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Research paper

# Complete genome sequences of two avian infectious bronchitis viruses isolated in Egypt: Evidence for genetic drift and genetic recombination in the circulating viruses

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ABSTRACT

Avian infectious bronchitis virus (IBV) is highly prevalent in chicken populations and is responsible for severe economic losses to poultry industry worldwide. In this study, we report the complete genome sequences of two IBV field strains, CU/1/2014 and CU/4/2014, isolated from vaccinated chickens in Egypt in 2014. The genome lengths of the strains CU/1/2014 and CU/4/2014 were 27,615 and 27,637 nucleotides, respectively. Both strains have a common genome organization in the order of 5′-UTR-1a-1b-S-3a-3b-E-M-4b-4c-5a-5b-N-6b-UTR-poly(A) tail-3′. Interestingly, strain CU/1/2014 showed a novel 15-nt deletion in the 4b-4c gene junction region. Phylogenetic analysis of the full S1 genes showed that the strains CU/1/2014 and CU/4/2014 belonged to IBV genotypes GI-1 lineage and GI-23 lineage, respectively. The genome of strain CU/1/2014 is closely related to vaccine strain H120 but showed genome-wide point mutations that lead to 27, 14, 11, 1, 1, 2, 2, and 2 amino acid differences between the two strains in 1a, 1b, S, 3a, M, 4b, 4c, and N proteins, respectively, suggesting that strain CU/1/2014 is probably a revertant of the vaccine strain H120 and evolved by accumulation of point mutations. Recombination analysis of strain CU/4/2014 showed evidence for recombination from at least three different IBV strains, namely, the Italian strain 90254/2005 (QX-like strain), 4/91, and H120. These results indicate the continuing evolution of IBV field strains by genetic drift and by genetic recombination leading to outbreaks in the vaccinated chicken populations in Egypt.



## 1. Introduction

Avian infectious bronchitis (IB) is a highly contagious viral disease of chickens (*Gallus gallus*) and is responsible for severe economic losses in the poultry industry around the world (Cavanagh, 2007). IB is one of the most prevalent diseases in poultry and is manifested clinically in three different forms affecting the respiratory, the reproductive and the renal systems (Cavanagh, 2007; Jackwood, 2012). Avian infectious bronchitis virus (IBV) has also been reported in other avian species including guinea fowl, partridge, peafowl and teal, but with no clinically detectable disease (Cavanagh, 2007; Liu et al., 2005; Sun et al., 2007).

IBV belongs to the genus *Gammacoronavirus* in the family *Coronaviridae* (King et al., 2011). IBV is an enveloped virus with a single-stranded, positive-sense RNA genome of about 27.6 kb (Masters and Perlman, 2013). The genome is organized in the order 5′-UTR-1a-1b-S-3a-3b-E-M-4b-4c-5a-5b-N-6b-UTR-poly(A) tail-3′. The 5′ two-third of the genome is made up of gene 1 (the replicase gene), separated into ORFs 1a and 1b and is translated into 1a and 1ab polyproteins, with 1ab resulting from −1 ribosomal frame shift at the ORF 1a/1b junction (Brierley et al., 1989). Gene 1 encodes non-structural proteins involved in proteolytic processing of polyprotein products, virus genome replication and transcription. The 3′ one-third of the genome codes for four structural proteins: Spike (S), envelope (E), membrane (M) and nucleocapsid (N), as well as, several accessory proteins that are not essential for viral replication but may play a role in antagonizing host innate immunity (Bentley et al., 2013; Cao et al., 2008; Hewson et al., 2011; Liu and Inglis, 1991, 1992) and serve as targets for rational attenuation of IBV (Casais et al., 2005; Cavanagh, 2007; Hodgson et al., 2006; Shen et al., 2003; Youn et al., 2005). The glycoprotein S is a surface protein that is post-translationally processed into S1 (N-terminal part) containing the globular head and S2 (C-terminal part) forming the stalk domain anchored in the viral membrane (Belouzard et al., 2012; Cavanagh, 2007). The glycoprotein S1 plays an important role in tissue tropism, induction of protective immunity as it contains the receptor binding sites

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and virus neutralizing and serotype-specific epitopes (Belouzard et al., 2012; Cavanagh et al., 1998; Niesters et al., 1987; Wickramasinghe et al., 2011). These epitopes can evolve rapidly, specifically within the hypervariable regions (HVRs) in the S1 gene to evade host immune response (Belouzard et al., 2012). The E and M proteins are required for the assembly and budding of the virus (Lai and Holmes, 2001; Lim et al., 2001). The N protein plays a key role in viral replication and assembly and in the cellular immunity (Lai and Holmes, 2001).

IBV is a continuously evolving virus. Many IBV serotypes and genotypes that exhibit limited cross protection to each other have been reported worldwide (Cavanagh, 2007). Mutations including insertions, deletions, and substitutions, as well as, recombination between different strains occur frequently in nature (Adzhar et al., 1997; Hewson et al., 2014; Jackwood, 2012) leading to emergence of new variant viruses (Abolnik, 2015; Abro et al., 2012; Cavanagh et al., 2007; Jackwood, 2012). The variant viruses tend to have increased virulence and trigger IB outbreaks. Recombination in IBV happens due to viral polymerase switching templates (copy choice mechanism) during RNA replication which contributes to genetic diversity (Lai, 1992; Lai and Holmes, 2001). The phylogenetic analysis of currently available S1 gene sequences has identified 6 IBV genotypes comprising 32 viral lineages (Valastro et al., 2016).

In Egypt, IBV is highly prevalent and is a major disease problem for the poultry industry. Several serotypes and genotypes of IBV are co-circulating in Egypt, leading to the emergence of variant viruses. Historically, the "classical" IBV vaccines H120 and Ma5, have been used as a routine method of disease prevention in Egypt. Since 2012, "variant" vaccine strains 4/91, CR88, and D274 were being used along with the classical vaccine strains to control IB outbreaks all over the country. Despite of intensive vaccination, classical H120-like viruses and variant viruses have been reported frequently in Egypt.

Studies on molecular epizootiology of IBV in Egypt are limited. In 2001, a variant strain Egypt/Beni-Suef/01, also known as, Egyptian variant I, was reported and it was closely related to the Israeli variant strain IS/720/99 (Abdel-Moneim et al., 2002). Another distinct variant strain, Egyptian variant II, was reported in 2011 (Abdel-Moneim et al., 2012). The Egyptian variants I and II were described based on the HVR3 sequences of the S1 gene. However, no complete S1 gene sequence is available for these variant strains. In 2003, Abdel Moneim et al. reported a full S1 gene sequence of strain Egypt/F/03 that showed 98% nucleotide (nt) sequence identity with the classical vaccine strain H120 (Abdel-Moneim et al., 2006). In 2016, Zanaty et al. reported full S1 gene sequences of four Egyptian variant strains that showed 91 to 95% nt sequence identity with the Israeli variant strain IS/1494/06 and one classical vaccine-like strain that showed 98% nt sequence identity with strain H120 (Zanaty et al., 2016). Till date, there is no information available on the complete genome sequence of any circulating IBV strain in Egypt.

In this study, we present the first record of complete genome sequences of two Egyptian IBV field strains: a "classical" vaccine-related strain belonging to GI-1 lineage and a "variant" strain belonging to GI-23 lineage. Our Egyptian variant strain genome is the first complete genome sequence of a lineage GI-23 IBV from the entire Middle East and Africa. Our results indicate that both mutations and recombination are involved in the evolution of the Egyptian field strains.

## 2. Materials and methods

### 2.1. Egyptian IBV field strains

In Egypt, IBV field strains IBV/Ck/EG/CU/1/2014 (CU/1/2014) and IBV/Ck/EG/CU/1/2014 (CU/4/2014) were isolated from the field samples received at the Cairo University and at the Beni-Suef University, respectively, in 2014, from 4-week-old broiler chickens from two different farms. Birds in both farms were vaccinated at day 7 of age using IBV vaccine H120. Strain CU/1/2014 was isolated from tracheal swab samples collected from birds showing cough, dyspnea and lacrimation. Strain CU/4/2014 was isolated from trachea and kidneys collected from birds showing severe respiratory signs in addition to whitish diarrhea. The post-mortem examination revealed caseous plug at tracheal bifurcation and swollen pale kidneys filled with urates. The Egyptian IBV strains were shipped to the University of Maryland (UMD) for complete genome sequencing following USDA/APHIS guidelines. These Egyptian IBV samples were first tested free of exotic Newcastle disease virus in the USDA Reference Laboratory, Ames, Iowa, USA before being sent to UMD. The IBV strains were propagated in 9 to 11-day-old SPF embryonated chicken eggs following standard procedures (OIE, 2013). Strains CU/1/2014 and CU/4/2014 were subjected to complete genome sequencing at the 11th and 8th egg passage, respectively.

### 2.2. Viral RNA extraction, RACE, and RT-PCR

The IBV genomic RNA was extracted from the infective allantoic fluid using QIAamp® viral RNA Mini Kit (Qiagen, Germany) per the manufacturer's instructions. Reverse transcription (RT) was performed using reverse transcriptase SuperScript® IV (SSIV) (Invitrogen, USA) following manufacturer's protocol. A PCR reaction was performed using the universal primer set; Oligo S1 5′ mod (forward): 5′-

**Table 1**
Consensus primer sets used for the amplification of the Egyptian IBV genomes.

| Amplicon[a] | nt position[b] | Forward primer sequence (5′-3′) | Reverse primer sequence (5′-3′) |
|---|---|---|---|
| 1 | 71–1669 | CTTAAATACCTACAGCTGGTCC | ATCTYCGGTGTWACACCATC |
| 2 | 1578–3012 | TAYGYRGCRCCTYTDTCWGG | TTRCHGGRTCTTGWTAAGAG |
| 3 | 2956–4717 | ARTGTGARGARGAGGAYGAGG | GGYTGAATACAAGCTTCAAGGCC |
| 4 | 4570–6157 | GTGAKTTYTCAGAYGCTAAYTGGC | GRAASASCAWATAMAGCCAATTCC |
| 5 | 6014–8120 | GTGVAGATGATTTTRYTYGTCDTG | ACACTACCYCTACAATAGCTGTC |
| 6 | 8021–9546 | AYTTBCAACCHAATGGTGTTAGGC | TTAGTAACTAAWTTRTCTGGTKGCAC |
| 7 | 9428–11,153 | CACCATCTDGAGTTRCCTARTGC | CTGACTTTGCAATRTTGGCRGC |
| 8 | 11,091–12,523 | ATGGTGGTGTWACACAGCAAG | ACATCATCAAAGGCTCGCTTTAC |
| 9 | 12,435–14,541 | TTTAAACGGGTACGGGGTAGC | RRCGCGCRACATTRGCAGATG |
| 10 | 14,250–16,323 | GMRGAYCCDRTYATGGGTTGGG | CRCAACTAACTTCHGGCAAGGC |
| 11 | 16,253–18,163 | RTTTAARGCWAATGAYACAGGC | CCARAACATACAAAGACCATCAGC |
| 12 | 18,004–19,270 | CTAYGAYATRGGCAACCCTAAAGG | CWCCATAHARTATGTGYTYAGACC |
| 13 | 18,990–20,572 | AGTBTCYACACAGTGTTAYAAGCG | GGYCTRWANKSRCTYTGGTAG |
| 14 | 20,819–22,009 | TTAAATCATTTCAGTGTGTTAATAAT | CATAACTAACATAAGGGCAA |
| 15 | 21,850–23,268 | GATGTCAACCAGCAGTTTGTAG | GCATACTGACTAGCATTAGCTG |
| 16 | 23,071–24,688 | SARAARATTAATGAGTGTGTHAARTC | ACCTACTGCWATGTTAAGGGG |
| 17 | 24,638–26,077 | CGADTTYCCNAARAACGGTTGG | RYTCTRCTTGTCCTGCTTTG |
| 18 | 25,971–27,836 | TAGTAAAGATAATCCTTTTCGCGG | TAGTGCTGTACCCTCGATCG |

[a] Amplicon refers to each of the 18 overlapping RT-PCR amplicons for IBV genome sequencing.
[b] nt position indicates corresponding nucleotide position of each RT-PCR amplicon in the aligned consensus of the 21 IBV genomes.