بسم الله الرحمن الرحيم

MONA MAGHRABY

شبكة المعلومات الجامعية

التوثيق الالكتروني والميكروفيلم

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شبكة المعلومات الجامعية

التوثيق الإلكتروني والميكروفيلم

جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

قسم

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MONA MAGHRABY

Cairo University
Faculty of Veterinary Medicine

Bacteriological and molecular studies on *Clostridium difficile* in small ruminants and poultry under desert conditions

A thesis submitted by

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For the Degree of PhD in Veterinary Medical Sciences (Microbiology)

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Supervision Sheet

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Abstract

Clostridium difficile is a leading cause of hospital-acquired diarrhea among humans. Meanwhile, it recently has a growing attention in the veterinary medicine. The present study investigates the performance of three different laboratory techniques for diagnosis of *C. difficile* infection in animals and poultry as well as the burden of *C. difficile* among diarrheic sheep and goats in rural settings. Accordingly, 90 fecal samples of animal origin (sheep, goats) and poultry were examined by conventional culture technique followed by PCR, GDH-ELISA and direct PCR for the detection of *C. difficile* from feces. The results revealed that direct PCR showed the highest detection rate (45.6%), followed by conventional culture technique with molecular confirmation (16.7%), while the lowest detection rate was obtained by GDH-ELISA (8.9%). Despite the high detection rate of direct PCR technique, three false negative results were recorded (positive by conventional culture technique followed by PCR). On the other hand, fecal samples from 60 diarrheic animals reared in rural settings (36 sheep and 24 goats) were pre-enriched in brain heart infusion broth and cultured on selective *C. difficile* agar medium. *C. difficile* isolates were identified using conventional, serological and molecular techniques. Moreover, the obtained isolates were examined for the occurrence of genes encoding toxin A and toxin B. The overall prevalence of *C. difficile* among the examined animals was 20% (12/60). However, only three ones (3/12) possessed toxigenic genes; *tcd*A in 2 isolates and *tcd*B in one isolate. The phylogenetic analysis of the obtained *tcd*A gene sequence from sheep showed high genetic relatedness to those of beef, pig and humans. In conclusion, direct PCR technique yielded a high detection rate for detection of *C. difficile* in animal fecal samples, whereas *C. difficile* may be a potential cause of diarrhea among sheep and goats in rural settings with public health implications.

Dedication

I dedicate this study to ***my mother and my Father****, who has always supported me morally and emotionally.*

Also I dedicate this study to ***my wife*** *who has always supported me practically and academically.*

Also I dedicate this study to ***my brother and sister*** *for their continuous encouragement.*

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BHI	brain, heart infusion broth
BLAST	basic local alignment search tool
Bp	base pair
CCFA	cycloserine-cefoxitin-fructose-agar
CCNA	Cell Cytotoxicity Neutralization Assay
CDI	*Clostridium difficile* infection
CDT	Binary toxin
CDTa	Binary toxin (enzymatic component)
CDTb	Binary toxin (binding component)
Cfu	colony forming unit
CPE	cytopathic effect
DNA	Deoxyribonucleic acid
EIA	enzyme immunoassays
ELISA	Enzyme-linked immunosorbent assay
GDH	glutamate dehydrogenase enzyme
G+C amount	guanine-cytosine amount
kDa	Kilodaltons
Mb	Megabase
µl	Microliter
PaLoc	pathogenicity locus
PCR	Polymerase chain reaction
***tcd*A**	Toxin A gene
***tcd*B**	Toxin B gene

Chapter (1)

Introduction

Clostridium difficile is a Gram positive, strictly anaerobic, spore forming, rod shaped bacterium. In 1935, *C. difficile* was firstly identified and isolated by Hall and O'Toole from healthy new-born infant stool **(Pasquale *et al.*, 2012; Troiano *et al.*, 2015).** *C. difficile* has the ability to colonize the intestinal tract of humans and animals **(Peláez *et al.*, 2013).** It is a nosocomial pathogen causing diarrhea that commonly occurred in patients after hospitalization and antibiotic treatments **(Rodriguez *et al.*, 2012).**

The most frequently predisposing factor for *C. difficile* infections is the usage of antibiotics for a long period in humans and animals as it can damage the normal bowel microflora. Accordingly, at this circumstance, *C. difficile* can colonize the intestinal tract to cause gastrointestinal symptoms. The symptoms of *C. difficile* infection (CDI) in human ranged from mild diarrhea to severe, life threatening pseudo-membranous colitis and toxic mega colon **(Thitaram *et al.*, 2016).**

In the last few years, some studies have pointed out to the occurrence of *C. difficile* among food animals. The identification of this bacterium among food producing animals highlights the possible zoonotic transmission and subsequently the public health implications **(Deng *et al.*, 2015; Hampikyan *et al.*, 2018).**

C. difficile virulence is generally owed to the presence of two major exotoxins. Toxin A (enterotoxin) and Toxin B (cytotoxin) which encoded by the *tcd*A and *tcd*B genes, respectively. However, some *C. difficile* strains do not have the ability for toxin production and accordingly, they do not cause any CDI symptoms **(Neely *et al.*, 2017; Hampikyan *et al.*, 2018).**

Kendrick, (2018) mentioned that there are different laboratory diagnostic techniques for *C. difficile* diagnosis as the follow: bacterial culture, enzyme immunoassays (EIA) and molecular assays as PCR are useful for diagnosis of CDI.

In the veterinary medicine, much remains unknown about the appropriate laboratory diagnostic tool to diagnose *C. difficile* infection among animals as well as the burden of such bacterium among diarrheic small ruminants.

Therefore, the current dissertation was aimed to:

1- Isolate and identify of *C. difficile* from small ruminants and poultry fecal samples.
2- Determine the appropriate laboratory diagnostic tool for CDI among animals and poultry by comparing different diagnostic methods.
3- Investigate the occurrence of *C. difficile* among diarrheic small ruminants.
4- Detect of toxigenic genes of *C. difficile* among isolated strains by molecular methods.
5- Investigate the phylogenetic analysis of some obtained strains to underscore the public health burden.

Chapter (2)

Review of literature

2.1. *Clostridium difficile* (classification, bacterium, pathogenesis and virulence)

Clostridium difficile is classificed as follow: Kingdom (Bacteria); Phylum (Firmicutes); Class (Clostridia); Order (Clostridiales); Family (Clostridiaceae); Genus (*Clostridium*); Species (*Difficile*) **(Sandhu and McBride, 2018).**

C. difficile is a Gram-positive, strictly anaerobic, spore-forming bacterium. It slowly grows compared to other anaerobes, and sometimes is overgrown by several micro-organisms making the laboratory isolation of *C. difficile* is very hard in mixed cultures. The bacterium's genome size (Mb) varies from 4.05 to 4.46, G+C amount varies from 28.4 to 29.2% **(Zhu *et al.*, 2018)**.

Approximately, about 11 % of their genome consists of transposons and prophages. Those movable genetic components can be horizontally transported among *C. difficile* strains that serve as vectors for different genes such as antibiotic-resistance genes **(Suzuki *et al.*, 2016)**.

Owing to the multitude adaptations and the bile salts withstanding capacity, *C. difficile* can survive in human and animal gut. The bacterium can subsequently generate and tolerate 4-methylphenol (para-Cresol) which is an organic molecule with bacteriostatic role. Many intestinal bacteria are sensitive to para-Cresol which expands *C. difficile* competitiveness toward them **(Meessen-Pinard *et al.*, 2012)**. Either the vegetative form or spore form, the organism is ingested and reaches the stomach. Its spores can simply stand stomach acidity, then pass into the intestine and colonize it under suitable micro-environmental conditions **(Crobach *et al.*, 2018)**.

After colonization, the bacterium produces and releases two exotoxins, toxin A (TcdA) and toxin B (TcdB) which are the major virulence factors of such bacterium **(Carter *et al.*, 2010; Davies *et al.*, 2011)**. TcdA and TcdB are high