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Chemical compositions of chicken eggs following infection with Infectious bronchitis virus.

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Abstract

IBV detection and isolation trials was done to set its relationship to the inner egg changes.Rapid hemagglutination (HA) activity after neuraminidase enzyme treatment of the concentrated allantoic fluid (AF) of inoculated embryonating chicken eggs (ECE) can give a positive indication for the presence of IBV. The specificity of rapid HA test was examined with a non-hemagglutinating avian viruses such as infectious bursal disease virus (IBDV). The sensitivity of the test was compared with reversed transcriptase-polymerase chain reaction. The results showed that this test was specific and had a sensitivity of 100% for IBV detection.

The detected IBV strain from Sharkia governorate was examined molecularly using polymerase chain reaction (PCR) and S-l partial gene sequence. Sequencing showed that this isolate is an **IBV variant 2** that resembles the Egyptian IBV strain (Eg /12120 S/2012 and IS/1494/2006) field strains with 99% identity.

The isolated virus designated **(IBV-EG/ SHARKIA – F-629-2015)** had showed (85.6%) similarity to the 4/91 variant vaccine, and (82.9 %) similarity to Dutch variants D-274 vaccinal strain, beside (82.2%) similarity to the classical vaccinal strains M-41. MA-5, H120.

In the present study the following parameters were investigated (Total Lipid, CholesterоL, Triglcerol, Phospholipids, NEFA, MDA, Albumin protein, Yolk protein and Whole protein beside Calcium, Phosphorus, Magnesium, Manganese, Potassium, Chloride and PH)

Chemical analysis of egg content may explain that deformed eggs had resulted from inappropriate shell deposition on an unstable watery albumen base helped by the contractility of the oviduct due the disturbance in sodium and potassium pump .

Watery albumen had resulted from an increase in PH and changes in sodium, potassium and chlorine concentrations, which leads to massive chemical changes in egg white and yolk.

As far we know. This is the first attempt to study the impact of IBV infection on chicken egg biochemical composition.

Key words: IBV, rapid HA, neuraminidase enzyme treatment for IBV, PCR, Sequencing, Egyptian IBV variant 2.

Introduction

IBV is a highly contagious acute viral disease of the upper respiratory tract of chickens, it can also replicate in epithelial tissues of kidneys, gonads and oviduct of chickens causing their pathology and affecting the performance **Lee et al. (2004).**

IBV causes high morbidity in all ages and high mortality in chickens less than 6 weeks old. In addition, poor egg production with poor quality follows the disease **(Cavanagh & Naqi 2003).**

The main objective behind this study was to set up and optimize a rapid, accurate, sensitive, specific and inexpensive test for detection of IBV based on observation of HA activity induced after neuraminidase enzyme ., and to determine the changes in chemical composition of eggs following IBV infection.

Materials and Methods

Deformed egg samples.

Thirty deformed egg samples showing (thin shelled, cracked, mottled, or with pale coloration) as (fig-1) were collected from a breeder flock suffering a drop in egg production % and quality.

Control eggs.

Thirty eggs from a healthy sibling of the previous flock that reared elsewhere were collected to serve as control.

Egg samples were submitted for chemical analysis without delay for the following parameters (Total Lipid, CholesteroL, TriglecerolI, Phospholipids, NEFA, MDA, Albumin protein, Yolk protein and Whole protein beside Calcium, Phosphorus, Magnesium, Manganese, Potassium, Chloride and PH at 24 ºC).

Embryonated chicken eggs (ECE).

Ten-day-old ECE were used for virus isolation trials **Cavanagh and Naqi (l997).**

Membrane filters.

Syringe membrane filter 450 nm Thermo scientific Nalgene. Cat .no .190-2545(8-0404-40493),

Infectious bursal disease virus (IBDV).

Virulent IBDV field isolate previously isolated and identified **Bayoumie and Mohamed (2008)**. was used in the present study, its titer was $10^{5.5}$ EID_{50}/0.1ml.

Chicken RBCS.

Chicken RBCS were obtained from three 28-day-old specific antibody negative chicken (SAN) raised for this purpose.

Saline.

Sodium chloride 0.9% **(ADWIC) ®, Sterile** Pyrogen free.

Neuraminidase enzyme.

Neuraminidase enzyme type V from *Clostridium perfringens* (Sigma, St. Louis, MO) N 2876 – 10 un. , Lot # SLBD9831 V, P code 1001685488, was used.

Dialysis hollow fiber role.

Visking dialysis role .SERVA electrophoresis Gmbh. 21 mm diameter lot.120573 with 1 nm pore size.

Polyethylene glycol.

Polyethylene glycol powder 6000 **(Alpha Chemika)** Serial.no. (AL 3120) Batch.no.(p 20911) mf g (2/ 2011), exp. (2/2016).

Sample preparation for EGE inoculation.

Watery egg albumen from the deformed eggsas seen in fig (1-3) were diluted to make 10 % w/v suspension in saline then filtrated though a 450 nm syringe membrane filter (Thermo scientific Nalgene) . 0.2ml of the filtered material was inoculated into 10 day old ECE via allantoic sac cavity (AS). Inoculated ECE were incubated at 37°C. The allantoic fluids (AFs) from the inoculated ECE were harvested 72 h post inoculation **Momayez et al., (2002).** In order to be sure that the sample was not contaminated with hemagglutinating viruses. The harvested AFs were tested for the lack of positive HA activity due to any other hemagglutinating virus before neuraminidase treatment.

Dialyses hollow fiber.

The harvested allantoic fluids (AFs) of the second passage from the inoculated ECE were placed in the dialyses hollow fiber role and legated then covered for overnight with Polyethylene glycol powder at 4°C for virus concentration **Trudel and Payment (1980).**

Neuraminidase enzyme treatment.

A working solution 1 U/ml of neuraminidase was prepared from the vial containing (10 U/ml) using PBS (pH7.2) as diluent. 25µl of the working solution was mixed with 25 µl of the dialysed AFs, and held at 37°C for 30 min, and then were placed at 4°C for 5 min **Momayez et al (2002)**.

Rapid HA test.

Twenty five µl of dialysed treated AFs were mixed with 25µl of 5% suspension of chicken red blood cells. HA reaction was read within 1min. Clear and consistent HA was considered as positive reaction.

Specificity and sensitivity.

IBVD of **Bayoumie and Mohamed (2008)** was propagated on 11 day old ECE via chorioallantoic membrane (CAM).,the infected CAMs were harvested , homogenized and clarified by centrifuge after three times of freezing and thawing ., then it was 450 nm membrane filterated (Thermo scientific syringe membrane filter). The supernatant fluid was treated with 1 U/ml of neuraminidase, as mentioned before then HA rapid test was done.

RNA extraction.

RNA extraction from the AF from ECE was performed using the QIAamp Viral RNA Mini kit (Qiagen, Germany, GmbH) according to their manufacturer's recommendations. primer of IBV strains is oligo S-1 5'-(TGA-AAA-CTG-AACAAA-

AGA-) 3' and reverse **Adzhar et al., (1996), Gelb et al., (2005)**. The reactions were performed in a T3 thermo cycler (Biometra). The amplicons were separated by electrophoresis on 1.8% agarose gel (Applichem, Germany, GmbH) along with 100- bp DNA Ladder (Qiagen, Germany, GmbH). Reaction products were stained with ethidium bromide, and visualized with ultraviolet trans illumination. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data were analysed by a computer software (Automatic Image Capture Software, Biosciences, and USA (fig-4).

S1 gene sequencing

Visualized bands in the agarose gel that are of similar in size to the positive control was excised from the gel. The PCR product is isolated from the agarose gel using a commercial gel extraction kit. Purified PCR products are run on a second 1.5% agarose nucleic acid stain gel to determine the quantity of product present. Approximately 20 µl of PCR product is required for sequencing. Sequencing was performed at NLQP sequencing facility. Assembly and analysis of sequence data were conducted using BioEdit 5.0 package .Nucleotide and amino acid deduced sequences were aligned using Clustal X software. Phylogenetic analysis was performed by the neighbour-joining method with 1000 bootstrap replicates with the software MEGA version 3.0 as described by **Kumar et al ., (2004).** Sequence chromatograms are edited using suitable analysis software. Edited IBV sequences were characterised using BLASTn for nucleotide or BLASTp for protein analysis

Biochemical analysis.

Lipids extraction for determination of total lipids, Cholesterol, triglycerides was determined by using the methods of **Hammad et al. (1996)** .Total lipids, total cholesterol and triglycerides were determined according to the method described by **Young** (**2001)**. Non esterified fatty acids **(NEFA)** were determined according to the method described by **Schuster (1979).** L-Mlondialdhyde **(MDA)** was estimated according to **Esterbauer et al., (1982).** Protein concentration in egg albumin, egg yolk and whole egg was done using Lowry method in which samples are digested in acid according to **Al-Ghais, (1995)** .Calcium, Phosphorus , magnesium, Sodium and Potassium were determined according to **Tietez (1986)** using spectrophotometer **Chem 7 geneses.** While chloride was estimated, using **Electrogeneses model 2000**. manganese was estimated by atomic absorption spectrophotometer **model 2380 (PERKIN-ELEMER)**.

Statistical analysis.

Data were statistically analyzed as described by **Snedecor and Cochran (1967)** using **SPSS -14 (2006).** Values were used to determine significance.

Results and Disscussion

In the present study detection of (IBV) was intended to insure that IBV had caused the chemical changes found in the examined eggs since different causative agents might be the cause for these changes such as NDV, EDS_{76}, AIV that might be incriminated with these changes **King and Cavanagh (1991), Cavanagh and Naqi (1997). Cavanagh and Naqi (2003).**

IBV grows well in the developing chicken embryos compared to chicken organ cultures like chicken kidney and tracheal culture **Cook et al.,(1976).** Upon inoculation by intra allontoic route, no visible changes were observed in first or second passage as previously found by **Wang et al., (1996), Arthur Sylvester et al., (2003)** and **Zanella et al., (2003).**

The induction of HA activity for IBV by neuraminidase enzyme is the unique property of Corona viruses **Naik et al., (2005)**. HA activity after treatment with neuraminidase enzyme was used in the present study to detect the presence of IBV in infected allantoic fluid (AF) of ECE after inoculation of IBV suspected materials in ECE via AS route. Clear and consistent HA observed after 30 min of incubation period with 1unit/ml of neuraminidase after the second passage without the need for further passages **Momayez et al., (2005). .Schultze et al., (1992)** mentioned that IBV contains Alpha 2, 3linked N-acetyl neuraminic acid that hinder the viral HA activity. When the virus is treated with crude filtrate of *Clostridium perfringens* culture, which is believed to contain neuraminidase enzyme, this enzyme, removes the neuraminic acid from the virus surface and induces HA activity. **Naik et al., (2005)** found that the allontoic fluid collected after 10th passage yielded HA titre of 1:16. This shows the value of virus concentrating of infected AS using the Dialysis hollow fiber role and Polyethylene glycol powder as used by **Trudel and payment(1980) and Eweis et al., (2008)**.

The specificity of rapid HA test was examined with IBDV which revealed non hemagglutinating virus as found also by **Momayez et al., (2005).**

The sensitivity of the rapid HA test was compared with RT-PCR **(fig-2)**. The results showed that this test was specific and had a sensitivity of 100% for IBV detection. The results of this study indicate that HA test for IBV after neuraminidase treatment is an accurate, sensitive, specific and inexpensive test for rapid detection of IBV these results are comparable to the previous work of **Kwon et al., (1993)**.

In the present study partial PCR for the S l gene sequence using universal primers succeeded to amplify the targeted sequence in the tested Sharkia isolates. S l partial sequence analysis resulted in a PCR product of 400 base pairs **(fig-2)** thus PCR succeeded to amplify the target sequence in the Sharkia isolates **Kingham et al., (2000).**

Based on blast analysis and multi sequence alignment of the Sl sequence of the successfully sequenced isolates together with 14 published IBV vaccinal strains, it was demonstrated that isolate is **IBV variant 2** resembles the Egyptian IBV strain **(Eg /12120 S/2012 and IS/1494/2006)** field strains with 99% identity **table (2), (fig 5-6).** This isolate was designated **(IBV-EG/ SHARKIA – F629-2015)** had showed **(85.6%)** similarity to the 4/91 variant vaccine, and **(82.9 %)** similarity to Dutch variants D-274 vaccinal strain, beside **(82.2%)** similarity to the classical vaccinal strains M-41. MA-5, H120 **table (2). El-Sayed Abd El Wahab (2015)** in a personal communication mentioned that the isolate **(IBV-EG/ SHARKIA – F629-2015)** formed a similar phylogenetic group with very close similarity to (4/91and also D-274) IBV.

The S1 sequences of nucleotide sequences of the isolate were aligned with published sequences and the dendrogram was generated to determine the phylogenetic position of these isolates among IBV strains **(fig-7).**

The obtained results presented in table (3) showed a high significant decrease in concentration of total lipids, triacylglycerol, Phospholipids and NEFA in IBV affected eggs. This was accompanied by very high significant decrease in yolk total cholesterol concentrations. Meanwhile, a high significant increase in L-malondialdehyde (MDA) concentration was recoded in affected egg group. This increase is a marker of lipid peroxidation and reflects the high production of free radical due to IBV infection. It also reflects the accumulation of free radicals in the blood and tissues of the infected birds **Elnile (2008)**. Further studies are necessary to clarify the effect of IBV in body fluids and tissues after the infection.

In the present study data presented in table (4) showed a high significant decrease in albumin, yolk and whole egg total proteins., while, the PH value of egg albumin showed a high significant increase at 24 C^{0} compared to the non-infected group**. Ivan (2004)** recorded that the reduction of albumen proteins changes the structural matrix of the albumen producing watery eggs. **Butler et al., (1972)** mentioned that microscopic changes such as reduction in the number and height of the epithelial cells., or the complete absence of the cilia, beside glandular hypoplasia caused by IBV may lead to the reduction in the synthesis of albumen proteins especially ovo-mucin, lysozyme and other major proteins which constitute the structural matrix of the thick albumen. Furthermore **Muneer et al, (1987)** explained that there is a decrease in the proportion of both thick and inner thin albumen, and an increase in the amount of outer thin albumen causing watery-whites and presence of blood or meat spots in the egg albumen.

Obtained data in table (5) in the present study revealed a very high significant decrease in the concentrations of calcium, phosphorus, magnesium and potassium. Moreover, a high significant decrease manganese and chloride concentration was reported. Meanwhile, the concentration of sodium revealed a very high significant increase in egg yolk if compared with the non-infected eggs table (5) .The dramatic

decrease in the concentrations of calcium, phosphorus, potassium, chloride and manganese concentration, and the very high significant increase in concentration of sodium are probably initiated by a depressed function of the sodium potassium pump and alteration of the activity of sodium potassium AT P ase. **Robinson and Monsey (1972). Solomon (2002)** Mentioned that changes observed in the uterine fluid of IBV infected hens could explains the fluidity and thinning of the egg albumin examined from the infected birds. There was deterioration in albumen quality which was reported in the infected hens this finding is attributed to the uterotropism of IBV for the fully functional oviduct **Leary (1999).**The functional disturbances which followed the virus infection are located in the surface epithelial cells of the uterine mucosa could be explain the depressed function **Chousalkar and Roberts (2007)**. In addition **Robinson and Monsey (1972)** Reported that the chemical reaction may take place naturally causing liquefaction of thick egg white gel at a relatively high pH value of 9.2 in egg white. The destruction of the gelatinous nature of thick egg white can occur due to ovomucin-lysozyme interaction as the pH of the albumen changes. It worth to mention that PH level in the examined infected eggs was 9.48 ± 0.19 table (5).

Acknowledgments

The authors are grateful to their colleagues at NLQP

Results of the present study is illustrated in tables (1- 5) and figs. (1-7).

Fig(1).Shows miss shaped chicken eggs

Fig(2).Shows fragile chicken egg

Fig(3).Shows liqued albumin

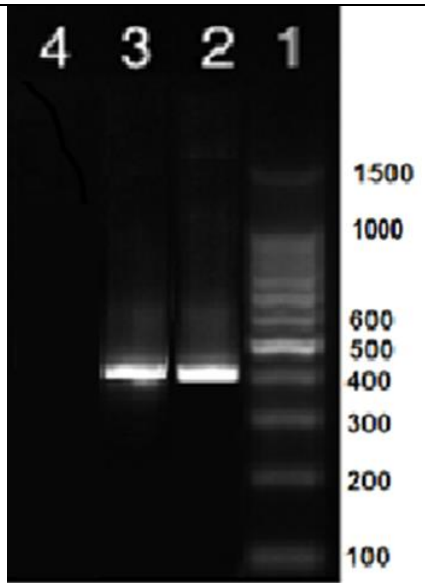

Fig (4).Shows PCR. Lane 400 bp using a ladder of 100 bp 1-ladder,2-positive control,3-sample

Table (1). Partial nucleotides sequence analysis 400 bp product of S1 gene of (IBV-EG/ SHARKIA –F629-2015).

AACGTATGAGTAGTTTTGTTTATAAACCTTCTGATTTTATGTATGGGTC
TTACCACCCGCAGTGTGATTTTAGACCAGAAACTATTAATAATGGTTT
GTGGTTTAATTCTCTATCTGTTTCACTAGCCTATGGGCCTCTACAAGGT
GGTTGTAAGCAGTCTGTCTTTAGCAATAGGGCAACGTGTTGTTATGCT
TATTCATACAATGGTCCTCATTTGTGTAAAGGTGTTTATACTGGTGAA
TTACAACAATATTTTGAATGTGGATTGCTGGTTTATGTAACTAAGAGT
GGTGGCTCTCGTATACAAACCAGGAATGAACCACTTGTGTTAACTCAT
CACAATTATAATAATATTACTTTGGATAGGTGTGTAGAGCATAATATA
TATGGCAGGGCCCGGGGGGGGGGGTGGGCCGGGTGAGGAAATTTTTT
TTTGAAAAACCCCCCCCCCCCCG

Fig (5).Nucleotides identities of (IBV-EG/ SHARKIA –F629-2015) with commonly used vaccine strains sequences. Dots indicate residues identical to (IBV-EG/ SHARKIA –F629-2015) Bold letters denotes codon areas. Shaded letters denote sites of differences.

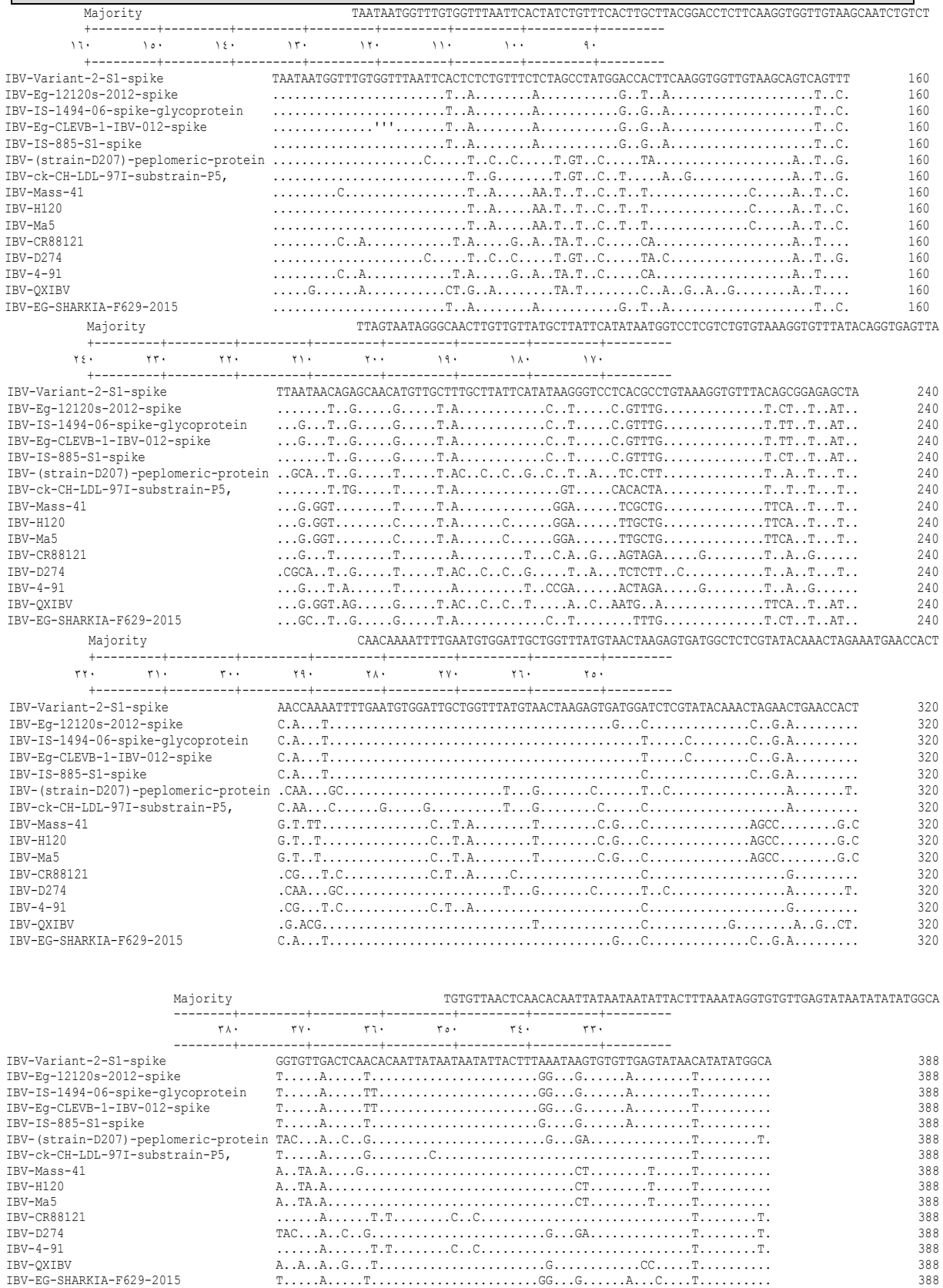

Fig (6). Amino acid identities of (IBV-EG/ SHARKIA –F629-2015) with commonly used vaccine strains sequences. Dots indicate residues identical to (IBV-EG/ SHARKIA –F629-2015). Potential glycosylation sites (NXS or NXT, except where X = P) are underlined. Shaded letters denote sites of differences. A:Alanine, C:Cysteine, D:Aspartic acid, E:Glutamic acid F:Pheny-lalanine,G:Glycine, H:Histidine, I:Isoleucine,K:Lysine, L:Leucine, M:Methionine, N:Asparagine, P:Proline, Q:Glutamine, R:Arginine, S:Serine, T:Threonine, V:Valine, W:Tryptophan,Y:tyrosine.

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Majority                          LSSFVYKASDFMYGSYHPSCDFRPETINNGLWFNSLSVSLAYGPLQGGCKQSVFSNRATCCYAYSYNGPSLCKGVYSGEL
                                  ---------+---------+---------+---------+---------+---------+---------+---------+
                                           10        20        30        40        50        60        70        80
                                  ---------+---------+---------+---------+---------+---------+---------+---------+
IBV-Variant-2-S1-spike            LSSFVYKQSDFMYGSYHPKCNFRPETINNGLWFNSLSVSLAYGPLQGGCKQSVFNNRATCCFAYSYKGPHACKGVYSGEL  238
IBV-Eg-12120s-2012-spike          .......P.........Q.D.....................................Y....N..RL.....T...  238
IBV-IS-1494-06-spike-glycoprotein .....................D.............................S......Y....N..RL.....I...  238
IBV-Eg-CLEVB-1-IBV-012-spike      .....................D.......... .....................S......Y....N..RL.....I...  238
IBV-IS-885-S1-spike               ..G....A............D.....................................Y....N..RL.....T...  238
IBV-(strain-D207)-peplomeric-protein ....I..A..Y.......S.K.KL..............G...I.........A......Y....N..SL.....R...  238
IBV-ck-CH-LDL-97I-substrain-P5,   ....R.VE............S..L..............G..............M....Y....S..TL.........  238
IBV-Mass-41                       .......E.N........S....L..............I..............SG.....Y....G..SL.........  238
IBV-H120                          .......E.N........S....L..............I..............SG.....Y....G..LL.........  238
IBV-Ma5                           .......E.N........S....L..............I..............SG.....Y....G..LL.........  238
IBV-CR88121                       .......P.........H.K....N.............T...I.........S......Y....Q..SR.....R...  238
IBV-D274                          ....I..A..Y.......S.K..L..............G...I.........A......Y....N..SL.....R...  238
IBV-4-91                          .......P.........N......N.............T...I.........S.K....Y....R..TR.....R...  238
IBV-QXIBV                         ..Q....A.........S.S.......S...........T.............SGK....Y.......M.........  238
IBV-EG-SHARKIA-F629-2015          M......P.........Q.D.....................................S......Y....N...L.....T...  238
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Majority                          QQYFECGLLVYVTKSDGSRIQTRNEPLVLTQHNYNNITLNRCVEYNIYGT
                                  ---------+---------+---------+---------+---------+
                                           90        100       110       120       130
                                  ---------+---------+---------+---------+---------+
IBV-Variant-2-S1-spike            NQNFECGLLVYVTKSDGSRIQTRTEPLVLTQHNYNNITLNKCVEYNIYGT                    388
IBV-Eg-12120s-2012-spike          Q.Y............G.......N......H........DR........I                    388
IBV-IS-1494-06-spike-glycoprotein Q.Y....................N......HY.......DR.........                    388
IBV-Eg-CLEVB-1-IBV-012-spike      Q.Y....................N......HY.......DR.........                    388
IBV-IS-885-S1-spike               Q.Y....................N......H.........R.........                    388
IBV-(strain-D207)-peplomeric-protein TKS.......F...T........N..FT...........DR.........                    388
IBV-ck-CH-LDL-97I-substrain-P5,   QKT.......F............N.....................                    388
IBV-Mass-41                       DL.............G......A...P.I.R.........T..D......                    388
IBV-H120                          DH.............G......A...P.I...........T..D......                    388
IBV-Ma5                           DH.............G......A...P.I...........T..D......                    388
IBV-CR88121                       T.Y....................S.......Y.................                    388
IBV-D274                          TKS.......F...T........N..FT...........DR.........                    388
IBV-4-91                          T.Y....................S.......Y.................                    388
IBV-QXIBV                         ST.............................Y.......D...A......                    388
IBV-EG-SHARKIA-F629-2015          Q.Y............G.......N......H........DR...H....N                    388
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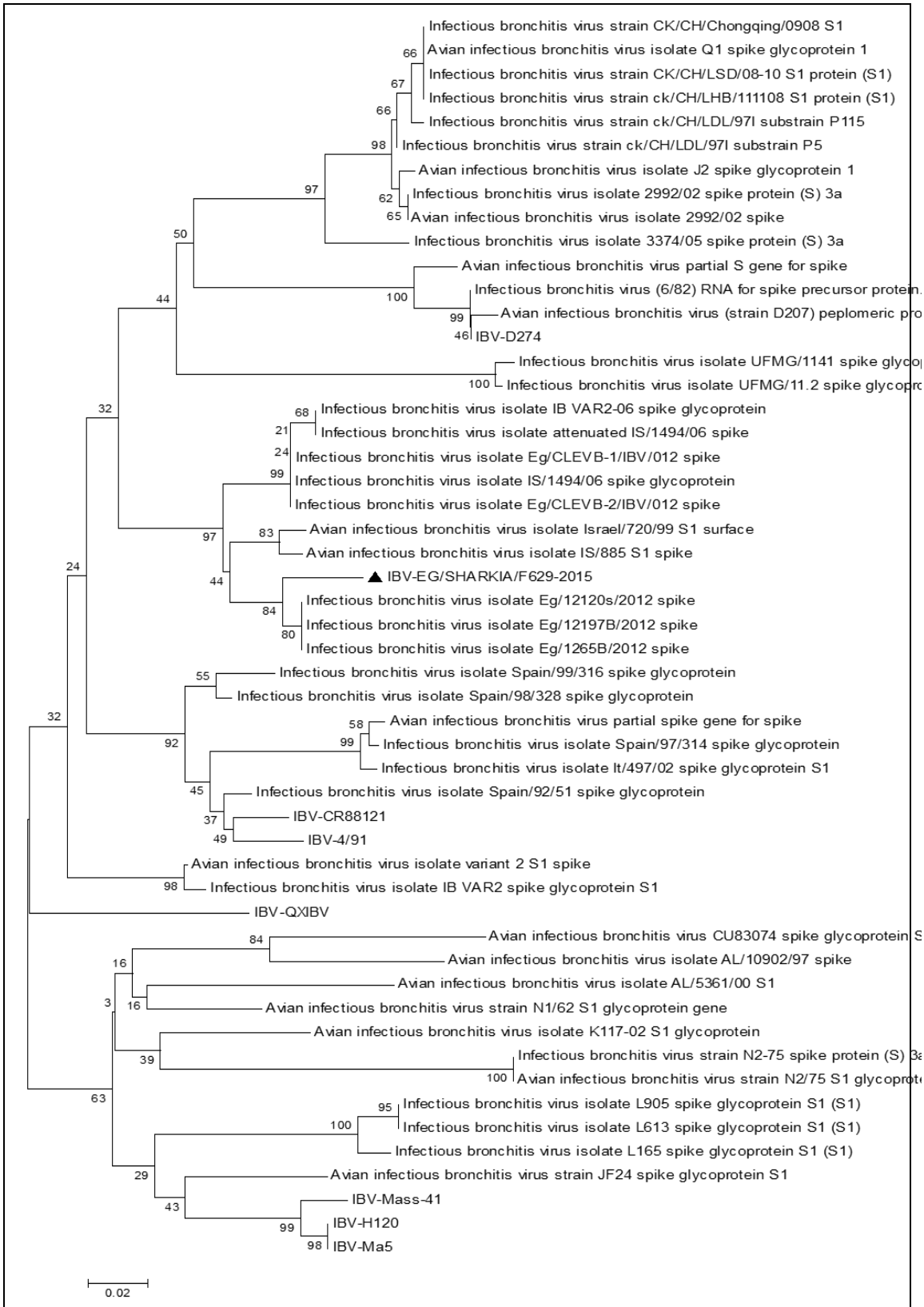
Table (2). Nucleotide (upper right) and amino acid(lower left) of (IBV-EG/ SHARKIA –F629-2015) with selected IBV Vaccines sequences

	Percent of identity																	
Divergence			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15		
		1		88.4	89.1	87.6	89.9	80.6	87.6	84.5	85.3	85.3	87.6	81.4	87.6	87.6	86.8	1	IBV-variant-2-S1-Spike
		2	12.7		95.3	93.8	96.1	83.7	86.0	82.9	82.9	82.9	86.8	84.5	86.0	82.9	96.9	2	IBV-Eg-12120s-2012-Spike
		3	11.8	4.8		98.4	95.3	83.7	86.0	82.2	82.2	82.2	88.4	84.5	87.6	85.3	93.8	3	IBV-IS-1494-06-Spikeglycoprotein
		4	13.6	6.5	1.6		93.8	82.2	84.5	80.6	80.6	80.6	86.8	82.9	86.0	83.7	92.2	4	IBV-Eg-CLEVB-1-IBV-012-Spike
		5	10.8	4.0	4.8	6.5		83.7	87.6	82.2	82.2	82.2	86.8	84.5	86.0	83.7	93.0	5	IBV-IS-885-S1-Spike
		6	22.5	18.4	18.4	20.4	18.4		84.5	78.3	78.3	78.3	83.7	99.2	81.4	79.1	82.2	6	IBV-(D207)-Peplomeric-Protein
		7	13.6	15.5	15.5	17.4	13.8	17.4		81.4	82.2	82.2	83.7	85.3	84.5	82.9	83.7	7	IBV-CK-LDL-971-Substrain-P5
		8	17.4	19.4	20.4	22.5	20.4	25.7	21.4		97.7	97.7	80.6	79.1	79.8	82.2	82.2	8	IBV-Mass-41
		9	16.4	19.4	20.4	22.5	20.4	25.7	20.4	2.4		100.0	80.6	79.1	80.6	82.9	82.2	9	IBV-H120
		10	16.4	19.4	20.4	22.5	20.4	25.7	20.4	2.4	0.0		80.6	79.1	80.6	82.9	82.2	10	IBV-Ma5
		11	13.6	14.5	12.7	14.5	14.5	18.4	18.4	22.5	22.5	22.5		84.5	96.1	85.3	86.0	11	IBV-CR88121
		12	21.4	17.4	12.4	19.4	17.4	0.8	16.4	24.6	24.6	24.6	17.4		82.2	79.8	82.9	12	IBV-D274
		13	13.6	15.5	13.6	15.5	15.5	21.4	17.4	23.5	22.5	22.5	4.0	20.4		86.0	85.3	13	IBV-4-91
		14	13.6	19.4	16.4	18.4	18.4	24.6	19.4	20.4	19.4	19.4	16.4	23.5	15.5		82.2	14	IBV-QXIBV
		15	14.5	3.2	6.5	8.2	7.3	20.4	18.4	20.4	20.4	20.4	15.5	19.4	16.4	20.4		15	IBV-EG-SHARKIA-F629-2015
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15		



Fig(7). IBV S1 gene sequence relationships expressed as a phylogenetic tree of (IBV-EG/ SHARKIA –F629-2015) isolate and selected IBV reference strains

Table (3).Concentrations of Total lipids, total cholesterol, triacylglycerol, Phospholipids and NEFA mg/gm, MDA nmolE/gm in egg yolk in IBV infected birds (N=5)

Parameters examined	Control	Infected
Total Lipid(mg/gm yolk)	**646.94 *± 50.22**	**502.66 ± 20.25**
CholesteroL(mg/gm yolk)	**161.62*** ± 10.30**	**79.31 ±3.07**
Triglecerol(mg/gm yolk)	**438.30** ± 28.51**	**298.18 ± 26.04**
Phospholipids(mg/gm yolk)	**9.24** ± 0.18**	**8.03 ± 0.24**
NEFA(mg/gm yolk)	**0.092** ± 0.003**	**0.066 ± 0.007**
MDA(nmolE/gm yolk **)**	**14.33** ± 0.67**	**19.14 ± 0.98**

Table (4). Concentrations albumin, yolk and whole egg total protein mg/gm, beside PH value at 24 ºC in IBV infected birds (N=5).

Parameters examined	Control	Infected
Albumin protein(mg/gm)	**12.20** ± 0.33**	**9.82 ± 0.49**
Yolk protein(mg/gm)	**14.98** ± 0.65**	**11.96 ± 0.62**
Whole protein(mg/gm)	**13.24** ± 0.31**	**11.98 ± 0.26**
PH at 24 ºC	**8.56* ± 0.24**	**9.48 ± 0.19**

Table (5). Concentrations of calcium, phosphorus, magnesium, sodium, potassium and chloride mg/gm, manganese ng/gm yolk in IBV infected birds (N=5).

Parameters examined	Control	Infected
Calcium(mg/gm yolk)	**1.35*** ± 0.054**	**0.90 ± 0.047**
Phosphorus(mg/gm yolk)	**5.95*** ± 0.27**	**4.43 ± 0.12**
Magnesium(mg/gm yolk)	**0.94** ± 0.17**	**0.44 ± 0.21**
Manganese(ng/gm yolk)	**1.60** ± 0.11**	**1.14 ± 0.02**
Sodium(mg/gm yolk)	**1.78 ***± 0.017**	**1.98 ± 0.026**
Potassium(mg/gm yolk)	**1.25*** ± 0.011**	**1.17 ± 0.007**
Chloride(mg/gm yolk)	**1.61** ± 0.064**	**1.39 ± 0.016**

* Represents statistical significant at P< 0.05 level.
** Represents statistical significant at P< 0.01 level.
*** Represents statistical significant at P< 0.001 level

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