بسم الله الرحمن الرحيم

MONA MAGHRABY

شبكة المعلومات الجامعية

التوثيق الالكتروني والميكروفيلم

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جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

قسم

نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها
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MONA MAGHRABY

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)

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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

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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 decreas 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 immunoglobuline 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 heterophillic 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.

DEDICATION

Dedicated to my parent's sole,

….my sister,

….my husband,

…my lovely sons, and my daughter

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LIST OF ABBREVIATION

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

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

INTRODUCTION

Necrotic enteritis (NE) is one of the most important disease in poultry and is very expensive for industry worldwide **(Bahram *et al.*, 2012).**

Necrotic enteritis is primarily caused by *C.perfringens* type A and to lesser extent type C strains, producing both alpha and beta toxins (**Van Immerseel *et al.*, 2004)**. It was first described in 1961 **(Parish, 1961)** and has since been found in all poultry producing countries.

Predisposing factors are required for the *C.perfringens* to colonize and cause disease in poultry. The most common predisposing factors are coccidiosis mucosal damage, and diets containing high level of indigestible water-soluble non starch polysaccharides, known to increase the viscosity of the intestinal contents **(Van Immerseel *et al.*, 2004).**

*C.perfringens*is a gram-positive, anaerobic, fermentative spore-forming bacillus, which classified into five types (A, B, C, D and E) according to the production of the four major toxins (alpha α, Beta β, epsilon ε and iotaι). Alpha toxins are produced by all strains and involved in disease pathogenesis (**Cato *et al.*, 1986).** Toxins B, NetB, were recently proposed as a new key virulence factor for the development of NE in broilers **(Abildgaard *et al.*, 2010).**

Infection with *C.perfringens* can cause poor production performance. The proper intestinal function is essential to achieve optimal feed conversation. The necrotic gut lesion and abnormal

clostridium dominance in gut microflora are factors likely to cause reduced feed conversion; together with intoxication with clostridial toxin; this might cause reduced productivity (**Lovland and Kaldhusdal, 2001)**.

The disease can occur in at least two forms. The acute form of NE typically results in mortality during the last weeks of rear of broilers (week 5-6). However, many cases of NE are associated with relatively mild clinical signs **(Brennan *et al.,* 2001a).** This subclinical form of NE results in decreased digestion and absorption of feedstuffs and consequently reduced weight gain **(Kaldhusdal *et al.,* 2001).** It is now believed that the subclinical form of NE is the most frequent form of the disease and cause the greatest economic losses to the poultry production industry **(Dahiya *et al.,* 2006).**

The acute disease leads to significant levels of mortality where as the chronic disease lead to loss of productivity and welfare concerns. It has been estimated that the disease cost the international poultry industry approximately 2 billion United States dollars A year **(Lovland and Kaldhusdal, 2001 and Van der sluis, 2000).**

Clinical signs include depression, dehydration, diarrhea, ruffled feathers and lower feed intake (**Songer 1996).** The gross lesions of the small intestine range from thin and friable walls to frank and extensive necrotic lesions **(Cooper *et al.*, 2010).**

Although it is clear that *C.perfringens* is etiologic agent of NE, a wide range of host and pathogen factors can influence the severity of the disease. These factors include the nature of the feedstuff,