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SOLATION AND IDENTIFICATION OF EGYPT/BENI-SEUF /01 A NOVEL GENOTYPE OF INFECTIOUS BRONCHITIS VIRUS

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SUMMARY

Infectious bronchitis virus (IBV) strain Egypt/Beni-Seuf/01 was isolated from 38-day-old broiler chickens suffering from respiratory and renal disease in the Beni-Seuf governorate of upper Egypt in 1998. The strain was identified using electron microscopy (EM), agar gel precipitation test (AGPT), and reverse transcriptase polymerase chain reaction (RT-PCR). S1 gene cycle sequencing of the RT-PCR product was used to determine the genotype of the strain. Three weeks old commercial chickens were vaccinated with IBV vaccine (H120) and protection level against challenge with Egypt/Beni-Seuf/01 was evaluated via viral reisolation attempts from tracheal scraping suspensions as well as tracheal histopathology at 4 days post challenge. Kideny histopathology at 4 & 17 days post challenge was examined. AGPT on the chorioallantoic membrane(CAM) homogenate of each passage using rabbit anti-IBV polyclonal serum showed positive result with all egg passages (1st to 7th). E/M examination revealed pleomorphic or round virions with widely spaced surface projections and 160nm average diameter. Alignment of the S1 sequence of Egypt\Beni-Seuf /01 with 22 published IBV strains demonstrated that the newly deposited sequence of Israel/720/99 in closely related to the Egyptian isolate. Vaccination of broiler-type chickens with the live H120 strain offerred only 20% protection against challenge with the Egyptian strain. Kidney histopathological lesions indicated that Egypt\Beni-Seuf/01 is a nephrogenic IBV. Results of S1 sequencing and the vaccination trial indicate that the Egypt\Beni-Seuf/01 strain is a novel genotype for which H120 vaccination does not provide protection.

INTRODUCTION

Avian infectious bronchitis virus (IBV) is a coronavirus in the new order Nidovirales (Cavanagh 1997). The virus has a tropism for the epithelial lining of the respiratory tract, oviduct and kidney of chickens. The IBV genome consists of single positive stranded RNA that encodes three major proteins; the phosphorylated nucleocapsid protein (N), the membrane glycoprotein (M), and the spike glycoprotein (S). The spike glycoprotein is post-translationally cleaved into two subunits, S1 and S2 (Cavanagh and Naqi 1997). The S2 protein contains the C-terminal portion of the sequence including the trans-membrane anchor and two long a helices that form the stalk of the peplomer (De Groot et al., 1987). The S1 protein forms the N-terminal portion of the peplomer and contains antigenic epitopes which induce virus neutralizing (VN) antibody used to define the virus serotype (Cavanagh et al., 1986; Cavanagh and Davis 1988).

Many different serotypes of IBV have been identified (Jungherr et al., 1956; Darbyshire et al., 1979; Cooke et al., 1987; Lee et al., 2001). The different serotypes, subtypes or variants of the IBV were thought to be evolved from point mutations (Kusters et al., 1987), and RNA recombination of the S-1 genes (Cavanagh and Davis 1988; Jia et al., 1995). AGPT detect group but not serotype specific antigens (Cavanagh and Naqi 1997). Differences in S1 sequences by restriction fragment length polymorphysim analysis (RFLP), (Lin et al., 1991; Kwon et al., 1993) or by sequencing RT-PCR products (Kingham et al., 2000), have been recently used for distinguishing between the genotypes of different IBV serotypes. Vaccination trials in chickens are still considered the best method for determining the level of protection against challenge with a heterologous strain (Gelb 1989).

In Egypt, the respiratory form of IBV was first reported in 1954 (Ahmed 1954). Although most farms routinely use H120 vaccination, IBV continues to be a common problem in broilers in Egypt. Isolates related to D3128, and D274 were isolated from different poultry farms (Sheble et al., 1986; El Kady 1989). Outbreaks of nephrogenic IBV have been reported in many governorates during the last few years. In the present study, Egypt/Beni-Seuf/01 was isolated from 38-day -old broiler chickens in Beni-Seuf governorate, identified by AGPT, E/M , sequenced to determine its S1 genotype, and used as a challenge virus vs. H120 vaccination.

MATERIALS AND METHODS

Embryonated chicken eggs (ECE). Specific-

pathogen-free (SPF) ECE obtained from Nile SPF (Koom Oshiem, Fayoum, Egypt) were used for isolation of the field isolate (Egypt/Beni-Seuf/01), serial passages, titration of the seed stocks of Egypt/Beni-Seuf/01 and vaccine strain H120, as well as virus reisolation attempts following challenge in the vaccination trial.

Chickens. Commercial broiler chickens (Integrated Co, Azab, Fayoum, Egypt) were reared under strict hygienic conditions in separate rooms and used in the vaccination trial.

Hyperimmune serum preparation. Rabbit polyclonal anti-IBV serum was prepared as described (Saifuddin and Wilks 1990). Briefly, equal volumes of 10^5 $TCID_{50}$ /ml IBV (H120) adapted to Vero cells (Vaccine & and Serum research and Production institute, Abbasia, Egypt) was mixed with complete Freund adjuvant (BACTO Difco, USA) and 2 ml were injected intramuscularly into each of two rabbits. Three weeks later, a second booster of H120 (vero adapted) dose and Freund incomplete adjuvant (BACTO Difco, USA) was injected. Three weeks following the booster immunization, blood samples were taken, serum was collected, and stored at -20°C until used.

Virus isolation and passage in SPF ECE: Egypt/Beni-Seuf/01 was isolated from 38-day-old broiler chickens suffering from both respiratory and renal disease from Beni-Seuf governorate in 1998. A kidney homogenate (10% in sterile phosphate buffer saline) and a tracheal scraping suspension were pooled, centrifuged at 500 x g for 10 min. The supernatant fluid was filtered through 0.45µ filter (Star, Cambridge, H. M., IK), and inoculated into chorioallantoic sac of 10-day-old SPF ECE. Allantoic fluid was harvested after 48 hrs and was used for repassage into ECE. Chorioallantoic membranes were harvested from each egg passage and homogenates were prepared for detecting IBV antigen by the AGPT. Five eggs of each passage were incubated till being 18-day-old and examined for typical lesions of IBV such as stunting, curling and urates in the kidney. A seed stock of Egypt/Beni-Seuf/01 was prepared following 7th serial passages in SPF ECE.

Virus titration: Egypt/Beni-Seuf/01 and H120 vaccine (Izavac, Italy) were titrated as described (Villegas and Purchase 1989). Virus titers were exspressed as 50% embryo infectious doses (EID_{50}) and calculated as described (Reed and Muench, 1938).

Agar Gel Precipitation Test: The AGPT was performed as described by (Woernle 1966). Briefly, 6 ml of 1.25% agar (SERVA, Feinbiochemica, Heidelberg, Germany) solution containing 8% NaCl was poured into sterile Petri dishes (60 mm). After solidification, 7 wells (six peripheral wells arround central one) were made 5.0 mm in diameter and 3.0 mm apart. H120 vaccine (Izavac, Italy) was reconstituted in 2 ml sterile PBS, and used as control positive antigen. Normal SPF CAM homogenate was used as control negative. Tween 20(SIGMA, Chemical Co., USA) was added to all samples including negative and positive control samples in concentraion of 0.05% (v/v). Tween treated samples were mixed thoroughly, centrifuged at 500xg for 3 min, and the clear supernatant fluid were used in the test. Fifty µl of rabbit polyclonal anti IBV serum was pipetted into the central well and tested samples were placed in the peripheral ones. Plates were incubated for 24 hrs at 37°C and examined for the presence of precipitation lines. The AGPT was used to identify the isolate as IBV and for detecting viral antigen in the CAM homogenates in each egg passage.

Electron microscopy: The EM examination was conducted according to the method adopted by (Cowen et al., 1987). Virus containing allantoic fluid was centrifuged at 1,000 x g for 15 min to clarify the virus suspension. The supernatant fluid concentrated by centrifugation at 40,000 x g for 2 hrs, and the resultant virus pellet was resuspended in 1 ml of distilled water. A copper grid coated with carbon film was floated on the surface of the virus suspension. After approximately 30 sec, the grid was transferred onto filter paper and left to air dry. The grid was transferred to a solution of 5% uranyl acetate for an additional 30 sec, and then allowed to air dry. The negatively stained preparation was then examined at 60 KV acceleration (Zeiss EM10, Germany). The appropriate virus size was estimated using the standard equation O= I\M, where M is the magnification power, I is the image height , and O is the object height (Bueche 1982).

Viral inactivation, polymerase chain reaction and S1 gene cycle sequencing: The Egypt/Beni-Seuf/01 strain was inactivated by treating approximately 2 ml of the allantoic fluids containing IBV with an equal volume of molecular biology grade of phenol (pH 4.3) (Fisher Scientific, Fair Lawn, NJ). Following inactivation, the isolate was shipped to the University of Delaware as stipulated by an USDA Veterinary Import Permit issued to J. Gelb, Jr. The phenol-treated allantoic fluid was vortexed and then centrifuged at 12,000

x g for 3 min. The supernatant was harvested and an additional treatment using phenol/chloroform with isoamyl alcohol (pH 4.3) (Fisher Scientific) was performed. Viral RNA was harvested from the aqueous layer and extracted using a Qiagen Viral RNA Mini Kit (Qiagen, Inc., Valencia, CA). The RNA was eluted in sterile diethyl pyrocarbonate (DEPC) -treated water and stored at -70°C..

RT was performed on the viral RNA using the GeneAmp RNA PCR Core Kit (Applied Biosystems, Foster City, CA). Approximately 2 µl of the extracted viral RNA was used to synthesize cDNA. Amplification of the S1 gene was performed using the forward primer S1 OLIGO5' (Kwon et al., 1993; Kwon and Jackwood 1995) and the reverse primer CK2 (Keeler et al., 1998). PCR was performed as described (Kingham et al., 2000) with the exception that extension was performed at 60°C. A PCR product was cut from 1.8% agarose gels, purified with the QIAquick Gel Extraction Kit (Qiagen, Inc.) and the DNA was quantitated as described (Kingham et al., 2000).

Purified RT-PCR products were sequenced in the forward direction using primer S1 OLIGO5' and in the reverse direction using primer CK2. Sequencing was performed as described (Kingham et al., 2000) Egypt/Beni-Seuf/01 sequence was deposited in the GenBank (access. No. AF395531).. A BLAST® analysis (Altschul et al., 1990) was initially performed using the S1 sequence of Egypt/Beni-Seuf/01 to establish its identity to GenBank accessions. Sequences representative to IBV S1 genotypes used for the alignments were obtained from the GenBank and EMBL databases. Sequences used in this study were Ark DPI (AF006624), BL-56 (AF352831), B1648 (X87238), Connecticut (L18990), CV-56 (AF027509), D1466 (X58001), D274 (X15832), DE/072/92 (U77298), H120 (M21970), Holte (L18988), India/1/00 (AY091551), Israel Variant 1 (AF093795), Israel Variant 2 (AF093796), Israel/720/99 (AY091552), JMK (L14070), Massachusetts 41 (M21883), Mex/1765/99 (AF 276300), N1/62 (AIU29522), PA/1220/98 (AF200685), PA/Wolgemuth/98 (AF305595), UK/4/91 (AF093794), and Vic S (U29519). A comparative analysis S1 protein sequences was performed using the CLUSTAL V (Higgins and Sharp 1988) package of DNAStar (DNAStar, Inc.; MegAlign; Version 1.03, 1993; Madison, WI).

Vaccination trial: Three groups of 15, 3-weeks-old chickens were used to evaluate the protection provided by H120 vaccination against challenge

with Egypt/Beni-Seuf/01. The H120 vaccinated chickens were vaccinated by eye drop with 10^3 EID50 of virus per bird. The non-vaccinated non-challenged control and non-vaccinated challenged control groups, did not receive H120. Four weeks post vaccination, both chickens in the H120 vaccinated group and the non-vaccinated challenged control group were challenged by eye drop with 10^5 EID_{50} per bird of Egypt/Beni-Seuf/01. The non-vaccinated non-challenged control were not challenged. Tracheas were collected four days post challenge(representative samples from each group) for virus reisolation attempts and histopathological examination. Tracheal scrapings were homogenized in 2ml of sterile PBS and centrifuged at 500 xg for 3min. The supernatant clear fluid of each sample was filtered through 0.45µm (Star, Cambridge, H.M., UK) . Virus reisolation attempts were performed by inoculating three, 10-day-old SPF ECE per sample as described (Gelb and Killian 1987). Embryos were examined for the specific pathological typical IBV lesions. Tracheas were fixed in formalin for histopathology and were processed routinely and stained with hematoxylin and eosin. The trachea from each bird was examined microscopically and assigned lesion scores of 0-3 with 0 = none, 1 = focal, 2 = multifocal, 3 = diffuse. Tracheas were scored for the amount of mucous, loss of cilia, epithelial hyperplasia, necrosis, lymphocyte infiltration, and heterophil infiltration, and the extent of tissue reaction. The scores for each bird were added, and the mean score for the bird in each group was calculated (Andrade et al., 1982). Kidney samples were also taken 4 and 17 days post challenge and examined microscopically for tubular degeneration and inflammation consistent with interstitial nephritis. Focal, multifocal, and . diffuse were used to assign kidney histopathology.

RESULTS

Virus isolation and passage in SPF ECE: The Egypt/Beni-Seuf/01 strain replicated in 10-day-old SPF ECE on the first passage as indicated by the results of (data not shown) as well as RT-PCR

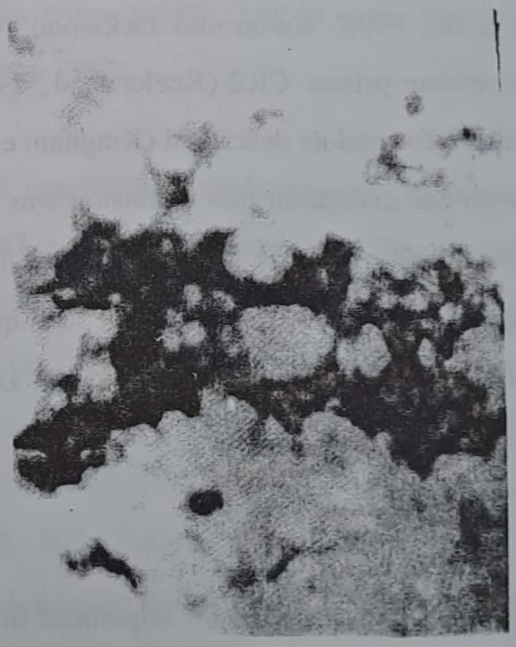

Fig.1 Electron microscopic picture showing negatively stained preparation of Egypt/Beni-Seuf/01/01(100,000x) illustrating pleomorphic particles with widely spaced, club-shaped surface projections.

The allantoic fluid was harvested at 48hr PI for rther passages till the 7th egg passage. The vi- s growth in each passage was ascertained by erforming AGPT on the CAM homogenate of e harvested egg set. Five eggs of the 7th passage ere incubated till being 18-day-old and all of em (100%) showed typical lesions of the IBV tunting and dwarfing), while the other passages id not show typical lesions in all embryoes (data ot shown).

CM: Negatively stained virions preparation of Egypt\Beni-Seuf /01particles showed to be pleo- morphic or round with widely spaced club-shaped urface projections (Fig.1) with an average diame- er of 160 nm, consitent with coronaviruses.

Polymerase chain reaction and S1 gene cycle equencing: RT-PCR of Egypt/Beni-Seuf/01/ RNA resulted in a product of 707 base pairs. us- ng S1 primers OLIGO 5' and CK2. BLAST BLAST analysis was performed every month ince the date of deposition of Egypt/Beni-Seuf/ 1 (26/5/2001). No sequence was related to Egypt/Beni-Seuf/01 till deposition of the Israel/ 20/99 (22/3/2002) that was very close to the ovel Egyptian genotype. Alignment of the S1 se- uence of Egypt\Beni-Seuf /01 with 22 published BV strains demonstrated it to be related (97.6%) o a strain recovered in Israel in 1999 (Table 1 nd Fig. 2). It was found that, Israel720/99 showed 6 amino acids and 8 nucleotides substitutions from Egypt/Beni-Seuf /01 (Table.2).Table 1 presents S1 similarity for 22 selected IBV reference strains and Egypt/Beni-Seuf/01/. The percentage of Similarities for other genotypes ranged from 30.1% (Mex/1765/99) to 71.9% (Israel Variant 2).

Vaccination trial: Chickens vaccinated with H120 showed 20% protection) 4/5 (by the virus reisolation procedure, and 0% (0/5) protection by histopathology after challenge with Egypt\Beni-Seuf /01(Table 3). High total tracheal scores were recorded in both H120 vaccinated and the non - vaccinated - Egypt\Beni - Seuf / 01challenged groups (11.6 and 11.2) in comparison to that of the non-vaccinated non-challenged Control group(2) (Table.3). On day four PI, the kidneys of all broilers in both the vaccinated (5/5) and nonvaccinated (5/5) Egypt\Beni - Seuf / 01challenged groups showed lesions of acute interstitial nephritis ranged from focal to diffuse lesion including, hyaline casts, vacuolar degeneration, and lymphocytic infiltration (Table 4). On day 17 PI, kidneys of H120 vaccinated (3/3) and non vaccinated-Egypt\Beni-Seuf /01challenged (3/3) groups showed chronic active interstitial nephritis including medullary interstitial lymphocytic infiltration, with plasma cells scattered between lymphocytes, degenerative changes in tubules, and fibroblastic proliferation .

Table 1.BV S1 protein sequence relationships expressed as percent similarities of Egypt/Beni-Seuf/01/01 and selected IBV reference strains in the GenBank database.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	IBV ISOLATE
	65.3	62.3	65.3	62.6	64.7	34.9	69.5	37.0	63.5	59.9	64.1	64.1	71.9	97.6	64.1	63.5	30.1	65.9	31.7	65.9	67.7	62.3	1 Egypt/Beni-Seuf/01
		72.7	68.4	62.6	74.3	35.5	75.0	36.4	64.1	67.6	61.9	66.3	75.0	65.3	72.3	64.1	33.6	75.9	31.7	73.1	67.5	73.3	2 Arkansas DPI
			66.7	61.3	69.0	33.1	72.0	35.2	64.7	66.3	60.7	67.5	73.2	62.3	68.6	64.1	32.2	75.6	34.8	66.7	68.6	72.7	3 B1648
				60.7	68.4	31.3	67.9	34.5	58.1	62.0	57.7	64.5	67.9	64.7	80.7	59.9	35.0	69.0	34.2	67.3	64.5	63.2	4 BL 56
					61.3	34.4	68.1	36.2	82.8	63.2	63.8	62.6	63.8	62.0	60.1	80.4	32.9	64.4	33.5	60.1	63.2	63.8	5 Connecticut
						28.3	75.0	32.1	59.3	65.5	59.5	65.7	69.0	64.1	69.6	59.3	29.4	80.7	32.3	84.8	65.1	73.7	6 CV-56B
							32.5	61.8	34.9	31.9	35.5	31.3	35.5	34.9	30.1	33.7	66.4	33.1	28.0	30.7	34.3	30.1	7 D1466
								37.6	67.1	702	66.1	71.4	85.7	69.5	73.8	65.9	34.3	75.0	32.9	70.2	70.8	74.4	8 D274
									37.0	35.8	37.6	32.7	37.6	37.0	34.5	36.4	65.7	32.1	30.4	32.7	33.9	31.5	9 DE/072/92
										61.1	65.9	65.3	65.9	63.5	62.3	92.8	35.0	67.1	31.7	57.5	65.3	66.5	10 H120
											58.3	59.8	70.2	58.7	67.6	64.1	31.5	69.4	29.2	60.8	59.2	73.3	11 Holte
												64.3	69.6	62.9	61.9	62.3	34.3	63.7	36.6	60.1	64.9	58.9	12 India/1/100
													71.4	64.7	69.2	64.7	29.4	68.6	32.3	62.7	89.3	63.9	13 Israel Variant 1
														72.5	73.2	65.3	34.3	71.4	32.3	67.9	70.8	71.4	14 Israel Variant 2
															64.7	63.5	30.8	65.3	32.9	66.5	68.3	62.9	15 Israel/720/99
																62.3	34.3	74.0	32.9	67.8	69.8	71.5	16 JMK
																	34.3	67.7	31.7	57.5	64.7	65.9	17 Massachusetts 41
																		30.8	28.0	29.4	32.2	28.7	18 Mexico/1765/99
																			31.7	77.2	68.0	76.6	19 N1/62
																				34.2	33.5	31.7	20 PA/1220/98
																					65.7	69.0	21 PA/Wolgemuth/98
																						65.1	22 UK/4/91
																							23 Vic S

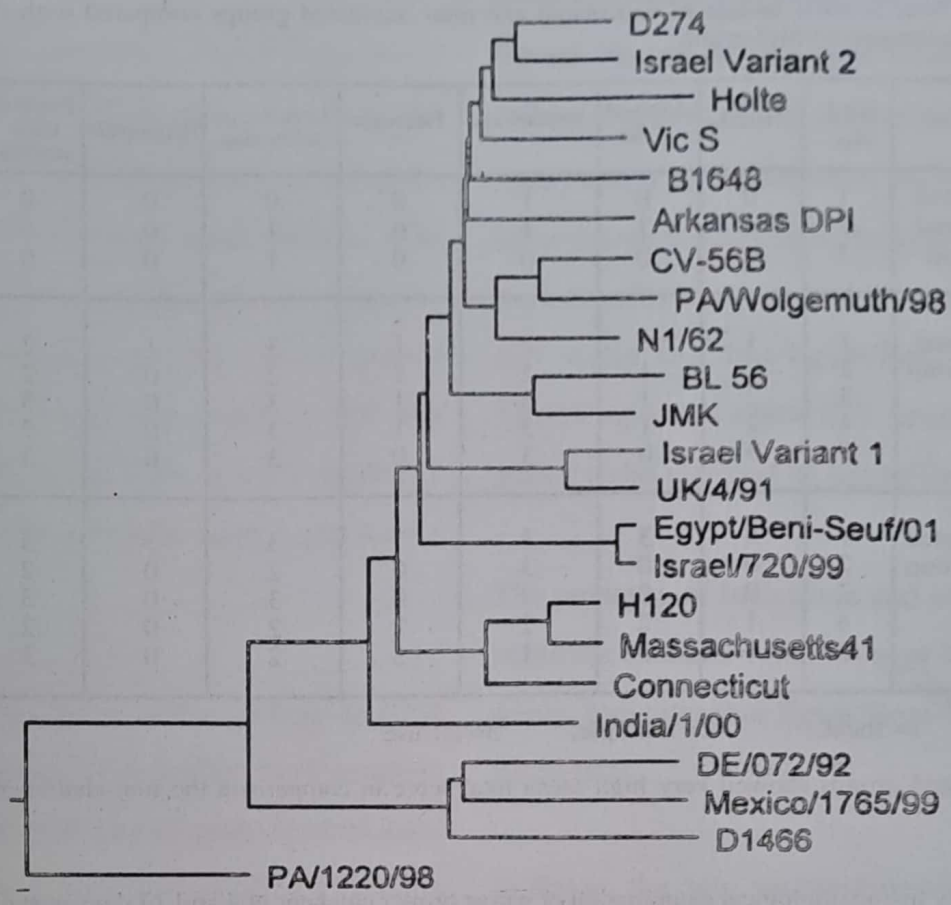

Fig.2. IBV S1 protein sequence relationships expressed as a phylogenetic tree of Egypt/Beni-Seuf/01/01 and selected IBV reference strains in the GenBank database.

Table 2: Nucleotide and amino acid substitutions of Egypt/Beni-Seuf/01/01 from Israel/720/99.

No.	Amino acid			Codon		
	Amino Acid Number	Egypt/Beni Seuf/01	Israel 720/99	Egypt/Beni Seuf/01	Israel 720/99	Mutation Type
1	3	E (Glu)	A (Gly)	GAG	G**G**G	Non-Silent
2	7	L(Leu)	S (Ser)	UUA	U**C**A	Non-Silent
3	62	T (Thr)	I (Ilu)	ACU	A**U**U	Non-Silent
4	81	M (Meth)	I (Ilu)	AUG	AU**C**	Non-Silent
5	105	I (Ilu)	F (Phen)	AUU	**U**UU	Non-Silent
6	135	V (Val)	A (Ala)	GUU	G**C**U	Non-Silent
7	139	N (Asn)	N (Asn)	AAC	AA**U**	Silent
8	169	L (Leu)	L (Leu)	UUA	**C**UA	Silent

Table 2 showed that Israel 720/99 have 6 amino acids and 8 nucleotides substitutions from Egypt/Beni-Seuf/01 from which the last two mutations were silent.

Table 3: Histopathological examination of tracheae of native broiler chickens at 4 days post challenge with Egypt/Beni-Seuf/01 isolate in vaccinated and non-vaccinated groups compared with the non-vaccinated non-challenged control group.

Group	Bird No.	Mucus	Loss of Cilia	Hyperplasia	Necrosis	Lymphocyte infiltration	Heterophils	Extent of tissue reaction	Total Score	Mean Score
Non-vaccinated non-challenged control group	1	0	0	1	0	0	0	0	1	2
	2	1	1	1	0	1	0	0	4	
	3	0	0	0	0	1	0	0	1	
Non-vaccinated challenged group	1	1	0	2	1	3	1	2	10	11.6
	2	2	1	2	1	2	0	2	10	
	3	2	1	3	1	3	0	3	13	
	4	2	1	3	1	3	0	3	13	
	5	3	0	3	0	3	0	3	12	
H120 Vaccinated challenged group	1	0	3	3	3	3	3	3	18	11.2
	2	0	0	3	0	2	0	2	7	
	3	0	0	3	0	3	0	3	9	
	4	1	2	2	2	2	0	2	11	
	5	1	1	1	3	2	0	3	11	

0 =none, 1= focal, 2= multiple, 3= diffuse

Both challenged groups showed very high mean total score in comparison the non-challenged control group.

Table 4: Kideny histopathological examination of native broiler chickens at 4 and 17 days post challenge with Egypt/Beni-Seuf/01 isolate in vaccinated and non-vaccinated groups compared with the non-vaccinated non-challenged control group.

Group	Severity of the lesion						
	Days post challenge	No. of birds	Normal	Focal	Multifocal	Diffuse	Type of Lesion
Non-vaccinated non-challenged control group	4	2	2	-	-	-	-
	17	2	2	-	-	-	-
Non-vaccinated challenged group	4	5	-	2	2	1	Acute diffuse interstital nephritis
	17	3	-	1	1	1	Chronic active interstital nephritis
H120 Vaccinated challenged group	4	5	-	-	3	2	Acute diffuse interstital nephritis
	17	3	-	1	2	-	Chronic active interstital nephritis

Acute interstitial nephritis and chronic active interstitial nephritis were observed 4 and 17 days post infection respectively in both challenged group whereas control non-vaccinated non-challenged group did not show any changes.

DISCUSSION

In this study, Egyptian strain Egypt\Beni-Seuf / 01was isolated from a tissue pool of kidney and trachea from H120 vaccinated broiler flock with a history of respiratory and renal disease. The strain was shown to produce embryo lesions in 100% by the seventh passage. The virus displayed characteristic coronavirus ultrastructure and was subsequently identified as IBV initially by AGPT and E/M and later by RT-PCR using universal S1 gene primers.

Scince the initial isolation of Egypt/Beni-Seuf/01 in 1998 from a commercial broiler flock in upper Egypt, another identical isolate (unpublished data) was isolated from a commercial broiler flock in Northen Egypt, that may denote the spread of Egypt/Beni-Seuf/01 to different parts of Egypt. BLAST analysis of Egypt/Beni-Seuf/01 S1 sequence found it to be related to Israel/720/99 (97.6%). The possible cause of existence of two closely related but not identical strains; Egypt/ Beni-Seuf/01 and Israel/720/99 in both Egypt and Israel in a subsequent manner is unknown. The close similarity between the two isolates indicates that Egypt/Beni-Seuf/01 and/or Israel 720/ 99 might be subjected to point mutation that resulted in this rapid change in the viral genome as subtypes of IBV were thought to be evolved from point mutations (Kusters et al., 1987) that is confirmed by our finding that Israel720/99 showed 2silent (because of the redundancy of the genetic code) and 6 non silent point mutations that resulted in 6 amino acids substitutions Table.2.. Egypt/ Beni-Seuf/01 was not highly related to any other IBV strain and thus Egypt/Beni-Seuf and Israel/ 720/99 represent a new IBV genotype that is suggested to be assigned as Egypt/Beni-Seuf/01.

The presence of both acute and chronic interstitial nephritis on days 4 and 17 post infection, respectively, indicates that Egypt\Beni-Seuf is a nephrogenic IBV.

In Egypt, the only permissable IBV living attenuated vaccine is H120 and M41, but H120 is more commonly applicable in broiler chickens at day-old. The reisolation of Egypt/Beni-Seuf/01 and the presence of tracheal and renal microscopic lesions in H120 vaccinated birds as shown in the vaccination trial denotes a lack of protection afforded by vaccination. The low S1 sequence similarity (63.5%) and high divergence (32.3%) (data not shown) between the Egyptian isolate and H120 may explain why the vaccine did not protect against challenge with Egypt/Beni-Seuf/01 / 01in the vaccination trial. The fact that heteroge-

nous vaccination produce variable protection (Cavanagh and Naqi 1997) together with the data presented in this study make it necessary to develop autogenous living attenuated vaccine in order to provide homologous protection to such new challenge to poultry industry in Egypt.

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