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Characterization and Genotyping of Avian Infectious Bronchitis Virus in Egypt from 2019 to 2022

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Abstract

Avian infectious bronchitis virus (IBV) causes a major problem in broiler chickens due to increasing mortality and lowering body weight. This group of gammacorona viruses has the ability to emerge frequent new variants. In the present study, 18 broiler chicken farms from 7 Egyptian governorates that showed respiratory signs were sampled from 2019 to 2022. There were 11 farms positive for detection of IBV with real time RT-PCR. The samples were inoculated in specific pathogen free (SPF) embryos for three successive blind passages and the obtained viruses were sequenced for hypervariable region of spike protein (S1) to study their genetic diversity. The results showed that the S1 gene was clustered into two major groups, the first group has only one virus belong to classical vaccine strain of GI-1 lineage 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, second subgroup contain one virus belong to genotype GI-23.2.2. The selection pressure analysis revealed episodic diversifying selection on multiple sites, with positive selection observed at five amino acid residues of the S1 protein, as demonstrated by FEL models. The recombination analysis of the S1 gene reveled two viruses with recombination events. The F1282-7-IB-2022 exhibited a slight recombination from IS/1494/2006 and a larger recombination from M41-2004. Meanwhile, the F1282-8-IB-2022 had a minor recombination of strain 4/91-1998 and a larger recombination from the Egyptian strain IBV-D1344/2/4/10-EG. The 3D structural models of hypervariable region HVR of S1 protein also showed that the recent viruses in this study from subgroup GI-23.2.1 (F1282-6-IB-2022) have high structural similarity with vaccine strain D274 and local vaccine seed virus IBV-EG/1212B-2012 than classic or variant GI-23.2.2 subgroup. These results can support efforts to compare the efficacy of local and imported vaccines both in-vivo and in-vitro and to help in controlling the disease.

KEYWORDS
Infectious Bronchitis Virus, Molecular Characterization, Spike protein, Selection pressure

INTRODUCTION

Avian infectious bronchitis (IB) is a contagious disease caused by the infectious bronchitis virus (IBV). It primarily affects the respiratory system of chickens, leading to reduced weight gain and increased susceptibility to bacterial infections. It is caused by the infectious bronchitis virus (IBV) of gammacoronavirus (Jackwood and de Wit, 2013). IBV is an enveloped, non-segmented, positive sense single stranded RNA virus. Its genome consists of about 27 kb and codes for three structural proteins: the spike (S), the membrane (M) glycoprotein, and the nucleocapsid (N) phosphoprotein. The spike glycoprotein (S) is attached to the viral envelope and is cleaved into two proteins designated S1 and S2 with three hypervariable regions (HVRs) in the S1 subunit (Moore *et al*., 1997).

Based on the sequence of the full-length S1 gene, the phylogenetic classification of IBVs has been standardized globally to comprise 6 genotypes and 32 lineages (Valastro *et al*., 2016). While the remaining genotypes only contain one viral lineage each (GII-1, GIII-1, GIV-1, GV-1, and GVI-1), genotype I contains 27 distinct viral lineages (temporarily ordered GI-1 to GI-27). (Ababneh *et al*., 2012). There are four different genetic lineages have been detected in Egypt. These are categorized into GI-1, which includes both the classical wild strains and vaccine-like strains. GI-23 which includes the Egyptian variant subgroups (Egy/Var-2 and Egy/Var-1). GI-16 which was first reported in China and is now prevalent in many countries in Asia, Africa, the Middle East and Europe. Lastly, GI-13 which includes 4/91-like strains and is believed to have arrived from the presently used 4/91 vaccine strain (Abozeid and Naguib, 2020).

Vaccination programs face challenges due to the diversity of antigenically different strains; because IBV presents the phenomenon of genetic recombination or the virus can acquire mutations, generating new strains (Bayry *et al*., 2005). Identification of these variant serotypes circulating together in the field is very important for screening the new variants as well as selecting the most appropriate vaccine strains (Cook *et al*., 1999).

Therefore, this study aimed to determine the genetic characterization of IBV field isolates in Egypt from 2019-2022 based on the S1 gene sequence.

MATERIALS AND METHODS

Ethical approval

The materials and protocols applied in this study were ac-

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cepted by the Scientific Research Ethics Committee of the Faculty of Veterinary Medicine, University of Suez, Ismailia, Egypt, approval No (201882).

Collection of samples and preparations

In this study, 18 broiler flocks showed respiratory manifestations and mortalities have been sampled from field observation during 2019-2022. The samples were collected from 7 Egyptian Governorates, including Damietta, Dakahlia, Beni Suef, Minia, Giza, Qalubia and Kafr-Sheikh. The samples were collected for molecular detection of IBV, virus isolation and histopathological examination. Tissue samples including tracheas, kidneys, and lungs were collected and pooled under hygienic conditions. The collected organs were washed in sterile saline and the tissue homogenates (10% w/v) were suspended in sterile saline (0.85% w/v) using sterile mortar and pestle, the homogenates were centrifuged at 3000 rpm for 10 min for molecular detection of IBV (Fehr and Perlman, 2015). Tissue samples, including trachea and kidneys, were collected from the examined birds for virus isolation and identification. Specimens from the tracheas and kidneys were fixed in neutral buffered formalin 10% for histopathological examination.

IBV detection by real time RT-PCR

Detection of IBV in collected samples and confirmation of the presence of virus after isolation in specific pathogen free embryonated chicken eggs (SPF-ECE) has been conducted using real-time reverse transcription-polymerase chain reaction (RT-PCR). RNA for RT-PCR was extracted from the supernatants of 10% w/v sample suspensions and allantoic fluid. The extraction of viral RNA was performed using a QiaAmp viral RNA mini kit (Qiagen, Germany) according to the manufacturer's instructions. Amplification of the specific target S1 gene was conducted 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 IBV-pan_Probe: 5-ACT GGA ACA GGA CCD GCC GCT GAC CT-3 (Naguib *et al*., 2017). Real-time RT-PCR was performed using Qiagen one step RT-PCR Kit (Qiagen, GmbH, Hilden, Germany) and the RT-PCR reactions were performed on Stratagene® thermal cycler machine.

Virus isolation

The supernatants of IBV-positive samples determined by RT-PCR were filtered with 0.22 um filter and the filtrate was treated with 10000 IU/mL penicillin, 1.0 mg/mL streptomycin then inoculated into five 10-day-old SPF-ECE (Koum Oshiem SPF chicken farm, Fayoum, Egypt) for each sample. The eggs were inoculated with 0.2 mL of the sample into the allantoic cavity then incubated at 37°C with daily candling. Allantoic fluids were harvested 96 h post inoculation. Three successive blind serial passages were performed. The allantoic fluids were harvested and stored at −70°C with examination of virus lesions in embryos like embryo mortality, curling and dwarfism (Fehr and Perlman, 2015).

Sequencing and phylogenetic analysis of the S1 gene

Eleven positive isolates were subjected to conventional RT-PCR using the Qiagen one step RT-PCR Kit (Qiagen, GmbH, Hilden, Germany) with the forward primer IBV-S1-F: 5-CACTGGTA-ATTTTTCAGATGG-3 and reverse primer IBV-S1-R: 5-CAGATTGCT-TACAACCACC-3 (Adzhar *et al*., 1997). S1 gene sequencing was conducted using the clear and specific band as selection criteria. Predicted IBV bands were observed at 450 bp in the gel. The amplicons were then purified using the QIA quick gel extraction kit (Qiagen, GmbH, and Hilden, Germany). Partial S1 gene sequencing was carried out using the genetic analyzer 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. The quality of obtained sequences was checked, assembled, and edited using BioEdit software version 7.0.4.1, and submitted to GenBank using BankIt tool (http://www.ncbi.nlm.nih.gov/WebSub/?tool=genbank), and the accession numbers were obtained. The S1 gene sequence was analyzed and compared with other IBV sequences available on GenBank, the National Center for Biotechnology Information (NCBI) (http://www.ncbi). Sequence similarities were computed using DNAstar software, and the nucleotide sequence phylogenetic tree was constructed using Mega 5. The retrievable sequences from the gene bank (http://ncbi/) are given in Table 1.

Estimation of selection pressure

Positive selection and positively selected sites within the S1 proteins were analyzed by the fixed effects likelihood (FEL) method of Data monkey version (http://www.datamonkey.org/26/08/2019) (Jackwood and Lee, 2017) to detect possible sites of amino acids that have undergone positive selection.

Recombination analysis

The current study used the Recombination Detection Program (RDP 4, Version 4.95) to detect potential recombination events of the S1 gene (Mo *et al*., 2013). This was achieved by implement-

Table 1. 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 GI-1	AY561711
H120	Netherlands	Classic GI-1	M21970
D274-1989	Netherlands	Variant 1 GI-12	X15832
IS/1494/2006	Israel	Variant 2 GI-23	EU780077
CR88-2014	Malaysia	Variant 1 GI-13	KM067900
strain4/91-1998	UK	Variant 1 GI-13	AF093794
IBV/CH/SA/6/2019	Saudi Arabia	Var II (G23.2.2)	MT270491
IB-54NB-chicken-LEBNAN	Lebanon	Var II (G23.2.1)	MH745418
MT081180._IS/64/2020	Israel	Var II (G23, 2.1)	MT081180
IBV/CK/EG/QENA-31/2018	Egypt	EGY variant 2GI-23	MN890132

ing various algorithms, which include RDP, Bootscan, Geneconv, MaxChi, Chimaera, SiScan, and 3Seq (Martin *et al*., 2015).

Predicted 3-dimensional structure of HVR of S1-glycoprotein

The 3D of HVR models of S1 proteins determinants of IB viruses from our study and vaccines viruses were generated using SWISSMODEL (https://swissmodel.expasy.org/) to find out the structural similarities between the HVR protein of our study virus and the vaccine viruses.

RESULTS

Clinical findings and postmortem lesions of infected chickens

The present study was focused on the targeted detection of IB in 18 chicken farms located in 7 different governorates (Damietta, Dakahlia, Beni Suef, Minia, Giza, Qalubia and Kafr-Sheikh) from 2019 to 2022. Respiratory signs were varied in severity and renal signs appeared in the form of chalky droppings in some flocks, resulting in varying mortalities across different breeds (Table 2). Post-mortem observations revealed tracheal congestion and exudation, tracheal and bronchial casts, rhinitis, serositis (pericarditis, perihepatitis and air sacculitis), splenic and hepatic congestion, and nephritis with ureates deposition in renal tissue and ureters.

Detection of IBV by real time RT-PCR

Upon conducting real time RT-PCR testing on 18 flocks, it was found that 11/18 flocks (61%) tested positive for IBV from 6 Governorates. Among the positive results, four out of seven flocks from Damietta, three out of six from Dakahlia and one each from Minya, Giza, Qalubia and Kafr-Skeikh were affected (Table 3).

Virus isolation

There were 10 samples positive for virus isolation from the 6 positive Governorates and the lesions were observed in the specific-pathogen-free embryos in the form of mortality of embryos, stunting, and curling. The lesions were clearly identified in inoculated embryos with positive samples by real time RT-PCR after the third passage.

Phylogenetic Analysis and genotyping of IBV

In this study, genetic characterization of the S1 gene of IBV was conducted using 10 isolates from the 6 studied governorates: three Damietta viruses (F1282-1-IB-2021, F1282-3-IB-2021 and F1282-7-IB-2022), three Dakahlia viruses (F1282-2-IB-2021, F1282-4-IB-2021 and F1282-9-IB-2022) and one each of Minya (F1282-5-IB-2021), Giza (F1282-6-IB-2022), Qalubia (F1282-8-IB-2022) and Kafr Sheikh (F1282-10-IB-2022). Our findings revealed that the S1 gene can be grouped into two categories. The first group consisted of one virus isolated from Damietta province (F1282-7-IB-2022), which was identified as the classical strain GI-1. The second group was identified as genotype GI-23, variant II, and included nine viruses that were divided into two subgroups. The first subgroup (GI-23.2.1) included eight viruses isolated from chickens in various governorates, while the second subgroup included one virus (GI-23.2.2) isolated from a flock in Dakahlia province (F1282-9-IB-2022). (Table 3 and Figure 1).

The comparison with other Egyptian and worldwide viruses retrieved from gene bank revealed that all viruses of Var II GI-23.2.1 exhibited a high nucleotide identity percentage of 93-100%. However, when compared with IBV var II GI-23.2.2, the identity percentage dropped to 81-85%, and from classical IBV strain with identity 78-81% (Fig. 2 and Table 4).

The obtained findings showed that classical GI-1 showed a significant amino acid sequence identity with classical vaccinal strains commonly used in Egypt including M41 and H120 vaccines with an identity of 81% and 88% respectively. also showed amino acid sequence identity with variant vaccinal strains com monly used in Egypt including D274, CR88, 4/91 and 1212B vaccines with an identity of 69%,61%,59% and 66% respectively (Table 4).

GI-23.2.1 (F1282-6-IB-2022) nucleotide sequences have a high identity percentage when compared to IBV/CK/EG/QENA-31/2018 strain isolated in Egypt 2018, IB-54NB-chicken-Lebanon strain isolated in Lebanon 2019 and IS/64/2020 strain isolated

Table 2. 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

in Israel 2020, with 99 %, 96 %, and 98 %, respectively. while the amino acid sequence identity ranged from 90-98 %,86-92 %, and 89-96 %, respectively (Table 4).

The nucleotide and amino acid sequences of GI-23.2.2 (F1282-9-IB-2022) have a high identity percentage of 98 % when compared to IBV/CH/SA/6/2019 strain isolated in Saudi Arabia in 2019 (Table 4).

Selection pressure analysis of S1 gene

The selection profile of S1 protein of IBVs was shown in Table 5 indicating that the S1 proteins of these IBV isolates had evolved under purifying selection. However, a few positively selected sites were detected although most sites were under neutral selection and purifying selection (Figure 3, Table 5). Aa residues 53, 68,

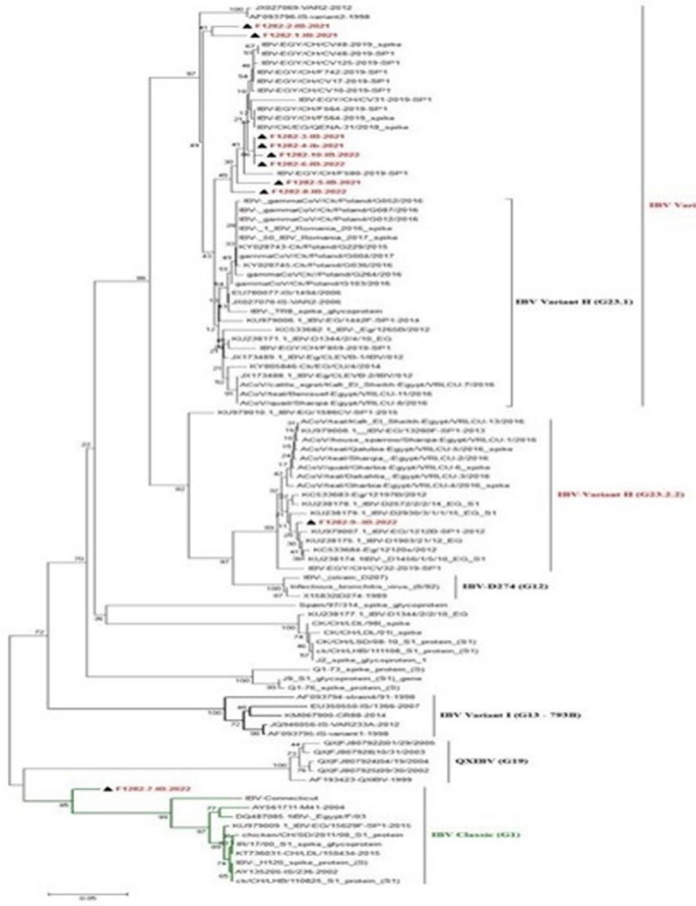

Fig. 1. Phylogenetic tree for the 10 IBV isolates based on S1 nucleotides.

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

130, 146 and 151 of the S1 protein were consistently highlighted by positive selection models (FEL) as positive selection sites. at p≤0.1 there are 5 Sites under diversifying positive selection, 62 Sites under purifying selection and 69 Sites under neutral selection.

Recombination analysis

Two strains of the S1 have been detected with recombination events. The F1282-7-IB-2022 exhibited a slight recombination from EU780077-IS/1494/2006 and a larger recombination from AY561711-M41-2004. Meanwhile, the F1282-8-IB-2022 had a minor recombination of AF093794-strain4/91-1998 and a larger recombination from the Egyptian strain KU238171.1. IBV-D1344/2/4/10 EG. (Figure 4).

Predicted 3D structure of HVR of S1-glycoprotein

The 3D models of HVR of S1 protein showed variation in their structures for classical and variant viruses. Maximum resemblance was found between study virus F1282-6-IB-2022 and the vaccine seed virus D274-1989 and the parent variant virus IBV-EG/1212B-SP1-2012. Whereas vaccine seed virus H120 spike protein and CR88-2014 showed less similarity. While the study virus F1282-7-IB-2022 that is belonging to classical group showed maximum resemblance with vaccine seed virus H120 spike protein and lower similarity with CR88-2014 and lowest similarity with vaccine strain D274-1989 and variant group viruses IBV-EG/1212B-SP1-2012 and study viruses of Egyptian G23-2.1 (Figure 5).

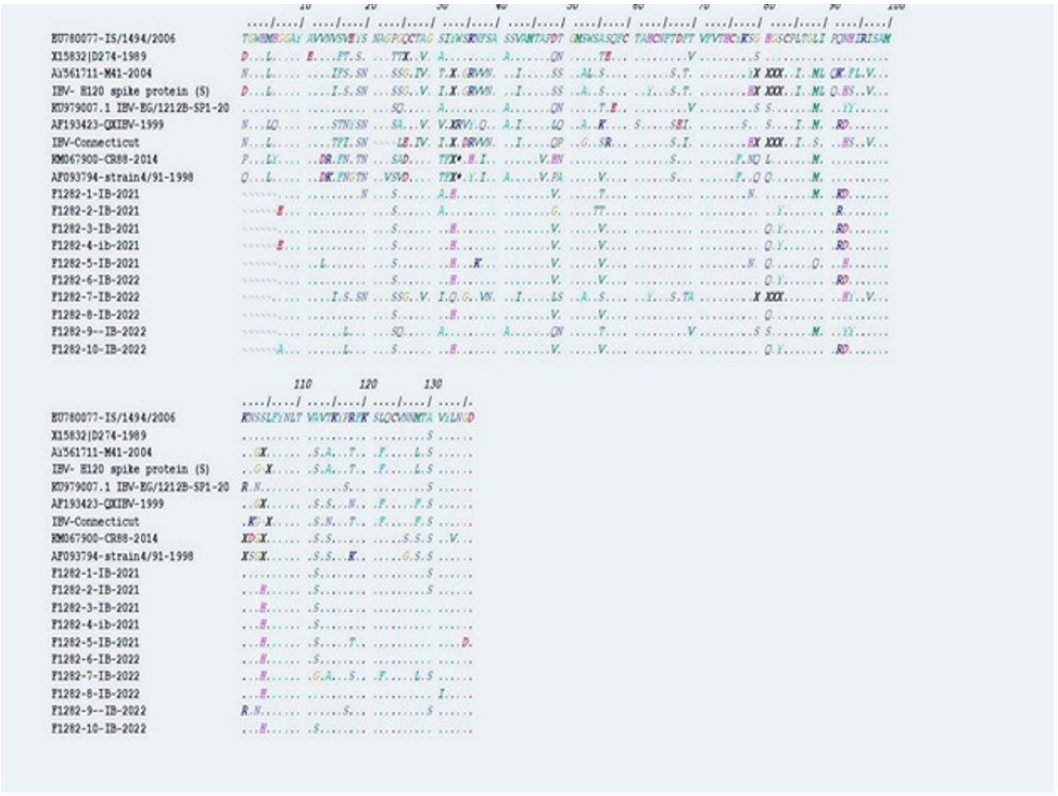

Fig. 2. Sequence alignment for the amino acids of HVR of S1 gene for the 10 isolates of the study.

Table 4. Nucleotide and amino acid identities of S1 gene sequence of Egyptian IBV compared to other selected strains and vaccine strains.

Similarity		Classical (F1282-7-IB-2022)		GI23-2.1 (F1282-1,2,3,4,5,6,8,10-IB)		GI23-2.2 (F1282-9-IB-2022)	
		Nt.	aa	Nt.	aa	Nt.	aa
Classical (F1282-7-IB-2022)				78-81%	67-71%	74%	67%
GI23-2.1 (F1282-1,2,3,4,5,6,8,10-IB)		78-81%	67-71%	93-100%	86-100%	81-85%	78-85%
GI23-2.2 (F1282-9-IB-2022)		74%	67%	81-85%	78-85%		
classical Vaccines	M41-2004	88%	81%	70-74%	60-66%	65%	57%
	H120	91%	88%	69-74%	63-68%	66%	62%
variant Vaccines	D274-1989	75%	69%	80-83%	76-84%	90%	88%
	CR88-2014	69%	61%	68-72%	70-77%	69%	72%
	strain4/91	69%	59%	68-72%	69-72%	66%	70%
	1212B-2012	74%	66%	81-85%	78-85%	99%	98%
IBV/CK/EG/QENA-31/2018		79%	70%	94-99%	90-98%	82%	83%
IBV/CH/SA/6/2019		73%	67%	81-85%	80-87%	98%	98%
IB-54NB-chicken-LEBNAN		78%	63%	93-96%	86-92%	82%	81%
IS/64/2020		80%	70%	94-98%	89-96%	83%	83%

Table 5. Detailed site-by-site results from the FEL analysis.

Codon site	Neutral model alpha=beta	Likelihood ratio LRT	Evidence of selection p-value
53	0.77	7.2	0.01
68	0.74	7.76	0.01
130	1.36	3.28	0.07
146	0.29	2.79	0.09
151	0.35	2.71	0.1

alpha=beta: The rate estimate under the neutral model; LRT: Likelihood ratio test statistic for beta = alpha, versus beta and neq; alpha; p-value: Asymptotic p-value for evidence of selection, i.e. beta and neq; alpha.

DISCUSSION

Infectious bronchitis (IB) continues to pose a significant threat to both vaccinated and non-vaccinated chicken flocks in Egypt, to address this issue, our study aims to assess the prevalence of the infectious bronchitis virus among Egyptian broiler chicken flocks in various governorates. Additionally, we seek to analyze the genetic evolution of circulating IBV strains and identify variation among variants emerged in the field.

The broiler chickens examined exhibited clinical symptoms such as gasping, rales, and ruffled feathers, along with depression and whitish droppings. The post-mortem examination of chickens infected with IBV revealed various symptoms like tracheal congestion and exudation, tracheal and bronchial casts, rhinitis, serositis as well as nephritis with ureates deposited in

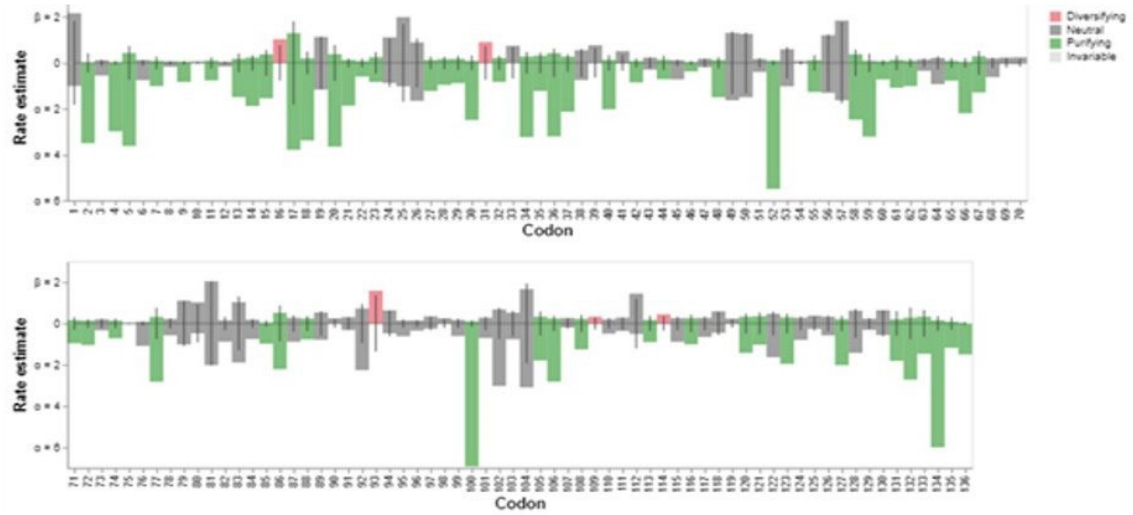

Fig. 3. Maximum likelihood estimates of synonymous (α) and non-synonymous (β) rates at each site. The line shows the estimates under the null model (α=β). Estimates above 10 are censored at this value.

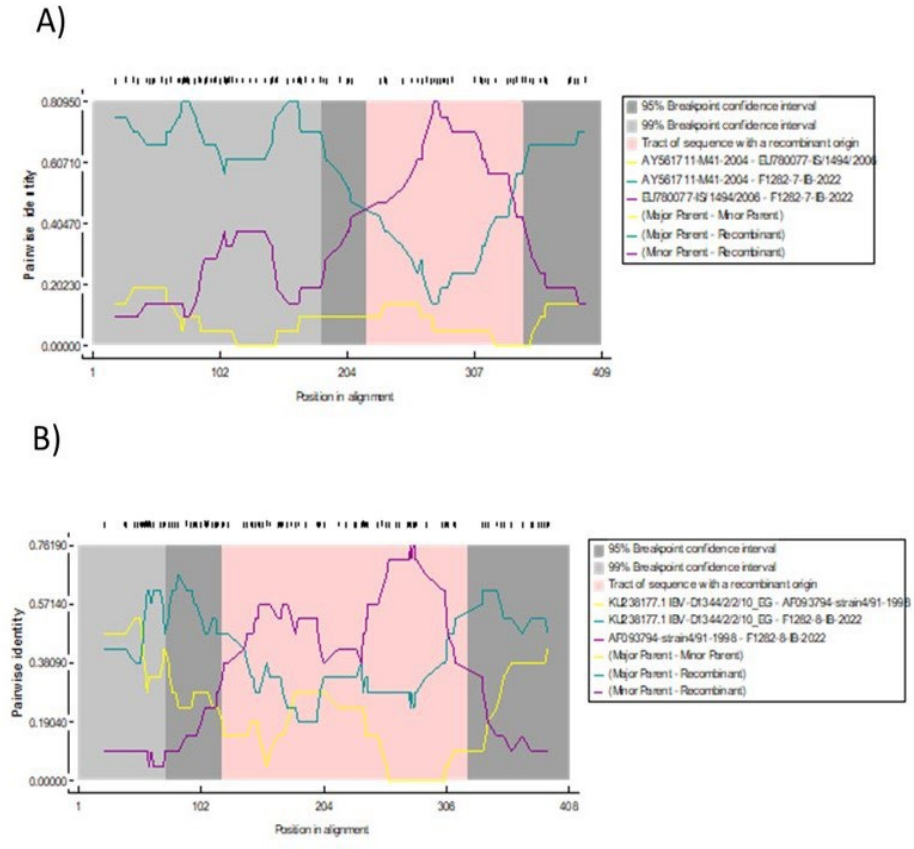

Fig. 4. Recombination analysis of S1 gene. a) Recombination event of F1282-7 from M41. b) Recombination event of F1282-8 from D1344/2/2/10-EG.

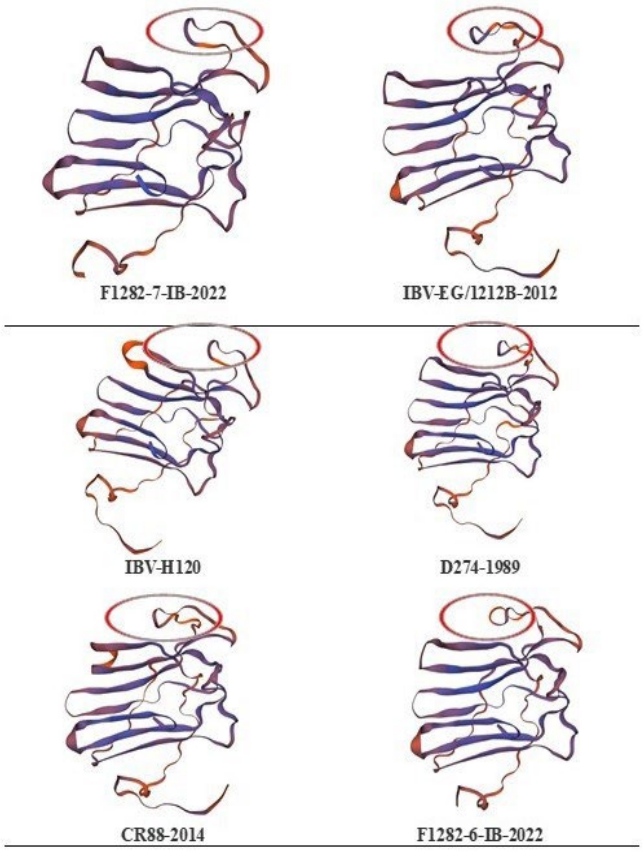

Fig. 5. Predicted 3-dimensional structure of HVR of S1-glycoprotein determinants of vaccine strains and study viruses of avian infectious bronchitis virus. Structures are drawn using SWISS homology modeller available online at http://swissmodel.expasy.org/.

renal tissue and ureters. These results are consistent with typical signs appeared on infected birds from previous infection with variant strains circulating in the field (El-Mahdy *et al*., 2010; Hassan *et al*., 2017).

According to real-time PCR analysis, 11 of the 18 (61%) tested flocks were positive for IBV. Specifically, four out of six flocks from Damietta province and two out of five flocks from Dakahlia province tested positive, as well as one each from Minya, Giza, Qalubia, Beni suef, and Kafr skeikh provinces in Egypt. These results indicate widespread occurrence of IBV that is closely like recorded with before (Zanaty *et al*., 2016; Setta *et al*., 2018; Esmaiel *et al*., 2019).

In this study, the successful isolation of the virus in SPF embryonated chicken eggs led to characteristic lesions like curling, stunting, and dwarfing, similar to previous findings with IBV Egyptian variant viruses. (Hassan *et al*., 2016). Further to virus isolation, we selected 11 samples that tested positive for IBV through real time PCR. These samples were subjected to amplification of S1 gene with RT-PCR and gel electrophoresis, as shown in Table 2.

The study conducted a genetic characterization of the S1 protein gene of IBV using 10 isolates. The phylogenetic analysis identified two major groups, the first group contained only one virus from Damietta province, which belonged to the classical GI-1 strain. The second group includes genotype GI-23 (variant II), with nine viruses occurring in two subgroups. The first subgroup, GI-23.2.1, had eight viruses isolated from chickens in Damietta, Dakahlia, Minya, Giza, Qualubia, and Kafr Sheikh provinces. The second subgroup contained one virus belonging to genotype GI-23.2.2 isolated from one flock in Dakahlia province. (Figure 1).

In this study, the genetic classification of Egyptian IBV, based on the S1 gene, is agreed with the results obtained previously by Zanaty *et al*., 2016; Abozeid *et al*., 2017. IBV is endemic in Egypt, and various serotypes and genotypes with minimal cross-protection are present, which co-circulate in the field (Abdel-Moneim *et al*., 2012). The evolution of IBV occurs in nature, often through genetic substitutions, insertions, deletions, or recombination. of different genes (Hassan *et al*., 2019), which can result in the emergence of highly virulent viruses with minimal cross-protection, leading to vaccination failure (Jackwood, 2012).

The Egyptian variant IBV can be grouped into GI-23 lineage based on the complete S1 sequence (Valastro *et al* 2016). The Egyptian variant-I strain was identified in various poultry farms in 2001 (Rohaim *et al*., 2019). In 2011, a new variant (Egyptian variant-II) was detected in both vaccinated and non-vaccinated flocks, causing intense virus infections (Abdel-Moneim *et al*., 2012). The Egyptian variant II strain can be differentiated from the classical vaccine H120 and Ma5 vaccine strains used in Egypt (Abd El Rahman *et al*., 2015). In 2012, an upgrade of the vaccines was introduced to control the outbreak in Egypt using "variant" vaccine strains 1/96, 4/91, CR88, and D274 (Abozeid *et al*., 2017).

Two Egyptian variant subgroups (Egyptian variant-1 and Egyptian variant-2) are circulating in all types of chicken flocks in Egypt until now (Ghetas *et al*., 2016). They were recently isolated from various wild bird species (house sparrow, teal, cattle egret, and quail) in Upper Egypt, indicating spillover transmission from domestic poultry to wild birds (Rohaim *et al*., 2019). This could explain the recent finding of GI-23 lineage strains in Europe (Lisowska *et al*., 2017) that had previously been identified as a Middle Eastern indigenous lineage (Valastro *et al*., 2016).

Natural selection often results in a decrease in detrimental mutations, consequently facilitating the development of beneficial mutations. Typically, genes that undergo positive selection through natural selection possess essential functions (Tang *et al*., 2006). This study found positive selection pressure to be present only in five locations of the S1 gene, which was expected due to the high usage of IBV vaccine, as reported earlier (Jahantigh *et al*., 2013). This selective pressure potentially alters the S1 gene's primary and secondary structures, leading to genetic and molecular changes in the virus. As a result, new strains may arise that evade the immune system, as previously reported (Dolz *et al*., 2008).

The emergence of new IBV genotypes was noticed due to the incidence of several recombination events within the same genotype or between different genotypes. Some of those events were observed between field and vaccine viruses (Jackwood *et al*., 2010; Li *et al*., 2016). The recombination was detected in the present study in two isolates from EU780077-IS/1494/2006, AY561711-M41-2004, AF093794-strain4/91-1998 and the Egyptian strain KU238171.1 IBV-D1344/2/4/10 EG (Fig.4). These findings highlight the need for further investigation and improvement in understanding the genetic makeup of these strains as also previously detected by Kiss *et al* (2016).

The 3D of HVR models of S1 proteins of IB viruses from our study and vaccines viruses were generated by using SWISSMOD-EL (https://swissmodel.expasy.org/) to find out the structural similarities between the HVR protein of our study virus and the vaccine viruses. The concept from the 3D modeling is that the similar proteins in their structure, they predicted to be functionally similar (Rother *et al*. 2011).

In the current study comparison of 3D structural models of HVR of S1 protein showed that vaccine strain D274-1989 and parent virus IBV-EG/1212B-SP1-2012 have more similarities with that of our study virus F1282-6-IB-2022. Many studies are required to compare the efficacy of local and imported vaccines both in-vivo and in-vitro as also has been conducted (Sultan *et al*. 2019).

CONCLUSION

The current study on isolated IBVs in Egypt from 2019 to 2022 revealed that they can be categorized into three groups. The 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 were found to cluster with the Egy/variant 2.2 of the GI-23 lineage, while F1282-9-IB-2022 was found to cluster with the Egy/variant 2.1 of the same lineage. Interestingly,

F1282-7 in 2022 showed similarities with IB vaccine strains, such as Mass-type (D274, M41, H120, CR88, 4/91 and 1212B) indicating the presence of divergent IBV strains in Egypt. To effectively control IBV, it recommends conducting more epidemiology studies to better understand the spreading nature of these viruses, as well as investigate the genetic relationship between circulating field strains and vaccine strains. Furthermore, the role of wild birds in IBV strain transmission from Egypt to other countries, as well as vice versa, should be investigated.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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