Journal homepage:
http://www.bsu.edu.eg/bsujournals/JVMR.aspx

Online ISSN: 2357-0520 Print ISSN: 2357-0512

Original Research Article

Molecular characterization and genotyping of recent isolate of Infectious Bronchitis Virus (IBV) in Egypt

Mostafa M. Hammouda, Abdelsattar M. Arafa and Hanafy M. Madbouly

Virology Department; Faculty of Veterinary Medicine; Beni Suef University; Beni-Suef, Egypt, National Laboratory for Veterinary Quality Control on Poultry Production; Animal Health Research Institute; Dokki, Giza, Egypt.

ABSTRACT

In this study a total number of 22 organ samples (including trachea, lung and kidney) from 22 broiler farms from northern Upper Egypt were collected from Mars 2017 to June 2018 from chickens showing clear clinical and pathological signs of Infectious Bronchitis. The samples were prepared and examined by real time RT-PCR for diagnosis of IBV. A total number of 11 samples were positive (50%) which were used for further isolation on SPF eggs by three blind serial passages. Positive samples that showed the pathogenic lesions of IB (curling and dwarfing of embryos) were collected and tested with real time RT-PCR (rRT-PCR) for more confirmation then a part from S1 gene sequence was amplified by RT-PCR and the product was sequenced and the data have been compared with other related IBV strains. The results indicate that the Egyptian virus in this study has an identity percent reached up to 89% with other recent Egyptian isolates. However, it reached 67% with classical vaccine strains like H120 and variant I like CR88 strain. The lowest identity was observed with M41 strain (59%) in this study. The phylogenetic tree compared to other isolates from Middle East and worldwide showed that this isolate is related to the IBV variant 2 group closely related to **IBVEg/1265B/2012** strain and the Israeli strain **IS/1494/06**.

ARTICLE INFO

Article history:

Received 3/2019

Accepted 4/2019

Online 4/2019

Keywords:

Molecular characterization, Genotyping, IBV, Egypt.

*Corresponding author. *Department* of Virology, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef 62511, Egypt.

Introduction

Infectious bronchitis virus (IBV) is a highly contagious viral disease of chickens causing high economic losses due to respiratory illness **(Jane et al**

2012). It mainly infects chickens causing a respiratory disease; however the virus can affects the female reproductive tract producing drop of egg production and poor egg quality. Some strains may infect the kidney of young chickens, leading to nephritis with significant mortality **(Jane et al 2012).**

Infectious bronchitis virus is a member of the Coronaviridae family ***(ICTV, 2019)***. IBV is pleomorphic, enveloped particles with diameter 80 to 160 nm with characteristic pear shaped projections on its surface "spikes"**.*(Berry et al 1964).*** IBV genome is single stranded RNA with molecular weight 8 X 10^6 Dalton consist of single continuous chain of about 23,000 mononucleotides. **(Lomniczi and Kennedy, 1977).** All coronaviruses maintain a set of essential genes, including those that encode the polymerase (Pol), spike (S), small membrane (E), membrane (M), and nucleocapsid (N) proteins, in the invariable order 5′-Pol-S-E-M-N3′ In addition to these essential genes, the genomes of all coronaviruses contain groupspecific, or accessory, genes, which encode small proteins of unknown function.**(Lai, and Cavanagh.1997).**

The molecular characterization of IBV is based on analysis of the S1 gene due to the presence of neutralizing and serotype-specific epitopes that are associated with hypervariable regions (HVR) in the S1 subunit **(Gough et al., 1992)**. The genetic variants are thought to be generated from few amino acid changes in the spike (S1) glycoprotein of IBV **(Kingham et al., 2000)**.

IBV was firstly detected in Egypt in 1954 by (**Ahmed 1954)** .Several IBV strains have been isolated recently in Egypt. Egypt/Beni-Seuf/01 (**Abdel-Moneim et. al. 2002).** In addition, novel genotypes called Ck/Eg/Bsu-2/2011, Egypt101-ck and Egypt/F/09 have been recently detected (**Abdel-Moneim et al., 2012).** Egyptian IBVs can be clustered within two different groups: One classic group resembling the vaccine strain GI-1 genotype and the second group is the variant group which was divided to two subgroups (Egy/var I and Egy/var II) resembling the Israeli variants IS/1494 and IS885 strains, respectively.(**Zanaty et. Al 2016)**. Other studies concluded that IBV in Egypt is commonly found to be in mixed infections with other viruses as New Castle Disease Virus and Avian Influenza Virus **(Sultan et al., 2015; and Hassan et al., 2016)**

The aim from this work is to study the molecular characterization of recent IBV isolates in Egypt with special reference to the north region of Upper Egypt in comparison to previous genetic data from Egypt. In addition, to compare with the commonly used vaccine strains in the field.

1- Materials and Methods

2.1 Sampling

In this study, 22 samples from IBV suspected cases were collected from different chicken farms located in El-Fayoum, Beni-seuif and Elmenya Governorates from Mars 2017 to June 2018. The age of chickens is ranged from 21 to 35 days.

Samples include Kidney, trachea and lung organs. The collected samples were transferred to the Reference Laboratory of veterinary quality control on poultry production (RLQP). The samples were placed in an isotonic phosphate-buffered saline (PBS), pH 7.0–7.4 with antibiotics penicillin , streptomycin (200 mg/liter), gentamycin (250µg/liter) and mycostatin (1000 units/ml).

Samples of each bird were pooled and examined using the rRT- PCR technique then the positive samples for IBV were subjected for virus isolation and sequencing for S1 gene of the virus.

3.2 Detection of IBV using rRT-PCR

The viral RNA was extracted by QiaAmp viral RNA mini Kit- Cat no 52904. (GmbH, Hilden, Germany) in accordance with manufacturer's instructions. After the extraction of viral RNA, the yield was collected and preserved at -70 for diagnosis by rRT-PCR.

Mastermix was prepared according to QuantiTect probe RT-PCR kit handbook. oligonucleotide primers and probe used in rRT- PCR according to **(Meir *et al*. 2010)**.

Primers/probes were obtained from Metabion with sequences (5'-3')

ATGCTCAACCTTGTCCCTAGCA, TCAAACTGCGGATCATCACGT, and taqman probe

(FAM-TTGGAAGTAGAGTGACGCCCA AACTTCA-TAMRA)

Thermo cycling rRT-PCR conditions were 50 °C for 30min for reverse transcription as one step, then 95 °C for 15 min as heat activation of polymerase followed by 40 cycles of denaturation step (94 °C for 15 sec), annealing (54 °C for 30 sec) and extension (72 °C for 10 sec).

3.3 Virus isolation

The positive samples by rRT-PCR were used for virus isolation in specific pathogen free embryonated chicken eggs (SPF) (obtained from KoumOshiem, Fayoum, Egypt) of 10-dayold. 0.2 ml of the sample was inoculated into the allantoic cavity (five eggs for each sample) for 3 successive blind passages. Allantoic fluids were harvested at 96 hours post inoculation.

The inoculated eggs were examined for daily embryo mortality and signs like curling and dwarfing of embryo. Confirmation of virus isolation was done using rRT-PCR for IBV.

3.4 Genetic characterization of Spike gene

The isolates were subjected for RT-PCR and then were used for the amplification of the of S1 gene. Using conventional PCR according

to Qiagen one step RT-PCR Kit (Qiagen,

GmbH, Hilden, Germany). With the forward primer IBV-S1-F

5′CACTGGTAATTTTTCAGATGG 3′ and reverse primer IBV-S1-R

5′CAGATTGCTTACAACCACC3′ according to **(Adzhar, et al 1997)**

Purification of amplicons were done using QIA quick gel extraction kit (Qiagen, GmbH, and Hilden, Germany).The sequence reactions were examined by 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 **(Meir *et al*. 2010)**. Specific softwares were used for sequence analysis and phylogenesis. For sequence analysis we used Seqscape® software for primary analysis of the raw data. A comparative analysis of sequences was performed using the CLUSTAL V multiple sequence alignment program, version 1.83 of MegAlign module of Lasergene DNAStar software (Madison, Wisconsin, USA). A phylogenetic tree of the nucleotide and amino acid sequences was constructed using MEGA5 software (**Tamura et al., 2011).**

2- Results

3.1 Results of rRT-PCR

Table (1) shows the results of rRT-PCR, the results revealed that there were 11 positive samples from a total of 22 flocks representing 50% of collected samples. The positive samples were two from Elmenya, one from Beni- suef and other samples were from Fayoum.

3.2 Results for isolation on ECE:

Only three isolates (samples number 13, 20 and 22) showed curling and dwarfing of inoculated embryos with subcutaneous haemorrhage. Other samples showed death of embryo before 48 hours in the second or third passage. One positive sample was from trachea and lung from birds with 35 days old, other two samples were from kidney with birds at age 30 and 28. Other isolated samples showed death of embryo before 48 hours at second or third passage.

Table 1: Results of IBV detection using rRT-PCR

Number	Age	locality	Sample type	Result
1	23	Fayoum	Trachea and lung	Negative
2	29	Fayoum	Trachea and lung	Negative
3	21	El-menya	Trachea and lung	Negative
4	21	El-menya	Trachea and lung	Positive
5	30	Fayoum	Trachea and lung	Negative
6	25	Fayoum	Trachea and lung	Negative
7	28	Fayoum	Trachea and lung	Negative
8	24	Fayoum	Trachea and lung	Negative
9	30	Beni- suef	Trachea and lung	Positive
10	24	Beni- suef	Trachea and lung	Negative
11	28	Fayoum	Trachea and lung	Negative
12	27	Fayoum	Trachea and lung	positive
13	35	Fayoum	Trachea and lung	Positive
14	28	Fayoum	Trachea and lung	Negative
15	28	Fayoum	Trachea and lung	positive
16	29	Fayoum	kidney	Positive
17	24	Fayoum	kidney	Positive
18	21	Fayoum	Trachea	Positive
19	31	Fayoum	kidney	Positive
20	30	Fayoum	kidney	Positive
21	22	Fayoum	Trachea and lung	Negative
22	28	Elmenya	kidney	Positive

Results of genetic analysis Amino acids identity%

Table (2) shows the results for analysis of amino acid identity.

The results indicate that the Egyptian virus in this study has an identity percent reached up to 89% with other recent Egyptian viruses. However, it reached 67% with old classical vaccine strains like H120 and variant I like CR88 strain. The lowest identity was observed with M41 strain (59%)

Table 2. Identity and diversity table.

Percent Identity

Divergence

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15		
1		96.3	96.3	95.6	65.4	72.8	74.3	64.7	61.8	82.4	63.2	68.4	83.8	89.7	89.7	1	IBVEg-CLEVB-2-IBV-012
2	3.8		96.3	95.6	65.4	73.5	75.7	64.7	61.8	83.1	64.0	69.9	84.6	91.2	89.0	2	IBVEg-CLEVB-1-IBV-012
3	3.8	3.8		99.3	64.7	72.1	73.5	64.0	61.0	83.8	64.0	69.1	84.6	90.4	89.0	3	IBVIB VAR2-06 spike glycoprotein
4	4.6	4.6	0.7		64.0	71.3	72.8	63.2	60.3	83.1	63.2	68.4	83.8	89.7	88.2	4	IBVattenuated IS-1494-06 spike
5	46.1	46.1	47.5	48.8		62.5	62.5	99.3	90.4	66.2	68.4	61.8	66.9	66.2	62.5	5	IBVstrain H120
6	33.8	32.7	35.0	36.1	51.6		91.2	61.8	61.8	71.3	66.9	89.0	70.6	74.3	67.6	6	IBVstrain CR88121
7	31.6	29.4	32.7	33.8	51.6	9.4		61.8	61.8	72.8	65.4	86.8	72.1	75.0	70.6	7	Avian IBVvariant 1 S1
8	47.5	47.5	48.8	50.2	0.7	53.0	53.0		91.2	65.4	67.6	61.0	66.2	65.4	61.8	8	IBV-Ma5-SP1
9	53.0	53.0	54.5	56.0	10.2	53.0	53.0	9.4		64.0	66.2	61.0	61.8	62.5	59.6	9	IBV-M41
10	20.2	19.2	18.3	19.2	44.8	36.1	33.8	46.1	48.8		65.4	69.9	96.3	83.1	77.9	10	IBV-D274
11	50.2	48.8	48.8	50.2	41.0	43.5	46.1	42.2	44.8	46.1		68.4	64.7	65.4	61.8	11	IBV-QXIBV-SP1
12	41.0	38.5	39.7	41.0	53.0	12.0	14.6	54.5	54.5	38.5	41.0		68.4	70.6	64.7	12	IBV-UK-4-91
13	18.3	17.3	17.3	18.3	43.5	37.3	35.0	44.8	53.0	3.8	47.5	41.0		84.6	80.1	13	Avian Avian IBV(strain D207)
14	11.1	9.4	10.2	11.1	44.8	31.6	30.4	46.1	51.6	19.2	46.1	37.3	17.3		83.8	14	IBVEg-1265B-2012
15	11.1	12.0	12.0	12.8	51.6	42.2	37.3	53.0	57.5	26.2	53.0	47.5	23.1	18.3		15	IBV-CH-EGY-1-Minya-2018
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15		

Phylogenetic analysis:

Figure (1) shows the phylogenetic tree of some IBV strains including the recent isolate in this study **IBV-CH-EGY-1-Minya-2018.**

The phylogenetic tree shows that our isolate is related to group variant 2 of IBV closely related to **IBVEg-1265B-2012.**

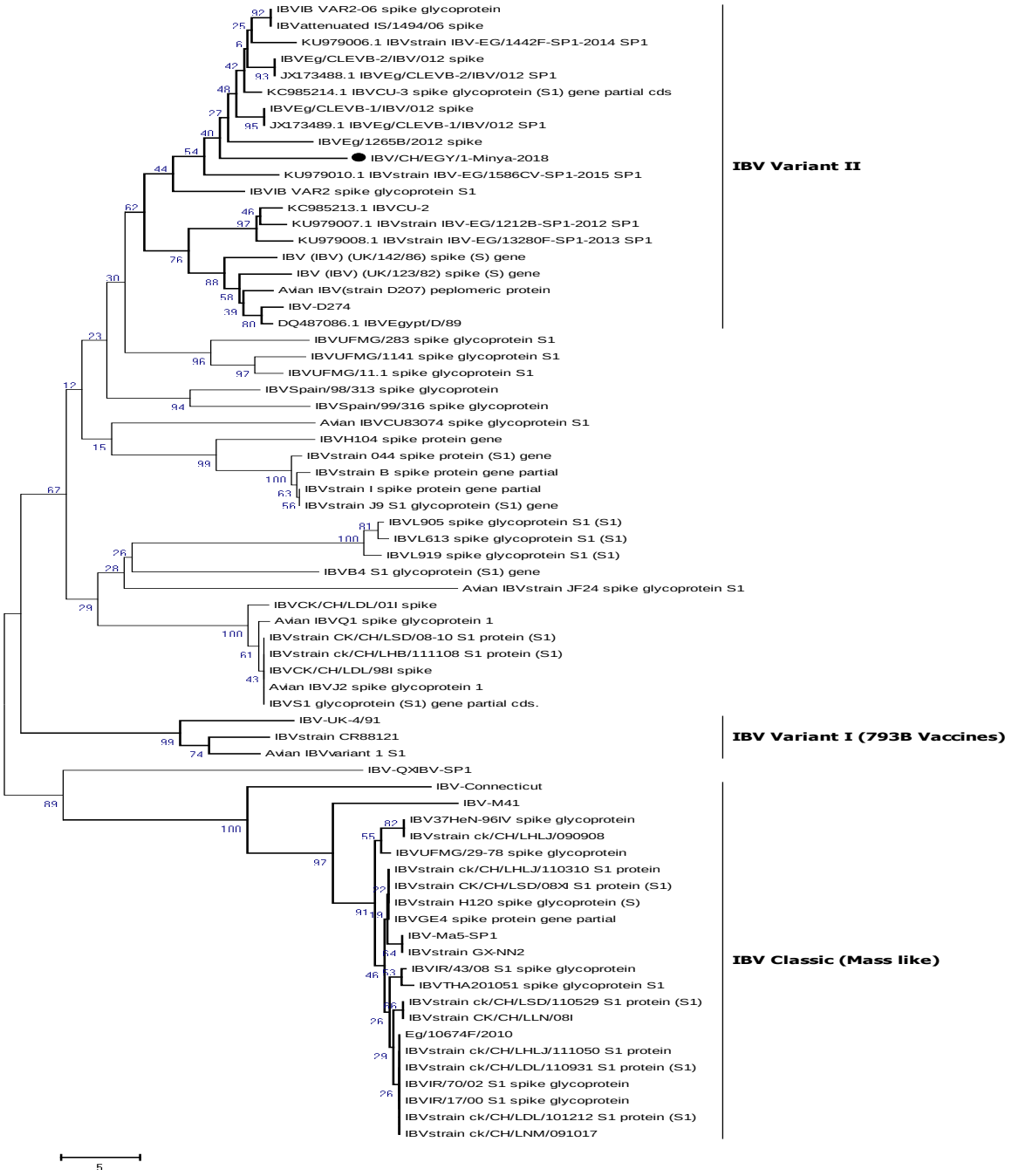

Figure 1. Phylogenetic tree of S1 gene. The black dots (•) refer to our isolated strain.

3- Discussion

Infectious bronchitis virus (IBV) is highly contagious viral disease of chickens causing high economic losses due to respiratory illness (**Jane et al 2012)**.Emerging of new IBV serotypes is mainly due to high rates of mutation of S1 gene **(Lee et. al. 2003 and Li et. al. 2013).** In this study, 22 samples collected from 22 chicken farms in Faoum, Beni seuif and Menya were examined by rRT-PCR for detection of IBV. The results showed that 11 samples were positive using rRT-PCR. Positive samples were isolated in SPF embryonated chicken eggs. Each sample was used for 3 blind passages. Only 3 samples showed the specific lesions which were confirmed for presence of IBV by rRT-PCR. Other samples showed early emberyonic death before 48 hours post inoculation, and when these samples were tested by rapid plate HA test, four of them were positive which may indicate a mixed infection with AI or NDV. In a previous study by **(Kareem E., et al. 2016)** mentioned that about 66% of viral respiratory infections of chickens in Egypt are caused by mixed viral infection and mixed infectious bronchitis (IBV) and avian influenza (AI)-H9N2 viruses were the most common infection (41.7%). One sample was used for partial sequencing for S1 gene. The sequencing result was compared to other strains for amino acid identity and a phylogenetic tree. In 2002, a strain Egypt/Beni-Seuf/01 was isolated and was closely related to Israel/720/99 with 62.9% identity percentage. **(Abdel-Moneim et. al. 2002 and Madbouly et. al. 2002)**. In 2005, a strain Egypt\F/03 was isolated and found to be closely related to Mass serotype with 99% nucleotide identity with GX1-98.China. Later, other isolate strains were showed close relation to D274 with 98.2% nucleotide identity. **(Sediek 2010)**. Recently, in Egypt, IBV was classified into two distinct groups; variant and classic(Mass like) , viruses belonged to a variant group, including 2 subdivisions Egy/Var-I and Egy/Var-II.**(Zanaty, Ali, et al. 2016).**

Our strain was named **IBV-CH-EGY-1-Minya-2018** with accession number is **MK673132**.

As shown in Table 1 The S1 gene sequence of the strain **IBV-CH-EGY-1-Minya-2018** showed about 90% amino acid identity compared to the strain **IBVEg-CLEVB-2-IBV-012** and 89% identity with **IBVIB VAR2-06** and **IBVEg-CLEVB-1-IBV-012**.The lowest identity percentage was with the classical strain **IBV-M41** was 59.6% and 61.8% for both strains **IBV-MA5** and **IBVQXIBV.** With 62.5% to **IBVstrain H120**, 64.7% to **IBV-UK-04-91** ,67.6% to **IBVstrain CR8812**, 70.6% for Avian **IBVvariant** 1 strain and 77.9% for **IBV-D274**. It reaches 80.1% with strain **D207**, 83.8% to **IBVEg-1265B-2012** and 88.2% with Israelian strains **IS-1494-06**.

Phylogenetic tree for the strain shows that it can be grouped with variant-2

group closely related to **IBVEg-1265B-2012.**

4- Conclusion

This work highlighted on the molecular characterization of recent IBV isolates in Egypt (from El-Fayoum, Beni-seuif and Elmenya Governorates) in comparison to previous genetic data from Egypt, Middle East, Europe and Asia. The study indicates high genetic diversity of recent field isolate with the commonly used vaccine strains in the field. This may help to increase our efforts for best vaccine seed selection to protect birds from field infection with IBV.

References

Abdel-Moneim, A. S., Madbouly, H. M., Gelb, J., & Ladman, B. S. (2002). Isolation and identification of Egypt/Beni-Seuf/01 a novel genotype of infectious bronchitis virus. veterinary medical journal-Giza, 50(4), 1065-1078.

Abdel-Moneim, A. S., Afifi, M. A., & El-Kady, M. F. (2012). Emergence of a novel genotype of avian infectious bronchitis virus in Egypt. Archives of virology, 157(12), 2453-2457.

Adzhar, A., Gough, R.E., Haydon, D., Shaw, K., Britton, P., and Cavanagh, D. (1997).Molecular analysis of the 793/B serotype of infectious bronchitis virus in Great Britain. Avian Pathology 26(3): 625-640.

Ahmed, H.N. (1954): Incidence and treatment of some infectious viral respiratory diseases of poultry in Egypt. M.Sc. Thesis, Cairo Univ.

Berry, D.M., Cruickshank, J.G., Chu, H.P. and Wells, R.J.H. (1964). The structure of infectious bronchitis virus. Virology, 23(3), pp.403-407.

Eissa, Y. M., Zaher, A. and Nafai, E. (1963).Studies on respiratory diseases: Isolation of infectious bronchitis virus.J. Arab. Vet. Med. Ass 23: 381-389.

El-Nahas, E. M., El-Sayed, H. S., Sawsan, S. & Gabr, F. A. (2017). Genotyping of a new avian infectious bronchitis virus isolated from chickens proventriculus in Egypt. J. of Virol. Sci., Vol. 1: 54-66.

Jackwood, M. W. (2012). Review of infectious bronchitis virus around the world. Avian diseases 56 (4): 634-641

Jane, K.A., Jackwood, M. and Jones R.C. (2012). The long view: 40 years of infectious bronchitis research. Avian Pathology 41 (3): 239-250.

Hassan, K. E., Salama, A.S. , Ali, A., Al-Hussien, M. D., Azza, A. E., and Magdy, M.F. (2016). Prevalence of avian respiratory viruses in broiler flocks in Egypt. Poultry science 95(6): 1271-1280.

International Committee on Taxonomy of Viruses [ICTV]. Virus Taxonomy: 2018b Release

Available from: https://talk.ictvonline.org/taxonomy/

Lai, M. M. C. and Cavanagh, D. (1997). The molecular biology of coronaviruses.Adv. Virus Res.48:1-100.

Lee, CW, Hilt, D.A., Jackwood, M.W. (2003). Typing of field isolates of infectious bronchitis virus based on the sequence of

the hypervariable region in the S1 gene. J
Vet Diagn. Invest. 15: 344–348

Li, M., Mo, M.L., Huang, B.C., Fan, W.S., Wei, Z.J., Wei, T.C., Li, K.R. and Wei, P., 2013. Continuous evolution of avian infectious bronchitis virus resulting in different variants co-circulating in southern China. Archives of virology, 158(8), pp.1783-1786. Lomniczi, B., & Kennedy, I. (1977). Genome of infectious bronchitis virus. Journal of virology, 24(1): 99-107.

Madbouly, H.M., Abdel-Moneim, A.S., Gelb, J., Ladman, B.S. and Rofael, N.B., 2002. Molecular characterization of three Egyptian infectious bronchitis virus isolates. Veterinary Medical Journal-Giza-, 50(4), pp.1053-1064.

Meir, R., Maharat, O., Farnushi, Y. and Simanov, L., 2010. Development of a real-time TaqMan® RT-PCR assay for the detection of infectious bronchitis virus in chickens, and comparison of RT-PCR and virus isolation. Journal of virological methods, 163(2), pp.190-194.

OIE Terrestrial Manual (2008): Infectious bronchitis. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Chapter 2. 3. 2. Journal of Veterinary Research, University of Shiraz, 6(2)

Sultan, H., Abdel-Razik A.G., Shehata A.A., Ibrahim M., and Talaat. S.(2013). Characterization of Infectious Bronchitis Viruses Circulating in Egyptian chickens during 2012 and 2013. J. Vet. Sci. Med. Diagn. 4 (5): 2.

Sediek, M. E. M.(2010). Further Studies on infectious bronchitis in chickens. Ph. D. Thesis, Poultry Dis, Alex. Uni.

Zanaty, A., Arafa, A. S., Hagag, N., & El-Kady, M. (2016). Genotyping and pathotyping of diversified strains of infectious bronchitis viruses circulating in Egypt. World journal of virology, 5(3): 125.

Zanaty, A., Naguib, M.M., El-Husseiny, M.H., Mady, W., Hagag, N. and Arafa, A.S. (2016). The sequence of the full spike S1 glycoprotein of infectious bronchitis virus circulating in Egypt reveals evidence of intra-genotypic recombination. Arch. Virol. 161(12).3583-3587