Cairo University
Faculty of Veterinary Medicine
Department of Virology

PhD Thesis

Molecular Analysis and Antigenic Mapping of Hemagglutinin of Selected Egyptian H9N2 Avian Influenza Virus

Presented by

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Supervision sheet

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Abstract

Avian influenza viruses of H9N2 subtype became widely distributed in most Middle Eastern countries, causing great economic losses in poultry industry especially when complicated with other pathogens. The H9N2 viruses in Egypt have a wide spread nature since its first occurrence in 2011. In this study, we collected Cloacal and tracheal samples from 19 flocks for detection, isolation and propagation of H9N2 virus using real-time RT-PCR and egg inoculation. We studied the molecular evolution of the hemagglutinin gene of H9N2 viruses by full HA gene sequencing, then the antigenic characterization was implemented using the cross HI assay and SVN assay. The antigenic relationships between the different viruses were clarified by the statistical analysis for results of the cross HI and SVN. 3D Bioinformatics cartography mapping software was used in analysis of HI results. The phylogenetic analysis of the HA gene of Egyptian H9N2 viruses clearly points out the presence of only one group (Egy/G1) of originally introduced viruses in 2011 related to the G1 lineage within group B, with the presence of multiple minor clusters includes viruses from 2011 to 2015. However, a new variant (Egy/G1var) cluster was detected in quails since 2012. Genetically, Egy/G1var viruses characterized by 20 amino acid substitutions within and adjacent to the antigenic sites in comparison to other Egyptian viruses. In addition, two glycosylation sites at amino acid residues 127 and 189 were determined in close to the receptor binding and antigenic sites. The antigenic analysis based on 3D antigenic mapping showed that the Egy/G1var cluster was clearly distinct from the original Egy/G1 viruses. One hundred serum samples of 5 vaccinated layers flocks were tested for sero conversion against different Egyptian viruses, and the results of seroconverion were analyzed statistically and found out a low response against the genetic and antigenic variant virus of quail origin. In conclusion, Egy/G1var is shown to be a new escape mutant variant cluster with an adaptive evolution in quails.

Keywords: LPAI H9N2, HA gene, Genetic characterization, Antigenic cartography, Glycosylation site, Antigenic sites, Receptor binding sites, Quails & Egypt

Dedication

For my family ….

My father, my mother

My sisters and my brother

For my dear friends ….

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