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Genetic characterization of infectious bursal disease Viruses during 2020 and 2021

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ABSTRACT

Infectious bursal disease (IBD) is caused by infectious bursal disease virus (IBDV) belonging to the family Birnaviridae. This virus causes an immunosuppressive disease in chickens. In case of subclinical infection of IBDV in young birds less than 3 weeks causes permanent immunosuppression. The current study aims to genetically characterize the IBDVs collected from 6 different governorates in Egypt within 2020 -2021. The forty samples were extracted and tested by RT-PCR for IBDV. The positive samples amplicons were purified and sequenced. The analysis included the alignment of the deduced amino acids and phylogenetic tree. The study revealed that the circulating IBDV strains include genotype A3 of the very virulent IBDV (vvIBDV). The comparison between the sequenced samples and the available IBD vaccines showed mutations in the antigenic sites of P domain loops. So it is important to evaluate the immunogenicity and the efficiency of the current used vaccines to select the most appropriate one. However, the selection of a seed virus from the circulating IBDV is considered the best choice after comprehensive surveillance.

INTRODUCTION

Infectious bursal disease virus (Gumboro) is a contagious immunosuppressive viral disease of chickens that targets the bursa of fabricius of young chickens **(Kibenge et al. 1988)**. IBDV belong to the family *Birnaviridae*, genus Avibirnavirus and the virus have two serotypes that have been identified; serotype I and serotype II. Serotype 1 have been classified into four pathotypes: classical virulent IBDV (cvIBDV), antigenic variant IBDV (vIBDV), Very virulent IBDV (vvIBDV) and attenuated IBDV **(Van Den Berg et al. 2004).** The vvIBDV is the acute form of IBDV that causes high economic losses to the poultry industry.

IBDV is a double-stranded RNA of two segments; segment A that encodes for VP2,

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VP3, VP4, and VP5 proteins while segment B encodes for VP1 protein (**Boudaoud et al. 2016)**.

The virus antigenicity and pathogenicity are contributed by VP2 protein **(Letzel et al. 2007)**, which contain the antigenic region that is responsible for the induction of the neutralizing antibodies. the molecular characterization and antigenic variation of IBDV are mainly based on the amino acid changes in the hypervariable region of VP2 (hvVP2) **(Jackwood and Sommer, 2005)**.

Egypt has faced several outbreaks of IBDV and several studies revealed the continuous circulation of vvIBDV in vaccinated flocks **(Khafagy et al. 1991 and Hala et al. 2019)**. Recently, **Tamer et al.(2019)** showed high mutation rate in the hypervariable domain of the VP2 of IBDV in Egypt.

So, this study aimed to genetically characterize the currently circulating strains of IBDV during 2020 and 2021 in commercial broiler farms to study the genetic evolution of IBDV in certain governorates of Egypt

2- MATERIAL and METHOD

2.1-Sample collection:-

Forty samples from vaccinated broiler farms at Giza, Fayoum, Benisuief, Monofeya, Sharkeya and kalyobeya governorates were collected. Each sample was consisting of 5 Bursae of Fabricius from suspected infected IBD chickens broiler farms. The samples were chopped and homogenized with sterile sand and phosphate buffer saline PBS containing 1000 µl /ml streptomycin and penicillin. The homogenized samples were centrifuged at 3000xg for 10 min. The supernatants were filtered using a 22µ filter to remove any bacterial contamination. {according to OIE Manuel chapter 3.3.1.2. (**OIE 2016**)}.

2.2- Identification of IBDV by conventional PCR:-

The IBDV RNAs were extracted using a commercial QIAamp® viral RNA mini kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. Then the partial amplification of the VP2 gene including the hypervariable region was done using EasyScript® One-Step RT-PCR SuperMix kit and according to the manufacturer's instructions. The sequence of primers used are Forward primer: AUS GU: 5-TCA CCG TCC TCA GCT TAC CCA CAT C-3, Reverse primer: AUS GL: 5-GGA TTT GGG ATC AGC TCG AAG TTG C-3. The thermal profile was 45ºC for 30 min for cDNA synthesis by reverse transcriptase enzyme, the Taq activation and initial denaturation were at 94Cº for 5 min followed by cycling 35x as follow; 94Cº for 30 sec for denaturation, then 57Cº for primers annealing and the extention was at 72Cº for 1 min. The final extention was at 72Cº for 10 min. The PCR amplicons were detected by electrophoresis using ethidium bromide-stained agarose gel with a 1.5% concentration. The visualization of the PCR product was carried out under UV rays using a gel documentation system (Biometra).

2.3- Molecular characterization by Nucleotide sequencing:-

The positive amplicons were purified using QIAquick® gel extraction kit. The sequence reactions were done using Bigdye® Terminator V3.1 cycle sequencing kit (PerkinElmer, Foster City, CA). The sequence reactions were purified using DyeEx® kit and finally installed in the Applied Biosystems 3500 xl genetic analyzer machine (ABI).

2.4- Phylogenetic and mutation Analysis:-

The nucleotide sequence was aligned and translated into the deduced Amino acids using Bioedit 7.2 software **(Hall, 1999).** While Mega 7.0.26 software **(Kumar et al. 2016)** was used to construct a nucleotide phylogenetic tree of the sequenced positive samples using the neighbor-joining method with 1000 bootstrap. The analysis of the sequenced samples was carried out in comparison with the most common vaccinal strains and some Egyptian IBDV strains from 2018 and 2019 retrieved from the Gene Bank.

3-RESULTS

3.1 Detection of IBDV by RT-PCR:-

The Positive samples were 32 out of 40 suspected chickens flocks representing 6 governorates within years of 2020 and 2021 by RT-PCR test and all amplicons were at 620 bp on the agarose gel (Figure 2).

3.2 Hypervariable region of VP2 gene (hrVP2) sequence analysis:-

The sequence analysis was carried out on the 32 positive samples for IBDV. About 417 nucleotides base from position 601 to position 1017 of VP2 gene and the deduced amino acids about 139 amino acids started from position 201 to position 339 including hrVP2. The nucleotide identity percent between the 32 samples varied from 92 to 100% while the amino acid identity percent varied from 94 to 100%. Regarding the phylogenetic tree, all the samples were clustered in the group of genotype 3 except the samples EGY-2-IBDV-2021, EGY-5-IBDV-2021, EGY-7-IBDV-2021, EGY-13-IBDV-2021, EGY-14-IBDV-2021, EGY-16-IBDV-2021, EGY-20-IBDV-2021, EGY-24-IBDV-2021, and EGY-27-IBDV-2021 were clustered with the classical vaccinal strains group of genotype 1. These samples got mutations at the following positions in comparison with genotype 3 of the sequenced samples; S217L, F220Y, A222P, I240V, S254G, T256V, and S299N. Moreover, the sample EGY-3-IBDV-2021 was different at position L282V, also the sample EGY-4-IBDV-2021 was different at position M325L and the sample EGY-8-IBDV-2021 was different at position I264M while the sample EGY-18-IBDV-2021 was different at position P291L.

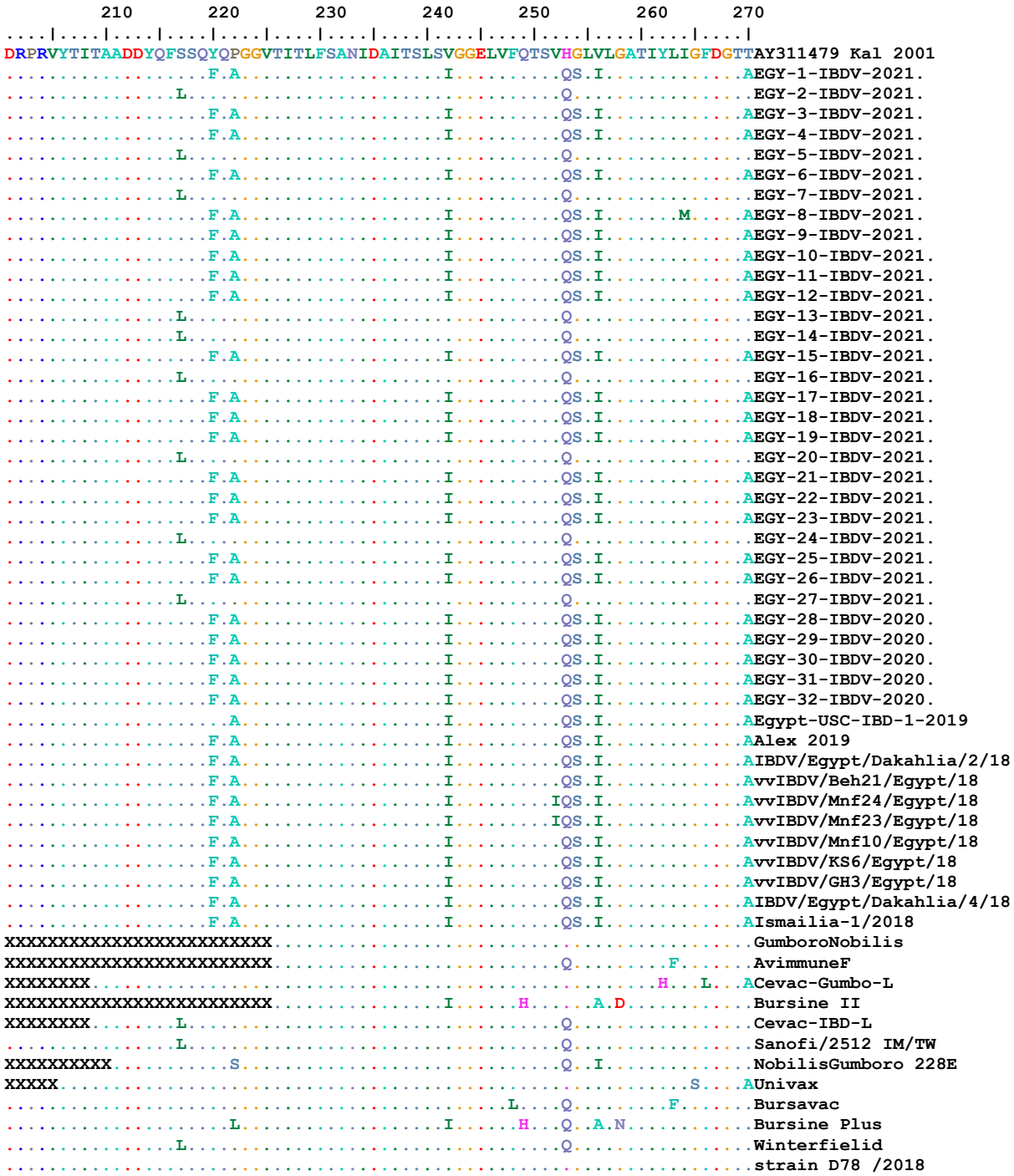

Figure 1:Shows the alignment of the amino acids between the 32 samples of the current study and samples from 2018 and 2019 in comparison with 12 vaccinal seed strain. X means that sequence is not available on the gene bank.

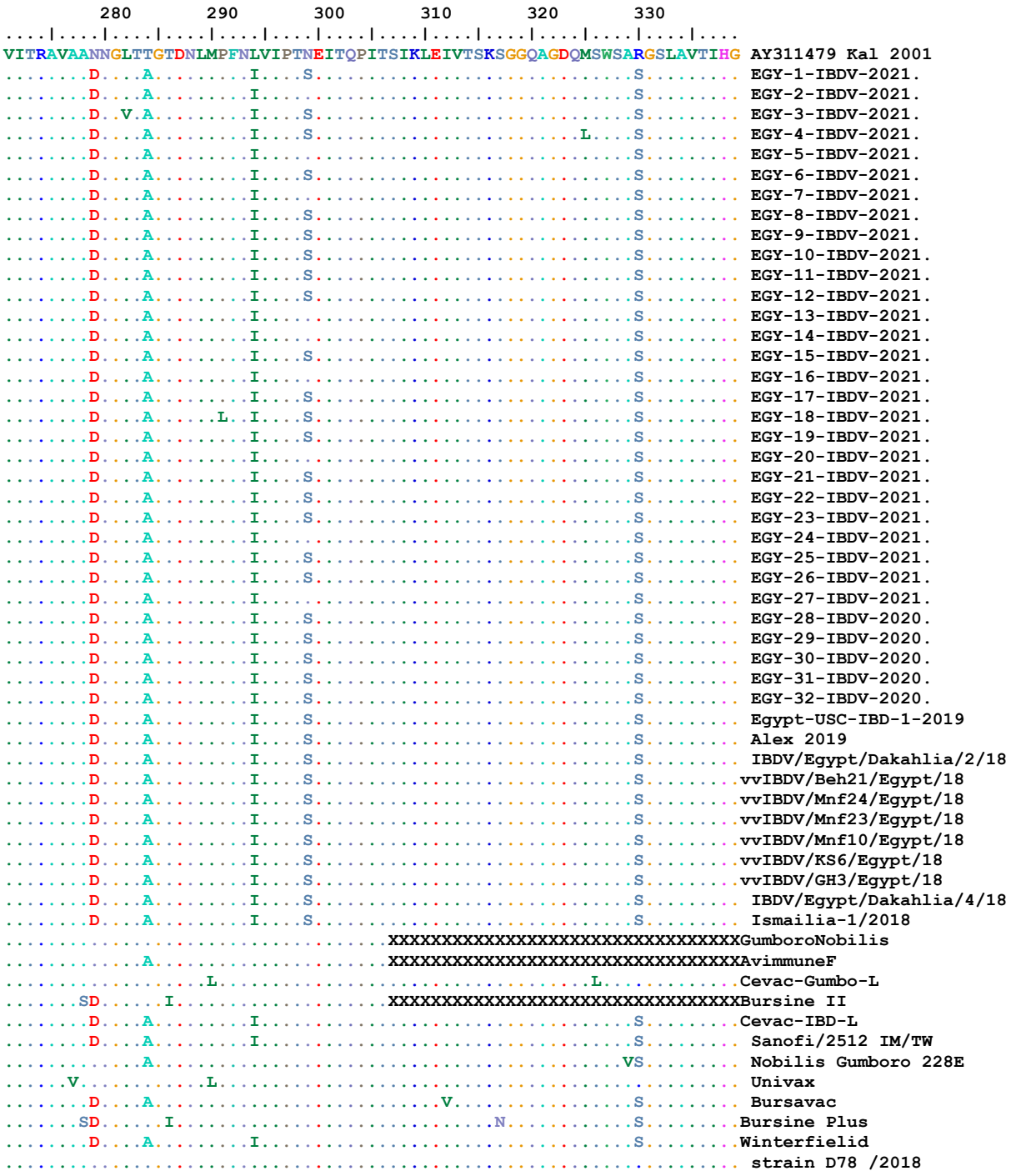

Figure 1: Continued……..The alignment of the amino acids between the 32 samples of the current study and samples from 2018 and 2019 in comparison with 12 vaccinal seed strain. X means that sequence is not available on the gene bank.

Table 1 Show the mutations in P domain loops (hvVP2) between the sequenced 32 samples in the current study and the 12 vaccinal strains

Strains	**loop P_{BC}**		**Loop P_{DE}**				**Loop P_{FG}**		**Loop P_{HI}**		
	217	222	249	253	254	256	284	286	312	317	325
G1/A1 classical IBDV of the study 2020-2021	L	P	Q	Q	G	V	A	T	I	S	M
G3/A3vvIBDV of the study 2020-2021	S	A	Q	Q	S	I	A	T	I	S	M/L*
GumboroNobilis	Nil	Nil	Q	H	G	V	T	T	I	S	M
AvimmuneF	Nil	Nil	Q	Q	G	V	A	T	I	S	M
Cevac-Gumbo-L	S	P	Q	H	G	V	T	T	I	S	L
Bursine II	Nil	Nil	H	H	G	A	T	I	I	S	M
Cevac-IBD-L	L	P	Q	Q	G	V	A	T	I	S	M
Sanofi/2512 IM/TW	L	P	Q	Q	G	V	A	T	I	S	M
NobilisGumboro 228E	S	S	Q	Q	G	I	A	T	I	S	M
Univax	S	P	Q	H	G	V	T	T	I	S	M
Bursavac	S	P	Q	Q	G	A	A	T	V	S	M
Bursine Plus	S	L	H	Q	G	V	T	I	I	N	M
Winterfielid	L	P	Q	Q	G	V	A	T	I	S	M
strain D78 /2018	S	P	Q	H	G	V	T	T	I	S	M

*Only one sample EGY-4-IBDV-2021 within G3/A3 vvIBDV clade of the study 2020-2021 showed 325L and the others showed 325M as the 12 vaccinal strains except for Cevac-Gumbo-L showed 325L

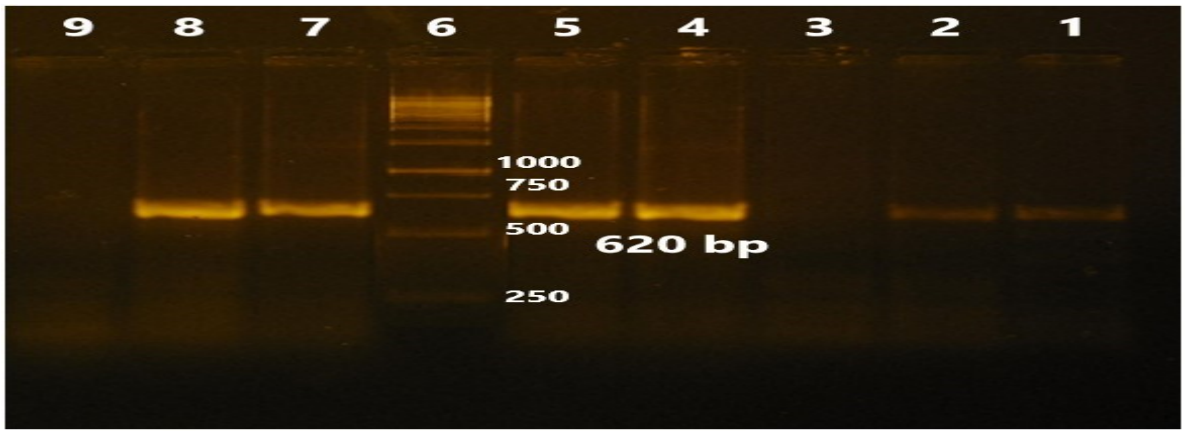

Figure 2: Shows agarose gel electrophoresis of RT-PCR result (1st group of tested samples in the study). The lanes No.1,2,4,5 and 7 are positive samples of specific weight 620bp. The lanes No. 3 is a negative sample. The lane No. 8 is the positive control and the lane No. 9 is the negative control. The Lane No. 6 is the 1kb DNA Ladder.

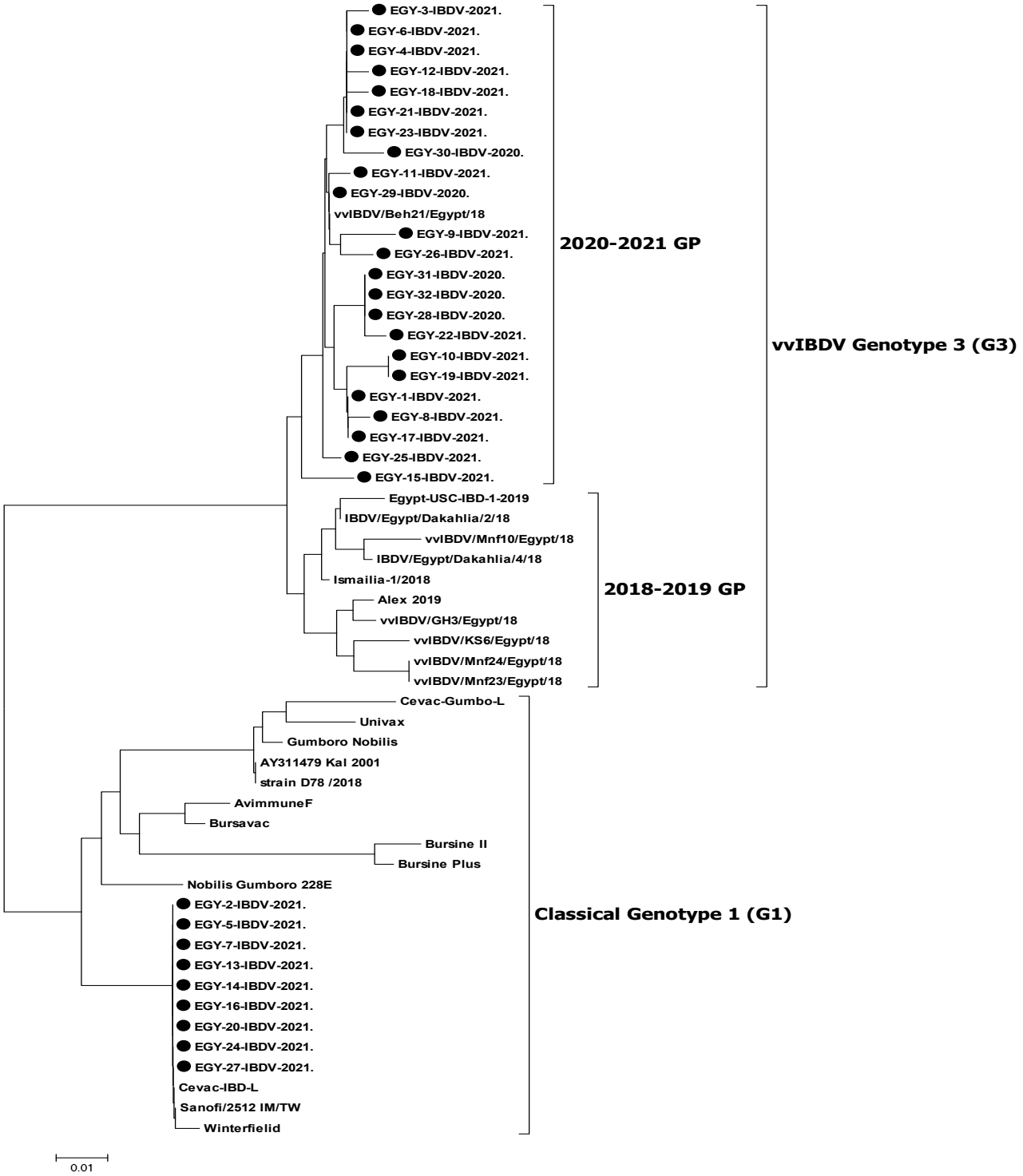

Figure 3: Phylogenetic tree of hvVP2 (nt601-1017) of 32 samples of the current study and 24 selected vaccinal and 2018-2019 Egyptian strains of IBDV. The tree was generated by the use of the Neighbor-Joining method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates). The. samples under the present study are marked with filled circles (●).

DISCUSSION.

The IBDV genome is composed of two segments A and B. The VP2 gene is encoded by segment A and historically, is targeted to evolutionary analyses of IBDV. Many studies have focused on the VP2 gene as the major determinant for virulence, antigenicity, and cell tropism **(van den Berg 2000 Brandt et al. 2001).**

The current study focused on the molecular characterization of the circulating IBDV from 6 different governorates in Egypt within 2020 and 2021 by genetic characterization of the hypervariable region of the VP2 (hvVP2) gene. The phylogenetic analysis revealed that 23 samples (5 samples in 2020 and 18 samples in 2021) were clustered in Genogroup 3 (G3) that concerned with very virulent IBDV (vvIBDV) and 9 samples (all in 2021) were clustered within classical and vaccinal strains Genogroup 1 (G1) as shown in figure 3 **(Jackwood 2018)**. Recent classifications of IBDV viruses were carried out based on the sequence of the 2 segments of the IBDV genome. Accordingly, the 23 samples(G3) were genotyped into A3 genotype with the vvIBDV strains and the 9 samples(G1) were genotyped into A1 genotype with the classical strains **(Islam et al. 2021 and Yu-long et al. 2021).**

The nucleotides identity between the A3 genotype samples (23) of the current study varied from 98-100% and the identity of the amino acids was also as the same percent. The nucleotides identity between the A1 genotype samples (9) varied from 99-100% and the amino acids identity showed the same percent. While the identity percent between the Genotype A1 samples and the Genotype A3 samples varied from 92-93% for nucleotides identity and 94% for amino acids identity**.** These findings approximately resemble identity between the 2012-2013 Egyptian field strains of IBDV **(Mawgod et al., 2014).** These differences could be exploited in diagnostic as about 6 mutations within 22 nucleotides at position 756 to position 777 could be invested to design multiplex real-time RT-PCR probes to differentiate between classical strains G1 /A1 genotype and circulating vvIBDV G3 /A3 genotype. The mismatches between the probes and the template decrease the sensitivity of PCR till the limit to prohibit the binding between them **(Hoang et al. 2019).** The high mutation rate of the double-stranded RNA IBDV relatively to the DNA viruses **(Domingo & Holland, 1997),** triggers the mutations of hvVP2 protein that is considered the major determinant for antigenicity **(Brandt et al. 2001).** The VP2 protein comprises the structural units of the viral capsid **(Coulibaly et al. 2010).** The P domain is one of the three domains of VP2 protein which consists of four loop structures termed P_{BC}, P_{DE}, P_{FG}, and P_{HI} which are exposed on the surface of the virion **(Coulibaly et al. 2005).** Regarding the loops of P domain of VP2 protein, the mutations analysis of them between the sequenced samples related to vvIBD (G3/A3) and the available vaccinal strains in the study revealed that Nobilis Gumboro 228E and Avimmune F strains showed 2 mutations only in P domain loops. Gumboro Nobilis, Cevac-IBD-L, Sanofi/2512 IM/TW, Bursavac and Winterfielid strains showed 4 mutations. Cevac -Gumbo-L, Univax, and strain D78 /2018 showed 5 mutations in P domain loop, Bursine II showed 6 mutations and Bursine Plus showed 7 mutations as shown in table 1 and Figure 1 in details. These amino acids changes in the hvVP2 region could result in antigenically diverse that can cause vaccinal failure even high maternal antibodies are present **(Ismail et al. 1990 Jackwood et al. 2001 Snyder et al. 1992).** So it is urgent to evaluate the immunogenicity and the efficiency of the current used vaccines to select the most appropriate and encourage its use as commercial vaccine of needed. However, the use of autogenous vaccines from killed local strains of the virus is the logic choice due to the limitation of the antigenic choices in the available vaccines for IBD **(Jackwood, 2017).**

In conclusion, the study revealed that the current circulating IBDV in 6 different governorates in Egypt within 2020 and 2021 include vvIBDV G3 genotype in old nomenclature and A3 genotype in recently proposed nomenclature. These strains could be antigenically diverse than the currently used vaccines due to continuous recording of IBDV outbreaks in vaccinated flocks and the presence of muta-

tions within the p domain loops that are concerned with antigenicity. This situation urgently triggers the efficacy evaluation of the used vaccines in the Egyptian field and updating the vaccinal seed to include strain from the currently circulating strains after comprehensive surveillance of IBDV in all Egyptian governorates.

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