Genetic Characterization of Egyptian Field Isolates of Infectious Bursal Disease Virus

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Abstract

Detection and characterization of field infectious bursal disease virus (IBDV) isolates circulating among different broiler breeds representing three Egyptian governorates with a history of vaccination against IBDV, was done during 2013- 2015. Reverse transcriptase polymerase chain reaction (RT-PCR) was used to amplify and produce clear band of 620 Base pair (bp) product within the hypervariable region of the IBDV viral protein (VP2) gene. The IBDV was prevalent in thirty out one hundred flocks (30%). Subsequently, chosen PCR products were subjected to further analysis by sequencing and phylogenetic studies. Amino acid substitution in VP2 region used to establish a clear framework on the epidemiology of isolated IBDV . Genetic analysis showed that all examined IBDV isolates characterized as vvIBDV had amino acids residues(222A, 242I, 256I, 294I, 299S) which showed to be unique for all vvIBDV strains and clustered phylogenetically with the previously Egyptian IBDVs (99.3-100%) identity. While, vaccinal IBDV strains (BURSA-VAC and UNIVAX) were clustered in another group with (91.1–91.9%), (91.9–92.6%) identity respectively which might leads to vaccination failure and reemergence of disease. Consequently, we recorded wide range of genetic similarities (94.8-95.6) with the recombinant vaccines (VP2 of Faragher 52/70) seed virus, which showed superior protective efficacy against recent Egyptian vvIBDV isolates. IBDV is still a threat against poultry industry. It is important to keep track on appearance and evolution of antigenically different IBDV circulating strains followed by regular updating of the vaccination strategy.

Key words: vvIBDV, phenotypic characterization, vaccination program, RT-PCR, Molecular characterization.

Introduction

Infectious bursal disease (IBD) is one of the most economically important diseases that affects commercially produced chickens worldwide. Infectious bursal

disease virus (IBDV) has two subtypes; the variant and the classical subtype that has been naturally range in virulence from attenuated to very virulent (vvIBDV) ***(Jackwood et al., 2008).*** These vv IBDV strains have ability to break through high levels of maternal antibodies (MAbs) in commercial flocks, causing high economic losses in chickens and producing lesion typical of IBDV ***(Xiumiao et al., 2012).*** To establish the proper control procedure, it is important to characterize as well as identify the viral strains prevalence in the geographic area which can be achieved by using reverse transcriptase polymerase chain reaction (RT-PCR) to amplify section of the IBDV genome. The VP2 gene is commonly studied because it contains important neutralizing antigenic sites, determinants for pathogenicity, which was highly variable among strains. The application of these techniques on more numbers of samples followed by further studies such as nucleotide sequencing have been extensively used by many workers for the molecular characterization of IBDV***(Sharma et al., 2005)*** and generating epidemiological information in order to formulate a vaccination strategy for control of the disease ***(Mittal et al ., 2005).*** This study was performed to detect the current status of IBDV prevailing in different commercially vaccinated broiler flocks using one-step RT-PCR during Jan 2013 to Jan 2015, then sequence hyper variable region (HVR) of VP2 of chosen isolates and compare with well characterized IBDV as well as vaccinal strains to better understand the progression of IBDV for designing suitable vaccination regimes.

Material and methods

History of examined flocks: One-hundred broiler chicken flocks from different Egyptian governorates (Ismailia, El Sewis and El-Sharqia) suspected to be infected with IBDV were examined during January 2013 to January 2015. The flocks were diagnosed according to criteria in the OIE manual ***(OIE, 2009)*** based on history, clinical findings, post-mortem lesions and RT-PCR techniques.

2. Virus Identification

2.1. Bursal tissue processing: Tissue pools of (5-10) bursa /flock were taken from the examined chickens flocks of 17 and 43 days of age. The specimens were taken under aseptic conditions, then kept in sterile labeled plastic bags and transported in ice containers to the laboratory where stored at - 80 °C until processing.

Frozen bursal pools tissues were prepared and used for virus detection ***(Hirai and Shimakura, 1972).***

2.2. Reverse Transcription Procedure: RT-PCR was done using QIAGEN One-Step RT-PCR to amplify a 620-bp product within the HVR of the IBDV VP2 gene with

the following forward and reverse primers: AUS GU-F5'-TCA CCG TCC TCA GCT TAC CCA CAT C-3'and AUS GL –R C5'-GGA TTT GGG ATC AGC TCG AAG TTG C-3**.** The reaction was done according to ***Metwally et al. (2009).***

2.3. Sequencing and Phylogenetic analyses of the HVR of VP2: PCR products of HVR VP2 gene at 620 bp were purified by QIAquick PCR (Qiagen Inc., Valencia, CA, and USA) following manufacturer's instructions. The purified PCR products were sequenced using a ready reaction Bigdye Terminator V3.1 cycle sequencing kit (Perkin-Elmer/Applied Biosystems, Foster City, CA) and 3130 genetic analyzer (applied Biosystems).

To set up a better framework for designing the molecular epidemiology of the chosen IBDV isolates, the nucleotide and amino acid sequences were analyzed in combination with sequence data from other strains obtained from GenBank (http://www.ncbi.nlm.nih.gov/GenBank). The VP2 of HVR that located between nucleotide 655 to 1060 bp sequences was determined.

The CLUSTAL V multiple sequence alignment program, version 1.83 of Meg Align module of Lasergene DNA Star software was used to determine nucleotide and amino acid sequence similarities and relationships as designed by ***Thompson et al. (1994).***

Phylogenetic trees were constructed using MEGA 7 program ***(Kumar et al., 2016)*** with neighbor- joining methods.

Results

Prevalence of IBDV among different examined chicken flocks: Using RT-PCR, thirty out of one-hundred examined broiler chicken samples were positive (30%) to IBDV in flocks vaccinated with different IBDV vaccination programs as shown in Table (1).

Sequence analysis: The deduced amino acid sequences included 131 amino acid residues (position 219 to 350) of chosen IBDV strains were aligned with IBDV reference strains of Egypt and other parts of the world based on VP2 region. It was found that all examined field isolates have amino acid identity ranged from 98.5-100% with each other and none of them are of vaccinal or attenuated origin due to absence of 253-Histidine and 284-Threonine mutations that are typically found in attenuated vaccine strains.

IBDV examined strains showed the characteristics of vvIBDV amino acid substitutions at residues 222A, 242I, 253Q, 256I, 279D, 284A, 299S and 330S except strains S9, S10, S11 and S12 whereas, S9 and S11 have alanine at (269A) in substitution of Threonine (269T) while, S10 and S12 have isoleucine (252I) in substitution of Valine (252V). The examined isolates have the serine-rich hepta-peptide SWSASGS that was found next to the second hydrophilic region 326–

332 that confirmed the nature of highly virulence among the chosen strains and summarized the differences between IBDV subtypes were more apparent in the minor hydrophilic peaks I and II than all other amino acid changes in the VP2 region(**Fig. 1).**

Amino acid substitution mutations were observed in the major at amino acid (220) and in the minor hydrophilic peaks at amino acids (252 & 254). In major hydrophilic peak A, all examined isolates have (220F) which is similar to reference Egyptian vvIBDV isolates (Beh 2003, Giza 2000 and Giza 2008) and differ from European vvIBDV strain (UK 661) which had (220Y).

In minor hydrophilic peak 1, both examined isolates S10 and S12 showed one mutation at position (252I) which differ from both vvIBDV European (UK 661) and Egyptian strains (Giza 2000, Giza 2008) that had (252V). Other amino acid mutation occurred at position 254, where thirteen local isolates had serine (254S) which were similar to vvIBDV Egyptian strains (Beh 2003, Giza 2000 and Giza 2008) and differ from European strain (UK 661) that had Glycine (254G).

Meanwhile in minor hydrophilic peak 2, it was found that all examined strains had the same aspartic acid (aa) at position 279 which were similar to both vvIBDV European (UK 661) and Egyptian strains (Giza 2008, Giza 2000) that had (279D).

*Alignment and phylogenetic tree analysis***:** Table (3) showed that field IBDV isolates had an aa identity ranged between (99.3-100%) to Egyptian vvIBDVs (Giza 2008 and Beh 2003), while aa identity ranged from 98.5-99.3% between old Egyptian strain (Giza 2000) and all examined isolates. Also, the percent of homology between field isolates and different vvIBDV isolates were 95.3–96.3% with Uk661, 97.3– 98.3% with Beh 2003 and 95.3–96.8% with the vvIBDV France 97.

Phylogenetic analysis of field isolates with other IBDVs reference (classical, very virulent, variant and vaccinal strains) strains revealed that field isolates were in a separate branch and they were clustered more close to the Egyptian vvIBDV (Giza 2000 and Giza 2008) followed by European, Nigeria and Israel reference strains, then, Faragher 52/70, Bursine Plus, UNIVAX and D78 but they were clustered at a far distance from IBD-BLEN (Fig. 2).

Table (1): *Effect of vaccination regimens' on occurrence of IBDV in examined flocks.*

Type of vaccines	Twice 7-14 day				Twice 14-24				Once (13)		Total	
	Foreign Broiler breeds		Baladi breeds		Foreign Broiler breeds		Baladi breeds		Baladi breeds		Foreign Broiler breeds	Bladi
	PCR-Positive IBDV /total samples tested	% of positive	PCR-Positive IBDV /total samples tested	% of positie	PCR-Positive IBDV /total samples tested	% of positie	PCR-Positive IBDV /total samples tested	% of positie	PCR-Positive IBDV /total samples tested	% of positive		
D78	9/20	45%	4/6	67%	5/15	33.3%	2/5	40%	2/2	100 %	(14/ 35) 40%	(8/13) 61.5%
D78+IBD - BLEN	6/7	86%	-	-	-	-	-	-	-	-	85.7% (6/7)	-
UNIVAX	0/14	0%	0/1	0%	0/6	0%	-	-	-	-	0%	0%
BURSAVAC	0/14	0%	0/2	0%	0/6	0%	-	-	-	-	0%	0%
*Recombinant	HVT + IBD by in day -old injection										0%	0%
Total	15/55 (27%)		4/9 (44.4%)		5/27 (18.5%)		2/5 (40%)		2/2 (100%)		47.6% 20/42)	61.5%

(-): no vaccination

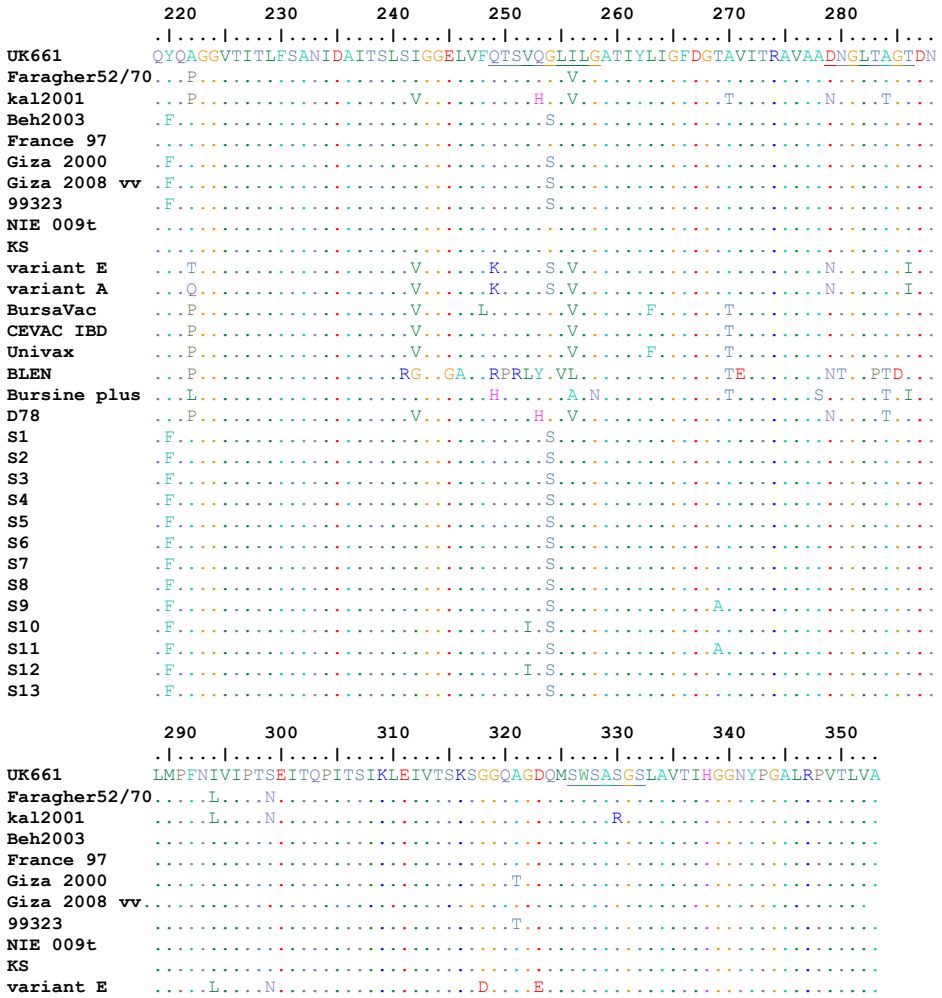

Figure (1): *Deduced amino acid sequences of VP2 variable domain from residues 219-350 (numbering according to* ***Bayliss et al., 1990).*** *Major* ***(Azad et al., 1985)*** *and minor* ***(Van Den Berg et al., 1996)*** *hydrophilic peaks are indicated.*

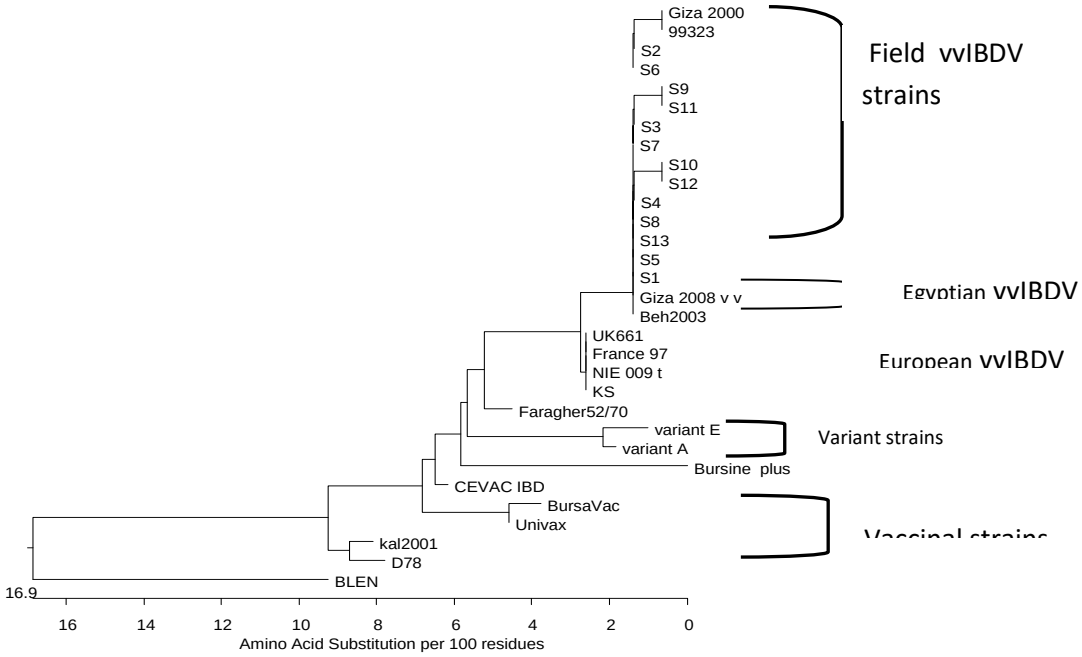

Figure (2): *Amino acid phylogenetic tree of the VP2 gene of the analyzed IBDV isolates and other strains published in GenBank.*

Table (2): *Amino acid substitution between thirteen examined isolates and Giza 2008.*

Amino acid position	Giza 2008	S9	S10	S11	S12
***252**	V	-	I	-	**I**
269	T	A	-	A	-

***Substitution of amino acid atcrucial site.
V: Valine A: Alanine I: Isoleucine T: Threonine (-)= no amino acid change

Table (3): *VP2 gene nucleotide and amino acid identity of examined vvIBDV isolates with other reference strains published in GenBank.*

IBDV VP2 gene GenBank accession no. (isolate)	**Virulence**	**Country of Origin**	**(Year of collection)**	**Field isolates identity(%) range**	
				`Nucleotide	**Amino acid**
UK-661 (AJ878898)	vv IBDV	Europe	89	95.3-96.3	97.8-98.5
France 97AL (DQ916194)	vv IBDV	France	97	95.3-96.8	97.8-98.5
NIE 009t (AY099456)	vv IBDV	Nigeria		95.3-96.3	97.8-98.5
KS (L42284)	vv IBDV	Israel	97	94.6-96.1	97.8-98.5
Beh 2003 (EU860092)	vv IBDV	Egypt	2003	97.3-98.3	99.3-100
Giza 2000(AY318758)	vv IBDV	Egypt	2000	97.00-98.00	98.5-99.3
Giza 2008(EU584433)	vv IBDV	Egypt	2008	97.5-99.00	99.3-100
*Faragher 52-70 (Y 14958)	Classical strain	Europe	70	92.9-93.8	94.8-95.6
BursaVac (AF498633)	Intermediate classical strain	USA		91.6-92.6	91.1-91.9
Cevac IBD (AJ632141)	Intermediate plus 2512 strain & IBD Antibodies)	Vaccinal strains		91.6-92.6	94.1-94.8
Univax (AF457106)	Mild strain	Vaccinal strains		91.6-92.6	91.9-92.6
Bursine plus (AF498632)	intermediate	Vaccinal strains		89.4-90.1	89.6-90.4
IBD blen (AY332560)	Winterfield2512 modified	Vaccinal strains		85.7-86.2	79.3-80.00
D78 (AF499929)	classical attenuated	Netherlands	1978	90.9-91.9	90.4-91.1

*52/70 seed virus of the recombinant vaccines (HTV-vector vaccine)

Discussion

IBDV is present in 95% of the member states, representing the most important disease that affects the commercial poultry (**Hussein *et al.*, 2003**).

Clinical signs and post mortem (PM) lesions in diagnosed flocks were completely agreed with various researchers **(Hamoud *et al*., 2007; Rathore *et al.*, 2013 and Grandia *et al*., 2014)** who mentioned that IBDV infected birds suffered from inappetence, dullness, depression, and reluctance to move. While in post-mortem examination, the haemorrhages were observed on leg muscles. The bursa of Fabricus was enlarged two times with severe pus accumulation in its lumen.

Sequence analysis and comparison of 131bp of the hypervariable region of VP2 with various strains revealed that all examined field isolates have amino acid identity ranged from 98.5-100% among each other, which indicates genetic homogeneity. These results agreed with **Neven et al., (2015)** who mentioned that most Egyptian IBDV isolate shave close relationships between the previously isolated Egyptian IBDVs and they are clustered together with (99-100%) identity.All of the examined field strains showed the characteristic of vvIBDV amino acid substitutions at residues at 222 (P→A), 256(V→I) and 294 (L→I) and 299S which have been implicated in increased virulence and used as molecular markers for vv IBDV strains(**Cao *et al.*, 1998** and **Brandt *et al.*, 2001**).The same results obtained by **Hernandez *et al.*, (2006**) and **Barathidasan *et al.*, (2013)** who noticed that four amino acid residues have conserved among most vvIBDV strains (222A, 256I, 294I, and 299S).

Our results revealed that, amino acid sequence identity was higher with vvIBDV (97.8-100%) strains than with classical or variant strains. Similar results were clarified by **Sara et al., (2014)**.

The amino acid change 222 P-A in first hydrophilic region of hypervariable sequence thought to be a genetic marker for very virulent viruses which is expected to prevent the binding of the MAbs 3 and 4. These results are in accordance with **Mardassi et al., (2004)** who reported that Tunisian vvIBDV strains contain an alanine at position 222 instead of proline which is expected to prevent the binding of the 3 and 4 neutralizing MAbs which might be explained occurrence of IBDV in vaccinated flocks.

Minor hydrophilic peak 1 showed that all local isolates have glutamine at position 253 which agreed with **Islam, *et al.*, (2012)** and **Negash, *et al.*, (2012)** who informed glutamine at position 253. A single amino acid mutation at position 253(H-Q) in VP2 markedly increased the virulence of an attenuated IBDV strains **(Jackwood *et al*., 2008).**

Other substitution that occurred at position 254 revealed that all local

isolates had serine at position 254 which agreed with **Kasanga et al., (2007)** who reported that vvIBDV isolated from vaccinated chickens vaccinated with classical attenuated vaccine with such aa residues at position 254. Exchanges of amino acid in this position may likely resulted in antigenic changes (**Durairajet al., 2011),** leading to a modified structure of the neutralizing epitopes (**Martin et al, 2007**) causing vaccination failure. Also, **Jackwood and Sommer-Wagner, (2011)** reported that amino acid mutations in VP2 in position 222 and 254 have been responsible for antigenic drift in IBDV.

In minor hydrophilic peak 2 showed that all examined strain had same aa at position 279 which is similar to both vvIBDV European (UK 661) and Egyptian strains (Giza 2000, Giza 2008) that had (279D). These results agreed with those of **Adamu et al., (2015)**.

Field isolates S10 and S12 have isoleucine (252I) instead of Valine (252V) when compared with Giza 2008. This result is in agreement with **Alejandro, (2002)** while it was showed Alanine (269A) instead of Threonine (269T) when compared isolates S9 and S11 with Giza 2008. This result agreed with **Jackwood et al., (2008)** who observed amino acid substitution 269 (T to A) in CS-2-35 isolates when compared to D78 vaccine.

It seems that most aa substitutions occurred at VP2 variables regions which is the major antigenic component that induced protective neutralizing antibodies.

Alignment and phylogenetic tree analysis: The observed identity percentages of the field isolates with reference vaccinal strains were 94.8-95.6 with 52/70, 94.1–94.8% with CEVAC- IBD, 91.9–92.6% with UNIVAX, 91.1–91.9% with BURSA-VAC 90.4%-91.1% with D78 and 89.6%-90.4% with Bursine plus while the lowest identity were observed with IBD-BLEN (79.3%-80%) **(Table 3; Figure2**). This result explains vaccination failure against vvIBDV field isolates. Similary, with **Mohamed *et al*., (2014)** mentioned that BURSA-VAC vaccine and CEVAC IBD were the nearest vaccines in sequence similarity to the local field examined IBDV strains (Bursine Plus and Nobilis Gumboro). The obtained results showed that isolated IBDVs that circulated in Egyptian flocks showed higher similarities to each other while they showed lower similarities to the used vaccinal strains. Therefore, comparison of IBDV sequences is important to identify new variants occurring in the field. Thus, the selection of VP2 amino acid sequence as closely as possible is important to get the best protection. Consequently, we recorded wide range of genetic similarities (94.8-95.6) of the European seed virus of recombinant vaccines (VP2 of Faragher (52/70) against the currently circulating field viruses

beside their ability to overcome difficulties in managing MAbs on vaccine uptake which explain no IBDV lesion in flock vaccinated with these vaccine. These results in accordance with **Sultan *et al.*, (2012)** who mentioned that HVT-IBD vaccine has the ability to induce an immune response in birds with high levels MAbs and could protect against recent Egyptian vvIBDV isolates. Also, agreed with **OIE (2008)** who reported that live recombinant vaccine (expressing the VP2 antigen of IBDV) produced protective immune responses in chickens better than the attenuated viral strains. These reflect the importance of continuous evaluation and updating of the vaccinal seed strains to determine its efficacy against currently circulating IBDV.

Monitoring chicken flocks against vvIBDV is essential and molecular methods can be used to identify and characterize the agent for vaccinal selection and formulating strategy. Further studies are needed for the unique two isolates which had amino acid substitution at aa (V252I) in minor peak 1.

References:

Adamu, J., Owoadeb,A. A. .; Abduc,P. A. ; Kazeema, H. M. and Fatihud, M. Y. (2015): Characterization of field and vaccine infectious bursal disease viruses from Nigeria revealing possible virulence and regional markers in the VP2 minor hydrophilic peaks. Avian pathol.,42(5): 420-433.

Alejandro, B. (2002): Characterization of field strains of infectious bursal disease virus (IBDV) using molecular techniques. Doctor of philosophy.

Azad, A.A.; Barett, S. A. and Fahey, K. J. (1985): Characterization and molecular cloning of the double stranded genome of an Australian strain of infectious bursal disease virus. Virol., 143:35–44.

Barathidasan, R.; Sin, S.D.; Asok Kumar , M.; Desingu, P.A.; Palanivelu, M.; Singh, M. and Dhama, K. (2013): Recurrent outbreaks of infectious bursal disease (IBD) in a layer farm caused by very virulent IBD virus (vv IBDV) in India: Pathology and Molecular analysis. South Asian J. of experim. Boil., 3(4): 200-206.

Bayliss, C.D.; Spies, u.; haw, k.; Petter, R.W.; Papageorgiou, A.; Muller, H. and Boursnell, M.G.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., 71:1303-1313.

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

Cao, Y. C.; Yeung, W.S.; Law, M.; Bi, Y.Z.; Leung, F. C. and

Lim, B. L. (1998): Molecular characterization of seven Chinese isolates of infectious bursal disease virus: classical, very virulent, and variant strains. Avian Dis., 42:340-351.

Durairaj, V.; Sellers, H.S.; Linnemann, E.G.; Icard, A.H. and Mundt, E. (2011): Investigation of the antigenic evolution of field isolates using the reverse genetics system of infectious bursal disease virus (IBDV). Archives of Virol., 156: 1717-1728.

Grandía, G. R.; Alfonso, M. A. and González, N. B. O. (2014): Characterization of a Gumboro disease outbreak in a poultry farm of Havana. 33%Revista de InvestigacionesVeterinarias delPerú (RIVEP), 25 (2): 333-339.

Hamoud, M.M.; Villegas, P. and Williams, S.M. (2007): Detection of infectious bursal disease virus from formalin-fixed paraffin-embedded tissue by immune-histochemistry and real-time reverse transcription-polymerase chain reaction. J. Vet. Diagn. Invest. (1):35-42.

Hernandez, M.; Banda, A.; Hernandez, D.; Panzera, F. and Perez, R. (2006): Detection of very virulent strains of infectious bursal disease virus (vvIBDV) in commercial broilers from Uruguay. Avian Dis., 50: 624-631.

Hirai, K. and Shimakura, S. (1972): Immuno-diffusion reaction to avain infectious bursal virus. Avian Dis., 16: 961-964.

Hussein, H.A.; Aly, A.M.; Sultan, H. and AL-Safty, M. (2003): Transmissible viral proventriculitis and stunting syndrome in broiler chickens in Egypt. Isolation and characterization of variant infectious bursal disease virus (IBDV). Vet. Med. J., Giza, 3:445-462.

Islam, M.T.; Hoa, T.; Rahman, M.M. and Islam, M.A. (2012): Molecular characterization of two Bangladeshi infectious bursal disease virus isolates using the hypervariable sequence of VP2 as a genetic marker. J. Vet. Sci.13 (4): 405-412.

Jackwood, D.J and Sommer-Wagner, S.E (2011): Amino acids contributing to antigenic drift in the infectious bursal disease Birnavirus (IBDV).Virol., 409: 33-37.

Jackwood, D. J.; Sreedevi, B.; LeFever, L.J. and Sommer-Wagner, S.E. (2008): Studies on naturally occurring infectious bursal disease viruses suggest that a single amino acid substitution at position 253 in VP2 increases pathogenicity. Virol., 377:110-116.

Kasanga, C.J.; Yamaguchi, T.; Wambura, P.N.; Maeda-Machang'u , A.D.; Ohya, K. and Fukushi, H. (2007): Molecular characterization of infectious bursal disease virus (IBDV): diversity of very virulent IBDV in Tanzania. Arch Virol ,152:783–790.

Kumar, S.; Stecher, G. and Tamura, K. (2016): Mega 7: Molecular evolutionary genetic analysis version 7.0 for bigger datasets. Molecular biology and evolution (doi): 10.1093/molbev/msw054.

Mardassi, H.; Khabouchi, N.;Ghram, A.; Namouchi, A. and Karboul, A. (2004): A very virulent genotype of infectious bursal disease virus predominantly associated with recurrent infectious bursal disease outbreaks in Tunisian vaccinated flocks. Avian Dis., 48:829–840.

Martin, A. M.; Fallacara,F.; Barbieri,I.; Tosi,G.; Rivallan, G. ; Eterradossi, N.; Cerutiti u, R. and Cordioli, P. (2007): Genetic and antigenic characterization of infectious bursal disease viruses isolated in Italy during the period 2002-2005.Avian Dis.,51: 863-872.

Metwally, A.A.; Ausama, A.Y.; Iman, B.S.; Walaa, A.M.; Attia, M.M. and Ismail, M.R. (2009): Re-emergence of very virulent IBDV in Egypt. Int. J. Virol., 5:1-7.

Mittal, D.; Jindal, N.; Gupta, S.L.; Kataria, R.S. and Tiwari, A.K. (2005): Detection of infectious bursal disease virus in field outbreaks in broiler chickens by reverse transcription-polymerase chain reaction. Int. J. Poult. Sci., 4 (4): 239-243.

Mohamed, M.A.; Kamal, E. S.; Bakhit, M. B. and Marwa M. S. (2014): Genetic characterization of infectious bursal disease viruses associated with Gumboro outbreaks in commercial broilers from Asyut Province, Egypt. Hindawi Publishing Corporation ISRN Veterinary Science, ID 916412, pages 9.

Negash T, Gelaye E, Petersen H, Grummer B, and Rautenschlein S (2012): Molecular evidence of very virulent infectious bursal disease virus in chickens in Ethiopia. Avian Dis. 56: 605-610.

Neven, R.; Shahera, Abdell-Fattah; Abdell-Dayem, M. (2015): Prevalence and characterization of Gumboro virus in chicken farms in Ismailia. Assiut, Vet. Med. J., 61(145).

OIE (2008): Infectious bursal disease (Gumboro disease). Chapter 2.3.12.Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. p. 549.

OIE (2009): Manual of standards for diagnostic test and vaccine, Office international Des Epizooties.

Rathore, S.; Kumar, A.; Thakur, P.; Kumari, N.; Meena, M. and Nimje, P. (2013): Pathological study of infectious bursal disease in broilers of Haryana. 15(1): 62-65.

Sara, A.; Abdel Satar, A. and Hussein A. H. (2014): Molecular genotyping of the infectious bursal disease virus (IBDV) isolated from broiler flocks in Egypt. Intern J. of Vet. Sci. and Med., 46–52.

Sharma, K.; Hair, B.M.; Omar, A.R. and Aini, I. (2005): Molecular characterization of infectious bursal disease virus isolates from Nepal based on

hypervariable region of VP2 gene. Acta. Virol.,49: 59–64.

Sultan, H.; Hussein A. H.; Abd El-Razik, A.G.; El-Balall, S.; Talaat, S.M. and Shehata, A.A. (2012) : Efficacy of HVT-IBDV vector vaccine against recent Egyptian vv IBDV in commercial broiler chickens. Inter. J. Poult. Sci., 11 (11): 710-717.

Thompson, J.D.; Higgins, D.G. and Gibson, T.J. (1994): Clustal W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acid Res., 22 (22): 4673-4680.

Van Den Berg TP, Gonze M, Morales D, and Meulemans G (1996): Acute infectious bursal disease in poultry: immunological and molecular basis of antigenicity of a highly virulent strain. Avian Pathol., 25: 751-768.

Xiumiao, H.; Dingming, G.; Ping, W.; Xiuving, Y.; Guijun, W. and Aijian, Q. (2012): Molecular epidemiology of infectious bursal disease viruses isolated from Southern China during the years 2000–2010. Virus Genes, 45: 246–255.

الملخص العربي
التوصيف الجزيئي لمرض الاتهاب الكيس الفيبريشي المعدي

أجريت هذه الدراسة لدراسة الوضع الحالي والتوصيف الجزيئي لمرض الجمبورو بين سلالات التسمين المختلفة التي تمثل ثلاث محافظات المصرية وذلك في الفترة من عام 2013 حتى عام 2015 . تم اختبار العينات باستخدام تفاعل البلمرة التصاعدي العكسي لبروتين الفيروس VP2. تم العثور على 30 (30٪) عينة احتوائها على IBDV الجينوم باستخدام RT-PCR الذي وظف لمقارنة معدلات الإصابة. وسجلت حالات إصابة الطيور على الرغم من التطعيم الروتيني ضد مرض التهاب غدة فا بريشية، مما يشير إلى احتمال وجود سلالة فتاكة جدا من أحدثت الإصابة.

بإجراء الاختبار الجيني للعينات , كشفت نسبه التماثل وتحليل شجرة النشوء والتطور أن جميع المعزولات قريبة جدا من سلالات IBDV الفتاكة حيث تحتوي علي الأحماض الأمينية (222 A، I 242، ، 256 I، ، 284 A، 294 Iو299 S) وتتجمع phylogenetically مع سلالات IBDVs المصرية المعزولة سابقا (3،99-100٪) . كانت نسبة التماثل بين المعزولات حين قورنت بالتحصينات الموجودة في الحقل 1،91-9،91٪ مع Bursavac، 91،92-9،6٪ مع Univex، 94،94-1،8٪ مع Cevac IBD، 79.3٪ 80-٪ مع Blen، 89.6٪- 90.4٪ مع Bursine بالإضافة إلى و90.4٪ -91.1٪ مع D78.

سجلت مجموعة واسعة من أوجه الشبه الجينية (94.8-95.6) مع الفيروس الأوروبي لقاحات المؤتلف (VP2 من 52/70) Faragher مما يثبت كفاءة هذه اللقاحات في الصد ضد المعزولات عاليه الضراوة في مصر.

لا يزال IBDV يشكل تهديدا ضد صناعة الدواجن. لذلك كان من المهم تتبع النطور الجيني الذي يحدث في السلالات الموجودة في الحقل لاختيار برامج التحصين المثلى للسيطرة على المرض .