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

By
ALY M. GHETAS¹, MOHAMMAD A. KUTKAT¹, MOHAMMAD M. AMER², and MOHAMMAD H. H. AWAAD²

Departments of Poultry Diseases¹,², National Research Centre¹, and Faculty of Veterinary Medicine, Cairo University², Egypt (*Correspondence: kutkat55@gmail.com)

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/F03, 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, determined 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-Oshiemi, 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 mortalities in 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+AAAGGAAGA TAGGCATGTAGCTT & reverse primer U TR1GCTTAACTCTATACCTAT.

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 bro-

mide and UV transillumination. Subsequently, IBV viral RNA was used to amplify portion of S1 gene using forward primer XCE1+ CACTGGTAATTTCAG ATGG and the reverse primer XCE3- CAG ATTGTCTTACAACCAC 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 in GATC Biotech Company, Germany by use ABI 373x1 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 database (Fig. 2).

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

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

Lane 1: Marker 50 bp; Lane 4: IBV S40; Lane 6: IBV S61; Lane 7: 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 broiler 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 that related to the Mass, D3128, D274, D-08880 and 4/91 genotypes were detected from different poultry farms in Egypt (Sult-tan et al., 2004; Abdel-Mo-neim 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 demonstrated that the continuous evolution of Egyptian IBV circulating in chickens despite vaccination using H120 live attenuated vaccine. Thus, development of IBV vaccine from Egyptian variants is required.
References


of SARS-coronavirus adaptation to human ACE2. EMBO 24:1634-43.


