Cairo University
Faculty of Veterinary Medicine

Studies on Combined Infection with Low Pathogenic Avian Influenza (H9N2) and Newcastle Disease Genotype VII in Chickens

A Thesis submitted by

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For Ph.D degree of Veterinary Science
Poultry diseases

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(2018)

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ABSTRACT

Co-infections of Newcastle disease (ND) and avian influenza (AI) were the major viruses causing high mortality and high economic losses. In this study 300 samples were collected from 50 chickens flocks suffering from respiratory symptoms , sever drop in egg production and mortalities from December,2013 to December, 2014. RT-PCR was performed to detect the M protein of H9 gene of avian influenza and F gene of velogenic NDV (vNDV) co-infections. H9 PCR results were 20 and 34 in coloacal and pharyngeal swabs with total percentage of 6.7 % and 11.3%; respectively. The H9 sequences showed low pathogenic RSSR/GLF motif. The phylogenetic tree showed that H9 sequences belong to the G1 lineage which clustered with 2015 -2016 Egyptian H9N2. Partial sequences of the Fusion gene revealed that the isolated NDV isolates belong to class II genotype VIIj. The cleavage site of the F protein showed the presence of a polybasic amino acid motif (^{112}RRQKRF^{117}) suggesting that our two isolates were vNDV strain. Out of molecular identified H9 (1 isolate) and NDV Genotype VII (2 isolates) selected from were named AI/CHICKEN/EGYPT/48Ob/NRC-CU/2014(H9) , NDV/CHICKEN/EGYPT/48Ob/NRC-CU/2014 , and NDV/CHICKEN/EGYPT/66Oc/NRC-CU/2014 , and nucleotide sequences were submitted to GenBank and given accession numbers MG966504, MG966505, and MG966506; respectively.

In This study we detect levels of protection induced by the commercial ND genotype II Hitchner B1, LaSota vaccination regime and inactivated H9N2 against challenge with vNDV, H9N2 separate and co-infection under experimental condition.

Newcastle disease (ND) HI (log2) mean titers in group received NDV vaccines 4.5 ± 1.2, 3.6 ± 1.1 and 4.0 ± 0.4) at 14, 21 and 29 days of age, respectively. Chicken group received ND vaccine and inactivated H9 showed relatively higher HI titres (5.0 ± 1.5, 3.5 ± 1.4 and 4.5 ± 1.3). HI titres against H9N2 in group given H9N2 inactivated vaccine are 3.7 ± 1.6, 2.5 ± 1.1 and 5.1 ± 1.3 at 14, 21 and 29 days; respectively. Challenge with NDV genotype VII at 33 days of age resulted in 100% mortality with severe signs as well as in non-vaccinated control sub-group challenged and 100% mortalities. Sub-groups (2a and 3a) vaccinated with ND vaccine and ND vaccine + H9N2 showed signs of depression, off food and moderate respiratory signs with protection rates is 70 and 75%; respectively. Control group challenged with H9N2 showed general signs with mild respiratory signs and 10% mortalities while, sub-groups given ND vaccine + H9N2 and

challenged with H9N2 show 100% survival. Chicken sub-groups vaccinated with ND vaccine or ND vaccine 2+ H9N2 and challenged with NDV+ H9N2 (Co-infection) show signs form the 3rd dpi with moderate respiratory signs and protection rate of 70%; respectively. Post-mortem lesions in vNDV challenged birds were septicemia, intestinal and respiratory lesion, while those challenged with both NDV and H9N2 showed more prominent lesions. The vaccinated groups showed unsatisfactory protection rates (75%). Simultaneous chicken challenge with H9N2 and vNDV pointed out that co-infection increased severity of clinical signs, mortality and gross lesions. No difference in virus shedding of vNDV were found among control unvaccinated and vaccinated groups. All groups shed virus in cloacal and oral swabs at days 4, 6 and 8 post challenge

Histopathological changes were reported in lung, intestine, and spleen of challenged non-vaccinated and vaccinated groups. The severity of tissue alteration was remarkably high in the non-vaccinated group and slightly mild tissue alteration in the vaccinated group. The histological changes ranged from severe congestion in blood vessels in tested organ with lymphoid depletion in spleen and hyperplasia of mucosal-associated lymphoid tissue in the intestine. With partial limited tissue change in the challenged vaccinated group.

key words: H9N2, NDV genotype VII, co-infection, RT-PCR, phylogenecity

vaccines, HI titres,

Dedication

to my family

my great father, my kind mother , my lovely sisters and brothers ,

my lovely husband and my sweety daughter "zeina"

Acknowledgement

Firstly, I want to express my deep thanks to the merciful ***ALLAH*** *who has given me everything I needed in life and the ability and patience to complete this work.*

I would like to express my deepest gratitude and cordial appreciation to ***my supervisors ;Prof. Dr. Mohammed Hussain Hassan AWaad,*** *Prof. of poultry diseases ,Faculty of veterinary medicine , Cairo University* ***and Prof. Dr. Mohammed Mahrous Amer ,****Prof. of poultry diseases, Faculty of veterinary medicine, Cairo University. No words are sufficient to express thanks to them for your kind supervision and reviewing the manuscript with a great care, editing my writings, and assisting me till completion.*

I would like to express my thanks and sincere gratitude ***my supervisor Prof. Dr. Mohammed abdelaziz kutkat,*** *Prof. of poultry diseases, veterinary research Division ,National Research Centre for suggesting the point of this work, kind supervision, valuable guidance, continuous support and your encouragement.*

My deepest heartfelt gratefulness and appreciation *to my senior* ***prof.Dr.mohammed ahmed ali,*** *Professor of Virology, Environment Research Division, National research for your support, opening the influenza research laboratory at national research center and possibilities you have given me in all practical parts of thesis.*

I would like to express my deep thanks to all my colleagues in Center of Scientific Excellence for Influenza Viruses laboratory, Environment Research Division, National Research Center and I would like to especially thank ***Ahmed Kandeil*** *for excellent technical assistance.*

I am heartfelt gratefulness to my sincere husband and my backbone ***Mohamed Helmy*** *and my dear daughter* ***Zeina*** *. a special thank you for being there especially in hard times of the work.*

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