

**EFFECT OF CERTAIN ANTIBIOTICS ON THE
IMMUNE RESPONSE OF CHICKENS TO
NEWCASTLE DISEASE AND AVIAN
INFLUENZA DISEASE VACCINES**

Thesis Presented

By

Lamiaa Ahmed Ali Mohamed Ali

B.V.Sc., Fac. Vet. Med., Alex. Univ., 2001

To

Department of Microbiology
Faculty of Veterinary Medicine
Alexandria University
EGYPT

For

The Degree of M.V.Sc.
MICROBIOLOGY

2010

UNDER THE SUPERVISION OF

Prof. Dr. Helmy Ahmed Torky

Professor of Microbiology

Faculty of Veterinary Medicine

Alexandria University

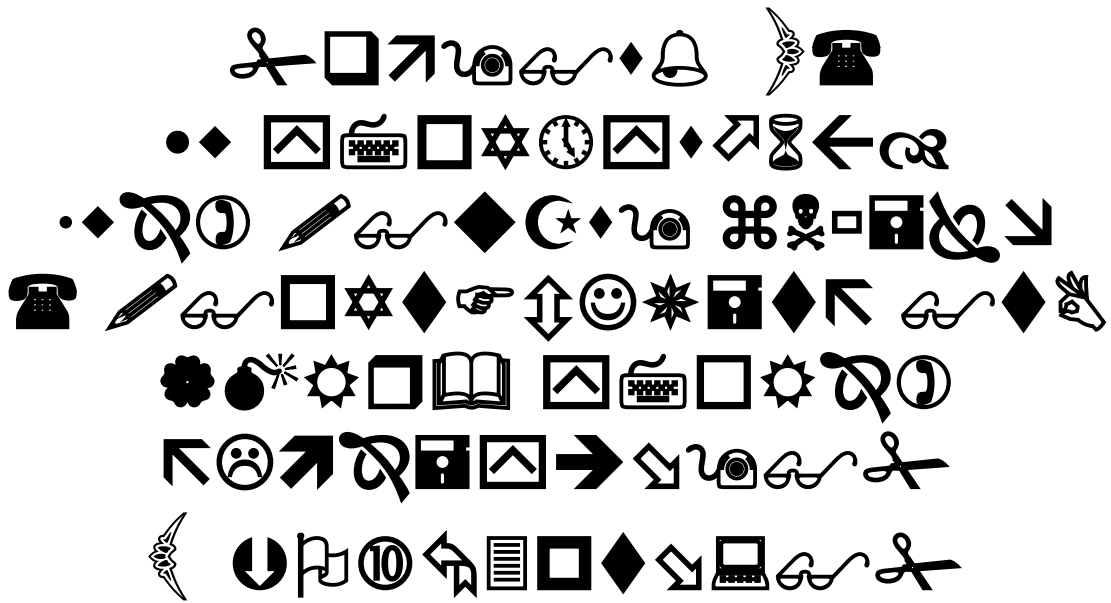
Prof. Dr. Samy Abd El Salam Khalil

Professor of Microbiology

Faculty of Veterinary Medicine

Alexandria University

j



صِدْقُ
العظيم

(سورة البقرة - آية 32)

ACKNOWLEDGEMENT

*First of all I am prayerful thanks to our merciful "ALLAH",
who gave me the ability to finish this work*

*Great thanks are offered to Prof. Dr. Helmy Ahmed torky,
Professor of Microbiology, Faculty of Veterinary Medicine, Alexandria
University for his kind supervision .*

*Great appreciation, profound gratitude and deepest thanks are offered
to Prof. Dr. Samy Abd El Salam Khalil Professor of
Microbiology, Faculty of Veterinary Medicine, Alexandria University
for his help, encouragement, continuous guidance, valuable advice
and the facilities provided during the course of the study.*

*I would like to express my thanks to Dr. Ashraf Mohamed Awad
, Assistant Professor of Poultry Diseases and Hygiene, Faculty of
Veterinary Medicine, Alexandria University for his help, cooperation
and encouragement during the course of the study.*

*I dedicate this thesis to
My family and my son*

Content

	Page
1. INTRODUCTION.....	1
2. REVIEW OF LITERATURE.....	4
3. MATERIALS AND METHODS	20
3.1. Materials	20
3.1.1. Chicks.....	20
3.1.2. Antibiotics	20
3.1.2.1. Spiramycin.....	20
3.1.2.2. Tylosin tartrate	20
3.1.2.3. Cefotan	21
3.1.3. Vaccines.....	21
3.1.3.1. Newcastle disease viral vaccine.....	21
3.1.3.1.1. Hitchner B1 strain	21
3.1.3.1.2. Lasota strain.....	21
3.1.3.1.3. Colone 30 strain.....	21
3.1.3.1.4. Oil emulsion inactivated NDV vaccine.....	21
3.1.3.2. Inactivated Avian Influenza Vaccine (H5N2).....	21
3.1.4. Chicken red blood cells (RBCs.....	22
3.1.5. Phosphate buffer saline	22
3.1.6. Antigen for HI test	22
3.1.6.1. NDV antigen.....	22
3.1.6.2. AIV antigen.....	22
3.1.7. Candida Albicans	22
3.2. Methods.....	22
3.2.1. Chickens	22
3.2.2. Antibiotics administration.....	22
3.2.3. Vaccination programme.....	22

3.2.3.1. Newcastle disease virus vaccine.....	23
3.2.3.2. Inactivated Avian Influenza virus Vaccine	24
3.2.4. Blood sample collection	24
3.2.5. Chicken red blood cells	24
3.2.6. Phosphate buffer saline	24
3.2.7. Weight of lymphoid organs	26
3.2.8. Antigen for HI test	26
3.2.8.1. NDV antigen	26
3.2.8.2. AIV antigen	26
3.2.9. Methods used for evaluation of immune response	27
3.2.9.1. Methods used for evaluation of humeral immune response of chickens to NDV and AIV.....	27
3.2.9.1.1. Haemagglutination inhibition test (H.I.) for NDV and AIV.....	27
3.2.9.2. Methods used for evaluation of cellular immune response of chickens.....	29
3.2.9.2.1. Determination of phagocytic activity and phagocytic index	29
3.2.10. Statistical analysis	29
4. RESULTS	30
1. Effect of antibiotics on ratio of bursa to body weight ...	30
2. Effect of antibiotics on ratio of thymus to body weight ...	30
3. Effect of antibiotics on ratio of spleen to body weight ...	35
4. Effect of antibiotics on H.I. titers of chickens to NDV vaccine.....	38
5. Effect of antibiotics on H.I. titers of chickens to AIV vaccine	41
6. Effect of different antibiotics on phagocytic activity of chickens at different intervals	44

7. Effect of different antibiotics on phagocytic index of chickens at different intervals	47
5. DISCUSSION	50
6. CONCLUSION	58
7. ENGLISH SUMMARY	60
8. REFERFNCE	63
ARABIC SUMMARY	

LIST OF TABLES

	Page
Table (1): Experimental design for vaccination of chicks with NDVV and AIVV.....	25
Table (2): Means of ratio of bursa to body weight.....	31
Table (3): Means of ratio of thymus to body weight.....	33
Table (4): Means of ratio of spleen to body weight.	36
Table (5): H.I. titers (\log_2) of chicks post vaccination with NDV vaccine.....	39
Table (6): H.I. titers (\log_2) of chicks post vaccination with AIV vaccine.....	42
Table (7): Effect of different antibiotics on phagocytic activity of chicks at different intervals.	45
Table (8): Effect of different antibiotics on phagocytic index of chicks at different intervals.	48

LIST OF FIGURES

	Page
Figure (1): Means of ratio of bursa to body weight.....	32
Figure (2): Means of ratio of thymus to body weight.....	34
Figure (3): Means of ratio of spleen to body weight.	37
Figure (4): H.I. titers of chicks post vaccination with NDV vaccine.....	40
Figure (5): H.I. titers of chicks post vaccination with AIV vaccine	43
Figure (6): Effect of different antibiotics on phagocytic activity of chicks at different intervals.	46
Figure (7): Effect of different antibiotics on phagocytic index of chicks at different intervals.	49

Introduction

1. INTRODUCTION

Poultry keeping is the form of poultry production in the developing world, so we must control the infections to prevent great economic losses. Controls of infectious diseases in poultry depend upon adequate flock immunity. Reduced immune responsiveness leads to increased diseases losses that can seriously damage the poultry industry (**Mohamed, 1997**). The immune system of chickens may be suppressed by Infectious agents and non-infectious causes (**Enrique Montile, 1999**). Infectious causes may include bacteria, viruses and internal parasites, while non-infectious causes include chemicals, hormones, antibiotics, toxins, environmental stresses and lack of dietary ingredient (**Mohamed, 1997**).

Many viral agents have been implicated in impressing the immune system of chickens; Newcastle disease (**Alexander , 1989; Calnek et al.,1991**) and infectious bursal disease (IBD) (**Sherma et al.,1976 ; Sivanandant et al.,1980**) as well as other viruses.

Newcastle disease (ND) still from the most important avian diseases because of its high economic impact on the poultry industry (**Leslie,2000**). Newcastle disease virus is synonymous with avian paramyxovirus type 1 (APMV-1) and has been classified in the order Mononegavirales, family paramyxoviridae, subfamily Paramyxovirinae, genus Rubulavirus (**Lamb et al., 1996; Alexander, 1997 and Alexander,1998**).

Over the past decade, the emergent avian influenza (AI) viruses have shifted to increase virulence for chickens. AI viruses typically produce a similar severe, systemic disease with high mortality in chickens). In Africa, H5N1 AI cases approved in February 2006 in several countries. It began in Nigeria then other African countries including Egypt (**Swayne, 2007**). Twenty six epizootics of AI have occurred in the world since 1995. The largest of these outbreaks has been the H5N1AI which has caused problems in poultry and some wild birds in over 60 countries of Asia, Europe and Africa since beginning in 1996. The spread of highly pathogenic avian influenza H5N1 viruses across Asia in 2003 and 2004 devastated domestic lethal H5N1 virus outbreak in humans to date (**Maines et al., 2005**) On 17 February 2006, the Egyptian Government confirmed that avian flu had broken out in the nation's poultry.

Avian influenza is caused by infection with viruses of the family Orthomyxoviridae placed in the genus influenza virus A. Influenza A viruses are the only orthomyxoviruses known to affect birds. Many species of birds have been shown to be susceptible to infection with influenza A viruses; aquatic birds form a major reservoir of these viruses, but the overwhelming majority of isolates have been of low pathogenicity for chickens and turkeys. Influenza A viruses have antigenically related nucleocapsid and matrix proteins, but are classified into subtypes on the basis of their hemagglutinin (H) and neuraminidase (N) antigens (**World Health Organization Expert Committee, 1980**).

In many of viral infections humeral and cell mediated immune responses play a pivotal role in protection against such diseases (**kumar et al.,1988**). Both humeral and cell mediated immune responses are essential for complete protection (**Chandrasekar et al., 1989**).

Some antibacterial drugs interference with the immune response to viral vaccines (**lavel, 1989**). Although many antimicrobial agents have been reported to cause immunosuppression in animals, macrolide antibiotics enhance immune function (**Baba et al., 1998**).

In the present study, we have chosen spiramycin and tylosin which widely used in poultry farms. We have also chosen cefotan as advanced generation of antibiotics.

In view of these facts, this study was attempted to investigate the effect of spiramycin, tylosin or cefotan on immune response of chickens to NDV and AI vaccines. To achieve this aim, we carried the following:

- a. Measuring the humeral immune response of chickens vaccinated with NDV and AIV vaccines by H.I. test.
- b. Measuring the cellular immune response of chickens vaccinated with NDV and AIV vaccines by phagocytic activity.
- C. Studying the effect of antibiotics (spiramycin, tylosin and cefotan) on the weight of lymphoid organs to body weight.

Review of literature