Cairo University

Faculty of Veterinary Medicine

Virological study of coinfection of Newcastle disease virus Genotype VII and H9N2 Avian influenza

A Thesis Submitted

Dalia Said Ibrahim El sayed

(B.V.Scs .Fac. Vet .Med. Cairo Univ., 2007; Master degree 2012)

For the PhD Degree in veterinary Medical sciences

(Virology)

Under supervision of

Prof.Dr. Ahmed Abd El Ghani El Sanousi
Professor of virology
Faculty of veterinary medicine
Cairo University

Dr. Ayman Hany Eldeeb
Assistant professor of virology
Faculty of veterinary medicine
Cairo University

Dr. Abd El Sattar A. Mohamed
Chief Researcher in Reference Lab. for
Quality control on poultry production
Animal Health research Institute

Dr. Abdullah Abdelzahir Selim
Chief Researcher in Reference Lab. for
Quality control on poultry production
Animal Health research Institute

(2020)

Cairo University

Faculty of veterinary medicine

Department of Virology

Supervision Sheet

Prof. Dr. Ahmed Abd El Ghani El Sanousi
Professor of virology
Faculty of Veterinary Medicine, Cairo University

Dr. Ayman Hany Eldeeb
Assistant Professor of virology
Faculty of Veterinary Medicine, Cairo University

Dr. Abd El Sattar Arafa
Chief Resarcher in Reference Lab for quality control on poultry production, Animal Heaalth Research Institute.

Dr. Abdullah Abdelzahir Selim
Chief Resarcher in Reference Lab for quality control on poultry production, Animal Heaalth Research Institute.

2020

Dedication

Dedicated to my family

….. Father,

….. Mother

…….. My Husband

Acknowledgment

First and always, all thanks to Allah, Almighty, the most merciful and the compassionate. His guidance and sustenance made this study a reality and came to the light.

I would like to express my deep appreciation and sincere gratitude to **Prof. Dr. Ahmed Abd El Ghani El Sanousi**, Professor of Virology, Faculty of Veterinary Medicine, Cairo University, for his help that is beyond my little words.

Grateful thanks and sincere gratitude are extended to**. Dr. Ayman Hany Eldeeb ,** Assistant Professor of Virology, Faculty of Veterinary Medicine, Cairo University, for his support.

Grateful thanks and sincere gratitude are extended to **Dr. Abdel-Satar Arafa Mohamed**,

Chief researcher**,** RLQP, Animal Health Research Institute for his support, and great help in this study.

Special thanks to **Dr. Abdullah Abd Elzahir selim**, Chief researcher, RLQP, Animal Health Research Institute for his advice and support.

Special thanks to **Dr. Ahmed Erfan and Dr. Ahmed Samy**, Biotechnology unit, RLQP, Animal Health Research Institute for their help during the study.

I wish to present my appreciation to **my colleagues in** the Reference Laboratory for Veterinary Quality Control on Poultry Production, Animal Health Research Institute, for their kind help during the study. I am greatly indebted to all.

Special thanks for all staff members of Department of Virology, Faculty of veterinary Medicine, Cairo University.

Faculty of Veterinary Medicine
Department of Virology
Cairo University

Name : **Dalia Said Ibrahim**

Nationality : **Egyptian**

Date of birth : **22 / 12 / 1985**

Place of birth : **Cairo**

Specification : **Virology**

Thesis title : **Virological study of coinfection of Newcastle disease virus Genotype and H9N2 Avian influenza**

Supervisors : **Prof. Dr. Ahmed Abd El Ghani El Sanousi.**

Dr. Ayman Hany Eldeeb

Dr. Abdel-Satar Arafa Mohamed.

Dr. Abullah Abdelzaher Selim

Abstract

In this study, we investigate the effect of coinfection with avian influenza (subtype H9N2) and velogenic Newcastle disease virus on the pathogenesis and virulence both viruses. In co-infection experiment, one strain of H9N2 by titer 10^6 EID_{50} and one strain of VNDV by titer 10^3 EID_{50} were used. In experimental design, 70 specific pathogen free chicks (four weeks old) were grouped into 7 groups (G1-G7) ten birds/group. Group 1 was uninfected control group, group 2 and 3 were positive control for H9N2 and VNDV respectively, group 4 infected firstly with VNDV and after 3days it was infected by H9N2 and vice versa in group 5 while in group 6 two studied viruses were inoculated simultaneously. The birds in group 7 were infected firstly by H9N2 and after 14 days were infected with vNDV. The obtained results showed that the mortality due to vNDV infection (G 3) and in group 7 started from 6 Days post infection (DPI) and reached 100% at 9DPI, while the mortalities started at 2 DPI in G4 and at 4DPI in G5,6 also the mortalities were reached 100% at 6,7,8DPI in G4,5and 6. The

VNDV shedding in control group (G3); G7; G6 and G4 started at 2DPI by titer $10^{2/}$/bird and it reached to 10^4/bird at 6DPI. While the VNDV shedding in group5 was reduced by 40% at 2DPI and at 4DPI the all tested birds were considered shedder by titer $5X10^5$/bird. The shedding of H9N2 in group infected with VNDV 3 days prior to H9N2 infection were reduced at 3days post infection (DPI) to 2/5 shedders (40% shedding) compared to 5/5 shedders (100% shedding) in positive control group with significant p value 0.0044 while there was no shedder birds in simultaneous infected group with significant P value < 0.0001. At 5DPI the shedding of H9N2 in group infected with VNDV 3 days prior to H9N2 infection was 100% (3/3 shedder) and was 20% (1/5 shedder) in simultaneous infected group with significant P value 0.046 while it was 40% (2/5 shedder) in positive control group. The shedding reached to 80% (4/5 shedders) in simultaneous infected group at 7DPI. VNDV post infection prolonged and enhanced shedding of H9N2 and reached to 100% at 5DPI compared to 40% in positive control group and completed to 100% at 7DPI while it was 20% in positive control group. . Immunohistochemical results showed that expression of H9 viral antigen recorded the peak in control H9 group in the lung tissue, meanwhile expression of ND viral antigen was maximum recorded in prior infection with H9 in the lung tissue. A significant increase in the H9 expression in the intestine of prior infection with NDV group, however simultaneous infection of both viruses scored the peak of ND expression in the intestinal tissue.

This study concluded that H9N2 prior infection interferes with pathogenesis and virulence of second VNDV infection while no significant interference in post or simultaneous infection. VNDV interfere with the pathogenesis and virulence of H9N2 in prior, post and simultaneous infections.

Key words

Influenza Virus; Newcastle virus; H9N2; Velogenic; Coinfection; Immunohistochemistry.

CONTENTS

List of tables

List of Figures