Co-circulation of different Infectious Bronchitis Virus serotypes In Egypt During 2012

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ABSTRACT

Variant strains of Infectious bronchitis virus cause a major problem in the Egyptian poultry industry. A mixture of genetic mutations and or recombination events has been occurred during replication of coronaviruses including IBV which called quasispecies. Serotypic and or genotypic classification of IBV is mainly based on the S1 subunit of Spike (S) gene. In the present study, thirty tracheal samples of broiler flocks suspected to be infected with IBV were collected from different Egyptian governorates during 2012. Isolation and genetic characterizations were carried out for only ten samples which were positive for IBVs. Multiple nucleotide and amino acids alignments revealed prominent nucleotide substitutions which affect amino acids composition of HVR3 of S1 subunit. Phylogenetic analysis revealed that seven strains were belonging to variant 2 group of IBV of great similarity with Israeli IB strains, two strains were belonging to IS/1494/06 group like and one strain was belonging to 4/91 group like. This study reports the co-circulation of different IBV serotypes in broiler sectors. Regular monitoring for evolution, epidemiology and genotyping of Egyptian IBV field strains is very important for screening the emergence of new variant strains as well as evaluating the existing vaccination programs.

Key words: Quasispecies; Serotypic; Genotypic; Co-circulation; Evolution.

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INTRODUCTION

Infectious bronchitis virus (IBV) causes a highly contagious disease that results in great economic losses to the commercial chicken industry. The disease frequently causes respiratory signs and decrease in egg production in layers (Gough et al., 2008). In addition, some virus strains have been associated with kidney lesions (Ziegler et al., 2002 and Liu and Kong, 2004). All ages of chickens are susceptible to IBV infection, but the clinical signs are more severe in young chickens (Liu and Kong, 2004). Furthermore, the disease is an important enhancing factor for secondary bacterial infections resulting in higher morbidity and mortality rate (Ziegler et al., 2002).

IBV belongs to genus Coronavirus of family *Coronaviridae* (Cavanagh et al., 1986). It is an enveloped virus with positive-sense single-stranded RNA genome of approximately 27 kb in length. The virion has three major structural proteins; the nucleocapsid (N) protein, the membrane (M) protein, and the spike (S) glycoprotein. The S glycoprotein is post-translationally cleaved into the S1 and S2 subunits (Cavanagh et al., 1988). The S1 subunit located outside the virion and was responsible for fusion between the virus envelope and the host cell membrane and induction of neutralizing serotype-specific

antibodies in chickens (Boursnell et al., 1987). The S1 subunit demonstrates more sequence variability than S2 (Kusters et al., 1989; Cavanagh et al., 1988). Neutralizing and serotype-specific epitopes are associated with the defined hypervariable region (HVR) in the S1 subunit; therefore, the molecular characterization of IBV is based on analysis of the S1 gene (Kingham et al., 2000).

IBV has a worldwide distribution, and many variant strains have been isolated (Davelaar, 1985; Wang et al., 1994; Liu and Kong, 2004). In general, coronaviruses including IBV have been shown mixture of genetic mutants and recombination events that occur during viral replication which called quasispecies as the viral RNA-dependent RNA-polymerase lacks the proofreading capabilities (Lai and Holmes, 2001; Jackwood et al., 2003; Zhang et al., 2007; Jackwood, 2011). Therefore, several studies were carried out to highlight the role of three factors in the development of IBV quasispecies: (1) lack of RNA polymerase proofreading (2) interference of continuous use of live and often multiple attenuated vaccines formulated with different IBV strains, and (3) vaccination pressure exerted on the circulating viruses by the persistence of partially immune bird populations (Ignjatovic and Spats, 2005, Vijaykrishna et al., 2007 and Montassier, 2010).

In Egypt, IBV was first described by Ahmed (1954), followed by several reports that emphasized the prevalence of the disease. Currently, the variant strains of IBV are extensively prevalent in commercial broiler chicken sectors in Egypt since the early months of 2010. Genotyping of IBV field strains is very important for screening the emergence of new variant strains as well as evaluating the existing vaccination programs (Hussein et al., 2013).

In the present study, we describe the evolutionary dynamic of IBVs infections circulating among the Egyptian broiler sectors during 2012 based on the molecular characterization, sequencing and phylogenetic analysis.

MATERIALS AND METHODS

Samples

Tracheal samples were collected from suspected clinically infected flocks associated with high mortality rates from different governorates in Egypt during 2012 showing characteristic clinical signs and pathognomic postmortem lesions **(**Table 1). Homogenized tissue samples of infected birds were inoculated in specific pathogen free (SPF) eggs for three passages according to OIE 2008.

Oligonucleotides

The primers were used according to Adzhar et al., (1997) and manufactured by Metabion ® Company (Germany). Primers were as follow; XCE1: 5'-CAC TGG TAA TTT TTC AGA TG-3' and XCE2: 5'-CTC TAT AAA CAC CCT TAC A-3', these primers target S1 gene of IBV and amplify a fragment with expected size 464 bp. The primers were received in lyophilized form and re-suspended in TE buffer making concentrated stock solution from which a working solution was made by dilution in nuclease free water to reach a final concentration of 10 µM and then stored at -80ºC.

Viral RNA extraction and RT-PCR assay

RNA was extracted from a tracheal homogenate using Trizol LS® reagent (Invitrogen, UK) according to the manufacturer's instructions. One-step RT-PCR was performed using the verso one

step RT-PCR kit (Thermo, UK). RT-PCR was performed in 25-ul reaction volumes containing 12.5 µl of the 2 X thermo RT-master mix, 1.25 µl enhancer, 0.5 µl Reverse Transcriptase (RT) enzyme, 0.5 µl of each primer (10 pmol of each primer) and 5 µl extracted RNA. The final volume was reached by adding 3.75 µl RNase- free water.

Thermal cycling profile was consisting of: reverse transcription at 50°C for 15 min, followed by an initial denaturation step at 95°C for 2 min. cDNAs were then amplified with 40 cycles of 95°C for 30 seconds, annealing at 48°C for 1 minute and extension at 72°C for 1 minute, followed by a final extension step at 72°C for 10 min. The amplified product (5µl) were loaded onto 1.5% agarose gel containing 0.5 µg/ml ethidium bromide for nucleic acid visualization. Electrophoresis was conducted using 1x TAE buffer and PCR products were visualized under UV trans-illumination.

Sequencing and sequence analysis

PCR products were purified separately from gels using QIAEX® Gel Extraction Kit (Qiagen Sciences, Maryland, USA). Purified PCR products were sequenced in both orientations by the dideoxy chain-termination method using the amplification primers which described above. Gene sequencing was carried out using a BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA) in an ABI PRISM® 3100. BioEdit software version 7.0.4.1 (Hall, 1999) was used to analyze and assemble the generated nucleotide sequences of the HA gene. The GenBank database was screened (BLAST) for closely related sequences. Comparative analysis of S1 gene sequences of the IBV viruses was carried out and compared with the available sequences using the National Center for Biotechnology Information (NCBI) IBV virus resources database.

Phylogenetic analysis of the newly obtained nucleotide sequences in this study was conducted using MEGA 4.0.2 software. The evolutionary history was inferred using the neighbour-joining method and the reliability of each tree branch was estimated by performing 1000 bootstrap replicates (Tamura et al., 2007).

Sequence submission to GenBank

The obtained sequence was submitted to NCBI GenBank by following instruction of the BankIt tool of the GenBank.

Agar gel precipitation test (AGPT)

AGPT was performed on the allantoic fluids of the third passage of three selected IBV isolates designated IBV/Egypt/VRLCU-01/2012 and IBV/Egypt/VRLCU-06/2012 (Variant-like group) and IBV/Egypt/VRLCU-10/2012. The procedure was performed according to (Witter, 1962) using reference antiserum for IBV (GD diagnostic company, Germany).

RESULTS

Nucleotide sequence analysis of partial S1 gene of IBV

To further identify the genetic characteristics of Egyptian IBVs, partial nucleotide sequence of S1 gene of IBV was carried out and compared with other representative circulating Egyptian strains among poultry sectors. Nucleotide Identity percentages for the isolates indicate that there are seven isolates were of high similarity with variant group 2 viruses of identity 99%; two isolates were highly similar ranged between 97% and 99% with IS/1494/06 variant group and only one isolate has great identity with 4/91 group which were ranged between 97% and 99%. These results indicate the

progressive and extensive genetic diversity and high evolution rate for the circulating IB Egyptian strains. Multiple nucleotide and amino acids alignment of the S1 subunit with geographically related strains were conducted to establish the genetic spectrum, origin and evolution of IBVs circulated in Egypt were conducted which revealed characteristic nucleotide substitutions at different sites on S1 subunit (Table 2).

Mutation trend analysis for hyper variable region 3 of IBV:

Multiple amino acids alignment revealed the presence of characteristic prominent mutations at S1 subunit (Figure 1). In the S1 subunit, HVR3 is located within S1 subunit (274–387 residue) which contains sequences associated with specific IBV serotypes as well as serotype specific neutralizing epitopes (Abdel-Moneim et al., 2006). The amino acids substitution sites were described in (Table 3).

Phylogenetic analysis:

Phylogenetic analysis of the isolated IBVs in this study revealed that there are 2 isolate designated as IBV/ Egypt/ Gharbia /VRLCU- 08/2012 and IBV/ Egypt/ Menofia/ VRLCU- 09/2012 are related to IS/1494/06 IBV group like viruses. The isolates that showed higher identity with variant group 2 like viruses were designated as follow; IBV/ Egypt/ VRLCU- 01/2012, IBV/ Egypt/ VRLCU- 02/2012, IBV/ Egypt/ VRLCU- 03/2012, IBV/ Egypt/ VRLCU- 04/2012, IBV/ Egypt/ Gharbia/ VRLCU- 05/2012, IBV/ Egypt/ Menofia/ VRLCU- 06/2012 and IBV/ Egypt/ Gharbia/ VRLCU- 07/2012. Only one isolate designated; IBV/ Egypt/ VRLCU- 10/2012 is belonging to 4/91 group like viruses (Figure 2).

GenBank accession numbers

GenBank accession numbers were received from web submission using the GenBank BankIt tool and were as (Table 4).

AGPT

The two isolates IBV/ Egypt/ VRLCU- 01/2012 and IBV/ Egypt/ VRLCU- 06/2012 which are identical to variant-like groups showed connected white line that indicate complete antigenic relationship between them.

Table (1): Data of the ten tracheal collected samples from different Egyptian broiler sectors during 2012

Lab code	Governorate	Age of flock	Flock total number
1	Cairo	27 day	15,500
2	El-Giza	30 day	20,000
3	El-Behira	35 day	15,300
4	EL-Gharbia	29 day	10,000
5	El-Giza	35 day	18,000
6	EL-Giza	33 day	20,500
7	EL-Gharbia	31 day	22,200
8	El-Behira	30 day	15,000
9	EL-Gharbia	32 day	11,000
10	El-Menoufia	25 day	10,500

*Morbidity rate was approximately 90%.

*Mortality rate was ranged between 60 %and 75%.

Table (2): Characteristic nucleotide substitution sites for Egyptian IBVs under study during 2012

Strain	Nucleotide substitution sites
IBV/Egypt/VRLCU-01/2012	C823T
IBV/Egypt/VRLCU-02/2012	C823T
IBV/Egypt/VRLCU-03/2012	C879T
IBV/Egypt/VRLCU-04/2012	C928T
IBV/Egypt/Gharbia/VRLCU-05/2012	C823T, C891T
IBV/Egypt/Menofia/VRLCU-06/2012	A761G, C823T
IBV/Egypt/Gharbia/VRLCU-07/2012	C823T, A922T, A924T

Table (3): Characteristic amino acids substitution sites for Egyptian IBVs under study during 2012

Strain	Amino acid substitution sites
KC292036.1 IBV/Egypt/VRLCU-01/2012	**H275Y**
KC292037.1 IBV/Egypt/ VRLCU -02/2012	**H275Y**
KC292032.1 IBV/Egypt/Gharbia/ VRLCU -05/2012	**H275Y, T297I**
KC292033.1 IBV/Egypt/Menofia/ VRLCU -06/2012	**H275Y**
KC292034.1 IBV/Egypt/Gharbia/ VRLCU -07/2012	**H275Y**

Table (4): GenBank accession numbers for Egyptian IBVs under study during 2012

Strain	Accession numbers
IBV/Egypt/ VRLCU-01/2012	KC292036.1
IBV/Egypt/ VRLCU-02/2012	KC292037.1
IBV/Egypt/ VRLCU-03/2012	KC292038.1
IBV/Egypt/ VRLCU-04/2012	KC292039.1
IBV/Egypt/Gharbia/ VRLCU-05/2012	KC292032.1
IBV/Egypt/Menofia/ VRLCU-06/2012	KC292033.1
IBV/Egypt/Gharbia/ VRLCU-07/2012	KC292034.1
IBV/Egypt/Gharbia/ VRLCU-08/2012	KC292030.1
IBV/Egypt/Menofia/ VRLCU-09/2012	KC292031.1
IBV/Egypt/ VRLCU-10/2012	KC292035.1

Figure 1

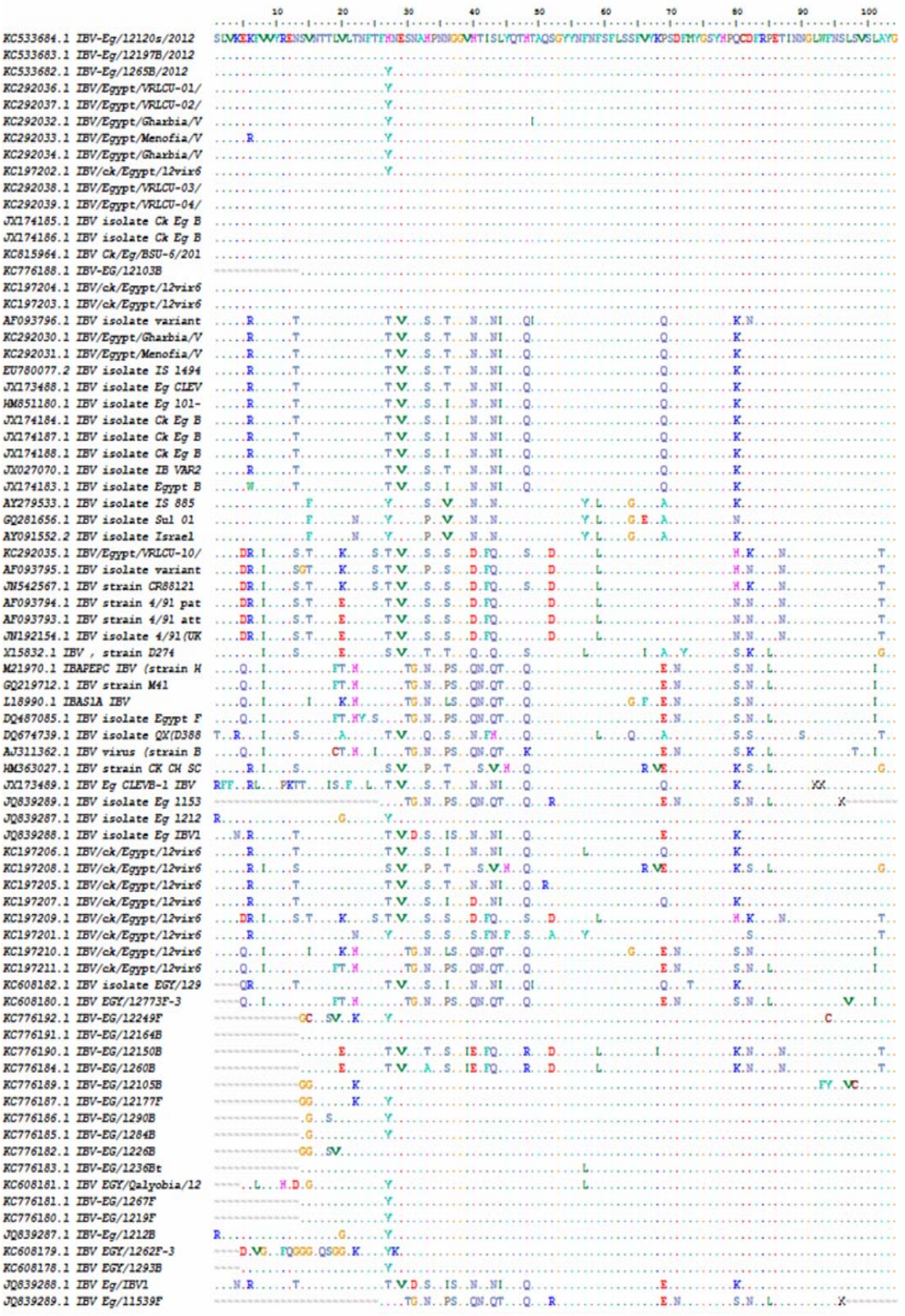
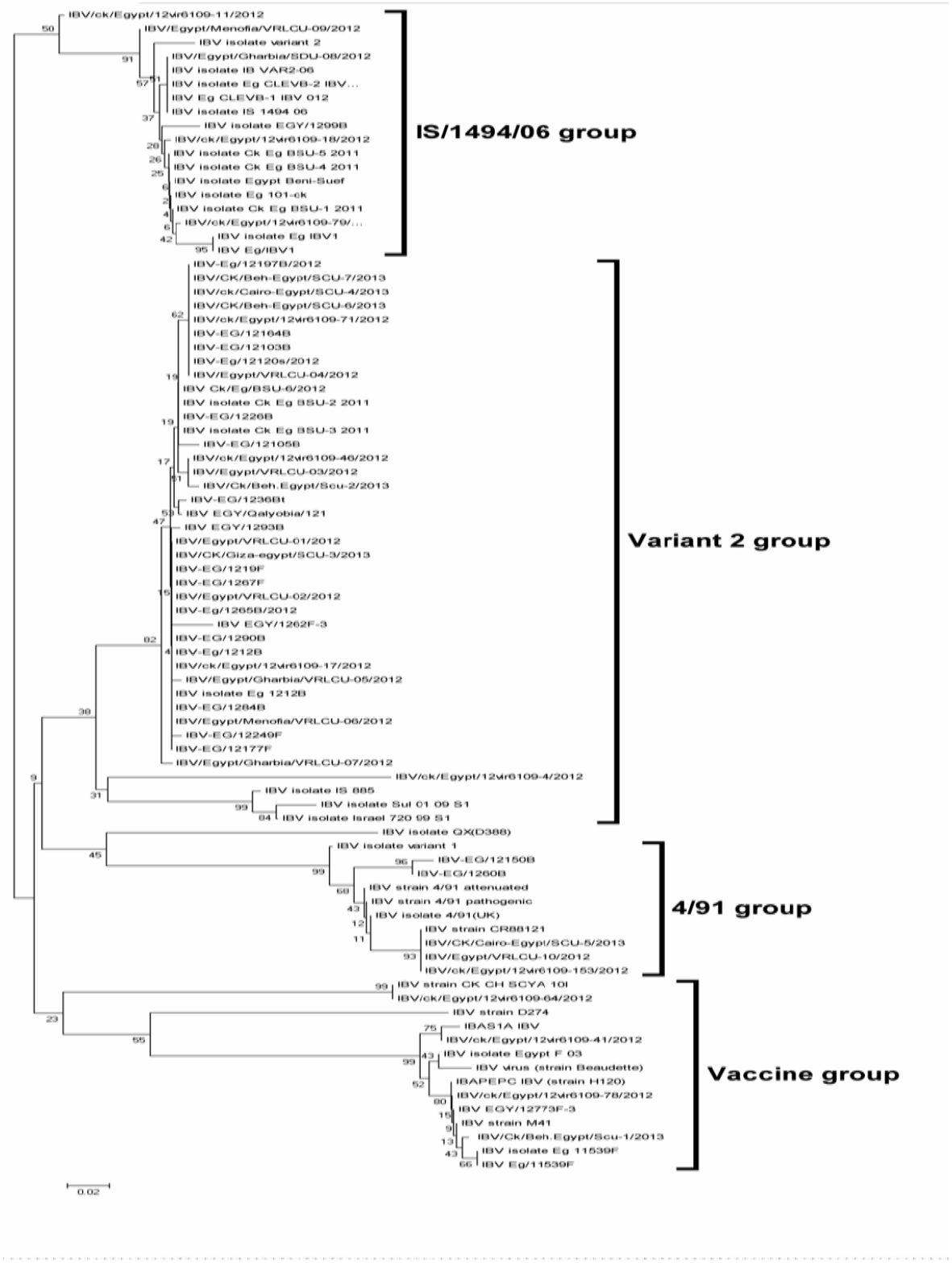

10
20
30
40
50
60
70
80
90
100
KC533684.1 IBV-Eg/12120s/2012
KC533683.1 IBV-Eg/12197B/2012
KC533682.1 IBV-Eg/1265B/2012
KC292036.1 IBV/Egypt/VRLCU-01/
KC292037.1 IBV/Egypt/VRLCU-02/
KC292032.1 IBV/Egypt/Gharbia/V
KC292033.1 IBV/Egypt/Menofia/V
KC292034.1 IBV/Egypt/Gharbia/V
KC197202.1 IBV/ck/Egypt/12vir6
KC292038.1 IBV/Egypt/VRLCU-03/
KC292039.1 IBV/Egypt/VRLCU-04/
JX174185.1 IBV isolate Ck Eg B
JX174186.1 IBV isolate Ck Eg B
KC815964.1 IBV Ck/Eg/BSU-6/201
KC776188.1 IBV-EG/12103B
KC197204.1 IBV/ck/Egypt/12vir6
KC197203.1 IBV/ck/Egypt/12vir6
AF093796.1 IBV isolate variant
KC292030.1 IBV/Egypt/Gharbia/V
KC292031.1 IBV/Egypt/Menofia/V
EU780077.2 IBV isolate IS 1494
JX173488.1 IBV isolate Eg CLEV
HM851180.1 IBV isolate Eg 101-
JX174184.1 IBV isolate Ck Eg B
JX174187.1 IBV isolate Ck Eg B
JX174188.1 IBV isolate Ck Eg B
JX027070.1 IBV isolate IB VAR2
JX174183.1 IBV isolate Egypt B
AY279533.1 IBV isolate IS 885
GQ281656.1 IBV isolate Sul 01
AY091552.2 IBV isolate Israel
KC292035.1 IBV/Egypt/VRLCU-10/
AF093795.1 IBV isolate variant
JN542567.1 IBV strain CR88121
AF093794.1 IBV strain 4/91 pat
AF093793.1 IBV strain 4/91 att
JN192154.1 IBV isolate 4/91(UK
X15832.1 IBV , strain D274
M21970.1 IBAPEPC IBV (strain H
GQ219712.1 IBV strain M41
L18990.1 IBAS1A IBV
DQ487085.1 IBV isolate Egypt F
DQ674739.1 IBV isolate QX(D388
AJ311362.1 IBV virus (strain B
HM363027.1 IBV strain CK CH SC
JX173489.1 IBV Eg CLEVB-1 IBV
JQ839289.1 IBV isolate Eg 1153
JQ839287.1 IBV isolate Eg 1212
JQ839288.1 IBV isolate Eg IBV1
KC197206.1 IBV/ck/Egypt/12vir6
KC197208.1 IBV/ck/Egypt/12vir6
KC197205.1 IBV/ck/Egypt/12vir6
KC197207.1 IBV/ck/Egypt/12vir6
KC197209.1 IBV/ck/Egypt/12vir6
KC197201.1 IBV/ck/Egypt/12vir6
KC197210.1 IBV/ck/Egypt/12vir6
KC197211.1 IBV/ck/Egypt/12vir6
KC608182.1 IBV isolate EGY/129
KC608180.1 IBV EGY/12773F-3
KC776192.1 IBV-EG/12249F
KC776191.1 IBV-EG/12164B
KC776190.1 IBV-EG/12150B
KC776184.1 IBV-EG/1260B
KC776189.1 IBV-EG/12105B
KC776187.1 IBV-EG/12177F
KC776186.1 IBV-EG/1290B
KC776185.1 IBV-EG/1284B
KC776182.1 IBV-EG/1226B
KC776183.1 IBV-EG/1236Bt
KC608181.1 IBV EGY/Qalyobia/12
KC776181.1 IBV-EG/1267F
KC776180.1 IBV-EG/1219F
JQ839287.1 IBV-Eg/1212B
KC608179.1 IBV EGY/1262F-3
KC608178.1 IBV EGY/1293B
JQ839288.1 IBV Eg/IBV1
JQ839289.1 IBV Eg/11539F

Figure 2


IBV/ck/Egypt/12vir6109-11/2012
IBV/Egypt/Menofia/VRLCU-09/2012
IBV isolate variant 2
IBV/Egypt/Gharbia/SDU-08/2012
IBV isolate IB VAR2-06
IBV isolate Eg CLEVB-2 IBV...
IBV Eg CLEVB-1 IBV 012
IBV isolate IS 1494 06
IBV isolate EGY/1209B
IBV/ck/Egypt/12vir6109-18/2012
IBV isolate Ck Eg BSU-5 2011
IBV isolate Ck Eg BSU-4 2011
IBV isolate Egypt Beni-Suef
IBV isolate Eg 101-ck
IBV isolate Ck Eg BSU-1 2011
IBV/ck/Egypt/12vir6109-79/...
IBV isolate Eg IBV1
IBV Eg/IBV1
IS/1494/06 group
IBV-Eg/12197B/2012
IBV/CK/Beh-Egypt/SCU-7/2013
IBV/ck/Cairo-Egypt/SCU-4/2013
IBV/CK/Beh-Egypt/SCU-6/2013
IBV/ck/Egypt/12vir6109-71/2012
IBV-EG/12164B
IBV-EG/12103B
IBV-Eg/12120s/2012
IBV/Egypt/VRLCU-04/2012
IBV Ck/Eg/BSU-6/2012
IBV isolate Ck Eg BSU-2 2011
IBV-EG/1226B
IBV isolate Ck Eg BSU-3 2011
IBV-EG/12105B
IBV/ck/Egypt/12vir6109-46/2012
IBV/Egypt/VRLCU-03/2012
IBV/Ck/Beh.Egypt/Scu-2/2013
IBV-EG/1236Bt
IBV EGY/Qalyobia/121
IBV EGY/1293B
IBV/Egypt/VRLCU-01/2012
IBV/CK/Giza-egypt/SCU-3/2013
IBV-EG/1219F
IBV-EG/1267F
IBV/Egypt/VRLCU-02/2012
IBV-Eg/1265B/2012
IBV EGY/1262F-3
IBV-EG/1290B
IBV-Eg/1212B
IBV/ck/Egypt/12vir6109-17/2012
IBV/Egypt/Gharbia/VRLCU-05/2012
IBV isolate Eg 1212B
IBV-EG/1284B
IBV/Egypt/Menofia/VRLCU-06/2012
IBV-EG/12249F
IBV-EG/12177F
IBV/Egypt/Gharbia/VRLCU-07/2012
IBV/ck/Egypt/12vir6109-4/2012
IBV isolate IS 885
IBV isolate Sul 01 09 S1
IBV isolate Israel 720 99 S1
Variant 2 group
IBV isolate QX(D388)
IBV isolate variant 1
IBV-EG/12150B
IBV-EG/1260B
IBV strain 4/91 attenuated
IBV strain 4/91 pathogenic
IBV isolate 4/91(UK)
IBV strain CR88121
IBV/CK/Cairo-Egypt/SCU-5/2013
IBV/Egypt/VRLCU-10/2012
IBV/ck/Egypt/12vir6109-153/2012
4/91 group
IBV strain CK CH SCYA 10I
IBV/ck/Egypt/12vir6109-64/2012
IBV strain D274
IBAS1A IBV
IBV/ck/Egypt/12vir6109-41/2012
IBV isolate Egypt F 03
IBV virus (strain Beaudette)
IBAPEPC IBV (strain H120)
IBV/ck/Egypt/12vir6109-78/2012
IBV EGY/12773F-3
IBV strain M41
IBV/Ck/Beh.Egypt/Scu-1/2013
IBV isolate Eg 11539F
IBV Eg/11539F
Vaccine group
0.02

DISCUSSION

Occurrence of frequent mutations in the IBV genome results in evolving viruses that have a mixed population called quasispecies. The continuous generations of mutant viruses or quasispecies subpopulations may result in adaptation of such viruses to different tissues in its host, evade the immune system and become transmissible to a new host (van Santen and Toro, 2008). Natural outbreaks of IBV are controlled through the use of vaccines. In spite of the routine use of vaccines, IBV outbreaks continue to occur in vaccinated poultry (Gelb et al., 1991; Wang and Tsai, 1996; Liu and Kong, 2004). Previous molecular studies revealed that the S1 subunit of Spike (S) gene is responsible for determining IBV serotypes (Cook et al., 1999). Furthermore, new IBV serotypes could arise as a result of few changes in the amino acids of the S1 subunit (Cavanagh and Naqi, 2003). S1 subunit has three HVRs, located within amino acids 38–67, 91–141 and 274–387 (Cavanagh et al., 1988 and Moore et al., 1997). The HVR was an essential determinant of coronavirus serotype specificity (Cavanagh and Naqi, 2003). HVR1 and HVR2 contain sequences that have been associated with specific IBV serotypes (Binns et al., 1986 and Kusters et al., 1989) as well as serotype specific neutralizing epitopes (Cavanagh et al., 1988; Koch et al., 1990 and Kant et al., 1992). Different serotypes, subtypes, and variant strains of IBV are thought to be generated by nucleotide point mutations, insertions and or deletion which are responsible for the emergence of IB outbreaks in vaccinated chicken flocks (Wang et al., 1994 and Jia et al., 1995).

One of the major problems of IBV is the frequent emergence of new variant strains (Yu et al., 2001). Different serotypes have been reported world wide and new variant serotypes continue to be recognized (Gelb et al., 1991 and Liu and Kong, 2004). Thus, it is necessary and important for diagnosis of these new serotypes. Molecular characterization using sequencing and phylogenetic analysis are effective methods for detection and characterization of recombination events among RNA viruses (Tamura et al., 2007). RT-PCR has been described previously as an efficient assay for detection of IBV and for further genotyping of IBV (Jackwood et al., 1997; Keeler et al., 1998; Handberg, et al., 1999 and Meulemans et al., 2001). RT- PCR was usually carried out on the S1 subunit, followed by sequencing for genotyping of IBV isolates (Adzhar et al., 1997). Therefore, recent genetic grouping of IBV has been performed mainly based on the nucleotide sequence of the S1 subunit (Keeler et al., 1998; Wang et al., 1996; Wang and Huang, 2000; Yu et al., 2001; Lee et al. 2004).

In the present study, RT-PCR was carried out on tracheal homogenate for amplification of internal part of S1 subunit 464bp using specific primers. The choice of this part of S1 gene of IBV as it encodes for the most important neutralizing epitopes of the virus, and so any change in its nucleotide sequence can lead to change in the antigenic sites and immunogenic capability. Multiple nucleotide alignment of S1 subunit of S gene revealed prominent nucleotide substitutions. Multiple amino acids alignment of S1 subunit revealed amino acids difference from the circulating Egyptian IBV strains.

Hussein et al., (2013) reported that most of the recent Egyptian isolates are clustered within at least 4 variants

strains (variant 1, variant 2, 4/91 like and Q-like) and vaccinal strains in the poultry sectors in Egypt. In this study, phylogenetic analysis of the ten IBV isolates revealed that the Egyptian isolates designated IBV/ Egypt/ VRLCU- 01/2012, IBV/ Egypt/ VRLCU- 02/2012, IBV/ Egypt/ VRLCU- 03/2012, IBV/ Egypt/ VRLCU- 04/2012, IBV/ Egypt/ Gharbia/ VRLCU- 05/2012, IBV/ Egypt/ Menofia/ VRLCU- 06/2012 and IBV/ Egypt/ Gharbia/ VRLCU- 07/2012 are closely related to the variant 2 circulating Egyptian isolates. While there are 2 isolates designated IBV/Egypt/Gharbia/VRLCU-08/2012 and IBV/Egypt/Menofia/VRLCU-09/2012 are closely related to IS/1494/06 group like that suggest the same origin of both viruses. IBV IS/1494/06 like strains were similar to the recently reported strains in Jordan, Israel and some Middle East countries suggesting the contribution of such strains in the mortalities in broiler sectors which started to increase by the end of 2011 and continue (Hussein et al., 2013). Only one isolate designated IBV/Egypt/ VRLCU-10/2012 is closely related to CR88 group like. These results revealed that progressive evolution of the circulating Egyptian IBVs which facilitate possible recombination events between different subgroups of IBVs.

Mutations and or recombination events enable IBV to shift host or change tissue tropism. Recombination may provide the evolutionary advantages through generation of diverse virus population that could be resistant vaccination and the host immune system (Thor et al., 2011). Previous studies reported that random recombination may occur between different IBV serotypes and also between field and vaccine viruses to produce mosaic progeny viruses (Mckinley, 2008; Thor et al., 2011). Also, contributions of mixed infection viruses augment the losses due to IBV infections and may contribute for he occurrence of IBV epidemic (Hussein et al., 2013). This study reports the co-circulation of different IBV serotypes with continuous evolution among the Egyptian poultry sectors. Regular monitoring and screening for the Egyptian poultry sectors must be conducted to study the evolution and epidemiology of such viruses.

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