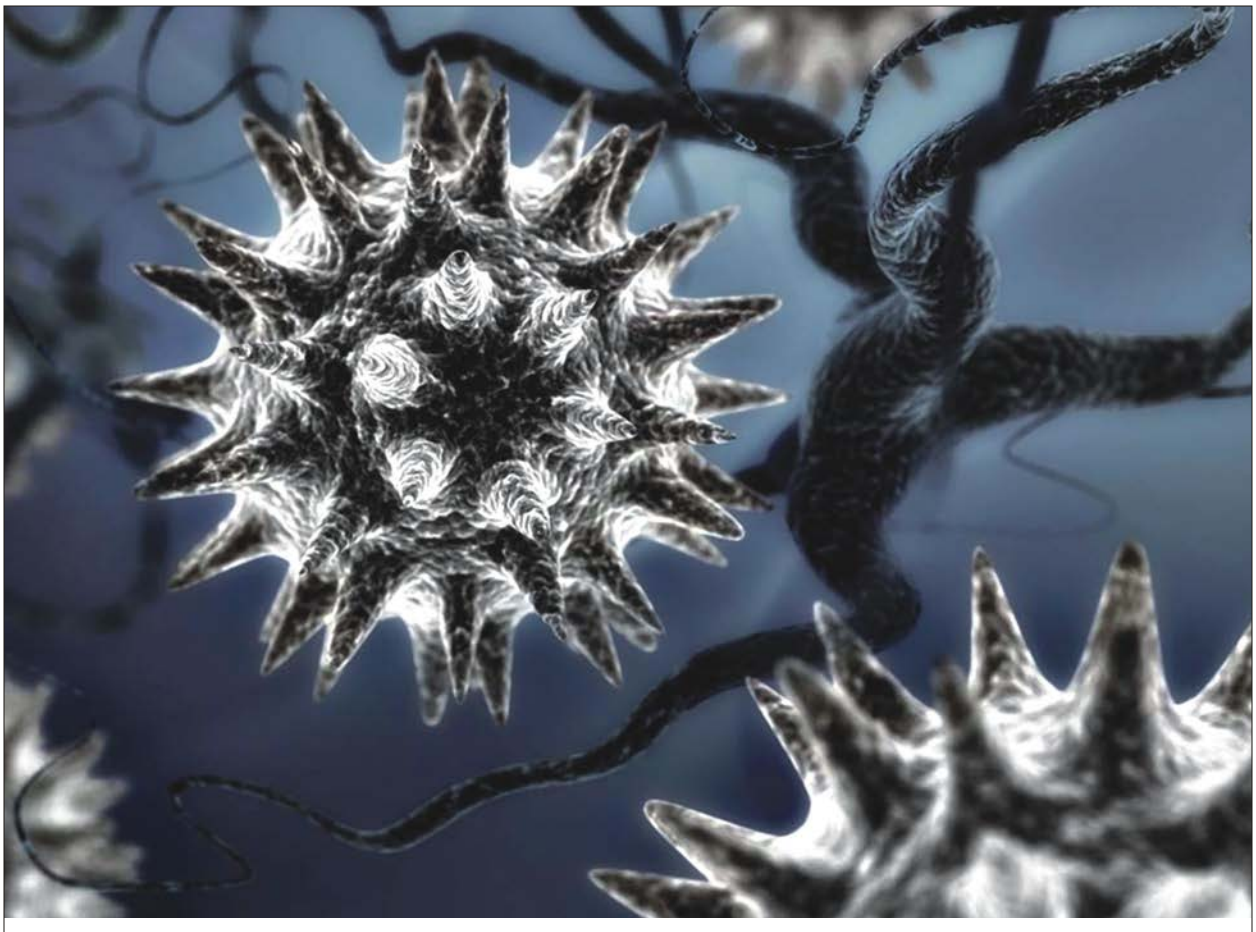

International Journal of Virology

ISSN 1816-4900

Academic Journals Inc.

www.academicjournals.com

International Journal of Virology 10 (1): 70-76, 2014
ISSN 1816-4900 / DOI: 10.3923/ijv.2014.70.76

Detection and Molecular Characterization of Infectious *Bronchitis virus* Isolated from Recent Outbreaks in Chicken Farms in Egypt 2012

[1]Karim Selim, [1]Abdelsatar Arafa, [2]Hussein A. Hussein and [2]Ahmed A. El-Sanousi
[1]National Laboratory for Veterinary Quality Control on Poultry Production, Animal Health Research Institute, P.O. Box 264, Dokki, 12618, Giza Egypt
[2]Department of Virology, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt

Corresponding Author: Karim Selim, National Laboratory for Veterinary Quality Control on Poultry Production, Animal Health Research Institute, P.O. Box 264, Dokki, 12618, Giza, Egypt

ABSTRACT

Egyptian viruses of infectious bronchitis of 13 isolates were collected between period from 1/1/2012 to 1/8/2012 from 200 samples were collected and examined with PT-PCR then choose positive sample from different governorates. Thirteen avian Infectious *Bronchitis virus* (IBV) isolates were isolated from broiler chickens showing respiratory signs. The isolated strains were characterized by reverse transcriptase polymerase chain reaction and sequence analysis of the hypervariable region of the S1 spike glycoprotein gene and study different and identity between them then compare between them by using phylogenetic analysis using Megalign software. Eleven out of thirteen isolates formed adistinct phylogenetic group with (IS/885) with identity 89.9%. Two of the thirteen isolates showed 97% amino acid sequence identity between them and 82.3% nucleotide sequence identity to the Egyptian variant 2 and the IS/1494/06 strains, respectively.

Key words: Infectious *Bronchitis virus*, PT-PCR, phylogenetic analysis, Egyptian variant

INTRODUCTION

Infectious Bronchitis (IB) is an acute and highly contagious respiratory disease of chickens. The disease is characterized by respiratory signs including gasping, coughing, sneezing, tracheal râles, andnasal discharge. In young chickens, severer espiratory distress may occur. In layers, respiratory distress, decrease in egg production and loss of internal egg quality and egg shell quality are reported. Some strains of the virus cause severe kidney damage and may be associated with high mortality (Butcher *et al*., 2011).

The virus is a member of the genus *Corona virus*, family Coronaviridae, in theorder Nidovirales. IBV and other avian coronaviruses of turkeys and pheasants are classified as group 3 coronaviruses, with mammalian corona viruses comprising groups 1, 2 and 4 (group 4 being the more recently identified Severe Acute Respiratory Syndrome (SARS) *Corona virus*) (Cavanagh, 2003).

IB affects chickens of all ages which, apart from pheasants are the only species reported to be naturally affected. The disease is transmitted by the air-borne route, direct chicken to-chicken contact and indirectly through mechanical spread (contaminated poultry equipment or egg-packing materials, manure used as fertilizer, farm visits, etc.) (OIE Terrestrial Manual, 2008). Infectious *Bronchitis virus* is an enveloped and positive sense single stranded RNA virus containing an unsegmented genome approximately 27.6 kb in size. The virion has four structural proteins:

Nucleocapsid protein (N), membrane glycoprotein (M) small envelope protein (E) and glycosylation spikeglycoprotein (S) (Su *et al*., 2012).

One of the major structure proteins of IBV is spike proteins that is cleaved into two smaller proteins namely S1 and S2. S1 gene contains two hypervariable regions that are responsible for the induction of neutralizing and serotype specific antibodies (Haqshenas *et al*., 2005).

Accordingly, genotyping of IBV field strains is very important for screening the emergence of new variants as well as evaluating the existing vaccination programs. IBV strains related to the Massachusetts D3128, D274, D-08880 and 4/91 genotypes have been detected at different poultry farms in Egypt (Abdel-Moneim *et al*., 2006; El-Kady, 1989; Sheble *et al*., 1986; Sultan *et al*., 2004).

Variation in S1 sequences has been recently used for distinguishing between different IBV serotypes. Diversity in S1 probably results from mutation, recombination and strong positive selection *in vivo*. Antigenically 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 (Abdel-Moneim *et al*., 2006).

MATERIALS AND METHODS

Sampling: In this study about 200 samples were collected during surveillance from 1/1/2012 to 1/8/2012 for study infection with IBV from different governorates.

Only 13 samples were selected for study based on sample from different governorates as show in Table 1.

RNA extraction: Viral RNA was extracted by using Viral Nucleic Acid Extraction Kit (Qiampviral RNA mini Kit- Cat No. 52904. Gmb H, Hilden, Germany) following the manufacturer's instructions directly from the supernatants of 10% w/v sample suspensions and from the allantoicfluid of embryonated chicken eggs used for virus isolation.

Virus isolation and propagation: For virus isolation, the supernatants of IBV positive samples determined by RT-PCR were inoculated into 10-day-old embryonated chicken eggs. For each sample to be examined, five embryonated chicken eggs were used. The eggs were inoculated with 0.2 mL of the sample into the allantoic cavity. The inoculated eggs were incubated at 37°C and candled

Table 1: Selected samples for sequencing

Isolate ID	Governorates
IBV-EG/1219F(CPC)-SP1	Al Sharkia
IBV-EG/1226B-SP1	Al Fayom
IBV-EG/12164B-SP1	Al Ismalia
IBV-EG/12105B-SP1	Al manya
IBV-EG/1290B-SP1	Luxor
IBV-EG/12249F(10D)-SP1	Al Behara
IBV-EG/1267F-SP1	Alexandria
IBV-EG/1284B-SP1	Diemetta
IBV-EG/12103B-SP1	Al Dakahlia
IBV-EG/12177F-SP1	Giza
IBV-EG/1236B-SP1	Monofia
IBV-EG/1260B-SP1	Bansuif
IBV-EG/12150B-SP1	Al Qaluobia

daily. Allantoic fluids were harvested at 96 h postinoculation. A further blind serial passage was performed in a similar way. All of the allantoic fluids were harvested and stored at -70°C and embryo examined for curling and dwarfism (Pohuang *et al*., 2009).

Product purification and sequencing: The RT-PCR products were cut from the gel and purified using the QIA quick gel extractionkit (Qiagen, Gmb H, Hilden Germany). Purified RT-PCR products were sequenced in a forward direction using primer IBV-S1 and in a reverse direction using primer IBV-S1 (Adzhar *et al*., 1997). Sequencing reactions were performed with the for performing gene sequencing using an Applied Biosystems 3130 genetic analyzer (HITACHI, Japan). Using Big dye Terminator V 3.1 cycle sequencing kit (Perkin-Elmer, Foster city, CA) and amplification 25 cycles at 96°C for 1 min then 50°C for 5 sec finally 60°C for 2 min.

Sequences and phylogenetic analysis: To identify the Egyptian IBV isolates, sequences of the S1gene of the Egyptian IBV isolates were compared with published IBV sequences deposited in the GenBank database using a BLAST search via the National Center of Biotechnology Information (USA). Sequence identities by BLAST analysis were included in alignment and phylogenetic construction. A phylogenetic tree of the nucleotide sequences was constructed using megalign gene sequences from the GenBank database were used for comparison or phylogenetic analysis in this study included M41, Ma5 and H120, Connecticut, CR88, QXIBV, 4/91, D41, D274, Egypt-F-o3, IR/4/2010-s1, IBV-Sul/01/09-S1, IS/1366-Sp1, IS/236-S1, IBV-S1-1494 and IS-885-S1.

RESULTS

Virus screening and isolation: After egg inoculation with serial blind passage and collect allantoic fluid resulted in (Fig. 1).

Results of conventional RT- PCR for S1 gene: All selected samples are positive as shown at 400 bp (Fig. 2).

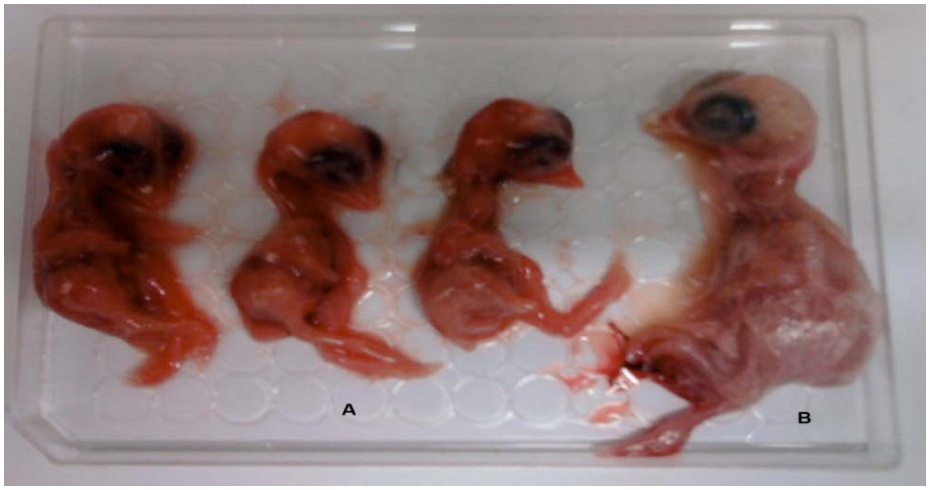

Fig. 1(a-b): Showing embryonic lesions (a) Postive with RT-PCR and (b) Curling, dwarfing and subcutaneous hemorrhage

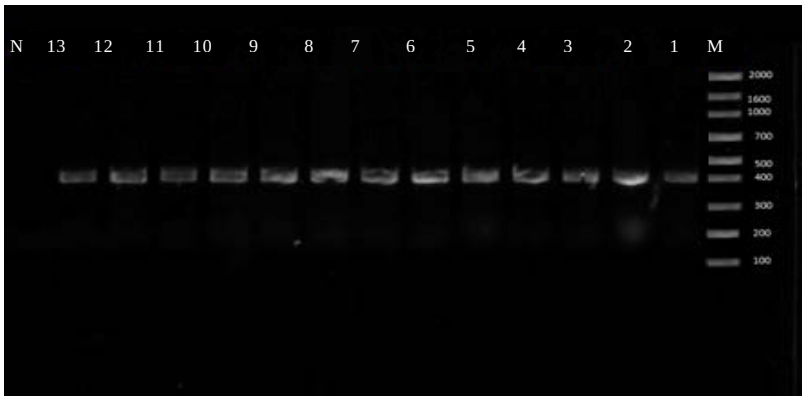

Fig. 2: Conventional PCR results for the 13 isolates for IBV gene

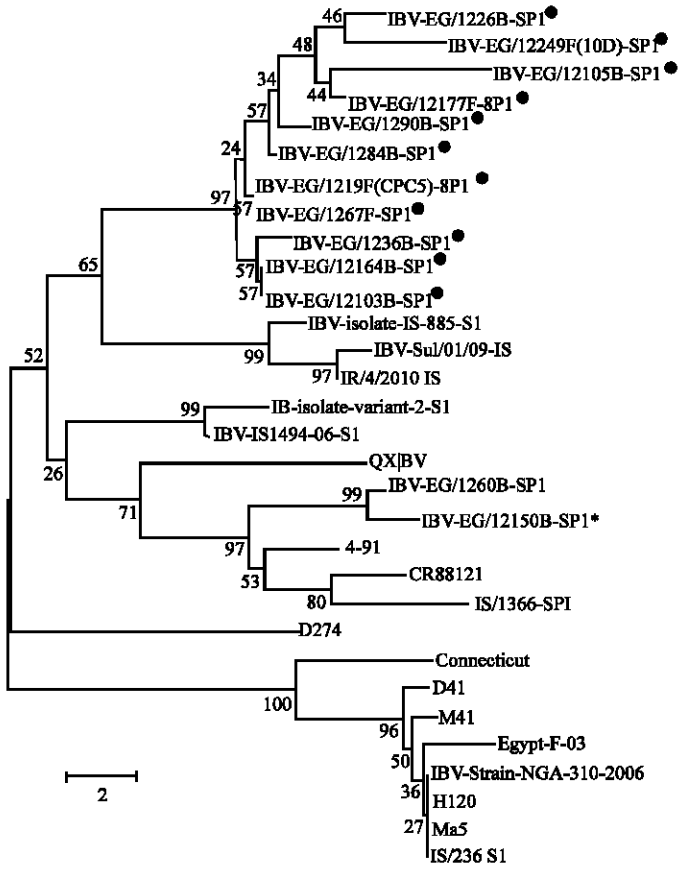

Fig. 3: Phylogenetic tree

Result of amino acid and nucleotides alignment: By using megaligen software compare thirteen selected sample with (variant 2-S1).We obtain result of position of mutation along sequenced area. From sequence we classified the 13 selected samples into to group according mutations in amino acids were recorded as shown in Table 2.

Phylogenetic analysis: To assess the genetic relationship among the IBV isolates, a phylogenetic tree was constructed from the nucleotide sequences of S1 genes. The results are shown in Fig. 3.

Table 2: Amino acid mutation in HVR of S1 gene of IBV

	S 20	T 23	V 26	N27	I29	N30	I31	Q 35	L36	S39	F46	Q46	Q56	K67	N69	T74	A89	L93
GP1	H	N		H		S	L	H	T			P	P	Q	D			
GP2	AT	S	I	E	f	Q	L	R	T	D	L	P	P			N	T	I

N.B: Abbreviation of amino acid at end of study, Group1 include 1st eleven samples shown in Table 1, Group 2 include last two samples shown in Table 1

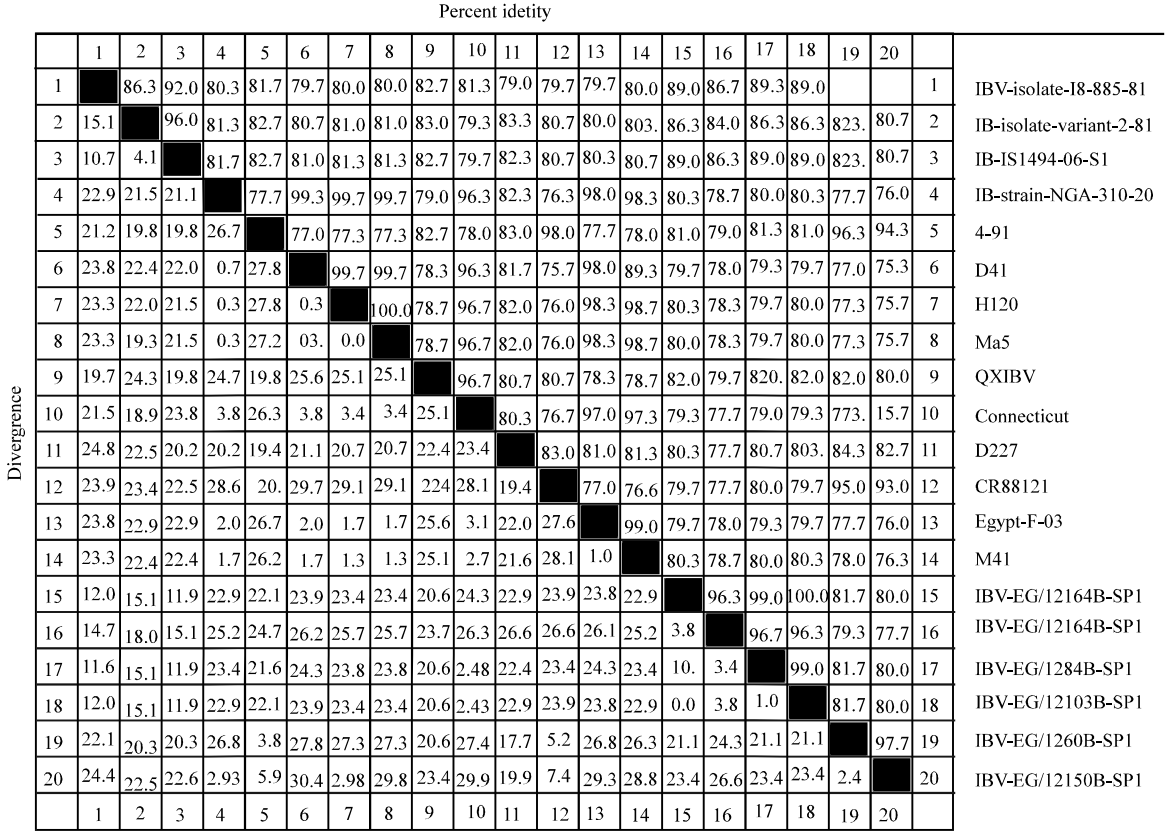

Fig. 4: Identity and divergence

The thirteen IBV isolates were separated into two distinct groups. Group I consisted of eleven isolates which include IBV-EG/219 F (CPC)-SP1, IBV-EG/1226B-SP1, IBV-EG/12164B-SP1, IBV-EG/12105B-SP1, IBV-EG/1290B-SP1, IBV-EG/12249F (10D)-SP1, IBV-EG/1284B-SP1, IBV-EG/1267F-SP1, IBV-EG/12103B-SP1, IBV-EG/12177F-SP1 and IBV-EG/1236B-SP1,the isolates in group I showed evolutionary distances from each other and close relation with variant-2 Iseraelian IBV (IS/885). Group II consisted of two isolates IBV-EG/1260B-SP1 and IBV-EG/12150B-SP1 which had a close relationship with variant-1 IBV (QXIBV, IBV-IS-1494-06-S1).

The Identity between the isolates depends on nucleotide sequence S1 gene of using DNA star software (Fig. 4).

DISCUSSION

IBV identification and genotyping was performed by the detection and analysis of a region in the HVR of the S1 gene (Pohuang *et al*., 2009). The homogeneity determined with the IS/1494/06

IBV isolates of the neighboring countries does not seem to be a sole coincidence. Although there is no information currently available howthe IS/1494/06 IBV was introduced to Turkey and how it disseminated among the countries of the Middle East, one possible source of introduction can be the wild birds based on the evidence that IBV may replicate inanseriformes (Bochkov *et al*., 2006). In addition to this, both the Middle East countries and Turkey has intense trade anduncontrolled movement of inhabitants and animal trade through borders which may cause clonal dissemination of an IS/1494/06 IBV strain in Turkey and in the neighbouring countries. Elucidation of the current clonal dissemination of this IS/1494/06 IBV strain (Mahmood *et al*., 2011). Two of the five isolates in Egypt showed 89 and 84% amino acid sequence identity and 89 and 88% nucleotide sequence identity to the Egyptian variant 1 and the IS/885 strains, respectively (Abdel-Moneim *et al*., 2012). The IS/1494/06 IBV is still a major variant involved in IBV infections in Israel and possibly in Jordan and Egypt and other countries in the Middle East. There is also a report, indicating the persistence of this Israeli Variant 2 isolate (IS/1494/06)- related problems in Egypt in spite of vaccinations of the broiler flocks with H120 which supports the argument of IS/1494/06 IBV presence in the Middle East countries. Also a nephropathogenic IBV related problem other than IS/1494/06 IBV has been reported in Iraq as a neighbouring country to Turkey (Mahmood *et al*., 2011).

Analysis of the hypervariable region of the S1 spike glycoprotein gene .Then compare between them by using phylogenetic analysis using Megalign software and Thirteen out of eleven isolates formed a distinct phylogenetic group with (IS/885) with identity 89.9%. Two of the thirteen isolates showed 97% amino acid sequence identity between them and 82.3% nucleotide sequence identity to the Egyptian variant 2 and IS/1494/06 strains.

Two group circulating in most Egyptian governorates without environmental limitation. Group 1% in samples isolated from Luxor, Al manya, Al Sharkia and Al Fayom. Group 2 pesent in samples isolated from Bansuif and Al Qaluobia.

In conclusion, recently thirteen isolated samples had mutation and by phylogenic analysis isolates divided into two groups; group 1 includes eleven isolates of thirteen similar to IS/885 about 89% identity but group 2 includes two isolates of thirteen similar to IS/1494-06 and Egyptian variant 2 with identity about 82.3%.

ACKNOWLEDGMENT

This work was supported by National Laboratory center for Quality Control of Poultry Disease (NLQP).

REFERENCE

Abdel-Moneim, A.S., M.A. Afifi and M.F. El-Kady, 2012. Emergence of a novel genotype of avian infectious *Bronchitis virus* in Egypt. Arch. Virol., 157: 2453-2457.

Abdel-Moneim, A.S., M.F. El-Kady, B.S. Ladman and J. Gelb, 2006. S1 gene sequence analysis of a nephropathogenic strain of avian infectious *Bronchitis virus* in Egypt. Virol. J., Vol. 3. 10.1186/1743-422X-3-78

Adzhar, A., R.E. Gough, D. Haydon, K. Shaw, P. Britton and D. Cavanagh, 1997. Molecular analysis of the 793/B serotype of infectious *Bronchitis virus* in Great Britain. Avian Pathol., 26: 625-640.

Bochkov, Y.A., G.V. Batchenko, L.O. Shcherbakova, A.V. Borisov and V.V. Drygin, 2006. Molecular epizootiology of avian infectious bronchitis in Russia. Avian Pathol., 35: 379-393.

Butcher, G.D., D.P. Shapiro and R.D. Miles, 2011. Infectious *Bronchitis virus*: Classical and variant strains. One of a Series of the Veterinary Medicine-Large Animal Clinical Sciences Department, Florida Cooperative Extension Service, IFAS. Publication#VM127. http://edis.ifas.ufl.edu/pdffiles/PS/PS03900.pdf

Cavanagh, D., 2003. Severe acute respiratory syndrome vaccine development: Experiences of vaccination against avian infectious bronchitis *Corona virus*. Avian Pathol., 32: 567-582.

El-Kady, M.F., 1989. Studies on the epidemiology and means of control of infectious bronchitis disease in chickens in Egypt. Ph.D. Thesis, Faculty of Veterinary Medicine, Cairo University, Egypt.

Haqshenas, G., K. Assasi and H. Akrami, 2005. Isolation and molecular characterization of infectious *Bronchitis virus* isolate shiraz 3. IBV, by RT PCR and restriction enzyme analysis. Iran. J. Vet. Res. Univ. Shiraz, 6: 9-15.

Mahmood, Z.H., R.R. Sleman and A.U. Uthman, 2011. Isolation and molecular characterization of Sul/01/09 avian infectious *Bronchitis virus*, indicates the emergence of a new genotype in the Middle East. Vet. Microbiol., 150: 21-27.

OIE Terrestrial Manual, 2008. Chapter 2. 3. 2: Avian Infectious Bronchitis. In: Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, OIE (Ed.). Vol. 6, University of Shiraz, Iran.

Pohuang, T., N. Chansiripornchai, A. Tawatsin and J. Sasipreeyajan, 2009. Detection and molecular characterization of infectious *Bronchitis virus* isolated from recent outbreaks in broiler flocks in Thailand. J. Vet. Sci., 10: 219-223.

Sheble, A., M.Z. Sabry, F.G. Davelaar, A.R. Burger and A.K. Khafagy *et al.*, 1986. Present status of infectious bronchitis in Egypt. J. Egypt. Vet. Med. Ass., 64: 393-411.

Su, J.L., Z.T. Zhou, Z.S. Guo, Q.R. Xu and Y.C. Xiao *et al.*, 2012. Identification of five *Bronchitis virus* (IBV) strainsisolated in China and phylogenetic analysis of the S1gene. Afr. J. Microbiol. Res., 6: 2194-2201.

Sultan, H.A., L.A. Tantawi, A.I. Youseif and A.A.S. Ahmed, 2004. Urolethiasis in white commercial egg laying chickens associated with an ifnectious *Bronchitis virus*. Proceedings of the 6th Science Conference Egyptian Veterinary Pollution Association, pp: 155-169.