Cairo University
Faculty of veterinary medicine
Department of Poultry Diseases

STUDIES ON NEWCASTLE DISEASE IN POULTRY FLOCKS IN EGYPT

Thesis Presented
By

Mohamed Adel Mohamed Abo Elfetouh M. El-Behairy
(B.V.Sc., Cairo University, 2012)

For the Master Degree of Veterinary Science
(Poultry Diseases)

Under Supervision of

Prof. Dr.
Mostafa Ahmed Bastami
Professor of poultry Disease
Faculty of Veterinary Medicine
Cairo University

Prof. Dr.
Manal Afifi Ali
Professor of poultry Disease
Faculty of Veterinary Medicine
Cairo University

2016

بِسْمِ ٱللَّهِ ٱلرَّحْمَٰنِ ٱلرَّحِيمِ

"وَمَا تَوْفِيقِي إِلَّا بِاللَّهِ عَلَيْهِ تَوَكَّلْتُ وَإِلَيْهِ أُنِيبُ"

سورة هود الأيه (88)

Cairo University
Faculty of veterinary medicine
Department of Poultry Diseases

Supervision Sheet

Prof. Dr. Mostafa Ahmed Bastami
Professor of poultry Disease
Faculty of Veterinary Medicine
Cairo University

Prof. Dr. Manal Afifi Ali
Professor of poultry Disease
Faculty of Veterinary Medicine
Cairo University

2016

Cairo University
Faculty of Veterinary Medicine
Department of Poultry Diseases

Name:	Mohamed Adel Mohamed Abo Elfetouh M. Elbehairy
Date of birth:	15/1/1991
Degree:	Master degree of veterinary science (poultry disease)
Nationality:	Egyptian
Specialization:	Poultry diseases
Title of the thesis:	Studies on Newcastle Disease in Poultry Flocks in Egypt.
Under supervision of	***Prof. Dr. Mostafa Ahmed Bastami*** Professor of Poultry diseases, Faculty of Veterinary Medicine, Cairo University ***Prof. Dr. Manal Afifi Ali*** Professor of Poultry diseases, Faculty of Veterinary Medicine, Cairo University

Abstract

Seventy four chicken, turkey and pigeon flocks were investigated for the presence of NDV using RT-PCR F-protein gene assay, 26 flocks tested positive for NDV. As a preliminary step for the exclusion of lentogenic vaccinal strain positive RTPCR samples were tested by real time reverse transcriptase polymerase chain reaction (rRTPCR) using a probe specific to velogenic strains. Nineteen samples tested positive for vNDV while seven samples tested negative. Twelve samples out of the positive samples were isolated on SPF ECE from eight different Egyptian governorates. Partial sequencing of the F-Protein gene revealed that all isolated strain have multiple basic A.A at the F-Protein cleavage site possessing the velogenic motif (112.**RRQKR*F**.117). Phylogenetic analysis of the F-protein 375 b.p. hypervariable region (nt 47– 422), revealed that all isolates are belonging to genotype VIId with (99%-100%) amino acid identity matrix with the Israelian **(Turkey-Israel-111-2011)** and Chinese **(Chicken-China-SDYT03-2011)** strain**,** Genotype VII. Single pure NDV isolated strain was selected **APMV1/chicken/EG-BH/POD.CU/2015** (**EG.BH/2015**) after testing negative for AI common gene, IB, and IBD virus. Titration of the selected strain reveals MDT of (44 hr.) and $10^{7.5}$ ELD_{50}/ 0.1 ml. In vivo vaccination protection study was done using different vaccination programs for protection against challenge with the locally isolated (**EG.BH/2015**). Commercial broiler chicks were divided in to 13 different vaccination groups including positive and negative non-vaccinated control groups. Results revealed that all groups showed 100% protection rate against mortality (except in groups no. VI, VII and XI), and the tracheal viral shedding was significantly decreased but not completely prevented in neither of the vaccinated groups. Group no.1 and 2 were showing a significantly high mean HI titer than other groups and least viral shedding following infection, while groups no. 3, 7, and 11 are showing a significantly lower mean HI titer and protection rate than other groups. Possible causes of these results were discussed in details.

Key words: Egypt, velogenic NDV, Gentype VIId, vaccination programs, protection study, shedding.

Dedication

To

My Father,

Mother,

Sister, and Brothers.

ACKNOWLEDGMENT

*First of all, prayerful thanks for our merciful **ALLAH**, for everything I had and supplied me with the power not only to carry out this work but also during my whole life. My endless prayers and thanks to the greatest person allover all eras; our prophet Mohammed who has been sent to us by the most important gift in our life that is our Islam.*

*I wish to express my sincere gratitude to **Prof.Dr. Mostafa Ahmed Bastami,** Professor of poultry Diseases, and previous dean of Faculty of Veterinary Medicine, Cairo University, for his wise planning, stimulating supervision, guidance, constructive criticism and valuable advices during supervising this work.*

*My grateful appreciation and thanks to **Prof.Dr. Manal Afifi Ali,** Professor of poultry Diseases, Faculty of Veterinary Medicine, Cairo University for her sincere supervision, offering materials and instruments, keen guidance, thesis editing, plan and endless support, throughout the whole work.*

*My grateful appreciation and thanks to **Prof.Dr. Amr Abd El Aziz** Professor of infectious disease Department, Faculty of Veterinary Medicine, Cairo University, for facilitating work in infectious disease department laboratory and supplying materials.*

*My grateful appreciation and thanks to **Prof.Dr. Ausama Abd El Raouf,** Professor and, Head of the virology Department, Faculty of Veterinary Medicine, Cairo University for facilitating work in Virology Department Research Laboratory.*

*I wish to express my sincere gratitude to **Prof.Dr. Kawkab Ahmed,** Professor of pathology, Faculty of Veterinary Medicine, Cairo University, for her effort in the histopathological examination studies in this work.*

*I wish to express my sincere gratitude to **Prof.Dr. Timm Harder**, head of the (OIE/FAO) National Reference Laboratory for Avian Influenza, Friedrich-Loeffler-Institute (FLI), Germany, for practicing and offering software for manipulating and editing my isolates nucleotide sequence.*

*I wish to express my sincere gratitude to **Dr., Abd-Elbary Prince**, **Dr. Ahmed Maher** and **Dr. Basem Abd Elhameed** for their help and offering materials.*

It is of great pleasure for me to express my thanks and gratitude to anyone offered me a help to during that work.

Contents

	Page
A. List of Tables	**I**
B. List of Figures	**III**
C. List of Appreviations	**V**
1. Introduction	**1**
2. Review of literature	**4**
3. Materials and methods	**50**
4. Results	**81**
5. Discussion	**125**
6. Conclusion	**139**
7. Summary	**140**
8. References	**143**
الملخص العربي	**١ - ٢**

LIST OF TABLES

No.	Title	Page
1.	Oligonucleotide primers used in RTPCR for detection of NDV F-protein gene.	54
2.	Oligonucleotide primers used in RTPCR for detection of AI common matrix Gene	54
3.	Oligonucleotide primers used in RTPCR for detection of IBD virus VP2 gene	54
4.	rRT-PCR Primer-probe set sequence	57
5.	The reaction mixture of RT-PCR for detection of APMV-1	63
6.	Thermal Profile for partial amplification of the F-Protein gene	63
7.	The reaction mixture of rRT-PCR for detection of NDV field strains	66
8.	Thermal profile for amplification of a part of the F-protein gene by rRT-PCR	67
9.	The reaction mixture of RT-PCR for detection of APMV-1	72
10.	Reaction mixture of RT-PCR for detection of AI matrix Gene	75
11.	Thermal profile for amplification of AI Matrix Gene by RT-PCR	75
12.	The reaction mixture of RT-PCR for detection of IBD	75
13.	Thermal profile for amplification of IBD VP2 Gene by RT-PCR	76
14.	The reaction mixture of rRT-PCR for detection of NDV field strains	76
15.	The thermal profile for amplification of S1 region of S protein gene for IBV by RRT-PCR	77