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Trials for Preparation of Vaccine against *Campylobacter*Species in Chicken

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Abstract:

Campylobacter is a worldwide infection, which has been estimated as the most significant economic burden by EFSA and ECDC in 2016. It caused mainly by either *Campylobacter jejuni* or *Campylobacter coli*. *Campylobacter* species are one of the main cause of gastroenteritis for humans in developed and developing countries. The disease is endemic in Egypt and is a major cause of diarrhea in children. Good understanding of epidemiology and surveillance of *Campylobacter* will help in the elimination and prevention of it among animals and humans. Several intervention strategies have been implemented to reduce the intestinal colonization in chicken and vaccination measures is one of the effective control methods. The main goal of the present study was to develop a vaccine for reducing the intestinal burden of *Campylobacter* in chickens. To achieve this, 290 samples were collected from broiler flocks and slaughter market from Cairo governorate, Egypt. Vaccination of chickens with a prepared killed whole-cell vaccine was carried out. The *Campylobacter* specific IgG were measured in collected serum samples using ELISA assay. Also, *Campylobacter* count in the cecal content and serum antibody which have been measured. The greatest reduction in *C.jejuni* colonization was determined. Production of *Campylobacter* specific IgG antibodies as well as a marked decrease in *C.jejuni* colonization were recorded in chickens. In conclusion, we approved that the vaccination with CWC provides 73.3% protection of chickens from *C.jejuni* colonization.

Keywords: *Campylobacter jejuni*, colonization, chicken, vaccination, killed whole cell vaccine.

Dedication

I am indebted to my family who endured with me the hardships of long hours necessary for completing this work. I am so grateful to my mother, she supported me in the hard times. I would like to thank my father, my brother Mohammed and my sisters Sara and Marwa for their prolonged support. Finally, I hope that they will be happy and proud.

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LIST OF ABBREVIATIONS

Abbreviation	Full Name
API	Analytical Profile Index
AFLP	Amplified Fragment Length Polymorphism
BB	Bolton Broth
BSA	Bovine Serum Albumin
CEB	*Campylobacter* Enrichment Broth
CFU	Colony Forming Unit
CPS	Capsular Polysaccharide
CWC	*Campylobacter* Whole Cell
ECDC	European Centere for Disease Prevention and Control
EFSA	European Food Safety Authority
EU	European Union
Fla	Flagellin Gene
GBS	Guillain Barre Syndrome
HL	Heat Labile
HRM	High Resolution Melting
HS	Heat Stable
IBD	Inflammatory Bowel Syndrome
MACPs/MCPs	Methyl Accepting Chemotaxis Proteins
MALDI-TOF/MS	Matrix Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry
mCCDA	Modified Charcoal Cefoperazone Deoxycholate
MLST	Multilocus Sequence Typing

MOMP	Major Outer Membrane Protein
MPCR	Multiplex PCR
ODN	Oligodeoxynucleotides
PB	Preston Broth
PBS	Phosphate Buffer Saline
PCR	Polymerase Chain Reaction
PFGE	Pulse Field Gel Electrophoresis
PNS	Peripheral Nervous System
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
RT-PCR	Real Time PCR
SDA	Sabroud Dextrose Agar
SECPs	Surface Exposed *C.jejuni* Proteins
SNP	Single Nucleotide Polymorphism
SVR	Short Variable Region
T3SS	Type III Secretion System
USDA	United states Department of Agriculture
VBNC	Viable But Non Culturable
VNTR	Variable Number of Tandem Repeats
WHO	World Health Organization

Chapter (1)

Introduction

Introduction

Campylobacter species are Gram-negative spiral, rod-shaped, or curved bacteria with a single polar flagellum, bipolar flagella, or no flagellum, depending on the species **(Man, 2011)**. *Campylobacter* species are non-spore-forming, are approximately 0.2 to 0.8 by 0.5 to 5 μm, and are chemoorganotrophs, which obtain their energy sources from amino acids or tricarboxylic acid cycle intermediates **(Vandamme *et al.*, 2005).**

Genus *Campylobacter* belongs to the family *Campylobacteraceae*, the order *Campylobacterales*, the class *Epsilonproteobacteria*, and the phylum *Proteobacteria*. In 2010, the number of species reached to 32, while the number of subspecies remained as 13 subspecies **(Euz´eby, 2010)**, with no change since **(Euz´eby, 2014).** The most common thermophilic species are; *C.jejuni*, *C.coli*, *C.lari*, and *C.upsaliensis*, particularly the first two species **(EFSA, 2013).**

Campylobacters have been known to be the cause of diseases in animals since 1909, but they have been generally recognized as a cause of human disease, only since about 1980. Public health services worldwide pay a great attention to *Campylobacter* spp. because these microorganisms are pathogenic to humans and commonly found in the gastrointestinal tracts of cattle, dogs, cats, and sheep, though poultry and pigs are the most common reservoirs **(USDA 2013).**

Domestic poultry (e.g., chickens, turkeys, ducks, and geese) and wild birds are frequently infected with thermophilic *Campylobacter* **(Sahin *et al.*, 2002; Zhang and Sahin, 2013; Golz *et al.*, 2014).** The prevalence rates of *Campylobacter*, especially in slaughter-age conventional broiler flocks, could reach as high as 100% on some farms worldwide.

Both *C.jejuni* and *C.coli* are well adapted to the avian host and reside mainly in the intestinal tract of birds. However, limited data suggest that *Campylobacter* colonization may be associated with disease production in poultry under certain conditions. For example, a very-recent study reported the production of intestinal inflammation and diarrhea in fast growing breeds of broiler chickens following experimental challenge **(Humphrey *et al.*, 2014).** In addition, vibrionic hepatitis with

high morbidity and mortality associated with *Campylobacter* infection was reported in laying hens and ostriches **(Stephens *et al.*, 1998 and Burch, 2005).**

Campylobacteriosis is the most common zoonotic and bacterial foodborne disease in humans **(Zendehbad *et al.*, 2015)**. Most *Campylobacter* infections are associated with consumption of contaminated or undercooked poultry and by-products that have been contaminated during processing **(Hermans *et al.*, 2011**; **Wagenaar *et al.*, 2013)**. The number of human campylobacteriosis cases has been dramatically increased worldwide, surpassing the number of cases of salmonellosis and shigellosis **(Cover *et al.*, 2014).** Although the number of human cases of campylobacteriosis in the European Union (EU) decreased in 2012 for the first time in over a 5 years' period **(EFSA, 2014).**

The epidemiology of *Campylobacter* infection in developed countries is totally different than in the developing world. Previously, *Campylobacter* infection is sporadic, the prevalence of asymptomatic infection is low, and marked seasonal variation is seen (**Nichols *et al.*, 2012**). While, *Campylobacter* is endemic in developed countries, asymptomatic infections that are usually limited to children are common and seasonality are less prominent or even absent **(Taniuchi *et al.*, 2013 and Lee *et al.*, 2013).**

The disease is endemic in Egypt and it is a major cause for pediatric diarrhea. Nonetheless, the epidemiology in animals and humans has not been fully characterized. The main source of *Campylobacter* transmission is the backyard chickens **(Khalifa *et al.*, 2013; El-Tras *et al.*, 2015; Omara *et al.*, 2015)**. From Zagazig governorate in Egypt, *C.jejuni* and *C.coli* were isolated from 47.5% of chicken samples and 2.7% of human samples (**Awadallah *et al.*, 2014).**

In recent years, isolates from both developed and developing countries have shown resistance to several antimicrobials, including fluoroquinolones, tetracyclines, beta-lactams, aminoglycosides and macrolides **(Luangtongkum *et al.*, 2009; Shobo *et al.*, 2016; Reddy and Zishiri, 2017),** which are the most frequently used antimicrobials for the treatment of campylobacteriosis **(Abdi-Hachesoo *et al.*, 2014; Shobo *et al.*, 2016; Reddy and Zishiri, 2017).** This has led the World Health Organization in 2017

to list *Campylobacter* spp. as one of the six high priority antimicrobial resistant pathogens **(WHO,2017).**

It is extremely difficult to keep poultry flocks free of *Campylobacter* which is commonly present in the poultry environment. Various intervention strategies have been implemented to reduce colonization rates, including on-farm biosecurity measures, vaccination, genetic selection, dietary manipulation, and the use of antimicrobial alternatives **(de Zoete *et al.*, 2007 and Lin, 2009)**. However, these strategies have been only partially effective in reducing the burden of *C.jejuni*. Among the above strategies, immunological interventions such as vaccines are of note.

There is no vaccine available to date to control *Campylobacter* infections in poultry. A successful chicken vaccine should prevent colonization or cause a strong reduction of *Campylobacter* numbers in chickens (>2 log units) **(de Zoete *et al.*, 2007)**.

The aim of the present work:

The main goal of the present study was to develop a vaccine for reducing intestinal burden of *Campylobacter* in chickens which can be achieved through:

1- Isolation and identification of *Campylobacter* species from collected chicken samples.
2- Confirmation of *Campylobacter* species using PCR technique.
3- Identification of the pathogenic (virulent) strains of *Campylobacter* species.
4- Detection of genetic similarity between isolates of different sources using sequencing technique.
5- Preparation of a vaccine from the selected *Campylobacter* strains.
6- Evaluation of the immunizing potential of the prepared vaccine by measuring *Campylobacter* specific IgG antibody titre.
7- Detection of *Campylobacter* load in the cecum of vaccinated chicken.

Chapter (2)

Review of Literature