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ISOLATION AND MOLECULAR IDENTIFICATION OF IBV ISOLATES IN DIFFERENT GOVERNORATES IN EGYPT

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Abstract

Tracheal swabs and different organs are collected from 17 chicken farms showing respiratory signs and variable mortalities in different governorates. Three successive blind serial passages were performed. Four IBV isolates are detected in vaccinated chickens by RT-PCR and are identified by sequence and phylogenetic analysis of portion of S1 gene. Two IBV isolates, IBV S40 and IBV S61, are related to Mass reference strains (Egypt/F/03, M41, H120, Ma5, and M52). However, IBV S78 and IBV S82 are related to Egyptian variant 2 IBV strains Ck/Eg/BSU-2/2011 and Ck/Eg/ BSU-3/2011.These results indicate the continuous evolution of Egyptian IBV circulating in chickens despite vaccination using H120 live attenuated vaccine.

Key words: IBV, isolation, identification, vaccine, Egypt

Introduction

Infectious bronchitis (IB) is an acute highly contagious respiratory disease of chickens (Cavanagh and Gelb, 2008). The disease is caused by infectious bronchitis virus (IBV), a single strand RNA virus. The IBV genome of 27.6 kb encodes non-structural proteins (NSP) and structural proteins. The replicase genes encode 15 non-structural proteins (NSP) including the RNA-dependent RNA polymerase. The remaining third of the genome encodes four structural proteins, interspersed with small nonstructural proteins, including the spike (S), envelope (E), membrane (M), and nucleocapsid (N) proteins (Cavanagh, 2003; Enjuanes *et al*, 2000a; Enjuanes *et al*, 2000b). The IBV S glycoprotein is post-translationally cleaved into two proteins S1 and S2. The S glycoprotein especially S1 subunit determines the serotype of IBV was responsible for virus attachment to cells, det- ermined species and tissue tropism (Wesley *et al*, 1991; Fazakerley *et al*, 1992; Hingley *et al,* 1994; Ballesteros *et al,* 1997; Baric *et al*, 1997; Leparc-Goffart *et al*, 1998; Phillips *et al*, 2002; Ontiveros *et al,* 2003; Casais *et al,* 2003;Li *et al,* 2005; Fang *et al*, 2005) and is a major determinant of host protective immune response by inducing virus neutralization (VN) and hemagglutination inhibition (HI) antibodies (Mockett *et al*, 1984; Koch *et al*, 1990).

In Egypt, different IBV strains which related to the Mass, D3128, D274, D-08880 and 4/91 genotypes have been detected from different poultry farms(Abdel-Moneim *et al*, 2006; El-Kady, 1989; Sultan *et al,* 2004). Also, Egyptian IBV variants related to Israeli variants have also been identified in Egypt based on sequence analysis of portion of S1 despite the extensive vaccination.

In this study, isolation of IBV isolates from different governorates in Egypt was done. Subsequently, IBV isolates were identified by sequence analysis of portion of S1 gene.

Materials and Methods

Embryonated chicken eggs (ECE): Specific-pathogen-free (SPF) ECE obtained from SPF chicken farm (Koom-Oshiem, Al-Fayoum, Egypt) were used for isolation of the IBV field isolates.

Virus isolation: Tracheal swabs and organs (trachea, liver, lung, spleen, and caecal tonsils) were collected from 17 chicken farms showing respiratory signs and variable mortalitiesin different governorates (Tab. 1). Pool of tracheal swabs and tissue homogenate of each sample was prepared and was inoculated by allantoic sac (0.1 ml/egg) into 10-day-old SPF ECE (Koum-Oshiem SPF

chicken farm) then incubated at 37°C with daily candling. Allantoic fluids were harvested 3 days post inoculation. Three successive blind serial passages were performed. The allantoic fluids were harvested and stored at -85°C.

Identification of IBV by conventional RT-PCR: Firstly, Extraction of viral RNA was performed on allantoic fluids by using the QIAamp® Viral RNA Mini Kit (Qiagen, Germany) following the manufacturer's protocol. Then, viral RNA was used to amplify portion of 3' untranslated region (3' UTR) by one step RT-PCR kit (Qiagen, Germany) using forward primer UTR2+AAGGAAGA TAGGCATGTAGCTT & reverse primer U TR1GCTCTAACTCTATACTAGCCTAT.

The RT-PCR cycle was performed with these primers **(**Williams *et al*, 1993)**.** The RT-PCR products were analyzed by electrophoresis in 1.5 agarose gels for product size approximately 300 bp. After electrophoresis the DNA was visualized with ethidium bromide and UV transillumination. Subsequently, IBV viral RNA was used to amplify portion of S1 gene using forward primer XCE1+ CACTGGTAATTTTTCAG ATGG and the reverse primer XCE3- CAG ATT-GCTTACAACCACC as described by Antarasena *et al*. (2008). Finally, the IBV cDNA was purified by Gene JET PCR Purification Kit (Thermo Fisher Scientific) following the manufacturer's protocol.

Sequencing of portion of S1 gene and phylogenetic analysis: The purified PCR product of four IBV isolates was sequenced sequencing in GATC Biotech Company, Germany by use ABI 3730xl DNA sequencer using the forward and reverse primers. The obtained sequences were initially identified by comparing with registered IBV sequences in GenBank database using BLAST analysis. Mega 7-software was used to construct a phylogenetic tree comparing the obtained sequences in this study to sequences registered in GenBank databased (Fig. 2).

Table 1: Sampling history from different vaccinated against IB governorates in Egypt.

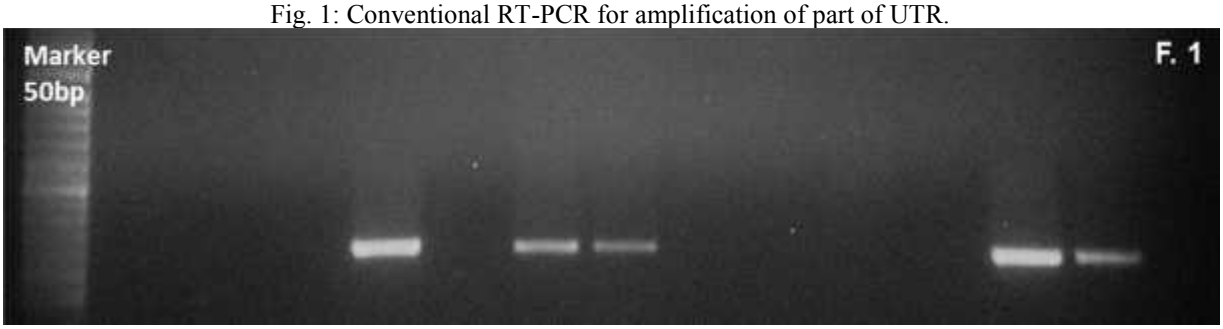
Serial No.	Governorate	Chicken type	Age/day	No.	PM
(1)	Qaliobia	Saso	63	8000	CRD & hemorrhage on proventricular glands
(2)	Qaliobia	layer	74	5000	CRD
(3)	Qaliobia	Layer	79	5000	CRD & hemorrhage on proventricular glands
(4)*	Sharkia	Broiler	32	6000	CRD
(5)	Cairo	Broiler	23	5000	CRD
(6)*	Qaliobia	Baladi (male)	29	5750	Airsaculitis (A.S), trachitis &congested liver
(7)*	Qaliobia	Saso	20	5750	CRD
(8)	Sharkia	layer	253	4000	A.S & hemorrhage on proventricular glands
(9)	Sharkia	layer	90	7000	A.S & hemorrhage on proventricular glands
(10)	Qaliobia	Saso	25	5000	Pneumonia & CRD
(11)	Qaliobia	Saso	41	5000	A.S & hemorrhage on proventricular glands
(12)	Qaliobia	Broiler	-	4500	CRD
(13)	Qaliobia	Saso	32	5000	Pneumonia & CRD
(14)	Qaliobia	Layer	74	2000	Pneumonia & CRD
(15)*	Cairo	Broiler	23	4500	Air saculitis & pneumonia
(16)	Qaliobia	Layer	78	7000	CRD
(17)	Sharkia	Baladi	57	9500	Trachitis, Ulcer on cecal tonsils

*Isolates' codes S61, S78, S84, and S40 respectively, # Chickens vaccinated with live attenuated vaccine, Chickens with respiratory signs and variable mortalities.

Results

Conventional RT-PCR: Four IBV isolates were detected by RT-PCR using universal primers which detect part of 3' UTR (approximately 300bp). Four IBV isolates coded S40, S61, S78, and S84 are detected. Amplification of part of S1 gene for these four isolates was performed and the expected PCR size (385 base) was obtained (Fig. 1).

Fig. 1: Conventional RT-PCR for amplification of part of UTR.



Lane 1: Marker 50 bp; Lane 4: IBV S40; Lane6: IBV S61; Lane7: IBV S78; Lane 13: IBV S84; Lane 14: control positive (IBV commercial live attenuated vaccine; lane 15: control negative. Expected size of RT-PCR product is 298 bp.

Sequencing and phylogenetic tree (Fig. 2): Two IBV isolates, IBV S40 & IBV S61, are related to Mass reference strains (Egypt/F/03, M41, H120, Ma5, and M52). However, IBV S78 and IBV S82 are related to Egyptian variant 2 IBV strains Ck/Eg/BSU-2/2011 and Ck/Eg/BSU-3/2011.

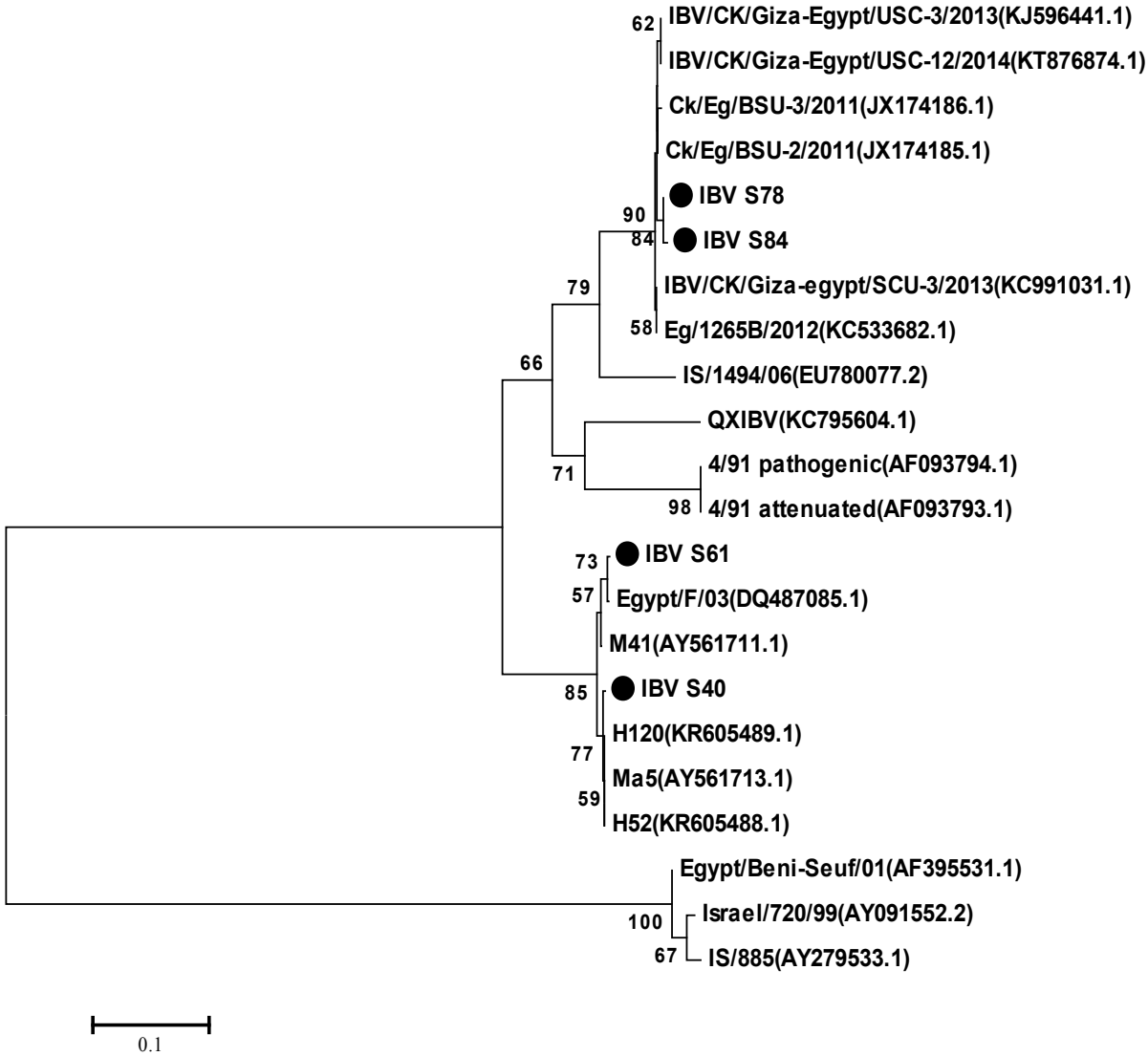


Fig. 2: Phylogenetic tree based on a partial sequence of the S1 gene. Black dots refer to viruses isolated in current study. Robustness of individual nodes of the tree was assessed using 1000 replications of bootstrap re-sampling of originally aligned nucleotide sequences.

Discussion

Avian infectious bronchitis virus (IBV) causes mild to acute respiratory disease in chickens, characterized by coughing, sneezing, tracheal rales and dyspnea (Cavanagh and Naqi, 2003). Worldwide, IBV causes huge economic losses in both broilers and layers. IBV has a tropism not only for the epithelium of the respiratory tract but also for the epithelium of kidneys, oviduct, gastrointestinal tract (oesophagus, proventriculus, duodenum, jejunum, bursa of Fabricius, caecal tonsils, rectum and cloaca) and testes (Woo *et al*, 2014). No doubt the estimation of the chicken zoonotic diseases are helpful for monitoring and improving public health and economy (Jackwood, 2012). To the authors knowledge, different IBV strains which related to the Mass, D3128, D274, D-08880 and 4/91 genotypes were detected from different poultry farms in Egypt (El-Kady, 1989; Sultan *et al,* 2004; Abdel-Moneim *et al,* 2006; Hassan *et al*, 2016). Furthermore, IBV variants related to Israeli variants have also been identified by genome analysis (Abdel-Moneim *et al*, 2002;2012; Al-Beltagi *et al*, 2014) despite the extensive vaccination.

In the present study, IBV S40 and IBV S61 strains related to Mass strains were identified in two broiler farms have respiratory signs and variable mortalities. IBV strain (Egypt/F/03) was previously isolated from unvaccinated broiler flock with the history of respiratory signs and renal disease (Abdel-Moneim *et al*, 2006). Thus, respiratory disease in this study could be caused by these IBV strains. However, mixed infections with other viral and/or bacterial pathogens caused the respiratory manifestations should be considered. The IBV strains related to the Israeli variants (IS/885 and IS/1494/06) ans Egyptian variants (Egypt/Beni-Seuf/01, Ck/ Eg/BSU-2/2011 and Ck/Eg/BSU-3/2011) were identified from chicken flocks have respiratory signs and renal disease (Al-Beltagi *et al,* 2014). The two IBV strains were identified, IBV S78 and IBV S82, which are related to the Egyptian variants 2 (Ck/Eg/BSU-2/2011 and Ck/Eg/BSU-3/ 2011).

These results indicate the continuous evolution of IBV circulating in chickens in Egypt. The two IBV strains were isolated from chickens vaccinated once at 7 day old by live attenuated vaccine (H120). Therefore, it seemed that vaccination did not confer a pro-per protection against IBV S78 and IBV S82 strains.

A live attenuated vaccine from Georgia 98 (GA98) variant strain, in the southeastern United States, was developed and evaluated. The vaccine protected against the homologous GA98 challenge as well as provided good protection against the DE072-type virus. In addition, the vaccine was shown to be adequately attenuated and safe at a 10 x dose (Jackwood *et al,* 2003). The GA98 live attenuated vaccine is commercially available in United States. Besides, a commercially live attenuated vaccine from IS/1494/06 strain is commercially used in Israel. Thus, the development of IBV live attenuated vaccine from Egyptian variants is required to protect chickens against these variants.

Conclusion

Generally speaking, the avian infectious bronchitis (IB) is one of the most important viral diseases of poultry, affecting chickens of all ages and causing major economic losses in poultry flocks. The IBV affects both the broiler and layer chickens. Although chicken flocks are routinely vaccinated with live vaccines, outbreaks of infectious bronchitis were observed in vaccinated flocks, as there was little or no cross-protection between different IBV serotypes. Consequently, the serological and molecular characterization of the Egyptian field isolates is very important in order to select the appropriate vaccine strains

The present study has demonstrated a distinct strain setting it apart from all strains reported so far in Egypt and other neighboring countries.

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