Molecular Characterization and Pathotyping of Avian Infectious Bronchitis Virus in some Egyptian provinces

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Abstract

Infectious bronchitis virus has been identified as a highly contagious disease in chickens. The molecular characterization and pathological lesions linked to IBV infection in naturally infected flocks from 7 Egyptian provinces from 2019 to 2022 were investigated in this study using the tissue samples from 18 broiler chicken flocks suspected of having respiratory disease. The reported isolates were detected by a real-time reverse transcriptase assay that targeted the s1 gene (HVR1-2), and they were characterized further by (S1) gene sequencing with histopathological examination of kidney and tracheal tissue. Phylogenetically, the S1 gene was clustered into two major groups, the first group has only one virus belonging to classical vaccine strain of GI-1 lineage, it had a specific character of 32 Amino Acids (AA) mutations compared to EU780077-IS/1494/2006 reference strain and the second group contain 9 viruses belong to genotype GI-23 (variant II). They were further separated in two subgroups, first subgroup GI-23.2.1, contains 8 viruses with 7-14 Amino Acids (AA) mutations, second subgroup contain one virus belong to genotype GI-23.2.2 which had 18 AA mutations, compared to EU780077-IS/1494/2006 reference strain, specifically in HVRs regions (HVRI, II). The histopathological examination revealed degenerative changes and loss of cilia of tracheal epithelium. The kidneys had necrosis with heterophil infiltrations, congestion, and tubular degeneration. In conclusion, the current study investigate the Characterization and Pathotyping of the infectious bronchitis virus in some Egyptian flocks. It is critical to ongoing monitoring to stop the spread of infections, as well as the creation and use of vaccines based on local viruses.

Keywords: Infectious Bronchitis Virus, Molecular Characterization, Genotype, sequencing.

Introduction

Poultry production in Egypt has evolved into an industry with a higher growth rate each year. Viral infections, particularly those that affect the respiratory tract, are an issue, and they have a significant negative impact on the economy ***(Hassan et al., 2016).***Infectious bronchitis virus (IBV) is one of the most common respiratory viruses affecting the global poultry industry because of its highly contagious nature, ongoing emergence of new variants, and evolution of a particular tissue tropism***.(Yang et al., 2018).*** Avian infectious bronchitis (IB) is a serious viral respiratory disease that affects all countries that raise poultry. IB causes serious respiratory problems, in addition to inducing reproductive and urinary lesions that, depending on the virus strain, age, and immune status of the birds. IBV are linked to high mortality. due to its significant virulence and rapid spread, as well as the existence of numerous serotypes with poor cross-protection among types. ***(Jackwood and De Wit, 2013).*** The virus is thought to replicate primarily in the ciliated epithelium and mucous-secreting cells of respiratory organs. The virus briefly causes viremia before spreading throughout the body to other organs, where it may continue to replicate depending on the virus strain and the host's immune system. ***(Owen et al., 1991 and Benyeda et al., 2010).*** IBVs are single stranded positive sense, enveloped RNA viruses that range in size from 27 to 32 kb. The virus has been identified as belonging to the family Coronaviridae, order Nidovirales, and genus Gammacoronavirus., the IBV genome is composed of structural and nonstructural proteins. Structural proteins include the spike [S] glycoprotein, envelope [E], matrix [M], and nucleocapsid [N]. Together, these proteins play a variety of roles in viral attachment, replication, and disease development. The S1 region of the spike glycoprotein has been identified as the key determinant of viral diversity and immune defense, and it plays a significant role in the attachment and entry of the virus into the cell via sialic acid receptors.***(Jackwood et al., 2012).***This protein has been used in recombinant IBV serotype vaccines and genotypic characterization. **(*Shi et al., 2011*** and ***Bande et al., 2015*).** Congestion and edema with serous catarrhal exudate in the tracheal lumen are two of the pathological changes that can be clearly seen. The distended tubules of the enlarged, pale, inflamed, mottled kidneys contained urates. Lung congestion has been present, with a focal area of consolidation. During the acute infection, air sacs may be foamy, but they could later turn cloudy and contain yellow caseous exudates. ***(Ziegler et al., 2002*** and ***Feng et al., 2012).*** After being examined under a microscope, the trachea showed loss of cilia, sloughing of the epithelial cells, serous exudation inside the

lumen, hypertrophy of the mucosal gland, and infiltration of heterophils and lymphocytes in the mucosa. The Bowman's capsule was swollen, the renal tubules were dilated and covered in urate crystals and cast, there was congestion and haemorrhage, and the kidneys displayed interstitial lymphocytic and heterophilic infiltrations, tubular degeneration, and necrosis. The lungs displayed congestion, haemorrhage, fibrin exudate, and lymphocyte and heterophil infiltration. Edema, epithelial desquamation, and fibrinous exudate were all visible in the air sac. The liver displayed congestion, hepatic cell necrosis, and degeneration along with hepatic cord distortion and inflammatory cell infiltration. ***(Gola et al., 2017).*** The most commonly used method for diagnosing IBV is RT-PCR, which amplifies the HVR of the S1 gene and then sequences the amplified amplicon, either with or without earlier virus isolation ***(Gallardo et al., 2010).*** IBV's primary issue is vaccination failure, which is primarily brought on by the regular appearance of new variants that are antigenically distinct from vaccine serotypes. ***(Yan, et al., 2011, and Selim, et al., 2013).*** Identification of these variant serotypes circulating in the Egyptian field is also crucial for screening new variants and choosing the best vaccine strains. ***(Cook, et al., 1999).*** This study aimed to describe the histopathological of suspected IBV infected flocks and characterization the diseased outbreak in commercial chicken flocks located in the Egyptian provinces during 2019-2022.

Material and Methods

Sample collection and preparation

Based on clinical symptoms and postmortem examination, 18 farms were suspected to be IBV-infected located in the Egyptian provinces during 2019-2022. Table 1. By collecting tissue samples, three birds per flock were sampled. (Kidney and trachea). for molecular detection of IBV ***(Lai,2001).*** While the entire samples were cut, immediately fixed in 10% neutral buffer formalin, ***(Bancroft et al., 2013).***

Molecular detection of IBV by RT- PCR

The supernatants of sample suspensions at a concentration of 10% by weight were used to extract RNA for RT-PCR. Using a QiaAmp viral RNA mini kit from Qiagen, Germany, we extracted viral RNA as directed by the manufacturer. The specific target genome was amplified using the forward primer.IBV-HVR1-2, 5- GTK TAC TAC TAC CAR AGT GC -3 and reverse primer IBV-HVR1-2,5- GAA GTG RAA ACR AGA TCA CCA TTT A -3 and probe IBV-pan_Probe, 5- ACT GGA ACA GGA CCD GCC GCT GAC CT -3 ***(Naguib et al., 2017).*** The Qiagen one step RT-PCR Kit was used to perform real-time RT-PCR (Qiagen, GmbH, Hilden, Germany) RT-PCR

was carried out using a Stratagene thermal cycler. Positive isolates underwent conventional PCR using the Qiagen one step RT-PCR Kit (Qiagen, GmbH, Hilden, Germany) with the forward primer IBV-S1-F 50-CACTGGTAATTTTTCAGATGG-30 and reverse primer IBV-S1-R 50-CAGATTGCTTACAACCACC-30 ***(Adzhar et al., 1997)***. After this, the amplicons were purified using the QIA quick gel extraction kit (Qiagen, GmbH, and Hilden, Germany). Partial sequencing of the S1 gene was then done using the Applied Biosystems 3500 XL (ABI, Foster City, CA, USA) and the Big dye Terminator V3.1 cycle sequencing kit (Perkin, Elmer, Foster City, CA, USA) with forward and reverse primers as previously mentioned***(Adzhar et al.,1997).*** With BioEdit software version 7.0.4 .1, the obtained sequences' quality was examined, assembled, and edited before being submitted to GenBank using the BankIt tool of GenBank (http://www.ncbi.nlm.nih.gov/WebSub/?tool=genbank), from which accession numbers were obtained.

Then, the S1 gene sequence was analyzed and compared with Egyptian IBV sequences available on GenBank, the National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/) ***(Thompson et al.,1994)***. Sequence similarities were calculated using DNAstar software, and a nucleotide sequence phylogenetic tree was constructed with Mega 5. The retrievable sequences from the gene bank (http://ncbi/) are provided in Table 2.

Histopathological evaluation

for histopathological evaluation of the lesions produced by naturally infected virus. Tracheal and kidney lesions were scored based on the severity: 1+ = mild epithelial hyperplasia, 2+ = moderate epithelial hyperplasia and subepithelial lymphoid infiltrates, 3+ = sever epithelial hyperplasia and subepithelial lymphoid infiltrate ***(Chen et al., 1996).***

Table (1): *Epidemiological information of the examined flocks:*

Flock/Y	No.	Province	Age/day	Breed	Vaccination	Mortality%
1 / 2019	10000	Damietta	28	Ross	HitchB1+IB	10.5
2 / 2020	5000	Dakahlia	26	Ross	H120	12.5
3 / 2020	3000	Beni suef	23	Cobb	Not vaccinate	14
4 / 2021	5000	Damietta	22	Cobb	HitchB1+IB	14.5
5 / 2021	9000	Damietta	27	Ross	H120	21.2
6 / 2021	5000	Damietta	31	Sasso	Not vaccinate	18.5
7 / 2021	8000	Dakahlia	18	Avian	H120	13.4
8 / 2021	5000	Damietta	24	Ross	HitchB1+IB	22.3
9 / 2021	7000	Dakahlia	25	Sasso	HitchB1+IB	7.4
10 / 2021	10000	Dakahlia	30	Cobb	HichB1+IB	13.5
11 / 2021	10000	Minya	28	Ross	Clone30+IB	20.5
12 / 2022	10000	Dakahlia	30	Cobb	HitchB1+IB	21.8
13 / 2022	10000	Damiata	25	Avian	HitchB1+IB	22.4
14 / 2022	10000	Giza	30	Avian	H120	8.9
15 / 2022	8000	Damietta	30	Ross	HitchB1+IB	14.2
16 / 2022	10000	Qalubia	29	Avian	Clone30+IB	16.7
17 / 2022	10000	Dakahlia	27	Sasso	HitchB1+IB	12.4
18 / 2022	5000	Kafr sheikh	22	Cobb	HichB1+IB	16.8

Table (2): *Retrieved sequences of IBV-S1 gene*

Strain	Country	Genotype	Accession No.
IBV-EG/1212B-SP1-2021	Egypt	EGY variant 2GI-23	KU979007
M41-2004	USA	Classic G1-1	AY561711
IBV-Connecticut	USA	Classic G1-1	L18990
H120	Nederland	Classic G1-1	M21970
D274-1989	Nederland	Variant 1 G1-12	X15832
IS/1494/2006	Israel	Variant 2 G1-23	EU780077
CR88-2014	Malaysia	Variant 1 G1-13	KM067900
strain4/91-1998	UK	Variant 1 G1-13	AF093794
QXIBV-1999	China	QXIBV variant	AF193423
IBV/CK/EG/QENA-31/2018	Egypt	EGY variant 2GI-23	MN890132

Results

1. Clinical signs

The most prevalent clinical symptoms included depression, decreased feed intake, ruffled feathers, crowding under a heat source, coughing, gasping, sneezing, and tracheal rales. and significant mortalities reach more than 20 %.

2. RT-PCR results

Real time RT-PCR offer a sensitive, rapid, and accurate results thus enabling the detection of IBV in examined organs of infected broilers within a short time. Out of 18 tested flocks, eleven flocks were positive for IBV using real time PCR with a percentage of 61%, four flocks out of 6 flocks from Damietta province, three flocks out of 5 flocks from Dakahlia province and one from Minya, Giza, Qalubia and Kafr skeikh province were positive (Table 3).

a. Partial S1 gene sequencing of IBV

Eleven positive samples were then amplified with RT-PCR and gel electrophoresis. S1 gene sequencing was conducted on 10 selected samples using the RT-PCR band intensities as selection criteria. Predicted IBV bands were observed at 450 bp in the gel. Subsequently, three Damietta strains, three Dakahlia strains and one each of Minya, Giza, Qalubia, and Kafr Sheikh strain sequences were analyzed for phylogeny. Phylogenetic analysis of 10 isolates revealed that infectious bronchitis genotypes were categorized into classical GI-1 and variant II strains (GI-23.2.1, and GI-23.2.2). (fig.1, table 3).

b. Amino acid alignments and variations:

Amino acid sequences of S1 gene of 10 IBV isolates in this study (classical, variant II GI-23.2.1 and GI-23.2.2) were aligned and compared to each other and with other Egyptian strains retrieved from gene bank showed that all strains of Var II GI-23.2.1 are closely related with a high identity percentage ranged from 87-100%. However, less identity percentages were observed when compared with IBV var II GI-23.2.2 with a percentage ranged from 81-86 % and more distant from classical IBV strain with identity of 70-73 % (Fig. 1 and table 3).

Egyptian variant II is subdivided into Egyptian IBV GI-23.2.1 isolates including isolates F1282-1-IB-2021, F1282-2-IB-2021, F1282-3-IB-2021, F1282-4-IB-2021, F1282-5-IB-2021, F1282-6-IB-2022, F1282-8-IB-2022 and F1282-10-IB-2022, these 8 isolates were very close to the Egyptian variant II isolate MN890132 IBV/CK/EG/QENA-31/2018 and other Egyptian variant II isolates available in the GenBank, Egyptian IBV GI-23.2.2 isolate including isolate F1282-9-IB-2022 was very close to the Egyptian variant II isolate KU979007.1 IBV-EG/1212B-SP1-2012. (Table 3, Fig. 1)

c. Amino acid mutations in S1 protein of IBV isolates

compared to EU780077-IS/1494/2006 reference strain, isolate (F1282-7-IB-2022) have 32 amino acids substitutions, isolate (F1282-9--IB-2022)) have 18 amino acids substitutions, and isolates F1282-1-IB-2021, F1282-2-IB-2021, F1282-3-IB-2021, F1282-4-IB-2021, F1282-5-IB-2021, F1282-

6-IB-2022, F1282-8-IB-2022 and F1282-10-IB-2022 have 12,11,10,11,14,10,7 and 12 amino acids substitutions. (Table 4).

d. Pathological findings

The trachea showed macroscopic pathological changes such as oedema, congestion, hemorrhagic inflammation, and a cavity filled with hemorrhagic exudate. (Fig 3,4). The kidneys were enlarged and inflamed with ureates accumulating in the ureters and renal tissue (Fig 5).

e. Histopathological findings:

Results of histopathology in our work exhibited a clear difference in severity and distribution of lesions in chickens naturally infected with different Egyptian strains of IBV, these differences showed in trachea and kidneys at 24hr and 48 hrs post infection than chickens died later. Significant lesions with variable scores were seen in trachea and kidney of chickens infected with Damietta, Dakahlia, and Qalubia strains than Giza and Minya but were less severe in chickens infected with Kafer Sheikh strain (Table 3). According to a histological report, normal birds had pseudostratified ciliated columnar epithelium in their trachea. (Fig. 6). The kidney of healthy birds showed healthy renal glomeruli and tubules. (Fig. 7). Trachea of birds with IBV Damietta and Dakahlia strain infections displayed distinct tracheitis symptoms (Fig.8 and 10). The interstitial nephritis in the kidneys was present in multiple locations and was accompanied by mononuclear cell infiltration. (Fig. 9 and 11). Birds with the IBV Minya strain and the IBV Giza strain had tracheitis, which was accompanied by blood capillary congestion in the lamina propria, hyperplasia of the lining epithelium, and infiltration of mononuclear inflammatory cells (Fig. 12, and 14). These birds' kidneys showed a small amount of mesangial cell proliferation with hyalinized matrix (Fig.13 and 15). IBV strains Qalubia and Kafr Sheikh-infected birds displayed mild to moderate tracheitis and renal tubular epithelial lining degeneration (Fig.16,17.18 and 19). The trachea and kidney of chickens infected with the Damietta, Dakahlia, and Qalubia strains showed significant lesions with varying scores compared to the Giza and Minya strains, but the Kafer Sheikh strain showed less severe lesions.Table 3, Table 5

Table (3): *Results of IBV by real time PCR:*

Flock No./Y	Province	Results	Genotype	Virus ID	accession No
1/2019	Damietta	Pos	Not done	-	-
2/2020	Dakahlia	Neg	-	-	-
3/2020	Beni suef	Neg	-	-	-
4/2021	Damietta	Pos	Var II (G23, 2.1)	F1282-1-IB-2021	OP585561
5/2021	Damietta	Neg	-	-	-
6/2021	Damietta	Neg	-	-	-

7/2021	Dakahlia	Pos	Var II (GI-23, 2.1)	F1282-2-IB-2021	OP585562
8/2021	Damietta	Pos	Var II (GI-23, 2.1)	F1282-3-IB-2021	OP585563
9/2021	Dakahlia	Neg	-	-	-
10/2021	Dakahlia	Pos	Var II (GI-23, 2.1)	F1282-4-IB-2021	OP585564
11/2021	Minya	Pos	Var II (GI-23, 2.1)	F1282-5-IB-2021	OP585565
12/2022	Dakahlia	Neg	-	-	-
13/2022	Damiata	Neg	-	-	-
14/2022	Giza	Pos	Var II (GI-23, 2.1)	F1282-6-IB-2022	OP585565
15/2022	Damietta	Pos	Classic GI-1	F1282-7-IB-2022	OP585567
16/2022	Qalubia	Pos	Var II (GI-23, 2.1)	F1282-8-IB-2022	OP585568
17/2022	Dakahlia	Pos	Var II (GI-23, 2.2)	F1282-9-IB-2022	OP585569
18/2022	Kafr sheikh	Pos	Var II (GI-23, 2.1)	F1282-10-IB-2022	OP585570

Pos: positive
Neg:negative

Table (4): *Amino acid substitutions of S1 gene of ten IBV isolates compared to reference strain EU780077-IS/1494/2006:*

No.	ID	Province	Genotype	Accession no.	Amino acids	
					substitution	identity %
1	F1282-1-IB-2021	Damietta	Var II (G23.2.1)	OP585561	12/130	87%
2	F1282-2-IB-2021	Damietta	Var II (G23.2.1)	OP585562	11/130	87%
3	F1282-3-IB-2021	Damietta	Var II (G23.2.1)	OP585563	10/130	88%
4	F1282-4-ib-2021	Dakahlia	Var II (G23.2.1)	OP585564	11/130	88%
5	F1282-5-IB-2021	Minia	Var II (G23.2.1)	OP585565	14/130	85%
6	F1282-6-IB-2022	Giza	Var II (G23.2.1)	OP585566	10/130	88%
7	F1282-7-IB-2022	Damietta	Classic GI-1	OP585567	32/127	70%
8	F1282-8-IB-2022	Qalubia	Var II (G23.2.1)	OP585568	7/130	90%
9	F1282-9--IB-2022	Dakahlia	Var II (G23.2.2)	OP585569	18/130	82%
10	F1282-10-IB-2022	Kafr Elsheikh	Var II (G23.2.1)	OP585570	12/130	87%

Table (5): *Scores of histopathological changes in organs of chickens naturally infected with IBV*

Genotype	Mortality %	Trachea	Kidney	Tropism and severity of signs
classical GI-1	8.9	++++	++	sever respiratory and mild renal lesions
variant II (GI-23.2.1)	16.8	+++	+++	moderate respiratory and renal lesions
variant II (GI-23.2.2)	16.5	+++	++++	moderate respiratory and sever renal lesions

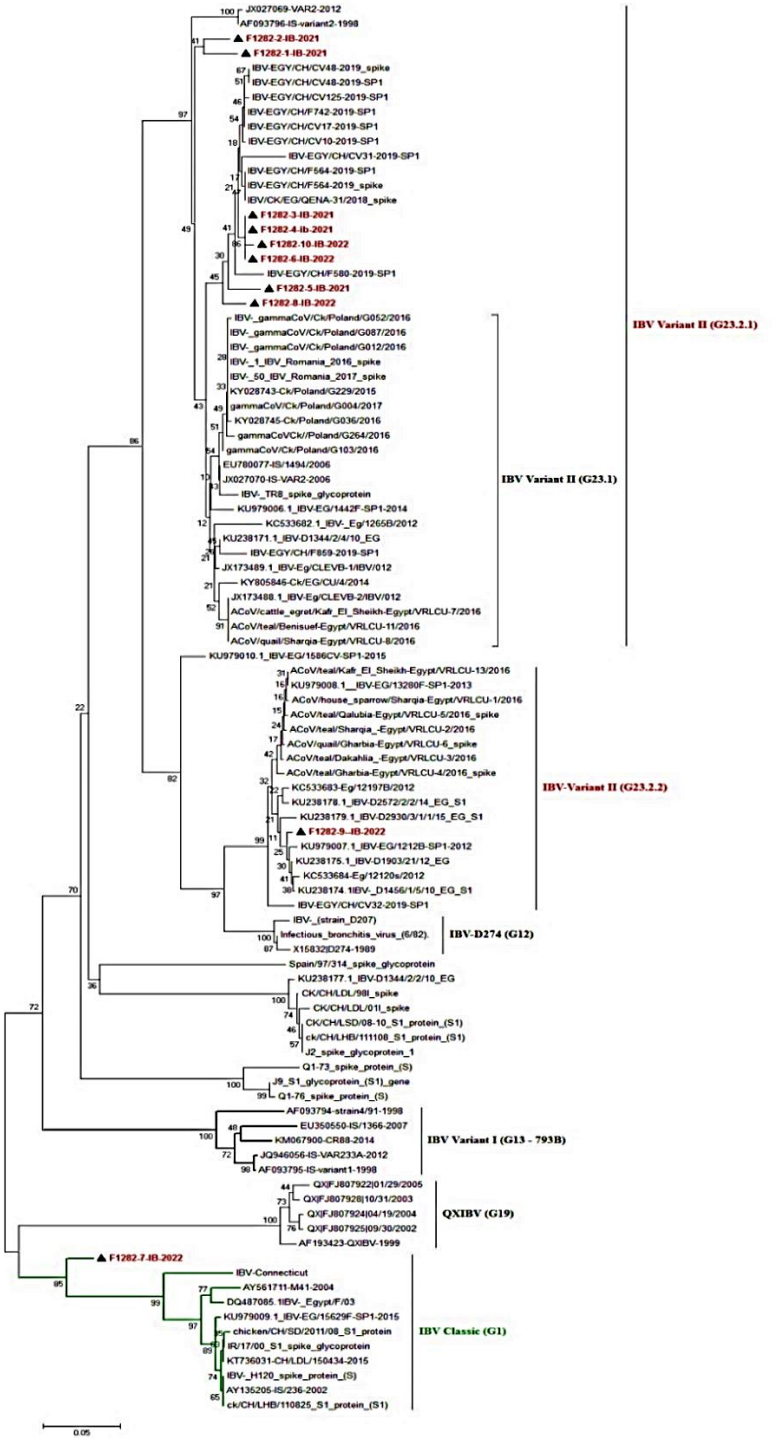

Fig. 1: Phylogenetic tree for the 10 IBV isolates based on S1

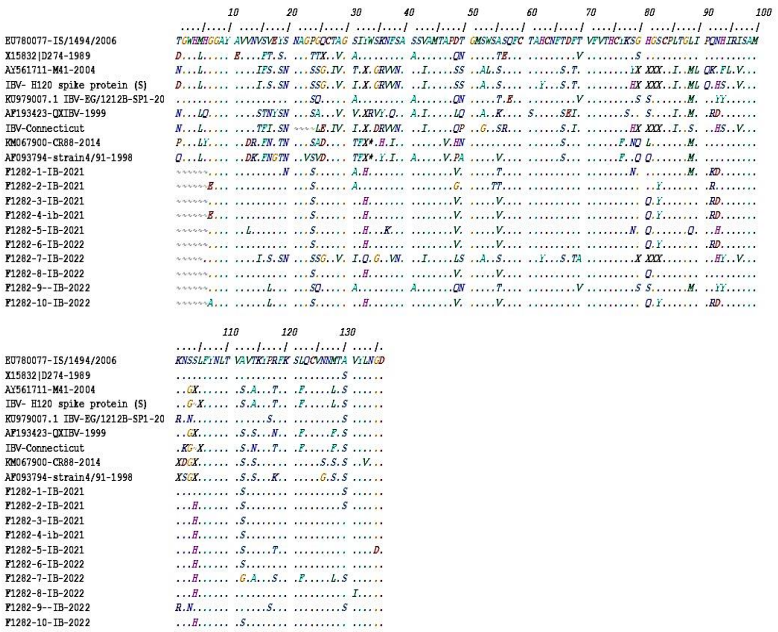

Fig. (2): alignment of the amino acid sequence of HVR of S1 gene for the selected 10 isolates of the study

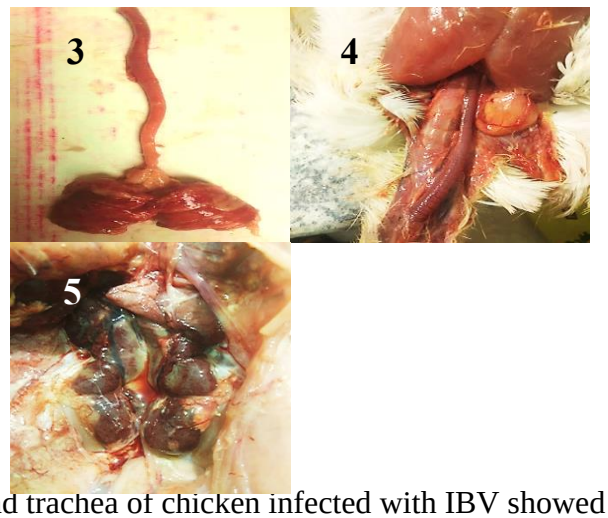

Fig. 3: Lung and trachea of chicken infected with IBV showed oedema, congestion and hemorrhagic inflammation.

Fig. 4: Trachea of chicken infected with IBV showed oedema, congestion and the cavity filled with hemorrhagic exudate.

Fig. 5: Trachea of chicken infected with IBV showed oedema, congestion and the cavity filled with hemorrhagic exudate.

Histopathological examination

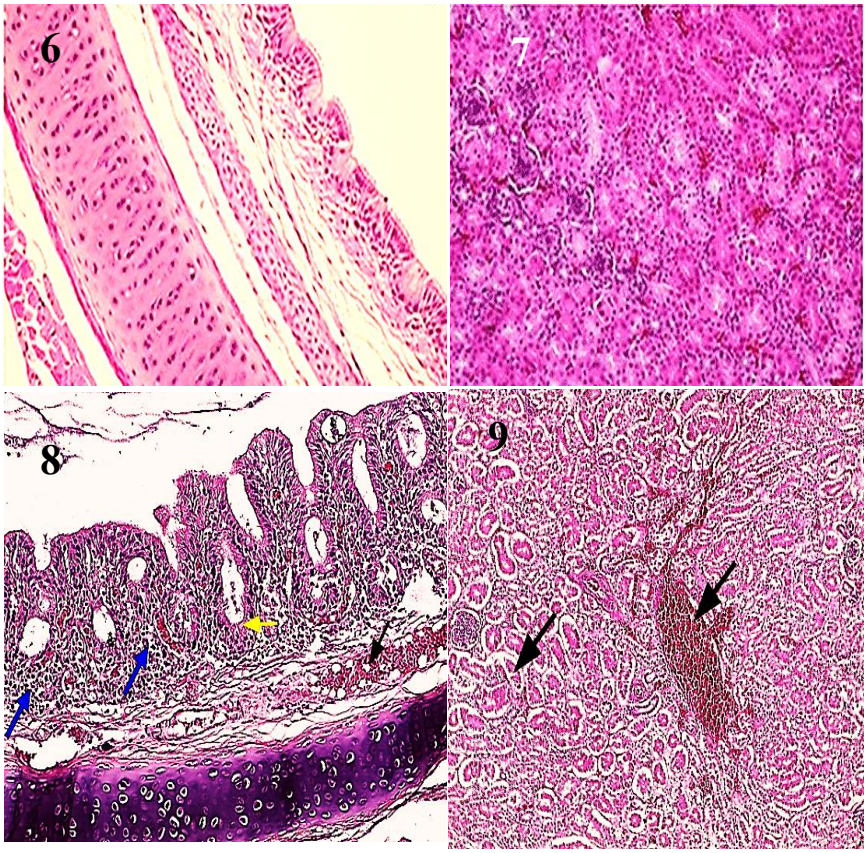

Fig. (6): Normal trachea show no obvious tracheal lesions.
Fig. (7): Normal kidney show no obvious kidney lesions.
Fig. (8): Trachea of chickens infected with IBV Damietta strain (classical GI-1) showed hyperplasia of glandular epithelium of trachea (yellow arrow), cellular exudates were observed in tracheal lumen (blue arrows), congestion of blood vessels and edema of submucosal layer was shown (black arrow).
Fig. (9): Kidneys of chickens infected with IBV Damietta strain (classical GI-1) showed diffuse necrosis of renal tubules associated with multiple hemorrhages.

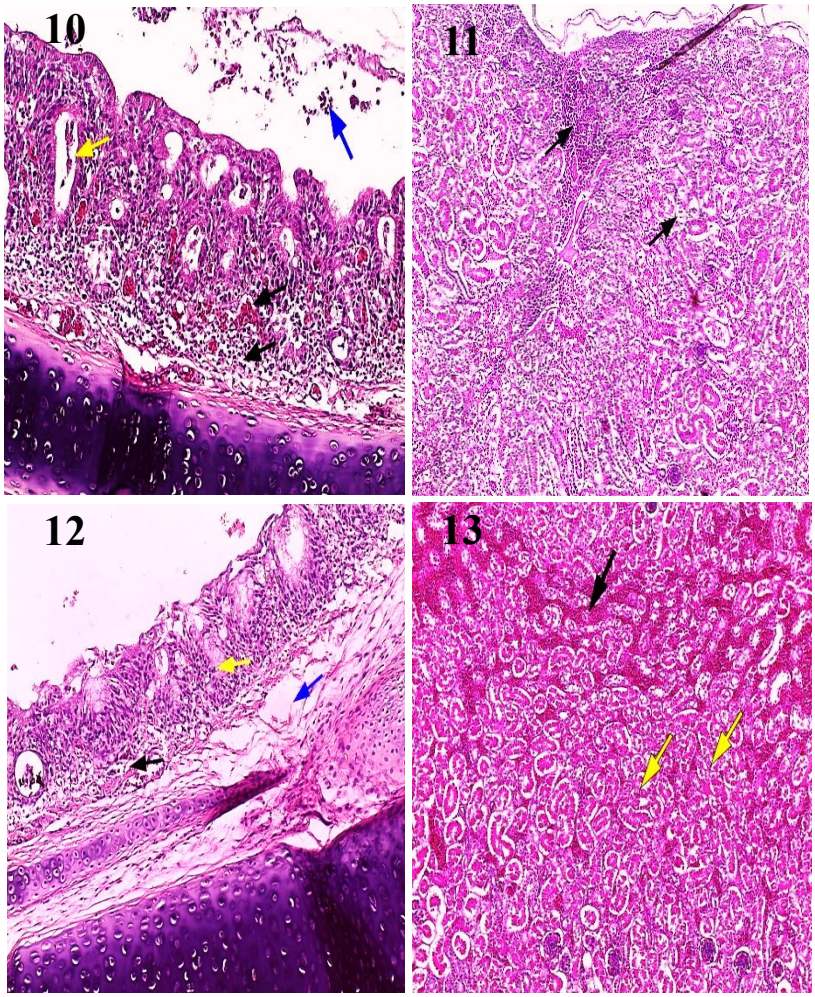

Fig. (10): Trachea of infected chicken with IBV Dakahlia strain variant II (GI-23.2.2) showed hyperplasia of glandular epithelium of trachea (yellow arrow), profuse infiltrations of mononuclear inflammatory cells in the tracheal mucosa (blue arrow), congestion of blood vessels and edema of submucosal layer was shown (black arrow).
Fig. (11): Kidney of infected chickens with IBV Dakahlia strain variant II (GI-23.2.2) showed interstitial nephritis characterized by multifocal infiltrations of mononuclear inflammatory cells in the renal interstitium.
Fig. (12): Trachea of chickens infected with IBV Minya strain variant II (GI-23.2.1) showed hyperplasia of goblet cells in the tracheal epithelium (yellow arrow) with mononuclear inflammatory cells infiltrations in the mucosal layer (black arrow). Edema of submucosal connective tissue was shown (blue arrow).
Fig. (13): Kidney of infected chickens with IBV Minya strain variant II (GI-23.2.1) showed Multifocal necrosis of renal tubules (yellow arrows) with multifocal hemorrhages was observed (black arrow).

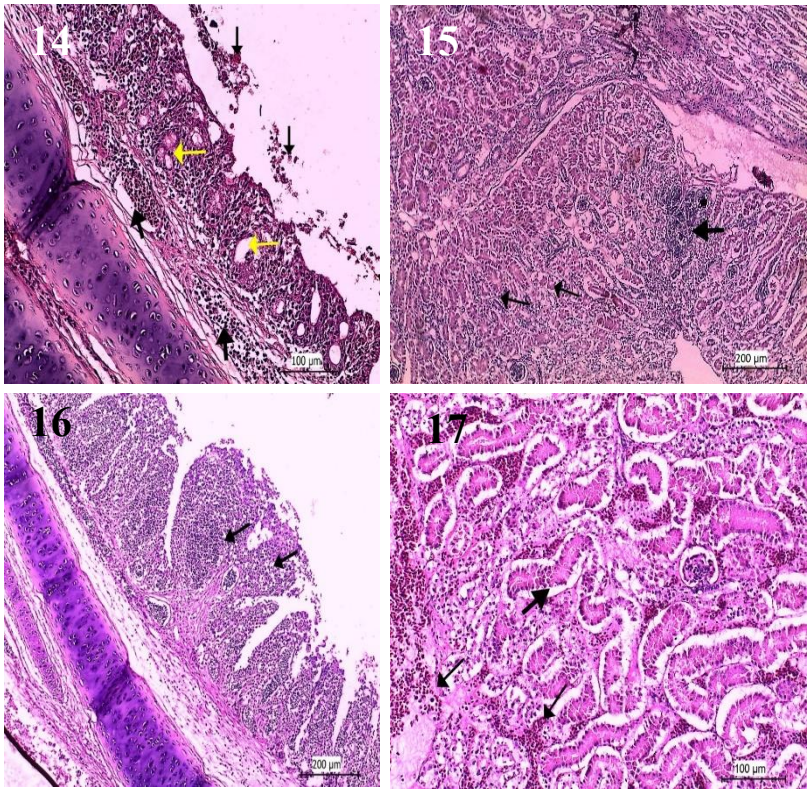

Fig. (14): Trachea of chickens infected with IBV Giza strain variant II (GI-23.2.1) showed tracheitis characterized by hyperplasia of glandular epithelium (yellow arrows) congestion of blood vessels and edema of submucosal layer was shown (thick arrows). Exudates with sloughed epithelium were shown in tracheal lumen (thin arrows).
Fig. (15): Kidney of chickens infected with IBV Giza strain variant II (GI-23.2.1) interstitial nephritis characterized by multifocal infiltrations of mononuclear inflammatory cells (thick arrow). Kidneys showed diffuse degenerated renal tubules (thin arrow).
Fig. (16): Trachea of chickens infected with IBV Qalubia strain variant II (GI-23.2.1) showed severe tracheitis characterized by hyperplasia of glandular epithelium, profuse infiltrations of mononuclear inflammatory cells in the tracheal mucosa (black arrow), congestion of blood vessels and edema of submucosal layer.
Fig. (17): Kidneys of chickens infected with IBV Qalubia strain variant II (GI-23.2.1) showed diffuse degenerated renal tubules (thick arrow) associated with multiple hemorrhages (thin arrows).

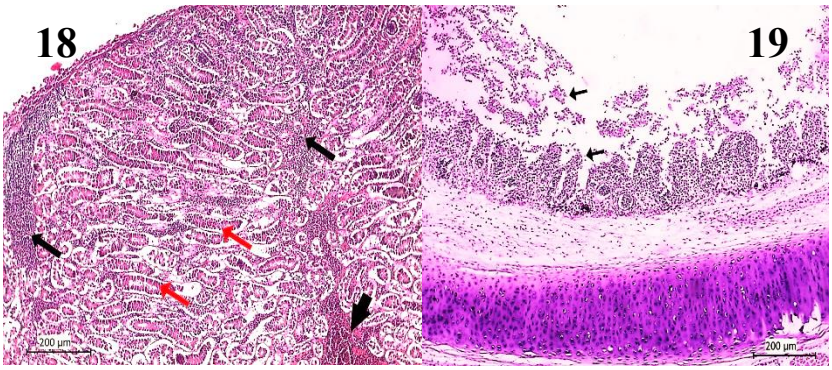

Fig. (18): Trachea of chickens infected with IBV Kafr sheikh strain variant II (GI-23.2.1) showed severe tracheitis characterized by severe degenerated glandular epithelium with sloughed necrotic tissue observed in tracheal lumen (black arrows).
Fig. (19): kidney of chicken infected with IBV Kafr sheikh strain variant II (GI-23.2.1) showed interstitial nephritis characterized by multifocal infiltrations of mononuclear inflammatory cells (thin black arrows). Kidneys showed diffuse degenerated renal tubules (red arrows) associated with multiple hemorrhages (thick black arrow)

Discussion

IBV is a common worldwide virus and could causes massive damage in poultry industry in vaccinated and non-vaccinated flocks ***(Villarreal, 2010).*** Different studies have focused on the epidemiology of IBV **(*Fathy et al., 2015; Sultan et al., 2019).*** In Egypt, despite of massive vaccination to IBV, multiple outbreaks were previously recorded ***(Abd El Rahman et al., 2015; Sultan et al., 2019).*** Clinical signs include whitish excretions, rales, respiratory sounds, peeping, ruffled feathers, and gasping. The mortality rate in IBV-infected flocks ranged between 25 and 30 percent, depending on the presence of secondary infections, flock age, immune status, management, and other environmental factors. The morbidity rate in IBV-infected flocks may reach as high as 100 percent. Depending on the virulence of the IBV infecting strain and the presence of other complicating viral and bacterial agents, mortality rates can reach 80%. ***(Cavanagh and Gelb, 2008).*** IBV-infected chickens had tracheal exudation, congestion, tracheal and bronchial casts, rhinitis, serositis (pericarditis, perihepatitis, and air sacculitis), splenic and hepatic congestion, and nephritis with ureate deposition in renal tissue and ureters. (Fig. 3, 4, and 5). These findings align with those made by ***Susan et al., 2010 and Hassan et al., 2017.*** Real time PCR was used commonly as screening and diagnostic test for detection of IBV

RNA in clinical samples ***(Cook et al., 2012)*.** Phylogenetically, the S1 gene was clustered into two major groups , the first group contain only one virus isolated from Damietta province (accession No.OP588567), belong classical strain GI-1and the second group contain genotype GI-23 (variant II), with nine viruses occurring in two subgroups, first subgroup GI-23.2.1, contains 8 viruses isolated from chickens in Damietta, Dakahlia, Minya, Giza, Qalubia and Kafr sheikh province respectively (accession No, OP585561, OP585562,OP585563, OP585564, OP585565, OP585566, OP585568 and OP585570), second subgroup contains one viruses belong to genotype GI-23, 2.2 isolated from one flock in Dakahlia province (accession No, OP585569) (Fig.1,table3).Several point mutations at the level of amino acid level were observed in Egyptian IBV isolated from different provinces. The number and percentages of substitutions were shown in table (4), Depending on the S1 amino acids sequence, IB isolates identified in this study grouped in three groups, the first group has only one virus belonging to classical vaccine strain of GI-1 lineage, it had a specific character of 32 Amino Acids (AA) mutations compared to EU780077-IS/1494/2006 reference strain and the second group contain nine viruses belong to genotype GI-23 (variant II). They are further separated in two subgroups, first subgroup GI-23.2.1, contains 8 viruses with 7-14 Amino Acids (AA) mutations, second subgroup contain one virus belong to genotype GI-23.2.2 which had 18 AA mutations, compared to EU780077-IS/1494/2006 reference strain. Results of histopathology in our work exhibited a clear difference in severity and distribution of lesions in chickens naturally infected with different Egyptian strains of IBV, these differences showed in trachea and kidneys. Significant lesions with variable scores (reference?) were seen in trachea and kidney of chickens infected with Damietta, Dakahlia, and Qalubia strains than Giza and Minya but were less severe in chickens infected with Kafer Sheikh strain (Table 5). Histopathological changes in trachea and kidneys are similar to the results of high nephropathogenic of IBV GD strain that caused the renal tubules and ureters to swell with urates, resulting in pale and swollen kidneys**.** In our research, nephropathogenic IBV strains result in nephritis, which is marked by kidney swelling and congestion (Figs. 9, 11, 13, 15, 17, and 19), along with the pallor of ureters that have urate deposits on occasion. Previous reports have described similar findings with nephropathogenic IBV strains that result in pale, swollen, and mottled kidneys. ***(Boroomand et al. 2012 &Cong et al. 2013)***, with tubular deterioration, interstitial nephritis, and heterophil infiltration. In some cases, urates and casts are found

inside necrotic and dilated tubules. ***(Cavanagh and Gelb 2008).*** Histopathological changes of trachea include cilia loss, oedema, epithelial cell rounding and sloughing, and lymphocyte infiltration. Trachea of birds infected with IBV Damietta and Dakahlia strain showed marked tracheitis features (Fig. 8 and 10). The same picture with severe degenerative changes of the tracheal mucosa, mononuclear cell infiltration and epithelial desquamation in the tracheal lumen was reported ***(Cavanagh et al., 1992 and Magouz et al. 2018).*** In our research, kidney damage was the most common lesion associated with IBV variants and was more easily visible on histology than on gross examination. The kidneys showed multifocal necrosis in renal glomeruli and tubules with mononuclear cell infiltration (Fig. 9, 11,13 and 15). Parallel to previous studies, Findings in the renal tubules were consistent with those reported by ***Chousalkar et al. (2007),*** who demonstrated mild mesangial cell proliferation with hyalinized matrix and severe renal tubular lining epithelial degeneration and proliferative glomerulonephritis because the virus may replicate more frequently in these epithelial cells. IBV-T-strain causes distension of the distal convoluted tubule and necrosis of the proximal convoluted tubule, according to experimental studies. Additionally, lymphocytes, heterophils, and necrotic foci are seen in the interstitial spaces. Spheroids and collecting ducts have both been reported to have granulocytic infiltration and Bowman's capsule edema. These birds' kidneys showed a slight proliferation of mesangial cells with a hyalinized matrix (Fig.13 and 15). ***(Chousalkar et al. 2007 & Chousalkar and Roberts 2007).*** The trachea of birds infected with IBV Minya strain and Giza strain showed tracheitis associated with hyperplasia of the lining epithelium, blood capillary congestion within the lamina propria, and infiltration of mononuclear inflammatory cells. (Fig. 12, and 14). The degree of tracheal damage following IBV growth in this tissue is frequently assessed using the ciliostasis test of the tracheal ciliated epithelium. ***(Cook et al., 1976; Tarpey et al., 2006).*** Both mild to moderate tracheitis and severe renal tubular epithelial lining degeneration were present in birds infected with the IBV strains Qalubia and Kafr Sheikh. (Fig.16,17,18 and 19). These histopathological changes are consistent with the findings of other researchers who suggested that the lesions developed as a result of the IBV's apoptotic effect on cells. ***(Han et al., 2017)***.

Conclusions

Our data showed that despite vaccination programmes, New IBV variants are spreading among flocks of chickens in Egypt's provinces. The high morbidity and mortality

rates caused by avian infectious bronchitis continue to pose a significant challenge for the chicken industry globally. Numerous regional and global variants of the virus, which is constantly changing, have been found. Along with this, it is crucial to acquire more knowledge about the pathological changes by the current and novel IB virus variants so that they can be distinguished from other poultry diseases. For IBV to be successfully controlled in Egypt, Continuous surveillance efforts and the development of local isolate-based vaccines were crucial components.

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الملخص العربى

التوصيف الجزيئى والتنميط الباثولوجى لفيروس التهاب الشعب الهوائية المعدي للطيور في بعض المحافظات المصرية

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فيروس التهاب الشعب الهوائية المعدي يعرف بأنه مرض شديد العدوى يصيب الدجاج. في هذه الدراسة تم دراسة التوصيف الجزيئي والتغيرات النسيجية المرتبطة بعدوى التهاب الشعب الهوائية المعدى في القطعان المصابة في الفترة من 2019-2022 من 7 محافظات مصرية باستخدام عينات لأنسجة من 18 قطيع من الدجاج يعانى من أعراض تنفسية. تم تحديد العزلات من خلال اختبار النسخ العكسي RT- PCR الذي يستهدف جين S1 ، بالإضافة إلى التغيرات النسيجية لأنسجة الشعب الهوائية والكلى،وأظهرت النتائج وجود مجموعتين رئيسيتين من الفيروسات ، المجموعة الأولى تشمل فيروس وحيد ينتمي إلى سلالة اللقاح الكلاسيكية GI-1 ، وتحوى طفرة في 32 من الأحماض الأمينية ، وشملت المجموعة الثانية على تسعة فيروسات تنتمي إلى النمط الجيني GI-23 (variant II), تم تقسيمها إلى مجموعتين فرعيتين: المجموعة الفرعية الأولى GI-23.2.1 تشمل 7 فيروسات تحوى طفرة في 14-8 من الأحماض الأمينية ، والمجموعة الفرعية الثانية GI-23.2.2 تحوى طفرة في 18 حمض أمينى في منطقة (HVRI, II)على وجه التحديد مقارنة مع السلالة المرجعية EU780077-IS/1494/2006. كشف الفحص النسيجي المرضي عن تغيرات تنكسية في ظهارة الشعب الهوائية وفقدان الأهداب. أظهرت الكلى نخرا ونزيفا واحتقانا وتنكس أنبوبي بالإضافة الى تسلل للخلايا المناعية المغايرة. في الختام ، تقدم الدراسة تحديثا حول انتشار فيروس التهاب الشعب الهوائية المعدي في قطعان الدواجن المصرية كأحد أهم أمراض الدواجن المؤثرة اقتصاديا نظرا لاستمرار تطور وتنوع فيروسات التهاب الشعب الهوائية المعدى. إن الرصد المستمر وإنشاء واستخدام اللقاحات القائمة على الفيروسات المحلية أمر مهم للغاية للسيطرة على انتشار العدوى .