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Isolation and Molecular Characterization of Novel IBV Isolates from Broiler Chicken Farms in Egypt

Ashraf M. Awad [α], Mahmoud E. Sediek [σ] & Mohamed E. El-Yamany [ρ]

Abstract- A survey of infectious bronchitis virus (IBV) genotypes in 25 commercial broiler flocks of various ages (20-35 days) raised in Al-Behera and Kafr-Elsheikh governorates and suffering from respiratory symptoms and pathological changes in kidney associated with high mortality rate. All flocks were vaccinated against IB disease. Tissue samples (trachea, lung and kidney) were collected aseptically from these flocks. Then virus propagation was performed in embryonated SPF chicken eggs via allantoic sac inoculation. Results of virus isolation trails from the collected organs revealed 15 IBV isolates (60%) out of 25 flocks as judged by antigen detection in CAMs by the AGPT against reference IBV Beaudette antiserum after 2-5 chicken embryo serial passages. However, three of 15 IBV isolates were also positive by the slide HA test. Moreover, 5 flocks gave positive slide HA test and negative by the AGPT. The other 5 flocks gave negative slide HA test and AGPT. Then selected ten IBV field isolated strains in this study were characterized by RT-PCR (all of ten selected isolates are positive for S1 gene), and then sequence analysis of partial S1 spike glycoprotein gene of seven IBV field isolates in this study (11, 15, 21, 13, 19, 22, 24) were made. The seven IBV field isolates showed 97% to 98.3 % and 96.7% to 98.3% nucleotide sequence identity to IBV-CU-2-SP1 and Eg/12120s/2012 strain (variant 2 like strain), respectively. Nucleotide identity between these seven IBV isolates ranged from 97.7% to 99% and between these isolates and vaccinal strain used in Egypt (M41, H120, Ma5, 4/91, CR88 and D274) ranged from 64.7% to 65.7%, 65.3% to 66.3%, 65.7% to 66.7%, 67.3% to 68.3%, 68.6% to 69.6% and 84.2% to 84.8%, respectively.

The presence of these strains may account for vaccination failure against IBV, since all IBV isolates were from vaccinated chickens. This study demonstrates a constant evolution of IBV in Egypt that necessitates continuous monitoring to control the spread of infections, and the development and use of vaccines based on indigenous viruses.

I. Introduction

Infectious bronchitis (IB) disease is an acute, highly contagious and infectious disease of poultry in worldwide, possess a major threat to the poultry industry and was first reported in North Dakota, USA, as a novel respiratory disease by Schalk and Hawn in 1931. The disease is characterized by respiratory signs including (sneezing, cough, tracheal rales, gasping and nasal discharge), reduction the growth rate of broilers, nephropathogenic strains causing acute nephritis, urolithiasis and may be associated by high mortality (The Merck Veterinary Manual, 2006).

IBV belongs to group III of the genus coronavirus of the coronaviridae family. It is an enveloped, non-segmented, positive sense single stranded RNA virus (Cavanagh, 2003). The nucleocapsid (N) protein is one of the major structural proteins of the virion, and since the N gene is highly conserved even among IBV isolates of different serotypes, it is often the target for nucleic acid based virus identification in diagnostic laboratories. The spike (S) glycoprotein is another major structural protein of the virion, and it is post-translationally cleaved into S1 globular and S2 stalk polypeptides (Cavanagh, 2007).While the S2 subunit is conserved, the S1 subunit generally varies by up to 23% at the amino acid level among viruses of the same serotype (Cavanagh et al., 2005). Three hypervariable regions (HVRs) have been identified in the S1 subunit (Moore et al., 1997). Diversity in S1 probably results from mutation, insertions, deletions, or RNA recombination of the S1 genes (Jackwood et al., 2012).

Detection of IBV infection in poultry flocks, as well as differentiation from other upper respiratory diseases, is amajor challenge and necessitates the use of appropriate diagnostic methods. Virus isolation in specific pathogen free (SPF) eggs, the reference standard, is time consuming and may require more than one passage before obtaining a result (Sediek, 2005). Reverse transcriptase-polymerase chain reaction (RT-PCR) assays are rapid, specific, and accurate, and when targeting the viral S1 gene, the amplification products can be used for further classification of the virus (Gelb et al., 2005 and Lee et al., 2000). Sequence analysis of the S1 portion of the genome of hundreds of isolates belonging to the many different serotypes of IBV worldwide has been carried out to study and determine phylogeny, evolution, antigenic, and genetic relatedness and virulence of this important poultry pathogen (Jackwood et al., 2007; Cavanagh, 2007).

IBV strains related to the Massachusetts, D3128, D274, D-08880 and 4/91 genotypes have been detected at different poultry farms in Egypt (Abdel-

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Moneim et al., 2006; Sultan et al. 2004; Sediek, 2010). The Egyptian variants which were closely related to the Israeli variant strain were isolated from different poultry farms (Abdel-Moneim et al. 2002; Sediek, 2010). The Egyptian variant IBV-CU-2-SP1and Eg/12120s/2012 were isolated by Afifi et al. (2013) and Arafa et al. (2013), respectively.

Control of the disease is mostly through the use of live attenuated vaccines, but antigentically different serotypes and newly emerged variants from field chicken flocks sometimes cause vaccine breaks. The generation of genetic variants is thought to be resulted from few amino acid changes in the spike (S) glycoprotein of IBV (Adzhar et al., 1997).

The aim of this study is to isolate and molecular characterization of novel IBV isolates from broiler chicken farms in Egypt. This is important for implementation of control measures especially for the future vaccination strategies.

II. Materials and Methods

a) Samples

Tissue samples (trachea, lung and kidney) were collected aseptically from 25 suspicious IBV chicken flocks (4-5 tissue samples per flock) of various ages (20-35 days) raised in Al-Behera and Kafr-Elsheikh governorates and suffering from respiratory symptoms and pathological changes in kidney associated with high mortality rate. All flocks were vaccinated against IB disease.

b) Virus propagation and isolation

Virus propagation was performed in 9–11 day-old embryonated SPF chicken eggs (Kom Oshim, Fayoum), Procedures were performed according to ***OIE terrestrial manual (2008)***. The allantoic fluid and CAM were harvested 48 h post-inoculation (PI). Three to five serial blind passages were performed in order to induce lesions typical of IBV in the chicken embryo.

c) Slide haemagglutination (HA) test

Slide HA was carried according to (Beard, 1980) on harvested AF collected from eggs inoculated at each serial passage with suspected field samples to rule out pathogens with HA activity. This test was done by placing one drop of 10% washed chicken RBCs suspension in sterile saline (0.8% sodium chloride) onto a clean microscopic slide and thoroughly mixed with one drop of the harvested AF. The result was recorded within one minute.

d) Agar Gel Precipitin Test

The test was carried out on a homogenate of the CAM of infected chicken embryos. The test was performed as described by (Lohr, 1980, 1981). Six peripheral wells surrounding a central well in a hexagonal form were made in the agar medium by a special appliance, the well size was 4 mm in diameter, and the distance between the central well and the evenly spaced peripheral wells was 4 mm. 30 µl of IBV Beaudette reference antiserum was placed into the central well, while 30 µl of antigens to be tested for precipitinogen were placed into the peripheral wells. The last peripheral two wells (NO. 6,5) in each slide served for positive control antigen (Beaudette antigen) and negative control (PBS) respectively. Readings were recorded after 24 h by observing the plate against an illuminated indirect light source with a dark background. Final readings were recorded after 48 h. An opaque precipitin line between the antigen- antibody wells was considered as a positive result.

e) Viral RNA Extraction

Extraction of viral RNA was carried out on allantoic fluids of selected ten IBV isolates according to the instructions for the QIAamp Viral RNA Mini Kit (Qiagen, Germany)

f) RT-PCR amplification of S1 gene

i. *-Oligonucleotide primers*

The RT-PCR was done for these selected ten IBV isolates using oligonucleotide primers encoding for S1 gene (Adzhar et al., 1997).

Table (1) : Primers for S1 gene amplification in conventional PCR

Primer ID	Nucleotide Sequence	Length	Position in S1 gene	Reference
IB-F	5'CACTGGTAATTTTTCAGATGG-3'	21 nt	729-749	Adzhar et al., 1997
IB-R	5' –CAGATTGCTTACAACCACC-3'	19 nt	1093-1111	Adzhar et al., 1997

ii. - *Qiagen one step RT – PCR kit:* was supplied by Qiagen, Germany (Cat. NO. 210212).

Thermal profile used in one step RT-PCR.

Stage	Temperature	Time	Cycles
Reverse transcription	50 oC	30 min	1
Primary denaturation	95 oC	15 min	1

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Amplification a) Secondary denaturation	95 oC	30 sec	40
b) Annealing	56 oC	45 sec	
c) Extension	72 oC	2 min	
Final Extension	72 oC	10 min	1
Storage		4 oC	

After the end of PCR run, Amplification products run in agar gel 1.5% which give specific band at 500 pb in weight measured against 100 pb ladder (Qiagen – Germany).

g) Sequencing of the S1gene

Seven purified PCR products were sent to NLQP, Animal Health Research institute, Egypt for sequencing.

h) Genetic analysis

A BLAST analysis of raw sequence data was initially performed to exclude sequence redundancy with the existing Gen Bank entries.

i. ***For sequence analysis:*** Bioedit software was used for analysis for the sequence of S1 gene of the isolates of this study.
ii. ***For Phylogenetic analysis:*** Software *MEGA* version 5 with a bootstrap resampling method (500 bootstraps) to make alignment for of S1 sequence and make a phylogenetic analysis for these isolates.
iii. ***Calculate the Sequence Distances:*** to display the divergence and identity percent values of each sequence pair in the alignment. Divergence is calculated by comparing sequence pairs in relation to the phylogeny reconstructed using MegAlign software. Percent Identity compares sequences directly, without accounting for phylogenetic relationships.

III. Results

a) Clinical signs and gross pathology

The clinical examination of investigated flocks revealed general signs of illness, respiratory signs and diarrhea in some flocks. The respiratory signs ranged from mild to severe and included conjunctivitis associated with lacrimation, gasping, sneezing, rales and coughing. Mortality rates during three days before flock visits (0.3-3.5 %). The examined affected chickens flocks aged 20- 35 days.

Gross pathological examination generally revealed mild to severe congestion of respiratory mucosa of trachea and small areas of pneumonia. Some flocks frequently showed mucous or caseated material in trachea and bronchi, and other showed fibrinous pericarditis, perihepatitis and airsaculitis. Pale or congested and enlarged kidneys with prominent tubules with urates deposition and slight to moderate distention of the ureters with urates were seen in some flocks.

b) Virus isolation and identification

Results of virus isolation trails from the collected organs revealed 15 IBV isolates (60%) out of 25 flocks as judged by antigen detection in CAMs by the AGPT against reference IBV Beaudette antiserum after 2-5 chicken embryo serial passages. However, three of 15 IBV isolates were also positive by the slide HA test and these 3 isolates were exclude from further identification. Moreover, 5 flocks gave positive slide HA test and negative by the AGPT against reference IBV Beaudette antiserum after 5 chicken embryo serial passages. The other 5 flocks gave negative slide HA test and AGPT against reference IBV Beaudette antiserum after 5 chicken embryo serial passages.

The 15 IBV isolates caused variably low embryonic death and or curling and dwarfing after 3-5 serial passages.

c) Results of conventional RT-PCR for S1 gene

All of selected ten isolates are positive for S1 gene (Fig1).

d) Results of sequence and Phylogenetic analysis

A phylogenetic tree was constructed from the nucleotide sequences of the S1 glycoprotein gene showing that the seven selected Egyptian IBV isolates (11, 13, 15, 19, 21, 22 and 24) present in the same group with IBV-CU-2-SP1, Eg/12120s/2012-SP1, Eg/12197B/2012-SP1 and Egypt/01-13. D274 strain is the nearest vaccinal strain present in Egypt to seven isolate (Fig 2).

e) Results of percent identity and divergence of seven Egyptian IBV isolates in this study in comparison to other Egyptian strains, reference strains and vaccinal strains present in Egypt

The seven IBV field isolates showed 97% to 98.3 % and 96.7% to 98.3% nucleotide sequence identity to IBV-CU-2-SP1 and Eg/12120s/2012 strain (variant 2 like strain), respectively. Nucleotide identity between these seven IBV isolates ranged from 97.7% to 99% and between these isolates and vaccinal strain used in Egypt (M41, H120, Ma5, 4/91, CR88 and D274) ranged from 64.7% to 65.7%, 65.3% to 66.3%, 65.7% to 66.7%, 67.3% to 68.3%, 68.6% to 69.6% and 84.2% to 84.8%, respectively table (2).

IV. Discussion

The present study is a trail to investigate the current status of IBV infection among broiler chickens in

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Al-Behera and Kafr-Elsheikh governorates. For this purpose, IBV isolation trails were accomplished by investigation of 25 broiler flocks, with history of vaccination against IB disease, suffering from general signs of illness, respiratory signs and diarrhea in some flocks. The respiratory signs ranged from mild to severe and included conjunctivitis associated with lacrimation, gasping, sneezing, rales and coughing. Mortality rates during three days before flock visits (0.3-3.5 %). The examined affected chickens flocks aged 20- 35 days. Gross pathological examination generally revealed mild to severe congestion of respiratory mucosa of trachea and small areas of pneumonia. Some flocks frequently showed mucous or caseated material in trachea and bronchi, and other showed fibrinous pericarditis, perihepatitis and airsaculitis. Pale or congested and enlarged kidneys with prominent tubules with urates deposition and slight to moderate distention of the ureters with urates were seen in some flocks. The clinical signs and gross lesions were suggestive of infectious bronchitis and were similar to those described by many authors (abdel- Moneim et al., 2002 and Sediek, 2005).

Trachea, lung and kidney were processed for virus isolation in SPF embryonated eggs. Five serial blind passages were performed to consider the sample to be IBV negative. Results of virus isolation trails from the collected organs revealed 15 field IBV isolates (60%) out of 25 flocks as judged by antigen detection in CAMs by the AGPT against reference IBV Beaudette antiserum after 2- 5 chicken embryo serial passages. However, three of 15 IBV isolates were also positive by the slide HA test and these 3 isolates were exclude from further identification. Moreover, 5 flocks gave positive slide HA test and negative by the AGPT against reference IBV Beaudette antiserum after 5 chicken embryo serial passages. The other 5 flocks gave negative slide HA test and AGPT against reference IBV Beaudette antiserum after 5 chicken embryo serial passages. The 15 IBV isolates caused variably low embryonic death and or curling and dwarfing after 3-5 serial passages, which were in agreement with those described by other researches (wang et al., 1997 and sediek, 2005).

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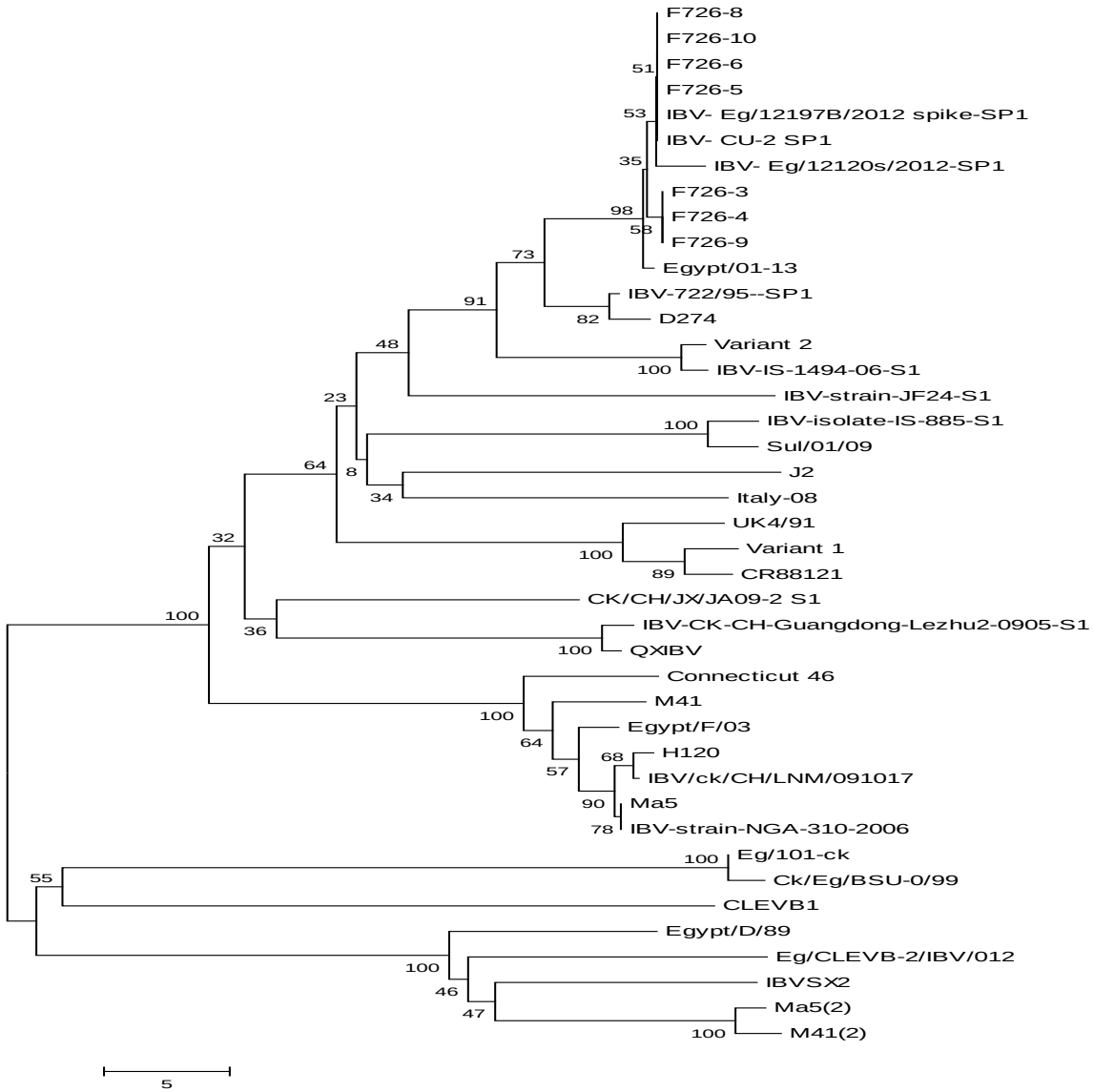

F726-3= isolate No 11 F726-4= isolate No 15 F726-5= isolate No 21 F726-6= isolate No 13
F726-8= isolate No 19 F726-9= isolate No 22 F726-10= isolate No24

Figure (2) : Phylogenetic tree based on a partial sequence of the S1 gene, showing the relationship between the seven Egyptian IBV isolates in this study, vaccinal strain present in Egypt and other reference IBV world circulated strains.

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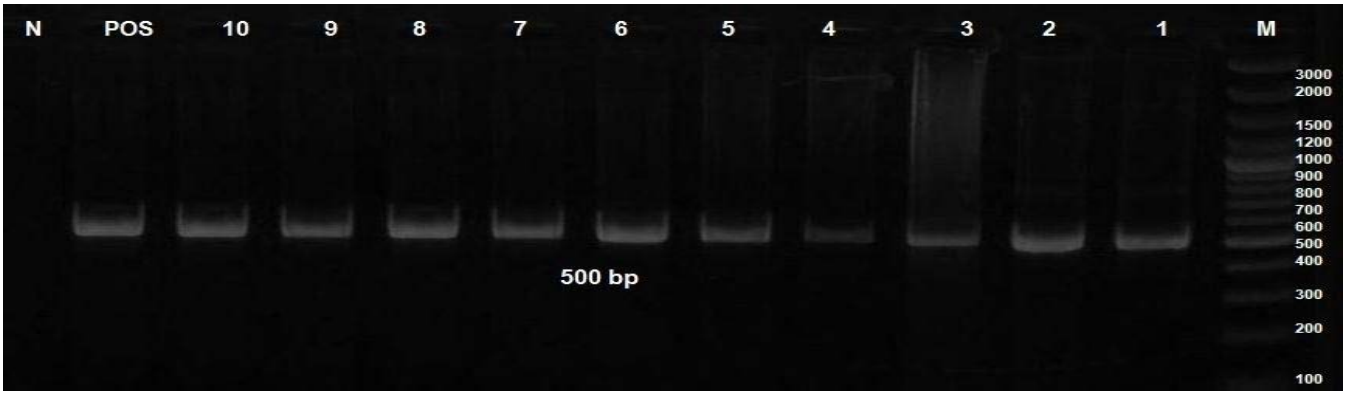

Figure 1 : Agarose gel electrophoresis of the 500 bp RT-PCR product of the selected ten isolates: lane 1, 2, 3, 4, 5, 6, 7, 8, 9, 10: the selected samples; lane M: DNA marker; Lane pos: positive control; lane N: negative control

Table 1 : Nucleotide identity percentage between the seven selected IBV isolates in this study and other reference IBV strains from gene bank

Isolates	Gene bank Accession No	Country	Year	Iden with 11	Iden with 15	Iden with 21	Iden with 13	Iden with 19	Iden with 22	Iden with 24
IBV CU-2 S1	KC985213.1	Egypt	2012	97%	97%	97%	97%	97%	97%	97%
Eg/12197B/2012 SP1	KC533683.1	Egypt	2012	97%	97%	97%	97%	97%	97%	97%
Eg/12120s/2012 SP1	KC533684.1	Egypt	2012	96%	96%	96%	96%	96%	96%	96%
IBV/Egypt/01-13/VIR9715/2012	KC527831.1	Egypt	2012	96%	96%	96%	96%	96%	96%	96%
IBV-11/99 S1	DQ449065.1	Russia	2006	91%	90%	90%	90%	90%	90%	90%
IBV-10/01 S1	DQ449064.1	Russia	2006	90%	90%	90%	90%	90%	90%	90%
NGA/295/2006.	FN182276.1	Nigeria	2006	90%	89%	89%	89%	89%	89%	89%
722/95	AF420320.1	Sweden	2001	90%	89%	89%	89%	89%	89%	89%
UK/123/82	X58067.1	UK	1991	90%	89%	89%	89%	89%	89%	89%
D3896	X52084.1	Netherlands	1990	90%	89%	89%	89%	89%	89%	89%
Egypt/D/89"	DQ487086.1	Egypt	1989	89%	89%	89%	89%	89%	89%	89%
D274"	X15832.1	Netherlands	1989	89%	90%	90%	90%	90%	90%	90%
RF/06/2008	HQ840489.1	Russia	2008	92%	92%	92%	92%	92%	92%	92%
IBV-05/00"	DQ449063.1	Russia	2006	89%	89%	89%	89%	89%	89%	89%
D207	M21969.1		1989	89%	89%	89%	89%	89%	89%	89%
RF/25/2009	HQ840496.1	Russia	2009	92%	92%	92%	92%	92%	92%	92%
RF/12/2008"	HQ840491.1	Russia	2008	92%	92%	92%	92%	92%	92%	92%
RF/11/2007	HQ840488.1	Russia	2007	92%	92%	92%	92%	92%	92%	92%
IS/378/97	AY789956.1	Israel	1997	84%	84%	84%	84%	84%	84%	84%
CU-4	KC985212.1	Egypt	2012	83%	83%	83%	83%	83%	83%	83%
variant 2"	AF093796.1	Israel	1998	83%	83%	83%	83%	83%	83%	83%
IS/572/98	AY789961.1	Israel	1998	83%	83%	83%	83%	83%	83%	83%
Eg/CLEVB-1/IBV/012	JX173489.1	Egypt	2012	83%	83%	83%	83%	83%	83%	83%
IS/1494/06"	EU780077.2	Israel	2006	83%	83%	83%	83%	83%	83%	83%
IS/223/96"	AY789950.1	Israel	1996	83%	83%	83%	83%	83%	83%	83%
IB VAR2-06	JX027070.1	Israel	2006	83%	83%	83%	83%	83%	83%	83%
Mans-1	KF856872.1	Egypt	2012	83%	83%	83%	83%	83%	83%	83%
Eg/CLEVB-2/IBV/012	JX173488.1	Egypt	2012	82%	82%	82%	82%	82%	82%	82%

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Eg/1265B/2012	KC533682.1	Egypt	2012	82%	82%	82%	82%	82%	82%	82%
NGA/293/2006	FN182275.1	Nigeria	2006	84%	84%	84%	84%	84%	84%	84%
RF/01/2010	HQ840511.2	Russia	2010	81%	81%	81%	81%	81%	81%	81%
3374/05	DQ402364.1	Taiwan	2006	76%	77%	76%	77%	77%	76%	76%
JP/Iwate-1/2011	AB858434.1	Japan	2011	77%	77%	77%	77%	77%	77%	77%
1494/06	JX104082.2	Turkey	2011	90%	90%	90%	90%	90%	90%	90%
Md27	FJ008695.1	USA	1976	77%	78%	78%	78%	78%	77%	78%
53XJ-99II	KC577391.1	China	1999	75%	76%	76%	76%	76%	76%	76%
13-078037-0002	KJ196257.1	Canada	2011	80%	80%	80%	80%	80%	80%	80%

Iden= Identity percent

Percent Identity

Divergence

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	
	78.9	77.9	78.2	80.5	95.0	77.1	74.9	75.2	74.9	74.1	76.3	86.2	74.4	77.9	77.6	77.6	78.2	77.6	78.2	77.9	1
21.6		97.7	98.0	89.8	79.9	71.0	68.6	66.0	66.3	65.0	70.0	85.1	67.3	97.4	97.4	97.0	98.3	97.7	97.4	97.7	2
23.0	2.4		98.7	89.8	78.9	70.3	68.0	66.0	66.3	64.4	69.3	84.5	67.0	97.7	96.7	98.0	97.7	97.4	98.3	98.3	3
22.6	2.1	1.4		89.8	79.2	71.0	68.6	66.0	66.3	65.0	70.0	84.8	67.3	97.7	97.4	98.0	98.3	97.4	98.3	99.0	4
19.5	11.4	11.4	11.4		80.2	70.3	68.0	68.6	69.0	68.0	70.0	94.4	67.3	90.1	88.8	89.4	89.4	89.1	89.8	89.4	5
5.1	20.3	21.6	21.2	19.9		76.3	74.7	73.8	73.6	72.7	75.8	85.7	73.6	78.9	78.5	78.5	79.2	78.5	79.2	78.9	6
27.4	33.5	34.6	33.5	34.7	28.5		92.8	70.2	70.5	70.0	96.1	75.8	71.1	70.0	69.6	69.6	70.6	70.0	70.3	70.3	7
30.7	37.3	38.4	37.2	38.6	31.0	7.6		71.3	71.6	71.1	93.9	72.7	73.6	67.7	67.3	67.3	68.3	67.7	68.0	68.0	8
30.3	42.0	42.0	41.9	37.3	32.4	38.4	36.6		99.2	95.9	71.1	72.7	69.4	65.7	65.3	65.3	66.3	65.7	66.0	66.0	9
30.7	41.4	41.4	41.3	36.7	32.9	38.0	36.1	0.8		96.4	71.3	73.0	69.7	66.0	65.7	65.7	66.7	66.0	66.3	66.3	10
32.0	43.8	45.0	43.8	38.4	34.2	38.9	37.1	4.3	3.7		70.8	72.7	68.9	65.0	65.0	65.0	65.3	64.7	65.7	65.0	11
28.8	35.3	36.4	35.3	35.3	29.4	4.0	6.3	37.1	36.7	37.6		74.9	70.8	69.0	68.6	68.6	69.6	69.0	69.3	69.3	12
15.4	13.4	14.2	13.8	2.8	16.1	29.6	34.4	34.3	33.9	34.3	30.9		71.3	84.8	84.2	84.2	84.8	84.2	84.8	84.5	13
31.8	39.5	40.0	39.5	39.6	33.1	36.8	32.8	39.4	38.9	40.4	37.2	36.6		66.3	66.7	67.0	67.0	67.0	66.7	66.7	14
23.1	2.8	2.4	2.4	11.0	21.7	35.2	39.0	42.6	42.0	43.8	37.0	13.8	41.3		98.7	98.7	98.0	98.3	99.0	98.7	15
23.5	2.8	3.5	2.8	12.6	22.1	35.8	39.6	43.3	42.7	43.9	37.7	14.6	40.7	1.4		98.7	98.3	98.7	98.3	98.3	16
23.5	3.1	2.1	2.1	11.8	22.1	35.8	39.6	43.3	42.7	43.9	37.7	14.6	40.1	1.4	1.4		97.7	98.7	99.0	99.0	17
22.6	1.7	2.4	1.7	11.8	21.2	34.1	37.8	41.4	40.8	43.2	35.9	13.8	40.1	2.1	1.7	2.4		99.0	98.0	98.7	18
23.5	2.4	2.8	2.8	12.2	22.1	35.2	39.0	42.7	42.1	44.6	37.1	14.6	40.1	1.7	1.4	1.4	1.0		97.7	98.3	19
22.6	2.8	1.7	1.7	11.4	21.2	34.6	38.4	42.0	41.4	42.6	36.5	13.8	40.7	1.0	1.7	1.0	2.1	2.4		98.7	20
23.0	2.4	1.7	1.0	11.8	21.6	34.6	38.4	42.0	41.4	43.9	36.5	14.2	40.7	1.4	1.7	1.0	1.4	1.7	1.4		21
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	

Variant-2
IBV- CU-2 (SP1)
IBV- Eg/12120s/2012 (SP1)
IBV- Eg/12197B/2012(SP1)
IBV-722/95-SP1
IBV-IS-1494-06-S1
Variant-1
4/91
H120
Ma5
M41
CR88121
D274
SUL/01/09
F726-3
F726-4
F726-5
F726-6
F726-8
F726-9
F726-10

F726-3= isolate No 11 F726-4= isolate No 15 F726-5= isolate No 21 F726-6= isolate No 13

F726-8= isolate No 19 F726-9= isolate No 22 F726-10= isolate No24

Table (2) : Nucleotide identities and divergences of the S1 partial sequence of seven Egyptian IBV isolated strains in this study with other Egyptian strains, reference strains and vaccinal strains present in Egypt.

Embryo mortalities increased with further passage, these findings may suggest that the IBV isolate are field viruses since, they were not embryo adapted (Difabio et al., 2000).

One of the major problems with IBV is the frequent emergence of new variants (Abdel-Moneim et al. 2002; Sediek, 2010). The detection and identification of these new variants is crucial to disease control *(Nakamura et al., 1996)*. So in this study set of primers mentioned by (Adzhar et al., 1997) are used for amplification of S1 gene in AF of ten selected isolates of study, all of selected isolates are positive for S1 gene, which were in agreement with Sarah, 2014. And then seven selected purified PCR products (11, 15, 21, 13, 19, 22, 24) were sent to NLQP, Animal Health Research institute, Egypt for sequencing.

Phylogenetic analysis revealed that the sequences of seven selected Egyptian IBV field isolates in this study (11, 15, 21, 13, 19, 22, 24) found in the same group with IBV-CU-2-SP1 (Afifi et al., 2013), Eg/12120s/2012-SP1 (Arafa et al., 2013), Egypt/01-13(Valastro et al., 2013) and Eg/12197B/2012-SP1(Arafa et al., 2013). The Phylogenetic analysis indicated that the seven selected Egyptian isolates are far from vaccine strains and D274 vaccinal strain is the nearest vaccinal strain present in Egypt to these seven isolates, this agree with Ali, 2013.

Nucleotides identity between the seven selected Egyptian IBV field isolates in this study (11, 15, 21, 13, 19, 22, 24) was ranged from 97.7% to 99%, between these isolates and vaccinal strain used in Egypt (M41, H120, Ma5, 4/91, CR88 and D274) ranged from 64.7%

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to 65.7%, 65.3% to 66.3%, 65.7% to 66.7%, 67.3% to 68.3%, 68.6% to 69.6% and 84.2% to 84.8%, respectively. And between these isolates and IBV-CU-2-SP1 and Eg/12120s/2012 (variant 2 like strain) ranged from 97% to 98.3 % and 96.7% to 98.3%, respectively. Our results are in agreement with the concept that IBV mutates commonly and that endemic variants 1, 2 are cocirculating in Egypt (**Abdel-Moneim et al., 2012 and Sarah, *2014***).

The recent seven Egyptian IBV field isolates in this study were distinctly different from vaccinal strain used in Egypt, M41, H120, Ma5, 4/91, CR88 and D274, (**Abdel-Moneim et al. 2002; Sediek, 2010 and Sarah, 2014**). So, vaccination with one serotype does not ensure complete protection against herterologous serotype (**Sediek, 2005 and 2010)** which emerge by changes in the IBV genome by point mutation, deletions, insertions or RNA recombination **(Zenella et al., 2000; Thor et al., 2011; Hong et., 2012)** which were responsible for outbreaks of IBV in the vaccination chicken flocks. And also differences in as few as 5% of the amino acid in S1 can decrease crossprotection **(Cavanagh, 2007)**, so developing vaccines from local strains is necessary for IBV control in Egypt. In addition to serotype changes, the genetic variation may result in changes of the tissue tropism and pathogenicity of the virus which lead to the generation of new IBV pathotype.

These findings showed no geographical restriction in the distribution of these isolates and their related isolates but the great homology with Israeli isolates still present. Although there is no geographical restriction, many countries share some common antigenic types, IBV strains within a geographic region are unique and distinct; examples of this include Europe, the United States, and Australia (***Ignjatovic, et al. 2002, Callison, et al. 2001and Adzhar, et al. 1997***).

Further epidemiological surveillance studies are needed in order to explain the mechanism of emergence of variants and their biological properties, including pathogenicity, along with developing suitable vaccines from endemic virus strains. Continuous surveillance of new IBV strains is important for understanding the molecular evolution of different genotypes and for selecting candidate virus strains for vaccination regimes.

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