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Full Length Article

Co-circulation of avian influenza viruses in commercial farms, backyards and live market birds in Egypt

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Abstract Cloacal and tracheal swab-samples were collected from commercial farms, backyards and live market birds (LBM) to identify the potential existence and genetic drifts of avian influenza subtypes (AI) H5 and H9 that are circulating among bird species in Egypt. The results revealed that, one sample out of 50 samples of chicken commercial farms was positive for the isolation of subtype H9N2 [KC699549, Influenza A virus: A/chicken/Egypt/VRLCU-R33/2012(H9N2)]; from Sharkeia province. Two samples out of 20 samples of Backyard ducks were positive for the isolation of 2 subtypes H5N1; [KC699547, Influenza A virus: A/duck/Egypt/VRLCU-R11/2012(H5N1), "backyard duck"] from El-Fayoum province and the other from Giza province [A/duck/Egypt/VRLCU-R28/2012(H5N1), "backyard duck"]. Analysis of haemagglutinin (HA) and the phylogenetic tree of the isolated viruses (H5N1) were fallen within the clade 2.2.1.1. Antigenic cartography for the isolated Egyptian H9N2 AI virus can intuitively be of group-B. The number of mutations in the amino acid sites (33, 47, 65, 90, 92, 143, and 150) and the Long Branch observed in the phylogenetic tree may suggest a rather long evolution period. The sequenced H9N2 Egyptian virus in the study was closely related to the previous Egyptian isolates.

1. Introduction

Avian influenza (AI) is a highly contagious respiratory disease affecting poultry caused by influenza A viruses of the family Orthomyxoviridae. Influenza A viruses are classified into 17 haemagglutinin (HA) subtypes and 10 neuraminidase subtypes

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[1]. The disease constitutes a major threat to poultry industry worldwide [2], where a high number of birds involved the extreme control costs of the disease and the gregarious consequences [3].

In 1996, A/goose/Guangdong/1/1996(H5N1), was identified in a geese farm in southern China. These H5N1 HPAIVs have been spread across Asia, Europe and Africa and presented a continuous threat to both animal and human health [4].

Highly pathogenic avian influenza (HPAI) virus of the H5N1 subtype descending from A/goose/Guangdong/1/96 lineage was first detected in Africa in 2006 [5].

In March 2006, vaccination of poultry using inactivated vaccines derived from either Mexican low pathogenic H5N2 or Asian H5N1 strains was sanctioned essentially [6].

In 2008, H5N1 HPAI infection perpetuated to fan out and became endemic in Egypt. Clade 2.2.1 was introduced into Egypt and spread rapidly in commercial and backyard flocks [7]. As a consequence of the persistence and extensive circulation of H5N1 HPAI viruses in Egypt, variant strains emerged evolving into distinct genetic subclades [8].

One of these variant strains is H9N2 AIVs which have circulated worldwide in poultry populations over the last decade, causing mild respiratory disease and reductions in egg production, resulting in great economic losses and co-infection with other pathogens [9–15].

H9 subtypes posses a mild nature which may provide them a great opportunity to turn more virulent through surreptitious spread, mutation and/or reassortment with other subtypes of influenza viruses [16–18].

Antigenic drift has been observed in type A influenza viruses resulting from point mutations which ultimately transmute the hemagglutinin (HA) protein epitope structure [19].

This study aimed to identify the potential existence and antigenic drift of Avian Influenza subtypes (AI) H5 and H9 circulating among chicken flocks, backyards and live bird markets in Egypt.

2. Materials and Methods

2.1. Samples

Cloacal and tracheal swab-samples were amassed from 50 Commercial farms (broilers, layers, breeders), 10 Backyard and LBM bird species (ducks, geese, turkeys, chickens) during 2012 in some Egyptian provinces [20].

2.2. Virus isolation, RT-PCR, sequencing and data analysis

2.2.1. Virus isolation

Specific pathogen free embryonated chicken eggs (ECE) of 9–11 day old were used for isolation and propagation of the avian viruses. The eggs were obtained from SPF engenderment project, Fayoum, Egypt.

2.2.2. Viral RNAs extraction

Viral RNAs were extracted by the use of QIAamp viral RNA Mini Kit (QIAGEN, Germany) Cat. No. 52904. The kit combines the selective binding properties of silica-gel-based membrane with the speed of micro spin technology. The kit contains: QIAamp mini spin columns, collection tubes (2 ml), buffer (AVL), buffer AW1, buffer AW2, and buffer AVE and Carrier RNA.

2.2.3. Amplification

Primers were designed for specific help in genetic characterization of AI strain. The design of primers was according to lab of Virology, Faculty of Veterinary Medicine, Cairo University and was manufactured by METABION® Company (Germany).

2.2.4. Sequencing

One-Step RT-PCR kit (QIAGEN, Germany) with primers specific for influenza virus. Cat. No. 210212, was used. The primer sequences and amplification conditions used were available upon request. The PCR products were separated on an agarose gel (Vivantis-Malaysia) by electrophoresis, and amplicons of the felicitous sizes were subsequently excised from the gel and extracted by use of a QIAGEN gel extraction kit.

2.2.5. Phylogenetic analysis of influenza virus genes

Phylogenetic and molecular evolutionary analyses were conducted using BIOEDIT version 7.0.4.1 (MEGA 5.05 software) (95/98/NT/2000/XP). MEGA is an integrated implement for conducting automatic and manual sequence alignment, inferring phylogenetic trees, mining web-based databases, estimating rates of molecular evolution, inferring ancestral sequences, and testing evolutionary hypotheses. MEGA is a multi-threaded windows application. It runs on all releases of Microsoft Windows operating system. Sequence submission to the Gen-bank (Sequence FASTA file): The frame adjusted, clean sequence which used in phylogenetic tree construction was submitted to Gen-bank. Sequence results were received via mail as text, BLAST report and AB1 file for both forward and reverse sequence of the sent sample.

2.2.6. Deduced amino acid sequence analysis

We analyzed the HA deduced amino acid sequences of 3 isolated strains and compared them with Egyptian H5N1 and H9N2 isolates available in the flu database.

3. Results

One sample out of 50 samples (2%) of chicken commercial farms was positive for the isolation of one subtype of H9N2; from Sharkeia province. Two samples out of 20 samples (10%) of Backyard species were positive for the isolation of 2 subtypes of H5N1; from ducks, one from El-Fayoum province and the other from Giza province, as indicated in Table 1.