

## Table of Contents

Object	Page No.
Table of contents	I
List of tables	VI
List of figures	VII
Dedication	VIII
Acknowledgment	IX
Abstract	X
ملخص الاطو حة	XII
Introduction	XIV
<b>Chapter (1) Literature Review</b>	
1.1. Diarrhoea	1
1.2. Pathogenesis of diarrhoea	2
1.3. Neonatal calf diarrhoea	3
1.3.1. Causes of calf diarrhoea	4
1.3.1.1. Viruses	4
a) 1.3.1.2. Helminth parasites	4
b) 1.3.1.3. Protozoa	4
c) 1.3.1.4. Bacteria	4
1.3.2. Epidemiology of calf Diarrhoea	5
1.3.1.1. Definition of <i>Enterobacteriaceae</i>	7
1.3.1.1.1. Tribe <i>Echericheae</i>	8
1.3.1.1.1.1. Definition of the genus <i>Echerichia</i>	8
1.3.1.1.1.1.1. <i>Escherichia fergusonii</i>	8
1.3.1.1.1.1.2. <i>Echerichia Hermanni</i>	9
1.3.1.1.1.1.3. <i>Escherichia vulneris</i>	9
1.3.1.1.1.1.4. <i>Escherichia blattae</i>	9
1.3.1.1.1.1.5. <i>Escherichia adecarboxylata</i>	10
1.3.1.1.1.1.6. <i>Escherichia coli</i>	10
1.3.1.1.1.1.6.1. Synonyms and history	10
1.3.1.1.1.1.6.2. Characterization of <i>E. coli</i>	10
1.3.1.1.1.1.6.2.1. Morphological characters	10
1.3.1.1.1.1.6.2.2. Cultural characteristics	11
1.3.1.1.1.1.6.3. Resistance	12
1.3.1.1.1.1.6.4. Biochemical characteristics	12

1.3.1.1.1.1.6.4.1. Carbohydrates fermentation	12
1.3.1.1.1.1.6.4.2. Other biochemical tests	13
1.3.1.1.1.1.6.5. Strains of <i>E. coli</i>	13
1.3.1.1.1.1.6.6. Serotyping of <i>E. coli</i>	13
1.3.1.1.1.1.6.6.1. Somatic (O) antigens	14
1.3.1.1.1.1.6.6.2. Capsular (K) antigens	14
1.3.1.1.1.1.6.6.2.1. Labile L antigens	15
1.3.1.1.1.1.6.6.2.2. A antigens	15
1.3.1.1.1.1.6.6.2.3. B antigens	15
1.3.1.1.1.1.6.6.3. Flagellar (H) Antigens	15
1.3.1.1.1.1.6.6.4. Other antigens	15
1.3.1.1.1.1.6.6.4.1. Fimbrial antigens	15
1.3.1.1.1.1.6.6.4.2. Common antigen (CA)	16
1.3.1.1.1.1.6.7. Pathogenesis and pathogenicity of <i>E. coli</i>	16
1.3.1.1.1.1.6.7.1. Capsules	17
1.3.1.1.1.1.6.7.2. Fimbria	17
1.3.1.1.1.1.6.7.3. Endotoxins	17
1.3.1.1.1.1.6.7.4. Verotoxins	18
1.3.1.1.1.1.6.7.5. Cytotoxic necrotizing factors	19
1.3.1.1.1.1.6.7.6. Haemolysins	19
1.3.1.1.1.1.6.7.6.1. Alpha haemolysin	20
1.3.1.1.1.1.6.7.6.2. Beta haemolysin	20
1.3.1.1.1.1.6.7.6.3. Enterohaemolysin	20
1.3.1.1.1.1.6.7.6.4. Cytolysin A	21
1.3.1.1.1.1.6.7.7. Colicins	21
1.3.1.1.1.1.6.7.8. Siderophores	22
1.3.1.1.1.1.6.8. Clinical infections of <i>E. coli</i>	22
1.3.1.1.1.1.6.8.1 Colibacillosis	22
1.3.1.1.1.1.6.8.1.1. Septicaemic colibacillosis	22
1.3.1.1.1.1.6.8.1.2 Enteric colibacillosis	23
1.3.1.1.1.1.6.8.1.2.1.1. Enterotoxigenic <i>E. coli</i> (ETEC)	24
1.3.1.1.1.1.6.8.1.2.1.2. Enteropathogenic <i>E. coli</i> (EPEC)	26
1.3.1.1.1.1.6.8.1.2.1.3. Enterohaemorrhagic <i>E. coli</i> (EHEC)	28
1.3.1.1.1.1.6.8.1.2.1.4. Necrotoxicogenic <i>E. coli</i> (NTEC)	29
1.3.1.1.1.1.6.8.1.2.1.5 Enteroinvasive <i>E. coli</i> (EIEC)	30
1.3.1.1.1.1.6.8.1.2.1.6. Enteroaggregative <i>E. coli</i> (EAggEC)	31
1.3.1.1.1.1.6.8.1.2.1.7. Diffusely adherent <i>E. coli</i> (DAEC)	32
1.3.1.1.1.1.6.8.1.2.1.8. Cell-detaching <i>E. coli</i> (CDEC)	32
1.3.1.1.1.1.6.8.1.2.1.9. Cytolethal distending toxin-producing <i>E. coli</i> (CLDTPEC)	32

1.3.1.1.1.6.8.1.2.2. Diagnostic procedures of Colibacillosis	33
1.3.1.1.1.6.8.1.2.3. Differential diagnosis of Colibacillosis	34
1.3.1.1.1.6.8.1.2.4. Treatment of Colibacillosis	35
1.3.1.1.1.6.8.1.2.4.1. Antimicrobial therapy	35
1.3.1.1.1.6.8.1.2.4.2. Immunoglobulin therapy	38
1.3.1.1.1.6.8.1.2.4.3. Alteration of the diet	38
1.3.1.1.1.6.8.2.5. Control of Colibacillosis	39
<b>Chapter (2) Materials and methods</b>	
2.2. Samples	40
2.2.1. Source of samples	40
2.2.2. Sampling procedure	40
2.2.2.1. Questionnaire	40
2.2.2.2. Faecal sample	40
2.2.3. Preparation of culture	41
2.2.3.1. Collection of blood	41
2.1. Materials	41
2.1.1. Media	41
2.1.1.1. Solid media	41
2.1.1.1.1. Blood agar	41
2.1.1.1.2. Blood agar slopes	42
2.1.1.1.3. Eosin Methylene Blue agar	42
2.1.1.1.4. MacConkey's agar	42
2.1.1.1.5. Nutrient agar	43
2.1.1.1.3. Triple Sugar Iron agar (TSI)	43
2.1.1.2. Semi-solid media	44
2.1.2.1. Gelatin	44
2.1.1.2.2. Hugh and Leifson's O.F medium	44
2.1.1.2.3. Simmons' citrate medium	44
2.1.1.2.4. Urea agar base	45
2.1.1.3. Liquid media	45
2.1.1.3.1. Brain Heart Infusion broth	45
2.1.1.3.2. MacConkey's broth	46
2.1.1.3.3. Moeller arginine decarboxylase broth base	46
2.1.1.3.4. Moeller KCN broth base	47
2.1.1.3.5. MRVP medium	47
2.1.1.3.6. Nitrate broth	47
2.1.1.3.7. Nutrient broth	48
2.1.1.3.8. Peptone water	48
2.1.1.3.9. Tryptophan broth	49
2.1.1.3.8. Api 20E suspension medium	49

2.1.1.4. Solutions and reagents	49
2.1.1.4.1. Oxidase test reagent	49
2.1.1.4.2. Hydrogen peroxide solution	49
2.1.1.4.3. Reagents used in Voges and Proskauer test	49
2.1.1.4.3.1. $\alpha$ -naphthol, 5%	49
2.1.1.4.3.2. Potassium hydroxide, 40%	49
2.1.1.4.4. Kovac reagent	50
2.1.1.4.5. Methyl red pH indicator	50
2.1.1.4.6. Reagent used in Nitrate reduction test	50
2.1.1.4.6.1. Reagent A	50
2.1.1.4.6.2. Reagent B	50
2.1.1.4.7. Reagents used in Api 20E	50
2.1.1.4.7.1. TDA	51
2.1.1.4.7.2. James	51
2.1.1.4.7.3. IND	51
2.1.1.4.7.4. NIT1	51
2.1.1.4.7.5. NIT2	51
2.1.1.4.7.6. VP1	51
2.1.1.4.7.7. VP2	52
2.1.1.4.8. Reagents used in Fimbrex kits	52
2.1.1.4.9. Reagents used in <i>Vibrio cholera</i> and <i>E. coli</i> Heat-labile enterotoxin test kit	52
2.2.4. Sterilization	52
2.2.4.1 Hot air oven	52
2.2.4.2. Autoclaving	52
2.2.4.3. Disinfection of bench	53
2.2.5. Cultural methods	53
2.2.5.1. Primary isolation	53
2.2.5.2. Incubation of culture	53
2.2.5.3. Examination of cultures	53
2.2.5.4. Gram's stain	53
2.2.5.5. Subculturing and purification	54
2.2.6. Biological and biochemical identification	54
2.2.6.1. Staining of smear	54
2.2.6.2. Catalase test	54
2.2.6.3. Oxidase test	54
2.2.6.4. Oxidation Fermentation test (O.F)	55
2.2.6.5. Motility test	55
2.2.6.6. Glucose breakdown	55
2.2.6.7. Fermentation of carbohydrates	55

2.2.6.8. Methyl red reduction (MR)	55
2.2.6.9. Voges-Proskauer reaction	56
2.2.6.10. Indole production	56
2.2.6.11. Urease activity	56
2.2.6.12. Citrate utilization	56
2.2.6.13. Gelatin hydrolysis	56
2.2.6.14. Eijkman's test	56
2.2.6.15. Arginine decarboxylase test	57
2.2.6.16. Potassium cyanide test	57
2.2.6.17. Nitrate reduction test	57
2.2.7. Confirmation of <i>E. coli</i> identification	57
2.2.7.1. API 20E	57
2.2.7.2. VITEK 2 identification system	61
2.2.8. Detection of pathogenic mechanisms of <i>E. coli</i>	67
2.2.8.1. FIMBEX kits	67
2.2.8.3. Detection of heat-labile (LT) enterotoxin	71
2.2.8.3.1. Principle of assay	72
2.2.8.2. Detection of STa enterotoxin	75
2.2.8.2.1. Suckling mouse test	75
2.2.8.2.1.1. Toxin preparation	75
2.2.8.2.1.2. Sterility of toxin	76
2.2.8.2.1.3. Mice inoculation	76
2.2.8.6 Antibiotic sensitivity test	76
<b>Chapter (3) Results</b>	
3.1. Questionnaire survey of dairy farms in Khartoum State	78
3.2. Epidemiology of calf diarrhoea in Khartoum State	81
3.2.1. Prevalence of calf diarrhoea in Khartoum State	81
3.2.2. Seasonality of calf diarrhoea	81
3.2.3. Mortality rates among diarrhoeic calves	81
3.3. Characteristics of samples collected	86
3.4. Microorganisms isolated from diarrhoeic samples	86
3.4.1. Identification of <i>E. coli</i>	92
3.4.1.1. Cultural characteristics	92
3.4.1.1.1. Blood agar	92
3.4.1.1.2. MacConkey agar	92
3.4.1.1.3. Nutrient agar	92
3.4.1.1.4. Eosin Methylene Blue agar	92
3.4.1.2. API 20E strips	95
3.4.1.3. VITEK 2 identification system	95
<b>3.4.1..</b> 3.4.1.3. Fimbrial antigens	100

3.4.1.4. Detection of STa enterotoxins	103
3.4.1.4.1. Suckling mouse test (SMT)	103
3.4.1.5. Detection of heat-labile (LT) enterotoxin	103
3.4.1.5.1. Reversed passive latex agglutination test (RPLA)	103
3.4.1.6. Sensitivity of <i>E. coli</i> to 10 antibiotics	107
<b>Chapter (4) Discussion</b>	
Discussion	112
Conclusion	124
Recommendations	126
<b>Chapter (5) References</b>	
References	127
<b>Appendixes</b>	
Appendixes	145

## List Of Tables

<b>Table No.</b>	<b>Title</b>	<b>Page No.</b>
<b>Table (1):</b>	API 20E Reading table	61
<b>Table (2):</b>	Test substrates on GN card	66
<b>Table (3):</b>	Zone size interpretation chart	77
<b>Table (4):</b>	Summary of the questionnaire survey responses by Five hundred owners of dairy farms in different localities of Khartoum state:	79
<b>Table (5):</b>	The incidence of calf diarrhoea among different localities of Khartoum State	82
<b>Table (6):</b>	Sex of diarrhoeic calves (the source of samples)	83
<b>Table (7):</b>	Order of diarrhoeic calves (the source of samples)	83
<b>Table (8):</b>	Seasonality of calf diarrhoea in Khartoum State	84
<b>Table (9):</b>	Mortality rates among 300 diarrhoeic calves with different age categories	85
<b>Table (10):</b>	Areas of samples collection in Khartoum State	87
<b>Table (11):</b>	Characteristics of the faecal samples collected from diarrhoeic calves	88
<b>Table (12):</b>	Microorganisms isolated from diarrhoeic samples	88

<b>Table (13):</b>	Cultural characteristics, bacterial morphology and biochemical tests of the isolated bacteria	90
<b>Table (14):</b>	API 20E identification system results of the isolated <i>Escherichia spp.</i>	96
<b>Table (15):</b>	VITEK2 identification system results	98
<b>Table (16):</b>	Detection of fimbrial antigens possessed by <i>E. coli</i> isolates	100
<b>Table (17):</b>	Detection of STa enterotoxin produced by <i>E. coli</i> isolates using suckling mouse test (SMT)	104
<b>Table (18):</b>	Detection of STa and LT enterotoxins elicited by <i>E. coli</i> isolates using CMT and RPLA in different localities of Khartoum State	105
<b>Table (19):</b>	Sensitivity of different <i>E. coli</i> isolates to 10 antimicrobials	108

### List of Figures

	<b>Object</b>	<b>Page No.</b>
<b>Figure (1)</b>	Vitek2 Compact autoanalyzer machine	65
<b>Figure (2)</b>	A sample of GN cards used for Vitek 2 autoanalyzer machines	65
<b>Figure (3)</b>	Fimbrex K99 test kit	70
<b>Figure (4)</b>	Fimbrex 987p test kit	70
<b>Figure (5)</b>	Fimbrex K88 test kit	71
<b>Figure (6)</b>	VET RPLA toxin detection kit for detection of heat-labile enterotoxin	75
<b>Figure (7)</b>	Treatment of calf diarrhoea in Khartoum State according to investigation forms	80
<b>Figure (8)</b>	The incidence of calf diarrhoea among different localities of Khartoum State	82
<b>Figure (9)</b>	Seasonality of calf diarrhoea in Khartoum State	83
<b>Figure (10)</b>	Mortality rates among 300 diarrhoeic calves with different age categories	85
<b>Figure (11)</b>	Microorganisms isolated from diarrhoeic samples	89
<b>Figure (12)</b>	Colonies of <i>E. coli</i> on blood agar	93
<b>Figure (13)</b>	Colonies of <i>E. coli</i> on MacConkey's agar	93
<b>Figure (14)</b>	Colonies of <i>E. coli</i> on Nutrient agar	94
<b>Figure (15)</b>	Colonies of <i>E. coli</i> on Eosin Methylene Blue agar	94
<b>Figure (16)</b>	Results of inoculation of Api 20E test strip with <i>E. coli</i>	95

<b>Figure (17)</b>	Two <i>E. coli</i> isolates (No. 31 and 33 up) showed positive results to F5	101
<b>Figure (18)</b>	<i>E. coli</i> isolate (No. 7 up) showed positive result to F6	101
<b>Figure (19)</b>	Two <i>E. coli</i> isolates (No. 02 and 03 up) showed positive results to F4	102
<b>Figure (20)</b>	Detection of LT enterotoxin elicited by <i>E. coli</i> isolates using RPLA in different Localities of Khartoum State	105
<b>Figure (21)</b>	Positively detected <i>E. coli</i> LT enterotoxins by using RPLA	106
<b>Figure (22)</b>	Sensitivity of different <i>E. coli</i> isolates to 10 antimicrobials	110
<b>Figure (23)</b>	<i>E. coli</i> on Nutrient agar tested using Sulphamethoxazole- trimethoprim	111

# Dedication

My father

My mother



My wife

My son

My sister

And my brothers

Dr. Mustafa El Madeih

## Acknowledgment

First and foremost my thanks to Almighty Allah, the Beneficent and the Merciful, which is impossible to capture in words for every thing that I know and which that he almighty know.

I wish to express my deep gratitude to my supervisor Professor Galal Eldin Elazhari Mohamed and co-supervisor Dr. Ali Abdel Ghani Elgaddal for their keen advice and critical supervision.

I am very indebted to DAAD organization in funding my research conduction. Also I am indebted to Dr. yahia the head of bacteriology

department at Suba Veterinary Research institute for his continuous guidance and invaluable advice.

I wish to thank the staff of the department of bacteriology at Suba Veterinary Research institute for their technical help.

It is pleasure to thank Dr. Mustafa El Madeih for his efforts in importing different kids and materials from outside Sudan.

No words can express my thanks to my family for everything.

Faithfully my thanks are due again and forever to Almighty Allah.