



Cairo University
Faculty of Veterinary Medicine
Department of Veterinary
Hygiene and Animal
Management



Role of Biosecurity in Reducing risk of Newcastle Disease

Thesis

Presented by

Ragia Sobhi Mohamed

(B. V.Sc.; 2004)

(Diploma, Faculty of Vet. Med, Cairo Uni, 2014)

For

M. V.Sc. Degree

Hygiene of Animal, Poultry and Environment

Under the Supervision of

Prof. Dr. Mamdouh Moustafa Hamoud

Emeritus Professor of Animal, Poultry and Environmental Hygiene

Faculty of Veterinary Medicine

Cairo University

Prof. Dr. Osama Mohamed
Kamel Zahran

Emeritus Professor of Animal, Poultry and
Environmental Hygiene
Faculty of Veterinary Medicine
Cairo University

Dr. Elshaimaa Ismael Sayed
Solayman

Lecturer of Animal, Poultry and
Environmental Hygiene
Faculty of Veterinary Medicine
Cairo University

2019

Cairo University
Faculty of Veterinary Medicine
Department of Veterinary Hygiene and Management



SUPERVISION SHEET

Prof. Dr. Mamdouh Moustafa Hamoud

Emeritus Professor of Animal, Poultry and Environmental
Hygiene
Faculty of Veterinary Medicine
Cairo University

Prof. Dr. Osama Mohamed Kamel Zahran

Emeritus Professor of Animal, Poultry and Environmental
Hygiene
Faculty of Veterinary Medicine
Cairo University

Dr. Elshaimaa Ismael Sayed Solayman

Lecturer of Animal, Poultry and Environmental Hygiene
Faculty of Veterinary Medicine
Cairo University

(2019)

Cairo University
Faculty of Veterinary Medicine
Veterinary Hygiene and Management

Name: Ragia Sobhi Mohamed

Date of birth: 17 /12 /1982

Site of birth: Cairo

Nationality: Egyptian

Speciality: Animal, Poultry and Environment Hygiene

Title of the research: Role of Biosecurity in Reducing risk of Newcastle Disease



Supervisors:

1. **Prof. Dr. Mamdouh Moustafa Hamoud** - Prof. of Animal, Poultry and Environmental Hygiene-Faculty of Vet. Med.-Cairo Univ.
2. **Prof. Dr. Osama Mohamad Kamel Zahran**- Prof. of Animal, Poultry and Environmental Hygiene-Faculty of Vet. Med.-Cairo Univ.
3. **Dr. Elshaimaa Ismael Sayed Solayman**- Assistant professor of Animal, Poultry and Environmental Hygiene-Faculty of Vet. Med.-Cairo Univ.

Abstract

Newcastle disease virus (NDV) is still a havoc to the poultry flocks and outbreaks have occurred even in vaccinated flocks. Commercial Newcastle disease virus vaccines can provide various levels of protection against challenge with different NDV genotypes, raising the importance of the relationship between vaccines and field strains. The aim of the current work is to evaluate the effect of moisture percentage and pH on the survivability of velogenic NDV genotype VII in the poultry manure which considers a major threat to the Egyptian poultry industry since 2011 onwards. In the present study, manure from specific pathogen free (SPF) chicks was contaminated with allantoic fluid of 8 log₂ NDV and kept at room temperature then daily examination of poultry manure for the survival of NDV was conducted on the basis of virus isolation and haemagglutination (HA) assay. Our results revealed that pH of the manure from time of its mixing with the NDV infected allantoic fluid till the 4th day was 7, then pH values changed progressively and recorded 8.5 till day 12. After that, pH increased towards the alkaline side and recorded pH = 9 till the 33rd day of the experiment. Regarding moisture %, after mixing the manure with allantoic fluid the moisture % of the mixture was 67%, and this wet manure remained moist (67%) till the 4th day; due to the humid environmental conditions applied at room temperature. Later, the moisture content of the treated manure decreased gradually and recorded 52.5%, 40% and 32% at the 8th, 28th and 33rd days of experiment; respectively. To recapitulate, both moisture % and pH of the poultry manure is greatly affect Newcastle disease virus survivability in the farm environment which is a critical point during cleaning and precleaning for the poultry farms to eliminate the viruses along with the use of effective disinfectants.

Key Words: Newcastle disease virus; Vaccines; Evolution; Manure; Disinfectants.

Acknowledgement

*The author would like to extend their appreciation and deepest thanks to the late **Prof. Dr. Mamdouh M. Hamoud**, Professor of Animal and Poultry Hygiene, Faculty of Veterinary medicine, Cairo University, for his guidance and advise. I would like to express my sincere gratitude and special thanks to **Prof. Dr. Osama M. K. Zahran**, Professor of Animal and Poultry Hygiene, Faculty of Veterinary medicine, Cairo University, for his valuable supervision, faithful guidance and assistance toward the achievement of this work. His willingness to give his time so generously has been very much appreciated. His help is far beyond what I can describe with words. I also like to extend my thanks and appreciation to **Dr. Elshaimaa Ismael**, Assistant professor of Animal and Poultry Hygiene, Faculty of Veterinary, Medicine, Cairo University, for her continuous help and encouragement. I am greatly grateful to **Prof. Dr. Manal M. Zaki**, Professor of Animal and Poultry Hygiene, Faculty of Veterinary medicine, Cairo University, for her generous help and the facilitation she offered during carrying out this thesis. I am greatly thankful to **Abdelrhman M. Gamal**, lecturer of Animal and Poultry Hygiene, Faculty of Veterinary, Medicine, Cairo University, for his co-operative efforts and appreciated team working.*

*Special thanks to **Mohammed Abdelmohsen**, lecturer of Virology, Faculty of Veterinary medicine, Cairo University for his continuous help.*

*Lastly, I would like to appreciate effort, help of **Dr. Mohamed Mamdouh Hamoud** Assistant Professor of Poultry Disease Department, Faculty of Veterinary medicine, Cairo University.*

Special thanks to Dr. Shimaa A.E. Nasr, Assistant Professor of Animal and Poultry Hygiene, Faculty of Veterinary medicine, Cairo University, for her generous help.

Special thanks to Dr. Samah E. Laban, lecturer of Animal and Poultry Hygiene, Faculty of Veterinary, Medicine, Cairo University, for her continuous help .

I would like to express my deepest gratitude and best thanks to all staff members of Hygiene and Management department for their valuable assistance.

CONTENTS

1. INTRODUCTION	1
2. REVIEW OF LITERATURE	4
2.1. Economic Importance of Newcastle disease virus	4
2.2. Newcastle disease virus control and vaccines potentiation	6
2.3. Evolution of Vaccine escape mutants and Vaccine Mismatching:.....	14
2.4. Newcastle disease virus shedding in the environment:.....	15
2.5. Survival of shed Newcastle disease virus in the environment:	17
3. PUBLISHED PAPERS	22
3.1. Evaluation of Moisture and pH in relation to Survivability of Escape Mutant Newcastle Disease Virus Genotype VII in Poultry Manure	22
3.2. Efficacy of Some Commercial Disinfectants against VND Genotype VII shed from poultry manure after Heterologous vaccination.	45
4. DISCUSSION.....	64
4.1. Evaluation of Escape Mutant Newcastle Disease Virus Genotype VII in Poultry Manure.....	64
4.2. Efficacy of Some Commercial Disinfectants against VND Genotype VII shed from poultry manure Heterologous vaccination ..	69
5. CONCLUSION.....	79
6. SUMMARY.....	74
7. REFERENCE	80
8. APPENDIX	87

List of Tables

N	Title	Page
1	Selected commercial disinfectants used against NDV for efficacy evaluation	53
2	Composition of the neutralizing broth for disinfectant Deactivation	54
3	Efficacy of Some Commercial Disinfectants against VND Genotype VII Shaded from poultry manure Heterologous vaccination	61
4	Survival of Mismatch (Genotype VII NDV) in poultry manure	87

List of figures

N	Title	Page
1	Amino acids mutation trend analysis for F protein of field strain velogenic NDV genotype VII in compare to commonly used vaccine strain of Genotypes I and II.	42
2	Phylogenetic analysis of the studied NDV isolate genotype VII and their clustering patterns with representative AAvV-1 isolates of each genotype.	43
3	Days interval for evaluation of moisture % and pH and their effect on velogenic NDV genotype VII survivability.	44
4	Evaluation of pH on the survival of velogenic NDV genotype VII in poultry manure.	44
5	Evaluation of days interval after disinfection on the supernatant HA titer on survival of velogenic NDV genotype VII in poultry manure.	62
6	Evaluation of days interval after disinfection on the supernatant HA titer & + ve HA ECE% on survival of velogenic NDV genotype VII in poultry manure	63
7	Changes in manure pH over time	88
8	Changes in manure moisture % over time.	88
9	Changes in HA activity (log 2) of manure supernatant over time.	89

List of Abbreviations

HPAIV	Highly pathogenic avian influenza virus
AIV	Avian Influenza virus
HI	Haemagglutination inhibition
OIE	World organization of animal health
WHO	World health organization
EMA	European-Middle Eastern-African clades
HA	Haemagglutinin/ Haemagglutination
VI	Virus isolation
ECE	Embryonated chicken eggs
SPF	Specific pathogen free
NA	Neuraminidase
RT-PCR	Reverse transcriptase- Polymerase chain reaction
PM	Post mortum
FAO	Food and agriculture organization
NDV	Newcastle disease virus
BPL	Beta-propiolactone
PBS	Phosphate buffered saline
Ag	Antigen
HAU	Haemagglutination units

EID	Egg infectious dose
VN	Virus neutralization
S/C	Sub-cutaneous
SE	Standard error

1. INTRODUCTION

1. INTRODUCTION

Newcastle disease virus (NDV) is a major threat to the poultry industry around the globe. The disease is endemic in many developing countries while the disease-free countries are prone to accidental outbreaks.

The overarching goal of biosecurity is to prevent, control and/or manage risks to life and health as appropriate to the poultry sector. In doing so, biosecurity is an essential element of sustainable poultry industry development.

The general biosecurity approaches to control Newcastle disease virus are depend mainly on hygienic measures and vaccination. Hygienic measures include cleaning, disinfection, limiting access of Visitors, wild birds, and personal hygiene for the farm staff. Vaccination in combination with appropriate hygiene measures, remains the most effective way to control NDV.

Research priorities are toward the improved diagnostics and better vaccine development while there is a need to study the effect of environmental farm conditions and focusing on novel biosecurity measures.

There are three main goals for NDV vaccination; i) decrease or eliminate clinical disease; ii) decrease the virulent virus shed; and iii) decrease the infectious dose of the challenge virus. Unfortunately, only the first goal is considered to be an objective of current control

strategies, as field veterinarians do not have the tools to assess the effectiveness of vaccination on the accomplishment of the second and third objectives.

Although the NDV vaccination is widely practiced in Egypt, the disease is still a havoc to the poultry flocks and outbreaks have occurred even in vaccinated flocks. Vaccination failure may result from inadequate method of vaccination, vaccination during incubation period of NDV, stress of extreme weather conditions (winter or summer), and transportation of birds after vaccination

Reports of vaccination failure from many countries and our field observations is mainly due to the reduced ability of the classical vaccines to decrease viral replication and shedding as most of the currently used vaccines are depending on viral strains which belongs to genotype I, II or III, which cannot fully prohibit the virus shedding against the prevalent genotype VII virulent strain attack, This creates an interest in developing vaccines formulated with genotypes homologous to the virulent NDV currently circulating in the field.

Biosecurity alone is not sufficient to prevent virus outbreaks however vaccination with NDV vaccines formulated with antigens homologous (of the same genotype) of the challenge circulating virus may significantly reduce the shedding compared to heterologous antigen, which has shown to be effective for increasing the efficacy against virulent challenge strains circulating in the field, and above all, on reducing the number of excreted viral particles.

The goals of this study were:

1. Isolation and identification of Newcastle Disease Virus currently circulating in the Egyptian poultry environment.
2. Evaluation of Moisture and pH in relation to Survivability of Escape Mutant Newcastle Disease Virus Genotype VII in Poultry Manure.
3. Efficacy of Some Commercial Disinfectants against VND Genotype VII shed from poultry manure Heterologous vaccination.

Chapter (2)

2.REVIEW OF LITERATURE