

## PHYLOGENETIC ANALYSIS OF HEMAGGLUTININ AND NEURAMINIDASE GENES OF HIGHLY PATHOGENIC AVIAN INFLUENZA H5N1 EGYPTIAN STRAINS ISOLATED FROM 2006 TO 2008 INDICATES HETEROGENEITY WITH MULTIPLE DISTINCT SUBLINEAGES

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### Important Findings

From the data in this study, researchers concluded that H5N1 in Egypt is continuously mutating and that multiple heterogenic strains persist inside Egypt. The data from this study identifies distinct genetic markers in both hemagglutinin (HA) and neuraminidase (NA) genes and suggests grouping of Egyptian isolates into 2 major HA sublineages of isolates from 2006 to 2008, and 3 smaller emergent subgroups. The NA phylogenetic and sequence analysis showed a similar pattern except that two of the emergent groups from the HA phylogenetic tree clustered together, evidence of likely reassortment. The different subgroups did not appear to segregate related to date of isolation, species of origin, nor geographic location of the viruses.

### Significance of Findings

In addition to finding that H5N1 is continuing to mutate with multiple heterogenic strains persisting in Egypt, this study also illustrates the important role of longitudinal gene analyses in endemic countries like Egypt, especially to help in the characterization of the newly emerged strains. Efforts to do whole-genome sequencing will aid in determining which the most important emerging viruses in the country are. Based on the available information, multiple lineages of viruses continue to circulate in Egypt, which can affect decisions on vaccination and control, but the reasons for the diversity within Egypt remain unclear.

### Additional Information

A unique highly pathogenic avian influenza (HPAI) H5N1 virus lineage first emerged in China in 1996, and in the past 5 years, it has become widespread, affecting poultry, wild birds, and humans in Asia, Europe, and Africa. Phylogenetic studies have identified, to date, nine major clades of H5N1 HPAIV of Asian origin. Clade 2 appears to be the most diversified, and members of subclade 2.2 have been responsible for the westward spread of H5N1 from Asia. Within subclade 2.2, three further subtypes have been distinguished and were designated with respect to their geographic origin—Europe, Middle East, or Africa.

HPAI H5N1 virus was first recognized to cause serious disease outbreaks in Egypt in 2006, and the virus has become enzootic in poultry throughout Egypt. Although outbreaks have a seasonal pattern, with most occurring during the cooler winter months, it remains unclear whether this seasonality reflects virus maintenance within Egypt or yearly introductions of the virus into the country. The aim of this work was to study the epidemiology of H5N1 HPAI in Egypt, based on the sequence analysis of the HA and NA genes of the Egyptian isolates from 2006–2008. The results revealed that most viruses isolated from 2006 and early 2007 were closely related to viruses isolated in the same time in the Middle East region with no distinct phylogenetic sublineage of viruses or unique amino acid markers being observed. However in late 2007 one variant cluster did emerge that was prominent based on number of amino acid changes in the HA and the high percentage of viruses that belonged to the group of those viruses isolated in 2007 and 2008.

This analysis will also aid in understanding which viruses circulating in Egypt are most important, assisting in the selection of viruses to be used for vaccine production and for challenge strains of existing vaccines.

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## EL ANÁLISIS FILOGENÉTICO DE LOS GENES DE HEMAGLUTININA Y NEURAMINIDASA DE LAS CEPAS EGIPCAS H5N1 ALTAMENTE PATOGENICAS DE INFLUENZA AVIAR AISLADAS DE 2006 A 2008 INDICAN HETEROGENECIDAD CON SUBLINAJES DISTINTOS MÚLTIPLES

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### Hallazgos Importantes

A partir de los datos en este estudio los investigadores concluyeron que la H5N1 en Egipto está mutando continuamente y que las cepas heterogénicas múltiples persisten dentro de Egipto. Los datos de este estudio identificaron distintos marcadores genéticos tanto en los genes HA, como en los NA y sugieren agrupar a los aislamientos egipcios en 2 sublinajes HA de 2006 a 2008, y a 3 subgrupos emergentes más pequeños. El análisis de secuencia y filogenético de NA mostró un patrón similar excepto que dos de los grupos

emergentes del árbol filogenético HA se agrupaban juntos, esto es evidencia de un posible reacomodo. Los diferentes subgrupos no parecieron segregarse en relación con la fecha de aislamiento, especies de origen o ubicación geográfica de los virus.

### Significancia de los Hallazgos

Además del hallazgo de que H5N1 está mutando continuamente con cepas heterogénicas múltiples persistentes en Egipto, este estudio también ilustra el importante papel del análisis longitudinal del gen en países endémicos como Egipto, especialmente para ayudar en la caracterización de cepas que surgieron recientemente. Los esfuerzos para hacer toda la secuenciación del genoma ayudarán a determinar

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