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**Occurrence and risk factors associated with pathogenic
E. coli in diarrheic calves**

A Thesis Presented By

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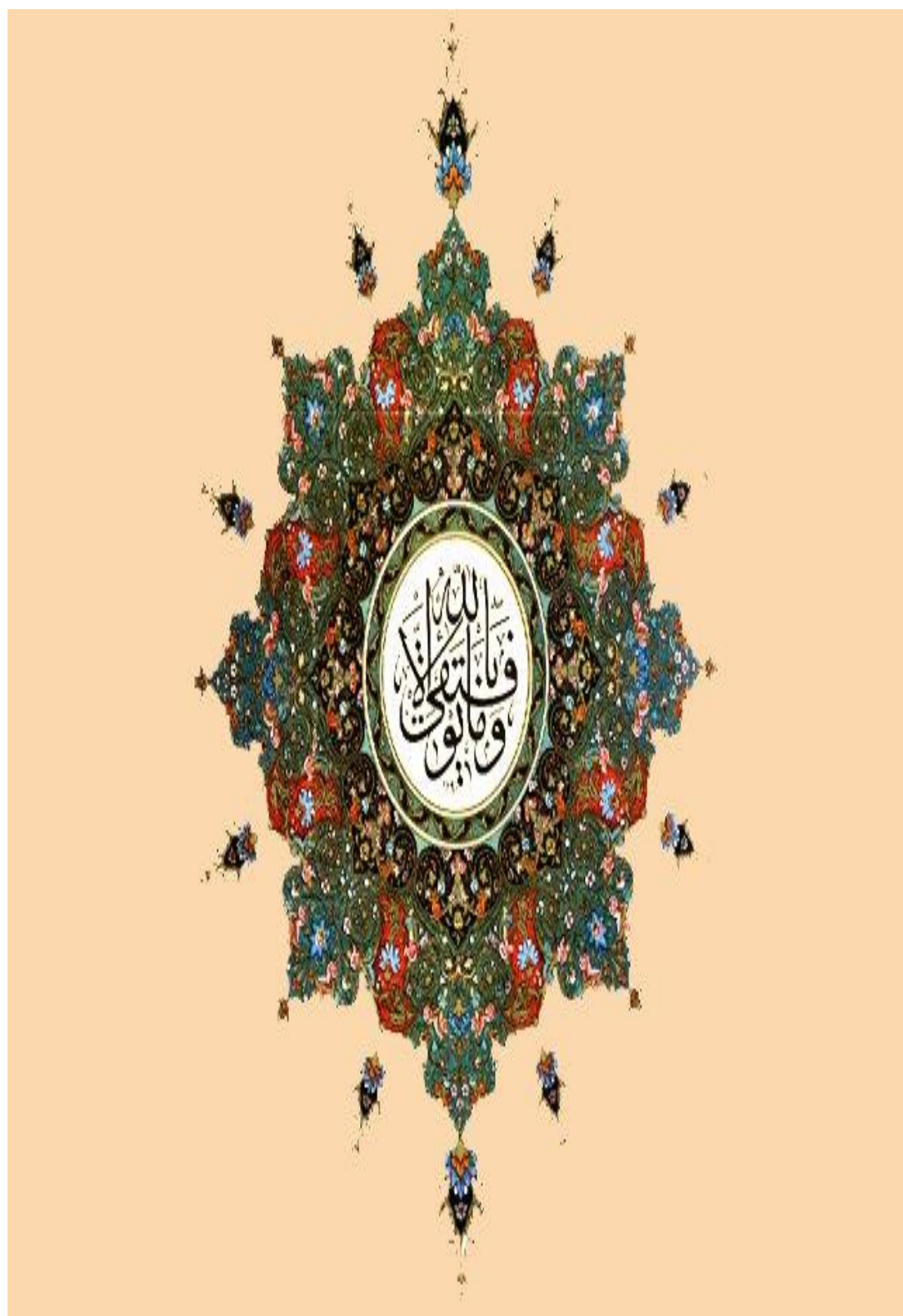
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Abstract

Bacteriological examination of fecal swabs collected from healthy (H) and diarrheic (D) calves, revealed isolation of 26 *E. coli* isolates from 56 calves with an incidence (46.4%). The serological tests for representative number of samples from every farm identified O1, O26, O44, O55, O115, O119, O125, O146, O151 and 2 samples were untyped. The isolates were sensitive to norfloxacin and gentamicin with 76.9% and resistant to Ampicillin and Cefotaxim with 100%. The PCR for 4 gene *ompA*, *stx1*, *stx2* and *eaeA*, detected *ompA* gene in all 26(100%) fecal samples, multiplies a region of 919bp, *stx1* gene multiplies a region of 614pb in 1(3.8%) isolate, *eaeA* gene was in 5 (19.2%) isolates, and no evidence for presence of *stx2* in all 26 *E. coli* isolates. Four colostrum samples were positive for *E. coli*. The isolates were serogroups O26, O55 and O119. All isolates were negative for *eaeA*, *stx1* and *stx2* except strain number 4 (O55) was positive for *stx1* only.
key words: examination- isolates- gene- sensitive- serogroups.

This work is dedicated to

My mother,

My brother Ahmed,

And my love Egypt.

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List of Abbreviations

AM	Ampicillin
AEEC	Attaching and effacing <i>E. coli</i>
BPW	Buffer peptone water
°C	Degree Centigrade
Cm	Centimeter
CTX	Cefotaxime
CN	Gentamicin
DN	Clindamycin
<i>eaeA</i>	Gene coding for attaching and effacing mechanism
EHEC	Enterohemorrhagic <i>E. coli</i>
<i>ehly</i>	Gene coding for hemolytic mechanisms
EIEC	Enteroinvasive <i>E. coli</i>
EPEC	Enteropathogenic <i>E. coli</i>
ETEC	Enterotoxigenic <i>E. coli</i>
(H)	Flagller Antigen
H ₂ S	Hydrogen sulphide
<i>invA</i>	Invasion gene A
K	Kanamycin
min.	Minute
MKTN	Muller Kauffmann Tetrathionate Novobiocin broth
N.	Number
NCD	Neonatal calf diarrhea
NOR	Norofloxacin
(O)	Somatic Antigen
PCR	Polymerase chain reaction
RVS	Rappaport-Vassiliadis Soya broth
SG	<i>Salmonella</i> Gallinarum
SMT	Standard microbiological technique
STEC	Shiga toxin-Producing <i>E. coli</i>
Stx	Shiga toxin
SXT	Sulphonamid-trimethoprim
TBE	Tis borate EDTA
TSA	Trypton soya agar
TSI	Triple sugar iron agar
XLD	Xylose Lysine Desoxycholate
TSI	Triple sugar iron agar
XLD	Xylose Lysine Desoxycholate

Chapter1

Introduction

1- INTRODUCTION

Neonatal calf diarrhoea (NCD) is one of the major health challenges in both beef and dairy cattle herds **(USDA, 2010)**. The prevalence and incidence risk for NCD has recently been reported to be 19.1 and 21.2%, respectively **(Bartels *et al.*, 2010; Windeyer *et al.*, 2014)**.

In USA, diarrhoea in neonatal calves accounts for more than 50% of unweaned dairy heifer deaths **(USDA, 2010)**. The herd veterinarian is the most adequate person to advice farmers how to treat and how to prevent NCD **(Smith, 2012)**.

However, calf diarrhea was perceived as a minor problem by dairy producers, while the beef producers did not consider it a problem at all **(Roderick and Hovi, 1999)**.

Diarrhoea in neonatal calves is a complex multifactorial and dynamic disease with the balance between the host's resistance (i.e. active and passive immunity) and the pathogen pressure being cardinal **(Lorenz *et al.*, 2011)**. Enterotoxigenic *Escherichia coli*, rota- and coronavirus and *Cryptosporidium parvum* are the four most important enteropathogens causing NCD world wide **(Gulliksen *et al.*, 2009; Bartels *et al.* 2010; Silverlas *et al.*, 2010)**. Rota virus and *Cryptosporidium parvum* are most frequently identified in faecal samples from calves with NCD:

prevalence in calves with diarrhoea ranges from 2.6 to 45.1%, 17.7 to 79.7%, 3.1 to 21.6% and 27.8 to 58.5%, for *E. coli*, rota- and coronavirus and *Cryptosporidium parvum* respectively **(Geurden et al., 2008; Ok et al., 2009; Bartels et al., 2010; Izzo et al. 2011)**. The simultaneous or consecutive presence of more than one than one of these pathogens often causes an increased morbidity and mortality rate **(Blanchard, 2012)**.

Each strategy to prevent NCD should begin with a confirmed diagnosis and the setup of a farm interview. The herd anamnesis addressing young stock management creates a list with potential critical control points. Key questions in this anamnesis should focus on: colostrum management, housing and hygiene, feeding of the calves, periods of stress, and drug used **(Blanchard, 2012; Smith, 2012)**.

Generally, calf diarrhea resulted from complex interaction of environment, infectious agents and the calf itself are the major constraints for raising replacement stock. The impacts of calf diseases could be direct and indirect through increased treatment expenses, decreased lifetime productivity and survivorship **(Randhawa et al., 2012)**.

Escherichia coli first described by a Bavarian Paediatrician, Theodor Escherich, in the late 19th century in a series of pioneering studies of the intestinal flora of infants he describe a normal microbial inhabitant of healthy individuals **(Kaper, 2005)**

The present investigation was aimed to study the risk factors associated with *E. coli* among diarrheic and apparent healthy calves.

Therefore, the following steps were carried out:

- 1-Collection of fecal samples from calves with and without diarrhea.
- 2-Trials for isolation and identification of *E. coli*.
- 3-Serotyping of the isolates.
- 4-Antimicrobial susceptibility testing of the isolates.
- 5-Measurment of virulence factors among the isolates.
- 6-Investigation of risk factors associated with calf diarrhea.