Egyptian Journal of Animal Health

P-ISSN: 2735-4938 On Line-ISSN: 2735-4946
Journal homepage: https://ejah.journals.ekb.eg/

Phylogenetic analysis of avian infectious bronchitis virus circulating in Egypt during 2021-2022

Hamdi Mohamed Sallam[*], *Mohamed Rady*[**], *Zienab Mosaad*[***], *Mostafa Mohamed Saleh*[****], *Ahlam E. Yonis*[*], *Ali Mahmoud Zanaty*[***]

[*]Reference Laboratory for Veterinary Quality Control on Poultry Production, Damanhour Branch, Animal Health Research Institute, Agricultural Research Center.
[**]Reference Laboratory for Veterinary Quality Control on Poultry Production, Fayoum Branch, Animal Health Research Institute, Agricultural Research Center.
[***]Reference Laboratory for Veterinary Quality Control on Poultry Production, Animal Health Research Institute, Agricultural Research Center.
[****]Animal Health Research Institute, Mansoura Branch, Agricultural Research Center.

Received in 16/1/2023
Received in revised from 9/2/2023
Accepted in 1/3/2023

Keywords:
Avian infection bronchitis
Phylogenetic Egypt

ABSTRACT

Avian infectious bronchitis virus (IBV) is still the cause of huge economic losses among poultry farms despite extensive vaccination. During this study, clinical samples were collected from four Egyptian governorates in the period 2021-2022. Samples with positive IBV real-time RT-PCR results were subjected to virus isolation on chicken embryos. IBV was identified by RT-PCR that was directed to the S1 gene. Partial sequencing of S1 gene revealed seven strains (HS1-7) that were submitted to Gen-Bank under the accession numbers (OQ349378-OQ349384). Phylogenetic analysis revealed clustering of five strains (HS1-5) with genotype I-lineage 23(GI-23), whereas the other two strains (HS6 and HS7) were clustered with genotype I- lineage 1 (GI-1). Strains HS1-3 were found to have a unique amino acid insertion in hypervariable region 1 (HVR1). GI-23-related (variant) strains shared less than 86.2% and 88.4% nucleotide and amino acid identities, respectively with the commonly used vaccine strains. The closest vaccine strain (EG/1212B) showed 9-15 amino acid substitutions within HVR1 and 2. Although GI-1-related strains shared close relation to Mass like vaccine strains, 5-13 amino acid substitutions were observed within HVR1 and 2. This study reports the identification of genetically distinct IBV strains in different Egyptian governorates with probable impact on the protection attracted by commercial vaccines.

*Corresponding author: Hamdi Mohamed Sallam, Reference Laboratory for Veterinary Quality Control on Poultry Production, Damanhour Branch, Animal Health Research Institute, Agricultural Research Center.
E-mail address: hamdysalam83@gmail.com
DOI: 10.21608 /EJAH.2023.291798

INTRODUCTION:

Avian infectious bronchitis virus (IBV), the *gammacoronavirus*, is the cause of highly contagious economically important viral disease of poultry (**Moharam et al. 2020 and Marandino et al. 2022).** IBV-infected chickens show respiratory signs (sneezing, coughing, tracheal coarse crackles), pro-ventriculitis, air sacculitis, enteritis, nephritis, and reduction in feed intake **(Yu et al. 2001; Cook et al. 2012; Al-Jallad et al. 2020 and Wu et al. 2022).**

IBV which belongs to family *Coronaviridae*, is an enveloped virus with positive sense single-stranded RNA of approximately 27.6 kb in size. This RNA encodes four structural proteins [namely; nucleocapsid protein (N), small envelope protein (E), membrane glycoprotein (M), and spike glycoprotein (S)] **(Cavanagh, 2003 and Su et al. 2011).**

The S protein is cleaved into amino-terminal (S1) and carboxyl-terminal (S2) glycopolypeptides that are involved in both virus attachment and activation of fusion between viral and host cell membranes in order to viral RNA release **(Cavanagh et al. 1988 and Cavanagh, 2007).** In addition, to be involved in virus infectivity, S1 protein contains three antigenic epitopes that are associated with three hypervariable regions (HVRs) (encompass aa 38–67, 91–141, and 274–387) that are the targets for neutralizing antibodies and have the highest nucleotide heterogeneities among IBV serotypes and can be used to determine genetic relationship between field strains, vaccines and novel variants **(Cavanagh, 2005; Gelb et al. 2005 and Al-Jalladet al. 2020).** Moreover, heterogeneities of S1 became the base for genotypic classification of IBV. Emerging new antigenic variant strains can occur due to genetic changes as a result of deletions, insertions, point mutations, or RNA recombination, especially in the S1 gene. In addition, the similarity of S1 gene between the used vaccine and field strains determines the efficacy of vaccination against IBV **(Lian et al. 2021).** Thus, new outbreaks in vaccinated chicken flocks often occur due to emerging of new variants **(Jia et al. 1995; Abdel-Moneim, et al. 2012; Valastro et al. 2016 and Lian et al. 2021).** Accordingly, continuous monitoring of genetic changes in S1 gene is important for determination of these new variants and essential for disease control via vaccination. Therefore, this study was carried out to identify the circulating IBV in four Egyptian governorates and to determine its genetic relation with the commonly used vaccine strains.

MATERIALS and METHODS

Samples:

Samples were collected from 30 broiler and five Broiler breeder farms in four Egyptian governorates **(Giza, Sharkia, Behera, and Gharbia)** during 2021-2022. These chickens suffered from nasal discharge coughing, gasping, sneezing, conjunctivitis, and watery whitish diarrhea. Tracheal swabs, trachea, kidney, and cecal tonsils were collected. The swabs specimens were suspended in 0.5 mL of phosphate-buffered saline (PBS) containing streptomycin and penicillin then vortexed for 20 min. Tissue samples were homogenized in PBS (a suspension of 20% w/v) containing also streptomycin (100µg/mL) and penicillin (100 U/mL) **(Lian et al. 2021).** Samples were stored at -80 ^{0}C until to be examined with RT-PCR.

Detection of viral RNA by real-time reverse transcription PCR (rRT-PCR):

RNA of IBV was extracted from samples using QiaAmp viral RNA mini kit (Qiagen, Valencia, Calif., USA), according to the manufacturer's instructions. Extracted RNA was reverse transcribed and amplified using Quantitect probe RT-PCR kit (Qiagen, Valencia, Calif., USA) following the manufacturer's instructions. Briefly, 12.5 µl of 2x Quantitect probe RT-PCR buffer, 4.5 µl of RNase - free water, 0.125 µl of Quantitect probe RT enzyme, 2.25 µl from each primer and 1µl of probe (table 1) were added to 5 µl of RNA template. Real-time PCR machine (Stratagen, MX 3005P, USA) was used to accomplish the reaction with the following thermal profile: 50 °C for 30 min (one cycle), 95 °C for 15 min (one cycle), 94 °C for 15 sec and 60 °C for 40 sec (40 cycles).

Table 1. Primers and probe were used in this study.

Name	Sequences (5′ - 3′)	Target regions	References
IBV5' GU391	GCT TTT GAG CCT AGC GTT		
IBV5' GL533	GCC ATG TTG TCA CTG TCT ATT G	5' untranslated region (5'–UTR)	Callison et al.,(2006)
IBV5' G probe	FAM- CAC CAC CAG AAC CTG TCA CCT C-BHQ1		
IBV-HVR1-2-FW	GTKTACTACTACCARAGTGC	S1	Naguib et al.,(2017)
IIBV-HVR1-2-RV	GAAGTGRAAACRAGATCACCATTTA		

Virus isolation:

Samples with rRT-PCR positive results were inoculated onto chorioallantoic sac of ten -day-old SPF (specific pathogen-free) embryonated chicken eggs. Embryos were checked for seven days post inoculation (PI) and that died within 24 hrs (PI) were discarded. The allantoic fluid was harvested for viral RNA identification and sequencing **(Momayez et al. 2002).**

Identification of IB viral RNA by RT-PCR:

Verso 1-Step RT-PCR ReddyMix Kit (Thermo Fisher Scientific, USA) was used for partial amplification of S1 gene of IBV according to the manufacturer's instructions. Briefly, 25 µl of 1-Step PCR Reddy Mix buffer, 2.5 µL of RT enhancer, 1 µl of Verso Enzyme Mix, 13 µl of Nuclease-free water, and 2 µl of each primer (table 1), were mixed to amplify 5 µl of RNA template (extracted from allantoic fluid of each isolate) in Applied biosystem 2720 thermal cycler (Germany). The following thermal profile was used: 50 °C for 15 min (one cycle), 95 °C for 2 min (one cycle), 95 °C for 20 sec, 52 °C for 30 sec and 72 °C for one min (40 cycles) then final extension at 72°C for 10 min. On 1.5% agarose gel containing ethidium bromide, PCR products were visualized with 100 bp DNA ladder (Fermentas, Thermo, Germany).

Partial sequencing of S1 gene:

QIA quick Gel Extraction Kit (Qiagen Inc. Valencia, CA, USA) was used for purification of DNA fragments that obtained by RT-PCR according to the manufacturer's instructions. Partial sequencing reaction was performed with sequencing kit (Big dye Terminator V3.1 cycle, Perkin-Elmer, Foster city, CA). Sequence reaction was purified using spin column (Centrisep). ABI automated sequencer (Applied Biosystems 3130 genetic analyzer, USA) was used for sequencing..

Phylogenetic analysis:

The obtained nucleotide sequences of S1 gene were subjected to basic local alignment search tool (BLAST) within GenBank database and aligned with reference and vaccine strains using CLUSTALW tool of BioEdit software (version 7.2.5). The pairwise distance was determined and Neighbor-joining (NJ) tree was constructed using maximum composite likelihood of MEGA11 software with 1000 bootstrap replications **(Tamura et al. 2021).**

Table 2. Epidemiological data of the seven IBV isolates.

Isolate name	Locality	year	Production type	Age (days)	Isolation source	Vaccine used	Accession nos.
HS1	Giza	2021	Broilers	23	Tracheal swabs	H120	OQ349378
HS2	Sharkia	2021	Broilers	28	Trachea and cecal tonsils	H120	OQ349379
HS3	Behera	2021	Broilers	19	Cecal tonsils	H120+D274	OQ349380
HS4	Giza	2022	Broilers	30	Kidney	Ma5	OQ349381
HS5	Behera	2021	Broilers	33	Trachea and cecal tonsils	H120	OQ349382
HS6	Gharbia	2022	Broiler breeders	56	Tracheal swabs	H120+D274	OQ349383
HS7	Giza	2022	Broilers	25	Trachea	H120+D274	OQ349384

Table 3. Nucleotide and amino acid identities of the seven tested IBV strains in relation to some vaccine and reference strains.

	Amino acid identity (%)																								
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	
1-Israel/720/99		69.5	70.4	57.9	61.2	62.3	64.4	67.5	56.8	63.3	65.4	97.7	95.5	71.4	59	61.2	61.2	70.4	67.5	70.4	70.4	70.4	71.4	59	57.9	1
2-EG/CU/4/2014	67.2		84.2	57.9	61.2	62.3	69.5	80.7	68.5	65.4	71.4	69.5	67.5	77.1	59	61.2	61.2	96.2	88.4	89.2	90	88.4	90	63.3	61.2	2
3-EG/1212B	65.2	84.1		54.5	56.8	60.1	71.4	85	66.4	67.5	73.3	71.4	69.5	77.1	54.5	56.8	56.8	86.7	79.8	81.6	84.2	81.6	88.4	59	57.9	3
4-Conn46	57	66.1	61.5		80.7	65.4	57.9	60.1	53.4	53.4	63.3	59	56.8	62.3	78.9	80.7	80.7	57.9	59	57.9	57.9	57.9	55.79	77.1	77.1	4
5-H52	57.8	66.8	61.8	91.4		64.4	57.9	61.2	55.7	55.7	62.3	62.3	57.9	61.2	91.6	100	100	60.1	63.3	61.2	61.2	62.3	60.1	93.2	95.5	5
6-QX	54.6	58.5	62.4	66.4	66.6		62.3	63.3	61.2	64.4	60.1	64.4	61.2	57.9	63.3	64.4	64.4	63.3	57.9	61.2	62.3	62.3	59	62.3	61.2	6
7-D-1	55.5	65.2	69	59.5	58.7	60.9		71.4	63.3	66.4	71.4	65.4	64.4	69.5	56.8	57.9	57.9	72.4	67.5	72.4	71.4	72.4	69.5	56.8	55.7	7
8-D274	62.3	83.5	88.8	64.4	66.2	64.5	70.4		67.5	68.5	71.4	67.5	64.4	71.4	59	61.2	61.2	84.2	74.3	77.1	78	77.1	78	57.9	56.8	8
9-CR88121	59	68.3	67.7	59.3	62.1	63.9	64.3	67.8		87.5	68.5	56.8	54.5	64.4	54.5	55.7	55.7	69.5	64.4	64.4	64.4	64.4	65.4	55.7	53.4	9
10-4/91	64	67.2	65.3	61.6	64.6	65.4	65.2	66.2	93.4		67.5	63.3	61.2	66.4	54.5	55.7	55.7	68.5	64.4	66.4	65.4	65.4	66.4	55.7	53.4	10
11-Q1	65.1	72.6	72.2	59.3	57.5	54.7	64.8	71.7	65.3	64.4		66.4	64.4	77.1	60.1	62.3	62.3	73.3	68.5	72.4	71.4	70.4	73.3	61.2	57.9	11
12-IS/885	98.4	67.3	65	56.8	58.5	55.4	56.7	61.6	58.3	62.5	66.9		95.5	72.4	59	62.3	62.3	70.4	68.5	72.4	72.4	72.4	71.4	60.1	59	12
13-Sul/01/09	97.9	66	63.6	55.6	56.4	52.9	54.7	60.5	58.4	62.9	64.4	97.4		71.4	55.7	57.9	57.9	68.5	66.4	69.5	69.5	69.5	69.5	56.8	55.7	13
14-D888	69.6	79.5	76.5	60.8	58.7	56.4	67.5	69.7	64	64.9	80.9	71.3	68.9		57.9	61.2	61.2	78	74.3	76.2	78	75.2	78	62.3	60.1	14
15-Mass41	57.7	66.9	62	91.6	96.4	65.9	60.4	65.5	61.4	63.9	59	57.9	55.9	60.9		91.6	91.6	57.9	59	60.1	60.1	62.3	57.9	90	88.4	15
16-H120	57.8	67.5	61.8	90.8	99.5	66.6	58.7	66.2	62.1	64.6	57.5	58.5	56.4	58.7	95.9		100	60.1	63.3	61.2	61.2	62.3	60.1	93.2	95.5	16
17-Ma5	57.8	66.8	61.8	91.4	100	66.6	58.7	66.2	62.1	64.6	57.5	58.5	56.4	58.7	96.4	99.5		60.1	63.3	61.2	61.2	62.3	60.1	93.2	95.5	17
18-IS/1494/06	67.6	97.9	85.1	67.1	67.8	60.1	67	85.1	70.3	69.1	73.8	67	66.4	79.6	67.9	68.5	67.8		89.2	91.6	92.4	90.8	92.4	62.3	60.1	18
19-HS1	68	93.7	81.6	66.2	68.3	56.6	65.8	79.9	65.1	65.9	70.4	67.8	67.2	78.3	67.4	68.3	68.3	93.9		92.4	93.2	91.6	90	66.4	64.4	19
20-HS2	70	94.3	83	65.8	68.7	59	68.4	80.8	67.1	67.9	73.1	70.1	69.6	80.3	69.3	69.4	68.7	94.5	96.6		97.7	97.7	92.4	64.43	62.3	20
21-HS3	70	94.5	83.3	65.5	68.3	59.1	68	80.4	66.8	67.1	72.7	70.1	69.6	80.6	69	69.1	68.3	94.8	96.9	98.7		97	94.7	64.4	62.3	21
22-HS4	69.1	94.2	82.3	65.5	68.4	59.2	67.5	80.1	66.8	67.2	72.3	69.3	68.8	79.6	69	69.1	68.4	94.5	96.6	99	99.2		91.6	65.4	63.3	22
23-HS5	71.1	93.8	86.2	65.3	67	58.9	65.7	80.3	67.4	67.5	74	70.4	69.2	81.1	67.1	67.7	67.0	94	94.5	95.6	96.9	96.1		63.3	61.2	23
24-HS6	57	70	65.2	89.6	95.6	66.5	57.1	64.4	62.1	63.6	56.7	57.7	56.1	60.5	95.6	95.1	95.6	70.2	72.7	73.2	72.8	72.9	71.5		94.7	24
25-HS7	55.4	69.1	63.4	88.4	96.6	65.4	56.5	62.6	60.6	62.1	54.2	56.1	54.4	59.0	93.7	97.2	96.6	69.3	71.1	72.3	71.9	72	70.6	96.9		25
	Nucleotide identity (%)																									

Table 4. Amino acid substitutions within HVR1 and 2 of the strains of variant group in relation to some IBV reference and vaccine strains.

	Amino acid substitutions																											
	HVR1											HVR2																
	38	41	50	54	55	57	60	62	63	64	67	94	96	105	108	116	117	118	119	125	126	127	128	129	130	137	138	139	140
Israel/720/99	D	-	V	L	A	A	-	Q	P	I	V	K	Q	S	T	S	S	G	Q	Q	L	Q	R	N	S	S	G	N	D
IS/1494/06	T	-	V	V	E	S	G	P	G	Q	A	A	Q	T	T	K	S	G	H	L	I	P	Q	N	H	K	N	S	S
EG/1212B	T	-	V	V	E	R	G	S	Q	Q	A	T	E	T	V	K	S	S	S	M	I	P	Q	Y	Y	R	N	N	S
EG/ CU/4/2014	T	-	V	V	E	S	G	S	G	Q	A	A	Q	T	T	K	N	G	Q	L	I	P	Q	N	H	K	N	S	S
HS1	T	L	L	V	E	S	G	S	G	Q	A	V	Q	T	T	K	N	G	Q	Q	I	P	Q	H	H	K	N	S	H
HS2	T	L	V	V	E	S	G	S	G	Q	A	V	Q	T	T	K	S	G	Q	L	I	S	K	D	H	K	S	S	H
HS3	T	L	V	V	E	S	G	S	G	Q	A	V	Q	T	T	K	S	G	Q	L	I	P	K	D	H	K	N	S	H
HS4	T	-	V	V	E	S	G	S	P	Q	A	V	Q	T	T	K	S	G	Q	L	I	S	K	D	H	K	N	S	H
HS5	T	-	V	V	E	S	G	S	G	Q	A	V	Q	T	T	K	S	G	Q	L	I	P	Q	N	Y	R	N	N	S

Numbers of amino acids are indicated according to strain Mass41 except No. 41, 118 and 119 that are amino acid insertions

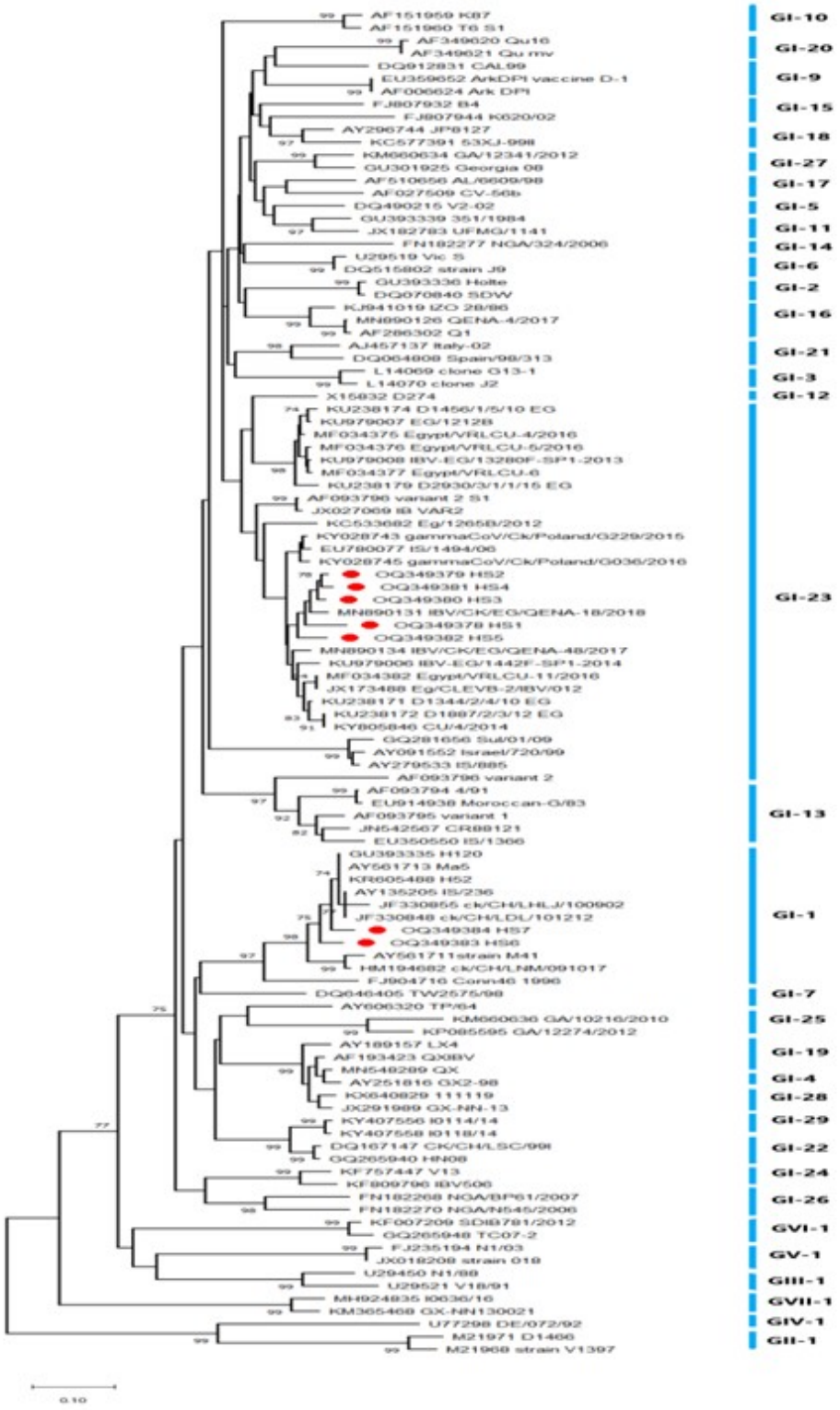

Figure 1. Neighbor-Joining tree based on partial amino acid sequences of S1 gene of the seven tested IBV strains (indicated with red circles) and other vaccine and reference strains.

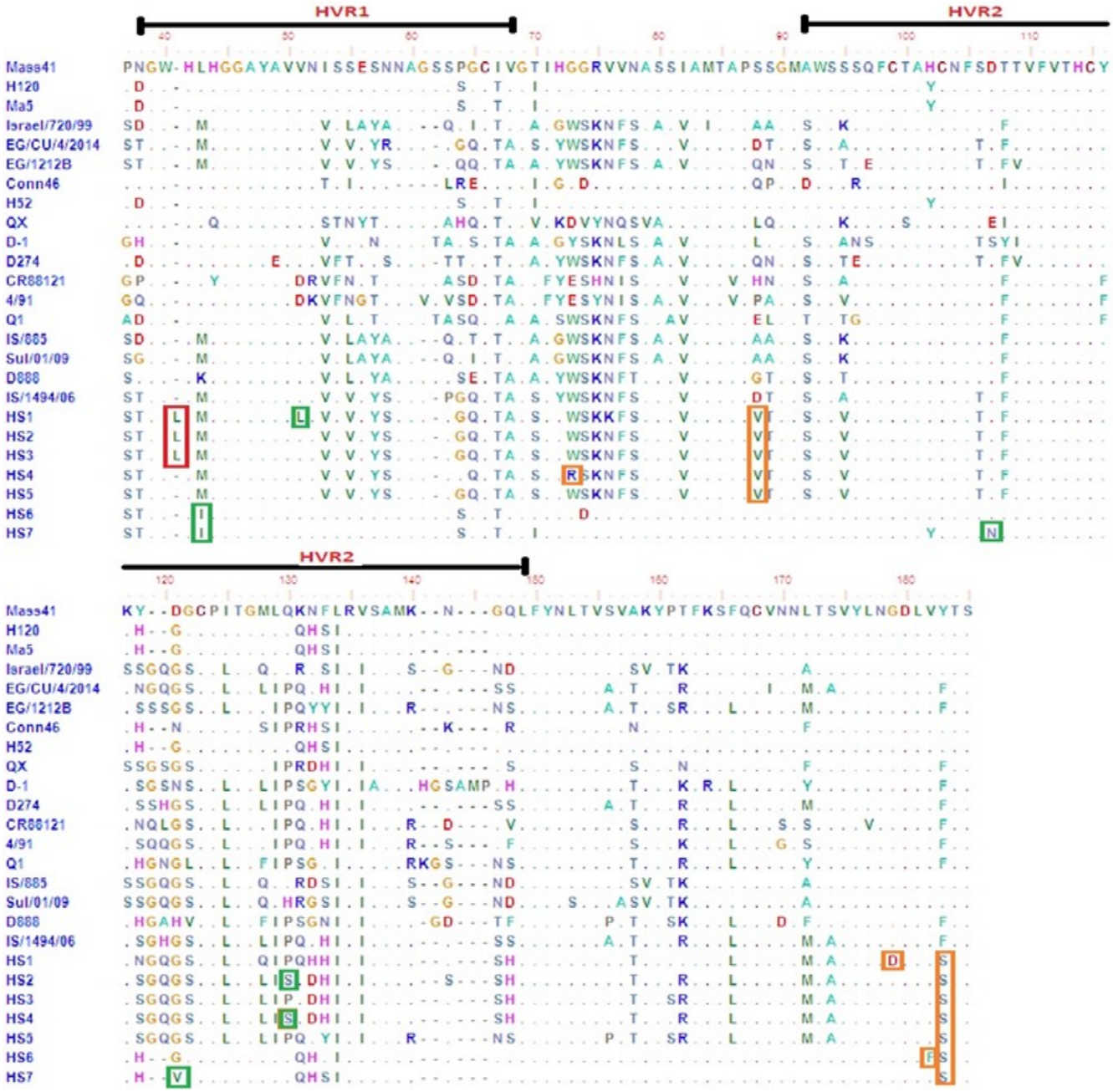

Figure 2. Unique deduced amino acid substitutions identified in the partially sequenced S1 gene. Green rectangular indicate amino acid substitutions within HVR1 and 2. Orange rectangular indicate amino acid substitutions out of HVR1 and 2. Red rectangular indicates amino acid insertion in three strains.

RESULTS:

Clinical examination:

The prevalent clinical signs were nasal discharge, coughing, gasping, sneezing, conjunctivitis, and watery whitish diarrhea.

Viral Identification and phylogenetic analysis:

Six isolates of IBV (HS1-5 and HS7) were identified in the samples collected from broiler farms in three Egyptian governorates (Giza, Sharkia and Behera) during 2021 and 2022. Additionally, one isolate (HS6) was identified in broiler breeder farm in Gharbia governorate during 2022. All these farms were vaccinated by different types of IBV vaccines (table 2). These isolates shared 70.6%-99.2% nucleotide identities and 61.2%-97.7% amino acid identities. Strains HS1-5 were found to be closely related and clustered within distict subclade which distantly related to the subclade of both HS6 and HS7 that were close to each others (table 3 and figure 1). Nucleotide and amino acid identities of 60.6%-97.2% and 53.45%-95.5%, respectively were identified with the commonly used vaccine strains (table 3).

A unique amino acid insertion was found in HVR1 of strains HS1-3. Moreover, HS1, HS6 and HS7 had an amino acid substitution within the same region. Additionally, within HVR2, a unique amino acid substitution was located in strains HS2 and HS4 and two unique amino acid substitutions in the strain HS7 (table 4 and figure 2).

DISCUSSION:

In Egypt, IBVs are usually identified and continue to circulate in commercial poultry production although of vaccination programs that are adopted with several viral strains in many poultry farms. It was suggested that the using of homologous vaccine strains can achieve the best protection against IBV challenge **(Ali et al. 2018; Sultan et al. 2019; Erfanmanesh et al. 2020; Houta et al. 2021).** Therefore, virus identification from infected chickens should be continuously undertaken in order to accomplish disease control. IBV strains were classified into seven genogroups (GI-GVII) with at least 32 distinct lineages, from which 29 lineages were confined to GI **(Houta et al. 2021 and Kariithi et al. 2022).** The genotype I- lineage 1 (GI-1) which represents the old Massachusetts (Mass) like strains, is widely distributed in all endemic countries and therefore is most commonly used for vaccine production. On the other hand, the genotype I- lineage 23 (GI-23) that includes IBV variant strains, continues to spread and circulate in the Middle East posing a major challenge the disease control **(Houta et al. 2021).**

During 2021-2022, samples were collected from chickens that suffered from respiratory signs and/or diarrhea in four Egyptian governorates (Giza, Sharkia, Behera and Gharbia) for identification and molecular characterization of IBV. Phylogenetic analysis that was based on partial sequences of S1 gene (including HVR1 and 2), revealed dividing of the isolated IBV strains into two groups; one (included HS1-5) was clustered with variant strains (GI-23), while the other one (included HS6 and HS7) was clustered with Mass like strains (GI-1). The two groups shared 70.6%-73.2% and 61.2%-66.4% nucleotide and amino acid identities, respectively (table 3).

The strains within each group shared high homology of nucleotide and amino acid identities that were interpreted in the amino acid-based neighbor-Joining tree by clustering of these strains together within distinct two monophyletic groups (figure 1). The variant group was clustered with IBV/CK/EG/QENA-18/2018 that was also assigned under a distinct subclade on the bases of sequencing of full-length S1 gene **(Sabra et al. 2020 and Houta et al. 2021).**

Nucleotide and amino acid identities of 65.1%-86.2% and 57.9%-88.4%, respectively, were estimated between the strains of the variant group and the commonly used vaccine strains, from which EGY/VAR II (EG/1212B) was the closest one. However, 9-15 amino acid substitutions were found between EG/1212B and the strains of the variant group (table 4). Moreover, Strains HS1-3 that were identified

from three different governorates **(Giza, Sharkia and Behera)** during 2021, were found to have the same unique amino acid insertion (leucine) within HVR1 in relation to vaccine strains (figure 2). Meanwhile, Mass like group shared wider ranges of nucleotide and amino acid identities (60.6%-97.2% and 53.4%-95.5%, respectively) with the commonly used vaccine strains, from which Mass like vaccine strains were the closest ones (table 3). However, 5-13 amino acid substitutions were observed between Mass like group and the closely related vaccine strains. Eight and six amino acid substitutions were identified in H120 in relation to HS6 and HS7, respectively, from which five were found within HVR1 and 2 that are the targets for neutralizing antibodies **(Cavanagh, 2005; Gelb et al. 2005 Al-Jallad et al. 2020),** and that might explain vaccination failure although of close relationship with the identified strain (figure 2).

CONCLUSION:

Collectively, this study indicated the circulation of both variant and Mass like strains of IBV in four Egyptian governorates. These strains were found to have some genetic variations from previously identified strains as well as commonly used vaccine strains. More investigations are needed to study the antigenicity of the tested IBV in relation to currently used vaccine strains to determine the proper protectotype .

REFERENCES:

Abdel-Moneim AS, Afifi MA, El-Kady MF. 2012. Emergence of a novel genotype of avian infectious bronchitis virus in Egypt. Arch. Virol., (157): 2453–2457.

Al-Jallad T, Kassouha M, Salhab M, Alomar A, AL-Masalma M, Abdelaziz F. 2020. Molecular characterization of isolated infectious bronchitis viruses from affected vaccinated broiler flocks in Syria. BMC Vet Res., 16:449.

Ali A, Kilany WH, Zain El-Abideen MA, Sayed ME, Elkady M. 2018. Safety and efficacy of attenuated classic and variant 2 infectious bronchitis virus candidate vaccines. Poult. Sci., 97(12):4238–4244.

Callison SA, Hilt DA, Boynton TO, Sample BF, Robison R, Swayne DE, Jackwood MW. 2006. Development and evaluation of a real-time Taqman RT-PCR assay for the detection of infectious bronchitis virus from infected chickens. J. Virol. Methods, (138): 60–65.

Cavanagh D. 2003. Severe acute respiratory syndrome vaccine development: experiences of vaccination against avian infectious bronchitis coronavirus. Avian Pathol., (32):567–582.

Cavanagh D. 2005. Coronaviruses in poultry and other birds. Avian Pathol., (34):439–448.

Cavanagh D. 2007. Review article: Coronavirus avian infectious bronchitis virus. Vet. Res., (38): 281-97.

Cavanagh D, Davis PJ, Mockett AP. 1988. Amino acids within hypervariable region 1 of avian coronavirus IB (Massachusetts serotype) spike glycoprotein are associated with neutralization epitopes. Virus Res., 11(2): 141-50.

Cook JKA, Jackwood M, Jones RC. 2012. The long view: 40 years of infectious bronchitis research. Avian Pathol., 41(3):239-50.

Erfanmanesh A, Ghalyanchilangeroudi A, Nikaein D, Hosseini H, Mohajerfar T. 2020. Evaluation of inactivated vaccine of the variant 2 (IS-1494/GI-23) genotype of avian infectious bronchitis. Comp. Immunol. Microbiol. Infect. Dis., (71):101497.

Gelb J, Weisman Y, Ladman BS, Meir, R. 2005. S1 gene characteristics and efficacy of vaccination against infectious bronchitis virus field isolates from the United States and Israel (1996 to 2000). Avian Pathol., 34(3): 194-203.

Houta MH, Hassan KE, El Sawah AA, Elkady MF, Kilany WH, Ali A, AbdelMoneim A. S. 2021. The emergence, evolution and spread of infectious bronchitis virus genotype GI-23. Arch. Virol., (166):9–26.

Jia W, Karaca K, Parrish CR, Naqi SA. 1995. A novel variant of avian infectious bronchitis virus resulting from recombination among

three different strains. Arch. Virol., (140): 259–271.

Kariithi HM, Volkening JD Leyson CM, Afonso CL, Christy N, Decanini EL, Lemiere S, Suarez DL. 2022. Genome sequence variations of infectious bronchitis virus serotypes from commercial chickens in Mexico. Front. Vet. Sci., (9): 931272.

Lian J, Wang Z, Xu, Z, Chen T, Shao G, Zhang X, Qin J, Xie Q, Lin W. 2021. Distribution and molecular characterization of avian infectious bronchitis virus in Southern China.Poult. Sci., (100): 101169.

Marandino A, Vagnozzi A, Tomás G, Techera C, Gerez R, Hernández M, Williman J, Realpe M, Greif G, Panzera, Y. 2022. Origin of new lineages by recombination and mutation in avian infectious bronchitis virus from South America. Viruses, (14): 2095.

Moharam I, Sultan H, Hassan K, Ibrahim M, Shany S, Shehata AA, Abo-ElKhair M, Pfaff F, Höper D, El Kady M. 2020. Emerging infectious bronchitis virus (IBV) in Egypt: Evidence for an evolutionary advantage of a new S1 variant with a unique gene 3ab constellation. Infect. Genet. Evol., (85): 104433.

Momayez R, Pourbakhs SA, Khodashenas M, Banani M. 2002. Isolation and identification of infectious bronchitis virus from commercial chickens. Arch. Razi Ins., (53): 1-10.

Naguib MM, El-Kady MF, Lüschow D, Hassan KE, Arafa A, El-Zanaty A, Hassan MK, Hafez HM, Grund C, Harder TC. 2017. New real time and conventional RT-PCRs for updated molecular diagnosis of infectious bronchitis virus infection (IBV) in chickens in Egypt associated with frequent co-infections with avian influenza and Newcastle disease viruses. J. Virol. Methods, (245): 19–27.

Sabra M, Abdellatif W, Ahmed AI, Osman N. 2020. Molecular characterization and phylogenetic analysis of full-length S1 gene of GI-16 and GI-23 infectious bronchitis virus in Qena, Egypt. J. World Poult. Res., 10 (1): 71 -80.

Su JL, Zhou ZT, Guo ZS, Xu, QR, Xiao YC, Li, ZL, Shi, DS, Bi, DR. 2011. Identification of five bronchitis virus (IBV) strains isolated in China and phylogenetic analysis of the S1 gene. Afr. J. Microbiol. Res., 6 (9): 2194–2201.

Sultan HA, Ali, A, El Feil WK, Bazid AHI, Zain El-Abideen MA, Kilany WH. 2019. Protective efficacy of different live attenuated infectious bronchitis virus vaccination regimes against challenge with IBV variant-2 circulating in the Middle East. Front. Vet. Sci., (6): 341.

Tamura K, Stecher G, Kumar S. 2021. MEGA11: Molecular evolutionary genetics analysis version 11. Mol. Biol. Evol., (38): 3022-3027.

Valastro V, Holmes EC, Britton P, Fusaro A, Jackwood MW, Cattoli G, Monne I. 2016. S1 gene-based phylogeny of infectious bronchitis virus: An attempt to harmonize virus classification. Infect. Genet. Evol., (39): 349-364.

Wu, Z, Fang H, Xu Z, Lian J, Xie Z, Wang Z, Qin J, Huang B, Feng K, Zhang X. 2022. Molecular characterization analysis of prevalent infectious bronchitis virus and pathogenicity assessment of recombination strain in China. Front. Vet. Sci., (9):842179.

Yu, L, Jiang Y, Low S, Wang Z, Nam S, J, Liu, W, Kwang J. 2001. Characterization of three infectious bronchitis virus isolates from China associated with proventriculus in vaccinated chickens. Avian Dis., 45 (2): 416-424.