Cairo University
Faculty of Veterinary Medicine

Trials for production of different types of *Salmonella* Typhimurium vaccine

A Thesis Presented By

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B.V.Sc., Faculty of Veterinary Medicine, Cairo University (2006)

M.V.Sc., Faculty of Veterinary Medicine, Cairo University (2011)

For the PhD. Degree of

Veterinary Medical Science, Microbiology
(Bacteriology, Immunology and Mycology)

Under Supervision of

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Supervision Sheet

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To the light of my life

My mother,

My husband

Hamza

&

Hlla

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Thesis Title:

"Trial for production of different types of *Salmonella* Typhimurium vaccines"

Abstract

The goal of this study is a trial to prepare different types of *Salmonella* Typhimurium vaccines "local isolate" (Formalized Inactivated, Gamma-Irradiated inactivated by three doses of radiation "3.5, 4.5 and 5.5 KGy" and Vector vaccines) and evaluate these prepared vaccines through performing quality control tests; purity, sterility and safety tests and assess their immunological efficacy in vaccinated birds, determination of the protection percentage, shedding of *Salmonella* Typhimurium in the intestinal content as well as the effect of these vaccines on internal organs (Liver, heart and spleen) as clearance test and finally estimation of the humoral immune response in vaccinated birds using ELISA and compare these findings with results of another imported living attenuated *Salmonella* Typhimurium vaccine. The obtained results showed that all the prepared vaccines are safe, pure and not contaminated with any extraneous contaminants. Challenge test after inoculation of the vaccinated different chicken groups with virulent *Salmonella* Typhimurium strain which revealed that the use of irradiated vaccines are the most effective and protective vaccines than other prepared vaccine. Shedding test revealed the use of irradiated vaccine (3.5 KGy) or vector one is more effective in reduction of cecal colonization than other types of the prepared vaccines. Clearance test classified the vaccinated groups into 3 categories; first one has the lowest value of reisolation which means the highest value of clearance represented by “4.5 KGy” irradiated vaccine group, followed by formalized, “3.5 KGy” irradiated, “5.5 KGy” irradiated and vector vaccines then finally the highest value of reisolation category is the living attenuated vaccine and control groups. Generally in could be concluded that “4.5 KGy” irradiated vaccine gave the best clearance value than other types of the prepared vaccines. Statistically, there is a significant difference between groups with special reference to group (4) (vaccinated with irradiated "4.5 KGy" inactivated *Salmonella* Typhimurium vaccine). Generally, although all the prepared *Salmonella* Typhimurium vaccines are proved to be safe, potent and protective but it could be concluded that the "4.5 KGy" irradiated inactivated *Salmonella* Typhimurium vaccine gave the best and highest values of the immunological parameters during assessment of the prepared vaccines through whole the length of the experiment and it could be used safely to immunize the chicken against infection with *Salmonella* Typhimurium.

LIST OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATION

(RT)-PCR	:	Real time PCR
+ve	:	Positive
µl	:	Microlitre
***aad*A2**	:	confers resistance to streptomycin
AmR	:	ampicillin resistant
APC	:	Antigen Presenting Cell
blaTEM	:	beta-lactamase TEM1
BPS	:	Phosphate buffer saline
CDC	:	Centers for Disease Control and Prevention
CFU	:	Colony Forming Unit
DC	:	Dendritic Cell
***dfr*A12**	:	dihydrofolate reductase type XII
DNA	:	Deoxyribonucleic Acid
ELISA	:	Enzyme Linked Immunosorbent Assay
***fli*C**	:	Full gene of flagellin

fljB	:	phase 2 flagellin
Fnr	:	Fumarate Nitrate Reduction
G	:	Group
GIT	:	Gastrointestinal Tract
IFCS	:	integrated food chain surveillance system
invA	:	invasion protein
LB	:	Luria-Bertani Broth
ml	:	Milli-litre
mRNA	:	messenger Ribonucleic Acid
OD	:	Optical Density
OIE	:	Office International des Epizooties
PCR	:	polymerase chain reaction
PFGE	:	Pulsed-field gel electrophoresis
RBCs	:	Red Blood Cells
RST	:	Rapid Slide agglutination Test
***S.* Enteritidis**	:	*Salmonella* Enteritidis
***S.* Typhimurium**	:	*Salmonella* Typhimurium
S/C	:	Subcutaneous

S/P	:	Sample Positive ration
SG	:	*Salmonella* Gallinarum
SM	:	*Salmonella* Montevideo
SPF	:	Specific Pathogen Free
S-S	:	Salmonella-Shigella
***tet*A**	:	tetracycline resistance protein A
TLR 5	:	Toll-like receptor 5
WHO	:	World Health Organization

1. INTRODUCTION

Worldwide, Salmonellosis is a serious medical and veterinary problem and raises great concern in the food industry. In the recent years, *Salmonella enterica* serovar Enteritidis has replaced serovar Typhimurium as the primary etiologic agent of *Salmonella* infections in many countries (**Rabsch *et al.*, 2001**). A likely source of serovar Enteritidis is the consumption of infected poultry (**Cox, 1995 and Rabsch *et al.*, 2001**).

Avian salmonellosis is an inclusive term designating a large group of acute and chronic diseases of poultry caused by any one or more member of genus *Salmonella*. However, particular *Salmonella* serovars may be encountered more frequently in one country than the other **(Liljebjelke *et al.*, 2005).** In Egypt several investigators **(Shouman and Moustafa, 1972 and Mervat, 1995)** have isolated many Salmonella species particularly, *S.* Typhimurium and *S.* Enteritidis from poultry.

Most human illnesses associated with the shell egg consumption are from *Salmonella* Enteritidis (SE). Several *Salmonella* serotypes have been isolated from egg products (**Cook *et al.*, 2004**). *Salmonella* Enteritidis colonizes the tissues of the chicken ovary and oviduct, presumably contaminating eggs and thereby contributing to human outbreaks of salmonellosis. The bacteria *Salmonella* are Gram negative, straight rods not exceeding 1.5 micrometers in width. They are facultative anaerobes usually motile by peritrichous flagella (**Mastroeni *et al.*, 2001, Pawsey, 2002**).