CairoUniversity
Faculty of Veterinary Medicine
Department of Poultry Diseases

FURTHER STUDIES ON BACTERIAL DISEASES CAUSING HIGH MORTALITY IN BROILER CHICKS

A thesis presented by

EMAN ANTER MORSY IBRAHIM

B.V. Sc. (2008) Cairo University
M.V. Sc. (2012) Cairo university

For the degree of Ph.D.

Veterinary Medical Sciences

(Poultry and Rabbits Diseases)

Under Supervision

Of

Prof. Dr. Diaa El-Dein Gad Ahmed Khelfa

Professor of Poultry and Rabbit Diseases
Department of Poultry Diseases
Faculty of Veterinary Medicine
Cairo University

Prof. Dr. Wafaa Abd El-Ghany Abd El-Ghany

Professor of Poultry and Rabbit Diseases
Department of Poultry Diseases
Faculty of Veterinary Medicine
Cairo University

(2015)

Name: Eman Anter Morsy Ibrahim
Nationality: Egyptian
Birth date:11/6/1986, Fayoum
Degree: Ph.D., 2015
Specification: Poultry and Rabbit Diseases
Supervisors:
Prof. Diaa El-Dein Gad Ahmed Khelfa
Prof. Wafaa Abd El-Ghany Abd El-Ghany

Thesis Title:

"FURTHER STUDIES ON BACTERIAL DISEASES CAUSING HIGH MORTALITY IN BROILER CHICKS"

ABSTRACT

A monitoring study was carried out on one hundred and two broiler flocks located in nine Egyptian governorates during the period of 2012 - 2014 in a trial for isolation of responsible aerobic bacterial agents causing high chicks mortality in such flocks. The highest mortality percent was recorded in Cobb breed on the 12th day of age in Beni-Suef while the lowest mortality percent recorded in Sasso breed on the 25th day of age in Sharkia. Bacteriological investigation revealed that the *Escherichia coli* isolates were the predominant organism (42.9%) followed by *Salmonella* (21.7%) *Klebsiella pneumonia*(10.1%), *Proteus mirabilis*(6.2%) and *Pseudomonas aeruginosa* (4.5%). Serological identification of *E. coli* isolates revealed that both O_{158} and O_{78} serogroups were the most predominant isolates (18.6%) each, followed by serogroups O_{27}(10.8%), O_{26} (6.8%), O_{18} and O_{55}(3.9%), each, O_6, O_{111} and O_{159} (2.9%), each, finally serogroup O_1, O_8, O_{15}, O_{44}, O_{114}, O_{119}, O_{142}, O_{153}, O_{166} and O_{169} (1.5%), each while un-typable strains were (13.7%). *Salmonella* serological identification revealed that *Salmonella Infantis* (SI) was the most predominant isolates (27.2%) followed by *Salmonella Virchow* (SV) (23.4%), *Salmonella Enteritidis* (SE)(20.8%), *Salmonella Gallinarum* (SG) (14.3%), *Salmonella Kentucky* (SK) (10.4%) and *Salmonella Typhimurium* (ST) (3.9%). *In vitro* testing of the phenotypic properties of the 204 *E. coli* isolates, it was found that all tested isolates were Congo red positive. Conventional and a real-time PCR assay were used for the detection of rfbS, flijB, fliC, stmm and sefA genes of SG, SI, SK, ST and SE respectively where they sequenced and submitted on gene bank with accession numbers KP730600, KP760484, KP760485, KP763723 and KP793717. The genetic diversity of the submitted genes was compared with sequences deposited in the NCBI database to infer phylogenetic relationships between them. The results of multiplex PCR for E. coli grouping were *E. coli* O_1 was grouped in Enterotoxogenic group, *E. coli* O_{26} and *E. coli* O_{158} were grouped in Enteropathogenic, also *E. coli* O_{158} was grouped in Enterotoxogenic group, *E. coli* O_{78} was grouped in Enterotoxogenic group, *E. coli* O_{27} grouped in Enteropathogenic, Enterotoxogenic and Shiga toxin producing *E. coli*. The results of pathogenicity of different isolated *Salmonella* species in day old SPF chicks revealed that, the clinical signs and PM lesions were variable in their time of onset, severity and duration in different *Salmonella* spp. The mortality percent was in ST (84%), SG (66%), SE (54%), SK (16%) and SI (8%). No mortalities were recorded in chicks inoculated with SV and un-inoculated controls. Analysis of the level of fecal shedding on 21 DPI revealed 100% in *Salmonella* re-isolation for all serogroups except *SV* (90.9%) and *SG* (27.3%), while on 28DPI *SE* was the highest in fecal re-isolation rate followed by *SV, SI, SK* and *SG* with a percentage of 100%, 70.7%, 66.7%, 25.6% and 0% respectively. The microscopic lesions revealed that, the lymphoid organs (bursa, thymus, spleen and cecal tonsils) were severely affected in SV, SK and SI infected groups while the lesions in S.T, S.G and SE infected groups were mostly related to heart, liver, cecum and intestine. The pathogenicity of five *E. coli* isolates (O_1, O_{26}, O_{27}, O_{78}, O_{158}) were investigated in day old SPF chicks by crop gavaged and subcutaneous inoculation (sc). Various clinical signs, post mortem and histopathological pictures were recorded. The mortality percent in sc inoculated group was 100% except in *E. coli* O_1 and O_{27} groups in which the mortality percent was 80%. The highest mortality percentage in orally inoculated *E. coli* group was recorded in O_{78} infected group, followed by O_{26} and O_{158}, O_{27} then O_1 group with a percentage of 60%, 53.3%, 53.3%, 40% and 13.3% respectively. *E. coli* and *Salmonella* infections in chicks significantly reduced feed intake, altered growth of the whole body. Study the effect of some *E. coli* and *Salmonella* isolates on broiler immune system showed that the gross observations of bursa of fabrcious, thymus and spleen were atrophied with variable degree in the different inoculated *Salmonella* and *E. coli* serovars during 3-4 post infection, which confirmed by measuring of lymphoid organs body weight ratio and histopathological examination. Also infected birds experienced depressed responsiveness to viral vaccines (Newcastle disease vaccine (NDV), infectious bronchitis (IB), avian influenza (AI) and infectious bursal disease (IBD) in comparing with uninfected controls for 2-3weeks post vaccination, indicating that E. coli and salmonella infection have a transient effect on immune system which interferes with the ability of the immune system to respond humorally to antigenic stimuli.

Keywords: Broiler chicks, *Salmonella*, *E. coli*, mortalities, PCR, pathogenicity, immune system.

Dedication

I dedicate this work to

My father soul

My husband Haitham, my kids Lojain &

Anas,

My mother and father in law,

My mother, my brother and sister

ACKNOWLEDGEMENT

First of all I thank ***Allah*** *who gave me this opportunity to achieve this work and I am praying to bless this work with his acceptance.*

I would like to express my gratitude and deepest thanks to ***Prof. Dr. Diaa El-Dein Gad Ahmed Khelfa****, Professor of Poultry Diseases, Faculty of Veterinary Medicine, Cairo University for his strict supervision, valuable help, kind cooperation and guidance in every step of this work and encouragement given to me during this work.*

I would like to express my gratitude and deepest thanks to ***Prof. Dr. M. H. Afify, Dr. Reham M. Abd-El Salam*** *and* ***Dr. Mrawa M. Salah Khattab****, Dep. of Pathology, faculty of Veterinary Medicine, Cairo University for their constant encouragement and support throughout the work.*

I would like to express my gratitude and deepest thanks to ***Dr. Khaled Sayed Shaban,*** *assistant prof., Dep. of Poultry Diseases, Faculty of Veterinary Medicine, Cairo University for his constant encouragement and support throughout the work.*

I would like to express my gratitude and deepest thanks to ***Prof. Dr. Fathi Farouk,*** *the previous dean of Faculty of Veterinary Medicine, Cairo University, for his constant encouragement and support throughout the work.*

I would like to express my gratitude and deepest thanks to ***Prof. Dr. Magdy El Kady,*** *Prof. of Poultry Diseases and dean of Faculty of Veterinary Medicine, Beni-swief University for his constant encouragement and support throughout the work.*

Best regards to my colleagues, ***Dr. Heba Mohammed Salem****,* ***Dr. Hassaneen Abo Zaid,*** ***Dr. Dalia Anwar Hamza****,* ***Dr. Esraa abd El-maged*** *and* ***Dr. Basem Ahmed****, Faculty of Veterinary Medicine, Cairo University for their constant encouragement and support throughout the work.*

Finally, my thanks are sent to every kind hand that extended to help me to allow the fulfillment of this work.

Contents

LIST OF ABBREVIATIONS

Abbreviation	Meaning
Ab.	antibody
ADH	Argenine dihydrolase
AI	Avian influenza.
AMY	amygdalin
APEC	Avian pathogenic *E. coli*
ARA	arabinose
BM	Bone marrow
bp	Base pair
b.wt.	Body weight
^{o}C	Celsius
CFU	Colony forming unit
CIT	citrate
CR	Congo red
C.S	Clinical signs
DNA	Deoxyribonucleic acid
dNTPs	Deoxy ribonucleotidestri phosphatase
DPI	Day post infection
D. W.	Distilled water
E. coli	*Escherchia coli*
ELISA	Enzyme linked immune-sorbent assay
EMB	Eosinemethylen blue
Fig.	Figure
GEL	gelatin
GLU	glucose
Gm.	Gram
H. gland	Harderian gland
H2S	Hydrogen sulide
gp.	group
HA	haemagglutination
HI	Haemagglutination inhibition
hrs.	Hours
IB	Infectious bronchitis
IBD	Infectious bursal disease
IND	indole
INO	inositol
Kg.	Kilogram
LDH	Lysine decarboxylase
MAN	mannitol
MBW	Mean body weight
MEL	melibiose
Mt.	Mortality

NCBI	National center of biotechnology information
ND	Not detected
NDV	Newcastle disease virus
No.	Number
ODC	Ornithine decaboxylase
ONPG	ß-galactosidase
P. aeruginosa	*Pseudomonas aeruginosa*
PCR	Polymerase chain reaction
P.I.	Post infection
PM	Post mortem
P. mirabilis	*Proteus mirabilis*
RHA	rhamnose
rpm	Revolution per minute
SAC	sucrose
sc	Subcutaneous
SE	*Salmonella Enteritidis*
SE	Standard error
SG	*Salmonella Gallinarum*
SI	*Salmonella infantis*
SK	*Salmonella Kentucky*
Spp.	species
SOR	sorbitol
SV	*Salmonella Virchow*
S.S agar	Salmonella shegilla agar
TDA	Tryptophan deaminase
Th.	thymus
URE	urease
VP	Voges-proskauer
Wk.	Week
Wt.	Weight
XLD	Xylose Lysine Desoxycholate Agar

LIST OF TABLES

Continue

Continue

LIST OF FIGURES

Continue

Continue

Continue

Continue

Continue