

**Cairo University**  
**Faculty of Veterinary Medicine**  
**Department of Poultry Diseases**



**MOLECULAR EPIDEMIOLOGICAL STUDIES ON THE CURRENT  
STATUS OF AVIAN INFLUENZA VIRUS (H5N1)  
IN POULTRY IN EGYPT**

A thesis presented by

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## Supervision sheet

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### **Abstract.**

This study has been pursued to better understand the epidemiological and genetic characters of HPAI viruses in the Egyptian commercial poultry flocks during the period beginning at Dec 2014 to May 2015 and to molecularly identify the predominant Highly pathogenic avian influenza (HPAI H5N1) strain(s) via field investigation for monitoring of different outbreaks of suspected AI infection in different poultry farms, the mortality rate ranged from 50 to 100 %, 39 out of 52 samples were positive for HPAI H5N1 (75%) with 9 highly positive samples with Cq ranged from 8.40 to 13.89, The same 39 samples caused deaths of the embryos within 18-36 hr , post inoculation (PI) with positive rapid HA test. The phylogenetic analysis revealed that the 9 HPAI H5N1 strains formed a new distinct monophyletic group within 2.2.1.2 clade. One strain out of 39 positive HPAI H5N1 cases, was free from other respiratory viral infection specially H9N2, NDV and IBV, it was titrated using SPF ECE and its titer was  $10^8$ /ml EID<sub>50</sub>. Then the pathogenic effect of the predominant HPAI H5N1 A/duck/Egypt/CLEVB-24\_N00238/2015 was tested in SPF chickens and the course of the disease was rapid and very severe as the mortality began at the 2<sup>nd</sup> DPI and reached to 100% at the 3<sup>rd</sup> DPI. The clinical signs , post mortem lesions, histopathological examination was recorded, as well as the virus was detected in all freshly dead chickens by real time RT-PCR using specific H5 gene primer. The efficacy of 5 commercially available inactivated AI (H5N1, H5N2 and H5N3) vaccines were evaluated against challenging with the current our recently isolated HPAI H5N1 (A/duck/Egypt/CLEVB-24\_N00238/2015) field strain using both SPF and broiler chickens. SPF groups were vaccinated at 21<sup>st</sup> day old while broiler groups were vaccinated at 10<sup>th</sup> day old, the serological response was evaluated on weekly bases using HI test also, virus shedding titer was detected by real time RT-PCR, result revealed that 3 vaccines poulvac H5N3, Nobilis H5N2 and Re-5 H5N1 AI vaccines were protective and – induced significant reduction in the titer of the shedding virus (more than 2 logs) compared to the non-vaccinated challenged groups in both broiler and SPF chickens. Finally the efficacy of the recombinant HVT-H5 avian influenza vaccine expressing the H5 gene from a clade 2.2 HPAIV H5N1 strain (rHVT-H5) was tested in both specific pathogen free (SPF) and commercial broiler chicks against challenging with the current recently isolated HPAI H5N1 field strain and our results declared that the protection percentage in both SPF and broiler groups were 70% and 60% .**Keywords:** Avian influenza virus (AIV), H5N1, Highly Pathogenic Avian Influenza (HPAI), pathogenicity, broiler, vaccine and vaccination.





# *Dedication*

*My Father,*

*My Mother,*

*My Sisters,*

*My brother and my best  
friends*







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