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## **FURTHER STUDIES ON BACTERIAL DISEASES CAUSING HIGH MORTALITY IN BROILER CHICKS**

A thesis presented by

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#### 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 12<sup>th</sup> day of age in Beni-Suef while the lowest mortality percent recorded in Sasso breed on the 25<sup>th</sup> 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<sub>158</sub> and O<sub>78</sub> serogroups were the most predominant isolates (18.6%) each, followed by serogroups O<sub>27</sub> (10.8%), O<sub>26</sub> (6.8%), O<sub>18</sub> and O<sub>55</sub> (3.9%), each, O<sub>6</sub>, O<sub>111</sub> and O<sub>159</sub> (2.9%), each, finally serogroup O<sub>1</sub>, O<sub>8</sub>, O<sub>15</sub>, O<sub>44</sub>, O<sub>114</sub>, O<sub>119</sub>, O<sub>142</sub>, O<sub>153</sub>, O<sub>166</sub> and O<sub>169</sub> (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*, *flixB*, *fliC*, *stx* 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<sub>1</sub> was grouped in Enterotoxogenic group, *E. coli* O<sub>26</sub> and *E. coli* O<sub>158</sub> were grouped in Enteropathogenic, also *E. coli* O<sub>158</sub> was grouped in Enterotoxogenic group, *E. coli* O<sub>78</sub> was grouped in Enterotoxogenic group, *E. coli* O<sub>27</sub> 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 28 DPI 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<sub>1</sub>, O<sub>26</sub>, O<sub>27</sub>, O<sub>78</sub>, O<sub>158</sub>) 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<sub>1</sub> and O<sub>27</sub> groups in which the mortality percent was 80%. The highest mortality percentage in orally inoculated *E. coli* group was recorded in O<sub>78</sub> infected group, followed by O<sub>26</sub> and O<sub>158</sub>, O<sub>27</sub> then O<sub>1</sub> 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 fabricius, 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 2-3 weeks 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*

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# LIST OF ABBREVIATIONS

Abbreviation	Meaning
<b>Ab.</b>	antibody
<b>ADH</b>	Argenine dihydrolase
<b>AI</b>	Avian influenza.
<b>AMY</b>	amygdalin
<b>APEC</b>	Avian pathogenic <i>E. coli</i>
<b>ARA</b>	arabinose
<b>BM</b>	Bone marrow
<b>bp</b>	Base pair
<b>b.wt.</b>	Body weight
<b>°C</b>	Celsius
<b>CFU</b>	Colony forming unit
<b>CIT</b>	citrate
<b>CR</b>	Congo red
<b>C.S</b>	Clinical signs
<b>DNA</b>	Deoxyribonucleic acid
<b>dNTPs</b>	Deoxy ribonucleotidetri phosphatase
<b>DPI</b>	Day post infection
<b>D. W.</b>	Distilled water
<b><i>E. coli</i></b>	<i>Escherchia coli</i>
<b>ELISA</b>	Enzyme linked immune-sorbent assay
<b>EMB</b>	Eosinemethylen blue
<b>Fig.</b>	Figure
<b>GEL</b>	gelatin
<b>GLU</b>	glucose
<b>Gm.</b>	Gram
<b>H. gland</b>	Harderian gland
<b>H<sub>2</sub>S</b>	Hydrogen sulide
<b>gp.</b>	group
<b>HA</b>	haemagglutination
<b>HI</b>	Haemagglutination inhibition
<b>hrs.</b>	Hours
<b>IB</b>	Infectious bronchitis
<b>IBD</b>	Infectious bursal disease
<b>IND</b>	indole
<b>INO</b>	inositol
<b>Kg.</b>	Kilogram
<b>LDH</b>	Lysine decarboxylase
<b>MAN</b>	mannitol
<b>MBW</b>	Mean body weight
<b>MEL</b>	melibiose
<b>Mt.</b>	Mortality

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

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