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Molecular Characterization of Infectious Bronchitis Virus in Chicken

Heba N. El-Behery[*] Sanaa S. Awad* and S.G. Kasem[**]
[*]Department of Poultry Diseases, Faculty of Veterinary Medicine, Mansoura University and [**]Department of Poultry Diseases, Faculty of Veterinary Medicine, Kafr El-Sheikh University, Egypt.

INFECTIOUS BRONCHITIS (IB) is a highly contagious disease of serious economic importance in the poultry industry worldwide. Diagnosis of Infectious Bronchitis Virus (IBV) infections in Egypt during 2013 - 2016 among poultry flocks. A total of 41 chicken flocks were examined (40 broiler flock and one breeder flock). Results revealed that 21 chicken flocks were found positive IBV by N gene conventional RT-PCR and real time RT-PCR. The samples were isolated and propagated in commercial embryonated chicken eggs (ECE) with result revealed that only one sample that showed curling and dwarfing of the embryo after the second passage while 10 samples were HA positive that suggest mixed infection of IBV with other viruses such as Newcastle Virus or Avian Influenza Virus. Furthermore, the allantoic fluids of the positive samples were subjected to specific S1 PCR to differentiate strains of IBV. The absence of positive reaction of S1gene conventional RT-PCR may confirm the involvements of other respiratory pathogens in chickens as Newcastle disease, avian influenza and mycoplasmosis that masked IBV infections. In conclusion, our results confirmed the detection of IBV in chicken flocks in Egypt which responsible for the respiratory problems in poultry farms and diagnosis with Real-time PCR because it is more rapid, sensitive, and reproducible, and the risk of carryover contamination is minimized compared to conventional PCR.

Keywords: IBV, Diagnosis, RT-PCR, Egypt.

Avian infectious bronchitis (IB) is one of the most important agents in chickens that causing respiratory disease in the world and causes extensive economic losses in the poultry industry (Boroomand *et al.,* 2012). IB is a common, acute and highly infectious disease in chickens that caused by coronavirus IBV (Jahantigh *et al.,* 2013 and Bentley *et al.,* 2013). Infectious bronchitis virus (IBV) cause disease in respiratory, renal and reproductive systems of chickens of all ages (Cook *et al.,* 2012). During 1950s, IB infection was first recognized in Egypt by Ahmed, 1954 from birds suffering from respiratory signs, subsequently variant strain identified by neutralization tests closely related to the Dutch variant D3128 (De Wit *et al.,* 2011). During 2015, Abdel-Rahman *et al.* (2015) could identify new variant strains of IBV in Northern Egypt. Those variant

* Corresponding author: Heba N. El Behery (hebanabilhassan@yahoo.com).

strains are closely related to each other and have the closest relatedness with the Egyptian Eg/CLEVB-1/IBV/012 and the Israeli IS/1494/06 strain, while, the Italy 02 strain sharing the closest relatedness of the "well-known" strains (79 and 77% on nucleotide and amino acid levels, respectively). Moemen and Awad identified the genetic changes in the IBVs isolated from chicken broilers using partial S1 gene sequences. This is the first study in Assiut-Egypt that indicated the variant natures of the circulating IBV strains which are genetically distinct from all other known reference IBV and vaccine strains (Moemen and Awad 2015). IBV belongs to family Coronaviridae, genus Gamma coronavirus (ICVT, 2015). IBV genome is a single stranded, positive sense RNA of 27.6 kb. IBV encodes four important structural proteins, the spike (S) glycoprotein, the membrane (M) glycoprotein, the nucleocapsid (N) protein and the envelope or small membrane (E) protein (Mo *et al.,* 2013 and Zanaty *et al.,* 2013).The IBV S gene is cleaved into two subunits, the N-terminal S1 subunit and the C-terminal S2 subunit (Cavanagh, 2007). The S1 protein is highly variable and this makes the S1 gene an ideal aim for typing IBV strains in molecular assays by reverse transcriptase polymerase chain reaction (RT-PCR) and sequencing (Ababneh *et al.,* 2012). Variation in S1 sequences has been used for differentiating IBV serotypes (Abdel-Moneim *et al.,* 2006).Inoculation of ECE is considered the best method for virus isolation that required several egg passages before the virus produce characteristic lesions which are dwarfed, curling, feather dystrophy (clubbing), kidney urates or embryo mortality (Swayne *et al.,* 1998, Cavanagh and Naqi, 2003). The molecular antigenic variation based on nucleotide sequencing of gene coding for the S1 subunit of the spike (S) protein because different serotypes have large differences (20-50%) in amino acid sequences of the S1 subunit (Kusters *et al.,* 1989 and Cavanagh, 2003). The objective of this study was isolation of IBV in different chicken breeds with respiratory signs in Egypt by application of RT-PCR and/or real-time PCR techniques on the collected field samples.

Material and Methods

Sampling

In this study forty one samples were collected from broiler and breeder chicken during the period from January 2013 till April 2016 which suffering from respiratory distress manifested by gasping, tracheal rales, swollen sinuses, ruffled feathers and depression. Post-mortem examination revealed lesions of tracheitis, Caseous plug in tracheal biforcation, pneumonia, air sacculitis, swollen and pale kidneys with distended tubules and ureters containing urate with high mortality rates. Samples including trachea, lung, kidney, liver and spleen collected in sterile Falcon tubes (15ml) containing phosphate buffer saline (PBS) with antibiotic and antifungal agents transported in ice box and stored at -4^{o}c until use.

Conventional RT- PCR for NP gene of IBV

The viral RNA was extracted from the field samples using Trizol Easy-Red^{TM} Total RNA extraction Kit according to the instructions of the manufacturer as

follows: The organ samples were cut into small pieces in a Petri dish with a sterile scalpel. Put samples in 2 ml microcentrifuge tube, then add 250 µl PBS, and then grind well by grinding bar till homogenized. Add 750µl of Easy-Red^{TM} and mix by vigorous vortex for 15 seconds and incubate at room temperature for 5min. Centrifuge at 13,000 rpm for 10 min. at 4°C. Add 200µl of chloroform and mix the sample at room temperature for 15 seconds by vigorous vortex then incubate the tube at room temperature for 5min. Centrifuge at 13,000 rpm for 15 min. at 4°C. Transfer 400µl of the resulting upper aqueous layer into a new sterile 1.5 ml microcentrifuge tube. Add equal volume of isopropanol and mix well by inverting the tube 4-5 times, and then incubate the tube for 10 min. at room temperature. Centrifuge at 13,000 rpm for 10 min. at 4°C. Carefully remove the supernatant without disturbing the pellet. Add 1ml of 70% ethanol and mix well by inverting the tube 4-5 times. Centrifuge the mixture at 13,000 rpm for 5 min. at 4°C. Carefully aspirate the supernatant without disturbing the pellet. Dry the RNA pellet at room temperature for about 5 min. Dissolve RNA pellet in 20-50 µl of RNAse free water and store at -80°C till use.

Reverse transcriptase (RT) prepared of 2 mixes in sterile PCR tubes as follows:

Mix I (8 µl)

Total RNA	6 µl
Oligo dT	1 µl
Sterile water	1 µl

Incubation at 70 °C for 10 min then place on ice for 5 min.

Mix II (20 µl)

5X reaction buffer	4 µl
dNTP mix (10 mM of each =40mM)	1 µl
RNAs inhibitor	1 µl
MMLV Reverase(200u/ µl)	1 µl
Sterile DNAse /RNAse free water	13 µl

Combine Mix I and Mix II and gently vortex. Incubate in heat block at 55°C for 90 min., then at 70°C for 10 min. for inactivation of the enzyme. Store at -80 °C for further applications.

The N gene were amplified using conventional PCR Maxime^{TM} RT PreMix Kit (Gene on) carried out in a total of 25 µl reaction volume using forward primer IBV-F GCTTTT GAG CCT AGC GTT and reverse primer IBV-R GCCATGTTGTCACTGTCTATT (Callison *et al.,* 2006).

5X Master Mix (Taq Master /High yield)	**5 µl**
IBV forward primer	1 µl
IBV reverse primer	1 µl
IBV cDNA	5 µl
PCR grade water	13 µl
Total reaction volume	25 µl

The PCR conditions were as follows: one cycle of initial denaturation at95°C for 5 min, 35 cycles (at 95°C for 1 min, 53°C for 1 min and 70°C for 1 min) and one cycle of final extension of 72°C for 10 min. After the end of PCR, amplification products run in agar gel 1.5% which give specific band at 149 bp in weight measured against gel 100bp plus blue DNA ladder **(**Gene ON, GmbH, Germany**)**.

IBV detection by real time RT-PCR

RNA extracted from the supernatants of 10% w/v sample suspensions by Bioflux viral RNA Mini Spin column. All steps were run in accordance with manufacturer‘s instructions using reagents provided in the kit. Load 250 µl of sample in an empty 1.5 ml lysis tube. Add 200 µl of Lysis Solution, and 50 µl of Proteinase K, mix thoroughly by pipetting. Incubate the sample for 15 min at room temperature. Add 300 µl of ethanol (96%) and mix by vortexing. Transfer the lysate to the prepared Spin Column preassembled within the wash tube. Centrifuge the column for 2 min at 12,000 rpm. Discard the Wash Tube containing flow-through. Place the Spin Column into a new 2 ml Wash Tube. Add 700 µl of Wash Buffer 1 supplemented with ethanol to the Spin Column. Centrifuge the column for 2 min at 12,000 rpm. Discard the Wash Tube containing flow-through. Place the Spin Column into a new 2 ml Wash Tube. Add 500 µl of Wash Buffer 2 supplemented with ethanol to the Spin Column. Centrifuge the column for 2 min at 12,000 rpm. Discard the Wash Tube containing flow-through. Place the Spin Column into a new 2 ml Wash Tube. Add 500 µl of Wash Buffer 2 supplemented with ethanol to the Spin Column. Centrifuge the column for 2 min at 12,000 rpm. Discard the Wash Tube containing flow-through. Place the Spin Column into a new 2 ml Wash Tube. Centrifuge the column for 2 min at 12,000 rpm. Discard the Wash Tube containing remaining flow-through. Place the Spin Column into a new 1.5 ml elution tube. Add 50 µl of Eluent to the center of Spin Column membrane. Incubate for 2 min at room temperature. Centrifuge the column for 2 min at 12,000 rpm. Discard the Spin Column. Keeps the elution tube containing pure viral nucleic acids. Store the purified nucleic acids at −20 °C.

Amplification of the specific target genome (5′ UTR of IBV) was conducted using the forward primer IBV5_GU391, AC GTATGACTACCCGCAGTATTCA and reverse primer IBV5_GL533, AGACCAGCCACCATGATTGC and probe IBV5_G, FAMCACCACCAGAACCTGTCACC TC-BHQ1 (Callison *et al.,* 2001).

Verso one step qRT-PCR kit plus ROX vial that composed of the following order and quantities:

Volume / reaction	Components
	Master Mix
3.75 µl	Nuclease free water
12.5 µl	2X 1-step PCR ready mix
1.25 µl	RT-enhancer
0.25 µl	Verso enzyme mix
1 µl	Forward primer
1 µl	Reverse primer
0.25 µl	Probe
5 µl	Extracted RNA
25 µl	Final volume of the reaction

The following IBV rRT- PCR thermal profile: 15 min at 50ºC and 15 min at 95ºC, followed by 40 cycles at 95ºC for 15 sec and 60 sec at 60ºC.

Isolation of IBV on ECE

For virus isolation, the tissue homogenate of IBV positive 14samples determined by RT-PCR were inoculated into allantoic fluid of commercial embryonated chicken eggs (four eggs 9-11 days old for each sample). The eggs were inoculated with 0.2 ml of the sample then incubated at 37°C with candling daily. Allantoic fluids were harvested at 96 h post inoculation. Two successive blind serial passages were performed. The allantoic fluids were harvested and stored at -20°C with examination of embryo for curling and dwarfism.

Slide Haemagglutination test (Rapid HA Test)

Rapid HA test was made by adding one drop of harvested allantoic fluid to one drop of 10% RBCs suspension on a slide and mixed thoroughly. The result observed macroscopically after 60 seconds as RBCs aggregates virus considered positive HA.

Conventional RT- PCR for S1 gene of IBV

The genomic RNA was extracted from the infected allantoic fluids using Thermo amp viral RNA Mini Kit #0731 (Thermo Fisher Scientific Inc.) according to the manufacturer's instructions as following: Transfer 15µl of infected allantoic fluid into a 1.5 ml microcentrifuge tube containing 170 µl of Lysis Buffer supplemented with β-mercaptoethanol Vortex for 10 s to mix thoroughly. Add diluted Proteinase K. Vortex to mix thoroughly and incubate at room temperature for 10 min. Centrifuge for 5 min at 12000 rpm. Add 225 µl of absolute ethanol and mix by pipetting. Transfer to the Gene JET RNA Purification Column inserted in a collection tube. Centrifuge the column for 2 min at 12000 rpm. Discard the flow through and place the purification column back into the collection tube. Add 350 µl of Wash Buffer 1 to the Gene JET RNA Purification Column and centrifuge for 2 min at 12000 rpm. Discard the flow-through and place the purification column back into the collection tube. Add 300 µl of Wash Buffer 2 to the Gene JET RNA Purification Column and

centrifuge for 2 min at 12000 rpm. Discard the flow through and place the purification column back into the collection tube. Add 150 µl of Wash Buffer 2 to the Gene JET RNA Purification Column and centrifuge for 2 min at12000 rpm. Discard the collection tube containing the flow-through solution and transfer the Gene JET RNA Purification Column to a sterile 1.5 ml RNase -free microcentrifuge tube. Add 100 µl of Water, nuclease-free to the center of the Gene JET RNA Purification Column membrane. Centrifuge for 2 min at 12000 rpm to elute RNA. Discard the purification column. Store the purified RNA at -20°C until use.

The RT-PCR was carried out used primer pair for amplification of genotypically diverse IBV strains is oligo S15' mod (forward): 5'-TGA-AAA-CTG-AACAAA-AGA-3' and CK2 (reverse): 5'-CNG-TRT-TRT-AYT-GRC-A-3' (Gelb et al., 2005) in a total of 50 µl reaction volume consisted of:

Reagent	**Volume/reaction**
2x MyTaq One-Step Mix	25µl
Forward primer	2 µl
Reverse primer	2 µl
Reverse transcriptase	0.5 µl
RiboSafe RNase Inhibitor	1 µl
Extracted RNA	5 µl
DEPC-H_2O	14.5 µl
Final volume	50 µl

The RT- PCR conditions were as follows: RT at 45°C for 20 min, one cycle at 95°C for 1 min, 40 cycles of heat denaturation at 95°C for 10 s, primer annealing at 60°C for 10 sec, primer extension at 72°C for 30 sec in advanced primus thermal cycler. The PCR products were analyzed on agarose gel according to the standard procedures (*Sambrook et al., 1989*). The PCR products were run on 1.5% agarose gel(with ethidium bromide) on 1X TAE buffer using 6 µl PCR product and 1 µl 6X loading dye per well. The gel runs on 95 volt for 60 min. in gel electrophoresis unit (cleaver scientific Ltd). The expected bands were detected in comparison with 100bp DNA ladder using UV transilluminator and photographed by gel documentation system (UVDI, Winpact Scientific).

Results

Clinical signs and postmortem findings of examined flocks

Forty one tissue samples were collected from clinically infected poultry of different breeds showing clinical signs of respiratory signs, high mortalities, gasping, tracheal rales, swollen sinuses, ruffled feathers and depression. Postmortem findings of freshly dead birds were tracheitis, Caseous plug in the tracheal bifurcation, pneumonia, air sacculitis, swollen and pale kidneys with distended tubules and ureters containing urate.

Results of N gene conventional RT-PCR : The IBV was detected by N gene RT-PCR in 7 samples out of 15 examined field samples as shown in Fig. 1 with percentage of (46.6%) according to the N gene PCR. The amplified products showed bands of 149bp belonging to nucleocapsid (N gene) of IBV.

Results of real time RT-PCR: the IBV was detected in 13 samples out of 25 examined field samples with a percentage of (52%).The results of the rRT-PCR of positive samples are shown in Fig. 2 and are summarized in Table 1.

Results of ECE inoculation: The RT PCR positive fourteen tissue samples processed, each sample homogenized separately and was inoculated via the allantoic fluid, Allantoic fluids were harvested at 96 h post inoculation. The embryo mortality at day 1 post infection was considered nonspecific and discarded. The IBV isolation trials revealed variably high embryonic death within 2days after inoculation. Congestion of the embryos was observed and curling and dwarfing after second serial passages as shown in Fig. 3. All the harvested allantoic fluids were used in Slide Haemagglutination Test with 10% washed RBCs suspension.

Results of rapid HA test: ten samples were tested by plate HA test giving positive result while two samples giving no heamagglutinating activity after 2nd passage in ECE.

Results of Conventional S1gene RT-PCR: this RT-PCR targeting the Spike-gene was performed on allantoic fluid of positive samples (21) to differentiate IBV serotypes. The results of Conventional RT-PCR of S1gene were negative. However, no results were obtained on this PCR and failed to produce a band. Due to failure of the Spike-gene RT-PCR, no sequences were generated. No genomic analysis or phylogenetic analyses were therefore possible.

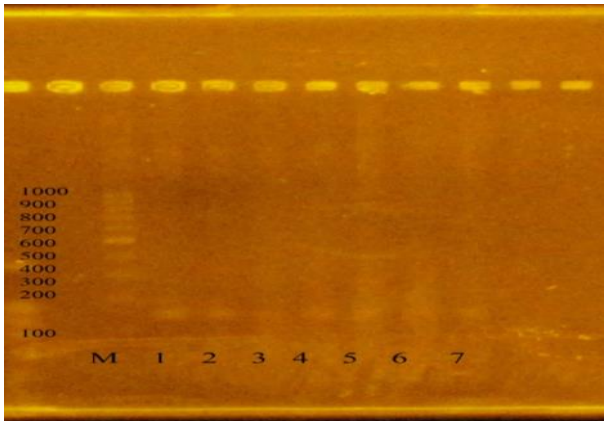

Fig .1. Agarose gel electrophoresis of PCR amplified products from purified RNA of IBV. The amplified products showed bands of 149 bp belonging to (NP gene) of IBV.

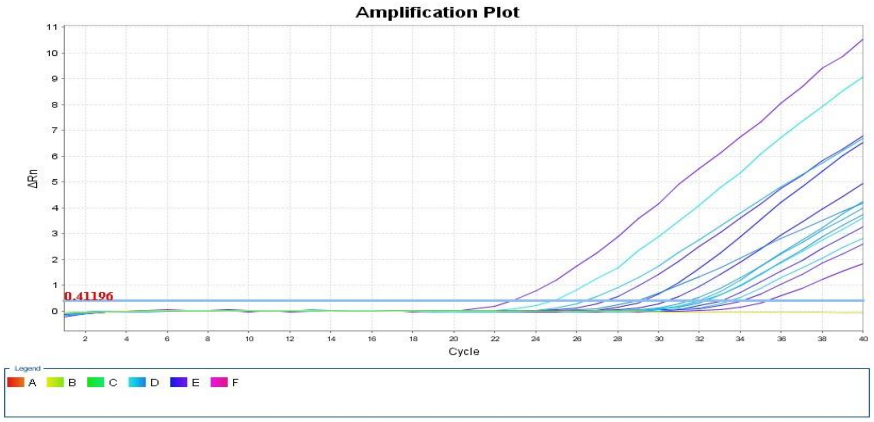

Fig. 2. Results show the positive curves of r RT-PCR that below 36 cycle threshold.

TABLE 1. Summary of the positive result of r RT-PCR.

Indication	Threshold cycle (ct)	Sample code
Positive	33	28
Positive	26	29
Positive	31	30
Positive	31	31
Positive	19	32
Positive	32	33
Positive	28	34
Positive	28	35
Positive	30	36
Positive	32	37
Positive	27	38
Positive	33	39
Positive	35	40

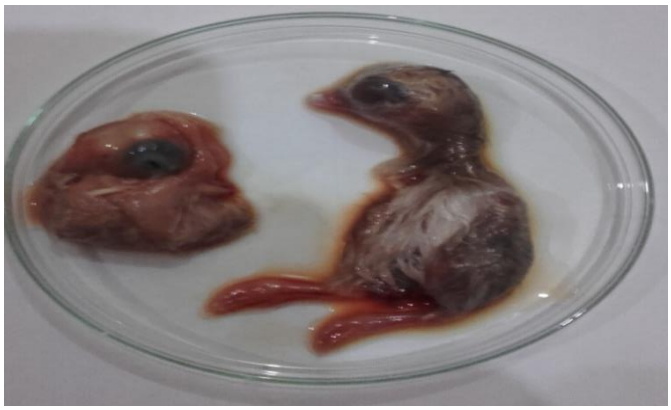

Fig. 3. Shows stunting and curling of embryos infected with IBV compared to a normal non inoculated 14 day old embryo.

Discussion

IB cause massive economic losses in poultry industry (Kwon *et al.,* 2003, Webster *et al.,* 2006 and Nguyen *et al.,* 2013). The IB virus has widely distributed around the world, and many variants have been isolated (Davelaar *et al.,* 1984, Wang *et al.,* 1994 and Liu *et al.,* 2003). A major problem in the poultry industry caused by the appearance of antigenic variants of IBV. Vaccine usage can control outbreaks of IBV but IBV outbreaks continue in vaccinated fowls (Gelb *et al.,* 1989, Wang &Tsai, 1996 and Liu *et al.,* 2003).

Diagnosis of poultry diseases in Egypt is based mainly on clinical signs and gross lesions with limited laboratory investigations used for confirmatory diagnosis. Conventional diagnosis of IB is commonly based on virus isolation and propagation in embryonating eggs, followed by haemagglutination test of the harvested allantoic fluids. Since two or three blind passages are often required for successful primary isolation of IBV, this procedure is time consuming and requires the use of specific polyclonal or monoclonal antibodies (Cook *et al.,* 2008). In this study, we performed common RT-PCR, which based on nucleocapsid (N) oligonucleotide pairs of N gene primers on the tissue samples (trachea, lung, kidneys, liver and spleen) from birds with a history of respiratory disease including coughing, sneezing, rales, and nasal discharge. The nucleocapsid gene sequence, unlike the remaining of the IBV genome is present on all IBV RNAs. We extended the sensitivity of the PCR, by RNA purification prior to the RT-PCR reaction to remove nonspecific inhibitors. Targeting the N gene has been postulated to be a better choice to improve the sensitivity of the PCR compared to the S1 gene, as the N protein is abundant in infected cells (Spencer & Hiscox, 2006 and Meir *et al.,* 2010).

In this study, Inoculation of 14 positive samples in the commercial embryonated chicken eggs resulted in stunted growth of the embryos, a characteristic sign for the IBV, in only one sample after second passage and 10 samples were HA positive so this agreed with Roussan *et al*. 2008 in that the isolation of IBV has been rare, probably due to the presence of other respiratory pathogens in chickens as Newcastle disease, Avian influenza and mycoplasmosis that masked IBV infections.

IBV serotype is classified according to feature of the S protein. As a result of molecular studies, it was now known that it was the S1 part of the IBV that is responsible for determining its serotype (Cook *et al.,* 1999). only a very few changes in the amino acid composition of the S1 part of the spike protein lead to emerge of a new IBV serotype (Cavanagh *et al.,* 1992). The greatest variation in the amino acid sequence was occurred between the residues 53 and 148 of the S1 (Niesters *et al.,* 1986, Wang *et al.,* 1994). Within the S1, there are two hypervariable regions (HVR) located at positions 56-69 and 117-133 from the beginning of the S1 were also defined (Niesters *et al.,* 1986). The HVR was an essential determinant of coronavirus serotype specificity (Cavanagh and Naqi,

2003). It is known that theS1 subunit was involved with infectivity and haemagglutinin activity and carries serotype-specific sequences (Cavanagh & Davis, 1986 and Cavanagh 1995).

All samples were tested by real-time RT-PCR and conventional RT-PCRbut serotypes were not determined. However, conventional RT-PCR is time consuming, prone to error, and is less sensitive than real-time RT-PCR. Because the real-time RT PCR assay targeting the highly conserved 5′UTR gene has a higher sensitivity than conventional RT-PCR in detecting IBV nucleic acids. (Callison *et al.,* 2007, Cavanagh & Gelb 2008, Meir *et al.,* 2010 and Acevedo *et al.,* 2013). Real-time PCR is considered the most important diagnostic tool because it is more rapid, sensitive, and reproducible, and the risk of carryover contamination is minimized compared to conventional PCR (Mackay, 2004). The increased sensitivity of real-time RT-PCR compared to conventional RT-PCR might be due to detection of the fluorescent signal emitted by specific amplification products (Acevedo *et al.,* 2013). Therefore, the real-time RT-PCR assay developed to be an important tool for the screening of samples during IB-suspected cases as the assay is rapid, simple, efficient, highly specific, and sensitive. Recently, real-time RT-PCR assays were described that can detect IBV either solely or in a multiplex set (Escutenaire *et al.,* 2007, Cavanagh & Gelb 2008, Fan *et al.,* 2012 and Acevedo *et al.,* 2013).

In this study, The conventional RT-PCR were applied to 16 clinical samples obtained from Dakahlia governorate and real-time RT-PCR were applied to 25 clinical samples obtained from Egyptian chicken flocks (23 from Dakahlia, 1 from Assuit and 1 from Matrouh) showing clinical signs of IB. IBV N gene amplification with conventional RT-PCR detected in 7 out of 15 (46.6%) ,whereas only 13out of 25 clinical samples (52%) were positive for IBV by 5′UTR amplification with real time RT-PCR. The frequency of IBV found in the sampled flocks of Egypt was high. The total percentage of positive samples was 51.2 % (21out of 41clinical samples).

Inoculation and multiplication of the virus in embryonated SPF eggs prior to RNA extraction in order to increase the viral titer in the initial sample material (De Wit, 2000).Using IBV RNAs extracted from allantoic fluid undergo RT-PCR technique that has been shown to be very efficient for detection and identification of IBV serotypes (Adzhar *et al.,* 1996, Jackwood *et al.,* 1997, Keeler *et al.,* 1998, Handberg, *et al.,* 1999 and Meulemans *et al.,* 2001).

The absence of positive reaction of S1gene conventional RT-PCR for the IBV detection of the test samples may suggest the involvement of other respiratory pathogens in the respiratory disease outbreaks in chickens in the study area. One of the major problems with IBV is the frequent emergence of new variants (Abdel-Moneim *et al.,* 2002). The detection and identification of these new variants is important to disease control (Nakamura *et al.,* 1996). In our

study, Although We could not identify any IBV strains may because of using different primers and subsequently differences of amplicon size of S1 gene IBV strains isolated by Abdel-Rahman and her group are closely related to each other and have the closest relatedness with the Egyptian Eg/CLEVB-1/IBV/012 and the Israeli IS/1494/06 strain, while, the Italy 02 strain sharing the closest relatedness of the "well-known" strains (79 and 77% on nucleotide and amino acid levels, respectively) (Abd El-Rahman *et al.,* 2015).

The emerging of new genotype strains may be due to the following, administration of live vaccines to IBV-infected birds, or infection of chickens with heterologous field isolates shortly after vaccination which is a favorable environment for natural recombination between vaccine and field strains, use of multiple strains for vaccination (Cavanagh, 2007). In addition to transcription of IBV RNA genomes has a high error rate (Lee *et al.,* 2004). So, it may partially explain one of the possibilities for failure of vaccines in the face of infection due to emerging new serotypes that push us to revise the Egyptian vaccination program against IB.

The initial plan to determine the genotypes of the isolated strains via DNA sequencing part of the S1-gene which did not succeed due to absence of positive results on the Spike gene RT-PCR. Possible reasons for failure of this RT-PCR include low virus titer in the sample. The protocol used for the S-gene RT-PCR was designed by OIE (2013). The oligo S15'mod/CK2 amplicon is beginning from the start of the S1 gene spanning two hypervariable regions used for genotyping. So, the reason of the failure may not due to "old" primers from which the field strains had emerged to the extent that the primers no longer would fit the strains.

There are several possible protocols that could have been made in order to increase the success of the S-gene RT-PCR. One includes the addition of a bigger amount of sample material to some or all of the steps in the process: more RNA to the cDNA, more cDNA to the PCR reaction or more PCR product to the electrophoresis. Without DNA sequencing, the exact genotype of these samples cannot be identified. All the positive samples for IBV may classify as "Variants" because the probability that they belong to the Egyptian Variant genotype is very high. With these facts in mind, it is probable that the unspecified genotypes called "Variants" in this study did belong to the Egyptian Genotype.

Conclusion

In conclusion, our results confirmed the detection of the IBV in chicken flocks in Dakahlia governorate which complicate the respiratory problems in affected flocks. From 41 examined flocks 21 samples were positive by RT-PCR and isolation of IBV is difficult because of mixed infection with other viral pathogens.

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التوصيف الجزيئى لفيروس الالتهاب الشعبى المعدى فى الدجاج

هبة نبيل البحيرى*، سناء سلامة عوض* وسامى جمعة قاسم**
*قسم امراض الدواجن - كلية الطب البيطرى- جامعة المنصورة و**قسم الفيروسات - كلية الطب البيطرى- جامعة كفر الشيخ - مصر.

تعتبر الاصابة بفيروس التهاب الشعب الهوائيه المعدى من أهم الامراض التنفسيه الاكثر انتشاراً فى مزارع الدجاج فى مصر مسببه خسائر اقتصاديه كبيره، هذا الفيروس يصيب جميع الاعمار كما يصيب الدجاج البياض والمنتج للحم. على الرغم من اتباع برامج التحصين المكثفة فى مزارع الدواجن فمازال هذا الفيروس يحدث خسائر اقتصادية لمربى الدواجن.

تهدف هذه الدراسة الى بحث امكانية عزل و توصيف هذا الفيروس من الدجاج. لقد جمعنا ٤١ عينة (قصبة هوائية و رئة و كلى و كبد و طحال) من دجاج علية اعراض تنفسية مثل الكحة و العطس وبعضهم لدية بعض الافرازات من الانف و الفم و اسهال ابيض و نسبة النفوق العالية من بعض الحالات التى تفحص فى العديد من العيادات البيطرية الخاصة خلال اربع سنوات، كما وجد اثناء اجراء الصفة التشريحية احمرار فى القصبة الهوائية و الرئة.

قمنا باستخراج الحامض النووى الريبوزى من العينات التى تم تجميعها، و قد قمنا بفحص مختلف العينات بواسطة اختبار تفاعل البلمرة المتسلسل مسبوقا بالنسخ العكسى للكشف عن وجود هذا الفيروس و ذلك باستخدام بوادئ عامة للكشف عن الحامض النووى الخاص بهذا الفيروس، ولقد قمنا ايضا باستخدام اختبار سلسلة تفاعل البلمرة الكمى التى اثبتت الدراسة قدرته و بنجاح على تحديد هذا الفيروس. لقد تم حقن عدد ١٤ عينة ايجابية باستخدام بيض مخصب عمر ٩-١١ يوم لعزل الفيروس ثم استخدام اختبار التلازن الدموى السريع. وقد اظهرت النتائج ان نسبة الاصابة بفيروس التهاب الشعب الهوائية المعدى ٥١.٢ ٪ ، و اظهرت النتائج ان اختبار تفاعل البلمرة الكمى اكثر حساسية واسرع من اختبار عزل وتصنيف الفيروس.