



**Cairo University**  
**Faculty of Veterinary Medicine**



# **Evaluation of the protective potentials of *Clostridium perfringens* NetB toxin-based vaccine in Broiler Chickens**

A thesis submitted by

**Amal Nader Awad El-Rasheed**

(BVSc, Cairo University, 2004; MVSc, Cairo University, 2012)

**For the degree of the (Ph.D)**

**Microbiology**

**(Bacteriolog, Immunology and Mycology)**

**Under Supervision of**

**Heidy Mohamed Shawky**

**Professor of Microbiology, Faculty of Veterinary Medicine  
Cairo University**

**Mahmoud El Hariri**

**Professor of Microbiology,  
Faculty of Veterinary Medicine  
Cairo University**

**Eman Fathy Farag**

**Senior Researcher, Anaerobic Unit, Bacteriology  
Department of Animal Health Research Institute,  
Dokki, Giza**

**2020**

# CONTENTS

<i>Subject</i>	<i>Page</i>
<b>Introduction</b> .....	1
<b>Review of literature</b> .....	8
<i>C.perfringens</i> microorganism .....	8
Incidence of necrotic enteritis in chickens .....	11
Incidence of necrotic enteritis in chickens in Egypt.....	16
Predisposing factors for necrotic enteritis .....	19
Characteristics of alpha clostridial toxins.....	25
Net B (Beta like toxin).....	27
Experimental infection of NE disease in chickens.....	31
Histopathological changes due to necrotic enteritis.....	34
Modulation of cellular and humeral immune response of <i>Clostridium perfringens</i> .....	34
<b>Puplished paper</b> .....	36
<b>Discussion</b> .....	45
<b>Conclusion and recommendation</b> .....	55
<b>Summary</b> .....	56
<b>References</b> .....	59
<b>Appendix</b> .....	80
<b>Arabic summery</b> .....	
المستخلص العربي .....	

## LIST OF TABLES

Tables	Title	Page
1	Primer sequences and amplicon sizes used in toxinotyping of <i>C.perfringens</i> isolates	39
2	Incidence of <i>C.perfringens</i> recovered from apparently healthy and diseased broiler chickens	39
3	Toxinotypes of <i>C.perfringens</i> isolates from apparently healthy and diseased birds	39
4	Toxinotyping of <i>C.perfringens</i> isolates collected from diseased chicken	86
5	Experimental animal groups and type of collected samples.	88
6	Sequence of primers used for real-time quantitative RT-PCR.	88
7	Effect of Toxoid on intestinal <i>C.perfringens</i> microbial load (log CFU /g).	93
8	Toxin genes screening <i>netB</i> and <i>tpeL</i> of <i>C. perferingens</i> isolates from apparently healthy and diseased birds	94
9	Protein profile bands and western blot comparison in Molecular Weight.	96
10	Effect of toxoids administration on intestinal secretory immunoglobulin (SIgA) concentration expressed as ng/ml.	98
11	Detection of specific immunoglobulin against <i>C.perfringens</i> in serum of chicks vaccinated and challenged with <i>C.perfringens</i> .	99
12	Effect of toxoid immunization on nitric oxide assay as a marker for macrophage activation.	102

## LIST OF FIGURE

<i>Figure</i>	<i>Title</i>	<i>Page</i>
1.	Agarose gel electrophoresis showing amplification of a 324 base pair fragment of Alpha-toxin gene from the extracted DNA of <i>C.perfringens</i> isolates.	40
2.	Agarose gel electrophoresis showing amplification of a 560and 280 base pair fragment of netB and <i>tpel</i> gense from the extracted DNA of <i>C.perfringens</i> isolates.	94
3.	Electrophoresis protein profile of <i>C.perfringens</i> three batchs culture supernatants.	95
4.	Western blot analysis of <i>C.perfringens</i> three batchs culture supernatants using the NE antibody positive controy.	96
5.	The mRNA expression levels of IL-4, observed in intestine of negative control (C), groups and other toxoid immunized groups at different time interval.	100
6.	The mRNA expression levels of IL-10, observed in intestine of negative control (C), groups and other toxoid immunized groups at different time interval.	101
7.	Intestine of chicken in –ve group (44 day old) showing normal structure (H and E stain X 100).	103
8.	Intestine of chicken in +ve group (44 day old) showing congested blood vessels, mononuclear cells infiltration in lamina propria and sloughing of the epithelial lining (H and E stain X 100).	103
9.	Intestine of chicken in group 2 (44 day old) showing normal tissue (H and E stain X 100)	103
10.	Intestine of chicken in group 3 (44-day old) showing normal tissue (H and E Stain X 100).	104
11.	Intestine of chicken in group 1 (44 day old) showing normal tissue (H and E stain X 100).	104

<i>Figure</i>	<i>Title</i>	<i>Page</i>
12.	Liver of chicken in +ve group (44 day old) showing mild vacuolation of hepatocytes (H and E stain X 200)	104
13.	Liver of chicken in -ve group (44 day old) showing normal histological structure (H and E stain X 200).	104
14.	Liver of chicken in group 3(44 day old) showing normal histological structure (H and E stain X 200).	104
15.	Liver of chicken in group 2 (44 day old) showing minute focus of mononuclear cells aggregation (H and E stain X 200).	104
16.	Liver of chicken in group 1 (44 day old) showing slight vacuolation (H and E stain X 200).	105
17.	Kidney of chicken in –ve group (44 day old) showing normal structure (H and E stain X 400).	105
18.	Kidney of chicken in +ve group (44 day old) showing mild degenerative changes in tubular epithelium (H and E stain X 400).	105
19.	Kidney of chicken in group 3 (44 day old) showing normal histological structure (H and E stain X 400).	105
20.	Kidney of chicken in group 2 (44 day old) showing interstitial edema (H and E stain X 400).	105
21.	Kidney of chicken in group 1 (44 day old) showing interstitial edema (H and E stain X 400).	105

## LIST OF ABBREVIATION

Abbreviation	Definition
<b>BHI</b>	Brain heart infusion
<b>BWG</b>	Body weight growth
<b><i>C.perfringens</i></b>	<i>Clostridium perfringens</i>
<b>cpa</b>	<i>Clostridium perfringens</i> alpha toxin
<b>cpb</b>	<i>Clostridium perfringens</i> beta toxin
<b>CPE</b>	<i>Clostridium perfringens</i> enterotoxin
<b>cDNA</b>	Complementary DNA
<b>CFU/gm</b>	Colony forming unit per gram
<b>CFU/ml</b>	Colony forming unit per milliliter
<b>CMM</b>	cooked meat medium
<b>CT</b>	Cycle threshold
<b>ELISA</b>	Enzyme-linked ImmunoSorbent Assay
<b>EM</b>	<i>Eimeria maxima</i>
<b>etx</b>	Epsilon toxin gene
<b>FCR</b>	Feed conversion ratio
<b>GAPDH</b>	Glyceraldehyde-3-phosphate dehydrogenase
<b>gDNA</b>	Genomic DNA
<b>GIT</b>	Gastrointestinal tract
<b>hrs</b>	Hours
<b>IgA</b>	Immunoglobulin A
<b>IgE</b>	Immunoglobulin E
<b>IL</b>	Interleukin
<b>ISI</b>	I See Inside

<b>LSD</b>	Least significance difference
<b>NE</b>	Necrotic enteritis
<b>NetB</b>	Necrotic enteritis toxin B
<b>ng/ml</b>	Nano gram per milliliter
<b>NK-cell</b>	Natural killer cell
<b>NOS</b>	Nitric oxide synthase
<b>NSP</b>	Non- starch polysaccharides
<b>OD</b>	Optical density
<b>PBS</b>	Phosphate buffer saline
<b>PCR</b>	Polymerase chain reaction
<b>PFT</b>	pore forming toxins
<b>PIgR</b>	Poly-Ig receptor
<b>plc</b>	Phospholipase C
<b>rNetB</b>	Recombinant Net B
<b>SBA</b>	Sheep blood agar
<b>SIgA</b>	Secretory immunoglobulin A
<b>SPC</b>	Soy protein concentrated
<b>SPF</b>	Specific pathogen free
<b>SPSS</b>	Statistical product and service solutions
<b>TGY</b>	Tryptone glucose yeast
<b>TPG</b>	Trypticase peptone glucose
<b>TSC</b>	Tryptose Sulphite Cycloserine

**Cairo University**  
**Faculty of Veterinary Medicine**  
**Department of microbiology**

**Name:** Amal Nader Awad El-Rasheed

**Nationality:** Egyptian

**Date of birth:** 24/ 7/1981, UAE

**Degree:** Ph.D in Veterinary Medical Science

**Title of thesis:** Evaluation of the protective potentials of *Clostridium perfringens* NetB toxin-based vaccine in Broiler Chickens.

**Supervisor:**

**Prof. Dr. Heidy Mohamed Shawky;** Cairo University, Faculty of Veterinary Medicine.

**Prof .Dr. Mahmoud El Hariri;** Cairo University, Faculty of Veterinary Medicine.

**Dr. Eman Fathy Ahmed Farag;** Anaerobic Unit, Animal Health Research Institute.

## **ABSTRACT**

A total of 200 samples representing intestinal content of apparently healthy and diseased broiler chickens showing enteric disorder symptoms and lesions suspected to be due to necrotic enteritis, were examined by conventional and molecular methods. *C.perfringens* was isolated with an incidence of 10% (10/100) from apparently healthy chickens, and with an incidence of 25% (25/100) from diseased chickens. Twenty isolates of *C.perfringens* were proved to be toxigenic with an incidence of 57.1% (20/35), while 42.8% (15/35) were non-toxigenic. Multiplex PCR was performed to toxinotype the 35 *C.perfringens* isolates, the result showed that all isolates were positive for the alpha toxin gene. Experimental infection with multiple doses of *Clostridium perfringens* toxoidtype A, C, Net B given S/C in chicken resulted in subclinical necrotic enteritis (NE) diagnosed by decreased body weight gain and histopathological lesions in intestine and liver. Intestinal samples were collected at 3,7,14 and 37 days after vaccination for enumerating *Clostridium perfringens* in all groups. Vaccinated groups showed a decrease in *Clostridium perfringens* count compared with negative and positive groups. Immune response to vaccination by toxoid of type A, type C and type A Net B positive, was estimated. The effect of toxoid administration on intestinal secretory immunoglobulin revealed significant increase in SIgA in all vaccinated groups. Moreover, detection of interleukin 4 also gave a high level in group (2) vaccinated with toxoid A+ NetB while Net B positive type C group (3) showed a steady regulated level. Regarding interleukin 10, regulation has been shown in all immunized groups compared with control –ve group. Histopathological changes in the intestine and liver of control positive group were estimated 14 days' post challenge and revealed focal necrotic areas with leukocytic infiltration and multifocal areas of mononuclear cells and or heterophilic infiltration in the portal area of liver. Moreover, severe epithelial and goblet cells hyperplasia of intestine have been detected, while immunized and control –ve group revealed normal histological structure.

**Keywords:** NE, *C.perfringens*, incidence, beta like toxin, broilers.