of Infectious Bronchitis Virus (IBV) in broiler and layer chicken flocks in Ismailia Governorate, Egypt, despite widespread vaccination. Samples were collected from 10 farms, each employing a vaccination regime of H120 (live attenuated vaccine) on the first day, followed by 4/91 vaccination after a week. Despite vaccination, clinical signs of IBV appeared after 25 days, with mortality rates of 20-30% and morbidity rates of 40%. Pooled trachea, lung, and kidney samples from the flocks underwent Reverse Transcription-Polymerase Chain Reaction (RT-PCR) analysis, focusing on a 457bp fragment within the hypervariable region of IBV. Out of 10 farm, two samples were positives for IBV. Nucleotide and amino acid sequence analyses were conducted on one of the positive samples. Phylogenetic analysis identified our isolates as belonging to the Genotype I clade 23 (GI-23), clustering with the VAR II strain previously isolated in Egypt, showing an 84% identity to S1 gene sequences with GenBank accessions MH021175 and KU79007. Histopathological examinations revealed significant changes in the infected birds. Tracheal samples displayed hyperplasia and degenerative

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changes in the epithelium, accompanied by edema. Lung tissues exhibited pneumonia, bronchitis, bronchiolitis, perivascular edema, and leukocyte infiltration. Kidney samples showed lymphocytic aggregation in the cortex and medulla, epithelial degeneration, and hemorrhage. These findings underscore the persistent challenge of IBV in poultry, even in vaccinated flocks, and the need for ongoing surveillance and vaccine development.

INTRODUCTION

Nowadays the disease is endemic in most of the countries that possess a developed commercial poultry industry. Its global economic impact has been estimated as the second most damaging poultry disease after highly pathogenic avian influenza **(TAFS-Forum, 2011).**

Infectious bronchitis virus (IBV) can replicate efficiently in a wide variety of epithelial cells of respiratory, renal, reproductive, and digestive tracts **(Carstens EB, Lefkowitz EJ, 2012).** The genome encodes 4 structural proteins, 15 nonstructural proteins (nsp 2–16), and at least 5 lineage-specific accessory proteins **(Dent et al. 2015).** IBV is a single-stranded, positive sense RNA virus of the *Coronaviridae* family, genus *Gammacoronavirus*[1]. The viral genome comprises two untranslated regions (UTR's) in its 5' and 3' ends, two overlapping reading frames (ORF's) encoding the structural polyproteins 1a and 1ab, and the region encoding the main structural proteins i.e. spike (S), envelope (E), membrane (M) and nucleocapsid (N). Finally, two accessory genes ORF 3 and ORF 5 encode for proteins 3a, 3b and 5a and 5b **(Ziebuhretal, 2000).** The S protein is located on the surface of the virus external membrane. The S1 portion of the S protein of IBV is responsible for viral attachment to host cells, virus variability and eliciting neutralizing antibodies in chickens **(Cavanagh 1983, Cavanagh and Davis 1986, Kusters et al. 1987, Casais et al. 2003).** At the genomic level, these differences can be greater or equal to 5% in the hypervariable region of the S1 gene. These variant strains are usually restricted to geographic regions and most of the time are transient **(Gallardo et al. 2016)**, reason why diagnostics and epidemiological surveillance are crucial to determine their existence and persistence. This strategy helps planning preventative measures in the field. The genome undergoes genetic recombination and spontaneous mutation leading to the emergence of new variants having low level of cross-protection and complicates the control program by vaccination **(Cavanagh and Gelb 2008; Jackwood et al. 2012**).

Vaccines capable of inducing cross protection against different genotypes of IBV are of paramount economic and practical importance **(Gelb, 2018).** Some commercially available vaccines are heterogenous, meaning that the predominant subpopulations in the vaccine are diverse and do not induce protective immune responses in chickens **(Ghetas et al. 2014).** Several conventional and molecular diagnostic methods have been described for the diagnosis of IB in chickens. Induction of apoptosis in kidney cells is a major contributor of pathogenicity for virulent nephron pathogenic IBV **(Liu et al. 2017**).

Infectious bronchitis virus (IBV) is a highly evolving avian pathogen that has increasingly imposed a negative impact on poultry industry worldwide. In the last 20 years, IBV has been continuously circulating among chicken flocks in Egypt causing huge economic losses to poultry production.

In 2019, IBV/CK/EG/Fadllah-10/2019 (GenBank accession number MK562092), a pathogenic 4/91 IBV strain belonging to GI-13 lineage IBV was isolated from Ma5-vaccinated layer flock and identified based on full S1 glycoprotein analysis. The isolated strain showed 88–90% amino acid identity with the currently used 793/B vaccine group (CR88 and 4/91) and was described as a new genotype-lineage expression (GI-13 lineage) in Egypt **(Rohim et al. 2019**).

The aim of the present study is to provide insight into the importance of surveillance pro-

grams and share our perspectives for better control of IBV circulating in Egypt.

MATERIALS and METHODS

1- Ethical approval

There is no experimental work was done on chicken during this study, it was only employed for samples collection. The usage of chicken and protocol were conducted in accordance with the European Communities Council Directive 1986 (86/609/EEC) guidelines and approved by the animal care the handling of chickens was based on the ethical role of the National Animal Health and Research Institute, Giza, Egypt.

This study did not involve direct experimentation on chickens. Rather, it focused exclusively on sample collection for research purposes. All procedures involving chickens adhered strictly to the guidelines of the European Communities Council Directive of 1986 (86/609/EEC). Additionally, the handling and care of the chickens complied with the ethical standards set forth by the Animal Health Research Institute (AHRI), Egypt

.2-Samples collection:

Throughout 2022 and 2023, 50 samples were obtained from various chicken flocks in Ismailia governorate, Egypt. These samples were sourced from poultry exhibiting clinical symptoms of respiratory distress. The chickens exhibited various respiratory symptoms, including nasal discharge, gasping, rales, and sneezing, along with instances of diarrhea., coupled with high mortality and morbidity. From each of the 10 farms, five birds were selected for virus isolation and necropsy. Samples including sections of the trachea, lungs, bronchi, liver, and kidney were collected from each bird. For virus isolation, these samples were pooled and aseptically placed in transport media. Another set of organ sample were preserved in 10% formalin for histopathological studies, while a third set was frozen for PCR analysis.

3-Histopathological examination:

For histopathological evaluation, specimens from the trachea, lungs, and kidneys were fixed in 10% neutral buffered formalin. These were then processed using standard paraffin embedding techniques. Paraffin-embedded sections, approximately five microns thick, were stained with hematoxylin and eosin for microscopic examination (**Suvarna et al. 2013**).

4-Virus isolation protocol

Virus isolation was carried out according to the method described by **(Momayez, et al. 2002).** Briefly, pooled samples were homogenized to give approximately 10% (w/v) suspension in PBS PH 7.2 containing 100IU/ml penicillin, 100ug/ml streptomycin, and 30 IU amphotericin B/ml. The homogenized samples were centrifuged at 1000g for 15min at 4c and then filtered through a 0.45um filter membrane. The supernatant was inoculated at 0.2ml via the allantoic cavity of groups of ten 9-11 day-old SPF eggs. Inoculated eggs were checked twice a day. Those that died within 24h after inoculation were discarded. Mortality between 2- and 7-days' post inoculation (PI) were considered to be virus specific. The allantoic fluid was harvested aseptically from embryos that died between 48 and 72h PI, if the fluid showed no Hemagglutination (HA) activity. Dead embryos were examined for the presence of embryo stunting, curling, urates in the mesonephrose, or focal necrosis in the liver. On day 3 PI, part of the eggs was removed from the incubator and were placed at 4c for 24h and the allantoic fluid was collected for the next passage.

Virus isolation followed the methodology described by **Momayez et al. (2002).** Briefly, pooled samples were homogenized to form a 10% (w/v) suspension in PBS (pH 7.2) with antibiotics. After centrifugation and filtration, 200 µl of the supernatant was inoculated into SPF eggs via the allantoic cavity of groups of ten 9-11 day-old. Eggs were monitored twice daily for specific mortality patterns post-inoculation, those that died within 24h after inoculation were discarded. Mortality between 2- and 7-days' post inoculation (PI) were considered virus specific. Embryonic anomalies post-mortem was also recorded. Dead embryos were examined for the presence of embryo

stunting, curling, urates in the mesonephrose, or focal necrosis in the liver. On day 3 PI, part of the eggs was removed from the incubator and were placed at 4c for 24h and the allantoic fluid was harvested for further analysis.

5- RNA Extraction and PCR Analysis:

Viral RNA was extracted from infected allantoic fluid of the third egg passage and from clinical pooled samples using a DNA/ RNA extraction kit (QIAampminkit, Qiagen, Germany) as recommended by the supplier.

Briefly, 140 µl of the sample suspension was incubated with 560 µl of AVL lysis buffer and 5.6 µl of carrier RNA at room temperature for 10 min. After incubation, 560 µl of 100% ethanol was added to the lysate. The sample was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 60 µl of elution buffer provided in the kit.

Viral RNA was extracted from infected allantoic fluid and pooled clinical samples using the QIAampminkit (Qiagen, Germany), following the manufacturer's protocol. After incubation with lysis buffer and ethanol, the lysate was washed, centrifuged, and the nucleic acids eluted. The extracted RNA was then used for both conventional and quantitative real-time RT-PCR (qRT-PCR). The qRT-PCR, targeting the conserved regions of the IBV genome, primarily served as a screening tool due to its inability to quantify strains concurrently present. Amplification of the IBV's S1 gene was performed using the Qiagen one-step test kit as per the manufacturer's instructions.

Quantitative Real-Time RT-PCR Methodology:

Quantitative real-time RT-PCR (qRT-PCR) allows the quantification of the targeted genetic material present in the sample. Real-time assays targeting conserved regions of IBV genome cannot quantify the respective contribution of simultaneously present strains, limiting their usefulness to screening purposes. Rt-PCR was used to amplify the S1 gene of IBV using Qiagen one step test Kit according to manufacturer's instructions.

The quantitative real-time RT-PCR (qRT-PCR) technique employed in our study enables the precise quantification of specific genetic sequences within the samples., For our purposes, qRT-PCR primarily served as a tool for screening targeting conserved regions of the IBV genome.

The S1 gene of IBV was amplified using the Qiagen One-Step Test Kit, adhering strictly to the guidelines provided by the manufacturer.

Table 1. Primers and probe used in qRT-PCR IBV amplification.

Primer	Type	Sequence (5′-3′)	References
IBV5_GU391	Forward	5-GCT TTT GAGCCT AGC GTT-3	(Callison et al., 2006)
IBV5_GL533	Reverse	5-GCC ATG TTG TCA CTG TCT ATT G-3	
IBV5-G probe	Probe	5-FAM-CAC CAC CAG AAC CTG TCA CCT C-BHQ1-3	

For the detection of IBV RNA, lyophilized primers and probes were first subjected to centrifugation. Subsequently, they were reconstituted in nuclease-free water or TE buffer to create a 100pmol concentration stock solution. This stock was then thoroughly mixed. Working solutions were prepared at a concentration of 50 pmol for primers and 30 pmol for probes and were gently mixed before use. Stock solutions were stored at -80ºC, while working solutions were maintained at -20ºC. The real-time PCR setup included the addition of a nuclease-free water negative control. Sealing of the negative-control tube was followed by the addition of a positive-control template, which consisted of either whole-virus RNA or transcribed RNA, the test was performed using the the thermal profile **table(2).**

For testing the RNA of the isolates of our studies for Avian infectious bronchitis virus (IBV) Lyophilized primers and probes were spin centrifuged, then they were reconstituted with nuclease-free water or TE buffer to prepare a stock solution in concentration 100pmol, then mixed and spin. The working solutions from the stock primers were prepared in concentration 50pmol, and from the stock probe in concentration 30 pmol, and then mixed gently to be ready for use. *N. B: the stock solutions were preserved in -80ºc freezer, while the working solutions were preserved in -20ºc freezer. Set up the real-time PCR instrument with the reaction conditions as following:

Table 2. Thermocycling Conditions for Gene-Specific Probe and Primer for IBV

Phase Step	Time	Temp	Number of Cycles
Reverse transcription	30 min	50 °C	1
Heatactivation of polymerase	15 min	95 °C	1
PCR Denaturation	10 sec	94 °C	40
Annealing	30 sec	60 °C	
Extension	10 sec.	72 °C	

The negative-control template (Nuclease-free water) was added to the appropriate tube then seal the negative-control tube, the positive-control template could be either whole-virus RNA ortranscribed RNA

Conventional PCR for SP1 gene.

A 457 bp segment of the spike protein SP1 was amplified using the primers listed in Table 3, adhering to the protocol established by **Naguib et al. 2007.** The PCR-generated products were subjected to separation using 1.5% agarose gel electrophoresis in a 1x TBE buffer solution, maintained at room temperature and employing a voltage gradient of 5V/cm. For gel analysis, each gel well was loaded with 15 µl of the PCR products. Size determination of the fragments was facilitated by a generuler 100 bp ladder (Fermentas, Thermofisher, Germany). The resolved gel was then captured using a gel documentation system (Alpha Innotech, Biometra), and the resulting images were subsequently analyzed using specialized computer software.

Table 3. Primers sequences, target genes, amplicon sizes

Target gene	Primer sequence (5'-3')	Length of amplified product (bp)	Reference
Spike SP1	IBV-HVR1-2-FW GTK TAC TACTAC CAR AGT GC IBV-HVR1-2-RV GAA GTG RAA ACR AGA TCA CCA TTT A	457 bp	Naguib *et al*., 2007

6-Sequencing of sp1 gene

7-Spike protein1 gene sequencing:

For genotyping of IBV viruses, a partial sequencing for the S1 gene was performed using primer sets and reaction conditions as described in PCR step.

Phylogenetic analysis of IBV Spike protein1 gene:

The obtained sequences From the S1 gene partial genome sequence were trimmed, consensus generated, and analyzed using the Uniport Ugene software version 47.0 **(Okonechnikov et al. 2012) .**Sequences were investigated using the National Center for Biotechnology Information (NCBI) online basic local alignment tool (BLAST at https:// blast.ncbi.nlm.nih.gov/Blast.cgi).For phylogenetic analysis, the sequence were aligned to other selected sequences hists retrieved from the GenBank in fatsa format. Multiple Sequence alignments (MSA) were performed using clustal omega algorism **(Sievers et al. 2011)**. And analyzed using the same software. IQ-TREE was used to create the phylogenetic tree **(Nguyen et al. 2014)**, best model finder and 1000 bootstrap repetitions were used to determine the most accurate model while using the maximum likelihood method. The tree was rescaled to indicate the number of substitutions persite by using branch length as a measure. Display, manipulation, and annotation of the phylogenetic tree were done with the Interactive Tree of Life (iTOL) **(Letunic and Bork, 2021).** The label Throughout the figures includes the accession number of retrieved sequence from GenBank, isolate names, sequence origin either inside Egypt or the country name where the virus was isolated and the year of isolation. The sequences retrieved for analysis are listed in **table (4).**

RESULTS

Postmortem Examination Results:

Upon examination of the deceased birds, several conditions were noted: Birds were generally found to be emaciated and dehydrated. A notable white chalky substance was observed at the vent area. Some birds exhibited facial swelling, along with serous, mucous, or viscid discharge in the nares and nasal passages. The tracheas appeared congested, with their lumens containing a range of secretions from clear to turbid mucus. At bronchial bifurcations, there was evidence of caseated material.

Virus isolation of IBV

We present the isolation and integrative genetic analysis that map the evolution of IBV in I. Typical IBV lesions such as embryo curling, and dwarfism were detected in embryonated chicken eggs (ECEs) after three successive passages.

In our research, we have successfully isolated the IBV, the virus demonstrated a characteristic pathological changes like embryo curling and dwarfism were observed in embryonated chicken eggs (ECEs), particularly after three rounds of virus passage, indicating typical IBV infection markers.

Table 4. Source modifier tabulates for IBV spike glycoprotein gene sequences of strains sequences retrieved from GenBank for alignment, phylogenetic analysis, and tree construction.

Country (location)	Sequence ID	GenBank Accession number	Collection date	Host and sources
Egypt	IBV-AHRI-ismailia2023 spike glycoprotein gene	OR361566.1	2021/2022	chicken
Egypt	F1282-3-IB-2021 spike glycoprotein gene	OP585563.1	2021/2022	chicken
Egypt	F1282-6-IB-2022 spike glycoprotein gene	OP585566.1	2021/2022	Chicken
Egypt	F1282-2-IB-2021spike glycoprotein gene	OP585562.1	2021/2022	Chicken
Egypt / /Dakahleya	IBV-EGY-ZU/CK/Dak-127/2021 spike glycoprotein S1 (S1) gene	OQ730214.1	2021	Chicken/ Lung and Kidney
Egypt/ Behira	HS4 spike glycoprotein (S1) gene	OQ349381.1	2022	Chicken/allantoic
Egypt	IBV-GI.23.3/ck/ERGY-Bens/AR97/19 S1 protein	MW072802.1	2022	chicken / allantoic fluid
Egypt/Behira	strain HS3 spike glycoprotein (S1) gene	OQ349380.1	2021	Chicken
Egypt	IBV-GI.23.3/ck/ERGY-Bens/AR97/19 S1 protein gene	MN967774.1	2019	chicken /trachea
Egypt/Qena	IBV/CK/EG/QENA-31/2018 spike glycoprotein (S1) gene	MN890132.1	2018	chicken/ allantoic fluid
Egypt	IBV/EGY/AY6/2020 spike gene	MW240846.1	2020	Chicken
Egypt	IBV-EGY/CH/CV48-2019 spike glycoprotein gene	MN651560.1	2019	chicken/ allantoic fluid
Egypt	IBV-EGY/CH/CV125-2019 spike glycoprotein gene	MN651565.1.	2019	chicken/ allantoic fluid
Egypt	IBV-EGY/CH/F742-2019 spike glycoprotein gene	MN651568.1	2019	chicken/ allantoic fluid
Chicken	IBV-EGY/CH/CV17-2019 spike glycoprotein gene	MN651562.1	2019	chicken/ allantoic fluid
Egypt /El-Gharbia	IBV-CH-EG-GH-VVT.NRC-2021 spike glycoprotein (S) gene	OL321756.1	2021	Chicken
Israel	IS/64/2020 spike glycoprotein S1 subunit gene	MT081180.1	2020	chicken /trachea
Chicken	strain HS1 spike glycoprotein (S1) gene	OQ349378.1	2021	Chicken
Egypt/ Qena	isolate IBV/CK/EG/QENA-48/2017spike glycoprotein (S1) gene	MN890134.1	2017	chicken/ allantoic fluid
Egypt/Qaliubiya	isolate IBV-CH-EG-QAL-VVT.NRC-2021 spike glycoprotein (S) gene,	OL415756.1	2021	Chicken
Egypt	isolate Mans-2 spike protein gene	KF856873.1	2012	Chicken
Turkey	isolate GammaCoV/Ck/TR/IBV1/2014 Spike glycoprotein S1 subunit (S1) gene	MN685714.1	2014	chicken /cloacal swap
Poland	isolate 12 spike S1 subunit (S) gene	MZ666005.1	2017	Chicken
Romania	isolate 732 spike S1 subunit (S) gene	MZ666077.1	2020	Chicken
Poland	isolate 1148 spike S1 subunit (S) gene	MZ666038.1	2020	Chicken
Poland	straingCoV/Ck/Poland/G259/2016spike protein S1 (S1) gene	MN887191.1	2016	Chicken
Egypt	IBV-GI.23.3/ck/EGY-Qual/AR545/18 S1 protein gene	MN967777.1	2018	chicken /trachea
Egypt /Fayoum	isolateIBV-GI.23.3/ck/EGY-Fayoum/AR593/18 S1 protein gene	MN967775.1	2018	chicken /trachea
Egypt /Fayoum	isolate IBV/ck/EGY-Monuf/NR725/16	MN987230.1	2016	chicken /trachea
Egypt/matrouh	strain Matrouh spike protein S1 (S1) gene	MT324521.1	2019	Chicken
Lebanon	isolate IB-54NB-chicken-LEBNAN spike protein (S1) gene	MH745418.1	2018	Chicken
Lebanon	isolate IBV_Duck_50NL spike protein (S1) gene	MH745419.1	2018	Duck
Egypt /Giza	isolate IBV-GI.23.3/ck/EGY-Giza/AR563/18 S1 protein gene	MN967776.1	2018	Chicken
Egypt	Isolate IBV-EGY/CH/F580-2019 spike glycoprotein gene	MN651566.1	2019	Chicken

IBV Detection via RT-PCR

The pooled samples from the trachea, lung, and kidney of these flocks were analyzed with RT-PCR. Were successfully amplified a 457 bp fragment within the hypervariable region of the IBV genome. Out of the 10 farms tested, two showed positive results for IBV. From these, one positive sample underwent further nucleotide and amino acid sequencing analysis, as depicted in Figure 1

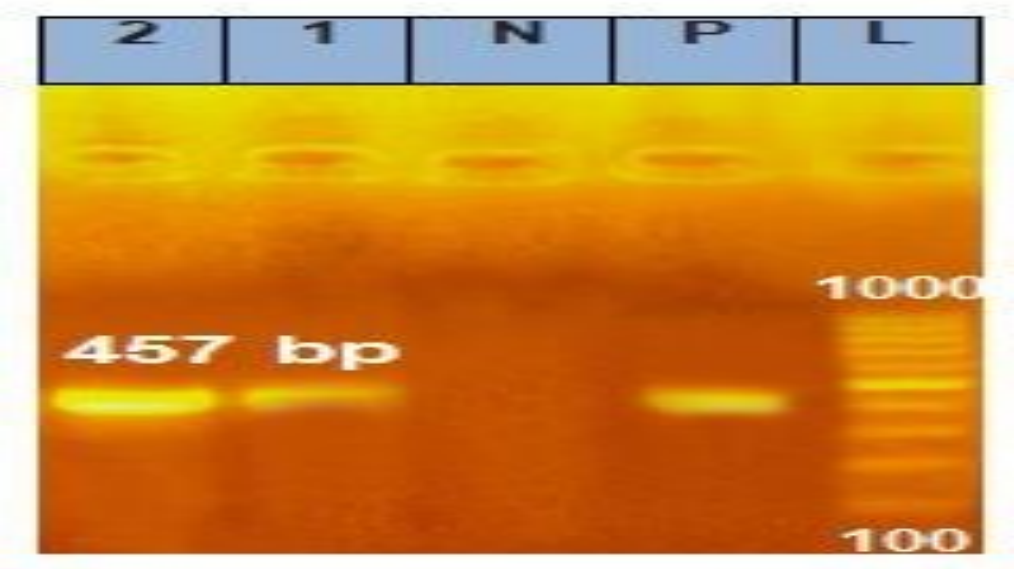

Figure 1. Gel electrophoresis of Rt-PCR showing 457bp band of Sp1 gene with positive control (P) and positive samples (lane 1, 2) and no band was observed in negative control (N). L= 100 bp ladder.

Result of Spike protein1 gene:

The nucleotide sequence of the spike protein 1 (S1) obtained from partial sequencing for selected isolate (isolate IBV-AHRI-ismailia2023) the isolated S1 sequence was annotated and submitted to the GenBank database where assigned the accession number (OR361566.1), bioinformatic analysis was conducted to characterize its genetic composition and potential implications based on nucleotides (391pb) where the phylogeny of the S1 gene. Phylogenetic analysis indicated that our isolate belong to genotype I clade 23 (GI-23) , and clustered with VAR II isolated from Egypt in the last year (fig.3) , Multiple sequence alignment was performed against reference sequences to identify conserved regions and assess sequence homology revealed 100 % nucleotides identity with some isolates recently identified in Egypt from chicken (OP585566.1) and (OP585563.1) isolates while other sequences from Egypt and another countries the homology ranged from 99 % to 95 %, the IBV showed a high similarity (99 %) to isolates (MN890132.1) genotype GI-23 from Qena , (OQ349380.1) from Behira and (MN967774.1) , also it was highly similar (98%) to isolates from Egypt, analysis revealed a similarity of 95 for sequence from Lebanon, Poland, Romania and some isolates in Egypt as they were clustered in a different group.

The identity to S1 our isolates vaccinal strains were around 84% with some vaccinal strains were around 84% with GenBank accessions (MH021175, KU979007).

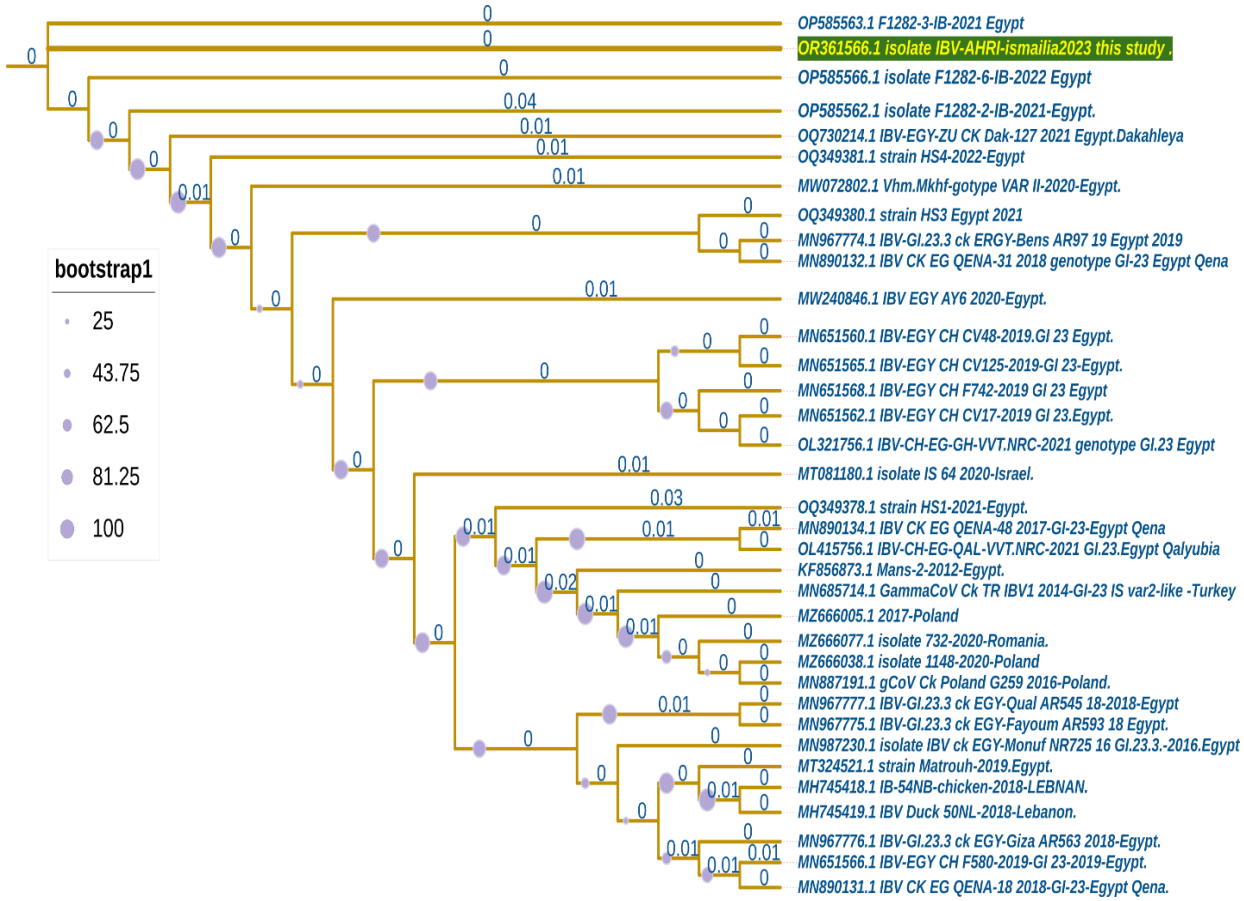

Fig 2. A phylogenetic analysis for IBV Based on S-protein partial sequence using maximum likelihood (ML) phylogenetic tree for isolated from chicken in Ismailia Governorate 2023. The tree was constructed in IQ-TREE with Model Finders. The accession number and brief GenBank ID are assigned to each used sequence from different lisolation sources and origins. The isolate was highlighted in yellow with a green background and the numbers above the branches are the branch length. The bootstrap values were computed from 1000 bootstrap repeats and branch length. The tree's roots are in the middle.

Histopathological findings

Histopathology has proven to be a vital tool for understanding the virulence and pathogenesis of Infectious Bronchitis Virus (IBV), as well as aiding in the diagnosis of both acute and uncomplicated cases of Infectious Bronchitis (IB) The lining epithelium of the trachea showed hyperplasia and metaplasia. The tracheal lumen contained sloughed, desquamated epithelium with few granulocytes (Figure 4). Tracheal submucosa was edematous with mild to moderate lymphocyte infiltration. Others Trachea showed focal thickening and degeneration of the lining epithelium associated with severe congested blood vessels and edema in L. propria (Figure 5). The serosal blood vessels showed endotheliosis . The lungs showed congestion, hemorrhage and edema with thickening of the interalveolar tissue Interstitial pneumonia with fibroblastic cell proliferation were recorded. The bronchial epithelium showed desquamation, necrotic masses and bronchiolitis (Figure 6&7).

The renal tissue showed focal aggregation of lymphocytes in the cortex and the medulla with hypercellularity of some glomeruli associated with perivascular and interstitial haemorrhage. The renal tubules showed desquamation, denudation, and degeneration of the lining epithelium and peritubular edema. Some renal tubules contained few tubular hyaline casts, and there was also inter tubular hemorrhage and congestion (Figure 8&9).

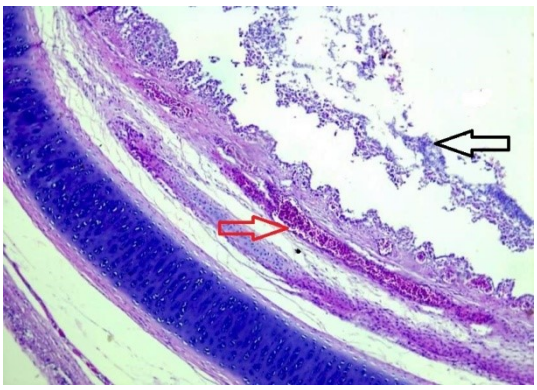

Fig.4: Trachea showing degenerated and desquamated epithelium (black arrow) and congested blood vessels (red arrow) .H&E X100.

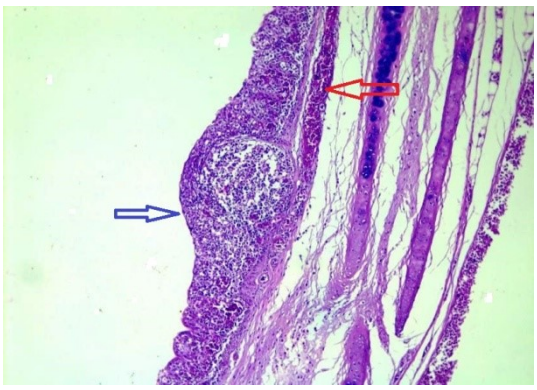

Fig. 5: Trachea showing hyperplasia of epithelium , leukocytic aggregation(blue arrow) and congested blood vessels(red arrow) .H&E X100.

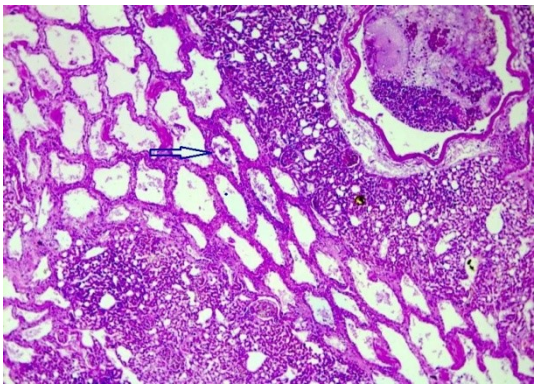

Fig.6: Lung showing pneumonia, thickening of interalveolar septa with esinophilic debris inside it (blue arrow) .H&E X100.

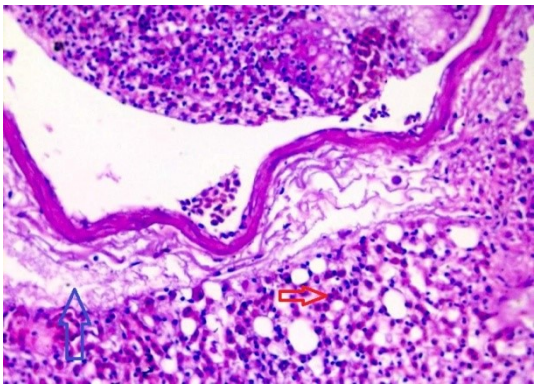

Fig7: Lung, high power of Fig. 3 showing edema (blue arrow) , hemorrhages and leuckocyttic infiltration (red arrow) . H&E X400.

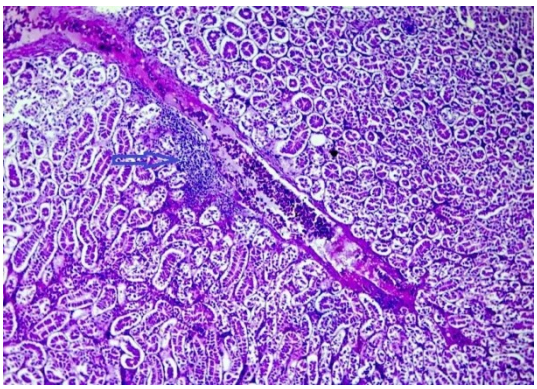

Fig. 8: Kidney showing congested blood vessels with perivascular leuckocytic aggregation (blue arrow) , denudation and hemorrhage between renal tubules .H&E X100.

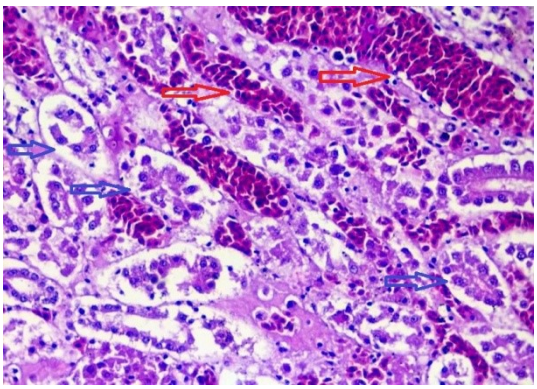

Fig. 9: Kidney high power of fig.8 showing denudation of renal tubules (blue arrow) and congestion between renal tubules (red arrow) .H&E X400.

DISCUSSION

In this study, we conducted a comprehensive phylogenetic analysis of the IBV) spike glycoprotein S1 gene within the Egyptian context to elucidate its genetic diversity and evolutionary relationships. The spike glycoprotein S1 gene, a pivotal component of IBV, plays a critical role in viral attachment and entry into host cells. Utilizing a dataset of S1 gene sequences obtained from diverse IBV isolates submitted in GenBank from different localities and isolation source in Egypt that revealed the sequence similarities at the nucleotide sequences analysis, multiple sequence alignment and phylogenetic reconstruction were performed. The resulting phylogenetic tree revealed distinct clades as in fig.2, illustrating the genetic heterogeneity and intricate evolutionary dynamics of IBV strains circulating within the region our isolates are identified within genotype I clade 23 (GI-23) as many isolates reported from different governorates in Egypt including Qena, Behera, El-Gharbia, Giza, Matrouh, Qaliubiya and Fayoum this result matching the progressive studies recently performed in Egypt **(Moharam et al. 2020)** .

Moreover, analysis of genetic variations within the S1 gene identified key mutations and sequence differences that may influence the virus's antigenicity and pathogenicity matching with result from other countries as, Romania, Lebanon, and turkey and Poland **(Lisowska et al. 2017)** indicating a possible link between Egyptian IBV viruses and European one . This phylogenetic approach sheds light on the evolutionary trajectory of IBV in Egypt, providing valuable insights into its genetic landscape and aiding in the development of targeted control strategies to mitigate the impact of this economically significant avian pathogen.

In our study, the identity of our isolates to the S1 region of vaccinal strains was determined to be approximately 84%. This level of identity suggests a robust conservation of key genomic features between our isolates and the reference vaccinal strains. The observed 84% identity is consistent with the inherent genetic variability that can be expected within viral populations. Viral strains, even those used in vaccines, may exhibit some degree of genetic diversity due to factors such as natural mutation rates and selective pressures. This is particularly relevant in the context of RNA viruses, where higher mutation rates are often observed.t is noteworthy that while an 84% identity was observed on average, there were variations among specific vaccinal strains. This variability could be attributed to the dynamic nature of RNA viruses, which undergo continuous evolution. Moreover, certain strains may have undergone adaptive changes during the vaccine development process or in response to environmental factors.

The histopathological findings in this study provide significant insights into the pathogenesis and virulence of Infectious Bronchitis Virus (IBV), corroborating the utility of histopathology in diagnosing both acute and non-complicated cases of Infectious Bronchitis (IB). These observations align with previous research indicating that histopathological changes are critical markers for understanding the progression and impact of IBV in affected avian populations.

Pneumonic area has been observed in the lungs. Kidneys were enlarged, inflamed and ureters were distended with urates which were similar to that result obtained by **Feng et al, (2012) and Abdel-Ghany H M and Elseddawy N M. (2019).** The specific histological lesions finding in all infected birds, exhibited tracheal lesions with mucosal thickening, hyperplasia of the surface epithelium, mononuclear inflammatory cell infiltrate of lamina propria. Primary and secondary bronchi, epithelial hyperplasia and mononuclear inflammatory cell infiltrate of the lamina propria were also observed by **Khataby et al. (2016)**

The observed hyperplasia and metaplasia in the tracheal lining epithelium, along with the presence of sloughed and desquamated epithelium, are indicative of the virus's direct impact on respiratory tissues. These changes, particularly the edematous submucosa with lymphocyte infiltration, are consistent with the inflammatory response typically seen in IBV

infections. The severity of these changes, including the thickening and degeneration of the epithelium and the congestion in blood vessels, further underscores the aggressive nature of the virus.

The pulmonary symptoms, notably congestion, hemorrhage, and edema, along with the interstitial pneumonia marked by fibroblastic cell proliferation, align with the known respiratory pathology of IBV. The consistency of these findings with existing literature emphasizes the respiratory system as a primary target for IBV, leading to significant impairment in gas exchange and respiratory function.

The renal findings, particularly the lymphocytic aggregation in both the cortex and medulla and the hypercellularity of glomeruli, highlight the systemic impact of IBV. These observations are particularly noteworthy as they suggest a more widespread effect of the virus beyond the respiratory system, potentially leading to renal dysfunction. The presence of tubular hyaline casts and intratubular hemorrhage and congestion further supports this view and aligns with previous studies that have documented renal involvement in IBV infections.

The Implications of histopathological changes observed in this study not only aid in the diagnosis of IBV but also have potential implications for the development of treatment strategies. Understanding the specific tissue alterations can guide veterinarians in predicting disease progression and potentially tailoring treatments to mitigate these effects. The histopathological examination remains a cornerstone in the study of IBV. The findings from this study contribute to the broader understanding of IBV pathogenesis and offer avenues for future research, particularly in exploring targeted therapeutic interventions. Future studies could focus on correlating these histopathological changes with clinical outcomes and response to treatment, thereby enhancing the management of IBV in poultry.

CONCLUSION

Despite substantial financial investments in controlling Infectious Bronchitis (IB), the poultry industry continues to face challenges due to the frequent outbreaks of both classical and newly emerging serotypes of the virus. Our study underscores the limited cross-protection offered by existing live attenuated vaccines against these new field strains, highlighting an urgent need for the development of more effective vaccines. These findings not only contribute to a deeper understanding of the pathogenesis and progression of IB but also emphasize the need for continual surveillance and the development of new, more effective vaccination strategies to combat the ever-evolving IBV strains. This study serves as a call to action for enhanced research and innovation in the field of avian health, particularly in the areas of vaccine development and disease management.

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