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*Faculty of Veterinary medicine*

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# Isolation and characterization of avian influenza viruses from different species of ducks in the delta region

***A thesis submitted***

**by**

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## Abstract

In this study, we have approached the epidemiological situation of avian influenza in duck species during the period between February 2013 to October 2014 collected from the Delta region governorates especially in domestic ducks which maintain the viruses in close proximity to other species and have been implicated in the spread of both LPAI and HPAI viruses to domestic poultry and terrestrial birds.

We have pursue HA gene evolution by sequencing of selected 6 isolates from 17 positively confirmed by real time RT-PCR which revealed that 5 isolates were H5 subtype and its phylogenetic analysis belong to clade 2.2.1while one isolate was H9 subtype and its phylogenetic analysis showed that it is related to G1 like viruses.

The sequence analysis of H5 nucleotides and predicted sequence amino acid revealed that all study isolates are closely related to each other while identity percentage from the first avian influenza isolate in Egypt was (95.4 to 98.5%) while H9 isolate show slightly different (96.7%) identity from the first H9N2 avian influenza isolated in Egypt.

Although Mutational rate differ between H5N1 isolates but all H5 isolates showed deletion of AA residue 129 and D43N substitution mutation while H9N2 isolate show S5P and S16N indicating continuous genetic evolution of avian influenza virus.

**Keywords:**Avian influenza, H5N1, H9N2, ducks, HA gene, HA protein, sequence analysis.

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# Introduction

# *1. Introduction*

Avian influenza (AI) is a highly contagious viral disease affecting several species including birds, which classified into highly pathogenic avian influenza (HPAI) and low pathogenic avian influenza (LPAI), depending on the severity of the disease in susceptible birds. HPAI outbreaks in birds have been caused mainly by H5 and H7 subtypes, while H9 and some strains of the H5 and H7 subtypes have been characterized as LPAI ***(Zhou et al., 1999).***

Avian influenza is caused by infection with viruses of the family *Orthomyxoviridae* placed in the genus influenzavirus A which categorized based on the relationship between the glycoprotein on the surface into 18 different haemagglutinin antigens (H1 to H18) and 11 different neuraminidase antigens (N1 to N11) where each virus has one haemagglutinin and one neuraminidase antigen in any combination ***(Tong, S. et al., 2013).*** Influenza A viruses is the only orthomyxoviruses known to naturally affect birds. Many species of birds have been shown to be susceptible to infection with influenza A viruses.

Ducks and other aquatic birds form a major reservoir of these viruses; in certain host species such as Pekin ducks some HPAI viruses do not necessarily provoke significant clinical disease. In addition, low pathogenicity avian influenza (LPAI) viruses, which normally cause only a mild or no clinical disease, may in certain circumstances produce a spectrum of clinical signs. Depending on the species, age and type of bird, specific characteristics of the viral strain involved, and on environmental factors (***OIE, 2012).*** Ducks have been shown to play a key role in avian influenza transmission.

During February 2006, H5N1 was reported in Egypt and the isolation of H5N1viruses in poultry was confirmed to be identical HPAI virus due to the presence of multiple basic amino acid sequences in HA0 cleavage site, where the virus widely extended in very short time and infected commercial poultry production sectors and backyards ***(Aly et al., 2006).***The virus is currently endemic among backyard poultry flocks in many provinces in Egypt. Surveillance is one of the most important tools to evaluate the current situation and essential to eradicate the disease according to national and international programs ***(Arafa et al., 2008).***

Egypt ranks second in cumulative number of avian influenza cases after Indonesia ***(WHO, 2013).*** And it was reported from the epidemiological analysis that the virus is still circulating in several countries in Asia and became only in Egypt for Africa ***(FAO, 2013).*** Ducks have been shown to play a key role in avian influenza transmission.

Avian influenza virus is enveloped, and pleomorphic with a size ranging from 80-120 nm. The genome of type A influenza is single –stranded, negative-sense RNA and contains eight genome segments that encode 11 proteins. (***spackman, 2008).***One of these gene products is HA protein which is the major surface antigen and an integral membrane glycoprotein .It is responsible for attachment to the host cell receptor as well as fusion between the virion envelope and host cell and plays a major role in determining virus pathogenicity. The HA protein also influences host specificity by preferentially binding to one of two different sialic acid receptors on the host cell surface. As found for sialic acid-containing receptors of the epithelial cells in duck intestine ***(Ito and Kawaoka, 2000).***

Several protocols based on different molecular methodologies, such as RT-PCR and real-time RT-PCR (RRT-PCR) used for rapid detection of influenza viral RNA. PCR-based and sequencing protocols are available to detect subtype and

pathotype of the virus in rapid turnaround time and faster characterization ***(Cattoli and Capua, 2006).***

Sequence analysis and phylogenetic characterization of the viral genes and their respective amino acids is a valuable tool for detection of any antigenic variation among viruses circulating in the field and also provides information about the source of the initial disease outbreak in a certain geographic district, The surface glycoprotein haemagglutinin (HA) helps the influenza A virus to evade the host immune system by antigenic variation and is a major driving force for viral evolution***(Duvvuri et al., 2009).*** Extensive circulation of the virus has resulted in a progressive genetic evolution and an antigenic drift ***(Cattoli et al., 2011).***

**The aim of this work:**

Approaching and studying the Epidemiological situation of avian influenza of both circulating H5 and H9 subtypes focusing on duck species in delta region by using the following methodology:

- Detection of AIV in duck samples by real time PCR for HA gene.
- Isolation of the positive samples in SPF embryonated chicken eggs for virus propagation then Haemagglutination assay performed.
- Sequencing of HA gene of isolates and study of genetic changes among Egyptian strains.
- Phylogenetic analysis of HA gene of duck AIV isolates.