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Isolation and molecular characterization of IBDV from Qualubyia governorate, Egypt, 2015.

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A B S T R A C T

Infectious bursal disease virus (IBDV) remerged frequently in Qualubyia was the cause of serious economic losses. Twenty field samples were collected from broiler chick farms in different localities of the governorate in January 2015, subjected for isolation on specific pathogen free-embryonated chicken eggs (SPF-ECE), detection using AGID and RT-PCR. Ten samples showed positive results with egg isolation, AGID and RT-PCR. Sequence analysis and characterization of the variable region of VP2 gene purified from the PCR product showed many amino acid substitutions that may affect IBDV antigenicity and virulence. Comparative phylogenetic analysis based on the sequence of the hypervariable region of VP2 gene clustered the isolated IBDV at a distance from the other reference IBDV from Egypt, Europe and other vaccinal strains. It was concluded that substitution mutations observed with the hypervariable region of VP2 gene of the isolated IBDV could affect the virus antigenicity and further studies on the efficacy of vaccines used locally against this variant IBDV strain have to be investigated.

Keywords: IBDV, Virus isolation, RT-PCR, VP2 Sequencing, phylogeny.

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1. INTRODUCTION

Infectious bursal disease (IBD) is an acute, contagious viral infection that causes immunosuppression in young chickens and disease and mortality in 3–6-week-old chickens; The virus infects actively dividing B lymphocytes within the bursa of Fabricius, leading to immunosuppression of varying duration and severity, and increased susceptibility to secondary viral and bacterial infections (van den Berg et al., 2000, and Mahgoub, 2012). IBDV is a member of the birnavirus genus, family Birnaviridae (Leong et al., 2000, Lukert and Saif, 2003).
It has a linear double-stranded RNA 6 kb in length that consists of two segments, segment A of 3.2 kb in length and segment B is 2.8 kb in length. Segment B encodes VP1 with polymerase activity while segment A codes for two open reading frames (ORF), the largest one encodes a polyprotein that is cleaved into two structural proteins VP2 as the major antigenic site eliciting neutralizing antibodies, VP3 eliciting antibodies that are very weakly neutralizing and VP4 with serine protease activity, while the small ORF encodes VP5 non-structural protein implicated in the induced bursal pathology (Fahey et al., 1985 and 1989, Mundt et al., 1995 and Lejal et al., 2000). VP2 is folded into three main domains, the base, shell and projection domains. The base and shell domains are formed by the conserved N- and C-termini while the projection domain is formed by the hyper variable region at amino acids (AAs) 206 to 350. VP2 hyper variable region contain two hydrophilic regions, region A spans AAs 212 to 224 and region B spans AAs 314 to 325. These regions

constitute four loops, PBC, PHI, PDE and PFG. PHI is the outmost part of the projection domain and represents neutralizing Ab-binding domains. Putative AAs responsible for virulence and cellular tropism were identified to be glutamine at AA position 253, aspartic acid at AA position 279, and alanine at AA position 284 (Azad et al., 1987, Bayliss et al., 1990, Brandt et al., 2001, Coulibaly et al., 2005 and Letzel et al., 2007).

IBDV has two different serotypes based on virus neutralization; serotype 1 contains the pathogenic strains and serotype 2 nonpathogenic strains that cannot protect against serotype 1 strain in chickens. Pathogenic serotype 1 IBDV in chickens are classified as classical virulent IBDV (cvIBDV), very virulent IBDV (vvIBDV), antigenic variant IBDV (avIBDV), and attenuated IBDV (Van den Berg et al., 2004). Classical IBD viruses occur worldwide (Becht, 1994). Very virulent IBD viruses (vvIBDVs) have been isolated in Asia, Central Europe, Russia, the Middle East, and South America (van den Berg, 2000). In Egypt, both vvIBDV strains and variant IBDV strains were reported and has been a serious problem circulating in flocks vaccinated using classical IBDV vaccines (Metwally et al. 2009, Helal et al., 2012, Mohamed et al., 2014 and Sara et al., 2014). Diagnosis of IBDV depends on isolation of the suspected virus then serological identification using FAT, ELISA, and AGPT but detection of IBDV using RT-PCR showed superior results and greater sensitivity and specificity than the serological techniques *(van den Berg, 2000).* The VP2 gene is commonly studied because it encodes for the major protective epitopes, contains determinants for pathogenicity, and is highly variable among strains (Abdel-Alim et al., 2003 and Tomas et al., 2012). Nucleotide sequencing is used to study the evolution of the virus in different geographic locations (Cortey et al., 2012).

The aim of our study was the isolation and molecular characterization of IBDV from suspected broiler chicks in Qualubyia governorate, Egypt.

2. MATERIAL AND METHODS

2.1. Viral samples:

Samples were collected from broiler chicks suspected for IBD with acute depression, nephritis and bursal enlargement. A total number of 20 samples were collected from Bursa of Fabricus of morbid and freshly dead chicks up to 3 weeks of age, during 2015 from broiler farms in Qualubyia governorate, Egypt. These samples were prepared according to El-Sanousi et al., (1994) and stored at – 20 °C till used for passage in Specific Pathogen Free-Embryonated Chicken Eggs (SPF–ECEs).

2.2. Reference IBD antisera:

It was purchased from Divesture Co., Holland, in a lyophilized form and reconstituted by addition of 1 ml sterile PBS buffer (according to the manufacturer). Reconstituted antisera stored at –20 ºC till used in agar gel precipitation technique.

2.3. Isolation of suspected IBDV on SPF – ECEs:

Prepared viral samples as bursal homogenates were inoculated (0.2 ml) on the chorioallantoic membrane (CAM) of 11 days old SPF–ECEs obtained from the SPF production farm, Koum Oshiem, Fayoum, Egypt. Inoculated eggs were collected after 96 hrs incubation at 37°C according to Hitchner (1970).

2.4. Identification of suspected IBDV isolates using Agar Gel Precipitation Test (AGPT):

Suspected isolates of IBDV were identified in prepared CAMs of inoculated ECEs using AGPT according to Hirai et al., (1972) using reference IBD antisera.

2.5. Identification of IBDV using Reverse Transcription-Polymerase Chain Reaction (RT-PCR):

Extraction of the viral RNA was done using RNeasy® (QIAGEN GmbH, Hilden, Germany) from the supernatant of prepared positive CAMs of inoculated SPF-ECEs according to procedure in the kit handbook.

A set of primers were used for the RT-PCR reaction and for the subsequent sequence analysis using forward and reverse PCR primers for amplification of 620 bp fragment IBDV on VP2: Forward primer: 5′-TCA CCG TCC TCA GCT TAC CCA CAT C-3′ and Reverse primer: 5′-GGA TTT GGG ATC AGC TCG AAG TTG C-3′. They were manufactured by metabion GmbH, (Lena-Christ-Strasse, Germany) after Metwally et al., (2009).

Preparation of 50 µl reaction mixture of 10 µl of extracted template RNA, 10 µl RT-PCR buffer, 2 µl of primer forward and 2 µl of primer reverse, 2 µl of dNTPs master mix containing 400 µM each dATP, dGTP, dCTP, dTTP and 2 µl of Qiagen One Step Enzyme Mix. PCR thermo cycling using (T3 Biometra-Germany) was as follow: 20 min at 50 °C (RT reaction); 95 °C for 15 min (initial PCR activation); 39 three-step cycles of 94 °C for 30 s (denaturation), 59 °C for 40s (annealing) and 72 °C for 1 min; then 72 °C for 10 min (final extension). After amplification, 5 µl of PCR products were analyzed by electrophoresis on a 1.5% agarose gel containing ethidium bromide with final concentration of 0.5 µg/ml at 95 V for 30 min in 1x TBE buffer, against GeneRulerTM100 bp Plus DNA Ladder (Fermentas). Images of the gels were photographed on BioDoc Analyze Digital Systems (Biometra, Germany).

2.6. Sequence analysis of VP2 of IBDV:

DNA band of the RT-PCR product was excised and purified from the gel using the QIAquick Gel Extraction Kit (Qiagen) according to the manufacturer instruction. The purified PCR products were sequenced using ABI PRISM® Big Dye TM Terminators v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) and the ABI PRISM® 3130 genetic analyzer (Applied Biosystems) with 80 cm capillaries. The sequences were edited with SeqScape Software Version 2.5 (Applied Biosystems), assembly of the consensus sequences and alignment trimming was performed with the Lasergene DNASTAR group of programs (DNASTAR Inc., Madison, WI), Using Clustal W method.

The alignment of the viruses, studying identity and divergence percent and the phylogram was carried out and drawn using DNAstar - MegAlign software according to Tamura et al., (2011). Egyptian viruses and other international reference strains were available from the Genbank, from the National Center for Biotechnology Information (NCBI) infectious bursal disease viruses resource (http://www.ncbi), as shown in table (1).

3. RESULTS

3.1. Isolation of suspected IBDV on SPF – ECEs:

Samples inoculated in SPF-ECE induced signs in 10 out of 20 samples by the 3rd passage as shown in table (2). Examination of the harvested egg embryos showed hemorrhage, edema of the head and necrosis of the liver (boiled appearance) while the harvested CAM were congested and thickened.

3.2. Identification of suspected IBDV isolates using AGPT:

Samples positive on isolation on SPF-ECE, showed positive results with AGPT using reference antiserum against IBDV as shown in table (2).

3.3. Identification of IBDV using RT-PCR:

After one step RT-PCR amplification of the VP2 gene of IBDV using Taq polymerase enzyme with the upstream and downstream specific primers, the genomic DNA products of the local IBDV isolates were subjected to electrophoresis which revealed the presence of the amplified products at the correct expected size of the VP2 encoding gene (620 bp) as shown in Figure No. (1). Results of RT–PCR confirmed the serological identification of IBD virus isolates using AGPT.

3.4. Sequence analysis of the hyper variable region of VP2 of IBDV:

A 330 bp fragment of the amplified hyper variable region of VP2 gene of the isolated IBDV-Qualubyia 2015 was subjected to sequencing and sequence alignment with other Egyptian and vaccinal IBDV strains. Multiple nucleotide substitutions were observed in comparison to other IBDV sequences showed that the isolated IBDV-Qualubyia 2015 would be a variant strain as shown in figure (2). Consensus of 110 amino acid residues were used for sequence analysis of the deduced amino acid sequences of the isolate (IBDV Qualubyia 2015) correspond to the region from AA residue 216 to AA residue 325. Substitution mutations were observed at 11 AA residues (230, 234, 238, 243, 255, 285, 295, 296, 297, 302 and 309) from which changes occurred in one AA residues in the major hydrophilic region (AA residue 285 K) which present in the PFG loop which represent the neutralizing Ab-binding domain. No changes were observed in AA residues responsible for virulence and cellular tropism were identified to be glutamine at AA position 253, aspartic acid at AA position 279, and alanine at AA position 284. All these results were shown in figure (3).

Studying the percent of divergence and homology between the isolated IBDV Qualubyia 2015 and other IBD viruses showed not less than 99.75% homology with local Egyptian IBDV isolates and other reference and vaccinal IBDV strains like IBDV K406/ Egypt/89, IBDV Nob2002/ Egypt/02, IBDV / Egypt/03, vvIBDV /Giza.Egypt/08, IBDV Hel2008MI /Egypt/08, IBDV Hel2008 /Egypt/08, vvIBDV / Egypt/09, IBDV / Egypt/Var-2009, IBDV / Egypt/S11-2013, vvIBDV UK661 /European, cvIBDV F52/70/ UK, IBDV K406/89/ German, cvIBDV Cu-1/ German, IBDV Variant A/ US, IBDV Bursa Vacc, IBDV Bursine Plus, IBDV Univax Strain, CEVAC IBDL and IBDV D78. Lower homology percent 98.50% was observed homology between the isolated IBDV Qualubyia 2015 and other IBD viruses as vvIBDV Lay./Kal. Egypt/98, vvIBDV Br./Men. Egypt/09, vvIBDV Lay./Beh. Egypt/10 and vvIBDV Br./Kal.Egypt/10 IBDV strain as shown in table (3). Phylogenetic analysis of isolated IBDV Qualubyia 2015 and other reference and vaccinal strains of IBDV revealed that IBDV Qualubyia 2015 was in a separate branch in the phylogenetic tree and it was originated from a common node and clustered more close to the classical Egyptian vvIBDV, German and the European strains but it was clustered at a far distance from vaccinal strains like Bursine Plus, Bursa vacc., Univax and other variant IBDV strains as shown in figure (3).

4. DISCUSSION

Our study was designed for isolation, identification and molecular characterization as well as phylogenetic analysis of a field isolate of IBDV in samples from infected broiler chicken farms at Qualubyia governorate. Isolation of suspected IBDV from bursal homogenates on specific pathogen free embryonated chicken eggs (SPF-ECE) was done for three passages. Samples inoculated in SPF-ECE induced signs in 10 out of 20 samples by the 3rd passage. Similar results were reported by Islam et al., (2005) and Sara et al. (2014).

Table (1): Reference sequences of IBDV strains and their accession number on Gene Bank based on VP2 gene sequence.

Reference Strain	Accession Number	Type
IBDV K406/ Egypt/89	AF159218.1	Egyptian IBDV
vvIBDV Lay./Kal.Egypt/98	JN983802.1	Egyptian vvIBDV
IBDV Nob2002/ Egypt/02	HM002752.1	Egyptian IBDV
IBDV / Egypt/03	AY311479.1	Egyptian IBDV
vvIBDV /Giza.Egypt/08	EU584433.2	Egyptian vvIBDV
vvIBDV / Egypt/09	AY318758.1	Egyptian vvIBDV
vvIBDV Br./Men.Egypt/09	JN983799.1	Egyptian vvIBDV
IBDV / Egypt/Var-2009	JN118617.1	Egyptian IBDV
vvIBDV Lay./Beh.Egypt/10	JN983801.1	Egyptian vvIBDV
vvIBDV Br./Kal.Egypt/10	JN983800.1	Egyptian vvIBDV
IBDV / Egypt/S11-2013	KF444834.1	Egyptian IBDV
IBDV Hel 2008	EU883569	Egyptian IBDV
IBDV Hel 2008 MI	FJ262538	Egyptian IBDV
IBDV K406-89	AF159218	German IBDv
vvIBDV UK661	NC-004178	European-like vvIBDV
IBDV F52/70	D00869	Classical virulent UK strain
IBDV Cu-1	X16107	Classical virulent German strain
IBDV Variant A	M64285	US variant
IBDV Bursa Vacc	Af498633	Vaccine
IBDV Bursine Plus	AF498632	Vaccine
IDV Univax Strain	Af457106	Vaccine
CEVAC IBDL	AJ632141	Vaccine
IBDV D78/2003	Y14962.1	Vaccine

Table (2): Laboratory diagnosis of suspected IBDV from broiler chicks in Qualubyia.

No. of Samples	*Positive samples.				
	Passages on SPF-ECE			**AGPT	***RT-PCR
	1st	2nd	3rd		
20	13	10	10	10	10

*Positive result represented by hemorrhagic and edema of embryo and thickened and congested CAM. **Positive result represented by appearance of white precipitin band at the interface between reference antiserum and the examined viral sample. ***Positive result represented by the presence of specific PCR product at the correct expected size of the VP2 gene (620 bp).

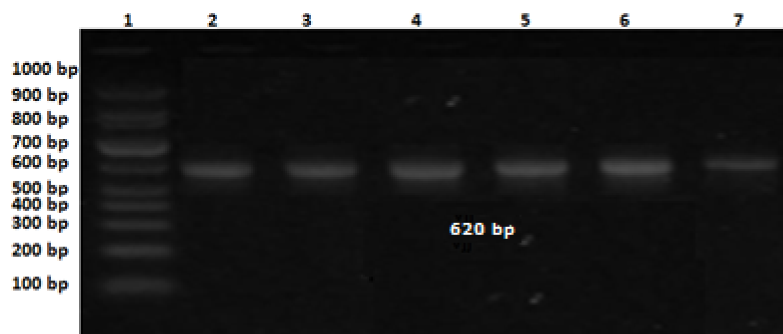

Figure No. (1) Electrophoresis of the amplified products for detection of the IBD virus VP2 gene for local isolates. It revealed the presence of specific PCR product at the correct expected size of the VP2 gene (620 bp). Lane 1: High base pair marker (100bp). Lanes 2, 3, 4, 5, 6 and 7: IBD virus isolates (from Qualubyia).

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                                           10        20        30        40        50        60
                                  ....|....|....|....|....|....|....|....|....|....|....|....|
IBDV/ Qualubyia.Egypt 2015        TTCCAAGCAGGTGGGGTACTAATCACACTGTTCTGCAGCCAAGATAGATGCTATCACAAG
IBDV K406/ Egypt/89               .A.................C......................T..C.....C........
vvIBDV Lay./Kal.Egypt/98          ...ATG..TCC....TC....TCGG.CG.A.....TGA...G.T.CTT...C.GTT..G.
IBDV Nob2002/ Egypt/02            ...................C......................T..C.....C........
IBDV / Egypt/03                   .A....C............C......................C..C.....C........
vvIBDV /Giza.Egypt/08             ..T................C......................C..C.....C........
IBDV Hel2008MI /Egypt/08          .A....TT..................................C..C..............
IBDV Hel2008 /Egypt/08            .A....TT..................................C..C..............
vvIBDV / Egypt/09                 ...................C......................T..C.....C........
vvIBDV Br./Men.Egypt/09           ...ATG..TCC....TC....TCGG.CG.A.....TGA...G.T.CTT...C.GTT..G.
IBDV / Egypt/Var-2009             ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~-..
vvIBDV Lay./Beh.Egypt/10          ...ATG..TCC....TC....TCGG.CG.A.....T.A...G.T.CTT...C.GTT..G.
vvIBDV Br./Kal.Egypt/10           ...ATG..TCC....TC....TCGG.CG.A.....TGA...G.T.CTT...C.GTT..G.
IBDV / Egypt/S11-2013             ...................C......................C..C.....C........
vvIBDV UK661 /European-like       .A.................C......................T..C.....C........
cvIBDV F52/70/ UK                 .A....C............C......................C..C..............
IBDV K406/89/ German              .A.................C......................T..C.....C........
cvIBDV Cu-1/ German               .A....C............C......................C..C.....C........
IBDV Variant A/ US                .A....CA............G.....................C..C.....C........
IBDV Bursa Vacc                   .A....C...................................C..C..............
IBDV Bursine Plus                 .A....TT..................................C..C..............
IBDV Univax Strain                .A....C...................................C..C..............
CEVAC IBDL                        .A....C...................................C..C..............
IBDV D78                          .A....C............C......................C..C.....C........

                                           70        80        90       100       110       120
                                  ....|....|....|....|....|....|....|....|....|....|....|....|
IBDV/ Qualubyia.Egypt 2015        CCTCAGCGTTGGGGGAGAATTTGTGTTTCAAACAAGCGTCCACGGCCTTATCAAGTTGAG
IBDV K406/ Egypt/89               .......A.C...........C....................A........A.T....GT
vvIBDV Lay./Kal.Egypt/98          AT.TG.GA.CA.CTCGA.G..GC.CACC.CGG.G.C......AC.A.AG....G....CC
IBDV Nob2002/ Egypt/02            .......A.C...........C....................AA.......A.T....GT
IBDV / Egypt/03                   ..................G..C...........................G.A.T....GC
vvIBDV /Giza.Egypt/08             .......A.C...........C....................AA.......A.T....GT
IBDV Hel2008MI /Egypt/08          .......A..........G..C.....C..T...........A......GCA.T...A.C
IBDV Hel2008 /Egypt/08            .......A..........G..C.....C..T...........A......GCA.T...A.C
vvIBDV / Egypt/09                 .......A.C...........C....................AA.......A.T....GT
vvIBDV Br./Men.Egypt/09           AT.TG.GA.CA.CTCGA.G..GC.CACC.CGG.G.C......AC.A.AG.........CC
IBDV / Egypt/Var-2009             .......A.C...........C....................A........A.T....GT
vvIBDV Lay./Beh.Egypt/10          AT.TG.GA.CA.CTCGA.G..GC.CACC.C.G.G.C......AC.A.GG.........CC
vvIBDV Br./Kal.Egypt/10           AT.TG.GA.CA.CTCGA.G..GC.CACC.CGG.G.C......AC.A.AG.........CC
IBDV / Egypt/S11-2013             .......A.C...........C.....C.....G........AA.......A.TT...GT
vvIBDV UK661 /European-like       .......A.C...........C....................A........A.T....GT
cvIBDV F52/70/ UK                 .......A..........G..C....................A......G.A.T....GC
IBDV K406/89/ German              .......A.C...........C....................A........A.T....GT
cvIBDV Cu-1/ German               ..................G..C...........................G.A.T....GC
IBDV Variant A/ US                ..................G.......................AA.....G.A.T....GC
IBDV Bursa Vacc                   ..................G..C...C................A......G.A.T....GC
IBDV Bursine Plus                 .......A..........G..C.....C..T...........A......GCA.T...A.C
IBDV Univax Strain                ..................G..C....................A......G.A.T....GC
CEVAC IBDL                        ..................G..C.................T..A......G.A.T....GC
IBDV D78                          ..................G..C...........................G.A.T....GC

                                          190       200       210       220       230       240
                                  ....|....|....|....|....|....|....|....|....|....|....|....|
IBDV/ Qualubyia.Egypt 2015        GATAATGGGGACACAACCGGCAGCGCCAAGCTTATGCCATTCAATCTTGTGATTATCTAC
IBDV K406/ Egypt/89               ..C........A..GG......CT.....C...............A........C..A..
vvIBDV Lay./Kal.Egypt/98          ...CG.CAC....AGG.TCC..CT...CGA.C....A...CTG...CCCC.TC....C..
IBDV Nob2002/ Egypt/02            ..C........T..GG......CT.....C..G............A........C..A..
IBDV / Egypt/03                   A.C........T..G.......C......C........................C..A..
vvIBDV /Giza.Egypt/08             ..C........T..GG......C......C...............A........C..A..
IBDV Hel2008MI /Egypt/08          ..C........T..G.......C......C...............A........C..A..
IBDV Hel2008 /Egypt/08            ..C........T..T.......T......T........................C..A..
vvIBDV / Egypt/09                 ..C...........GG......C......C...............A........C..A..
vvIBDV Br./Men.Egypt/09           ...CG.CAC.....GG.TCC..CT...CGA.C....A...CTG...CCCC.CC....C..
IBDV / Egypt/Var-2009             ..C...........GG......CT.....C...............A........C..A..
vvIBDV Lay./Beh.Egypt/10          ...CG.CAC..T..GG.TCC..CT...CGA.C....A...CTG...CCC..CC....C..
vvIBDV Br./Kal.Egypt/10           ...CG.CAC..T..GG.TCC..CT...CGA.C....A...CTG...CCCC.CC....C..
IBDV / Egypt/S11-2013             ..C...........GG......C......C...............A........C..A..
vvIBDV UK661 /European-like       ..C........T..GG......CT.....C...............A........C..A..
cvIBDV F52/70/ UK                 ..............GG......C......T....................C...C..A..
IBDV K406/89/ German              ..C........T..GG......CT.....C...............A........C..A..
cvIBDV Cu-1/ German               A.C........T..G.......C......C...........................A..
IBDV Variant A/ US                A.C........T..GG......T......T........................C..A..
IBDV Bursa Vacc                   ..C........T..GG......C......T........................C..A..
IBDV Bursine Plus                 ..C........T..T.......T..A...T........................C..A..
IBDV Univax Strain                ..C........T..GG......C..A...T........................C..A..
CEVAC IBDL                        ..C........T..GG......C..A...T...............A........C..A..
IBDV D78                          A.C........T..........C..A...C........................C..A..
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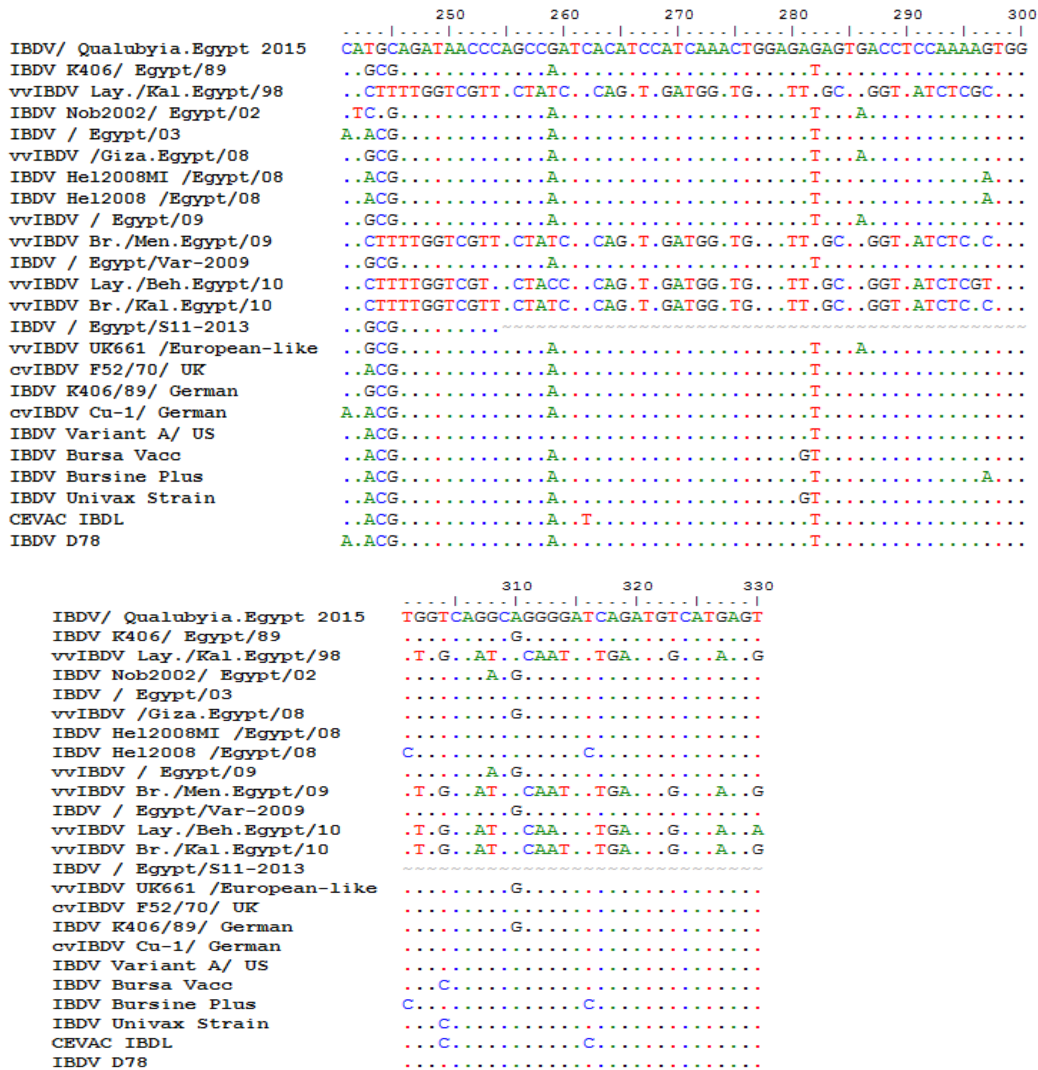

Figure (2): Nucleotide sequences of the VP2 variable domain in the IBDV Qualubiya 2015 aligned with other reference and vaccinal IBDV strains. Dots indicate position where the sequence is identical to the consensus.

Positive signs of inoculated egg embryos were hemorrhage, edema, and liver necrosis with boiled appearance and harvested CAMs were congested and thickened. These signs became more pronounced from the 3rd passage. The results agreed with those obtained with Nadia (2011). Serological identification of the suspected viral isolates showed that all samples positive on isolation on SPF-ECE, showed positive results with AGPT using reference antiserum against IBD virus. These results agreed also with that of Sara et al., (2014). Molecular identification of IBDV using RT-PCR for amplification of the VP2 gene using Taq polymerase enzyme with the upstream and downstream specific primers, revealed the presence of the amplified products at the correct expected size (620 bp) on electrophoresis. Results of RT-PCR as a sensitive test for IBDV detection confirmed the results of AGPT and agreed with those of Abdel-Alem et al. (2003) and Sara et al., (2014). When a 330 bp fragment of the amplified hyper variable region of VP2 gene of the isolated IBDV-Quqlubyia 2015 was subjected to sequencing and sequence alignment with other reference and vaccinal IBDV strains showed that the isolated IBDV-Qualubia 2015 strain could be have variations from some of these IBDV strains.

Table (3): Identity and diversity of the isolated IBDV Qualubyia 2015 with the other reference and vaccinal IBDV strains. .

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1		99.81	98.56	99.79	99.82	99.80	99.80	99.79	99.81	98.58	99.82	98.63	98.59	99.76	99.81	99.83	99.81	99.82	99.80	99.81	99.79	99.81	99.80	99.82
2	0.19		98.57	99.94	99.88	99.95	99.86	99.83	99.96	98.58	99.99	98.69	98.57	99.93	99.99	99.91	99.99	99.87	99.86	99.87	99.82	99.87	99.87	99.87
3	1.44	1.43		99.84	99.94	99.82	99.78	99.95	98.57	99.94	98.64	98.58	99.91	99.96	99.88	99.95	99.82	99.84	99.83	99.78	99.83	99.83	99.84	99.94
4	0.21	0.06	1.44		99.87	99.89	99.86	99.87	98.46	99.87	98.54	98.49	99.83	99.88	99.92	99.89	99.99	99.8	99.93	99.86	99.93	99.91	99.87	99.89
5	0.19	0.12	1.55	0.16		99.89	99.81	99.97	98.54	99.96	98.61	98.54	99.96	99.96	99.89	99.96	99.86	99.87	99.86	99.8	99.86	99.86	99.85	99.81
6	0.20	0.05	1.47	0.06	0.13		99.97	99.84	98.48	99.87	98.55	98.51	99.81	99.86	99.9	99.87	99.87	99.86	99.89	99.96	99.89	99.89	99.97	99.84
7	0.20	0.14	1.54	0.18	0.11	0.15		99.8	98.43	99.82	98.5	98.46	99.78	99.83	99.89	99.83	99.85	99.86	99.88	99.99	99.88	99.88	99.8	98.43
8	0.21	0.17	1.58	0.22	0.14	0.19	0.03		98.57	99.97	98.61	98.55	99.96	99.97	99.89	99.96	99.86	99.85	99.85	99.8	99.85	99.85	98.57	99.97
9	0.19	0.04	1.45	0.05	0.13	0.03	0.16	0.20		98.57	99.97	98.61	98.55	99.96	99.97	99.89	99.96	99.86	99.85	99.85	99.8	99.85	98.57	99.97
10	1.42	1.42	0.03	1.43	1.54	1.46	1.52	1.57	1.43		98.55	99.93	99.98	98.66	98.57	98.57	98.58	98.46	98.51	98.52	98.42	98.51	98.55	99.93
11	0.19	0.01	1.47	0.06	0.13	0.04	0.13	0.18	0.03	1.45		98.59	98.52	99.93	99.99	99.92	99.99	99.89	99.87	99.88	99.82	99.88	98.59	98.52
12	1.37	1.31	0.08	1.36	1.46	1.39	1.45	1.50	1.39	0.07	1.41		98.65	98.58	98.55	98.59	98.5	98.52	98.55	98.45	98.54	98.5	98.65	98.58
13	1.42	1.43	0.04	1.42	1.51	1.46	1.49	1.54	1.45	0.02	1.48	0.07		98.65	98.58	98.55	98.59	98.5	98.52	98.55	98.45	98.54	98.65	98.58
14	0.24	0.07	1.34	0.09	0.17	0.04	0.19	0.22	0.04	1.34	0.07	1.29	1.35		99.93	99.86	99.93	99.81	99.83	99.82	99.77	99.82	99.93	99.88
15	0.19	0.01	1.44	0.04	0.12	0.04	0.14	0.17	0.03	1.43	0.01	1.36	1.42	0.07		99.8	99.99	99.87	99.86	99.87	99.82	99.87	99.8	99.99
16	0.17	0.09	1.45	0.14	0.08	0.11	0.10	0.11	0.11	1.43	0.08	1.39	1.45	0.14	0.20		99.91	99.91	99.92	99.94	99.88	99.94	99.91	99.91
17	0.19	0.01	1.43	0.05	0.11	0.04	0.13	0.17	0.04	1.42	0.01	1.35	1.41	0.07	0.01	0.09		99.87	99.87	99.88	99.83	99.88	99.87	99.87
18	0.19	0.13	1.55	0.18	0.01	0.14	0.13	0.15	0.14	1.54	0.15	1.46	1.50	0.19	0.13	0.09	0.13		99.89	99.91	99.84	99.91	99.89	99.91
19	0.20	0.14	1.50	0.16	0.20	0.13	0.14	0.14	0.15	1.49	0.13	1.41	1.48	0.17	0.14	0.08	0.13	0.11		99.9	99.86	99.91	99.9	99.89
20	0.20	0.13	1.50	0.17	0.07	0.14	0.11	0.12	0.15	1.48	0.12	1.40	1.45	0.18	0.13	0.06	0.12	0.09	0.10		99.87	99.99	99.95	99.91
21	0.21	0.18	1.89	0.22	0.14	0. 20	0.04	0.01	0.20	1.58	0.18	1.51	1.55	0.23	0.18	0.12	0.17	0.16	0.14	0.13		99.89	99.89	99.86
22	0.19	0.13	1.50	0.17	0.07	0.14	0.11	0.12	0.15	1.49	0.12	1.41	1.46	0.18	0.13	0.06	0.12	0.09	0.09	0.01	0.11		99.97	99.93
23	0.20	0.13	1.55	0.17	0.09	0.14	0.11	0.12	0.15	1.53	0.12	1.46	1.50	0.17	0.13	0.07	0.12	0.10	0.10	0.05	0.11	0.03		99.91
24	0.19	0.13	1.57	0.17	0.01	0.14	0.13	0.14	0.14	1.56	0.15	1.49	1.53	0.19	0.14	0.09	0.13	0.02	0.11	0.09	0.14	0.07	0.09	

No.	Strain
1	IBDV/Qualubyia.Egypt2015
2	IBDV K406/ Egypt/89
3	vvIBDV Lay./Kal.Egypt/98
4	IBDV Nob2002/ Egypt/02
5	IBDV/ Egypt/03
6	vvIBDV/Giza.Egypt/08
7	IBDV Hel2008MI/Egypt/08
8	IBDV Hel2008 /Egypt/08
9	vvIBDV/ Egypt/09
10	vvIBDV Br./Men.Egypt/09
11	IBDV/ Egypt/Var-2009
12	vvIBDV Lay./Beh.Egypt/10
13	vvIBDV Br./Kal.Egypt/10
14	IBDV/ Egypt/S11-2013
15	vvIBDV UK661 /European
16	cvIBDV F52/70/ UK
17	IBDV K406/89/ German
18	cvIBDV Cu-1/ German
19	IBDV Variant A/ US
20	IBDV Bursa Vacc
21	IBDV Bursine Plus
22	IBDV Univax Strain
23	CEVAC IBDL
24	IBDV D78

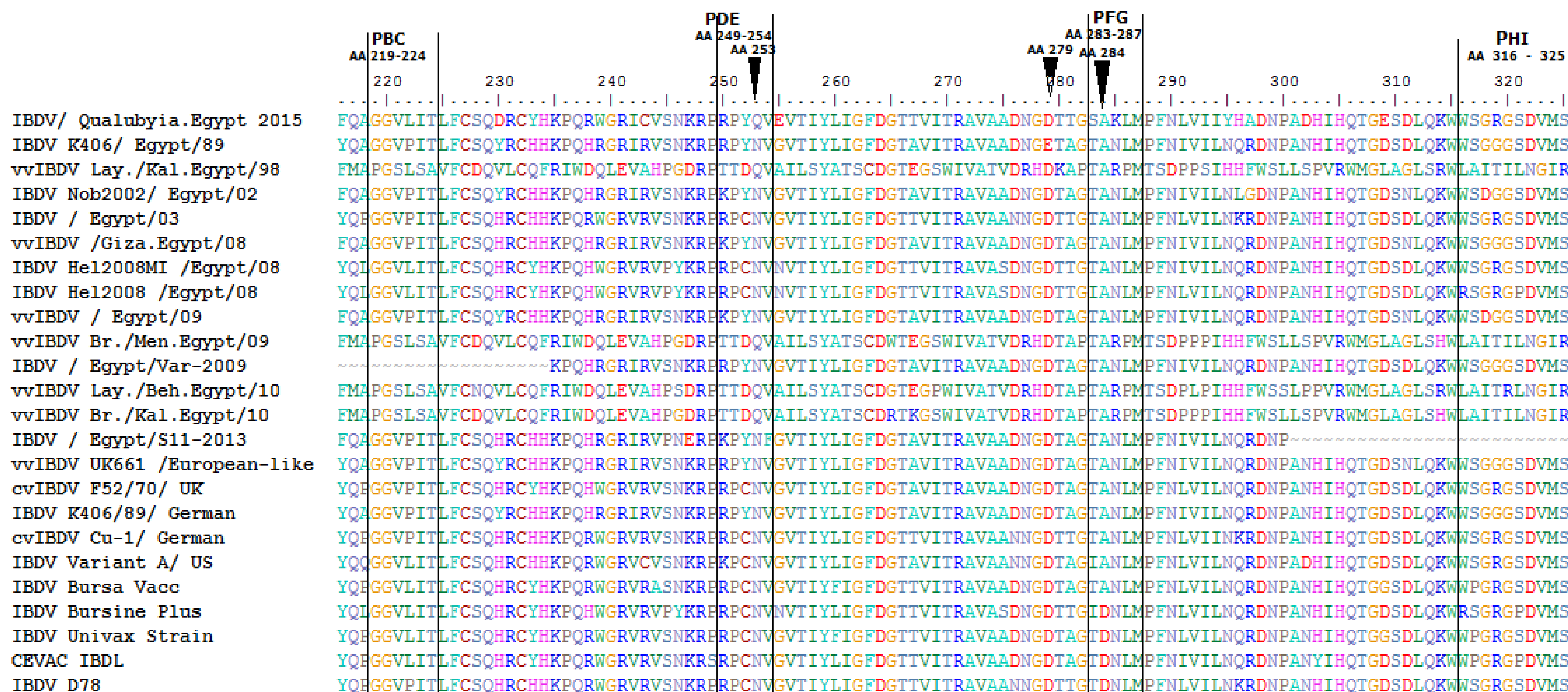

Figure (3): Clustal W multiple sequence alignment of the deduced amino acid sequence of the IBDV Qualubyia 2015 VP2 in comparison to previously characterized Egyptian and reference strains. Hydrophilic region A extends between AA 212 -224, PBC (AA 219-224) and PHI loops (AA 316-325) constitute neutralizing Ab-binding domains, Other PDE loop (AA 249-254) and PFG loop (AA 283-287) are additional loop projection domain. AA residues responsible for virulence and cellular tropism were identified to be glutamine at AA position 253, aspartic acid at AA position 279, and alanine at AA position 284.

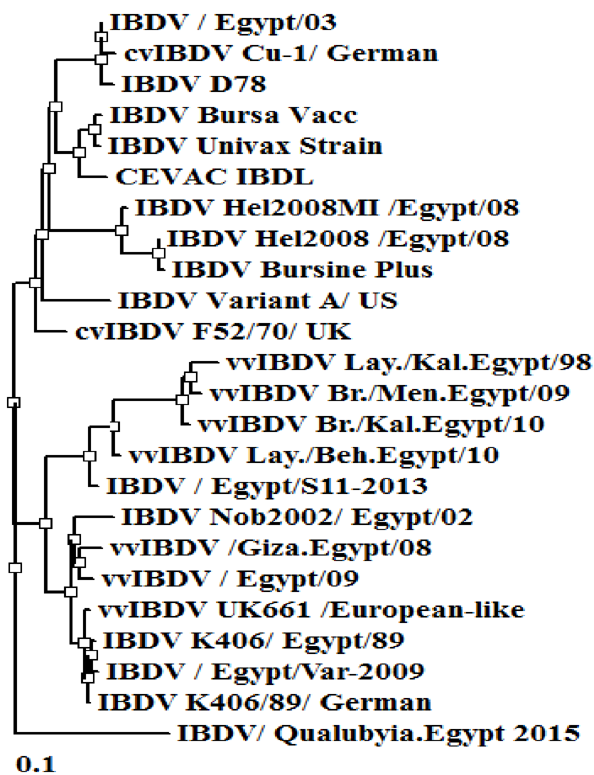

Figure (4): Phylogenetic tree of VP2 deduced amino acid sequences of IBDV Quqlubyia 2015 and other reference and vaccinal strains of IBDV.

Studying and analysis of consensus 110 amino acids (from AA residue 216 to AA residue 325) of the isolated IBDV (Quqlubyia 2015). Substitution mutations were observed at 11 AA residues (230, 234, 238, 243, 255, 285, 295, 296, 297, 302 and 309) from which changes occurred in one AA residues in the major hydrophilic region (AA residue 285 K) which present in the PFG loop which represent the neutralizing Ab-binding domain. No changes were observed in AA residues responsible for virulence and cellular tropism were identified to be glutamine at AA position 253, aspartic acid at AA position 279, and alanine at AA position 284.

Studying the percent of divergence and homology between the isolated IBDV Qualubyia 2015 and other IBD viruses showed not less than 99.75% homology with local Egyptian IBDV isolates and other reference and vaccinal IBDV strains like IBDV K406/ Egypt/89, IBDV Nob2002/ Egypt/02, IBDV / Egypt/03, vvIBDV /Giza.Egypt/08, IBDV Hel2008MI /Egypt/08, IBDV Hel2008 /Egypt/08, vvIBDV / Egypt/09, IBDV / Egypt/Var-2009, IBDV / Egypt/S11-2013, vvIBDV UK661 /European, cvIBDV F52/70/ UK, IBDV K406/89/ German, cvIBDV Cu-1/ German, IBDV Variant A/ US, IBDV Bursa Vacc, IBDV Bursine Plus, IBDV Univax Strain, CEVAC IBDL and IBDV D78. Lower homology percents 98.50% was observed homology between the isolated IBDV Qualubyia 2015 and other IBD viruses as vvIBDV Lay./Kal.Egypt/98, vvIBDV Br./Men.Egypt/09, vvIBDV Lay./Beh. Egypt/10 and vvIBDV Br./Kal.Egypt/10 IBDV. These results were seen in a comparative view with those of Mahgoub (2012) and Sara et al., (2014). Phylogenetic

analysis of isolated IBDV Qualubyia 2015 and other reference and vaccinal strains of IBDV revealed that IBDV Qualubyia 2015 was in a separate branch in the phylogenetic tree and it was originated from a common node and clustered more close to the classical Egyptian vvIBDV, German and the European strains but it was clustered at a far distance from vaccinal strains like Bursine Plus, Bursa vacc., Univax and other variant IBDV strains. Results of studying divergence and homology between the isolated IBDV Qualubyia 2015 strain and other IBDV strains and the phylogenetic analysis were agreed with those of Abdel-Alem et al. (2003) and Sara et al., (2014) which denoted to the continuous evolution and mutation of IBDV, which may affect the virus antigenicity and pathogenicity. This amino acid substitution may change antigenicity and virulence of the virus, which may attribute to the intensive vaccination programs performed in the field with live attenuated viruses (Van den Berg et al., 2004). It is concluded that this study made a successful genomic characterization of an IBDV isolated from Qualubyia governorate, Egypt 2015 that indicate progressive evolution and persistence of IBDV in Egypt, but additional studies on the virus pathogenicity and antigenicity are required.

5. REFERENCES

Abdel-Alim, G.A., Awad, M.H.H., Saif, Y.M. 2003. Characterization of Egyptian field strains of infectious bursal disease virus. Avian Dis. 47, 1452-1457.

Azad, A.A., Jagadish, M.N., Brown, M.A., Hudson, P.J., 1987. Deletion mapping and expression in Escherichia coli of the large genomic segment of a birnavirus. Virology 161:145–152.

Bayliss, C.D., Spies, U., Shaw, K., Peters, R.W., Papageorgiou, A., Muller, H., Boursnell, M.E., 1990. A comparison of the sequences of segment A of four infectious bursal disease virus strains and identification of a variable region in VP2. J Gen Virol 71(6):1303–1312.

Brandt, M., Yao, K., Liu, M., Heckert, R.A., Vakharia, V.N., 2001. Molecular determinants of virulence, cell tropism, and pathogenic phenotype of infectious bursal disease virus. J Virol 75:11974–11982.

Becht, H, 1994. Birnaviruses - Animal. In: Webster RG, Garnoff A, eds. Encyclopedia of virology Vol. 1. London, UK: Academic Press, Harcourt Brace & Company, 143-149.

Cortey, M., Bertran, K., Toskano, J., Majó, N., Dolz, R., 2012. Phylogeographic distribution of very virulent infectious bursal disease virus isolates in the Iberian Peninsula. Avian Pathology, 41(3):277-284.

Coulibaly, F., Chevalier, C., Gutsche, I., Pous, J., Navaza, J., Bressanelli, S., Delmas, B., Rey, F.A., (2005). The birnavirus crystal structure reveals structural relationships among icosahedral viruses. Cell 120:761–772.

Fahey, K.J., O'Donne, I.J., Bagust, T.J. 1985. Antibody to the 32K structural protein of infectious bursal disease virus neutralizes viral infectivity in vitro and confers protection on young chickens. Journal of General Virology 66, 2693-2702.

Fahey, K.J., Erny, K., Crooks, J., 1989. A conformational immunogen on VP-2 of infectious bursal disease virus that induces virus-neutralizing antibodies that passively protect chickens. J Gen Virol 70(6):1473–1481.

Helal, A.M., Susan, S. El-Mahdy, Manal, A. Afify, 2012. Study the Prevalence of Variant IBD Strains in Some Egyptian Chicken Farms. New York Science Journal, 5(6):8-11.

Islam, M.A., Khatun, M.M., Rahman, M.M., Hossain, M.T., 2005. Serologic and pathogenic characterization of

infectious bursal disease virus isolated from broiler chickens Bangladesh. Veterinarian, 22(2):57-64.

Lejal, N., Da Costa, B., Huet, J.C., Delmas, B., 2000. Role of Ser-652 and Lys-692 in the protease activity of infectious bursal disease virus VP4 and identification of its substrate cleavage sites. J Gen Virol 81:983–992.

Leong, J.C., Brown, D., Dobos, P., Kibenge, F.S.B., Ludert, J.E., Muller, H., Mundt, E. Nicholson, B. 2000. "Family Birnaviridae." In Virus Taxonomy. Seventh Report of the International Committee on the Taxonomy of Viruses, pp. 481-490. Edited by M.H. V. van Regenmortel, C.M. Fauquet, D. H.L. Bishop, E.B. Carstens, M.K. Estes, S.M. Lemon, J. Maniloff, M.A. Mayo, D. J. McGeoch, C.R. Pringle & R.B. Wickner. San Diego: Academic Press.

Letzel, T., Coulibaly, F., Rey, F.A., Delmas, B., Jagt, E., van Loon, A.A., Mundt, E., 2007. Molecular and structural bases for the antigenicity of VP2 of infectious bursal disease virus. J Virol 81:12827–12835.

Lukert, P.D., Saif, Y.M. 2003. Infectious bursal disease. In: Diseases of Poultry, 11th edition, Saif YM, Barnes HJ, Glisson JR, Fadly AM, McDougald LR and Swayne DE (eds), Iowa State Press, Ames, Iowa, 161–179.

Mahgoub, H.A., 2012. An overview of infectious bursal disease. Archives of Virology, 157(11):2047-2057

Metwally, A.M., Yousif, A.A., Shaheed, I.B., Mohammed, W.A., Samy, A.M., Reda, I.M., 2009. Re-emergence of very virulent IBDV in Egypt. Int J Virol; 5:1–17.

Mohamed, M.A., Elzanaty, K.E.S., Bakhit, B.M., and Safwat, M.M., 2014. Genetic Characterization of Infectious Bursal Disease Viruses Associated with Gumboro Outbreaks in Commercial Broilers from Assiut Province, Egypt. ISRN Veterinary Science, Volume 2014, Article ID 916412, 9 pages. http://dx.doi.org/10.1155/2014/916412

Mundt, E., Beyer, J., Muller, H., 1995. Identification of a novel viral protein in infectious bursal disease virus-infected cells. J Gen Virol 76(2):437–443.

Nadia M. Ibrahim, 2011. Immunological studies on the local Infectious Bursal Disease (IBD) Virus adapted on specific pathogen free embryonated chicken eggs (SPF-ECE). Egypt. J. Agric. Res., 89(2):709-729.

Sara Abdel Mawgod, Abdel Satar Arafa, Hussein A. Hussein, 2014. Molecular genotyping of the infectious bursal disease virus (IBDV) isolated from broiler flocks in Egypt. International Journal of Veterinary Science and Medicine, 2:46–52.

Tomás G, Hernández M, Marandino A, Panzera Y, Maya L, Hernández D, Pereda A, Banda A, Villegas P, Aguirre S, Pérez R, 2012. Development and validation of a TaqMan-MGB real-time RT-PCR assay for simultaneous detection and characterization of infectious bursal disease virus. Journal of Virological Methods, 185(1):101-107.

van den Berg, T.P., Eterradossi, N., Toquin, D., Meulemans, G. 2000. Infectious bursal disease (Gumboro disease). Revue Scientifique Et Technique Office International Des Epizooties 19(2):527–543.

Van den Berg, T.P., Morales, D., Eterradossi, N., Rivallan, G., Toquin, D., Raue, R., Zierenberg, K., Zhang, M. F., Zhu, Y.P., Wang, C.Q., Zheng, H.J., Wang, X., Chen, G.C., Lim, B.L. and Muller, H., 2004. Assessment of genetic, antigenic and pathotypic criteria for the characterization of IBDV strains. Avian Pathology, 33(5):470-476.