AEROBIC BACTERIA AND YEAST ASSOCIATED WITH DIARRHOEA AMONG CALVES

A Thesis

Presented to the Graduate school

Faculty of Veterinary Medicine, Alexandria University

In partial fulfillment of the Requirements for the Degree

of

Master of Veterinary Medical Science

Specialization

(bacteriology&mycology)

By

Asmaa Mohamed El-sayed Badawy

2010

البكتيريا الهوائيه و الفطريات المسببة للاسهال في العجول

رسالة علمية

مقدمة الى الدراسات العليا بكلية الطب البيطري – جامعة الاسكندرية

استيفاء للدراسا ت المقررة للحصول على درجة

ماجستير العلوم الطبية البيطرية

تخصص

(بكتريولوجيا & فطريات)

مقدمة من

ط.ب./ اسماء محمد السيد بدوي

٢٠١٠

لجنة الاشراف

الاستاذ الدكتور/ محمد علي عقيلة

استاذ الميكروبيولوجيا
رئيس قسم الميكروبيولوجيا
كلية الطب البيطري
جامعة الاسكندرية

الاستاذ الدكتور/ احمد ابراهيم الشيخ

استاذ التربية و الانتاج الحيواني
كلية الطب البيطري
جامعة الاسكندرية

الاستاذ الدكتور/ عادل محمد خضر

استاذ امراض الحيوان المعديه
كلية الطب البيطري
جامعة الاسكندرية

الاستاذ الدكتور/ احمد ابو المجد بخيت
مدير معهد بحوث صحة الحيوان
فرع دمنهور

ACKNOWLEDGMENT

First of all prayerful and deepest thanks to our merciful ALLAH who gave me every thing I have and gave me the power and chance to make this work. I wish to express my greetings to the greatest man in this world to our Prophet Muhammad (peace and blessings be upon him)

I am deeply grateful and wish to express my sincere appreciation and heartily thanks to **Prof. Dr. Mohamed Ali Akiela** , professor of microbiology and head of microbiology department , faculty.Vet. Med. Alex. Univ. for his constant help, kind advices and valuable discussion. Whose help supervision had provided all facilities for production of this work.

I would like to express my profound gratitude to **Prof. Dr. Helmy Ahmed Torky,** professor of microbiology.Vet. Med. Alex. Univ. for his kindness and interest encouragement and stimulating supervision. Who has gave me continuous advices and constructive criticism throughout the work

I would like to express my deepest thank to **Prof. Dr. Samy Khalil** professor of microbiology.Vet. Med. Alex. Univ. for his indispensable help.

I am heartily thanks to **Prof. Dr. Ahmed Ibrahim El-Sheikh,** professor of animal breeding and health production, faculty. Vet. Med. Alex. Univ. for his great guidance.

My sincere gratitude to **Prof. Dr. Adel Mohamed Khadr,** professor of infectious diseases, faculty. Vet. Med. Alex. Univ. for his kind cooperation and help during this work.

Cardial thank to **Prof. Dr. Ahmed Abou El-Majd Bekheit,** director of animal health research institute, Damanhour branch, for his help and support.

Also, special thanks to **Prof. Dr. Riad Hassan Khalil,** professor of fish diseases and hyagiene, department of poultry and fish diseases, faculty. Vet. Med. Alex. Univ. for his direct and effective advises during the course of this work.

I would like to thank all my professors and colleagues who in one way or another have helped to bring this work to light.

Finally I would like to express my special thank to my family for their continuous encourage and support me to fulfill this work.

List of content

LIST OF ABBREVIATIONS

EPEC	Enteropathogenic Escherichia coli
ETEC	Enterotoxigenic Escherichia coli
EHEC	Enterohaemorrhagic Escherichia coli
E. coli	Escherichia coli
STEC	Shiga toxin-producing Escherichia coli
VTEC	Verocytotoxin-producing Escherichia coli
K_{99}	Fimbrial antigen of E. coli
F5	Fimbrial antigen of E. coli
O	Somatic antigen of E. coli
H	Flagellar antigen of E. coli
Hly	Hemolysin
bfpA	Bundle-forming pili
CNF	Cytotoxic necrotizing factor
EHly	Enterohaemolysin
eae	Intimin
LT	Heat labile toxin of E. coli
STa	Heat stable toxin a of E. coli

stx	Shiga toxins
SLTII	Shiga like toxin type 2
PCR	Polymerase chain reaction
bp	Base pair
TE	Tris-EDTA
dNTPs	Deoxynucleotide triphosphate solution
EDTA	Ethylene diamine tetra-acetic acid
TPE	Tris-phosphate EDTA
DDW	Double-distilled water
TM	Melting temperature
SDA	Sabouraud's Dextrose Agar
Spp.	Species

LIST OF FIGURES

Figure **Page**

LIST OF TABLES

1. INTRODUCTION

Diarrhoea was considered as one of the major problems facing livestock production not only in Egypt, but also all over the world. **(Farid et al., 2001** and **Ibrahim, 2007).**

Enteritis among newlyborn calves causes high morbidity and mortality rates which resulted as large economical losses in Egypt. **(Novert and Hammad, 2001** and **Ashraf, 2007)**

Each year thousands of neonatal calves are suffering from diarrhoea, resulting in economic losses, these losses not only by increasing calf fatality but also by decrease in the calf's ability to gain weight, treatment cost, time spent on care as well as subsequent chronic ill thrift and poor growth **(Bazeley, 2003).**

Non infectious causes such as colostrums intake, birth weight, age susceptibility, housing factors, climatic condition, as well as the virulence of etiological pathogens, all have been incriminated as predisposing factors of enteritis **(Meltzer and Shpigal, 1996).**

The family Enterobacteriaceae that includes inter-related Gram negative microorganisms may constitute a prime cause of enteritis among newly born calves. E. coli and Salmonella take the major importance as a cause of diarrhoea **(Ashraf, 1996).**

There were at least four major categories of diarrhoegenic E. coli, namely: enterotoxigenic E. coli (ETEC), enteropathogenic E. coli (EPEC), enteroinvasive E. coli (EIEC) and enterohaemorrhagic E. coli (EHEC) **(Levin, 1987).**

Enterotoxigenic E. coli (ETEC) was well recognized as an important cause of neonatal diarrhoea among calves **(Moon et al., 1978; Zaman et al., 1989, Quinn et al., 1994 and Rodney and David**, **2010)**. Also, ETEC isolated from calves was known to posses K_{99} (F5) adhesion antigen and to produce stable toxin (STa) but not labile toxin **(Acres, 1985; Mainil et al., 1986; Holland, 1990; Wray et al., 1993 and Ok et al. 2009)**

Since verotoxigenic E. Coli (VTEC) was isolated from cattle with or without diarrhoea (**Holland, 1990; Wray et al., 1993; Burnens et al., 1995 and Eriksson and co-workers 2005)**, many reports indicated that VTEC were responsible for haemorrhagic enterocolitis and diarrhoea with characteristic lesions described as attaching and effacing lesions **(Moxley and Francis, 1986; Janke et al., 1990 and Agbodaze, 1999).** In addition, the occurrence of VTEC in young farm animals makes them candidate reservoirs of zoonotic agents (**Holland, 1990 and Agbodaze, 1999).**

Polymerase chain reaction (PCR) is a valuable and sensitive method for determining the virulence factors of E. coli strains. PCR seemed to give good results in epidemiological investigation of diarrheogenic E. coli especially fimbrial and toxin (LT1, LT2, STa, STb, SLT1, SLT2) genes. **(Osek et al., 1999 and Salvadori et al. 2003**)

For detection of ETEC several PCR assays are developed that are quite sensitive, rapid and specific when used directly on clinical samples or in isolated bacterial colonies **(Rich et al., 2001).**

(Herrera-Luna et al., 2009; Ok et al., 2009 and **Mahdi et al., 2010)** used Polymerase chain reaction to characterize E. coli isolates and toxins isolated from diarrhoeic calves.

The fungi, particulary yeasts and moulds are always neglected although they are well known to cause diseases of all animal species predisposing by their insensitivity to antibiotics, so they usually flourish following prolonged antibiotic therapy **(Ibrahim, 2007)**

Candida albicans may be isolated in form of mixed infection with EPEC and Salmonella spp. through examination of 40 diseased buffalo calves with diarrhoea and 20 healthy calves **(Manna et al., 1993)**.