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RESEARCH

A genotyping of a new avian infectious bronchitis virus isolated from chickens proventriculus in Egypt

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ABSTRACT

Background: A novel avian infectious bronchitis virus (IBV), Egypt/Qal/014p was isolated from 14-day-old broiler chickens with proventriculitis in Qaluobia province of Egypt in 2014.
Objective: The isolate was identified by a real time RT-PCR targeting the nucleocapsid (N) gene with further genetic and antigenic characterization.
Methods: Beside a real time RT-PCR; sequence analysis of partial S1 gene, cross-neutralization and the challenge experiment for H120 vaccinated broiler chickens were also performed.
Results: Egypt/Qal/014p had close relation with that of the Massachusetts prototypes. Comparisons nucleotide and amino acid sequence of partial S1 gene showed that the recent isolate had 98.2% and 95.2% nucleotide similarities and 96.8% and 91.4% amino acid similarities with the commonly used IBV vaccine Massachusetts strains H120 and M41 respectively. Egypt/Qal/014p had a unique amino acid substitution at residues 39 (Serine), 40 (Tyrosine), 41 (Lysine), 64 (Glutamate) and 69 (Valine) of the S1 proteins from H120 and M41.The cross-neutralization test revealed antigenic relatedness of Egypt/Qal/014p with Massachusetts serotypes. The commercial H120 vaccine conferred partial protection against proventriculitis induced by Egypt/Qal/014p.
Conclusion: IBV strains exhibiting proventriculitis were found co-circulating in broiler chickens in Egypt and optimum control of IBV in Egypt require preparation of vaccine from indigenous isolates beside periodic evaluation of cross-protective capabilities of such vaccine.

Keywords: Antigenic relatedness; Egypt/Qal/014p; Partial S1 gene analysis; Protection test

BACKGROUND

Avian infectious bronchitis virus (IBV), is a member of genus Gammacoronavirus, family Coronaviridae, order Nidovirales (de Groot et al., 2011). Many different IBV strains have been reported in chickens worldwide (Jackwood, 2012), with pathology ranging from mild respiratory symptoms to severe kidney and oviduct disease, resulting in poor weight gain in broilers, and serious egg yield drop and poor egg quality in layers and breeders (Cavanagh, 2007). Thus, IBV has a significant economic impact on the modern poultry industry. Natural outbreaks of IBV are often the result of infections with strains that differ serologically from the vaccine strains (Cavanagh and Gelb, 2008).

The IBV genome consists of a single positive-stranded RNA of approximately 27 kb in length that encodes four main structural proteins: the phosphorylated nucleocapsid (N) protein, the membrane (M) glycoprotein, small envelope (E) protein and the spike (S) glycoprotein. The S protein is post-translationally cleaved into two subunits, S1 and S2 (Masters, 2006). It has been known that the S1 subunit is involved in infectivity and hemagglutinin activity and contains virus-neutralizing epitopes and serotype-specific sequences (Casais et al., 2003; Sjaak de Wit et al., 2011). The different serotypes, subtypes, or variants of IBV were thought to be generated by nucleotide point mutations, insertions, deletions

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(Cavanagh and Gelb, 2008; Sjaak de Wit et al., 2011), or RNA recombinations of S1 genes (Cavanagh, 2007; Liu et al., 2013),which were responsible for outbreaks of infectious bronchitis (IB) in vaccinated and non vaccinated chicken flocks. In addition to serotype changes, genetic variation may result in changes in tissue tropism and pathogenicity of the virus that lead to the generation of new IBV pathotypes. (Casais et al., 2003; Fang et al., 2005; Ammayappan et al., 2008; Cavanagh et al., 2005) Thus, the molecular identification of IBV has been focused on analysis of the S1 protein gene.

A number of IBV variant genotypes have been reported in Egypt, including isolates related to Massachusetts, D3128, D274, D-08880, 4/91, Israel/720/99, Israel/885/00, IS/1494/06 and the novel genotype Egypt/Beni-Seuf/01, Egypt/F/03, Egypt/D/89 (Sultan et al., 2004; Abdel-Moneim et al., 2006; Selim et al., 2013). Some of these genotypes in particular IS/885/00 and IS/1494/06, have become dominant in the majority of farms in Egypt and the Middle East countries, causing respiratory and renal diseases (Meir et al., 2004; El-Mahdy et al., 2012; Selim et al., 2013). The commonly used IBV attenuated vaccine is H120 while the M41 strain is commonly used in inactivated vaccines.

During March 2014, an outbreak of a disease associated with proventriculus had been occurred in 14-day-old commercial broiler farm with previous IBV vaccination in Qaluobia province of Egypt. In this study, the Egypt/Qal/014p was isolated from the proventricular tissue of affected broiler chickens, the isolate was characterized genotypically, serotypically and the challenge experiment for H120 vaccinated broiler chickens were also performed.

MATERIALS AND METHODS

Samples

During March 2014, suspicious IBV infections were found in 14-day-old commercial broiler farm with previous IBV vaccination in Qaluobia province, Egypt. The flock was vaccinated against IB and Newcastle disease viruses at one day of age using H120 and B1 vaccines respectively. The sick birds presented with respiratory symptoms and the pathological changes in proventriculus (enlarged, filled with fluid, and its mucosa was thickened and exuded a milky fluid when squeezed at postmortem) without renal lesion. Tissue samples of swollen proventriculus were collected and frozen at -70 °C for further analysis.

Embryonated chicken eggs and chickens

Specific pathogen free embryonated chicken eggs (SPF ECEs) and commercial one day old chickens were obtained from Nile SPF and El-Waddi Co, Egypt respectively. Embryonated eggs were used for primary isolation of field strain, production and titration of seed stocks of Egypt/Qal/014p and IBV vaccine strain (H120), cross neutralization and re-isolation attempts of IBV following challenge experiment. SPF chickens and commercial chickens were reared under strict hygienic conditions in separate rooms and used in production of antisera and challenge experiment respectively.

The study was approved by the Ethics Committee of animal research unit, Faculty of veterinary medicine, Benha University, Egypt.

Virus isolation on SPF ECEs

Samples were prepared as 10% tissue suspensions in phosphate-buffered saline (PBS), clarified by centrifugation at 1500 g at 4 °C for 10 min, and filtered through 0.22 µm membrane filters (Millipore) before inoculation into the allantoic cavities of 10 day-old embryonated SPF eggs. Three passages were performed and characteristic embryo changes, such as dwarfing,

stunting, or curling of the embryos, were observed between post-inoculation days 2 and 7. The virus isolate was designated as Egypt/Qal/014p. The viral stocks of the Egypt/Qal/014p was produced by inoculating the virus into embryonated SPF eggs via the allantoic cavity and collecting the infectious allantoic fluid 72 h post-inoculation and stored at -70 °C as previously described (OIE, 2008). Real time RT-PCR didn't detect Newcastle disease and infectious bursal disease viruses in the allantoic fluid (Data not showed).

Viral RNA Extraction

Viral RNA was extracted from allantoic fluid and proventriculus suspension taken from naturally infected and challenged chickens. Extraction of RNA was performed using a QiaAmp viral RNA mini kit (Qiagen) following the manufacturer's instructions.

Detection of IBV by Real time RT-PCR

Detection of Egypt/Qal/014p in sample suspension and allantoic fluid was conducted using real-time RT-PCR. The real time RT-PCR was done using Quantitect probe RT-PCR kit (Qiagen) as previously described (Meir et al., 2010). Briefly, a conserved region of 336 b located at nucleotide position 741–1077 of the H120 strain N gene sequence (GenBank accession no. AM260960) was used to design primers and probe for the real time RT-PCR assay.

A downstream primer AIBV-fr (5-ATGCTCAACCTTGTCCCTAGCA-3) located at nucleotide position 811–832; an upstream primer AIBV-as, located at nucleotide position 921–941 (5-TCAAACTGCGGATCATCACGT-3), and a TaqMan probe AIBV-TM (FAM-TTGGAAGTAGAGTGACGCCCAAACTTCA- BHQ1) located at nucleotide position 848–875 were synthesized to amplify a 130-bp fragment. Both primers and probe were manufactured by Metabion. The 25 µL real-time RT-PCR reactions contained 12.5 µL 2x QuantiTect probe RT-PCR Master Mix, 0.125 µL RT-PCR enzyme mix, primers to a final concentration of 400 nM, probe to a final concentration of 120 nM, 6 µL RNA template and nuclease free water. The reaction was carried out in Stratagene MX3005P real-time PCR system (Applied Biosystems) at 45 °C for 10 min, 95 °C for 10 min, and 40 cycles of 95 °C for 15 s, and 60 °C for 45 s. Amplification plots were recorded, analyzed, and the cycle threshold (Ct) determined with the Stratagene software (Applied Biosystems). Specificity of the assay was tested with RNA from H120 IBV vaccine strain and negative controls were obtained from allantoic fluid of non inoculated SPF egg.

RT-PCR and cycle sequencing of S1 gene

Partial amplification of the S1 gene hyper-variable region (HVR) was performed on viral RNA using one step RT-PCR Kit (Qiagen). Sequences of the primers used for amplification were as follows: forward primers IBV-S1-F 5\- ACTACTACCAAAGTGCCT -3\ and reverse primer IBV-S1-R 5\ - ACATCTTGTGCAGTACCATTAACA -3\ (Bochkov et al., 2007). The amplicons (572 base pairs, bp) were purified using the QIAquick gel extraction kit (Qiagen) and the DNA was quantitated as described (Kingham et al., 2000). The sequence reactions were performed using genetic analyzer Applied Biosystems 3130 (ABI, USA) by Big Dye Terminator V3.1 Cycle Sequencing kit (Perkin, Elmer, Foster city, CA) using forward and reverse primers as previously mentioned.

Nucleotide sequence accession number

Partial S1 gene sequence of IBV- Egypt/Qal/014p isolate was submitted to GenBank and assigned accession number KM065535.

Phylogenetic analysis and nucleotide comparison

Multiple sequence alignments were carried out with Clustal W (Thompson et al 1994), and phylogenic tree was constructed with MEGA 5 software (Tamura et al 2011), using the Neighbor-joining tree method with 1000 bootstrap replicates to assign confidence levels to branches. The IBV sequences were aligned and compared with reference and vaccine strains that were found or used in the Middle East. The sequences were retrieved from GenBank (National Centre of Biotechnology Information) (table 1) and BLAST search was carried out.

Table 1: IBV strains included in the phylogenetic analysis of the S1 gene in this study

Strain	serotype	Geographic origin	GenBank accession no.[a]
H120	Massachusetts	Holland	M21970
M41	Massachusetts	USA	M21883
Beaudette.US	Beaudette	USA	AJ311362
GX1-98.China	unknown	China	AY319302
Connecticut	Connecticut	USA	L18990
D274	D274	Netherlands	X15832
Israel/720/99	unknown	Israel	AY091552
IS/188/96/Var.1	unknown	Israel	AY789949
IS/385/97	unknown	Israel	AY789957
IS/585/98/Var.2	unknown	Israel	AY789962
Q1	unknown	China	AF286302
T3	unknown	China	AF227438
J2	unknown	China	AF286303
It/497/02_spike	unknown	Italy	DQ901377
Egypt/F/03	unknown	Egypt	DQ487085
Egypt/Beni-Suef/01	unknown	Egypt	JX174183
Egypt/VRLCU-04/2012	unknown	Egypt	KC292039
Egypt/VRLCU03/2012	unknown	Egypt	KC292038
Egypt/VRLCU-02/2012	unknown	Egypt	KC292037
Egypt/Gharbia/VRLCU-07/2012	unknown	Egypt	KC292034
Egypt/Menofia/VRLCU-06/2012	unknown	Egypt	KC292033
Egypt/VRLCU154/2012	unknown	Egypt	JX893950
Egypt/01-13/VIR9715/2012	unknown	Egypt	KC527831
IBV/CK/Cairo-Egypt/SCU-4/2013	unknown	Egypt	KF731612
IBV/CK/Beh-Egypt/SCU-6/2013	unknown	Egypt	KF158999
IBV/CK/Giza-Egypt/SCU-3/2013	unknown	Egypt	KC991031
IBV/ck/Egypt/12vir6109-78/2012	unknown	Egypt	KC197211
Egypt/Zag/07-02	unknown	Egypt	EU368593
Egypt/Zag/07-03	unknown	Egypt	EU368594
Egypt/Zag/07-01	unknown	Egypt	EU368592
Egypt/D/89	unknown	Egypt	DQ487086
CU-2	unknown	Egypt	KC985213
CU-4	unknown	Egypt	KC985212
CU-1	unknown	Egypt	KC985211
EGY/Qalyobia/121	unknown	Egypt	KC608181
Ck/Eg/BSU-5/2011	unknown	Egypt	JX174188
Ck/Eg/BSU-3/2011	unknown	Egypt	JX174186
Egypt/Beni-Seuf/01	unknown	Egypt	AF395531
Mans-1	unknown	Egypt	KF856872
Mans-2	unknown	Egypt	KF856873
Mans-3	unknown	Egypt	KF856874
Eg/CLEVB-1/IBV/012	unknown	Egypt	JX173489
Eg/10674F/2010	unknown	Egypt	KC533681
Eg/12120s/2012	unknown	Egypt	KC533684
EGY/12773F-3	unknown	Egypt	KC608180
IBV-EG/12249F	unknown	Egypt	KC776192

[a] Based on S1 gene sequence

Production of antisera for virus neutralization assay

Monospecific antisera to the field isolate Egypt/Qal/014p was raised following a specific immunization protocol previously described (Gelb and Jackwood, 1998). Briefly, 4-week-old SPF chickens were intratracheally immunized with about 105 EID50 per bird. Two and 5 weeks later chickens were reinoculated with the same dosage by the intravenous route. Blood samples were collected from chickens 2 weeks after the last inoculation. Sera were harvested and frozen before being used in the virus neutralization procedure. Antisera to vaccine strains H120 and M41 were prepared by similar protocol.

Chicken embryo cross-neutralization assays

Reciprocal virus neutralization assay (VN) was performed on Egypt/Qal/014p and vaccine strains (H120 and M41) and their respective sera. The test was modified from a previously described procedure (Cowen and Hitchner, 1975). Ten-fold dilutions of serum were reacted with 100 EID50 of IBV at room temperature for 1 h. The end points of the homologous and heterologous neutralization titers were determined as the dilution, which showed no signs of dwarfing (complete neutralization) after incubation of inoculated eggs for 7 days. The antigenic relatedness values (r) were calculated by using homologous and heterologous titers (Archetti and Horsfall, 1950).

Protection from proventriculitis using H120 vaccine

Forty five commercial 1-day-old chickens were used to evaluate the protection provided by H120 vaccination against challenge with Egypt/Qal/014p. Birds were divided into three groups; GI, GII and GIII each of fifteen chickens. Vaccination was performed at day 1 by eye drop application. Single dose of H120 vaccine was used for each bird in GI and GII according to manufacturer's instructions while birds in the GIII were kept as unvaccinated control. Four weeks post vaccination, chickens in GI and GIII were challenged by eye drop with Egypt/Qal/014 (105 EID50 per bird) while birds in GII were not challenged and kept as control. Four days post-challenge the birds were killed humanely and sampled for the proventriculus. The samples were processed as described previously for virus re-isolation and real time RT-PCR detection of the N gene.

RESULTS

Virus isolation on SPF ECEs

IBV (Egypt/Qal/014p) was isolated from the sampled proventriculus through several blind passages. The isolated virus was shown to induce pathological changes in embryos including dwarfing and death of embryos between 48 and 144 h post-inoculation. Embryo dwarfing increased with the number of serial passages, so that 100% of the embryos were stunted by the fourth passage.

IBV detection and identification by Real time RT-PCR

Egypt/Qal/014p was detected by real-time RT-PCR targeting the N gene before and after virus isolation and challenge experiments.

Sequence analysis of Partial S1 genes

Isolate Egypt/Qal/014p belongs to Mass-type IBV. The partial S1 nucleotide sequences of Egypt/Qal/014p isolate was aligned with the published S1 glycoprotein sequences representing Massachusetts, Beaudette, Connecticut, Israelian, Chinese and Egyptian strains. The dendrogram was generated to determine the phylogenetic position of the isolate among IBV strains (Fig. 1). The Egypt/Qal/014p isolate formed a phylogenetic group with IBV strains H120,

GX1-98, IS/385/97, Beaudette US and M41, CU-1 and Egypt/Zag/07-02. Also partial S1 gene nucleotide sequences showed that Egypt/Qal/014p shared identities 98.2% with Mass-type H120, 96.2% with GX1-98, 95.9% with IS/385/97 strains and 95.2% with Mass-type M41 and Beaudette US strains. Percent identities of the deduced amino acid of partial S1protein revealed that Egypt/Qal/014p had 96.8%, 94.1%, 93.0%, 92.4%, 91.4% similarities with IBV strains H120, GX1-98, IS/385/97, Beaudette US and M41 respectively (table. 2) indicating that the Egypt/Qal/014p isolate was clearly of the Mass genotype.

Fig. 1: Phylogenetic tree for partial S1 nucleotide sequence of Egypt/Qal/014p. The tree was constructed by use of MEGA 5 software and the clustal alignment algorithm. The length of the branch represents the distance between sequence pairs. The reference sequence of IBV obtained from GenBank database.

Table 2: Nucleotide and amino acid identities of Egypt/Qal/014p with selected IBV sequences from GenBank based on partial S1 gene analysis.

Nucleotide sequence percent identity

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15		
1		96.6	71.3	71.3	71.3	96.6	90.5	97.7	71.3	74.3	97.7	77.2	76.8	72.7	98.2	1	**H120**
2	93.0		71.8	71.8	71.8	97.1	91.0	98.0	71.3	74.0	97.3	77.2	76.7	72.7	95.2	2	**M41**
3	68.1	68.1		99.8	99.8	71.6	69.8	72.0	73.8	74.0	71.8	78.5	76.8	77.0	70.6	3	**Q1**
4	68.1	68.1	99.5		100.0	71.6	69.7	72.0	74.0	74.0	71.8	78.3	77.0	77.2	70.6	4	**T3**
5	68.1	68.1	99.5	100.0		71.6	69.7	72.0	74.0	74.0	71.8	78.3	77.0	77.2	70.6	5	**J2**
6	94.6	93.0	68.1	68.1	68.1		90.5	97.8	71.5	74.3	97.5	77.4	77.2	73.1	95.2	6	**Beaudette_US**
7	83.8	82.2	67.6	67.6	67.6	82.2		91.4	69.1	71.5	90.7	75.2	74.9	71.1	89.4	7	**connecticut**
8	95.7	94.6	67.7	69.2	69.2	95.1	84.3		71.6	75.2	98.6	77.7	77.4	73.1	96.2	8	**GX1-98**
9	67.6	66.5	69.2	69.2	69.2	67.0	64.3	67.6		72.0	72.4	76.3	73.2	73.4	70.6	9	**Israel/720/99**
10	67.6	66.5	71.9	71.9	71.9	68.6	65.9	69.7	67.6		75.2	78.1	77.2	76.1	73.8	10	**IS/188/96_var1**
11	95.7	93.5	68.1	68.1	68.1	95.7	83.2	97.3	69.2	69.7		77.9	77.2	73.1	95.9	11	**IS/385/97**
12	70.3	70.3	76.8	77.3	77.3	71.4	68.1	71.9	74.1	74.6	72.4		85.6	79.5	76.7	12	**IS/585/98_var2**
13	72.4	70.8	76.2	76.8	76.8	72.4	71.9	73.0	70.8	75.1	72.4	85.9		81.1	75.8	13	**D274**
14	68.1	68.6	76.8	77.3	77.3	68.6	68.1	69.7	71.4	71.4	69.2	78.4	80.0		71.6	14	**It/497/02_spike**
15	96.8	91.4	67.0	67.0	67.0	92.4	82.7	94.1	65.9	66.5	93.0	68.6	70.8	67.0		15	**Egypt/Qal/014p**
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15		

Amino acid percent identity

The genetic similarities of Egypt/Qal/014p with Mass-type H120 and M41strains

Compared with the Mass-type H120 and M41 published sequences, the partial S1 sequences of the isolates Egypt/Qal/014p showed 10 point mutations from H120; 2 silent and 8 non silent mutations. On the other hand, it showed 27 point mutations; 7 silent and 20 non silent mutations from M41 (table. 3, 4). Egypt/Qal/014p contain 17 amino acid substitutions, including 38 aspartate, 39 serine, 40 tyrosine, 41 lysine, 63 serine, 64 glutamate, 66 threonine, 69 valine, 101 tyrosine, 117 histadine, 118 valine, 128 glutamine, 129 histadine, 130 serine, 131 isoleucine, 213 glutamate and 215 lysine. Among these substitutions, eleven were shared by H120 and six amino acid substitutions including 39 serine, 40 tyrosine, 41 lysine, 64 glutamate, 69 valine and 215 lysine were unique to the isolate.

Table 3: Pairwise comparison of partial nucleotide sequences of the S1 gene between H120, M41 and isolate Egypt/Qal/014p

strain	**Position of nucleotide in S1 gene**													
	112	114	115	119	120	121	123	124	129	138	147	162	187	191
H120	G	T	G	G	G	C	T	T	T	G	T	T	T	G
M41	A	T	G	G	G	C	T	T	C	T	A	C	C	G
Egypt/Qal/014p	G	**C**	**A**	**A**	**T**	**A**	**A**	**C**	T	G	T	T	T	**A**
	Position of nucleotide in S1 gene													
	197	205	206	301	345	349	353	382	385	389	391	426	607	614
H120	C	A	T	T	C	C	T	C	C	C	A	T	G	G
M41	T	A	C	C	T	T	A	A	A	T	T	C	A	A
Egypt/Qal/014p	C	**G**	T	T	C	C	T	C	C	C	A	T	G	A

The unique nucleotides substitution in strain Egypt/Qal/014p are highlighted in bold

Table 4: Pairwise comparison of deduced amino acid sequences of the S1 gene between H120, M41 and isolate Egypt/Qal/014p

strain	Position of amino acid in S1 protein								
	38	**39**	**40**	**41**	**63**	**64**	**66**	**69**	**101**
H120	D	G	W	H	S	G	T	**I**	Y
M41	N	G	W	H	P	G	I	T	H
Egypt/Qal/014p	D	**S**	**Y**	**K**	S	**E**	T	**V**	Y
	Position of amino acid in S1 protein								
	117	**118**	**128**	**129**	**130**	**131**	**213**	**215**	
H120	H	V	Q	H	S	I	E	R	
M41	Y	D	K	N	F	L	K	K	
Egypt/Qal/014p	H	V	Q	H	S	I	E	**K**	

The unique amino acid for Egypt/Qal/014p are highlighted in bold, aspartate (D), asparagines (N), glycine (G), serine (S), tryptophane (W), tyrosine(Y), histadine (H), lysine (K), proline (P), glutamate (E), threonine (T), isoleucine (I), valine (V),glutamine(Q), phenylalanine (F), leucine (L),and arginine (R).

Chicken embryo cross-neutralization assays

The percentage of antigenic relatedness values (r) was determined by VN for the field isolate Egypt/Qal/014p and vaccine strains H120 and M41. The Egypt/Qal/014p shared 80% with H120 and 50% with M41. This confirmed that relatedness of our isolate to Massachusetts's serogroup.

H120 protects chickens from proventriculitis induced by Egypt/Qal/014p

Challenged chickens with Egypt/Qal/014p four weeks post vaccination with IBV H120 strain were monitored by virus re-isolation and real time RT-PCR (Table 5). In group GI, H120 protects chickens against Egypt/Qal/014p induced proventriculitis by 73.3% (11/15) and 60% (9/15) as monitored with virus re-isolation and real time RT-PCR respectively. Egypt/Qal/014p was not detected in the proventriculi of group GII either with virus re-isolation or real time RT-PCR as it was vaccinated unchallenged group. Chickens in group GIII were not protected against Egypt/Qal/014 and the virus detected in 100% (15/15) of challenged chickens based on virus re-isolation or real time RT-PCR as it was unvaccinated challenged group.

Table 5: Challenged chickens with Egypt/Qal/014p following vaccination with IBV H120 strain monitored by virus re-isolation and Real time RT-PCR from proventriculus

Groups	Number of chicken	vaccination	challenge	Virus re-isolation		Real time RT-PCR	
				Positive	negative	Positive	negative
GI	15	+	+	4	11	6	9
GII	15	+	-	0	15	0	15
GIII	15	-	+	15	0	15	0

Only group GI and GII were vaccinated with IBV H120 vaccine at 1-day of age. Group GI and GIII were challenged by eye drop with $10^5 EID_{50}$ per chicken of Egypt/Qal/014p

DISCUSSION

The Egyptian IBV isolate, Egypt/Qal/014p recovered from the proventriculi of 14-day-old broiler chicks that had a history of respiratory and proventricular manifestation, were characterized. The isolate was shown to cause dwarfing in infected embryo and were subsequently specified as IBV by real time RT-PCR targeting the highly conserved N gene (Meir et al., 2010). Severe proventriculitis associated with respiratory and enteric signs, whereas no kidney lesions were reported either in the field or in challenged birds was in agreement with (Yu et al., 2001; Pantin-Jackwood et al., 2005) who confirmed the presence of IBV viral antigens in such organ.

Egypt/Qal/014p isolate was genotyped based on partial amplification of S1 gene hyper-variable region and sequence analysis as indicated by Wang and Huang, 2000; Jones et al., 2005. Phylogenetic analysis showed that the isolate belong to Mass-type IBV. Also, serotype analysis by cross-neutralization tests also confirmed them as Mass serotype. Thus emphasis the fact that Mass-related isolates are widely distributed and predominant in many countries around the world and could be recovered from gastrointestinal tract (Fabricant, 1998; Benyeda et al., 2009). Our findings confirmed that Mass.-related isolate is still prevalent among Egyptian poultry flocks.

The existence of variable genotypes of IBV in Egypt was recognized and strains related to the Massachusetts D3128, D274, D-08880 and 4/91 genotypes have been detected at different poultry farms in Egypt (Cavanagh, 2003; Abdel-Moneim et al., 2006). The phylogentic pattern of our isolate to other Egyptian isolates revealed relatedness to CU-1 and Egypt/Zag/07-02 strains indicating transfer of the virus between provinces of Egypt. Really these results need further work to imply this relatedness as CU-1 and Egypt/Zag/07-02 strains were pneumotropic but our isolate was provenriculotropic.

Taken in our consideration; chickens at one-day of age (time of vaccination in challenge experiment) are not fully immunocompetent, possibility of challenge virus to overcome immunity produced by H120 as a mild vaccine and partial S1 nucleotide and amino acid sequence analysis of Egypt/Qal/014p. However, routine vaccination of commercial broiler chickens possess maternally derived antibodies at one-day of age was recommended (Cook et al., 1999; Bijlenga et al.,2004) without apparent interference by the maternal derived antibodies in the development of active immunity, at least in the respiratory tract that measured by challenge (Cavanagh, 2003). As well as it is possible that some strains with high degrees of S1 identities may not cross-protect against challenge (Ladman et al., 2006). A very few amino acid differences located in major immunodominant regions of the gene may be sufficient to cause a discrepancy between sequence and protective relatedness values (PRV) correlations (Cavanagh et al., 1992). The S1 proteins of the Beaudette and M41 strains of IBV have 95% amino acid identity. When chickens were inoculated with a recombinant IBV Beaudette expressing the spike protein of the M41 strain, good protection was induced against challenge with M41, as assessed by ciliary activity and snicking (Hodgson et al., 2004). In contrast, inoculation with Beaudette induced very poor protection against challenge with M41, suggesting that some of the few amino acids that differed between the two strains were associated with protection-inducing epitopes.

In fact, serotypic determinants have been identified in the first 395-amino acid region of the S1 subunit, which contains three major hypervariable regions (HVRs). In the European IBV strains, these HVRs were associated with virus-neutralizing antigenic sites and located between

the amino acid residues 56-69 (HVR-1), 117-131 (HVR-2), and 274-387 (HVR-3) (Wang and Huang, 2000; Cavanagh et al., 2005). Also It has been suggested that the amino acids located between residues 99 and 127 in the deduced S1 protein sequence may play a role in the different pathogenesis of these viruses (Sapats et al., 1996; Moore et al., 1997). Egypt/Qal/014p possesses unique amino acid substitutions glutamate and valine at position 64 and 69 instead of glycine and isoleucine in H120 and glutamate and threonine in M41 respectively within S1 hypervariable region. Also, the isolate possesses a unique substitutions at residues 39 (Serine), 40 (Tyrosine), 41 (Lysine) and 215 (Lysine) of the S1 protein. Mutations and or recombination events enable IBV to shift host or change tissue tropism (Lim et al., 2011), resulting in virus population resist vaccination and host immune response (Dolz et al., 2008). Our data may provide crucial information about genetic and antigenic characterization and protectotyping of IBV Egypt/Qal/014p isolated from proventriculitis in broiler chickens flock.

CONCLUSION

Egypt/Qal/014p is a closely related Mass-serotype associated to proventriculitis in broiler chicken flock in Egypt. Sequence similarity between isolate and H120 does not simply equate with complete protection ability and optimum control of IBV in Egypt requires preparation of vaccine from indigenous isolates beside periodic evaluation of cross-protective capabilities of such vaccine.

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