

**CLINICO - DIAGNOSTIC AND THERAPEUTIC STUDIES
ON LOWER URINARY TRACT INFECTIONS
IN DOGS**

By

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I.D.No: GVM/2018-027

THESIS SUBMITTED TO THE
SRI VENKATESWARA VETERINARY UNIVERSITY
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE AWARD OF THE DEGREE OF

MASTER OF VETERINARY SCIENCE
(VETERINARY MEDICINE)
IN THE FACULTY OF VETERINARY SCIENCE



**DEPARTMENT OF VETERINARY MEDICINE
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APRIL 2021

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No part of the thesis has been submitted by the student for any other degree or diploma. The published part has been fully acknowledged. All the assistance and help received during the course of investigations have been duly acknowledged by the author of the thesis.

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TABLE OF CONTENTS

CHAPTER	TITLE	Page No.
I	INTRODUCTION	1-4
II	REVIEW OF LITERATURE	5-36
2.1	OCCURRENCE	5-6
2.1.1	Occurrence in relation to age	6-7
2.1.2	Occurrence in relation to breed	8-10
2.1.3	Occurrence in relation to gender	10-11
2.2	CLINICAL SIGNS	11-12
2.3	DIAGNOSIS	13
2.3.1	Urinalysis	13-14
2.3.2	Urine sediment examination	15-16
2.3.3	Radiography	16-17
2.3.4	Ultrasonography	17-19
2.3.5	Isolation and identification of bacteria	19-22
2.4	ANTIBIOTIC SUSCEPTIBILITY TESTING	22-24
2.5	UNDERLYING OR ASSOCIATED FACTORS	24-26
2.6	HAEMATOLOGY	26
2.6.1	Haemoglobin	26-27
2.6.2	Packed cell volume	27
2.6.3	Total leucocyte count	28
2.6.4	Differential leucocyte count	28-29
2.7	SERUM BIOCHEMISTRY	29
2.7.1	Total protein	29-30
2.7.2	Albumin	30
2.7.3	Blood urea nitrogen	31-32
2.7.4	Creatinine	32-33
2.8	THERAPY	33-36
III	MATERIALS AND METHODS	37-55
3.1	MATERIALS	37
3.1.1	Animals	37
3.1.1.1	Control animals	37

3.1.1.2	Clinical cases	37
3.1.2	Clinical materials	37-38
3.1.3	Laboratory material	38
3.1.3.1	Urinary dipsticks	38
3.1.3.2	Chemicals	38
3.1.3.3	Culture media	38
3.1.3.4	Reagents	38-39
3.1.3.5	Antibiotic discs	39
3.1.3.6	Equipment	39
3.1.3.7	Biochemical kits	39-40
3.1.3.8	Glass ware	40
3.1.4	Therapeutic agents	40
3.2	METHODS	40
3.2.1	Occurrence of lower urinary tract infections in dogs	40
3.2.2	Clinical examination	40
3.2.3	Diagnosis	41
3.2.3.1	Urinalysis	41
3.2.3.2	Urine sediment examination	41
3.2.3.3	Cultural examination of bacteria	43
3.2.3.3.1	Preparation of glass ware	43
3.2.3.3.2	Preparation of culture media	43
3.2.3.3.3	Collection of material	43
3.2.3.3.4	Isolation and identification of bacteria	43-44
3.2.3.3.4.1	Haemolysis pattern on blood agar	44
3.2.3.3.4.2	Gram's staining	44
3.2.3.3.4.3	Catalase test	45
3.2.3.3.4.4	Oxidase test	45
3.2.3.3.4.5	Growth on Mannitol salt agar	45
3.2.3.3.4.6	Growth on Edwards agar	45
3.2.3.3.4.7	Growth on EMB agar	48
3.2.3.3.4.8	Growth on MacConkey agar	48
3.2.3.3.4.9	Brain heart infusion agar	48

3.2.3.3.4.10	Indole test	48
3.2.3.3.4.11	Methyl red test	49
3.2.3.3.4.12	Voges proskauer test	49
3.2.3.3.4.13	Citrate utilization test	49
3.2.4	Antibiotic susceptibility testing	49-50
3.2.5	Haematology	50
3.2.6	Serum biochemistry	50-51
3.2.6.1	Total protein	51
3.2.6.2	Albumin	51
3.2.6.3	Blood urea nitrogen	51
3.2.6.4	Creatinine	51
3.2.7	Abdominal radiography	51
3.2.8	Abdominal ultrasonography	52
3.2.8.1	Instrumentation	52
3.2.8.2	Preparation and restraint of the patient	52
3.2.8.3	Abdominal ultrasonographic technique	52
3.2.9	Therapeutic trial	54
3.2.9.1	Group I	54
3.2.9.2	Group II	54
3.2.9.3	Group III	54
3.2.9.4	Evaluation of therapeutic regimen	54
3.2.10	Treatment of associated/underlying factors	55
3.2.11	Statistical analysis	55
IV	RESULTS	56-110
4.1	OCCURRENCE	56
4.1.1	Occurrence of lower urinary tract infections	56
4.1.1.1	Occurrence in relation to age	57
4.1.1.2	Occurrence in relation to breed	57
4.1.1.3	Occurrence in relation to gender	57
4.2	CLINICAL SIGNS	57 & 63
4.3	DIAGNOSIS	63
4.3.1	Urinalysis	63 & 65

4.3.2	Urine sediment examination	65
4.3.3	Abdominal radiography	65
4.3.4	Abdominal ultrasonography	65
4.3.5	Isolation and identification of bacteria	75
4.3.5.1	Haemolysis pattern on blood agar	75
4.3.5.2	Gram's staining	77
4.3.5.3	Catalase test	77
4.3.5.4	Oxidase test	77
4.3.5.5	Growth on Mannitol salt agar	79
4.3.5.6	Growth on Edwards agar	79
4.3.5.7	Growth on EMB agar	79
4.3.5.8	Growth on MacConkey agar	79
4.3.5.9	Brain heart infusion agar	79
4.3.5.10	Indole test	81
4.3.5.11	Methyl red test	81
4.3.5.12	Voges proskauer test	81
4.3.5.13	Citrate utilization test	81
4.4	ANTIBIOTIC SUSCEPTIBILITY TESTING	84
4.5	UNDERLYING OR ASSOCIATED FACTORS IN LOWER URINARY TRACT INFECTIONS	84
4.6	HAEMATOLOGY	84
4.6.1	Haemoglobin	84
4.6.2	Packed cell volume	91
4.6.3	Total leucocyte count	91
4.6.4	Differential leucocyte count	91
4.7	SERUM BIOCHEMISTRY	91
4.7.1	Total protein	91
4.7.2	Albumin	92
4.7.3	Blood urea nitrogen	92
4.7.4	Creatinine	92
4.8	THERAPEUTIC TRIAL	92-93
4.8.1	Group I	93-94
4.8.1.1	Haematology	94 & 97

4.8.1.2	Serum biochemistry	97-98
4.8.2	Group II	98-99
4.8.2.1	Haematology	100 & 103
4.8.2.2	Serum biochemistry	103
4.8.3	Group III	104-105
4.8.3.1	Haematology	105-106
4.8.3.2	Serum biochemistry	106 & 109
4.9	RECOVERY PATHWAY	109
V	DISCUSSION	111-134
5.1	OCCURRENCE OF LOWER URINARY TRACT INFECTIONS	112-113
5.1.1	Occurrence in relation to age	113
5.1.2	Occurrence in relation to breed	114
5.1.3	Occurrence in relation to gender	114-115
5.2	CLINICAL SIGNS	115-118
5.3	DIAGNOSIS	119
5.3.1	Urinalysis	119
5.3.2	Urine sediment examination	120
5.3.3	Abdominal radiography	120-121
5.3.4	Abdominal ultrasonography	121-122
5.3.5	Isolation and identification of bacteria	122-123
5.4	ANTIBIOTIC SUSCEPTIBILITY TESTING	123-124
5.5	UNDERLYING OR ASSOCIATED FACTORS	124-125
5.6	HAEMATOLOGY	125-126
5.7	SERUM BIOCHEMISTRY	126-127
5.8	THERAPEUTIC TRIAL	127-128
5.8.1	Group I	128-130
5.8.2	Group II	130-132
5.8.3	Group III	132-134
VI	SUMMARY	135-140
	LITERATURE CITED	141-149
	APPENDIX	150-158

LIST OF TABLES

Table No.	Particulars	Page No.
1	Identification of bacteria by morphology of colonies on specific agar medium in dogs affected with lower urinary tract infections	47
2	Age wise occurrence of lower urinary tract infections in dogs	58
3	Breed wise occurrence of lower urinary tract infections in dogs	59
4	Gender wise occurrence of lower urinary tract infections in dogs	60
5	Clinical signs in dogs affected with lower urinary tract infections	61
6 -8	Urinalysis findings in dogs affected with Lower UTI	66-68
9	Bacteria isolated from dogs affected with lower urinary tract infections	83
10	Antimicrobial susceptibility testing in dogs affected with bacterial LUTI	87
11	Underlying or associated factors in dogs affected with lower urinary tract infections	88
12	Haemato biochemical findings in dogs affected with lower urinary tract infections	89
13	Haemato biochemical findings in Group I dogs before and after therapy	95
14	Haemato biochemical findings in Group II dogs before and after therapy	101
15	Haemato biochemical findings in Group III dogs before and after therapy	107
16	Recovery pathway in dogs affected with bacterial lower urinary tract infections	110

LIST OF FIGURES

Figure No.	Particulars	Page No.
1	Isolation and identification of bacteria in dogs affected with lower urinary tract infections	46
2	Age wise occurrence of lower urinary tract infections in dogs	58
3	Breed wise occurrence of lower urinary tract infections in dogs	59
4	Gender wise occurrence of lower urinary tract infections in dogs	60
5	Clinical signs in dogs affected with lower urinary tract infections	61
6	Bacteria isolated from dogs affected with lower urinary tract infections	83
7	Antimicrobial susceptibility testing in dogs affected with bacterial LUTI	87
8	Underlying or associated factors in dogs affected with lower urinary tract infections	88
9	Haemato biochemical findings in dogs affected with lower urinary tract infections	90
10	Haemato biochemical findings in Group I dogs before and after therapy	96
11	Haemato biochemical findings in Group II dogs before and after therapy	102
12	Haemato biochemical findings in Group III dogs before and after therapy	108
13	Recovery pathway in dogs affected with bacterial lower urinary tract infections	110

LIST OF PLATES

Plate No.	Particulars	Page No.
1	Diagnostic techniques	42
2	Diagnostic imaging techniques	53
3	Clinical signs observed in dogs affected with lower urinary tract infections	62
4	Variations in urine colour and transparency in dogs affected with lower urinary tract infections	64
5,6 &7	Microscopic examination of urine sediment in dogs affected with lower urinary tract infections (40X)	69-71
8	Radiographic findings on lateral view of abdomen in dogs affected with lower urinary tract infections	72
9&10	Trans abdominal B mode ultrasonographic findings in dogs affected with lower urinary tract infections	73-74
11	Haemolysis pattern of bacterial isolates on blood agar in dogs affected with lower urinary tract infections	76
12	Microscopic examination of morphology of bacterial uropathogens on grams staining (100X)	78
13	Growth of isolated bacterial uropathogens on specific agar medium	80
14	Biochemical tests of bacterial uropathogens	82
15&16	Antimicrobial susceptibility testing	85-86

Acknowledgement

All praises are the Almighty who bestowed on me the courage, patience and mental peace so that this piece of work could come to completion.

*I take this privilege to express my deepest sense of gratitude originating from the core of my heart to my honorable and enthusiastic guide, Chairman of Advisory Committee, **Dr. K. Suresh**, Professor and Head, Department of Veterinary Clinical Complex, NTR College of Veterinary Science, Gannavaram, the best advisor and teacher I could have ever wished for, to work under his noble guidance for his unstinted support, worthy counsel, indefatigable guidance, prudent planning, innovative ideas, parental encouragement, whenever needed and last but not the least for being the first choice of clarifying my doubts have made his an unforgettable personality from whom I have and shall continue to imbibe essential qualities. His trust and scientific excitement inspired me in the most important moments of making right decisions. I feel extremely honored for the opportunity bestowed upon me to work under his meticulous guidance.*

*I owe my special thanks to **Dr. N. Lakshmi Rani**, Professor and Head, Department of Veterinary Medicine NTR College of Veterinary Science, Gannavaram and member of my advisory committee for her motivation, timely cooperation, attention to minute details, involvement, affectionate encouragement, precious suggestions, guidance, persistent encouragement, positive attitude and never ending moral support under all the circumstances during my course work. I consider myself very fortunate to have worked under such a caring, focused and wholesome member of Advisory Committee for her valuable advice and generosity. Without your endless encouragement and untiring interest this work would not have materialized.*

*I wish to record my sincere thanks to **Dr. Makkena Sreenu**, Professor and Head, Department of Veterinary Surgery and Radiology, NTR College of Veterinary Science, Gannavaram and member of my advisory committee for his wholehearted support, sustained encouragement, care, unceasing help, valuable suggestions and timely cooperation constructive guidance, providing facilities and valuable help during the tenure of this study.*

*I owe special gratitude to **Dr. T. Srinivas Rao**, Professor and Head, Department of Veterinary public health and Epidemiology, **Dr. Aswini Kumar**, Professor and Head, Department of Veterinary Biochemistry, NTR College of Veterinary Science, Gannavaram, for providing facilities in their respective departments and suggestions during my research work,*

It is my pleasure and privilege to express my sincere thanks and gratitude to **Dr. K. Rajesh, Dr. Y. Chaitanya, Dr. K. Sai Krishna**, Assistant professors, Department of Veterinary medicine, **Dr. V. Devi Prasad**, Professor, Department of Veterinary surgery and Radiology *NTR College of Veterinary Science, Gannavaram* for their valuable suggestions, affectionate encouragement and moral support during the course of my study.

I also express my sincere gratitude to **Dr. Laimi Elizabeth Shaju**, Assistant Professor Contractual, Department of Veterinary Medicine and **Dr. L. Narayana Rao** Assistant Professor Contractual, Department of Veterinary Clinical Complex, *NTR College of Veterinary Science, Gannavaram* for extending their constant encouragement, suggestions and support during the prosecution of course and research work.

I owe my special thanks to **Dr. Srihari**, Assistant Professor Contractual, Department of Veterinary Biochemistry for his help and support in successful completion of my research work.

I am very much thankful to **Dr. Vittal**, Associate Librarian, *NTR College of Veterinary Science, Gannavaram* for library facility while collecting the articles as part of my research work.

My dear colleagues **Dr. K. Sandeep Raju, Dr. K.F.S Sreekanth and Dr. P. Radhika** deserve special thanks and applauses for always being there creating a healthy environment and to initiate scientific discussions. I would really miss colleagues like them when it comes to research. The mutual understanding and passion for research which we shared is really inexpressible.

I am very much thankful to my juniors **Dr. Akhila, Dr. Likhitha sri, Dr. Nagaveni, Dr. Lakshmi Prasanna, Dr. Surendra, Dr. Vishnu Vardhan and Dr. Tharangini** for their immense help, cooperation during my research work.

My heartily thanks to my friends **Dr. Bhavana, Dr. Lavanya, Dr. Prathyusha, Dr. Keerthana, Dr. Charitha, Dr. Anjali, Dr. Soujanya, Dr. Aafreen, Dr. Chaithanya, Dr. Sri ram murthy, Dr. Hussaine, Dr. Bhargav** for unceasing encouragement, help, perpetual support and sharing happiest moments of joy throughout my research work as well as my stay at *Gannavaram*.

I express my sincere gratitude to my seniors **Dr. B. Niveditha, Dr. Sri Sandhya, Dr. Sreelakshmi, Dr. Bhargav kumar, Dr. Ravi subhramanyam, Dr. Rajesh, Dr. Siva Rama krishna, Dr. Krishna Chaithanya** for their constant encouragement.

*Instant availability without continuous presence is probably the best role a parent can play. Behind my success, there is my family. It is my immense pleasure to put on record my deep sense of gratitude towards my parents **Kurli Narsi Reddy, Kurli Usha Rani and Bhaskar Reddy, Sreedevi** for their love, affection, blessings, goodwill, encouragement, inspiration and sacrifice. I cannot weigh my feeling with my words and something that is inexpressible in my heart for my sisters **Lavanya, Nithya Sree, Lekha Sree**, my brother in law **Akshay Kumar Reddy** and my brother **Dinesh Kumar Reddy** for their support in each and every step of my success, constant love and affection.*

*I want to remember my friends who are like family and gave constant support and suggestions throughout, caring, love & affection towards me **Dr. Vishnu Vardhan Chowdary, Dr. Saisree, Dr. Kiranmay, Dr. Lavanya and Dr. Bindhu.***

*The help rendered by Veterinary Assistant **Mr. Aaditya** and Non-teaching staff **Mr. Yeshaaya, Mr. Giri, Mr. Satish, Mr. Anil and Mrs. Radha rani** Department of Veterinary medicine and Department of Veterinary Clinical complex, N.T.R.C.V.Sc, Gannavaram is gratefully acknowledged.*

I would like to thank The Vice Chancellor and Dean SVVU and the Associate Dean, NTR College of Veterinary Science for giving me this opportunity to spend two fruitful years in this prestigious institution with amiable environment.

I would like to acknowledge all the teachers I learnt from since my childhood, I would not have been here without their guidance, blessing and support.

Many people have directly contributed their energies and ideas, to all I am especially grateful. I apologize to all the people I have missed. I am indebted always to those people who do their best to improve on my best.

*Place:-**Gannavaram***

Date:-

*(**Dr. Kurli Madhavi**)*

CONTENTS

Chapter No.	Title	Page No.
I	INTRODUCTION	1-4
II	REVIEW OF LITERATURE	5-36
III	MATERIALS AND METHODS	37-55
IV	RESULTS	56-110
V	DISCUSSION	111-134
VI	SUMMARY	135-140
	LITERATURE CITED	141-149

DECLARATION

I, **KURLI MADHAVI**, I.D.No: **GVM/2018-027** hereby declare that the thesis entitled “**CLINICO - DIAGNOSTIC AND THERAPEUTIC STUDIES ON LOWER URINARY TRACT INFECTIONS IN DOGS**” submitted to **SRI VENKATESWARA VETERINARY UNIVERSITY** for the Degree of **MASTER OF VETERINARY SCIENCE** is a result of original research work done by me. It is further declared that the thesis or any part thereof has not been published earlier in any manner.

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Degree to which it is Submitted : **Master of Veterinary Science**

Faculty : **Faculty of Veterinary Science**

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University : **Sri Venkateswara Veterinary University, Tirupati.**

Year : **2021**

ABSTRACT

The present study was undertaken to record the occurrence of lower urinary tract infections and also to study the clinical symptomatology, haemato-biochemical findings, antibiotic susceptibility pattern as well as to assess the therapeutic regimen in dogs affected with lower urinary tract infections. The occurrence of urinary tract infections among 2930 cases presented to the small animal medical ward recorded was 8.39 (246) per cent of which lower urinary tract infections constituted 33.33 (82/246) per cent of the total urinary tract infection cases. Thirty five dogs affected with lower urinary tract infections were diagnosed preliminarily by clinical signs and urinalysis which were subjected to cultural examination in which 33 (94.28%) dogs were positive for bacterial isolates and in 2 (5.71%) dogs no bacterial growth was observed upon culture. Occurrence of lower urinary tract infections in the present study was recorded in 35 dogs while therapeutic trial was undertaken in only 24 dogs affected with bacterial LUTI. The occurrence of lower urinary tract infections was highest in the age group of zero to four years, Labrador Retriever breed and Spayed female dogs in the present study. The clinical signs observed in dogs affected with lower urinary tract infections were fever, haematuria, inappetence/anorexia, pollakiuria, oliguria, urinary incontinence, stranguria, vomiting, pyuria, polyuria, anuria, polydipsia and dribbling of urine. Diagnosis of lower urinary tract infections was done based on the clinical signs, urinalysis findings, microscopic

examination of urine sediment, isolation and identification of bacteria and by advanced diagnostic imaging modalities i.e., radiography and ultrasonography. Identification of different bacterial isolates was done by randomly picking the colonies from blood agar which were subjected for gram's staining, catalase test, oxidase test, biochemical test and confirmation was done based on morphological appearance of colonies on specific agar medium i.e., Mannitol salt agar, Eosin methylene blue agar, Edwards agar, MacConkey agar and Brain heart infusion agar. Out of 33 samples *Escherichia coli* was identified as a major uropathogen (33.33%) followed by *Staphylococcus* spp (21.21%), *Streptococcus* spp (18.18%), *Pseudomonas* spp (12.12%), mixed infection of *E.coli* and *Staphylococcus* spp (9.10%) and least identified was *Klebsiella* spp (6.06%). Antibiotic susceptibility test revealed highest susceptibility to amikacin (100%) followed by gentamicin (93.94%), ceftriaxone + tazobactam (90.91%), amoxicillin sodium + sulbactam and nitrofurantoin (81.82% each), trimethoprim-sulphamethoxazole (78.79%) and least to ciprofloxacin (75.76%). Underlying factors noticed in dogs affected with lower urinary tract infections were prostate enlargement (8.57%), cystoliths and long term use of glucocorticoids (5.71%) each and urethroliths (2.86%). Haemato biochemical findings in dogs affected with LUTI revealed significant decrease in mean values of Hb, PCV, lymphocytes, total protein and albumin while the mean values of TLC, neutrophils, blood urea nitrogen and creatinine were increased significantly. Therapeutic trial was conducted in three groups of dogs with eight dogs in each group using three different antibiotics viz., amoxicillin sodium + sulbactam, ceftriaxone + tazobactam and amikacin. All the three antibiotics were found to be effective in the treatment of lower urinary tract infections based on resolution of clinical signs, urinalysis findings, negative bacteriological culture of urine after therapy and improvement in the haemato-biochemical parameters.

LIST OF SYMBOLS AND ABBREVIATIONS

%	-	percent or percentage
/	-	per
@	-	at the rate of
<	-	less than
>	-	greater than
±	-	plus or minus
≤	-	less than or equal to
≥	-	greater than or equal to
µg	-	microgram
µl	-	micro liter
ABST	-	Antibiotic sensitivity testing
AMR	-	Antimicrobial resistance
A: G	-	Albumin to globulin
b. wt	-	body weight
BHI	-	Brain heart infusion
BID	-	Twice in a day
BUN	-	Blood urea nitrogen
CFU	-	Colony forming unit
CLSI	-	Clinical and Laboratory Standards Institute
DLC	-	Differential leukocyte count
EDTA	-	Ethylene Diamine Tetra acetic acid
EMB	-	Eosin methylene blue

<i>et al.</i>	-	and others
Fig.	-	Figure
g	-	gram
g/dl	-	grams per deciliter
g/L	-	grams per liter
GSD	-	German Shepherd
Hb	-	Haemoglobin
hpf	-	high power field
hrs	-	Hours
I/M	-	Intramuscular
I/V	-	Intravenous
i.e.,	-	that is
Inj.	-	injection
IU	-	International units
lb	-	Pound
Ltd.	-	Limited
LUTI	-	Lower urinary tract infection
M/S	-	Messers
MAC	-	MacConkey Agar
MDR	-	Multidrug- resistance
mg	-	Milligram
mg/dl	-	Milligram per deciliter
mg/kg	-	Milligram per kilogram
mg/ml	-	milligram per milliliter

MHA	-	Muller Hinton Agar
MHz	-	Megahertz
min.	-	Minutes
ml	-	milliliter
mm	-	millimeter
mm ³	-	Cubic millimeter
MS	-	Mass spectrometry
MSA	-	Mannitol salt agar
N	-	Normality
n	-	Number
NAD	-	No abnormality detected
nm	-	Nanometer
No.	-	Number
°	-	Degree
°C	-	Degree centigrade
PBP	-	Penicillin binding protein
PCV	-	Packed cell volume
PO	-	Per os
PU	-	Polyuria
PD	-	Polydipsia
Pvt.	-	Private
RBC	-	Red blood cell
rpm	-	revolutions per minute
rRNA	-	Ribosomal RNA.

S/C	-	Subcutaneous
SE	-	Standard Error
sec	-	Seconds
SID	-	Once in a day
spp.	-	Species
SPSS	-	Statistical Package for Social Sciences
TLC	-	Total leukocyte count
TSB	-	Tryptic soy broth
UPC	-	Urine protein to creatinine
UTI	-	Urinary tract infection
Viz.	-	Namely
WBC	-	White blood cell

CHAPTER- I

INTRODUCTION

The urinary system is one of the most important systems of the dog consisting of a pair of kidneys, a pair of ureters, urinary bladder and urethra. The function of the urinary system is to excrete the metabolic waste products from the body, maintain the electrolyte, water, other solute balance and to regulate the acid-base balance of the body. The urinary tract has a great capacity to avoid infection despite its proximity to anus and potential for faecal contamination. Normal host defenses aids in preventing urinary tract infection and these include – normal micturition, normal anatomic structures, mucosal defense barrier, normal microflora, antibacterial properties of urine, surface glycosaminoglycan's, local antibody production and systemic immunocompetence (Somu *et al.*, 2015). Any alterations in these normal host defense mechanisms results in development of UTI in dogs (Smee *et al.*, 2013a).

A urinary tract infection (UTI) is defined as either a temporary or permanent breach in host defense mechanisms that allows microbes to adhere, multiply and persist within the urinary tract. UTI is thought to be most common infectious disease in dogs. Infection of urinary tract may affect more than one organ or may be localized to the upper tract (kidney and adjacent ureter) causing bacterial pyelonephritis, to the lower tract (bladder) causing bacterial cystitis; or adjacent urethra causing bacterial urethrocystitis. Infection of lower urinary tract in intact male dogs are considered to be concomitantly affecting the prostate gland causing bacterial prostatitis. UTI may occur as a primary disease, secondary to other conditions (e.g. metabolic diseases, stress and urinary retention) or iatrogenic (e.g. immunosuppressive drug therapy, transurethral catheterization and nosocomial infection) (Hall *et al.*, 2013).

The urinary tract infections in dogs are classified as subclinical bacteriuria in which bacteria are identified on urine culture with no lower urinary tract signs, sporadic cystitis in which there is inflammation of bladder with exhibition of clinical signs and recurrent UTI in which dog experiences 3 or more episodes within 12 months period. The recurrent UTI is further classified as reinfection when dog experiences UTI by different organism within 6 months of discontinuation of antibiotic therapy and relapse when dog experiences UTI by same organism within 6 months of discontinuation of antibiotic therapy (Weese *et al.*, 2019).

UTIs are caused by wide variety of pathogens which includes bacteria, fungi, mycoplasma, parasites and rarely viruses among which bacterial UTIs are most common. It is estimated that 5-27 per cent of dogs experience UTIs at some point during their life time. Bacterial UTIs results due to accession of bacteria either through haematogenous route which is relatively uncommon or ascending infection from surrounding tissue i.e vulva and prepuce which is quite common for development of infection (Smee *et al.*, 2103a). The presence of highly pathogenic bacteria increases the risk of persistent UTI (Lulich and Osborne, 2004).

Bacterial urinary tract infections are the most common cause of urinary tract diseases in dogs. The most common bacteria isolated from dogs affected with urinary tract infections (UTI) include *Escherichia coli*, *Staphylococcus* spp, *Proteus* spp, *Streptococcus* spp, *Klebsiella* spp and *Pseudomonas* spp (Hariharan *et al.*, 2016). Bacterial urinary tract infections are most common and are responsible for affecting up to 14 per cent of all dogs during their lifetime and infection with a single bacterial species are more prevalent than mixed infections (Ettinger and Feldman, 2010; Westropp *et al.*, 2012).

UTIs have been found in female dogs more often than in males and older animals have been thought by some investigators to be at increased risk of UTI (Weese *et al.*, 2011; Hall *et al.*, 2013 and McMeekin *et al.*, 2017). The predisposing causes such as bladder stones, tumors, metabolic disorders (Diabetes mellitus, Cushing's syndrome) and medications (cortisone-like drugs, anti-cancer drugs) make the dog prone to bacterial UTI. The patient with UTI may show multifarious clinical signs like pollakiuria, dysuria, stranguria, urinary incontinence, haematuria, frequent licking of vulvar or preputial area, dribbling of urine and various systemic signs like fever, anorexia, depression and vomiting.

Diagnosis of bacterial urinary tract infections is based on clinical signs, haemato-biochemical findings, urinalysis (physical, chemical and microscopic examination of urine sediment), microbiological culture, antimicrobial susceptibility, other imaging techniques like radiography and ultrasonography. The microbiological culture associated with antimicrobial susceptibility aids as gold standard tests for treatment of bacterial lower urinary tract infections (Bartges *et al.*, 2004; Ball *et al.*, 2008 and Rampacci *et al.*, 2018).

Urinary tract infection (UTI) is a major reason for antibiotic prescription in small animal practice. A wide range of antimicrobials are in use in treatment of UTIs such as penicillins, cephalosporins, tetracyclines, chloramphenicol, aminoglycosides and fluoroquinolones. Despite UTI being a common condition, management of UTIs in dogs has become more complicated as prevalence of antibiotic resistant strains of various pathogens has increased (Jessen *et al.*, 2015). Antimicrobial resistance (AMR) is currently one of the greatest threats to global health (Wong *et al.*, 2015 and Waller *et al.*, 2018). The choice of antimicrobial agent include different factors like causative

pathogens, patients history, urinary concentration of antimicrobial agent, route of administration and local microbial resistance and susceptibility patterns.

In small animal practice UTIs are among the most common conditions which require diagnostic and therapeutic intervention. It has been estimated that as many as 10 per cent of all canine patients seen by veterinarians for any reason have UTI in addition to the problems for which they are presented (Ling 2001; Cetin *et al.*, 2003 and Somu *et al.*, 2015). Furthermore, increasing emergence of multidrug-resistance (MDR) bacteria causing urinary tract infections (UTI) in dogs and cats is of great veterinary public health concern, which itself reflects the relevance of this study. The present study was designed to study in detail about lower urinary tract infections in dogs with the following objectives.

1. To study the occurrence of lower urinary tract infections in dogs.
2. To record the clinical signs and haemato-biochemical findings in dogs affected with lower urinary tract infections.
3. To isolate and identify bacteria causing lower urinary tract infections.
4. To evaluate the suitable therapeutic regimen in dogs affected with lower urinary tract infections caused by bacteria.

CHAPTER-II

REVIEW OF LITERATURE

2.1 OCCURRENCE

Kivisto *et al.* (1977) conducted a study on 237 dogs over a period of one year and documented with an incidence of 32.8% of dogs affected with bacterial lower urinary tract infections.

Kogika *et al.* (1995) in a study on 51 dogs affected with urinary tract infections reported the occurrence of upper and lower urinary tract infections as 74.5 per cent (38/51) and 25.5 per cent (13/51) respectively.

Forrester *et al.* (1999) reported that out of 42 dogs affected with UTI only 4.76 (2/42) per cent exhibited clinical signs and positive urine cultures (2/42).

Seguin *et al.* (2003) examined 100 dogs over a period of 10 years and recorded occurrence of recurrent or persistent urinary tract infections with anatomical defect as 71 per cent (71/100) and dogs without any predisposing anatomical defect in development of recurrent or persistent urinary tract infections as 29 per cent (29/100).

Torres *et al.* (2005) documented that up to an 18-39 per cent of the dogs receiving long term glucocorticoids were at risk of developing lower urinary tract infections.

Stiffler *et al.* (2006) in their study on 92 dogs with lower urinary tract infections recorded 27 per cent (25/92) were identified to be suffering with urinary tract infections.

Bubenik *et al.* (2007) in their study to evaluate frequency of urinary tract infections in catheterized and non-catheterized dogs documented incidence of infection as 59.8 per cent (147/246) and 40.2 per cent (99/246), respectively.

Olby *et al.* (2010) recorded the occurrence of lower urinary tract infections as 36 per cent (9/25) out of 25 dogs examined with signs of urinary tract infections.

Yehia and Salem (2015) examined 32 dogs and documented the occurrence of upper urinary tract infections and lower urinary tract infections as 25% (8/32) and 75% (24/32), respectively.

Hariharan *et al.* (2016) in a study on 151 dogs with signs suggestive of urinary tract infections observed an incidence of 34.43% (52/151) of dogs affected with bacterial lower urinary tract infections.

Adamama *et al.* (2017) examined 8893 dogs over a period of 3 years and recorded the occurrence of urinary tract infections as 1.82 per cent (162/8893) of which 90.7 per cent (147/162) were affected with lower urinary tract infections.

Liebelt and Pigott (2019) reported that the occurrence of lower and upper urinary tract was 85 per cent and 15 per cent respectively in their study on urinary tract infections in dogs.

2.1.1 Occurrence in relation to age

Corriere *et al.* (1972) described the mechanism of urethrovesical reflux resulting in development of lower urinary tract infections in young age dogs.

Kivisto *et al.* (1977) documented that dogs of 4-10 years age group are more commonly affected with urinary tract infections.

Kogika *et al.* (1995) recorded higher incidence of bacterial urinary tract infections in dogs of 4-8 years of age.

Ling *et al.* (2001) documented highest prevalence of urinary tract infections in dogs of 8-10 years age group (27%) followed by greater than 11 years (26.4%), 0-4 years (23.3%), 5-7 years (21.9%) and least in dogs of unknown age group (1.3%).

Cohn *et al.* (2003) reported that dogs of 7-8 years age group were commonly affected with bacterial urinary tract infections.

Macotpet *et al.* (2009) documented highest occurrence of lower urinary tract infections in dogs of 5-10 years age group (68.21%) followed by above 10 years (18.2%) and least was recorded in 3 months to 3 years (13.6%).

Wong *et al.* (2015) documented highest occurrence of urinary tract infections in dogs of above 7 years (64.3%) age group followed by 1-7 years (30.4%) and least in below 1 year (5.2%).

Kumar and Kamran (2016) documented highest incidence of urinary tract infections in dogs of 5-7 years (63.6%), followed by 8-10 years (27.27%) and least in 10-12 years (9.09%) age group.

Adamama *et al.* (2017) documented highest age group wise incidence of urinary tract infections in dogs of 5-10 years (49.4%) followed by below 5 years (29.6%) and least in above 10 years (21%) of age.

Biasibetti *et al.* (2019) in their study reported that dogs in the age group of 4-11 years old were more commonly affected with urinary tract infections.

Liebelt and Piggot (2019) documented highest occurrence of urinary tract infections in dogs in age group of 7-8 years.

Lippi *et al.* (2019) reported that middle to old age group dogs are more commonly prone for development of urinary tract infections.

2.1.2 Occurrence in relation to breed

Kogika *et al.* (1995) in a study on breed wise occurrence of urinary tract infections documented highest occurrence in Cocker Spaniels (19.6 per cent) followed by German Shepherd (16.68 per cent), Mongrel dogs (9.8 per cent), Dalmatian (8.84 per cent), Great Dane, Pekingese and Beagle (5.88 per cent each), Daschund and Old English Sheepdog (3.92 per cent each) and least in Doberman Pinscher, Boxer, Basset Hound, Fox, Afgan Hound, Fila Brasileiro, Poodle, Teneriff, Schnauzer, Miniature Pinscher (1.96 per cent each).

Norris *et al.* (2000) conducted a study on urinary tract infections in dogs reported highest occurrence in German Shepherd followed by Miniatures, Labrador Retriever, Mixed breed, Dachshund, Doberman Pinscher, Golden Retriever, Dalmatian, Great Dane, Hound, Rottweiler and least in Shetland Sheepdog.

Gaeta *et al.* (2009) in a study on breed wise occurrence of urinary tract infections in dogs reported highest occurrence in Mixed breed (13.6%), followed by Cocker Spaniel (13.6%) and least in Golden Retriever, Bichon Fries (9.1% each).

Macotpet *et al.* (2009) in a study on breed predisposition for urinary tract infections reported highest incidence in Mixed breed (54.4%) followed by Dalmatian (22.7%), Poodles (13.6%) and least in German Shepherd (9.3%).

Olby *et al.* (2010) conducted a study on breed wise occurrence of urinary tract infections recorded highest occurrence in Dachshunds (46.2 per cent) followed by Beagle (15.38 per cent), Miniature Poodle, Shih Tzu (7.69 per cent each); Dalmatian, Mixed breed, Boykin Spaniel, Cocker Spaniel, Pekingese, and Pembroke Welsh Corgi (3.84 per cent each).

Rowlands *et al.* (2011) recorded highest incidence of urinary tract infections in Boxer (13.1%) followed by Labrador Retriever (10.52%), Crossbreed and West Highland White Terrier (7.89% each), Staffordshire Bull Terrier, Bernese Mountain dog, Border Collie, American Bulldog, Beagle, Lhasa Apso, Basset Hound, Cocker Spaniel, Alaskan Malamute and New Foundland (6.06% each).

Wong *et al.* (2015) studied about the breed predisposition of dogs and reported highest incidence in Labrador Retrievers followed by German Shepherds, Golden Retrievers while least incidence was recorded in Dachshunds.

Yogeshpriya *et al.* (2015) stated that incidence of urinary tract infection was more common in sporting group dogs followed by working group and least in herding group dogs.

Wynn *et al.* (2016) stated that Beagle, Doberman Pinscher, Border Collie, Shih Tzu and Russell Terrier are at high risk of developing urinary tract infections.

Adamama *et al.* (2017) in a study on breed wise occurrence of urinary tract infections reported highest occurrence in medium breeds (33.9%), followed by large (32.1%) breeds, small (27.8%) breeds and least in giant breeds (6.2%).

Sorensen *et al.* (2018) in a study on breed predisposition for urinary tract infections in dogs reported highest occurrence in Labrador Retriever followed by Mixed breed dogs and least in Cocker spaniel

Liebelt and Pigott (2019) stated that Mixed breed, Labrador Retriever, Golden Retriever, German Shepherd, Corgi and Shih Tzu are at higher risk of developing urinary tract infections.

Sanchez *et al.* (2019) in a study on breed predisposition for urinary tract infections reported highest incidence in Labrador retriever followed by Cocker Spaniel, Golden Retriever, German Shepherd and least in Crossbreeds

2.1.3 Occurrence in relation to gender

Ling *et al.* (2001) in their study on 8354 dogs recorded higher incidence of urinary tract infections as 58.34 per cent (4873/8354) in female dogs and 41.66 per cent (3481/8354) in male dogs.

Swenson *et al.* (2004) in their study on 441 dogs documented higher incidence of bacterial urinary tract infection in spayed females (51.2%) followed by castrated males (28.5%), intact females (10.6%) and least in intact males (9.7%).

Gaeta *et al.* (2009) recorded higher incidence of urinary tract infections in castrated male (59.1%) and spayed females (40.9%) when compared to those of intact males and females.

Neil *et al.* (2013) in their study reported that females (81.82%) were more affected with urinary tract infections when compared to males (18.18%).

Wan *et al.* (2014) conducted a study in 101 healthy dogs and reported an incidence of subclinical bacteriuria as 8.9 per cent (9/101) in females.

Wong *et al.* (2015) in their study on 1028 dogs reported incidence of urinary tract infections was more in female dogs (74%) when compared to male dogs (26%). In the same study he also reported that the incidence of infection was more in spayed females (66.5%) and castrated males (21%) over that of intact females (7.4%) and intact males (5.1%).

Wynn *et al.* (2016) recorded higher incidence of bacterial urinary tract infections in spayed females (66.66%) than neutered males (33.34%).

Merkel *et al.* (2017) in their study on 27 dogs observed the incidence of emphysematous cystitis was more common in spayed females (62.96 %) when compared to neutered males (37.03 %).

Sorensen *et al.* (2018) reported that female dogs (73%) were comparatively more affected with urinary tract infections than males (27%)

Fumeo *et al.* (2019) reported higher incidence of urinary tract infections in female dogs compared to male dogs.

Lippi *et al.* (2019) reported that females (55.55 %) were more commonly affected with urinary tract infections compared to males (44.45%)

2.2 CLINICAL SIGNS

Bartges (2004) recorded dysuria, pollakiuria, urinary incontinence, signs of abnormal detrusor reflex, haematuria at end of micturition and cloudy urine with abnormal odour as common findings in dogs affected with lower urinary tract infections.

Weese *et al.* (2011) reported that in dogs affected with lower urinary tract infections clinical signs include fever, inappetence, dysuria, pollakiuria, increased urgency of urination, haematuria, and pyuria.

Dunning and Stonehewer (2012) observed dysuria, pollakiuria, stranguria, haematuria, nocturia, polyuria, polydipsia, abdominal discomfort, altered urine colour, pyrexia, lethargy, anorexia, vomiting and prostatic enlargement in dogs affected with lower urinary tract infections in dogs.

Smee *et al.* (2013_b) recorded the clinical signs such as pollakiuria, stranguria, dysuria, haematuria and inappropriate urination in dogs affected with lower urinary tract infections.

Chigozie (2015) observed haematuria, dysuria, pollakiuria and pain on palpation of bladder in dogs affected with haemorrhagic cystitis.

Kumar and Kamran (2016) recorded the clinical signs like pollakiuria, dysuria, stranguria, haematuria, urinary incontinence, polyuria and polydipsia in dogs affected with urinary tract infections.

Merkel *et al.* (2017) recorded the clinical signs such as haematuria, stranguria, dysuria, pollakiuria, fever, fetid urine, abdominal pain, dribbling of urine and vomiting in dogs diagnosed as cystitis.

Sorensen *et al.* (2018) documented that the suggestive clinical signs in dogs affected with lower urinary tract infections viz., dysuria, pollakiuria, haematuria, stranguria, malodorous urine, periuria, urinary incontinence, lethargy, anorexia/inappetence, pyrexia and painful abdomen.

Yogeshpriya *et al.* (2018) reported that anorexia, pyuria, stranguria, haematuria, pollakiuria, foul smelling urine and dark yellow colour urine in dogs affected with lower urinary tract infections.

Lippi *et al.* (2019) reported abdominal pain, nausea, vomiting, fever, haematuria, pneumaturia, pollakiuria, stranguria, urinary incontinence and polyuria/polydipsia (PU/PD) in dogs with lower urinary tract infections.

2.3 DIAGNOSIS

2.3.1 Urinalysis

Aizenberg and Aroch (2003) performed urinalysis and recorded turbid and dark brown urine, haematuria, proteinuria, low pH of 5.5 and high specific gravity of 1.040 in dogs with signs of urinary tract infections.

Cetin *et al.* (2003) reported cloudy appearance of urine, decreased urinary specific gravity (1.010-1.0150), alkaline pH of urine (6-9), proteinuria and presence of nitrates upon urinalysis in dogs with signs suggestive of urinary tract infections.

Vaden *et al.* (2004) in a study conducted on 70 urine samples of dogs documented that there was no significant change in levels of urinary albumin in dogs showing symptoms of pyuria alone (71%) where as in dogs with concurrent haematuria and bacteriuria (39%) there was significant increase in urine albumin levels. In the same study they also stated that 81% of samples with symptoms of lower UTI showed no significant difference in urine protein to creatinine ratio (UPC) when compared with healthy dogs.

Macotpet *et al.* (2009) demonstrated the presence of leukocytes, RBC, proteins, high urinary pH and high specific gravity (>1.03) in urine of dogs affected with urinary tract infections.

Dunning and Stonehewer (2012) documented that bacterial urinary tract infection by *Staphylococcus* and *Proteus* spp resulted in high alkaline pH of urine on performing urinalysis by dip stick. In the same study they also reported that increased urinary urea concentrations contributed to resistance to lower urinary tract infections with exception to urease producing *Staphylococcus* and *Proteus* spp which colonize at high urinary urea concentrations resulting in the development of UTI in dogs.

Jasim (2012) recorded the presence of turbidity, erythrocytes, leukocytes (+2), protein (+2), nitrates (+1) and increased specific gravity (> 1.02) as common findings upon conducting urinalysis in dogs affected with urinary tract infections.

Parrah *et al.* (2013) documented the urinalysis findings in healthy dogs as pale yellow to brown yellow colour of urine with clear transparency, garlicky odour, pH of 5.5-7.0, specific gravity of 1.001-1.065 and negative for protein, glucose, ketones, bilirubin, urobilinogen, blood and leukocytes.

Smee *et al.* (2013_a) in a study reported that the antibacterial property of urine in healthy dogs is due to low urinary pH, high specific gravity and high urea concentrations.

Chigozie (2015) performed urinalysis in dogs with haemorrhagic cystitis and reported presence of turbid urine, decreased urinary pH (6.0), proteinuria and presence of nitrates in urine collected from dogs affected with urinary tract infections.

Somu *et al.* (2015) conducted a study in 66 dogs suggestive of urinary tract infections out of which 28 were positive for urinary tract infections and reported the urinalysis findings as no change in colour, specific gravity and urinary pH while proteinuria was noticed in 67.85% (19/28) and occult blood in 28.57% (8/28).

Merkel *et al.* (2017) conducted urinalysis in dogs with signs of urinary tract infections and recorded the findings as increased specific gravity (> 1.021), haematuria (> 30 red cells/hpf), pyuria (20 white cells/hpf) in dogs affected with bacterial urinary tract infections.

Grimes *et al.* (2019) performed urinalysis on 451 urine samples and revealed relatively high specific gravity, high urine pH and high urine protein to creatinine ratio (UPC) as compared with that of healthy dogs.

2.3.2 Urine sediment examination

Guire *et al.* (2002) conducted a study in dogs affected with urinary tract infections and reported that examination of urine sediment from dogs with positive urine cultures revealed no significant change in the number of red blood cells, white blood cells, epithelial cells, casts and crystals when compared to that of healthy control group.

Aizenberg and Aroch (2003) conducted a systematic study and found the presence of rod shaped bacteria and pyuria as microscopic findings on performing urine sediment examination in dogs affected with bacterial urinary tract infections.

Cetin *et al.* (2003) stated that microscopic examination of urine sediment revealed the presence of leukocytes, erythrocytes, epithelial cells, epithelial casts and leukocytic casts in dogs affected with lower urinary tract infections.

Swenson *et al.* (2004) compared the accuracy of urine sediment examination by unstained and stained method recorded the presence of cocci (34.9%, 12.3%), rods (15.8%, 58.9%) and mixed population (49.3%, 28.8%) respectively in dogs affected with urinary tract infections.

Dunning and Stonehewer (2012) conducted a study and reported that urine sediment examination from sample collected by cystocentesis with increased red blood cells and white blood cells ($> 3/\text{hpf}$) is an indicative of urinary tract infection in dogs.

Jasim (2012) conducted a study in 7 dogs and stated the presence of crystals, epithelial cells and casts on examination of urine sediment as an indicative of lower urinary tract infections in dogs.

Smee *et al.* (2013_b) identified pyuria and bacteriuria on performing urine sediment examination in dogs affected with lower urinary tract infections.

Somu *et al.* (2015) performed urine sediment examination and found haematuria and pyuria in all positive samples (28) whereas bacteriuria noticed in 78.57% dogs (22/28) and granular casts in 7.14%% (2/28) in their study on 66 dogs suggestive of lower urinary tract infections.

Biasibetti *et al.* (2019) conducted urine sediment cytology and reported with increase in the number of leukocytes (above 5/hpf) and epithelial cells as common finding in dogs with urinary tract infections and inflammation.

Grimes *et al.* (2019) performed urine sediment examination in their study on 451 dogs and recorded pyuria (53.3%), bacteriuria (46.7%) and haematuria (13.3%) in dogs affected with lower urinary tract infections.

2.3.3 Abdominal Radiography

Aizenberg and Aroch (2003) identified the presence of gas in bladder wall and lumen of bladder on radiographic examination in dogs affected with emphysematous cystitis.

Forrester (2004) identified the presence of radiopaque urocystoliths on lateral abdominal radiograph in a 12 year-old poodle dog affected with lower urinary tract infection.

Petite *et al.* (2006) reported with the radiographic findings in dogs affected with emphysematous cystitis as a gas stripe in lumen of bladder with an incidence of 25% (1/4).

Merkel *et al.* (2017) conducted radiographic examination in dogs and observed the presence of gas in the wall and lumen of the urinary bladder (51.9%), in only the wall

of the bladder (33%), and in only the lumen of the bladder (14.8%) in dogs affected with cystitis.

Fumeo *et al.* (2019) documented radiographic findings as presence of line of gas around bladder wall on plain radiography in dogs affected with emphysematous cystitis.

Lippi *et al.* (2019) in their study recorded the radiographic findings such as small round areas of increased radiolucency and gas localization at the wall of the bladder in dogs affected with cystitis.

2.3.4 Abdominal Ultrasonography

Aizenberg and Aroch (2003) performed ultrasonographic examination in dogs affected with emphysematous cystitis and recorded the findings as thickened bladder wall, bladder wall with increased acoustic shadow, echogenic foci within bladder representing gas in bladder wall or lumen.

Forrester (2004) documented the presence of urocystoliths, mass in trigone of bladder on conducting ultrasonographic examination in a poodle dog affected with urinary tract infections.

Kundu and Gosh (2006) in their study documented the ultrasonographic findings as cystic calculi with hyper echogenicity in bladder and acoustic shadow of calculi in dogs affected with lower urinary tract infections.

Petite *et al.* (2006) in a study conducted on 4 dogs with emphysematous cystitis identified hyper echogenicity of bladder, decreased size of bladder and its contents by using a 7.5-MHz micro convex probe in dorsal recumbency and in a standing position on conducting ultrasonography.

Mantis and Lamb (2008) reported an increased bladder wall thickness ($> 2\text{mm}$) in dogs affected with urinary tract infection when compared with that of healthy dogs ($< 2\text{mm}$) by ultrasonographic studies.

Kealy *et al.* (2010) identified presence of hyper echogenic area of bladder wall in 42% of dogs affected with urinary tract infections on performing ultrasonography in dogs.

Biegen *et al.* (2013) conducted ultrasonographic examination in dogs and found mild distended urinary bladder with an eccentrically thickened bladder wall (8.4mm thickness detected at the apex), hyperechoic mucosal surface and irregular shape of bladder as common findings in dogs affected with cystitis.

Robotti and Lanfranchi (2013) documented the presence of debris in lumen of bladder, moderate thickening of bladder wall, hyperechoic calculi in lumen of bladder, tumor growth in lumen of bladder and enlargement of prostate gland on ultrasonographic examination in dogs affected with lower urinary tract infections.

Dinesh *et al.* (2015) in their study in dogs with signs of urinary tract infections identified spheroid bladder, thick and distinct three layered hyperechoic bladder wall and anechoic lumen, hyperechoic calculi in lumen of bladder as common findings on 2D ultrasonogram.

Yehia and Salem (2015) performed ultrasonography in 32 dogs affected with urinary tract infections and recorded ultrasonographic findings as increase in bladder wall thickness ($> 6\text{mm}$), hyperechoic masses in lumen of bladder and clots in lumen of bladder as hyperechoic lace like appearance.

Kumar and Kamran (2016) in their study conducted on 22 dogs reported distended bladder wall thickness of $> 3\text{mm}$, hyperechoic focal echogenicity that shadow in

dependent portion of bladder with urinary sediments, hypoechoic thickening of bladder wall as common ultrasonographic findings in dogs affected with cystitis.

Yogeshpriya *et al.* (2018) conducted a systematic review in their study on bladder wall thickness by performing ultrasonography and identified 3 out of 12 dogs with thickness of bladder wall of $> 2\text{mm}$ while in remaining 9 dogs had bladder wall thickness of $< 2\text{mm}$.

Biasibetti *et al.* (2019) in their study on 10 dogs identified thickening of bladder wall and irregularity of bladder mucosa as a common finding in dogs with signs of urinary tract infections.

Lippi *et al.* (2019) in their study conducted on 36 dogs identified diffuse thickening of bladder wall in 39.5 per cent and focal thickening of bladder wall in 10.5 per cent among dogs affected with urinary tract infections.

2.3.5 Isolation and identification of bacteria

Kivisto *et al.* (1977) isolated *Escherichia coli* (67%), *Micrococci* (15%) *Streptococci* spp (6%), *Staphylococci* spp (6%) *Enterobacter* spp (3%) and *Proteus* spp (3%) from 237 dogs affected with cystitis.

Brearley *et al.* (1986) isolated *Escherichia coli* (46.87%) as predominant pathogen followed by *Staphylococcus* spp (34.37%), *Proteus* spp (12.5%) and *Streptococcus* spp (6.25%) in 32 dogs affected with lower urinary tract infections.

Lobetti and Goldin (1998) isolated and identified that *Escherichia coli* and *Clostridium* as most frequent pathogens for development of emphysematous cystitis in dogs.

Ling *et al.* (2001) identified *Escherichia coli* (44.1%), *Staphylococcus* spp (11.6%), *Proteus* spp (9.3%), *Klebsiella* spp (9.1%), *Enterococcus* spp (8.0%), and *Streptococcus* spp (5.4%) as common bacterial isolates from dogs affected with urinary tract infections.

Cetin *et al.* (2003) examined 100 dogs affected with urinary tract infections and isolated *Escherichia coli* (23.68%), *Streptococcus* spp (15.68%), *Micrococcus* spp (11.76%), *Staphylococcus* spp, *Corynebacterium* spp (9.8% each), *Klebsiella pneumoniae* (7.84%), *Proteus* (5.88%), *Pseudomonas aeruginosa*, *Citrobacter freundii*, *Acinetobacter* spp and *Enterobacter* spp (3.92% each).

Oxenford *et al.* (2006) conducted cultural studies in 64 dogs with signs of urinary tract infections and isolated 45.3 per cent *Proteus* spp followed by 24.5 per cent *Escherichia coli*, 11.3 per cent *Staphylococcus* spp, 7.55 per cent *Pseudomonas aeruginosa*, 5.66 per cent *Klebsiella pneumoniae* and 5.69 per cent other species of bacteria.

Papini *et al.* (2006) examined 114 dogs with signs of urinary tract infections and isolated *Escherichia coli* (34.2%), followed by *Proteus mirabilis* (32.46%), *Pseudomonas aeruginosa* (14.03%), *Staphylococcus aureus* (13.16%), *Streptococci* (4.39%) and *Klebsiella* (1.75%).

Bubenik *et al.* (2007) identified *Escherichia coli* and *Proteus* spp as most frequent pathogens isolated from 147 non catheterized dogs, whereas *Enterobacter* spp and *Staphylococcus* spp as most common pathogens from 99 catheterized dogs.

Ball *et al.* (2008) isolated *Escherichia coli* (63.6%), *Staphylococcus intermedius* (14.5%), *Enterococcus* spp, *Proteus* spp (5.5% each) and other gram negative organisms (10.9%) from 473 dogs affected with urinary tract infections.

Jasim (2012) isolated *Escherichia coli* alone in 5 dogs (71.42%) and mixed infection of *Escherichia coli* as well as *Streptococcus* spp in 2 dogs (28.57%) in their study on 7 dogs affected with bacterial lower urinary tract infections.

Hall *et al.* (2013) in a study conducted on culture of 1037 urine samples identified 85% of samples with single bacterial isolate, 12.4% of samples with two bacterial species, 2.1% of samples with three bacterial species and 0.3% of samples with four bacterial species

Smee *et al.* (2013_a) identified *Escherichia coli* as a common pathogen on culture of urine which accounts for one third to one half followed by *Staphylococcus* spp, *Streptococcus* spp, *Enterococcus* spp, *Proteus*, *Klebsiella*, *Pasteurella*, *Mycoplasma*, *Enterobacter* and *Pseudomonas* in dogs with urinary tract infections . In the same study they also stated that from the positive urine samples single bacterial isolate was isolated (75%), followed by two bacterial isolates (20%) and three isolates (5%) in dogs with urinary tract infection.

Windahl *et al.* (2014) in their study on urine samples of 961 dogs reported 625 dogs were showing positive bacterial growth of which *Escherichia coli* was frequent organism isolated (68.9%) followed by *Staphylococcus* spp (11.2%), *Proteus* (8.8%), *Streptococcus* spp (5.6%), *Enterococcus* spp (3.7%) and *Klebsiella* (1.8%) .

Chigozie (2015) isolated and identified *Streptococcus* spp as most common cause for haemorrhagic cystitis in dogs.

Wong *et al.* (2015) conducted a study on 1028 dogs affected with urinary tract infections and isolated *Escherichia coli* (52.5%), *Staphylococcus* spp (13.6%) and *Enterococcus* spp (13.3%) as common uropathogens in dogs

Punia *et al.* (2018) isolated *Escherichia coli* (29.62%) and *Streptococcus spp* (29.62%) as predominant pathogens followed by *Staphylococcus spp* (22.22%), *Klebsiella* (11.11%), *Pseudomonas spp* (3.7%) and *Bacillus spp* (3.7%) in 22 dogs out of 35 dogs affected with urinary tract infections.

Yogeshpriya *et al.* (2018) identified *Escherichia coli* (78.57%) as the predominant pathogen followed by *Proteus mirabilis* (7.14%), *Staphylococcus aureus* (7.14%), *Klebsiella pneumoniae* (3.57%) and *Streptococci spp* (3.57%) in their study on 32 dogs out of 2545 dogs affected with urinary tract infections.

Fumeo *et al.* (2019) identified *Escherichia coli* as a predominant pathogen that causes emphysematous cystitis in dogs.

Lippi *et al.* (2019) collected 36 urine samples from dogs affected with cystitis out of which 35 (97.22%) samples were positive for bacterial isolates. Of these 35 samples *Escherichia coli* was isolated from 25 dogs (71.4%), *Proteus mirabilis* was isolated from three dogs (8.6%), mixed infection of *Enterococcus* along with *Klebsiella pneumoniae* was isolated from three dogs (8.6%), mixed infection of *Staphylococcus* along with *Klebsiella pneumoniae* was isolated from three dogs (8.6%) and *Klebsiella pneumoniae* in one dog (2.8%).

2.4 ANTIBIOTIC SUSCEPTIBILITY TESTING

Cetin *et al.* (2003) noticed that 46% of bacterial strains isolated from urinary tract infections were sensitive to amoxicillin/clavulanic acid (90.19%), 40% of isolates sensitive to gentamicin (78.43%), 39% of isolates to ampicillin/sulbactam (76.47%), 31% of isolates to enrofloxacin (60.78%), 30% of isolates to nitrofurantoin (58.82%), 29% of isolates to kanamycin (56.86%), 28% of isolates to oxytetracycline (54.9%), 26% of isolates to cephalothin (50.98%), and least sensitivity to sulphamethoxazole (45.09%).

Oxenford *et al.* (2006) conducted antibiotic sensitivity testing from 85 urine samples and identified maximum sensitivity of *Escherichia coli* (100%) to gentamicin and least sensitivity to amoxycillin (18.2%), while *Proteus* was found to be 100% sensitive to gentamicin and least sensitivity to tetracyclines (3.6%).

Ball *et al.* (2008) conducted antibiotic sensitivity testing in 473 isolates of *Escherichia coli*, *Streptococcus intermedius* and *Proteus* spp of which 93.3% were sensitive to gentamicin, 91.1% to enrofloxacin, 90.1% to amoxicillin-clavulanic acid, 89.1% to ceftiofur, 87.8% to chloramphenicol, 86.7% to trimethoprim-sulfamethoxazole and 62.2% to cephalothin.

Macotpet *et al.* (2009) in a study on canine urinary tract infections conducted antimicrobial susceptibility testing against isolates of *Escherichia coli*, *Klebsiella*, *Pseudomonas*, *Proteus*, *Staphylococcus* and *Streptococcus* of which 100% were sensitive to ciprofloxacin and norfloxacin with least sensitivity towards metronidazole (20%).

Thompson *et al.* (2011) noticed that 80% of *Escherichia coli* isolates have shown increased resistance to fluoroquinolones, third generation cephalosporins and clavulanic acid potentiated β -lactams in their study conducted over a period of 9 years.

Windahl *et al.* (2014) conducted antibiotic susceptibility testing in 429 urine samples with *Escherichia coli* isolates and found maximum susceptibility of all isolates to cefotaxime (99.8%) while least susceptibility was reported with amoxicillin and clavulanic acid. In the same study he also documented 11 urine samples with *Klebsiella* isolates and found maximum susceptibility (100%) to cefotaxime and least susceptibility to ampicillin.

Wong *et al.* (2015) in a study on urinary tract infections in canines conducted antibiotic susceptibility testing in 1028 urine samples with *Escherichia coli*,

Streptococcus spp and *Enterococcus* spp and found maximum sensitivity to trimethoprim-sulfamethoxazole (85.7%) and least susceptibility towards ampicillin (59.4%).

Punia *et al.* (2018) conducted antibiotic susceptibility testing in 35 suspected urine samples of dogs with urinary tract infections and recorded that 22 samples have shown presence of *Escherichia coli*, *Streptococcus* spp as predominant uropathogens with overall maximum sensitivity towards ceftriaxone + tazobactam (88.88%) and least sensitivity towards amoxicillin and cloxacillin (29.62%) followed by amoxicillin and clavulanic acid (76%), enrofloxacin (74%), cephalexin (66%) and least towards amoxicillin (59%).

McGovern *et al.* (2019) conducted antibiotic susceptibility testing in 208 urine samples of dogs with *Escherichia coli*, *Staphylococcus* and *Streptococcus* as predominant pathogens with overall maximum sensitivity towards chloramphenicol (100%) followed by trimethoprim sulphamethoxazole (95.2%), enrofloxacin (92.5%), amikacin (90.2%) and least sensitivity towards cephalothin (60.5%).

2.5 Underlying or associated causes

Greene (1992) stated that immunocompromised dogs and dogs receiving long term glucocorticoids as underlying factors for development of urinary tract infections in dogs.

Aizenberg and Aroch (2003) stated that long term use of glucocorticoids as an underlying cause for development of emphysematous cystitis in dogs.

Torres *et al.* (2005) in a study on 127 dogs showing pruritis and receiving long term glucocorticoids for > 6 months identified that 18.1 % (23) of dogs showed the presence of asymptomatic urinary tract infections.

Gaeta *et al.* (2009) in their study from 1998-2011 over a period of 12 years identified 22 dogs with sterile haemorrhagic cystitis due to long term use of oral cyclophosphamide which is one of the underlying cause in development of disease.

Weese *et al.* (2011) conducted a study and documented about risks of complications by urolithiasis, ascending or systemic infections in addition to elimination of infection aids in successful treatment of urinary tract infections.

Smee *et al.* (2013_a) in their study on 101 dogs with systemic disease receiving long term glucocorticoid therapy reported the occurrence of urinary tract infection in 42 dogs (41.6%).

Smee *et al.* (2013_b) documented that the underlying causes in urinary tract infections in dogs included vulvar involution, perivulvar dermatitis, vaginal stenosis, chronic kidney disease, diabetes mellitus, hyperthyroidism and long term glucocorticoid therapy.

Dinesh *et al.* (2015) identified the underlying conditions associated with recurrent urinary tract infections as urine retention due to calculi, prostatic enlargement and presence of short urethra in females.

Olin and Bartges (2015) reported that ascension of bacteria from perineal, rectal, and genital areas as underlying or associated causes in obese dogs affected with bacterial urinary tract infections.

Wynn *et al.* (2016) in a study conducted on 46 dogs recorded obesity with body fat % >45 as one of the underlying factor in the development of bacterial urinary tract infections.

Fumeo *et al.* (2019) reported that diabetes mellitus as the most underlying risk factor in development of emphysematous cystitis in dogs.

McGovern *et al.* (2019) in their study on 208 dogs with 63 dogs showing signs of lower urinary tract infections recorded the comorbid conditions as long term use of immunosuppressive drugs and chronic recurrent UTI (19% each), followed by urinary incontinence, anatomical defects (13% each), urinary tract neoplasia, restricted mobility (8% each), renal azotemia (6%) and endocrinopathies, pyometra (2% each).

2.6 HAEMATOLOGY

2.6.1 Haemoglobin

Sravanthi *et al.* (2014) stated that the haemoglobin values in dogs affected with lower urinary tract infections were within the normal range (11.2 ± 0.60 g/dl).

Ukwueze (2015) reported a significant decrease in haemoglobin level in dogs affected with urinary tract infections.

Das *et al.* (2017) stated a nonsignificant decrease in haemoglobin levels in dogs affected with lower urinary tract infections (11.21 ± 0.45 g/dl) compared with healthy control group (11.58 ± 0.32 g/dl).

Merkel *et al.* (2017) reported a significant decrease in haemoglobin levels in dogs affected with urinary tract infections.

Roopali *et al.* (2018) recorded a highly significant decrease in values of haemoglobin in dogs affected with urinary tract infections (9.52 ± 1.12 g/dl) compared with healthy control group (12.18 ± 0.04 g/dl).

Yogeshpriya *et al.* (2018) documented that the haemoglobin levels in dogs affected with urinary tract infections were within normal range (13.11 ± 2.70 g/dl) as compared to healthy control group (13.42 ± 2.30 g/dl).

Liebelt and Pigott (2019) recorded a significant decrease in haemoglobin levels in dogs affected with urinary tract infections.

2.6.2 Packed cell volume

Beigen *et al.* (2013) in their study reported a significant increase in packed cell volume in dogs affected with cystitis.

Mukherjee *et al.* (2014) conducted a study on urinary tract infections in canines to find out haemato-biochemical changes and reported marked increase in PCV in dogs affected with cystitis and urolithiasis.

Das *et al.* (2017) reported that the packed cell volume levels in dogs affected with lower urinary tract infections were within the normal range (34.34 ± 3.71 %) as compared with that of healthy control group (34.88 ± 0.81 %).

Merkel *et al.* (2017) documented that there was significant decrease in packed cell volume in dogs affected with cystitis when compared with that of healthy dogs.

Roopali *et al.* (2018) recorded a significant decrease in packed cell volume (32.22 ± 1.36 %) in dogs affected with urinary tract infections in comparison with healthy control group (42.49 ± 0.03 %).

Yogeshpriya *et al.* (2018) recorded a significant decrease in packed cell volume levels in dogs affected with urinary tract infections (33.00 ± 1.70 %) when compared to healthy control group (35.09 ± 2.30 %).

2.6.3 Total leukocyte count

Cetin *et al.* (2003) reported a significant increase in total leukocyte count in dogs affected with urinary tract infections.

Sravanthi *et al.* (2014) stated that the total leukocyte count in dogs affected with lower urinary tract infections were within normal range ($9.17 \pm 1.22 \times 10^3/\text{mm}^3$).

Roopali *et al.* (2018) documented a significant increase in mean total leukocyte count ($17.76 \pm 1.30 \times 10^3/\text{mm}^3$) in dogs affected with urinary tract infections compared to that of healthy control group ($10.58 \pm 0.17 \times 10^3/\text{mm}^3$).

Yogeshpriya *et al.* (2018) recorded a high significant increase in total leukocyte count ($15571.88 \pm 4909.2/\text{mm}^3$) in dogs affected with bacterial urinary tract infections against healthy control group ($11813.13 \pm 2354.2/\text{mm}^3$).

Biasibetti *et al.* (2019) documented a significant increase in total leukocyte count in dogs affected with urinary tract infections.

2.6.4 Differential leukocyte count

Cetin *et al.* (2003) recorded neutrophilia (80-90%) with shift to left in dogs affected with urinary tract infections.

Das *et al.* (2017) reported a nonsignificant increase in neutrophils, significant increase in eosinophils and significant decrease in lymphocytes levels in dogs affected with lower urinary tract infections.

Merkel *et al.* (2017) documented neutrophilia with left shift and lymphopenia in their study on dogs with lower urinary tract infections.

Roopali *et al.* (2018) observed a significant increase in values of neutrophils, significant decrease in eosinophils, basophils and lymphocytes and no significant difference in levels of monocytes in dogs suffering with urinary tract infections.

Yogeshpriya *et al.* (2018) reported a significant increase in levels of neutrophils ($76.22 \pm 10\%$) and a decrease in level of lymphocytes ($19.98 \pm 0.80\%$) and nonsignificant decrease in eosinophils ($1.94 \pm 1.50\%$) and monocytes ($1.28 \pm 0.60\%$) in dogs affected with urinary tract infections when compared to healthy control group with mean neutrophil levels ($66.78 \pm 5.40\%$), lymphocytes ($24.56 \pm 10.20\%$), eosinophils ($2.33 \pm 4.30\%$) and monocytes ($2.66 \pm 0.40\%$).

2.7 SERUM BIOCHEMISTRY

2.7.1 Total protein

Polzin and Jeraj (1980) recorded nonsignificant increase in values of total protein in dogs affected with urinary tract infections.

Throop *et al.* (2013) observed a significant decrease in A:G ratios and significant increase in globulin levels in dogs affected with urinary tract infections in canines compared to healthy control animals.

Fransson *et al.* (2007) reported decreased levels of total protein in dogs affected with ascending urinary tract infections.

Das *et al.* (2017) recorded significant decrease in total protein levels (4.31 ± 0.52 g/dl) in dogs affected with lower urinary tract infections compared to that of healthy control group dogs (6.12 ± 0.56 g/dl).

Focak *et al.* (2017) recorded no significant change in levels of total protein in Maltese (6.92 ± 0.77 g/dl) and Belgian Shepherd dogs (6.80 ± 0.74 g/dl) affected with urinary tract infections.

Roopali *et al.* (2018) documented a significant decrease in total protein levels (4.11 ± 1.84 g/dl) in dogs suffering with urinary tract infections compared to healthy control dogs (6.52 ± 0.18 g/dl).

Yogeshpriya *et al.* (2018) recorded a nonsignificant decrease in mean values of total protein in dogs affected with urinary tract infections (7.08 ± 0.70 g/dl) when compared with healthy control group (7.21 ± 0.30 g/dl)

2.7.2 Albumin

Squires (1994) noticed a significant decrease in albumin levels in dogs with urinary tract infections

Andonova *et al.* (2010) documented a decrease in mean albumin levels (1.46 ± 0.11 g/dl) in dogs affected with complicated urinary tract infections compared to that of healthy control group (3.70 ± 0.24 g/dl).

Focak *et al.* (2017) recorded no significant change in levels of albumin in Maltese (3.80 ± 0.10 g/dl) and Belgian Shepherd dogs (3.35 ± 0.50 g/dl) affected with urinary tract infections.

Roopali *et al.* (2018) observed a significant decrease in values of albumin (1.53 ± 1.28 g/dl) compared with the mean levels in healthy control dogs (3.22 ± 0.22 g/dl).

Yogeshpriya *et al.* (2018) observed a significant decrease in the mean values of albumin (2.90 ± 0.60 g/dl) in dogs affected with urinary tract infections as compared with the mean levels in healthy control dogs (3.87 ± 0.22 g/dl).

2.7.3 BUN

Cetin *et al.* (2003) in their study conducted in dogs affected with urinary tract infections recorded a marked increase in serum urea concentrations (82-156 mg/dl).

Jasim (2012) reported a significant increase in blood urea nitrogen levels in dogs affected with bacterial lower urinary tract infections in dogs.

Biegen *et al.* (2013) recorded a significant increase in blood urea nitrogen levels in dogs affected with bacterial cystitis.

Sravanthi *et al.* (2014) stated that the blood urea nitrogen levels in dogs affected with lower urinary tract infections were within normal range (16.70 ± 2.70 mg/dl).

Dinesh *et al.* (2015) observed a significant increase in blood urea nitrogen levels in dogs affected with urinary tract infections when compared with that of healthy control group.

Das *et al.* (2017) stated a nonsignificant increase in blood urea nitrogen levels in dogs affected with lower urinary tract infections (57.90 ± 0.54 mg/dl) compared with healthy control group (42.50 ± 0.74 mg/dl).

Roopali *et al.* (2018) observed a significant increase in blood urea nitrogen levels in dogs affected with urinary tract infections (54.18 ± 0.20 mg/dl) as compared with healthy control dogs (22.11 ± 0.11 mg/dl).

Grimes *et al.* (2019) documented a significant increase in blood urea nitrogen levels in dogs affected with urinary tract infections.

Yogeshpriya *et al.* (2018) documented a significant increase in blood urea nitrogen levels (42.28 ± 0.32 mg/dl) in dogs affected with lower urinary tract infections compared to healthy control group (23.67 ± 0.26 mg/dl).

2.7.4 Creatinine

Blanco and Bartges (2001) stated that there was statistical increase in creatinine levels in dogs with lower urinary tract infections (1.80 ± 1.50 mg/dl) when compared to healthy dogs (1.02 ± 0.90 mg/dl).

Cetin *et al.* (2003) observed a significant increase in values of creatinine (3.20-4.50 mg/dl) in dogs affected with urinary tract infections as compared to healthy dogs.

Jasim (2012) documented a significant increase in serum creatinine levels in dogs affected with bacterial lower urinary tract infections.

Biegen *et al.* (2013) observed that there was a significant increase in serum creatinine concentrations in dogs affected with cystitis.

Sravanthi *et al.* (2014) stated that the serum creatinine levels in dogs affected with lower urinary tract infections were within normal range (0.95 ± 0.02 mg/dl).

Dinesh *et al.* (2015) documented a significant increase in serum creatinine concentrations in dogs affected with urinary tract infections.

Das *et al.* (2017) stated a nonsignificant increase in serum creatinine levels in dogs affected with lower urinary tract infections.

Focak *et al.* (2017) recorded a significant increase in serum creatinine levels in dogs affected with urinary tract infections (1.40 ± 0.23 mg/dl) as compared to healthy dogs (0.97 ± 0.18 mg/dl).

Roopali *et al.* (2018) documented a significant increase in serum creatinine levels in dogs affected with urinary tract infections (4.12 ± 2.56 mg/dl) compared with healthy control group (1.02 ± 0.12 mg/dl).

Yogeshpriya *et al.* (2018) recorded a significant increase in mean creatinine levels in dogs affected with urinary tract infections (1.80 ± 1.50 mg/dl) when compared to healthy control group (1.02 ± 0.90 mg/dl).

Grimes *et al.* (2019) reported a significant increase in serum creatinine levels in dogs affected with urinary tract infections.

Liebelt and Pigott (2019) stated a mild increase in serum creatinine levels in dogs affected with lower urinary tract infections.

2.8 THERAPY

Ling and Ruby (1979) reported that intramuscular injection of gentamicin @ 6.6 mg/kg body weight IM divided into equal parts and given at 8 hours interval for a period of 4-14 days was effective in treatment of recurrent urinary tract infections.

Rogers *et al.* (1988) reported that amikacin @ 18-20 mg/kg body weight is effective in treatment of lower urinary tract infections in dogs when compared to trimethoprim – sulphamethaxazole.

Mitra *et al.* (1994) conducted a study on 24 dogs to rule out comparative efficacy of gentamicin, norfloxacin and nitrofurantoin against urinary tract infection in canine and concluded that gentamicin and norfloxacin are 100% effective whereas nitrofurantoin was found to be 87.5% effective.

Cotard *et al.* (1995) conducted therapeutic trial in dogs suffering from urinary tract infections into two groups. One group was treated with marbofloxacin @ 2mg/kg

body weight orally once daily for 14 days and the other group was treated with amoxicillin sodium + sulbactam @ 12.5mg/kg body weight twice daily PO for 10-28 days and found success rates as 83% and 70%, respectively.

Gupta *et al.* (2007) in their study on urinary tract infections stated that use of oral cranberry extracts as a supportive therapy for a period of 60 days helps to prevent the recurrence of clinical signs of urinary tract infections in dogs

Passmore *et al.* (2007) in their study on 129 dogs compared cephalixin @15mg/kg body weight twice daily orally for 14 days and cefovecin @ 8mg/kg body weight single S/C injection for 14 days in dogs affected with urinary tract infections and found success rates of 36.4% to cephalixin and 79.1% to cefovecin in treatment of urinary tract infections in canines.

Thirunavukkarasu *et al.* (2010) conducted a study in 10 dogs affected with cystitis and reported use of ceftriaxone-tazobactam @ 20mg/kg bwt. bid I/V for a period of 7 days is more effective than sulbactam –clavulanic acid in treatment of lower urinary tract infections in dogs.

Weese *et al.* (2011) reported that amoxicillin sodium + sulbactam @ 12.5-25 mg/kg PO is effective in treatment of lower urinary tract infections in dogs.

Westropp *et al.* (2012) compared enrofloxacin at dose rate of 18-20mg/kg once daily PO and amoxicillin-clavulanic acid at the dose rate of 13.75-25mg/kg twice daily PO and found both were effective in the treatment of uncomplicated urinary tract infections in canines with success rates of 89% and 88% respectively.

Clare *et al.* (2014) divided dogs suffering from urinary tract infections in to two groups, One group was treated with trimethoprim-sulphamethoxazole @ 15 mg/kg body

weight PO twice daily for 7 days and other with cefalexin @ 20 mg/kg body weight PO twice daily for a period of 10 days and found the recovery rates of 85% in trimethoprim-sulphamethoxazole treated group and 72% in cefalexin treated group .

Torres and Cooke (2014) in their study on lower urinary tract infection in 9yr old golden retriever caused by multidrug-resistant strain of *Pseudomonas aeruginosa* concluded that intravesical instillation of amikacin @ 15mg/kg diluted in 30ml of 0.9% normal saline for 14 days resulted in successful therapy.

Jessen *et al.* (2015) conducted a systemic review to study the effect of antibiotic treatment in canine urinary tract infections and concluded that trimethoprim-sulphamethaxazole at the dose rate of 15mg/kg PO twice daily for 3 days or high dose enrofloxacin at the dose rate of 18-20 mg/kg PO for a period of 7 days were effective in treatment of uncomplicated urinary tract infections in canines.

Colakoglu *et al.* (2017) reported that single dose of ceftriaxone @ 25 mg/kg I/V resulted in disappearance of clinical signs in 1-5 days when compared to enrofloxacin @ 5 mg/kg S/C sid for 14 days which resulted in subsidence of clinical signs in 4-9 days and concluded that ceftriaxone is more efficacious in the treatment of lower urinary tract infections in dogs.

Punia *et al.* (2018) conducted a study to compare therapeutic efficacy of ceftriaxone-tazobactam and amoxicillin-clavulanic acid in dogs suffering from urinary tract infections The dogs were divided into two groups, one group was treated with ceftriaxone-tazobactam @ 20 mg/kg once daily I/M for a period of 5 days and second group was treated with amoxicillin-clavulanic @ 20 mg/kg twice daily I/M for a period of 7 days and concluded that the dogs treated with ceftriaxone-tazobactam showed a recovery rate of 100% with remission of clinical signs and improvement in haemato-

biochemical parameters after therapy when compared to second group treated with amoxicillin-clavulanic acid with a recovery rate of 83.33% in dogs.

Riviere and Papich (2018) described the mechanism of action of amoxicillin sodium sulbactam, ceftriaxone tazobactam and amikacin in treatment of lower urinary tract infections in dogs.

Leuin *et al.* (2020) conducted a study in 14 dogs affected with lower urinary tract infections and recorded nitrofurantoin @ 4.3mg/kg q 8h PO for a period of 14 days showed complete recovery with resolution of clinical signs and negative bacteriological culture after 14 days of therapy in 12 dogs (85.71%) and no response to therapy in 2 dogs (14.29%).

CHAPTER-III

MATERIALS AND METHODS

Description of various materials and details of different methods in the present study are as follows.

3.1 MATERIALS

3.1.1 Animals

3.1.1.1 Control animals

Ten apparently healthy dogs of different breeds aged between one to nine years were selected as control group for obtaining normal data for comparison of parameters under study.

3.1.1.2 Clinical cases

The dogs presented to the Veterinary Clinical Complex, NTR College of Veterinary Science, Gannavaram during the period of January 2020 and December 2020 with the symptoms suggestive of lower urinary tract infections formed the basis for the present study. Dogs with symptoms suggestive of diabetes were not included in the present study. The dogs diagnosed to be affected with lower urinary tract infections formed the basis for the present study.

3.1.2 Clinical materials

The following clinical samples were collected from the dogs affected with lower urinary tract infections and were subjected to laboratory examination.

- Urine sample
- Whole Blood

- Serum

3.1.3 Laboratory materials

3.1.3.1 Urinary dipsticks

- Reagent strips for urinalysis

3.1.3.2 Chemicals

- Hydrogen peroxide (M/S Agarwal pharmaceuticals, Delhi).
- Oxidase disc

3.1.3.3 Culture media

- Agar-agar powder
- Mannitol salt agar (MSA)
- Eosin Methylene blue agar (EMB)
- Edwards medium
- MacConkey agar (MAC)
- Blood agar base
- Mueller-Hinton Agar (MHA)
- Simmons citrate agar
- Tryptic soy broth (TSB)
- Brain heart infusion (BHI) broth
- Brain heart infusion (BHI) agar

3.1.3.4 Reagents

- WBC diluting fluid
- RBC diluting fluid
- 0.1N Hydrochloric acid
- Leishman's stain solution
- Gram's staining kit
- Kovac's reagent

- Methyl red
- Barritt's reagent A & B

3.1.3.5 Antibiotic discs

S. No.	Name of the antibiotic disc	Symbol	Concentration
1	Ceftriaxone with tazobactam	CIT	30/10 Mcg
2	Amoxicillin sodium with sulbactam	AMS	30/15 Mcg
3	Gentamicin	GEN	10 Mcg
4	Ciprofloxacin	CIP	5 Mcg
5	Nitrofurantoin	NIT	300 Mcg
6	Trimethoprim-sulphamethaxazole	COT	25 Mcg
7	Amikacin	AK	30 Mcg

The reagent strips for urinalysis used in the present study were supplied by M/S ARKRAY Healthcare Pvt. Ltd., Gujarat.

All the reagents used in the present study were supplied by M/S Thermo Fisher Scientific India Pvt. Ltd., Mumbai.

All the culture media and antibiotic discs used in this study were procured from M/S Himedia Laboratories, Mumbai.

3.1.3.6 Equipment

- Compound microscope (M/S Olympus India Pvt. Ltd., New Delhi).
- Sahli's Haemoglobinometer (M/S Singhla scientific industries, Haryana).
- Haemocytometer (M/S Rohem instruments Pvt. Ltd., Maharashtra).
- Micro centrifuge (M/S Remi lab instruments Ltd., Mumbai).
- Semi auto analyzer (M/S Chem -5 plus, ERBA, Mumbai).

3.1.3.7 Biochemical kits

The diagnostic kits used in the present study for the estimation of serum total

protein, albumin, BUN and creatinine were supplied by M/S ERBA diagnostics Mannheim Pvt. Ltd., Mumbai.

3.1.3.8 Glass ware

All the glass ware used in the present study was obtained from M/S Borosil Ltd., Mumbai.

3.1.4 Therapeutic agents

- a) Inj. Amoxirum forte 300 mg (M/S Virbac pharmaceuticals Ltd.) consisting of 200 mg of amoxicillin and 100 mg of sulbactam.
- b) Inj. Intacef Tazo 562.5 mg (M/S Intas pharmaceuticals Ltd.) consisting of 500 mg of ceftriaxone and 62.5 mg of tazobactam.
- c) Inj. Venkacin 10ml ((M/S Venkys pharmaceuticals Ltd.) containing 250 mg of amikacin in each ml.

3.2. METHODS

3.2.1 Occurrence of lower urinary tract infections in dogs

The occurrence of lower urinary tract infections was recorded in 35 dogs exhibiting the clinical signs of lower urinary tract infection which were confirmed preliminarily by urinalysis. The occurrence in relation to age, breed and gender was analyzed in dogs which were diagnosed as affected with lower urinary tract infection.

3.2.2 Clinical examination

Dogs with symptoms suggestive of lower urinary tract infection like fever, haematuria, stranguria, pollakiuria, foul smelling urine, urinary incontinence, oliguria, polyuria, dribbling of urine, vomiting, anuria and/or pyuria were subjected to thorough clinical examination (Appendix A).

3.2.3 Diagnosis

3.2.3.1 Urinalysis

Dogs presented with clinical signs suggestive of urinary tract infections were subjected to clinical examination and confirmed after performing urinalysis. Urine samples from these dogs were collected by cystocentesis by placing the animal in either dorsal or lateral recumbency. The bladder should be palpated and is immobilized against body wall. The site is cleaned with antiseptic solution after which 5 ml of urine is collected in a 5 ml syringe with a 22 gauge needle. The collected sample is transferred into a sterile container and then kept in refrigerator at 4-8°C for further analysis. Physical and chemical examination of urine such as colour, pH, transparency, proteins, nitrates, glucose, bile pigments, ketones and occult blood were detected by urine dipsticks (Rose 2014) and specific gravity is detected by refractometer (Chew *et al.*, 2010 and Latimer 2011) (Plate 1B).

3.2.3.2 Urine sediment examination

Microscopic examination of urine sample was done by taking 5 ml of urine sample in a sterile tube and was centrifuged at 1000 rpm for 5 min. The supernatant was discarded and sediment was transferred onto a slide and examined under 10X and 40X power of microscope. The sediment is transferred on to a slide and examined for presence of erythrocytes, leucocytes, epithelial cells, casts, crystals and bacteria with number of more than or equal to 2-5 per high power field which is indicative of urinary tract infection (Swenson *et al.*, 2004; Chew *et al.*, 2010; Latimer 2011 and Somu *et al.*, 2015).

Plate- 1: Diagnostic techniques

A. Collection of urine by cystocentesis in a Pug dog



B. Urine dipsticks and Refractometer

3.2.3.3 Cultural examination of bacteria

3.2.3.3.1 Preparation of glass ware

The glass ware used in this study were initially dipped in 1% hydrochloric acid for 24 hours, then washed under running tap water and again soaked in teepol solution for 24 hours, cleaned with brush and washed under running tap water. Then the glass ware were rinsed in double distilled water which were dried carefully and packed followed by sterilization at 160°C for two hours in hot air oven. These glass ware were used for cultural and biochemical examination.

3.2.3.3.2 Preparation of culture media

All the media obtained as dehydrated powders were rehydrated as per the manufacturer's instructions by adding double distilled water to dissolve the powder. Then the media were sterilized by autoclaving at 121°C, 15 lb pressure for 15 minutes.

3.2.3.3.3 Collection of material

Urine samples were collected from dogs (n=35) with clinical signs suggestive of lower urinary tract infections. The urine was collected by cystocentesis as per the procedure described by Yogeshpriya *et al.* (2018). All the aseptic precautions were taken during the procedure. The collected samples were then transferred to sterile containers (Plate 1A). The samples were immediately placed in a refrigerator at 4-8°C and were later transported to the laboratory within two hours in ice packets for further analysis.

3.2.3.3.4 Isolation and identification of bacteria

Isolation of causative bacteria has been carried out from urine samples of dogs affected with lower urinary tract infections using standard microbiological procedure as

depicted in Fig 1. The urine samples collected from these dogs were simultaneously streaked on blood agar, incubated at 37° C for 24 hours. Growth characteristics on Blood agar and morphology in gram's stained smears and biochemical tests were taken into account for the presumptive identification of the causative bacteria (Punia *et al.*, 2018 and McGovern *et al.*, 2019). Isolation and identification of bacteria was done in 35 dogs with clinical signs suggestive of LUTI. Bacterial uropathogens were isolated in pure cultures from these dogs and the isolates were characterized by conventional microbiological procedures. Primary identification of bacterial isolates was done based on colony morphology on blood agar, gram's staining, catalase test, oxidase test and biochemical tests.

Single colonies were picked from blood agar and inoculated on Tryptic soy broth which were further streaked on Eosin methylene blue, Edward's medium, MacConkey agar, Brain heart infusion agar and Mannitol salt agar for confirmation of bacterial isolates.

3.2.3.3.4.1 Haemolysis pattern on blood Agar

The collected samples were streaked on blood agar plate and incubated at 37° C for 24 hrs which helps in differentiation of haemolytic and non-haemolytic bacterial isolates (Dunning and Stonehewer 2012) (Table 1).

3.2.3.3.4.2 Gram's staining

Bacterial smears were prepared by mixing 24 hour old cultures with sterile saline on a clean microscopic slide. The smears were air dried, heat fixed over flame, stained with gram's staining kit and examined under oil immersion.

3.2.3.3.4.3 Catalase test

A loopful of bacterial colony and one drop of three percent hydrogen peroxide were placed on a microscopic slide and mixed thoroughly. A positive reaction was indicated when there was production of gas bubbles which is an indication of effervescence of nascent oxygen within 1-2 minutes whereas absence of production of gas bubbles was taken as negative.

3.2.3.3.4.4 Oxidase test

Fresh bacterial growth was taken from the agar plate with a sterile inoculation loop and gently rubbed onto the paper which contains oxidizing reagent (tetramethyl-p-phenylenediamine dihydrochloride). Observations were recorded after 10 sec. A blue-violet colour was interpreted as a positive reaction.

3.2.3.3.4.5 Growth on Mannitol Salt Agar

The collected samples were streaked on Mannitol salt agar containing 7.5 per cent sodium chloride and incubated at 37° C for 24 hrs. The fermentation of mannitol was observed after 24 hours which was visible in the form of yellow colouration of the media and colonies (Table 1).

3.2.3.3.4.6 Growth on Edwards Agar

The collected samples were streaked on Edwards medium containing 5 per cent sodium chloride and incubated at 37° C for 24 hrs. The utilization of thallium salts was observed after 24 hours which was visible in the form of pin point brown colouration of the colonies (Table 1).

Fig 1: Isolation and identification of bacterial uropathogens in dogs affected with lower urinary tract infections

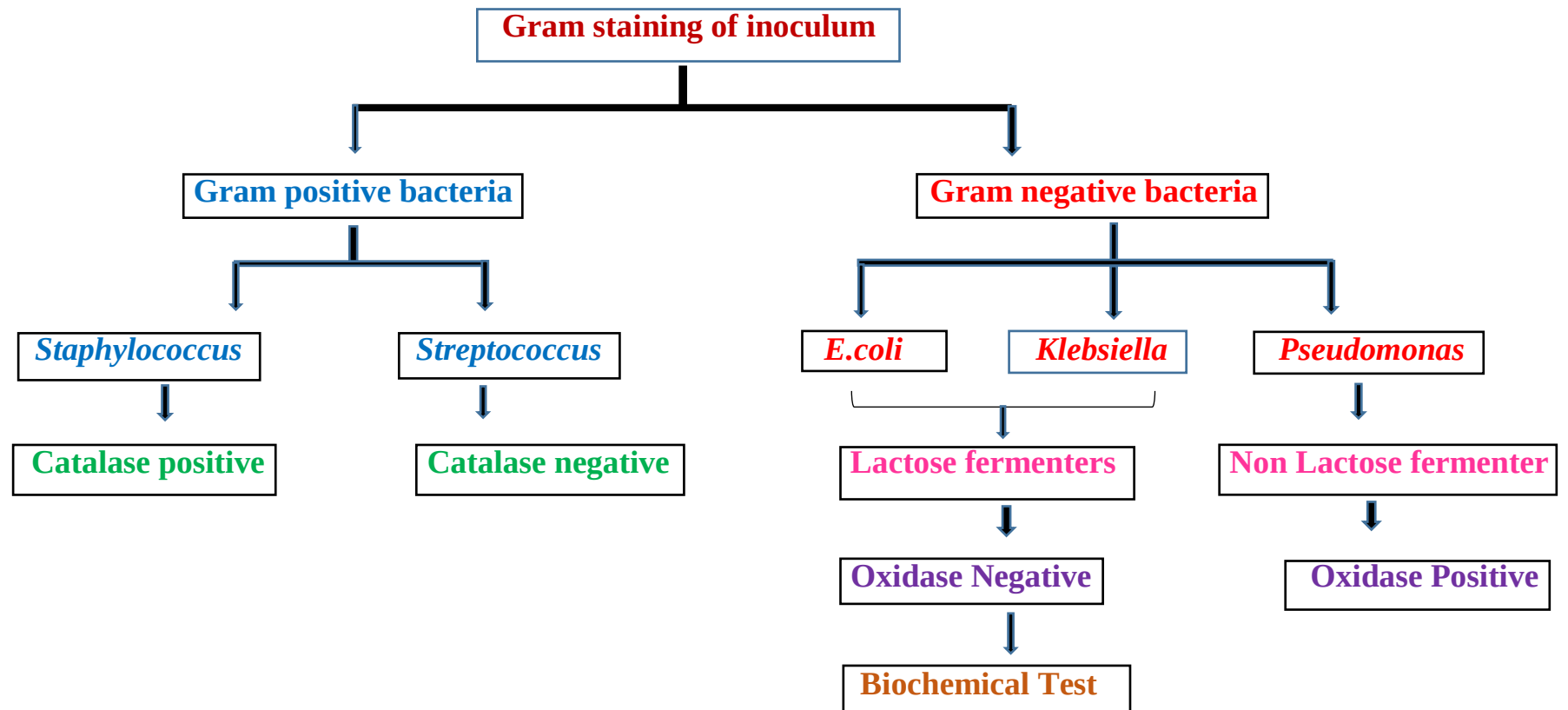


Table 1: Identification of bacteria by morphology of colonies on specific agar medium in dogs affected with lower urinary tract infections

SNo	Bacterial isolate	Mannitol salt agar	Edwards agar	EMB agar	MacConkey agar	Brain heart infusion Agar	Blood agar
1	<i>Stahylococcus</i> spp	Golden yellow colour colonies	---	---	---	---	Colonies with clear zone of haemolysis
2	<i>Streptococcus</i> spp	---	Pin pointed brown colour colonies	---	---	---	Small mucoid colonies with zone of haemolysis
3	<i>E.coli</i>	---	---	Growth of colonies greenish metallic sheen	Pink colour colonies	---	Zone of translucence around the colony with haemolysis
4	<i>Klebsiella</i> spp	---	---	---	Pink colour colonies	---	Mucoid and grey white colonies
5	<i>Pseudomonas</i> spp	---	---	---	White colour colonies	Flat green colour colonies	Large flat grey blue colour colonies

3.2.3.3.4.7 Growth on EMB Agar

The collected samples were streaked on Eosin methylene blue agar (EMB) and incubated at 37° C for 24 hrs. The growth was observed after 24 hours which was visible in the form of metallic sheen colouration of the colonies (Table 1).

3.2.3.3.4.8 Growth on MacConkey agar

The collected samples were streaked on MacConkey agar and incubated at 37° C for 24 hrs which aids in differentiation of lactose fermenters and lactose non-fermenters among gram negative bacteria. The fermentation of lactose was observed after 24 hours which was visible in the form of red or pink colouration of the colonies and non –lactose fermenters as colourless transparent colonies (Table 1).

3.2.3.3.4.9 Brain heart infusion agar

The collected samples were streaked on Brain heart infusion agar (BHI) and incubated at 37° C for 24 hrs. The growth was observed after 24 hours which was visible in the form of green colouration of the colonies (Table 1).

3.2.3.3.4.10 Indole Test

This test demonstrates the ability of certain bacteria to decompose the aminoacid tryptophane to indole which accumulates in the medium containing peptone (10gm), sodium chloride (5 gm) making the total volume to 500 ml. The medium is sterilized by autoclaving at 121°C for 15 minutes. The sample is then inoculated into the medium and incubated for 48 hours at 37°C. 0.5 ml of Kovac's reagent is added and shaken gently. Formation of red colour ring indicates a positive reaction.

3.2.3.3.4.11 Methyl red Test

This test determines the ability of acid production by fermentation of glucose in the medium which contains peptone (5 gm), dipotassium hydrogen phosphate (5 gm), Glucose (10%) for a total volume of 1 liter. The medium is sterilized at 121°C for 15 minutes. The sample is then inoculated and incubated at 37°C for 48 hours. Few drops of methyl red reagent is added and mixed gently. Bright red colouration indicates a positive test and yellow colour indicates a negative test.

3.2.3.3.4.12 Voges – proskauer Test

Sample is inoculated into a medium and incubated at 37°C for 48 hours. Then 1ml of 40% potassium hydroxide and 3 ml of 5% solution of α – naphtholin absolute ethanol is added to medium. A positive reaction is indicated by the development of a pink colour in 2-5 minutes which turns crimson in 30 minutes.

3.2.3.3.4.13 Citrate utilization Test

This test determines the ability of organisms to utilize citrate as source of carbon and ammonium salt as a source of nitrogen. The sample is inoculated into a medium containing agar (20gm) and 0.2 % bromothymol blue (40ml) making the total medium volume to 1 litre. Medium is transferred to test tubes which were autoclaved at 121°C for 15 min and allowed to set into slopes. Sample is inoculated and incubated for 96 hours at 37°C. Positive reaction is indicated by blue colour and streak of growth whereas negative reaction is indicated by original colour (green) and absence of growth.

3.2.4 Antibiotic susceptibility testing

ABST was performed in all the 33 dogs affected with bacterial LUTI by the standard Kirby Bauer disc diffusion method on Mueller Hinton Agar according to Clinical

and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2017). A single colony from the solid medium was suspended in sterile distilled water, the turbidity of the suspension was matched to Mc Farland standard tube number 0.5 (equivalent to an approximate cell density of 1.5×10^8 CFU/ml having absorbance of 0.132 at wavelength of 600 nm). This was used as inoculum to seed the MHA surface using sterile cotton swab and allowed to dry for 5 minutes with lid in place. The antibiotic discs were placed on MHA plates using sterile forceps with a minimum distance of 24 mm between the disc centers and incubated at 35° C for 16-18 hrs. Diameter of zone showing complete inhibition of growth of the organism under test was measured. Results were interpreted according to CLSI recommendations.

The bacterial isolates were tested for susceptibility to the following antibiotics viz., ceftriaxone with tazobactam (CIT 30/10), amoxicillin sodium with sulbactam (AMS 30/15), ciprofloxacin (CIP 5), amikacin (AK 30), trimethoprim-sulphamethaxazole (COT 25), nitrofurantoin (NIT 300) and gentamicin (GEN 10).

3.2.5 Haematology

Two milliliters of whole blood was collected from the cephalic or cephalic vein of the dog using sterile disposable syringes into vacutainers with EDTA for estimating haematological parameters such as haemoglobin, packed cell volume, total leukocyte count and differential leukocyte count (Jain, 1993). Care was taken during collection and processing of blood samples to avoid haemolysis.

3.2.6 Serum biochemistry

For separation of serum, 3 ml of blood was collected in clot activator vacutainer. Serum was separated immediately after clotting by centrifugation at 2000 rpm for 5 minutes.

All the serum biochemical parameters were estimated by semi auto analyzer (M/S Chem. -5 plus, ERBA, Mumbai) employing the kits supplied by M/S ERBA diagnostics, Mumbai.

3.2.6.1 Total protein

Total protein was estimated by end point method (Modified Biuret) and the values were expressed as g/dl.

3.2.6.2 Albumin

Albumin was estimated by end point method (Bromo cresol Green) and the values were expressed as g/dl

3.2.6.3 BUN

Blood urea nitrogen was estimated by initial rate assay (GLDH-UREASE) and the values were expressed in mg/dl.

3.2.6.4 Serum creatinine

Serum creatinine was estimated by initial rate assay (Modified Jaffes reaction) and the values were expressed in mg/dl.

3.2.7 Abdominal radiography

Abdominal radiographs were taken by placing dog in lateral and dorsal recumbency with computerized radiographic system (Core Stream Ltd.) {Plate 2A}.

3.2.8 Abdominal ultrasonography

3.2.8.1 Instrumentation

Abdominal ultrasound was performed by using ALOKA PROSOUND α 6LT ultrasound machine. A linear array 3.5 MHz, 5.0 MHz, and 7.5 MHz probes were used for large, medium and small dogs to perform abdominal ultrasonography.

3.2.8.2 Preparation and restraint of the patient

The hair over the abdomen was clipped and shaved. Ultrasonographic examination was performed by placing the animals in dorsal/lateral recumbency. A liberal amount of coupling gel was applied to provide sufficient contact (Mannion, 2008).

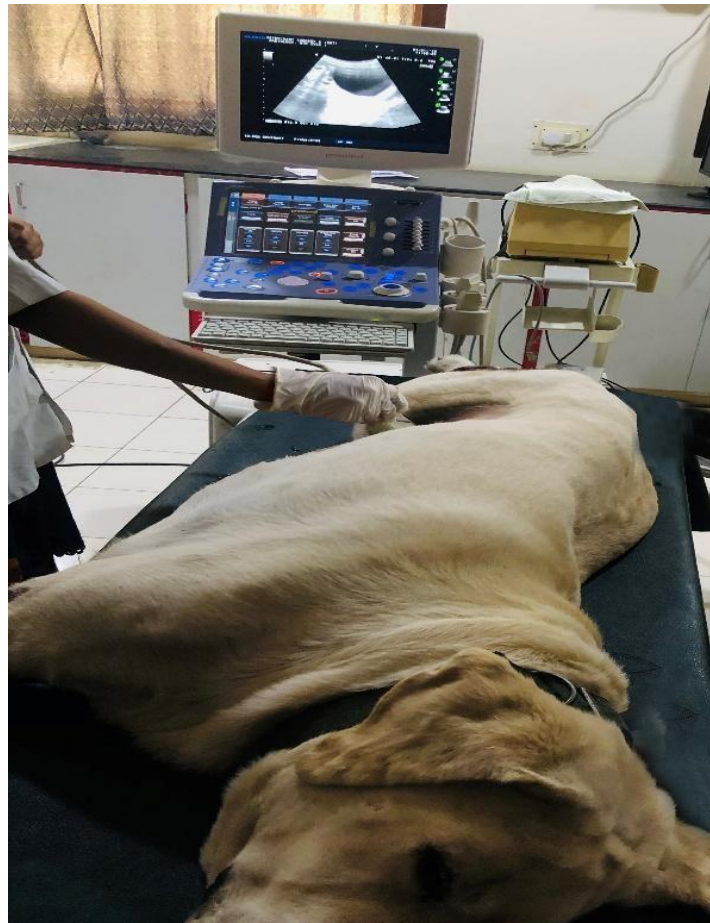
3.2.8.3 Abdominal ultrasonographic technique

After patient preparation, both sagittal and transverse scans of the bladder were performed. The sagittal scan plane is achieved by placing the transducer in the caudal abdominal area cranial to the pubis with the indicator mark pointing cranially. The urinary bladder was identified as an anechoic round or oblong structure with a thin, echogenic wall. The transducer swept slowly back and forth, left to right, to image the entire width of the bladder. In case of distended bladder, the transducer was positioned cranially and caudally to image the entire length of the bladder. Rotating the transducer 90 degrees counterclockwise produced a transverse image. The transducer was then moved cranially and caudally to image the entire length of the bladder in cross section. In male dogs the transducer was moved caudally to gain access to the prostate gland, which may be partially within the pelvic inlet. In the sagittal plane, the prostate was seen adjacent to the neck of the bladder as a hyperechoic, homogeneous, finely textured organ {Plate 2B}.

Plate -2: Diagnostic Imaging techniques



**A. Conventional X ray machine with computerized radiographic unit
[Printer(P), Monitor(M), Console(C)]**



B. Trans abdominal ultrasonography in a Labrador dog. Note the patient positioning, transducer placement and ultrasound unit

3.2.9 Therapeutic trial

Therapeutic trial was done in only 24 dogs affected with bacterial lower urinary tract infection. The dogs were randomly divided into three groups *i.e.*, Group I, Group II and Group III each having eight dogs with different therapeutic regimen. The dogs in which underlying factors have been identified were treated accordingly.

3.2.9.1 Group I

Dogs in this group were subjected for treatment with Inj. amoxicillin sodium and sulbactam @ 20mg/kg body weight twice daily I/M (Weese *et al.*, 2011).

3.2.9.2 Group II

Dogs in this group were subjected for treatment with Inj. ceftriaxone + tazobactam @ 20mg/kg I/M (Punia *et al.*, 2018)

3.2.9.3 Group III

Dogs in this group were subjected for treatment with Inj. amikacin @ 20mg/kg I/M (Rogers *et al.*, 1988).

In all the three groups of dogs antibiotic therapy was continued for a period of one week beyond clinical recovery.

3.2.9.4 Evaluation of therapeutic regimen

Response to therapy was evaluated based on the resolution of clinical signs, recovery pathway and negative bacteriological culture of urine after therapy. The dogs were examined at weekly intervals until resolution of clinical signs and negative bacteriological culture of urine.

3.2.10 Treatment of underlying/associated Factors

Dogs with cystoliths/uroliths as underlying cause in development of urinary tract infections were subjected for surgical correction whereas the dogs with prostate enlargement as an underlying cause in development of UTI were treated with antibiotic and advised for castration to prevent recurrence of infection.

3.2.11 Statistical analysis

The results obtained were subjected to statistical analysis as per the method described by Snedecor and Cochran (1994) by using SPSS 20.00 version.

CHAPTER IV

RESULTS

The present study was undertaken to record the occurrence, clinical signs, haemato-biochemical findings, isolation and identification of bacteria, antibiotic sensitivity testing in dogs affected with lower urinary tract infections and to evaluate suitable therapeutic regimen in dogs affected with bacterial lower urinary tract infections which were presented to the Veterinary Clinical Complex, NTR College of Veterinary Science, Gannavaram. A total number of 2930 dogs were screened during the period of January 2020 to December 2020 of which 246 dogs exhibited the signs of urinary tract infections which accounted for 8.39 per cent of total number of cases. Of the 246 cases, 82 (33.33%) were showing clinical signs suggestive of lower urinary tract infections. Thirty five LUTI cases which were preliminarily diagnosed by clinical signs and urinalysis were subjected to cultural examination of which 33 dogs were diagnosed to be affected with bacterial etiology and remaining two dogs were found negative for bacterial growth. The occurrence, clinical signs, haemato biochemical changes were recorded in 35 dogs affected with LUTI. However therapeutic trial was restricted to only 24 dogs affected with bacterial lower urinary tract infection.

4.1 OCCURRENCE

4.1.1 Occurrence of lower urinary tract infections

Occurrence was recorded in 35 dogs diagnosed to be affected with LUTI based on clinical signs and urinalysis selected for the study which was further analysed with respect to age, breed and gender.

4.1.1.1 Occurrence in Relation to Age

Age wise occurrence of lower urinary tract infections revealed highest per cent occurrence in dogs of 0-4 years (45.71%) age group followed by 4-7 years (31.43%), 8-10 years (14.29%) and least in greater than 10 years age group (8.57%) (Table 2 and Fig 2).

4.1.1.2 Occurrence in Relation to Breed

Out of 35 dogs affected with lower urinary tract infections highest occurrence of 28.57 per cent (10/35) was noticed in Labrador Retriever breed of dogs followed by 14.29 (5/35) per cent in Rottweiler; 11.43 (4/35) per cent each in German Shepherd and Pug; 8.57 (3/35) per cent each in Shih Tzu, Non-Descript and Pomeranian; 5.71 (2/35) per cent in Great Dane and 2.86 (1/35) per cent in Boxer (Table 3 and Fig 3).

4.1.1.3 Occurrence in Relation to Gender

Gender wise occurrence of lower urinary tract infections in the present study revealed highest occurrence in spayed female dogs (45.72%) followed by castrated male dogs (37.14 %), intact female dogs (11.43%) and least in intact male dogs (5.71 %) (Table 4 and Fig 4).

4.2 CLINICAL SIGNS

Clinical symptomatology of the 35 dogs affected with lower urinary tract infections include fever in 26 dogs (74.29%), haematuria in 24 dogs (68.57%), inappetence/anorexia in 18 dogs (51.43%), pollakiuria in 14 dogs (40.00%), oliguria in 13 dogs (37.14%), urinary incontinence in 11 dogs (31.43%), stranguria and vomiting in 10 dogs each (28.57%), pyuria, polyuria and anuria in 6 dogs each (17.14 %) and

Table 2: Age wise occurrence of lower urinary tract infections in dogs (n=35)

S.No	Age group	Number of dogs affected with lower UTI	Per cent of dogs affected with lower UTI
1.	0-4 years	16	45.71
2.	4-7 years	11	31.43
3.	7-10 years	5	14.29
4.	10 years and above	3	8.57
	TOTAL	35	100

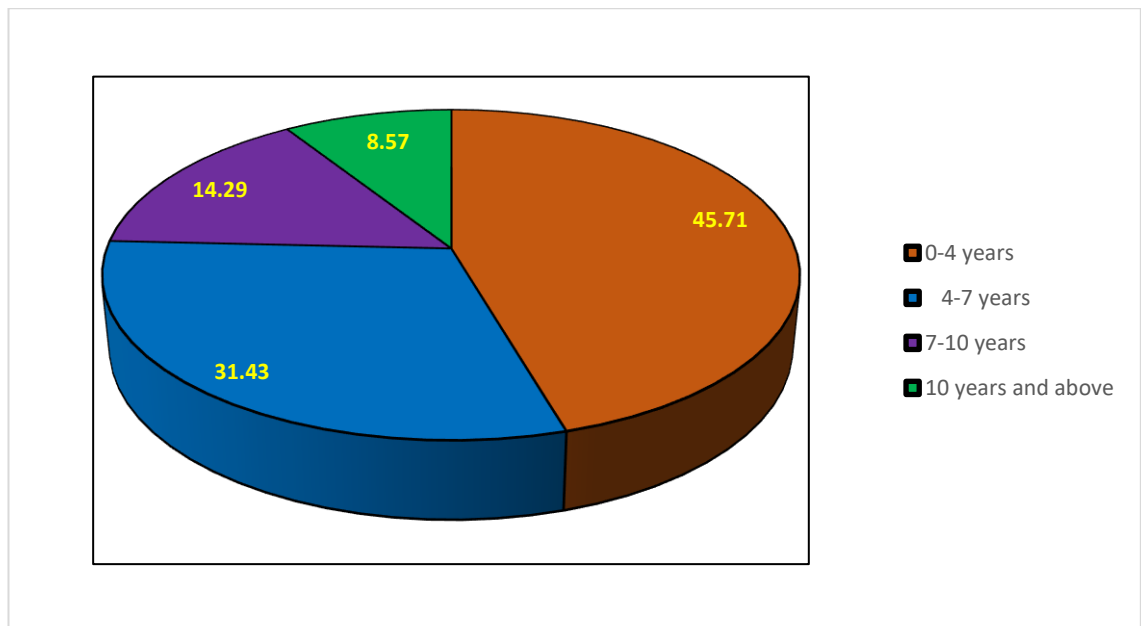
Fig 2: Age wise occurrence of lower urinary tract infections in dogs (n=35)

**Table 3: Breed wise occurrence of lower urinary tract infections in dogs
(n=35)**

S.No	Breed of dog	Number of dogs affected with UTI	Per cent of dogs affected with UTI
1.	Labrador Retriever	10	28.57
2.	Rottweiler	5	14.29
3.	German Shepherd	4	11.43
4.	Pug	4	11.43
5.	Non - Descript	3	8.57
6.	Pomeranian	3	8.57
7.	Shih Tzu	3	8.57
8.	Great Dane	2	5.71
9.	Boxer	1	2.86
	TOTAL	35	100

**Fig 3: Breed wise occurrence of lower urinary tract infections in dogs
(n=35)**

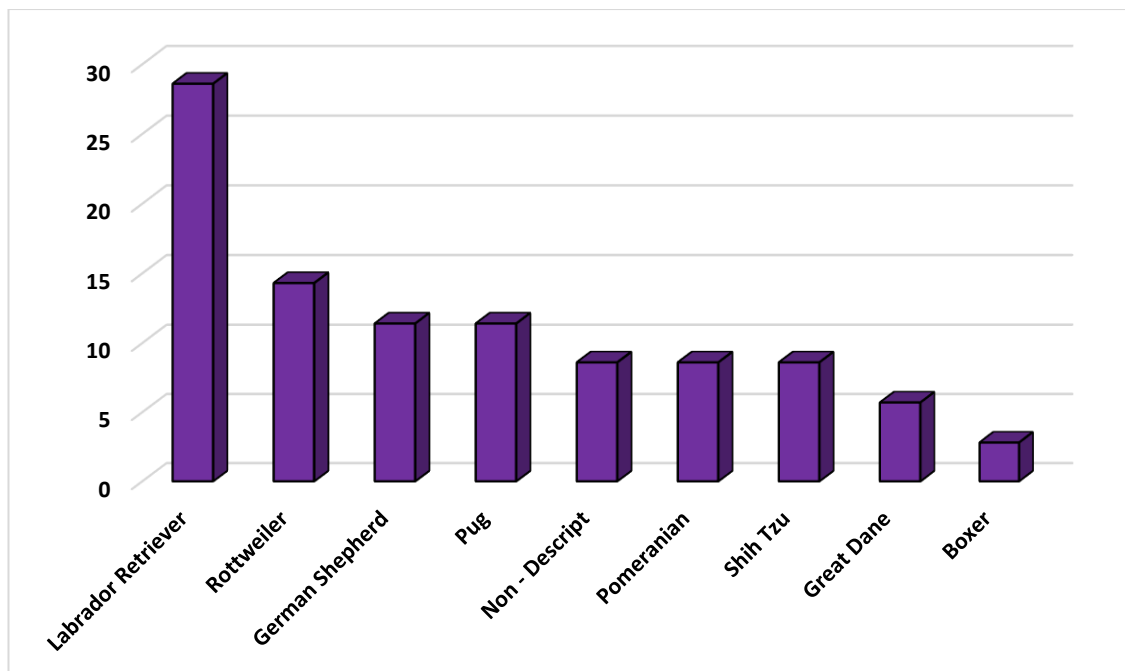


Table 4: Gender wise occurrence of Lower urinary tract infections in dogs (n=35)

SNo	Gender	Number of dogs affected with UTI	Per cent of dogs affected with UTI
1.	Castrated Male	13	37.14
2.	Intact Male	2	5.71
3.	Spayed Female	16	45.72
4.	Intact Female	4	11.43
	Total	35	100

Fig 4: Gender wise occurrence of lower urinary tract infections in dogs (n=35)

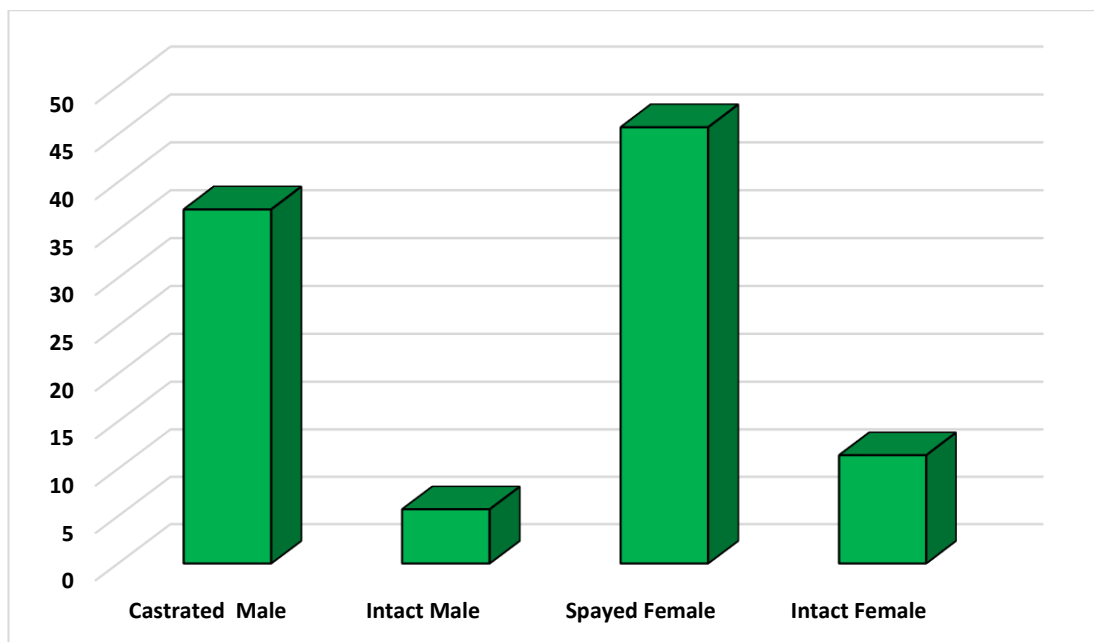


Table 5: Clinical signs in dogs affected with lower urinary tract infections (n=35)

S.No	Clinical sign	Number of positives (n=35)	Per cent
1.	Fever	26	74.29
2.	Haematuria	24	68.57
3.	Inappetence/Anorexia	18	51.43
4.	Pollakiuria	14	40.00
5.	Oliguria	13	37.14
6.	Urinary incontinence	11	31.43
7.	Stranguria	10	28.57
8.	Vomiting	10	28.57
9.	Pyuria	6	17.14
10.	Polyuria	6	17.14
11.	Anuria	6	17.14
12.	Polydipsia	4	11.43
13.	Dribbling of urine	4	11.43

Fig 5: Clinical signs in dogs affected with lower urinary tract infections (n=35)

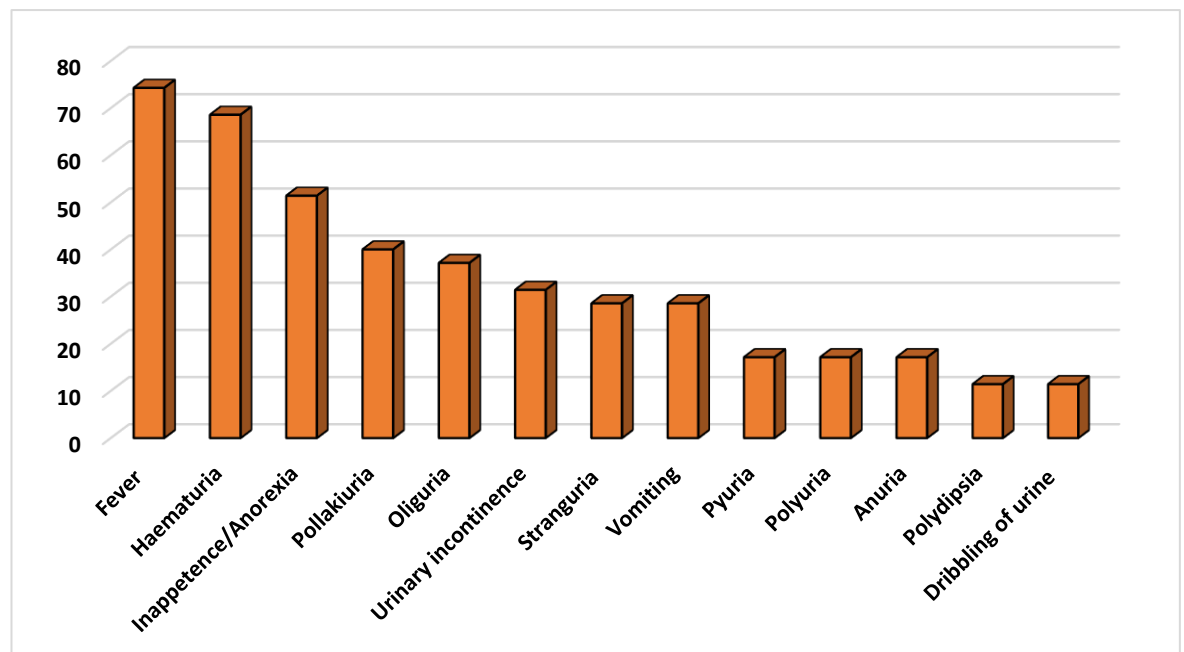
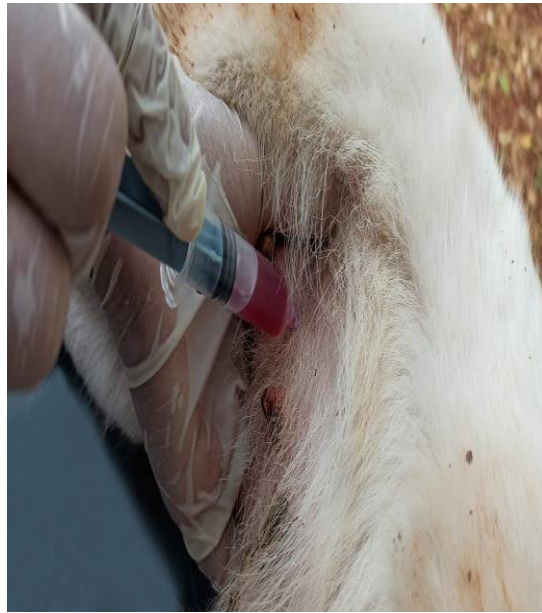


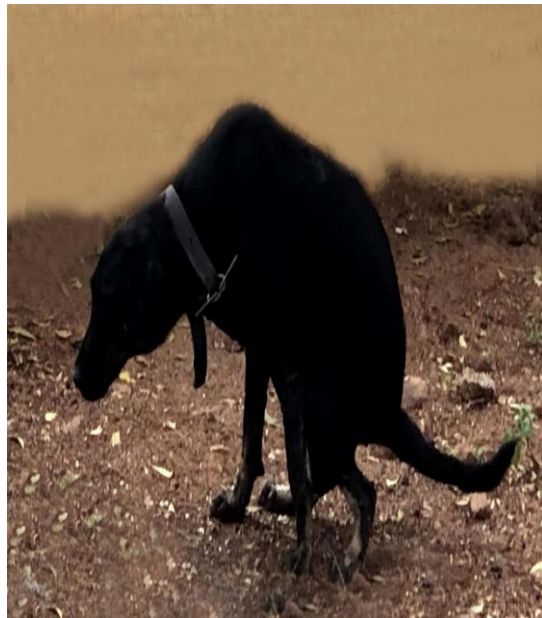
Plate -3: Clinical signs observed in dogs affected with lower urinary tract infections



A. Haematuria in a Pomeranian dog affected with lower urinary tract infection



B. Pyuria in a Pug dog affected with lower urinary tract infection



C. Stranguria in a Labrador retriever dog affected with lower urinary tract infection



D. Male dog urinating like female dog in cystitis

polydipsia as well as dribbling of urine in 4 dogs each (11.43%) (Table 5, Fig 5 and Plate 3).

4.3 DIAGNOSIS

Diagnosis of lower urinary tract infections was done based on urinalysis, abdominal radiography, abdominal ultrasonography as well as by isolation and identification of bacteria by cultural examination of urine.

4.3.1 Urinalysis

Urine samples were collected from dogs affected with lower urinary tract infections by cystocentesis. The samples were analysed for physical and chemical examination by using urinary dipsticks. Further the specific gravity is measured by using refractometer.

As depicted in Table 6, 7 & 8 the urinalysis findings in dogs affected with lower urinary tract infections varied individually. The colour of urine varied from pale yellow to red colour. Pale yellow colour of urine was observed in 9 dogs while the colour was dark yellow in 9 dogs, yellow in 10 dogs, red colour and green colour in 3 dogs each and amber coloured in 1 dog (Plate 4).

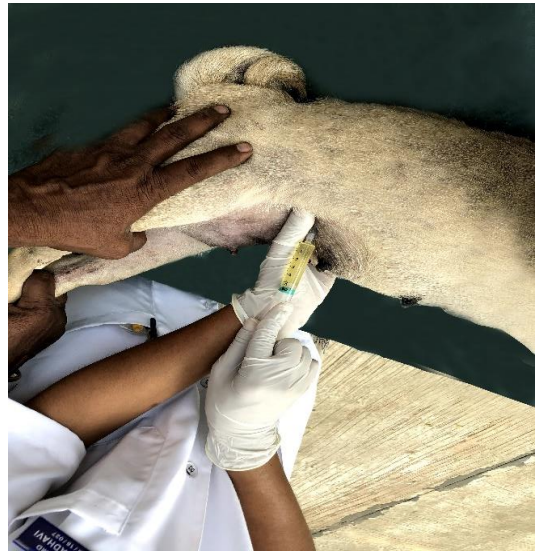
Urinary pH ranged from 5.0 to 9.0 in dogs affected with LUTI while the specific gravity ranged between 1.01 to 1.05 with a mean specific gravity of 1.03 and transparency varied from clear to turbid urine which was clear, mildly turbid, moderately turbid and severely turbid in 18, 5, 4 and 8 dogs respectively (Plate 4).

The chemical examination of urine revealed presence of proteins with a concentration of 1.0 g/L in thirteen dogs, 0.3 g/L in nine dogs, 0.15g/L in seven dogs, 3.0 g/L in one dog and no protein concentration in five dogs. The presence of bile salts was

Plate -4: Variations in urine colour and transparency in dogs affected with lower urinary tract infections



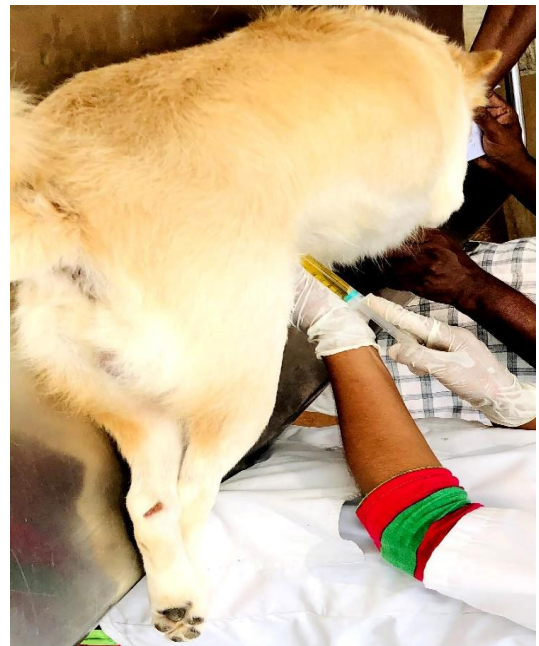
A. Pale yellow colour of urine in dog affected with bacterial LUTI



B. Yellow coloured urine in a Pug affected with bacterial LUTI



C. Turbid urine in a German Shepherd dog affected with bacterial LUTI



D. Dark yellow coloured urine in a Pomeranian dog affected with bacterial LUTI

noticed in 5 dogs, bile pigments in 3 dogs and occult blood in 8 dogs. All the dogs taken up for the study were negative for sugars, nitrates and ketones.

4.3.2 Urine sediment examination

Microscopic examination of urine sediment in dogs affected with lower urinary tract infections in dogs revealed the presence of epithelial cells, RBC, struvite crystals, granular casts, WBC, hyaline casts, calcium oxalate crystals, renal tubular epithelial casts, triple phosphate crystals, bilirubin crystals, tyrosine crystals and bacteria either alone or in combination (Table 6,7&8; Plate 5, 6 & 7).

4.3.3 Abdominal Radiography

Abdominal radiography performed in all the 35 dogs affected with lower urinary tract infections revealed prostate enlargement in 3 dogs (8.57%), urethroliths in 1 dog (2.85%), cystoliths in 2 dogs (5.71%), distension of bladder and distension of bladder with a strip of gas in lumen in 12 dogs (34.28%) each and increased radioopacity of the bladder wall in remaining 5 (14.29%) dogs (Plate 8).

4.3.4 Abdominal Ultrasonography

The abdominal ultrasonography findings in dogs affected with lower urinary tract infections revealed thickened bladder wall (> 2mm) in 16 dogs (45.71%), hyperechoic calculi in lumen and neck of bladder with acoustic shadow in 2 dogs (5.71%), sludge in lumen of bladder in 11 dogs (31.44%), irregularity of bladder mucosa with hyperechoic bladder wall in 3 dogs (8.57%) while the remaining 3 dogs (8.57%) had prostate enlargement (Plate 9&10).

Table 6: Urinalysis findings in dogs affected with Lower UTI (n=12)

SNo	Urine colour	pH	Transparency	Specific gravity	Protein (g/L)	Blood	sugars	Ketones	Bile salts	Bile pigments	Nitrates	Microscopic Examination of urine sediment (/ hpf)
1.	Yellow	5.5	Clear	1.02	-	-	-	-	-	-	-	3-4 epithelial cells
2.	Dark yellow	9.0	Moderately turbid	1.02	0.3	-	-	-	+	-	-	2-4 hyaline casts and 3-5 epithelial cells
3.	Dark yellow	7.0	Clear	1.01	1.0	-	-	-	-	-	-	3-6 hyaline casts and 2-3 epithelial cells
4.	Red	8.0	Severely turbid	1.03	1.0	+	-	-	+	+	-	3-5 RBC's
5.	Pale yellow	6.0	Clear	1.02	0.3	-	-	-	-	-	-	NAD
6.	Pale yellow	9.0	Clear	1.04	0.15	-	-	-	-	-	-	2-5 granular casts
7.	Dark yellow	9.0	Moderately turbid	1.03	1.0	-	-	-	-	-	-	3-6 epithelial cells
8.	Green	8.0	Severely turbid	1.05	3.0	-	-	-	+	-	-	3-6 Struvite crystals
9.	Dark yellow	8.0	Severely turbid	1.04	0.15	+	-	-	-	-	-	2-5 Epithelial cells and 2-4 RBC
10.	Yellow	6.0	Clear	1.05	0.3	-	-	-	-	-	-	3-5 Calcium oxalate crystals
11.	Yellow	5.0	Clear	1.02	0.15	-	-	-	-	-	-	2-3 WBC, 1-2 Epithelial cells and 1-
12.	Yellow	8.5	Clear	1.03	1.0	-	-	-	-	-	-	>5 Struvite crystals

NAD – No abnormality detected

/hpf – high power field

+/- Present/absent

Table 7: Urinalysis findings in dogs affected with Lower UTI (n=12)

S.No	Urine colour	pH	Transparency	Specific gravity	Protein (g/L)	Blood	sugars	Ketones	Bile salts	Bile pigments	Nitrates	Microscopic Examination of urine sediment (/ hpf)
13.	Green	9.0	Severely turbid	1.04	-	-	-	-	-	-	-	NAD
14.	Dark yellow	9.0	Mildly turbid	1.04	1.0	+	-	-	+	-	-	> 5 Renal tubular epithelial casts
15.	Dark yellow	8.0	Severely turbid	1.03	0.3	-	-	-	-	-	-	2-4 RBC, Triple phosphate crystals
16.	Pale yellow	7.0	Clear	1.01	0.15	-	-	-	-	-	-	NAD
17.	Yellow	6.5	Clear	1.02	-	-	-	-	-	-	-	NAD
18.	Red colour	6.0	Moderately turbid	1.05	0.3	+	-	-	-	-	-	> 5 RBC
19.	Amber	8.0	Severely turbid	1.04	0.15	+	-	-	-	-	-	3-4 Epithelial cells, RBC and WBC
20.	Dark yellow	5.5	Moderately turbid	1.02	1.0	-	-	-	-	-	-	Calcium oxalate crystals, Triple phosphate crystals
21.	Yellow	9.0	Clear	1.04	1.0	-	-	-	-	-	-	2-4 RBC and Epithelial cells
22.	Yellow	6.0	Clear	1.02	1.0	-	-	-	-	-	-	Motile Bacteria and 3-5 Renal tubular epithelial
23.	Pale Yellow	9.0	Clear	1.03	0.15	-	-	-	-	+	-	Bilirubin and tyrosine crystals
24.	Pale yellow	9.0	Clear	1.02	-	-	-	-	-	-	-	NAD

NAD – No abnormality detected

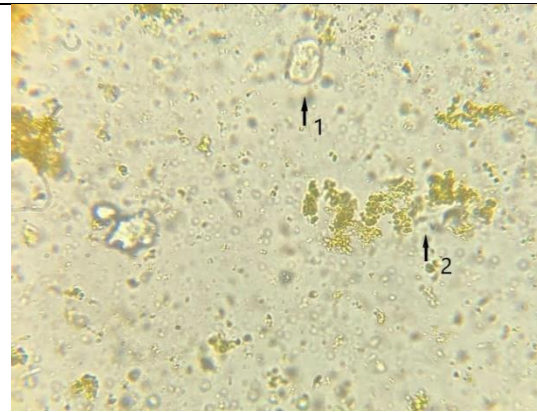
/hpf – high power field

+/- Present/absent

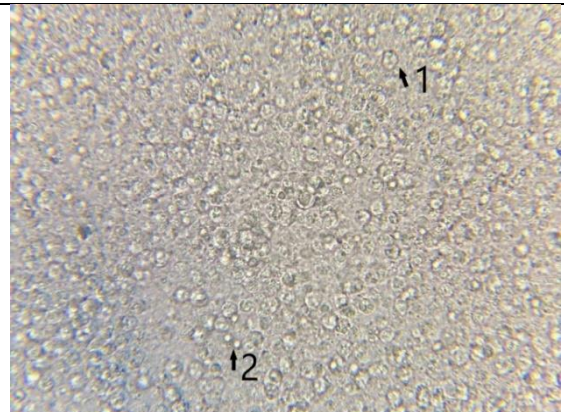
Table 8: Urinalysis findings in dogs affected with Lower UTI (n=11)

S.No	Urine colour	pH	Transparency	Specific gravity	Protein (g/L)	Blood	sugars	Ketones	Bile salts	Bile pigments	Nitrates	Microscopic Examination of urine sediment (/ hpf)
25.	Yellow	8.0	Clear	1.02	0.3	-	-	-	-	-	-	NAD
26.	Dark yellow	9.0	Mildly turbid	1.04	1.0	+	-	-	-	-	-	2-4 RBC > 5
27.	Yellow	7.0	Mildly turbid	1.03	0.3	-	-	-	-	-	-	> 5 Renal tubular epithelial casts
28.	Pale yellow	7.0	Clear	1.02	1.0	-	-	-	-	+	-	NAD
29.	Pale yellow	7.5	Clear	1.04	-	-	-	-	-	-	-	NAD
30.	Green	6.0	Severely turbid	1.05	1.0	-	-	-	+	-	-	> 5 RBC
31.	Pale yellow	9.0	Mildly turbid	1.04	0.15	-	-	-	-	-	-	Calcium oxalate crystals
32.	Red colour	6.5	Clear	1.02	0.3	+	-	-	-	-	-	3-4 Epithelial cells
33.	Dark yellow	9.0	Severely turbid	1.04	1.0	+	-	-	-	-	-	2-4 RBC and Epithelial cells
34.	Yellow	6.0	Mildly turbid	1.02	0.3	-	-	-	-	-	-	Motile Bacteria and 3-5
35.	Pale yellow	8.0	Clear	1.03	1.0	-	-	-	-	-	-	NAD

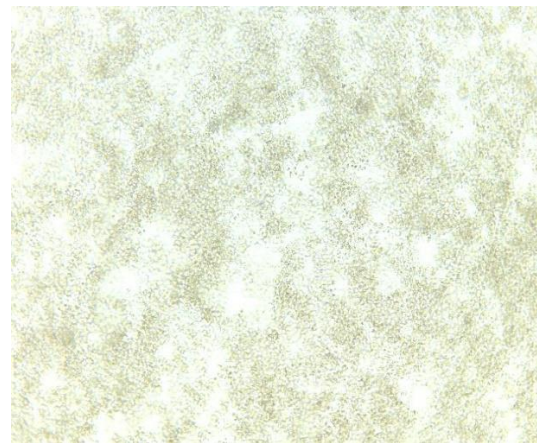
Plate -5: Microscopic examination of urine sediment in dogs affected with lower urinary tract infections (40X)



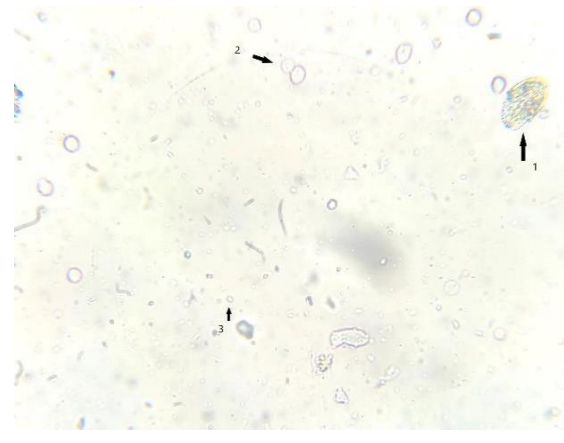
A. Epithelial cells(1) and granular casts (2)



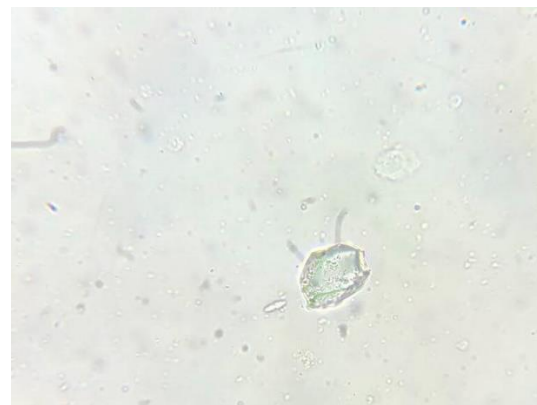
B. WBC (1) and RBC (2)



C. RBC cast



D. Epithelial cell (1), WBC (2) and RBC (3)

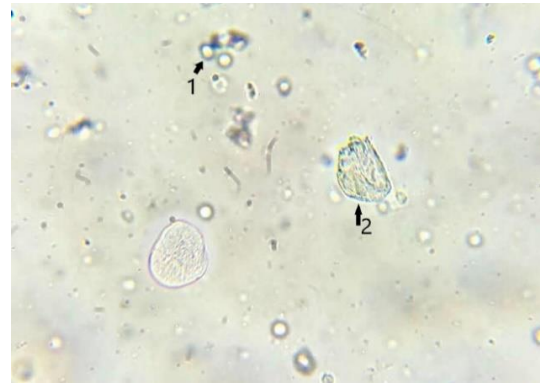


E. Uric acid cast in urine sediment

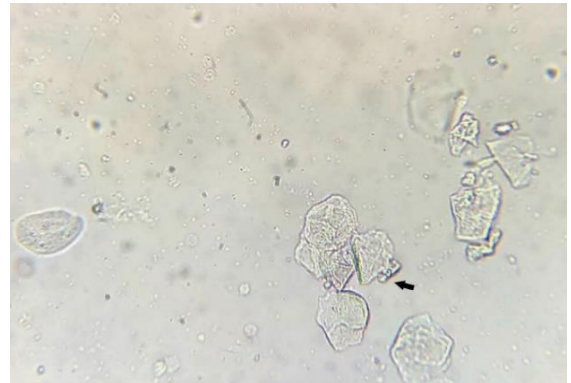


F. Granular cast in urine sediment

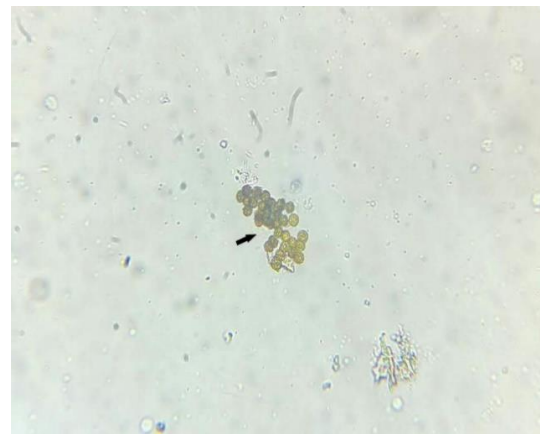
Plate -6: Microscopic examination of urine sediment in dogs affected with lower urinary tract infections (40X)



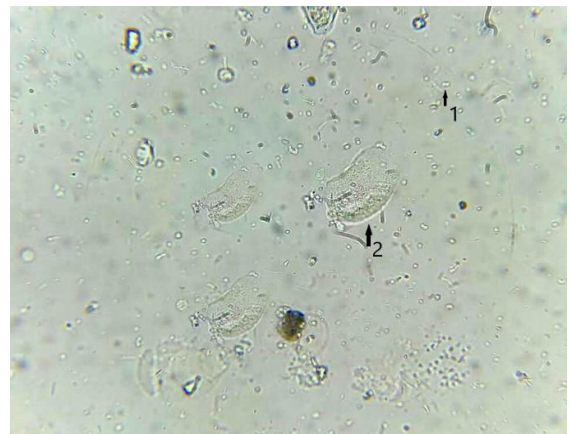
A. Fat droplets(1) and Epithelial cells(2)



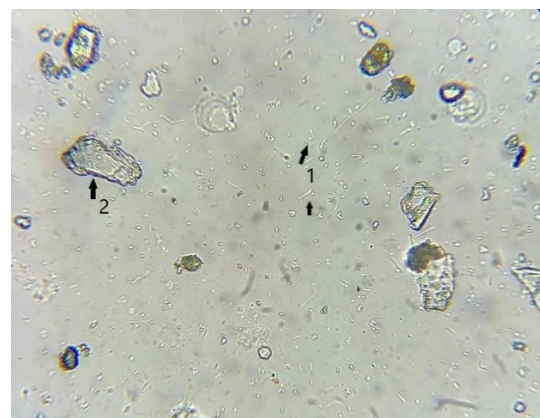
B. Epithelial cast in urine sediment



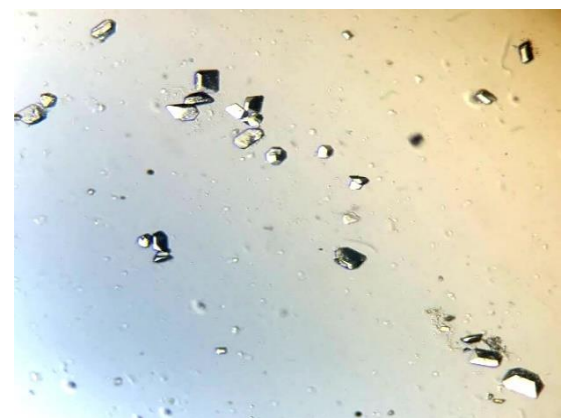
C. Renal tubular epithelial cast



D. Bacteria (1) and Hyaline cast (2)

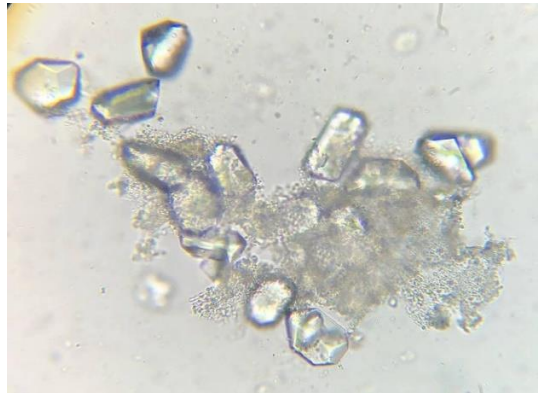


E. Bacteria (1) and Epithelial cell (2)



F. Calcium oxalate dihydrate crystals

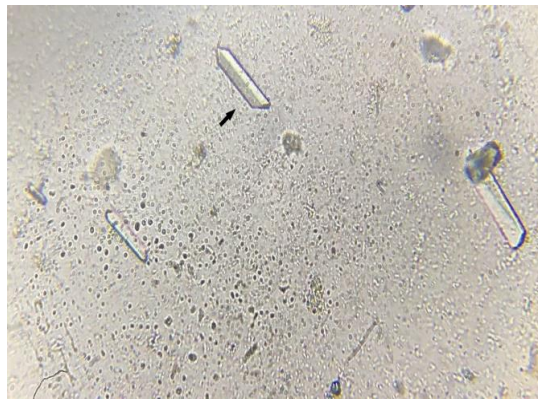
Plate -7: Microscopic examination of urine sediment in dogs affected with lower urinary tract infections (40X)



A. Triple phosphate crystals in urine sediment



B. Waxy casts in urine sediment



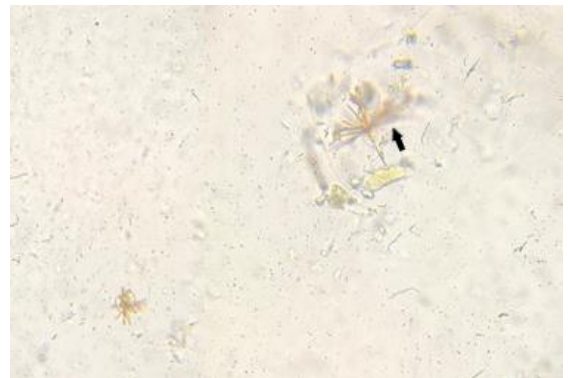
C. Calcium oxalate monohydrate crystals



D. Struvite crystals in urine sediment



E. Bilirubin crystals in urine sediment



F. Tyrosine crystals in urine sediment

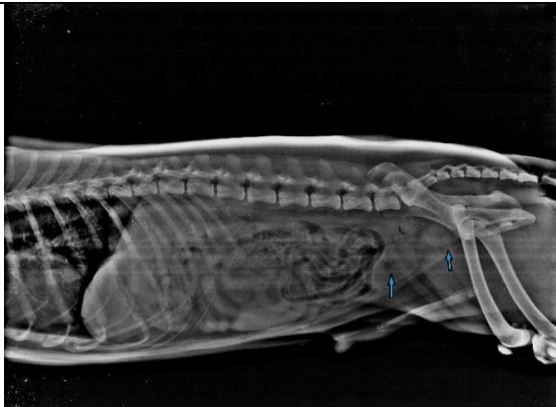
Plate -8: Radiographic findings on lateral view of abdomen in dogs affected with lower urinary tract infections



A. Skiagram showing distension of urinary bladder in dog affected with LUTI



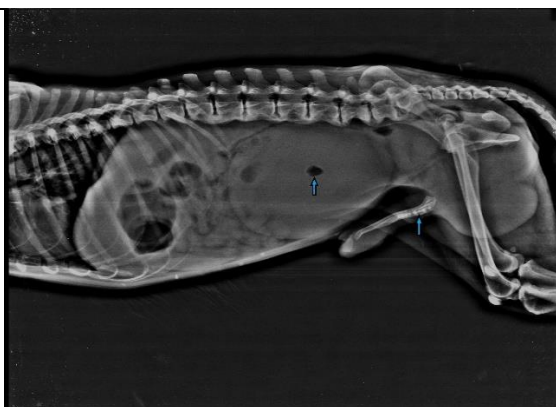
B. Skiagram showing presence of strip of air and cystoliths in a dog affected with lower urinary tract infection



C. Skiagram showing prostate enlargement and air in the lumen of bladder in a dog affected with cystitis



D. Skiagram showing radio opaque calculi in lumen of bladder in dog affected with LUTI

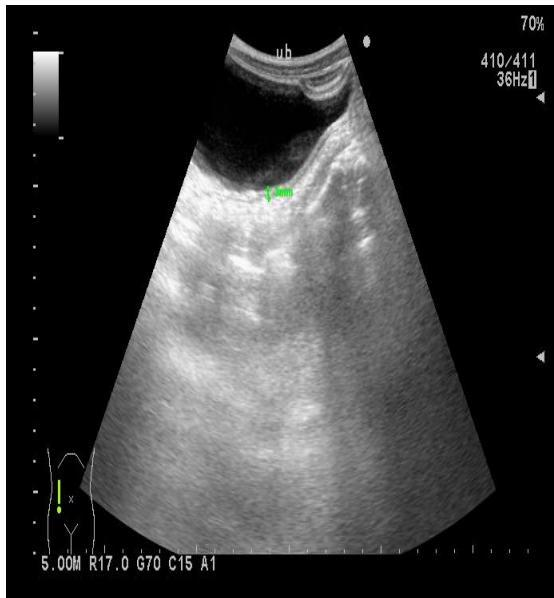


E. Skiagram showing distension of urinary bladder with presence of air in lumen of bladder and radio opaque urethroliths in penile urethra



F. Skiagram showing distension of urinary bladder with prostate enlargement in a dog affected with cystitis

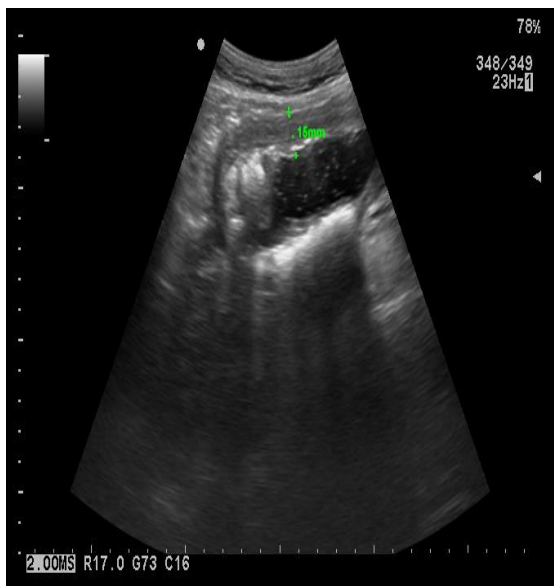
Plate-9: Trans abdominal B mode ultrasonographic findings in dogs affected with lower urinary tract infections



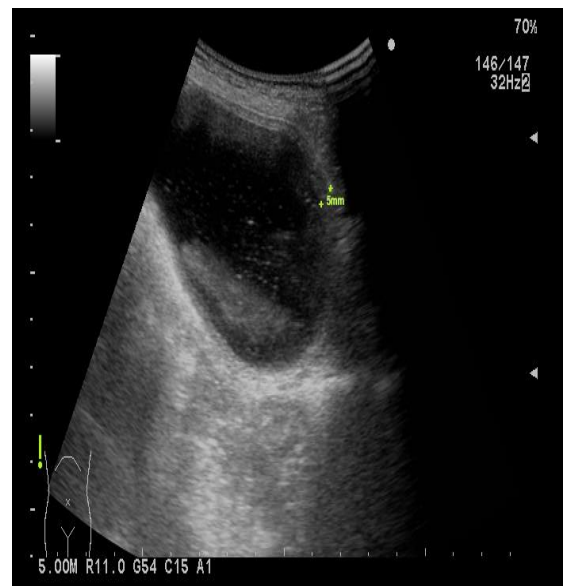
A. Sonogram showing thickened bladder wall with presence of sludge in bladder in a dog affected with cystitis



B. Sonogram showing bladder wall thickening and presence of sediment in dog affected with LUTI

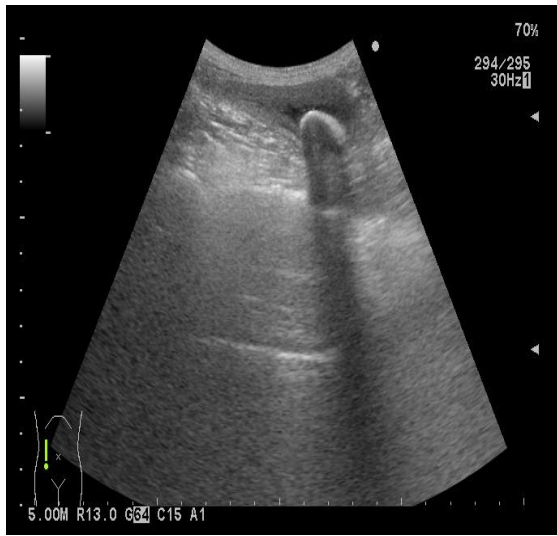


C. Sonogram showing severe thickening with hyper echogenicity of bladder wall and hyperechoic minute calculi in lumen of bladder



D. Sonogram showing thickened bladder wall with presence of echogenic minute calculi in lumen of bladder

Plate-10: Trans abdominal B mode Ultrasonographic findings in dogs affected with lower urinary tract infections



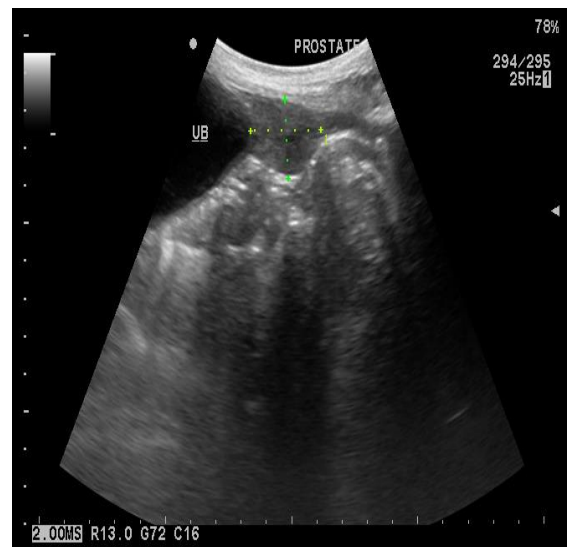
A. Sonogram showing single calculi in lumen of urinary bladder in a female dog with acoustic shadow



B. Sonogram showing presence of emphysema in lumen of urinary bladder and hyper echogenic calculi with acoustic shadow in a dog affected with emphysematous cystitis



C. Sonogram showing prostate enlargement in a dog affected with LUTI



D. Sonogram showing enlargement of prostate in a dog affected with lower urinary tract infections

4.3.5 Isolation and identification of bacteria

Thirty five samples from dogs affected with lower urinary tract infections were subjected for bacterial isolation and identification. The colonies were randomly picked from blood agar and were subjected for grams staining, catalase test, oxidase test and biochemical tests while confirmation was done based on morphology of the colonies on specific agar medium. Out of 35 samples in 33 samples bacterial growth was seen while in the remaining 2 samples no bacterial growth was observed.

Out of thirty five samples collected from dogs affected with lower urinary tract infections which were subjected for cultural examination of which 33 samples (94.28%) were found positive for bacterial isolates and 2 (5.71%) samples were found negative for bacterial growth on culture. Of the 33 positive samples 11 (33.33%) were positive for *E.coli*, 7 (21.21%) were positive for *Staphylococcus* spp, 6 (18.18%) were positive for *Streptococcus* spp, 4 (12.12%) were positive for *Pseudomonas* spp, 3 (9.10%) were positive for mixed infection of *E.coli* and *Staphylococcus* spp and 2 (6.06%) were positive for *Klebsiella* spp. The occurrence of lower urinary tract infections was assessed in 35 dogs diagnosed as affected with bacterial etiology (Table 9 and Fig 6).

4.3.5.1 Haemolysis pattern on Blood agar

As depicted in Plate 11 in the 33 samples with positive bacterial growth taken up for the study haemolysis pattern on blood agar revealed large colonies with clear zone of haemolysis on blood agar indicating *Staphylococcus* spp in 7 dogs, haemolysis on blood agar with small mucoid colonies indicating *Streptococcus* spp in 6 dogs, haemolysis with zone of translucence around the colony indicating *E.coli* in 11 dogs, mucoid and grey white colonies is indicating *Klebsiella* spp in 2 dogs , 1 large flat grey blue colour colonies indicating *Pseudomonas* spp in 4 dogs and in 3 dogs both *E.coli* and *Staphylococcus* were seen with haemolysis.

Plate-11: Haemolysis pattern of bacterial isolates on blood agar in dogs affected with lower urinary tract infections



A. Colony morphology of *Pseudomonas* spp on blood agar



B. Colony morphology of *Staphylococcus* spp on blood agar



C. Colony morphology of *E.coli* on blood agar



D. Colony morphology of *Streptococcus* spp on blood agar



E. Colony morphology of *Klebsiella* spp on blood agar

4.3.5.2 Gram's staining

Samples from all the 33 dogs taken up for detailed study were stained by gram stain revealing the presence of purple appearance of only gram positive organisms in 13 dogs while in the 17 dogs pale reddish appearance of only gram negative organisms were observed and in remaining 3 dogs mixed appearance of gram positive and gram negative organisms were observed. Gram positive organisms observed in the present study were *Staphylococci* (bunches of cocci) in 7 samples while in the other 6 samples presence of chains characteristic of *Streptococci* was seen. The gram negative organisms with rod shaped morphology observed in the present study were *E.coli* (11), *Klebsiella* (2) and *Pseudomonas* (4) and in remaining 3 dogs mixed infection of gram positive *Staphylococci* and gram negative *E.coli* were observed (Plate 12).

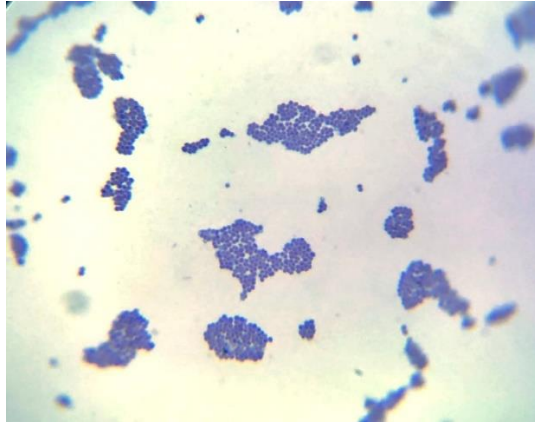
4.3.5.3 Catalase test

Among the 33 samples of dogs with LUTI 13 samples alone and 3 mixed infection samples were found to be gram positive which were further subjected for catalase test to differentiate *Streptococcus* and *Staphylococcus*. Out of these 7 alone and 3 mixed samples were found positive for catalase test which was evident in the form of production of gas bubbles on addition of culture to 3 per cent hydrogen peroxide which is due to the release of nascent oxygen indicative of *Staphylococci* while the remaining 6 were catalase negative and indicative of *Streptococci* (Plate 14A).

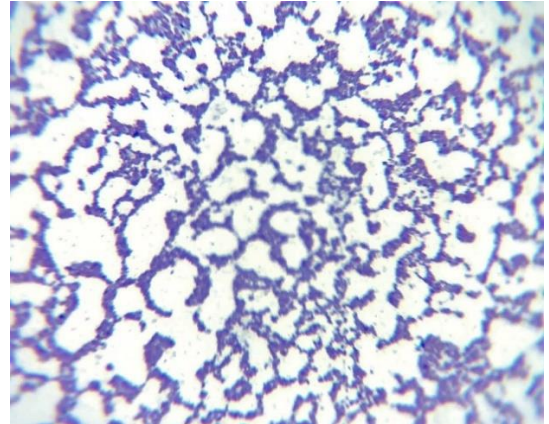
4.3.5.4 Oxidase test

Among the 33 samples of dogs with LUTI 17 samples were found to be gram negative alone and 3 with mixed infection samples which were further subjected for oxidase test to differentiate *E.coli*, *Pseudomonas* and *Klebsiella*. Out of these 4 were found positive for oxidase test which was evident in the development of oxidase disc from colourless to blue-violet colour in 5-10 seconds on addition of culture due to oxidation of cytochrome c enzyme which releases oxidase enzyme indicating *Pseudomonas* organisms while the remaining 13 samples and 3 mixed infection samples were negative for oxidase test. (Plate 14B).

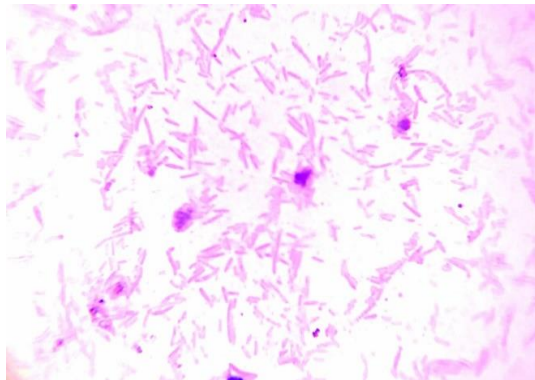
Plate-12: Microscopic examination of morphology of bacterial uropathogens on gram staining (100x)



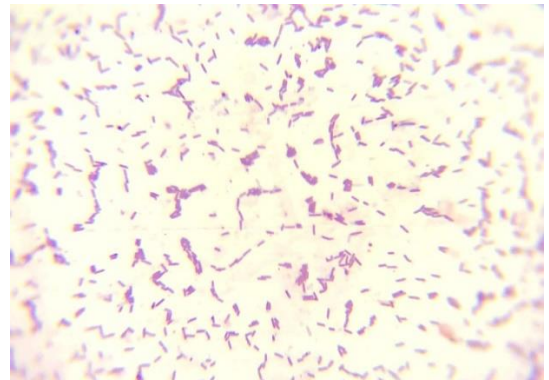
A. Clusters of gram positive *Staphylococcus* on gram's staining



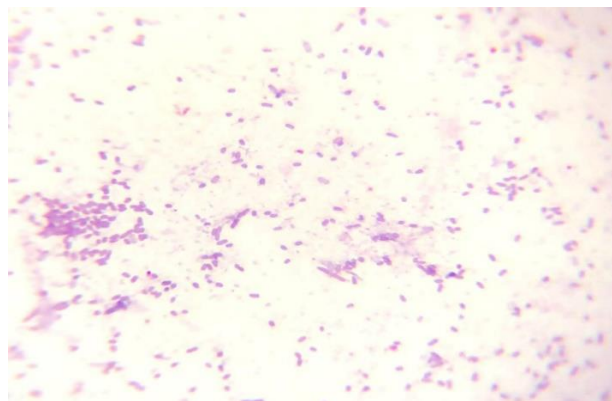
B. Clusters of gram positive *Streptococcus* on gram's staining



C. Clusters of gram negative *Klebsiella* on gram's staining



D. Clusters of gram negative *Pseudomonas* on gram's staining



E. Clusters of gram negative *E.coli* on gram's staining

4.3.5.5 Growth on Mannitol salt agar

All the 7 dogs alone affected with *Staphylococcus* spp and 3 dogs with mixed infection were found to ferment mannitol which was observed in the form of yellow colour discolouration on MSA (Plate 13A).

4.3.5.6 Growth on Edward's agar

Out of 33 dogs affected with lower urinary tract infections 6 were found positive for *Streptococcus* spp which was visible in the form of pin pointed brown colour colonies by utilization of thallium salts in Edwards medium (Plate 13B).

4.3.5.7 Growth on EMB agar

Among the 33 dogs affected with LUTI 11 alone and 3 mixed infection samples were found to be positive for *E.coli* which was evident in the form of greenish metallic sheen growth of colonies on EMB agar (Plate 13C).

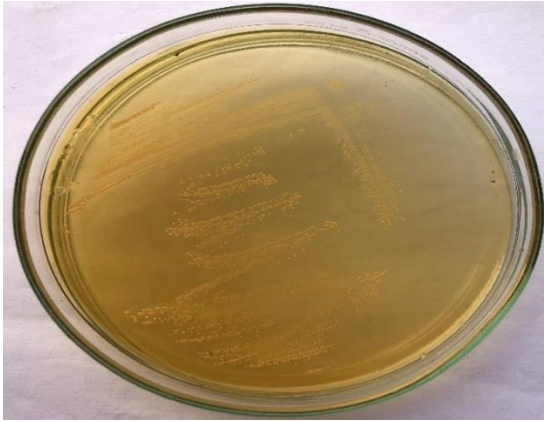
4.3.5.8 Growth on MacConkey agar

All the 17 alone gram negative samples and 3 mixed infection samples were streaked on MacConkey agar. Out of 17 *E.coli* spp (11) , *Klebsiella* spp (2) and 3 mixed infection samples have developed pink colour colonies on MacConkey agar by fermentation of lactose whereas *Pseudomonas* spp (4) developed white colour colonies on MacConkey due to absence of fermentation of lactose (Plate 13D).

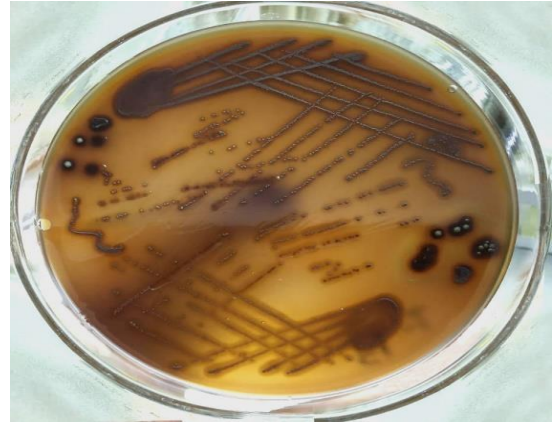
4.3.5.9 Brain heart infusion Agar

Among all the 33 samples of dogs with LUTI 4 samples were found positive for *Pseudomonas* spp which developed flat green colour colonies on streaking onto BHI agar medium (Plate 13E).

Plate-13: Growth of isolated bacterial uropathogens on specific agar medium



A. *Staphylococcus* spp on MSA



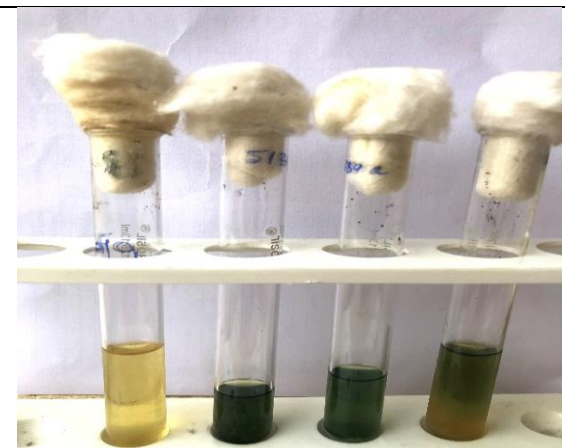
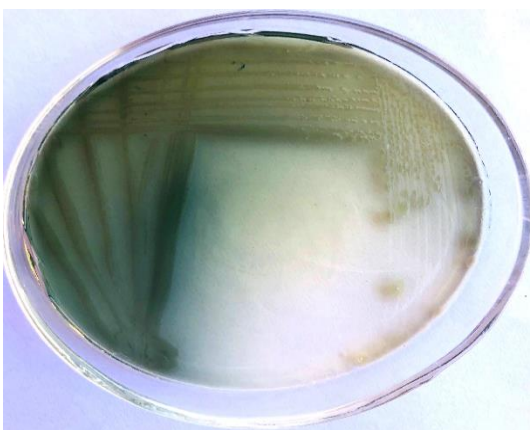
B. *Streptococcus* spp on Edwards medium



C. *E.coli* on EMB agar



D. *Klebsiella* spp on MacConkey agar



E. *Pseudomonas* spp on BHI agar and BHI broth

4.3.5.10 Indole test

Among all 33 samples of dogs with LUTI the 11 samples detected with *E.coli* alone and 3 mixed infection samples were found positive for indole test by development of red colour ring due to decomposition of amino acid tryptophan to indole which accumulates in medium whereas 2 *Klebsiella* samples were found negative to indole due to absence of decomposition of tryptophan to indole (Plate 14C & 14D).

4.3.5.11 Methyl Red test

Among all 33 samples of dogs with LUTI the 11 samples detected with *E.coli* alone and 3 mixed infection samples were found positive for methyl red test by development of bright red colour on addition of methyl red reagent to the medium due to acid production by fermentation of glucose which accumulates in medium whereas 2 *Klebsiella* spp were found negative to methyl red by persistence of yellow colour even after addition of methyl red reagent to the culture medium (Plate 14C & 14D).

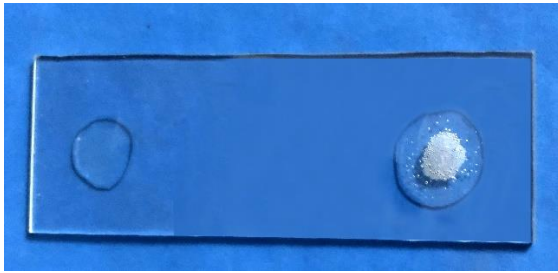
4.3.5.12 Voges proskauer test

The two *Klebsiella* isolates were found to develop pink colour which turns to crimson by addition of reagent A and reagent B which contains 40% potassium hydroxide and 5% solution of α – naphthol in absolute ethanol indicating a positive reaction. On the other side the eleven *E.coli* isolates alone and 3 mixed infection samples were found to be negative for voges proskauer test without any colour development in the medium even after addition of reagent A and B (Plate 14C & 14D).

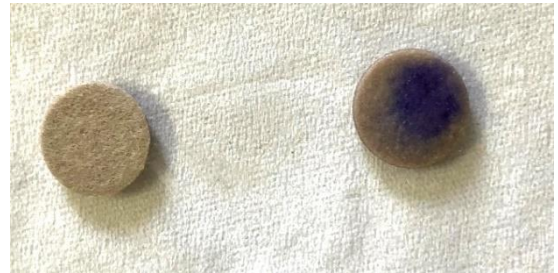
4.3.5.13 Citrate utilization test

The two *Klebsiella* isolates were found positive for citrate utilization test with presence of streak of growth and development of blue colour citrate slant due to utilization of citrate and bromothymol blue in the medium. The eleven *E.coli* isolates alone and 3 mixed infection samples were found to be negative for citrate utilization test and absence of growth which is evident as green colour slant (Plate 14C & 14D).

Plate-14: Biochemical tests of bacterial uropathogens



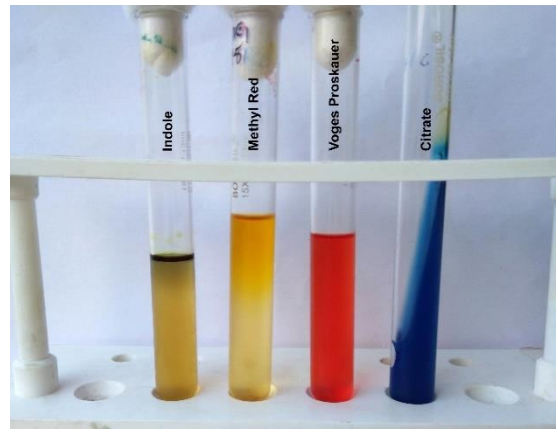
A. Catalase test left negative and right positive



B. Oxidase test left negative and right positive



C. IMViC Tests for *E.coli*

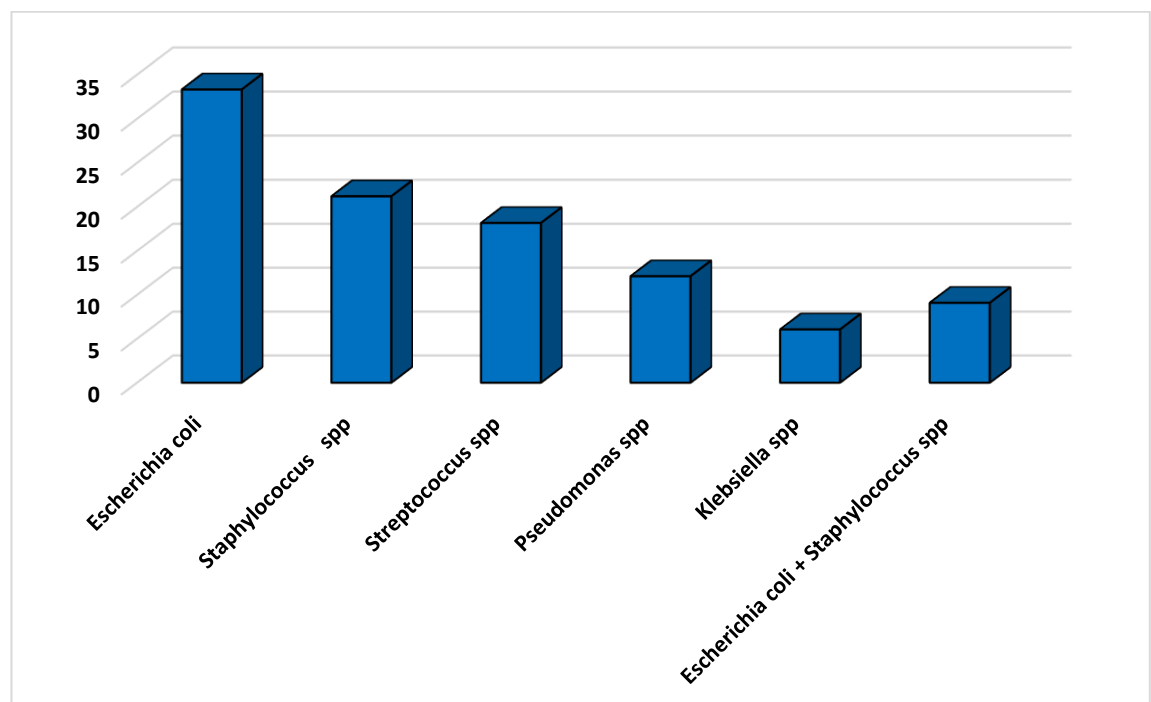


D. IMViC Tests for *Klebsiella*

Table 9: Bacterial isolated from dogs affected with lower urinary tract infections (n=33)

S.No	Bacterial isolate	Proportion of bacteria isolated in dogs affected with lower UTI (n=33)	
		Number	Percentage
1.	<i>Escherichia coli</i>	11	33.33
2.	<i>Staphylococcus</i> spp	7	21.21
3.	<i>Streptococcus</i> spp	6	18.18
4.	<i>Pseudomonas</i> spp	4	12.12
5.	<i>Klebsiella</i> spp	2	6.06
6.	<i>Escherichia coli</i> + <i>Staphylococcus</i> spp	3	9.10

Fig 6: Bacterial isolated from dogs affected with lower urinary tract infections



4.4 ANTIBIOTIC SUSCEPTIBILITY TESTING

As depicted in Table 10 and Fig 7 all the 33 lower urinary tract infection cases were tested for susceptibility to seven antibiotics viz., ceftriaxone + tazobactam (CIT 30/10), amoxicillin sodium + sulbactam (AMS 30/15), gentamicin (GEN 10), ciprofloxacin (CIP 5), nitrofurantoin (NTT 300), trimethoprim-sulphamethaxazole (COT 25) and amikacin (AK 30).

Out of 33 samples susceptibility to amikacin was noticed in all 33 samples (100%), whereas susceptibility to gentamicin was noticed in 31 samples (93.94%), 30 samples (90.91%) to ceftriaxone + tazobactam, 27 samples (81.82%) to amoxicillin sodium + sulbactam and nitrofurantoin each, 26 samples (78.79%) to trimethoprim-sulphamethaxazole and 25 samples (75.76%) to ciprofloxacin (Plate 15&16).

4.5 UNDERLYING OR ASSOCIATED FACTORS IN LOWER URINARY TRACT INFECTIONS

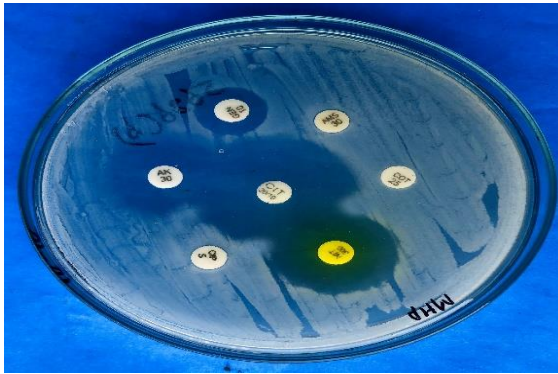
Among the 35 dogs affected with lower urinary tract infections underlying factors were noticed in 8 dogs which included prostate enlargement in 3 dogs (8.57%), long term use of glucocorticoids and cystoliths in 2 dogs (5.71%) each and urethroliths in 1 dog (2.86%) (Table 11 and Fig 8).

4.6 HAEMATOLOGY

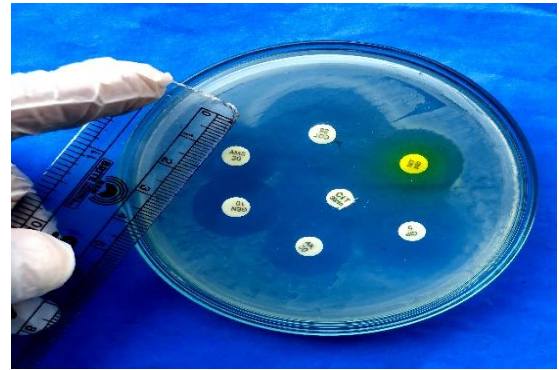
4.6.1 Haemoglobin (g/dl)

The mean haemoglobin concentration in apparently healthy dogs recorded was 15.05 ± 0.33 g/dl. In dogs affected with lower urinary tract infections Hb concentration varied between 10 and 14 g/dl with the mean Hb concentration of 11.96 ± 0.40 g/dl which was significantly ($P < 0.01$) less when compared with healthy control group of dogs (Table 12 and Fig 9).

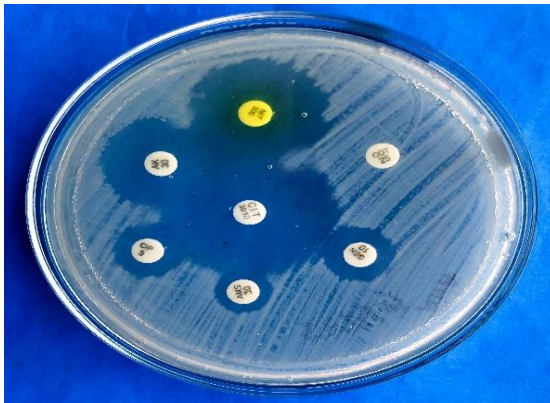
Plate-15: Antimicrobial susceptibility testing



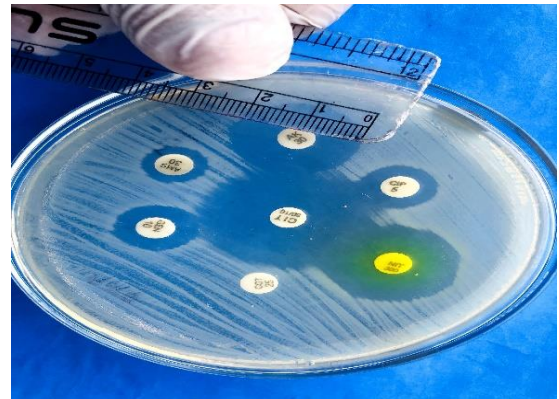
A. Isolate of *E.coli* showing resistance to amoxicillin sodium and sulbactam



B. Isolate of *Staphylococcus* spp showing sensitivity to amoxicillin sodium and sulbactam



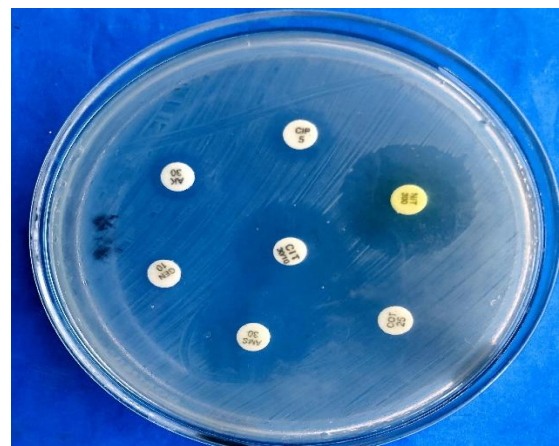
C. Isolate of *Klebsiella* spp showing sensitivity to amikacin



D. Isolate of *E.coli* showing sensitivity to amikacin

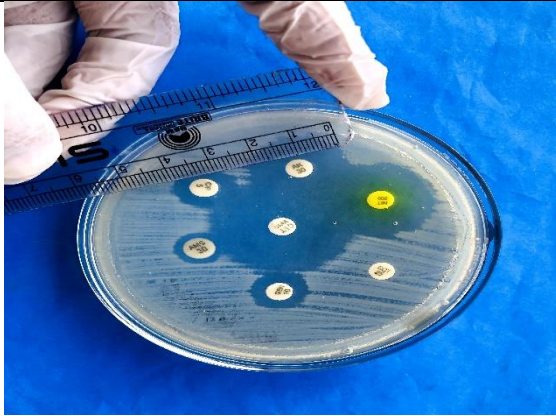


E. Isolate of *Staphylococcus* spp showing sensitivity to gentamicin

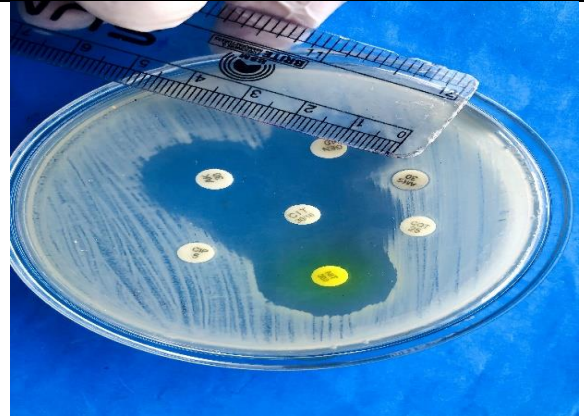


F. Isolate of *Staphylococcus* spp showing resistant to gentamicin

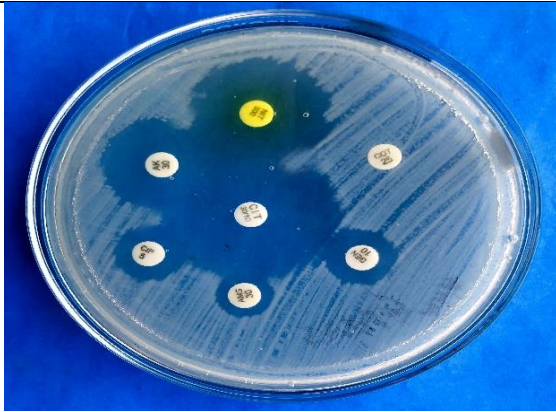
Plate-16: Antimicrobial susceptibility testing



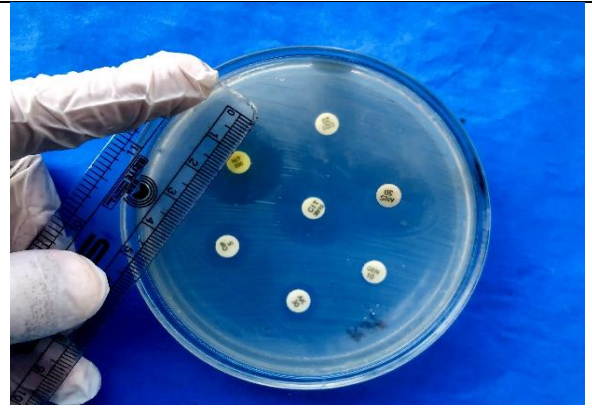
A. Isolate of *Streptococcus* spp showing sensitivity to ciprofloxacin



B. isolate of *E.coli* showing resistance to ciprofloxacin



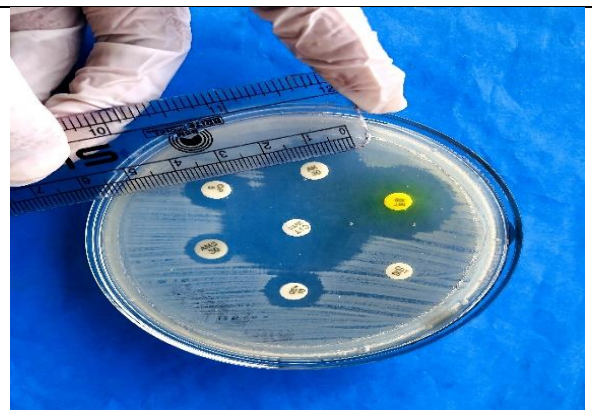
C. Isolate of *Pseudomonas* spp showing sensitivity to ceftriaxone and tazobactam



D. Isolate of *E.coli* showing sensitivity to nitrofurantoin



E. Isolate of *Staphylococcus* spp showing sensitivity to amoxicillin sodium and sulbactam, resistant to trimethoprim-sulphamethaxazole and gentamicin



F. Isolate of *Pseudomonas* spp showing sensitivity to ciprofloxacin, amikacin and resistant to trimethoprim-sulphamethaxazole

Table 10: Antimicrobial susceptibility testing in dogs affected with bacterial LUTI (n=33)

Antibiotic disc	No. of Resistant isolates	No. of Intermediate isolates	No. of Sensitive isolates	Resistant per cent	Sensitive per cent
Ceftriaxone Tazobactum (CIT)	3	6	24	9.09	90.91
Amoxicillin sodium Sulbactam (AMS)	6	4	23	18.18	81.82
Gentamicin (GEN)	2	4	27	6.06	93.94
Ciprofloxacin (CIP)	8	-	25	24.24	75.76
Nitrofurantoin (NTT)	6	2	25	18.18	81.82
Trimethoprim-sulphamethaxazole (COT)	7	-	26	21.21	78.79
Amikacin (AK)	-	5	28	0.00	100

Fig 7: Antimicrobial susceptibility testing in dogs affected with bacterial LUTI (n=33)

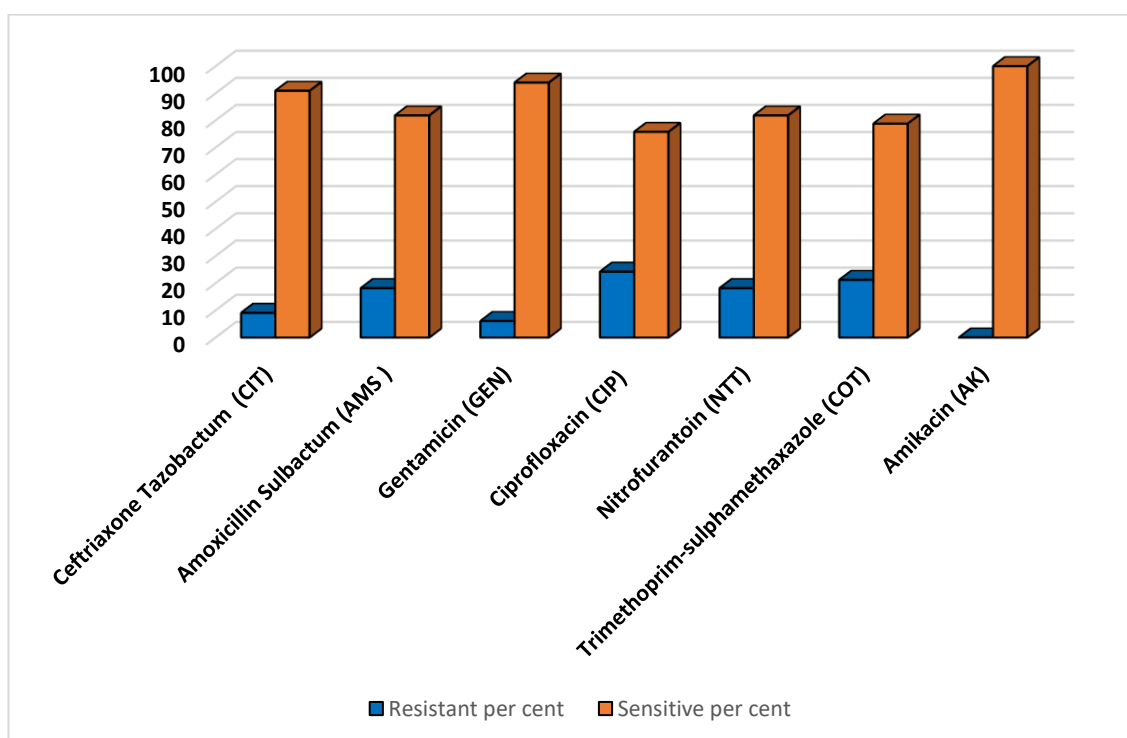


Table 11: Underlying or associated factors in lower urinary tract infections (n=35)

S.No	Condition	Number positive	Percent
1	Cystoliths	2	5.71
2	Urethroliths	1	2.86
3	Long term use of glucocorticoids	2	5.71
4	Prostate Enlargement	3	8.57

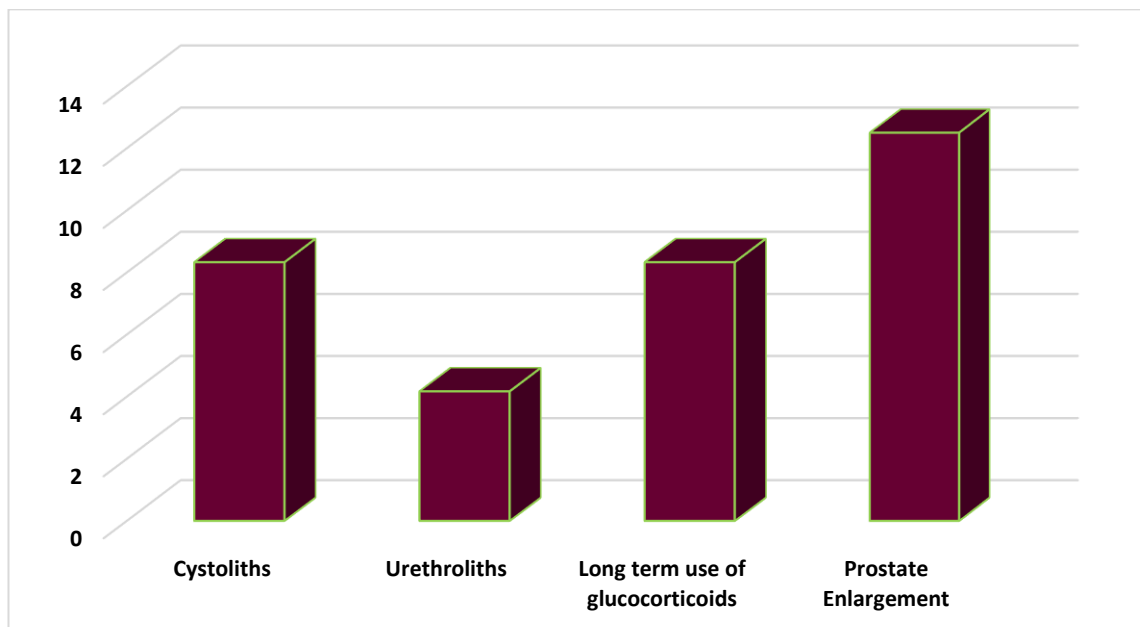
Fig 8: Underlying or associated factors in lower urinary tract infections (n=35)

Table 12: Haemato biochemical findings in dogs affected with lower urinary tract infections (Mean $\pm$ SE) (n=35)

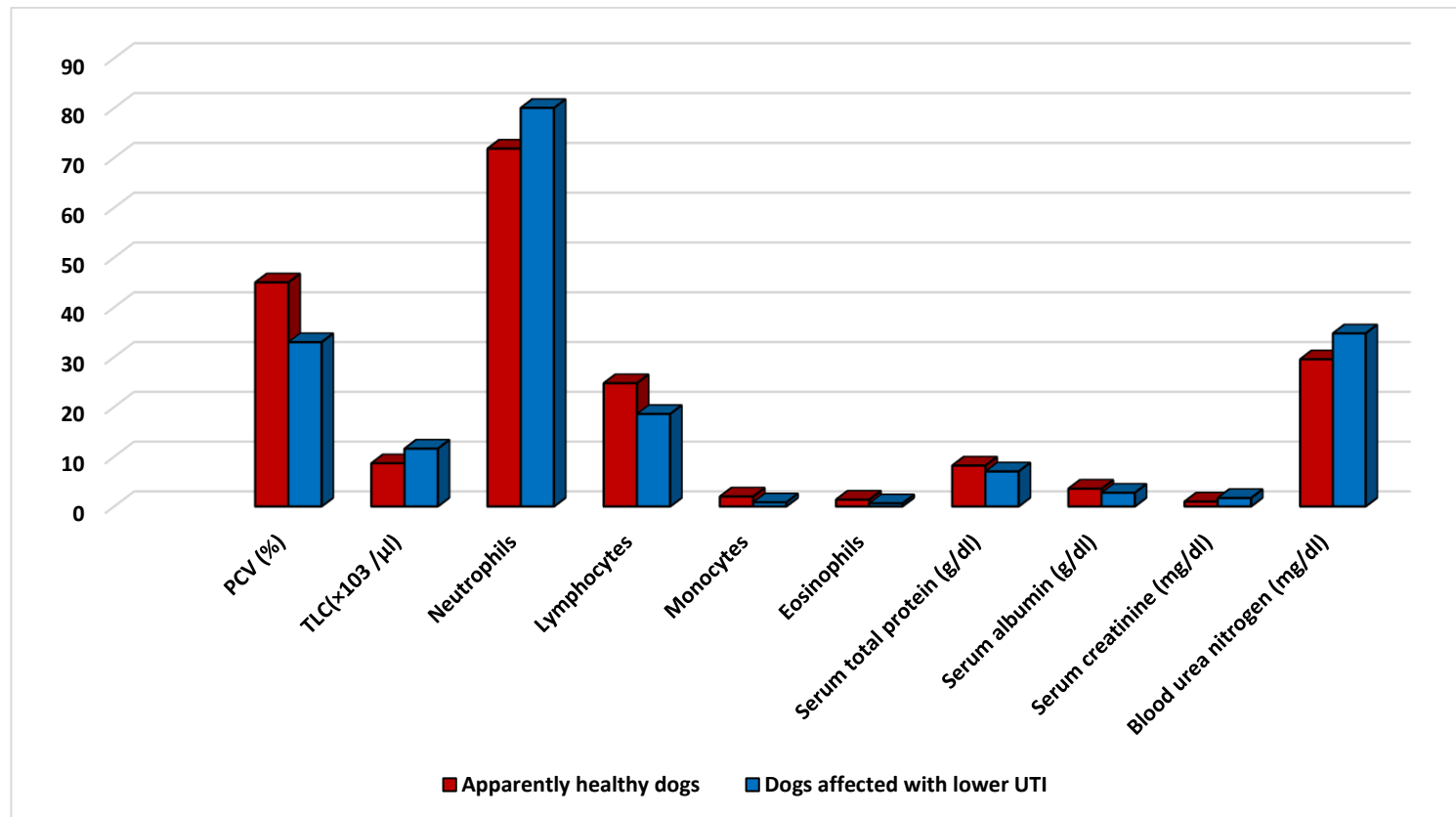
S.No.	Parameter	Apparently healthy dogs (n=10)	Dogs affected with lower UTI (n=35)
1.	Hb (g/dl)	15.05 $\pm$ 0.33	11.96 $\pm$ 0.40 ^{**}
2.	PCV (%)	45.00 $\pm$ 0.94	33.00 $\pm$ 0.89 ^{**}
3	TLC($\times 10^3$ / μ l)	8.68 $\pm$ 0.09	11.62 $\pm$ 0.26 ^{**}
4	DLC (%)		
	Neutrophils	71.87 $\pm$ 0.69	80.04 $\pm$ 1.04 ^{**}
	Lymphocytes	24.75 $\pm$ 0.64	18.58 $\pm$ 1.01 ^{**}
	Monocytes	2.00 $\pm$ 0.27	0.83 $\pm$ 1.16 ^{NS}
	Eosinophils	1.38 $\pm$ 0.14	0.63 $\pm$ 1.14 ^{NS}
	Basophils	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
5.	Serum total protein (g/dl)	8.22 $\pm$ 0.08	7.04 $\pm$ 0.07 ^{**}
6.	Serum albumin (g/dl)	3.58 $\pm$ 0.13	2.76 $\pm$ 0.07 ^{**}
7.	Blood urea nitrogen (mg/dl)	29.56 $\pm$ 0.64	34.76 $\pm$ 0.07 ^{**}
8.	Serum creatinine (mg/dl)	0.99 $\pm$ 0.03	1.70 $\pm$ 0.07 ^{**}

^{**} : Means differ significantly (P < 0.01) with healthy control group

^{*} : Means differ significantly (P < 0.05) with healthy control group

NS : Not significant (P > 0.05)

Fig 9: Haemato biochemical findings in dogs affected with lower urinary tract infections (Mean $\pm$ SE)



4.6.2 Packed Cell Volume (%)

The mean PCV in apparently healthy dogs was 45.00 ± 0.94 per cent (Table 12 and Fig 9). The PCV in dogs affected with lower urinary tract infections was significantly ($P < 0.01$) less ($33.00 \pm 0.89\%$) when compared to healthy control group of dogs.

4.6.3 Total leukocyte count ($\times 10^3/\mu\text{l}$)

The total leukocyte count ranged from 8.00 to $16.25 \times 10^3/\mu\text{l}$ in dogs affected with lower urinary tract infections with a mean value of count $11.62 \pm 0.26 \times 10^3/\mu\text{l}$ which was significantly ($P < 0.01$) higher when compared to apparently healthy dogs ($8.68 \pm 0.09 \times 10^3/\mu\text{l}$) (Table 12 and Fig 9).

4.6.4 Differential Leukocyte count (%)

The mean differential leucocyte counts of neutrophils, lymphocytes, monocytes and eosinophils in dogs affected with lower urinary tract infections were 80.04 ± 1.04 , 18.58 ± 1.01 , 0.83 ± 1.16 and 0.63 ± 1.14 per cent respectively and in healthy dogs these values were 71.87 ± 0.69 , 24.75 ± 0.64 , 2.00 ± 0.27 and 1.38 ± 0.14 per cent respectively which was depicted in Table 12 and Fig 9. There was a significant ($P < 0.01$) increase in neutrophils, significant ($P < 0.01$) decrease in lymphocytes and a nonsignificant decrease in the value of eosinophils and monocytes when compared to healthy control group.

4.7 SERUM BIOCHEMISTRY

4.7.1 Total protein (g/dl)

The mean serum total protein value recorded in dogs affected with lower urinary tract infections was 7.04 ± 0.07 g/dl while the mean value in healthy dogs was 8.22 ± 0.08 g/dl indicating a significant ($P < 0.01$) decrease in affected dogs when compared to healthy control group of dogs (Table 12 and Fig 9).

4.7.2 Albumin (g/dl)

In dogs of control group the mean serum albumin recorded was 3.58 ± 0.13 g/dl. From Table 12 and Fig 9 it was evident that the mean serum albumin level in dogs suffering from lower urinary tract infections was significantly ($P < 0.01$) less when compared with apparently healthy control group of dogs with the mean value of 2.76 ± 0.07 g/dl.

4.7.3 Blood urea nitrogen (mg/dl)

The mean blood urea nitrogen value recorded in dogs affected with lower urinary tract infections was 34.76 ± 0.07 mg/dl while the mean BUN value in healthy dogs was 29.56 ± 0.64 mg/dl indicating a significant ($P < 0.01$) increase in affected dogs when compared to healthy control group of dogs (Table 12 and Fig 9).

4.7.4 Creatinine (mg/dl)

The mean serum creatinine value recorded in dogs affected with lower urinary tract infections was 1.70 ± 0.07 mg/dl while in healthy dogs the mean value recorded was 0.99 ± 0.03 mg/dl indicating a significant ($P < 0.01$) increase in affected dogs when compared to healthy control group (Table 12 and Fig 9).

4.8 THERAPEUTIC TRIAL

Therapeutic trial in the present study was taken up in 24 dogs affected with bacterial lower urinary tract infections by dividing the dogs randomly into three groups with 8 dogs each for evaluation of efficacy of three different antibiotics. Antibiotic therapy in all the dogs was continued for one week beyond the clinical cure. Underlying factors were treated accordingly. Fever, haematuria, inappetence/anorexia, pollakiuria,

oliguria, urinary incontinence, stranguria, vomiting, pyuria, polyuria, anuria, polydipsia and dribbling of urine either alone or in combination were the clinical signs noticed in dogs taken up for therapeutic trial. All the dogs were monitored at weekly intervals for the resolution of clinical signs, urinalysis findings and negative bacteriological culture.

4.8.1 Group I

Dogs in this group were treated with amoxicillin sodium + sulbactam at the dose rate of 20 mg/kg body weight twice daily I/M. Six dogs showed improvement by 7th day with remission of clinical signs like fever, haematuria, pollakiuria, oliguria and complete recovery by 14th day by subsidence of clinical signs, normal urinalysis findings and negative bacterial culture. Remaining two dogs in this group showed improvement by 14th day with remission of clinical signs like fever, inappetence, pollakiuria, urinary incontinence, anuria, polydipsia and dribbling of urine with complete recovery by 21st day and normal urinalysis findings, negative bacterial culture.

Among two dogs in this group which recovered by 21st day, one dog was diagnosed with prostate enlargement and the other with cystolithiasis as the underlying factor. The dog which was affected with prostate enlargement showed improvement by 14th day but culture of urine samples revealed positive bacterial growth on 14th day so the treatment was further continued and urine sample was cultured which showed negative bacterial growth with subsidence of clinical signs of lower urinary tract infection by 21st day.

The other dog diagnosed as affected with bacterial LUTI had cystolithiasis as underlying factor which recovered after surgical removal of cystoliths and the dog was

examined at weekly intervals for resolution of clinical signs like fever, inappetence, pollakiuria, urinary incontinence, anuria, polydipsia, dribbling of urine and negative bacterial growth on urine culture. The dog showed improvement in subsidence of clinical signs by 14th day but urine culture revealed positive bacterial growth and thereby treatment was further continued and urine sample revealed negative bacterial growth by 21st day of therapy.

Based on the recovery pathway in dogs affected with lower urinary tract infections in this group the average number of days taken for recovery was 15.75 ± 1.07 which was depicted in Table 16 and Fig 13.

4.8.1.1 Haematology

The pre therapeutic mean values of haemoglobin, packed cell volume, total leukocyte count, neutrophils, lymphocytes and monocytes varied significantly when compared to mean values of healthy control group.

The mean haemoglobin (g/dl) and packed cell volume (%) in Group I dogs before treatment were 12.63 ± 0.92 and 30.37 ± 2.04 respectively which increased significantly ($P < 0.05$) to 13.67 ± 0.55 and ($P < 0.01$) 40.62 ± 3.20 respectively after therapy. However after therapy values of haemoglobin and packed cell volume increased when compared to before therapy values indicating improvement in affected dogs. (Table 13 and Fig 10).

In dogs affected with lower urinary tract infections of this group the mean value of TLC recorded was $11.72 \pm 0.50 \times 10^3/\mu\text{l}$ which upon treatment was found to be significantly ($P < 0.01$) decreased to $8.83 \pm 0.36 \times 10^3/\mu\text{l}$ indicating response to therapy. (Table 13 and Fig.10).

**Table 13: Haemato biochemical findings in Group I before and after therapy
(Mean $\pm$ SE)**

S. No.	Parameter	Apparently healthy dogs (n=10)	Before therapy (n=8)	After therapy (n=8)
1.	Hb (g/dl)	15.05 $\pm$ 0.33	12.63 $\pm$ 0.92**	13.67 $\pm$ 0.55 [#]
2.	PCV (%)	45.00 $\pm$ 0.94	30.37 $\pm$ 2.04**	40.62 $\pm$ 3.20 ^{##}
3	TLC ($\times 10^3$ / μ l)	8.68 $\pm$ 0.09	11.72 $\pm$ 0.50**	8.83 $\pm$ 0.36 ^{##}
	DLC (%)			
	Neutrophils	71.87 $\pm$ 0.69	78.26 $\pm$ 2.34**	72.63 $\pm$ 0.80 [#]
	Lymphocytes	24.75 $\pm$ 0.64	19.87 $\pm$ 2.23*	25.78 $\pm$ 1.04 [#]
	Monocytes	2.00 $\pm$ 0.27	0.87 $\pm$ 0.29*	0.86 $\pm$ 0.35 ^{NS}
	Eosinophils	1.38 $\pm$ 0.14	1.00 $\pm$ 0.26	0.63 $\pm$ 0.26 ^{NS}
	Basophils	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
5.	Serum total protein (g/dl)	8.22 $\pm$ 0.08	6.85 $\pm$ 0.14**	7.85 $\pm$ 0.23 ^{##}
6.	Serum albumin (g/dl)	3.58 $\pm$ 0.13	2.61 $\pm$ 0.10*	3.35 $\pm$ 0.15 ^{##}
7.	Blood urea nitrogen (mg/dl)	29.56 $\pm$ 0.64	35.43 $\pm$ 1.73**	29.40 $\pm$ 2.00 ^{##}
8.	Serum creatinine (mg/dl)	0.99 $\pm$ 0.03	1.81 $\pm$ 0.08*	1.10 $\pm$ 0.04 ^{##}

** : Means differ significantly (P < 0.01) in comparing before therapy with healthy control group

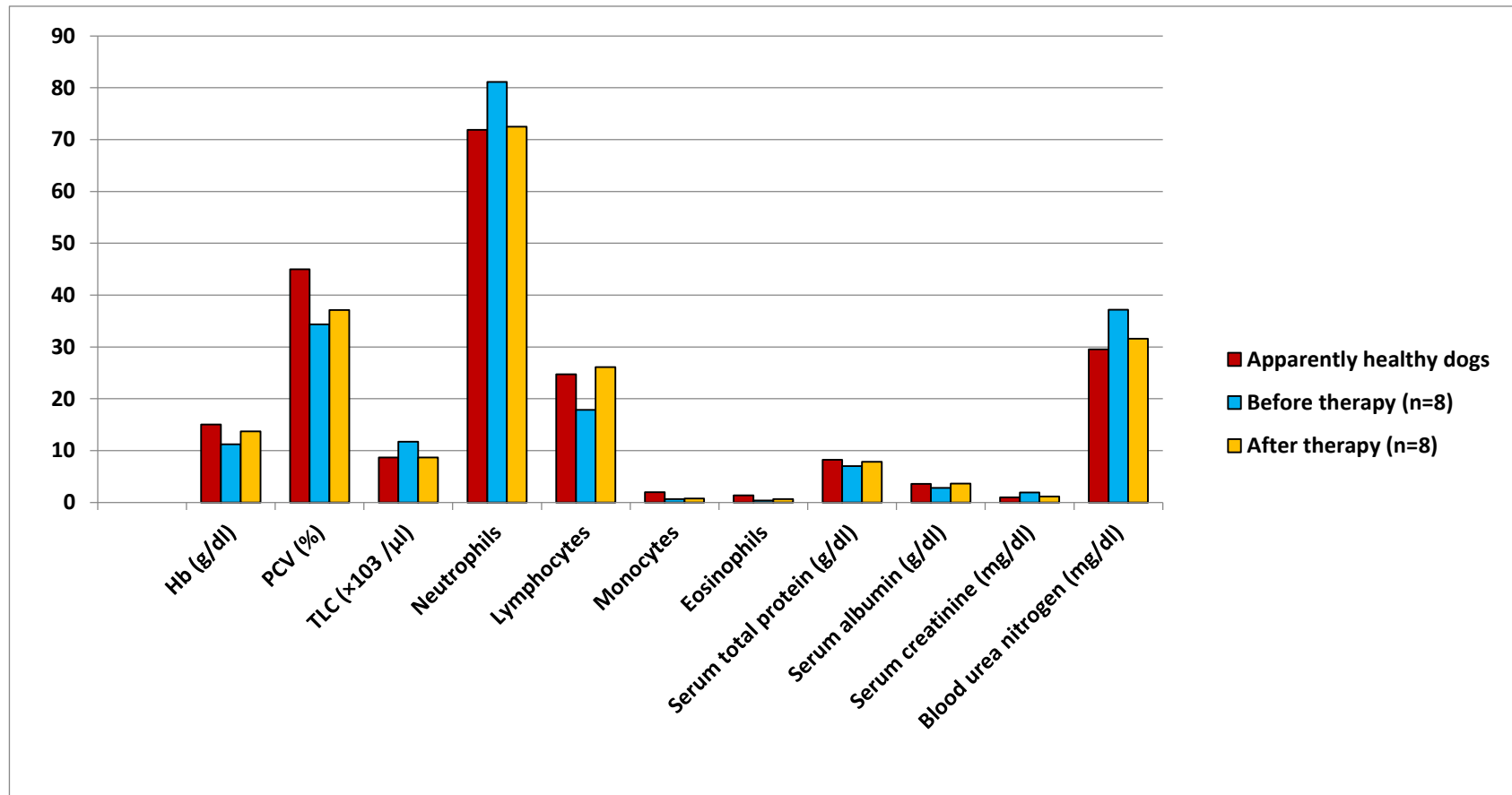
* : Means differ significantly (P < 0.05) in comparing before therapy with healthy control group

: Means differ significantly (P < 0.01) in comparing after therapy with before therapy

: Means differ significantly (P < 0.05) in comparing after therapy with before therapy

NS : Not significant (P > 0.05)

Fig 10: Haemato biochemical findings in Group I dogs affected with lower urinary tract infections (Mean $\pm$ SE)



The pre therapeutic mean values of neutrophils, lymphocytes, monocytes and eosinophils in the dogs of this group were 78.26 ± 2.34 , 19.87 ± 2.23 , 0.87 ± 0.29 and 1.00 ± 0.26 per cent respectively. After therapy a significant ($P < 0.05$) decrease in the mean values of neutrophils ($72.63 \pm 0.80\%$) and a significant ($P < 0.05$) increase in the mean value of lymphocytes ($25.78 \pm 1.04\%$) and a non significant decrease in the mean value of monocytes ($0.86 \pm 0.35\%$) and eosinophils ($0.63 \pm 0.26\%$) were recorded when compared with pre therapeutic mean values. There was a decrease in post therapeutic mean values of neutrophils, monocytes and eosinophils; increase in post therapeutic mean value of lymphocytes when compared to pre therapeutic mean value indicating response to therapy (Table 13 and Fig10).

4.8.1.2 Serum biochemistry

There was a significant variation in pre therapeutic mean values of total protein and albumin, blood urea nitrogen and creatinine when compared to that of healthy control group.

There was a significant increase ($P < 0.01$) between pre therapeutic mean value (6.85 ± 0.14 g/dl), (2.61 ± 0.10 g/dl) and post therapeutic mean value (7.85 ± 0.23 g/dl), (3.35 ± 0.15 g/dl) of serum total protein and albumin respectively in this group of dogs. However after therapy mean values of total protein and albumin were increased when compared to before therapy values indicating improvement which reached to near normalcy as that of apparently healthy dogs (Table 13 and Fig 10).

In the dogs of this group the mean value of blood urea nitrogen before therapy was 35.43 ± 1.73 mg/dl which was significantly ($P < 0.01$) decreased to 29.40 ± 2.00 mg/dl

after therapy. However after therapy mean value of blood urea nitrogen was decreased when compared to before therapy value indicating improvement (Table 13 and Fig 10).

The mean value of serum creatinine (1.1 ± 0.04 mg/dl) after therapy showed a significant decline ($P < 0.01$) when compared with pre therapeutic mean value (1.81 ± 0.08 mg/dl) in this group of dogs affected with lower urinary tract infection. The post therapeutic mean value of serum creatinine was decreased when compared to pre therapeutic mean value indicating of improvement in affected dogs (Table 13 and Fig. 10).

4.8.2 Group II

Dogs in this group were treated with ceftriaxone + tazobactam at the dose rate of 20 mg/kg body weight once daily I/M. Five dogs showed improvement in subsidence of clinical signs like fever, vomiting, inappetence, stranguria by 7th day and complete recovery with remission of all clinical signs normal urinalysis findings and negative bacterial growth by 14th day. Two dogs in this group showed response to therapy with subsidence of all clinical signs like haematuria, inappetence, pollakiuria, urinary incontinence, anuria and negative bacterial growth by 7th day indicating complete recovery with normal urinalysis findings and negative bacterial culture. Remaining one dog showed improvement in subsidence of clinical signs like stranguria, vomiting, pyuria and polyuria by 14th day and complete recovery with remission of all clinical signs, normal urinalysis findings and negative bacterial growth by 21st day of therapy.

One dog which recovered by 21st day in this group was diagnosed to be affected with cystolithiasis as an underlying cause resulting in the development of lower urinary

tract infection. The dog showed improvement by 14th day after surgical correction by remission of clinical signs like stranguria, vomiting, pyuria and polyuria but urine culture revealed positive bacterial growth on 14th day. So the treatment was further extended and urine culture was done on 21st day of therapy which revealed negative bacterial growth and complete subsidence of clinical signs on 21st day of therapy.

Out of 5 dogs which recovered by 14th day in this group 2 dogs were diagnosed to be affected with prostate hypertrophy and urolithiasis as underlying cause in development of lower urinary tract infection. The dog with prostate hypertrophy showed improvement in subsidence of clinical signs like fever, vomiting, stranguria by 7th day of therapy but urine culture revealed positive bacterial growth and hence treatment was continued and dog was reexamined on 14th day of therapy which showed complete remission of clinical signs and negative bacterial growth on urine culture by 14th day of therapy.

The dog with urolithiasis showed improvement in subsidence of clinical signs like fever, inappetence, vomiting, stranguria on 7th day of surgical correction but urine culture revealed positive bacterial growth on 7th day of therapy. So the treatment was continued and dog was reexamined on 14th day of therapy which showed complete remission of clinical signs exhibited by dog and negative bacterial growth on urine culture on 14th day of therapy.

Based on the recovery pathway in dogs affected with lower urinary tract infections in this group the average number of days taken for recovery was 13.13 ± 1.48 which was depicted in Table 16 and Fig 13.

4.8.2.1 Haematology

The pre therapeutic mean values of haemoglobin, packed cell volume, total leukocyte count, neutrophils, lymphocytes and monocytes varied significantly when compared to mean values of healthy control group.

In this group of dogs the mean haemoglobin (g/dl) and packed cell volume (%) before therapy were 11.25 ± 0.24 and 34.37 ± 1.10 respectively and after therapy the mean values increased significantly ($P < 0.05$) to 13.71 ± 0.46 and 37.12 ± 1.34 respectively when compared to pre therapeutic values. However the mean values after therapy were increased when compared to mean values before therapy indicating improvement in dogs affected with LUTI (Table 14 and Fig 11).

The mean value of TLC before therapy in this group of dogs was $11.75 \pm 0.39 \times 10^3/\mu\text{l}$ which on treatment showed a significant ($P < 0.01$) decrease with the mean value of $8.69 \pm 0.38 \times 10^3/\mu\text{l}$ when compared to pre therapeutic mean value. However the post therapeutic mean value of total leukocyte count was decreased when compared to pre therapeutic mean values indicating response to therapy (Table 14 and Fig 11).

The pre therapeutic mean values of neutrophils, lymphocytes, monocytes and eosinophils are 81.13 ± 1.75 per cent, 17.87 ± 1.66 per cent, 0.63 ± 0.26 per cent, 0.37 ± 0.18 per cent respectively. After therapy there was a significant ($P < 0.01$) decrease in the mean value of neutrophils ($72.52 \pm 1.21\%$), significant ($P < 0.01$) increase in lymphocytes ($26.1 \pm 1.16\%$), nonsignificant increase in monocytes ($0.75 \pm 0.25\%$) and mean value of eosinophils ($0.63 \pm 0.18\%$) when compared with the pre therapeutic values. However there was a decrease in post therapeutic mean values of neutrophils, monocytes and

**Table 14: Haemato biochemical findings in Group II before and after therapy
(Mean $\pm$ SE)**

S. No.	Parameter	Apparently healthy dogs (n=10)	Before therapy (n=8)	After therapy (n=8)
1.	Hb (g/dl)	15.05 $\pm$ 0.33	11.25 $\pm$ 0.24**	13.71 $\pm$ 0.46 [#]
2.	PCV (%)	45.00 $\pm$ 0.94	34.37 $\pm$ 1.10**	37.12 $\pm$ 1.34 [#]
3	TLC ($\times 10^3$ / μ l)	8.68 $\pm$ 0.09	11.75 $\pm$ 0.39**	8.69 $\pm$ 0.38 ^{##}
4	DLC (%)			
	Neutrophils	71.87 $\pm$ 0.69	81.13 $\pm$ 1.75**	72.52 $\pm$ 1.21 ^{##}
	Lymphocytes	24.75 $\pm$ 0.64	17.87 $\pm$ 1.66**	26.1 $\pm$ 1.16 ^{##}
	Monocytes	2.00 $\pm$ 0.27	0.63 $\pm$ 0.26*	0.75 $\pm$ 0.25 ^{NS}
	Eosinophils	1.38 $\pm$ 0.14	0.37 $\pm$ 0.18	0.63 $\pm$ 0.18 ^{NS}
	Basophils	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
5.	Serum total protein (g/dl)	8.22 $\pm$ 0.08	7.00 $\pm$ 0.11	7.87 $\pm$ 0.18 ^{NS}
6.	Serum albumin (g/dl)	3.58 $\pm$ 0.13	2.79 $\pm$ 0.10*	3.62 $\pm$ 0.23 [#]
7.	Blood urea nitrogen (mg/dl)	29.56 $\pm$ 0.64	37.18 $\pm$ 0.88**	31.57 $\pm$ 1.57 ^{##}
8.	Serum creatinine (mg/dl)	0.99 $\pm$ 0.03	1.92 $\pm$ 0.10**	1.16 $\pm$ 0.06 ^{##}

** : Means differ significantly (P < 0.01) in comparing before therapy with healthy control group

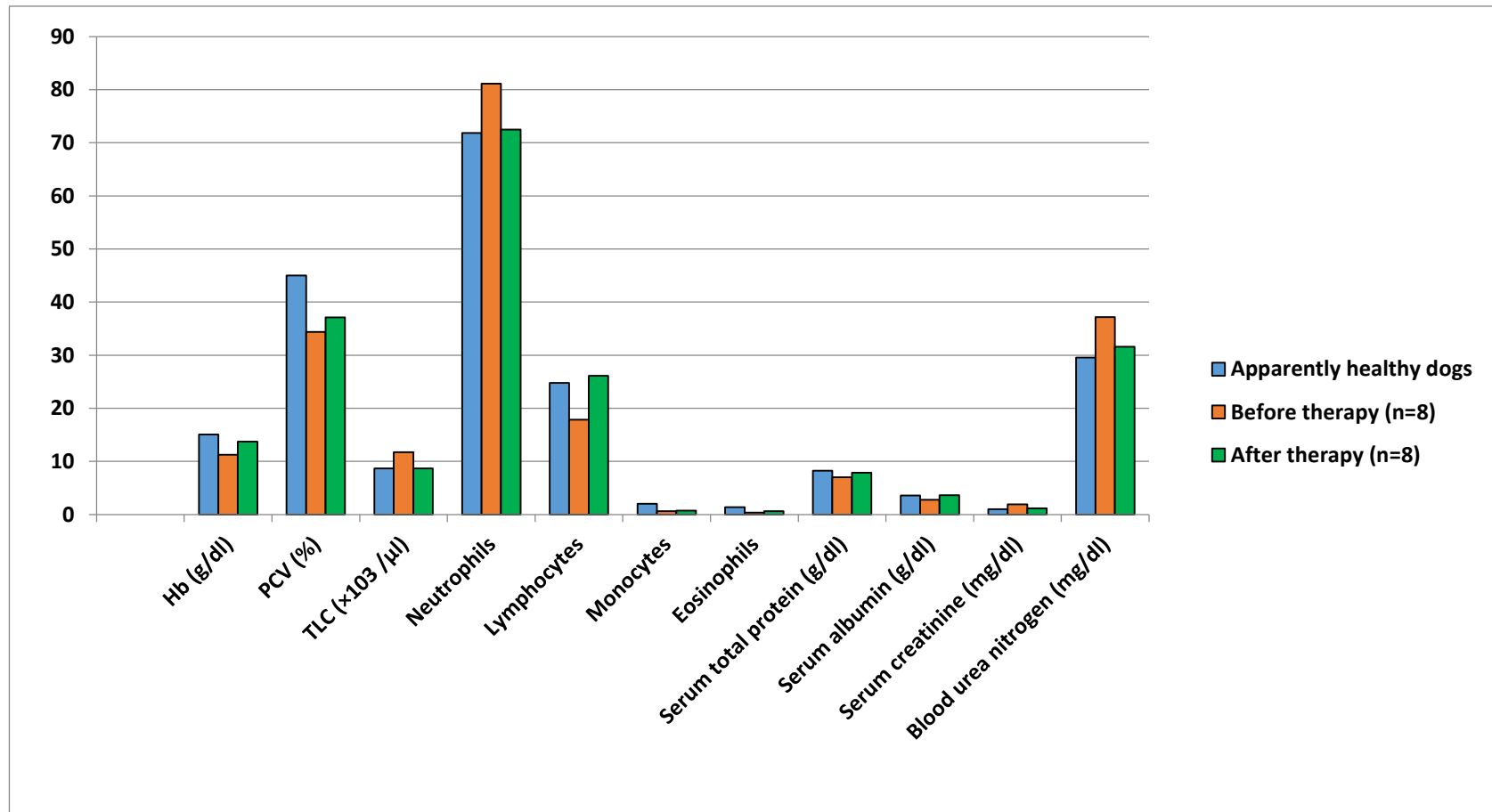
* : Means differ significantly (P < 0.05) in comparing before therapy with healthy control group

: Means differ significantly (P < 0.01) in comparing after therapy with before therapy

: Means differ significantly (P < 0.05) in comparing after therapy with before therapy

NS : Not significant (P > 0.05)

Fig 11: Haemato biochemical findings in Group II dogs affected with lower urinary tract infections (Mean $\pm$ SE)



eosinophils; increase in post therapeutic mean value of lymphocytes when compared to pre therapeutic mean value indicating improvement in affected dogs with bacterial UTI (Table 14 and Fig 11).

4.8.2.2 Serum biochemistry

There was a significant variation in pre therapeutic mean values of albumin, blood urea nitrogen and creatinine when compared to that of healthy control group.

A nonsignificant increase in the post therapeutic mean value of serum total protein (7.87 ± 0.18 g/dl) was observed when compared with pre therapeutic mean value (7.00 ± 0.11 g/dl) in this group of dogs. The post therapeutic mean value (3.62 ± 0.23 g/dl) of serum albumin (g/dl) in this group of dogs showed a significant increase ($P < 0.05$) when compared to pre therapeutic mean value (2.79 ± 0.10 g/dl). However after therapy mean values of total protein and albumin were increased when compared to before therapy values indicating response to therapy (Table 14 and Fig 11).

The post therapeutic mean value of blood urea nitrogen in this group of dogs (31.57 ± 1.57 mg/dl) was significantly ($P < 0.01$) decreased when compared with pre therapeutic value (37.18 ± 0.88 mg/dl). However after therapy mean value of blood urea nitrogen was decreased when compared to before therapy indicating improvement (Table 14 and Fig 11).

The mean value of serum creatinine after therapy was 1.16 ± 0.06 mg/dl which showed a significant decrease ($P < 0.01$) when compared with pre therapeutic value (1.92 ± 0.10 mg/dl) in this group of dogs. The post therapeutic mean value of serum creatinine was decreased when compared to pre therapeutic mean value indicating improvement in affected dogs (Table 14 and Fig 11).

4.8.3 Group III

Dogs in this group were treated with amikacin at the dose rate of 18 mg/kg body weight once daily I/M. Five dogs in this group showed improvement in subsidence of clinical signs like fever, haematuria, normal urinalysis findings and negative bacterial growth by 7th day of therapy. Remaining three dogs showed improvement in subsidence of clinical signs like haematuria, inappetence, oliguria, urinary incontinence, pyuria, polyuria by 7th day of therapy but urine culture revealed positive bacterial growth on 7th day. The dogs were reexamined on 14th day of therapy which revealed complete remission of clinical signs, normal urinalysis findings and negative bacterial growth by 14th day of therapy.

Three dogs in this group which recovered by 14th day of therapy were diagnosed with prostate enlargement and long term use of glucocorticoids as an underlying cause in development of lower urinary tract infection. The dog with prostate enlargement showed improvement in remission of clinical signs by 7th day of therapy but urine culture revealed positive bacterial growth on 7th day. So the treatment was continued and urine sample was cultured on 14th day of therapy which revealed negative bacterial growth with complete subsidence of clinical signs on 14th day of therapy.

Two dogs with long term use of glucocorticoids as an underlying cause in development of lower urinary tract infection showed improvement by 7th day of therapy with improvement noticed in remission of clinical signs like haematuria, inappetence, oliguria, urinary incontinence, pyuria, polyuria, but urine culture revealed positive bacterial growth. So the treatment was continued and dog was examined on 14th day of

therapy which revealed subsidence of clinical signs and negative bacterial growth on urine culture on 14th day of therapy.

Based on the recovery pathway in dogs affected with lower urinary tract infections in this group the average number of days taken for recovery was 9.62 ± 1.19 which was depicted in Table 16 and Fig 13.

4.8.3.1 Haematology

The pre therapeutic mean values of haemoglobin, packed cell volume, total leukocyte count, neutrophils and lymphocytes varied significantly when compared to mean values of healthy control group.

The mean values of haemoglobin (g/dl) and packed cell volume (%) in the dogs of this group before treatment were 12.00 ± 0.51 and 34.25 ± 0.90 respectively. The post therapeutic mean value of haemoglobin showed a significant increase ($P < 0.05$) to 12.92 ± 0.29 g/dl and post therapeutic mean value of packed cell volume showed a significant increase ($P < 0.01$) to 43.27 ± 0.65 %. However the post therapeutic mean values of haemoglobin and packed cell volume were increased when compared to pre therapeutic mean values indicating response to therapy (Table 15 and Fig 12).

In the dogs of this group the mean value of TLC was recorded as $11.4 \pm 0.52 \times 10^3/\mu\text{l}$ before therapy which on treatment was found to be significantly ($P < 0.01$) decreased with the mean value of $8.48 \pm 0.52 \times 10^3/\mu\text{l}$. However the post therapeutic mean value of total leukocyte count was decreased when compared to pre therapeutic mean values indicating response to treatment (Table 15 and Fig 12).

The mean values of neutrophils, lymphocytes, monocytes and eosinophils before therapy in this group of dogs were 80.75 ± 1.19 per cent, 18.00 ± 1.46 per cent, 1.00 ± 0.32

per cent and 0.5 ± 0.26 per cent respectively. After therapy a significant ($P < 0.01$) decrease in the mean value of neutrophils ($74.52 \pm 0.90\%$), significant ($P < 0.05$) increase in lymphocytes with the mean value of 24.22 ± 0.81 per cent as well as nonsignificant decrease in the mean values of monocytes ($0.88 \pm 0.23\%$) and eosinophils ($0.38 \pm 0.18\%$) respectively were recorded when compared with pre therapeutic mean values. However there was a decrease in post therapeutic mean values of neutrophils, monocytes and eosinophils; increase in post therapeutic mean value of lymphocytes when compared to pre therapeutic mean value indicating response to therapy in dogs affected with LUTI (Table 15 and Fig 12).

4.8.3.2 Serum biochemistry

The pre therapeutic mean values of total protein, albumin, blood urea nitrogen and creatinine varied significantly when compared to that of healthy control group.

There was a significant increase ($P < 0.01$) in the post therapeutic mean value of serum total protein (8.67 ± 0.26 g/dl) when compared with pre therapeutic mean value (7.27 ± 0.07 g/dl). After therapy a significant increase ($P < 0.01$) in the mean value of serum albumin (3.55 ± 0.08 g/dl) was recorded when compared with pre therapeutic mean values of 2.89 ± 0.15 g/dl. The after therapy mean values of total protein and albumin were increased when compared to before therapy mean values indicating improvement in dogs affected with bacterial LUTI (Table 15 and Fig 12).

The mean value of serum blood urea nitrogen before therapy in this group of dogs was 31.69 ± 1.30 mg/dl and the mean value decreased significantly ($P < 0.05$) to 28.68 ± 0.65 mg/dl after therapy when compared with the mean value before therapy. However the after therapy mean value of blood urea nitrogen was decreased when compared to before therapy mean value indicating improvement (Table 15 and Fig. 12).

**Table 15: Haemato biochemical findings in Group III before and after therapy
(Mean $\pm$ SE)**

S. No.	Parameter	Apparently healthy dogs (n=10)	Before therapy (n=8)	After therapy (n=8)
1.	Hb (g/dl)	15.05 $\pm$ 0.33	12.00 $\pm$ 0.51**	12.92 $\pm$ 0.29 [#]
2.	PCV (%)	45 $\pm$ 0.94	34.25 $\pm$ 0.90**	43.27 $\pm$ 0.65 ^{##}
3	TLC ($\times 10^3$ / μ l)	8.68 $\pm$ 0.09	11.4 $\pm$ 0.52**	8.48 $\pm$ 0.52 ^{##}
4	DLC (%)			
	Neutrophils	71.87 $\pm$ 0.69	80.75 $\pm$ 1.19**	74.52 $\pm$ 0.90 ^{##}
	Lymphocytes	24.75 $\pm$ 0.64	18.00 $\pm$ 1.46*	24.22 $\pm$ 0.81 [#]
	Monocytes	2.00 $\pm$ 0.27	1.0 $\pm$ 0.32	0.88 $\pm$ 0.23 ^{NS}
	Eosinophils	1.38 $\pm$ 0.14	0.5 $\pm$ 0.26	0.38 $\pm$ 0.18 ^{NS}
	Basophils	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
5.	Serum total protein (g/dl)	8.22 $\pm$ 0.08	7.27 $\pm$ 0.07*	8.67 $\pm$ 0.26**
6.	Serum albumin (g/dl)	3.58 $\pm$ 0.13	2.89 $\pm$ 0.15**	3.55 $\pm$ 0.08 ^{##}
7.	Blood urea nitrogen (mg/dl)	29.56 $\pm$ 0.64	31.69 $\pm$ 1.30*	28.68 $\pm$ 0.65 [#]
8.	Serum creatinine (mg/dl)	0.99 $\pm$ 0.03	1.36 $\pm$ 0.13	1.03 $\pm$ 0.05 ^{NS}

** : Means differ significantly (P < 0.01) in comparing before therapy with healthy control group

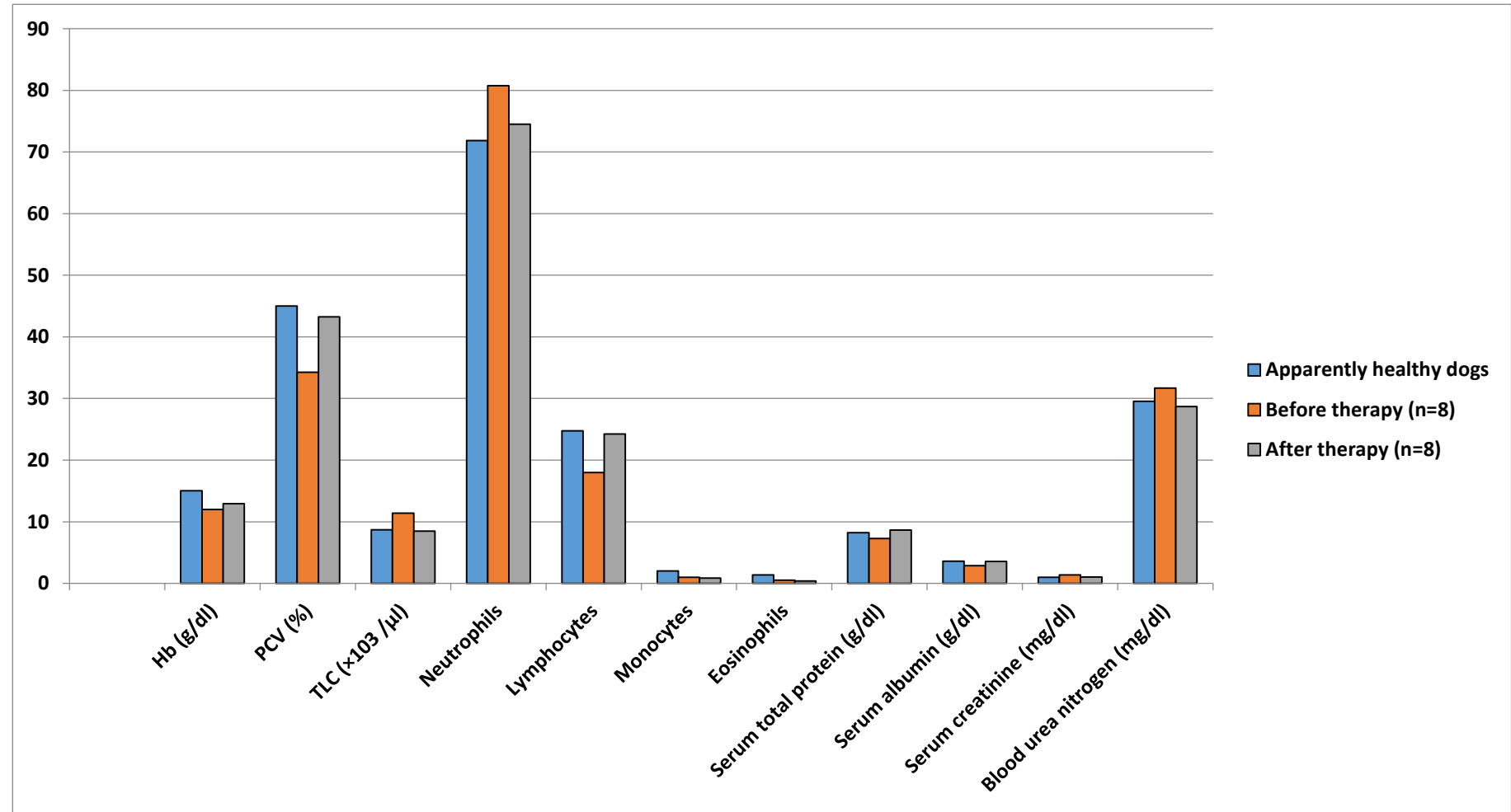
* : Means differ significantly (P < 0.05) in comparing before therapy with healthy control group

: Means differ significantly (P < 0.01) in comparing after therapy with before therapy

: Means differ significantly (P < 0.05) in comparing after therapy with before therapy

NS : Not significant (P >0.05)

Fig 12: Haemato biochemical findings in Group III dogs affected with lower urinary tract infections (Mean $\pm$ SE)



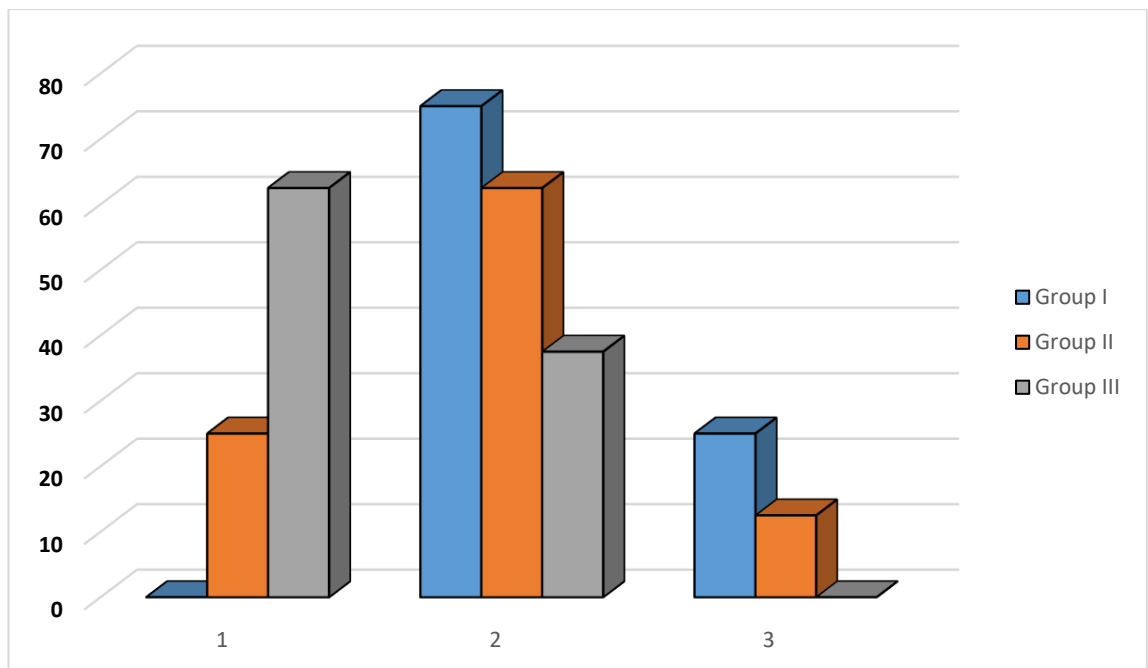
The mean value of serum creatinine in dogs affected with lower urinary tract infection before therapy was 1.36 ± 0.13 mg/dl which on therapy showed a nonsignificant decrease with the mean value of 1.03 ± 0.05 mg/dl. There was nonsignificant decrease in post therapeutic mean value of serum creatinine when compared to pre therapeutic mean value indicating improvement (Table 15 and Fig12).

4.9 RECOVERY PATHWAY

Recovery pathway in dogs affected with lower urinary tract infections revealed that 62.5 per cent of the dogs in Group III and 25 per cent of dogs in Group II showed complete recovery by 7th day whereas none of the 8 treated dogs recovered by 7th day in Group I; 75 per cent of dogs in Group I, 62.5 per cent of dogs in Group II and 37.5 per cent of dogs in Group III showed complete recovery by 14th day; 25 per cent of dogs of Group I, 12.5 per cent of dogs of Group II showed complete recovery by 21st day whereas none of the 8 treated dogs recovered by 21st day in Group III.

Table 16: Recovery pathway in dogs affected with lower urinary tract infections

Group	Number of dogs recovered						Average number of days taken for recovery
	7 th day		14 th day		21 st day		
	Number	Per cent	Number	Per cent	Number	Per cent	
I	0	0	6	75	2	25	15.75 ± 1.07
II	2	25	5	62.5	1	12.5	13.13 ± 1.48
III	5	62.5	3	37.5	0	0	9.62 ± 1.19

Fig 13: Recovery pathway in dogs affected with lower urinary tract infections

CHAPTER V

DISCUSSION

The urinary system is one of the most important systems of the dog consisting of a pair of kidneys, a pair of ureters, urinary bladder and urethra. The function of the urinary system is to excrete the metabolic waste products from the body, maintain the electrolyte, water, other solute balance and to regulate the acid-base balance of the body. Normal host defenses aids in preventing urinary tract infection and these include – normal micturition, normal anatomic structures, mucosal defense barrier, normal microflora, antibacterial properties of urine, surface glycosaminoglycan's, local antibody production and systemic immuno competence (Somu *et al.*, 2015). Any alterations in these normal host defense mechanisms results in development of UTI in dogs (Ball *et al.*, 2008, Chew *et al.*, 2011 and Smee *et al.*, 2013a,). UTIs are caused by wide variety of pathogens which includes bacteria, fungi, mycoplasma, parasites and rarely viruses among which bacterial UTIs are most common which occurs due to accession of bacteria by haematogenous route or by ascending infection from surrounding tissues. The invasion and colonization of bacteria in the urinary bladder/ or upper segment of urethra results in development of bacterial lower urinary tract infection (LUTI). It is estimated that approximately 14% of all dogs experience UTIs at some point during their lifetimes (Ettinger and Feldman, 2010 and Hariharan *et al.*, 2016). The most common bacterial isolates which aids in development of urinary tract infections in dogs include *E.coli*, *Enterococcus* spp, *Klebsiella* spp, *Pseudomonas* spp, *Staphylococcus* spp and *Streptococcus* spp (Rampacci *et al.*, 2018). The present study was undertaken to record the occurrence of lower urinary tract infections, to study the clinical symptomatology, haemato-biochemical findings, antibiotic sensitivity pattern and to assess the therapeutic

regimen in dogs affected with lower urinary tract infections that are presented to the Veterinary Clinical Complex, NTR College of Veterinary Science, Gannavaram.

5.1 OCCURRENCE OF LOWER URINARY TRACT INFECTIONS

Out of a total number of 2930 dogs presented to the Veterinary Clinical Complex, NTR College of Veterinary Science, Gannavaram from January 2020 to December 2020, 246 dogs (8.39%) were affected with urinary tract infection by exhibiting clinical signs. This was in partial accordance with that of Forrester *et al.* (1999) who recorded a lower (4.76%) occurrence rate of urinary tract infections with exhibition of clinical signs when compared to the present study. The reason for the higher occurrence of urinary tract infections in the area under study might be due to poor hygienic conditions maintained which aids in the development of UTI by ascending route of entry of pathogen, change in the urination habits, feeding of commercial foods resulting in development of uroliths due to high mineral content and change in the pH of urine making dogs more prone for development of urinary tract infections. On the contrary Adamama *et al.* (2017) recorded the occurrence of urinary tract infections in 1.82 per cent of dogs which might be due to better hygiene conditions maintained in that area under study and type of food offered to the dogs in that area.

Among the dogs screened in the present study lower urinary tract infections was noticed in 33.33 per cent of dogs in the present study which was in accordance with findings of Kogika *et al.* (1995), Stiffler *et al.* (2006) and Olby *et al.* (2010) who recorded the occurrence of lower urinary tract infection in 25.5 per cent, 27 per cent and 36 per cent of dogs respectively. On contrary Yehia and Salem (2015) and Liebelt and Pigott (2019) recorded the incidence of lower urinary tract infections in dogs as 75 per cent and 85 per cent respectively. In the present study on lower urinary tract infections in 35 dogs,

samples were subjected for cultural examination of which 33 (94.28%) were positive for bacterial growth. This was in accordance with Adamama *et al.* (2017) who isolated bacterial from 96.88 per cent of cystocentesis urine samples on culture of urine. On the contrary Kivisto *et al.* (1977), Hariharan *et al.* (2016) and Mendoza *et.al.* (2017) recorded the occurrence of positive bacterial culture of urine as 32.8 per cent, 34.02 per cent and 34.43 per cent respectively in dogs affected with lower urinary tract infections.

5.1.1 Occurrence in relation to age

Age wise occurrence of lower urinary tract infections in the present study revealed highest (45.71%) occurrence in the age group of 0-4 years followed by 31.43 per cent in age group of 4-7 years, 14.29 per cent in dogs of the age group 8-10 years and 8.57 per cent in dogs of more than 10 years age. The high incidence of LUTI in the age group of 0-4 years in the present area under study might be due to relative higher presentation of this age group dogs when compared to remaining age group of dogs and urethrovesical reflux in young dogs which was in accordance with Kivisto *et al.* (1977). Urethrovesical reflux causes retrograde closure of urethra and bladder neck at the end of micturition which aids in colonization of bacterial and development of lower urinary tract infection (Corriere *et al.* 1972). The incidence of 31.43 per cent in the age group of 4-7 years in present study was in accordance with Kogika *et al.* (1995), Kumar and Kamran (2016) and Liebelt and Piggott (2019) who recorded highest occurrence of bacterial lower urinary tract infections in the age group of 5-7 years followed by 7-8 years and 4-8 years respectively. In contrast to the findings of the present study Macotpet *et al.* (2009) and Wong *et al.* (2015) recorded occurrence of bacterial urinary tract infections in 3 months to 3 years age group and dogs of less than 1 year group respectively.

5.1.2 Occurrence in relation to breed

Breed wise occurrence of lower urinary tract infections in the present study revealed that Labrador Retriever (28.57%) breed of dogs are mostly affected followed by Rottweiler (14.29%); German Shepherd and Pug (11.43% each), Non – Descript, Pomeranian, Shih Tzu (8.57% each), Great Dane (5.71%) and Boxer (2.86%). This was in accordance with Wong *et al.* (2015) who recorded higher incidence of urinary tract infections in Labrador Retrievers and Adamama *et al.* (2017) who recorded an incidence of 32.1 per cent in the same breed. The high incidence of LUTIs in German Shepherd was in accordance with Kogika *et al.* (1995) and Macotpet *et al.* (2009) who recorded an incidence in 16.68 and 9.3 per cent of dogs respectively. The highest per cent occurrence of lower urinary tract infections in the present study may be due to more number of Labrador breeds and German Shepherd breeds in the area under study. In addition to that, the high occurrence of lower urinary tract infections in Labrador Retriever in the area under study may be due to feeding habits and obesity noticed in these breeds which was in accordance with Karlsson and Beck (2010) who recorded obesity as a cause for predisposing dogs in development of lower urinary tract infections.

5.1.3 Occurrence in relation to gender

The highest per cent occurrence of lower urinary tract infections was recorded in spayed female dogs (45.72%) followed by neutered male dogs (37.14 %), intact female dogs (11.43%) and least in intact male dogs (5.71 %). This was in accordance with Swenson *et al.* (2004), Wong *et al.* (2015), Wynn *et al.* (2016) and Merkel *et al.* (2017) who recorded that the occurrence of lower urinary tract infections was more common in spayed females than neutered males due to shorter urethra which offers less degree of protection against ascending infections. The high incidence in spayed females and

neutered males over intact females and intact males is due to decreased bladder tone, less efficient urethral seal due to reduction in expression of gonadotrophin receptors making the dogs more prone to lower urinary tract infections which was in accordance with Pegram *et al.* (2019). The findings are in contrast with Gaeta *et al.* (2009) who reported that the occurrence of lower urinary tract infections was more common in castrated males rather than spayed females due to various stress factors such as systemic diseases, poor immune status of dogs making them more prone to urinary tract infections.

5.2 CLINICAL SIGNS

In dogs affected with lower urinary tract infections clinical signs included fever in 26 dogs (74.29%), haematuria in 24 dogs (68.57%), inappetence/anorexia in 18 dogs (51.43%), pollakiuria in 14 dogs (40.00%), oliguria in 13 dogs (37.14%), urinary incontinence in 11 dogs (31.43%), stranguria and vomiting in 10 dogs each (28.57%), pyuria, polyuria and anuria in 6 dogs each (17.14%); polydipsia, dribbling of urine in 4 dogs each (11.43%).

In the present study fever was observed in the 26 dogs (74.29%) which was in accordance with Weese *et al.* (2011), Dunning and Stonehewer (2012), Sorensen *et al.* (2018) and Lippi *et al.* (2019). The reason for development of fever in dogs affected with urinary tract infections may be due to release of toxins by bacteria which spreads in the circulation causing an increase in thermal set point.

In the present study haematuria was observed in the 24 dogs (68.57%) which was in accordance with Bartges (2004), Forrester (2004), Smee *et al.* (2013_b), Kumar and Kamran (2016), Sorensen *et al.* (2018), Yogeshpriya *et al.* (2018) and Lippi *et al.* (2019). The presence of haematuria in the present study might be due to damage of the mucosal surface/barrier of the urinary tract by various causes viz., long term use of

glucocorticoids, uroliths causing leakage of red blood cells into the urinary space resulting in haematuria. Initial haematuria in the present study is due to diseases of urethra while haematuria at the end of urination is due to lesion of bladder making red blood cells to remain in the dependent part and hence voided at last of urination.

Inappetence/Anorexia in the present study was noticed in 51.43 per cent of dogs affected with UTI which was in accordance with Weese *et al.* (2011), Dunning and Stonehewer (2012), Sorensen *et al.* (2018) and Yogeshpriya *et al.* (2018). The presence of inappetence/anorexia in the present study is due to elicit of pain due to the abdominal discomfort in dogs affected with lower urinary tract infections.

Pollakiuria in the present study was noticed in 14 dogs (40.00%) which was in accordance with Bartges (2004), Weese *et al.* (2011), Dunning and Stonehewer (2012), Smee *et al.* (2013_b), Chigozie (2015), Kumar and Kamran (2016), Merkel *et al.* (2017), Sorensen *et al.* (2018), Yogeshpriya *et al.* (2018) and Lippi *et al.* (2019). The presence of pollakiuria in the present study is due to uroliths, poor sphincter muscle tone, damage of the mucosal epithelial barrier of lower urinary tract, long term use of medications which causes irritation and exhibition of clinical sign of pollakiuria in dogs affected with lower urinary tract infections.

The presence of oliguria in the present study was noticed in 13 dogs (37.14%) which was in accordance with Barsanti *et al.* (2012). The uroepithelium composes of umbrella cells which secretes glycosaminoglycan's (GAGs) and proteoglycans which are hydrophilic and responsible for bladder filling and distension which further decreases adherence of bacteria and crystals due to formation of water layer on apical membrane of bladder. Any chemical/mechanical damage to uroepithelium disrupts GAG layer resulting in increased adherence and permeability of bacteria to bladder mucosa causing irritant

substances to pass through and leading to submucosal inflammation. The presence of oliguria in the present study is due to dehydration, urethral obstruction and decreased filling capacity of bladder due to inflamed uroepithelium which decreases voiding frequency, volume and adherence of bacteria to damaged mucosal epithelium that predisposes dogs for the development of lower urinary tract infections in dogs.

Urinary incontinence in the present study was noticed in 11 dogs (31.43%) which was in accordance with Bartges (2004), Sorensen *et al.* (2018) and Lippi *et al.* (2019). The urinary incontinence in the present study is due to weak sphincter tone of bladder, prostate disorders and uroliths causing small amount of urine leak to inadvertent voiding of a large amount of urine.

The presence of stranguria in the present study was noticed in 10 dogs (28.57%) which was in accordance with Dunning and Stonehewer (2012), Smee *et al.* (2013b), Kumar and Kamran (2016), Merkel *et al.* (2017), Sorensen *et al.* (2018), Yogeshpriya *et al.* (2018) and Lippi *et al.* (2019). The stranguria observed in the present study is due to inflamed bladder wall, urolithis and prostate enlargement causing difficulty in urination and prone for lower urinary tract infection.

The presence of vomiting as a clinical sign in the present study was noticed in 10 dogs (28.57%) which was in accordance with Dunning and Stonehewer (2012) and Lippi *et al.* (2019).

In the present study pyuria was noticed in 6 dogs (17.14%) which was in accordance with Weese *et al.* (2011) and Yogeshpriya *et al.* (2018) who recorded pyuria as a clinical sign in dogs affected with lower urinary tract infections. The reason for pyuria in LUTI in the present study might be due to inflammation of any part of lower urinary tract i.e bladder, urethra and prostate or cystoliths and urethroliths which further leads to

infection and inflammation which causes neutrophils to migrate from blood vessels through tissue space and cross epithelial cell layer to enter urinary space resulting in the presence of a large number of white blood cells in the urine of dogs affected with lower urinary tract infections.

Polyuria as a clinical sign in the present study was noticed in 6 dogs (17.14%) which was in accordance with Dunning and Stonehewer (2012), Kumar and Kamran (2016) and Lippi *et al.* (2019) who recorded polyuria in dogs affected with lower urinary tract infections.

In the present study anuria was noticed in 6 dogs (17.14 %) which might be due to, poor bladder tone, cystoliths obstruction at neck of bladder or urethroliths which decreases the luminal width for outflow of urine causing affected dogs prone for exhibition of clinical sign of anuria.

Polydipsia as a clinical sign was noticed in 4 dogs (11.43%) which was in accordance with Dunning and Stonehewer (2012) and Kumar and Kamran (2016) who recorded polydipsia as a clinical sign in dogs affected with lower urinary tract infections.

In the present study dribbling of urine was noticed in 4 dogs (11.43%) which was in accordance with Punia *et al.* (2018). The reason for dribbling of urine in the present study might be due to inflamed mucosal epithelium of bladder and urethra or small sized cystoliths, uroliths causing obstruction and voiding of drop by drop urine in dogs affected with lower urinary tract infections.

5.3 DIAGNOSIS

5.3.1 Urinalysis

In the present study the preliminary diagnosis of bacterial lower urinary tract infections was based on urinalysis of freshly collected urine samples by cystocentesis in sterile manner which was in accordance with Somu *et al.* (2015) who confirmed the lower urinary tract infections in dogs by urinalysis.

In the present study the urinalysis findings in dogs affected with lower urinary tract infections revealed that the colour of urine varied from pale yellow to red colour, pH ranged from 5.0 to 9.0, transparency varied from clear to severely turbid, specific gravity ranged between 1.01 to 1.05 with a mean specific gravity of 1.03, protein concentration ranged from 0.3 g/L to 3.0 g/L, presence of mild bile salts, bile pigments and occult blood as well as absence of sugars, ketones and nitrate. This was in accordance with Dunning and Stonehewer (2012), Jasim (2012), Somu *et al.* (2015), Merkel *et al.* (2017) and Grimes *et al.* (2019) who recorded alterations in physical and chemical examination findings in urine of dogs affected with lower urinary tract infections.

In the present study the presence of turbidity of urine in the affected dogs might be due to large concentrations of bacteria, fat, crystals or mucus in urine resulting in cloudy appearance of urine. The high urine specific gravity in the present study might be due to relatively high protein concentration and low urine specific gravity might be due to relatively less protein concentration in urine of affected dogs. The alkaline pH of urine in the present study might be due to dietary cause or urease producing *Staphylococcus* which break down ammonia to urea leading to alkaline pH of urine. Proteinuria and occult blood in the present study might be due to infection and inflammation of urinary tract resulting in presence of protein and blood in dogs affected with lower UTI.

5.3.2 Urine sediment examination

Urine sediment examination in dogs affected with lower urinary tract infections revealed the presence of epithelial cells, RBC's, struvite crystals, granular casts, WBC's, hyaline casts, calcium oxalate crystals, renal tubular epithelial cells, triple phosphate crystals, bilirubin crystals, tyrosine crystals and bacteria . This was in accordance with Guire *et al.* (2002), Cetin *et al.* (2003), Swenson *et al.* (2004), Bartges and Polzin (2011), Dunning and Stonehewer (2012), Jasim (2012), Palma *et al.* (2013), Smee *et al.* (2013_b), Somu *et al.* (2015), Yogeshpriya *et al.* (2015), Biasibetti *et al.* (2019) and Grimes *et al.* (2019) who recorded presence of crystals, casts and bacteria in urine sediment of dogs affected with lower urinary tract infections.

The presence of RBCs, WBCs and epithelial casts on examination of urine sediment in affected dogs in the present study might be due to damage to the mucosal defence barrier of uroepithelium. The presence of various crystals and casts in the present study might be due to feeding of commercial diet rich in magnesium and calcium, decreased water intake, dehydration, in appropriate urination habits like oliguria, anuria making the urine more concentrated and causing deposition of minerals, sediments due to retention of the concentrated urine which further gets accumulated on ventral wall of bladder and urethra.

5.3.3 Abdominal Radiography

In the present study abdominal radiographic examination was done in 35 dogs affected with lower urinary tract infections of which urethroliths and cystoliths were noticed in 2.85 per cent and 5.71 per cent of dogs respectively which was in accordance with Forrester (2004) and Grimes *et al.* (2019) who recorded radiographic findings as urocystoliths in dogs affected with lower urinary tract infections. The prostate

enlargement in the present study was noticed in 8.57 per cent of dogs on radiographic examination which might be due to extension of infection from urinary tract and difficult to eradicate bacteria from prostate due to blood prostate barrier which re infects the urinary tract and cause local infection in the prostate which eventually turns to abscess (Cunto *et al.* 2019).

In the present study distension of bladder, distension of bladder with a strip of gas in lumen was noticed in 34.28 per cent each and increased radioopacity of the bladder wall was noticed in 14.29 per cent of dogs respectively which was in accordance with Aizenberg and Aroch (2003), Petite *et al.* (2006), Merkel *et al.* (2017), Fumeo *et al.* (2019) and Lippi *et al.* (2019) who recorded the radiographic findings as gas localization in lumen and wall of bladder. The reason for gas localization in the present study might be due to rapid catabolism of sugars, albumin causing accumulation of carbondioxide and hydrogen resulting in localized gas in lumen and wall of bladder.

5.3.4 Abdominal Ultrasonography

In the present study ultrasonographic findings in dogs affected with lower urinary tract infections revealed thickened bladder wall (> 2mm) in 45.71 per cent of dogs which was in accordance with Dinesh *et al.* (2015), Yehia and Salem (2015), Kumar and Kamran (2016), Yogeshpriya *et al.* (2018), Lippi *et al.* (2019) and Biasibetti *et al.* (2019) who recorded thickening of bladder wall on performing ultrasonography in dogs affected with lower urinary tract infections. The reason for thickening of bladder wall in the present study is due to damage of mucosal defence barrier of bladder due to infection and leading to inflammation causing increase in the thickness of bladder mucosa.

The presence of hyperechoic calculi in lumen and neck of bladder with acoustic shadow in the present study was noticed in 5.71 per cent of dogs. This was in accordance

with Aizenberg and Aroch (2003), Forrester (2004), Kundu and Gosh (2006), Robotti and Lanfranchi (2013) and Dinesh *et al.* (2015) who recorded the ultrasonography findings as calculi in lumen and neck of bladder with their acoustic shadow in dogs affected with lower urinary tract infections.

The presence of sludge in lumen of bladder in the present study was noticed in 31.44 per cent of dogs which was in accordance with Kumar and Kamran (2016). The reason for sludge in the present study in dogs affected with LUTI might be due to concentrated urine resulting in deposition of urine sediments in lumen of bladder.

The irregularity of bladder mucosa with hyperechoic bladder wall in the present study was noticed in 8.57 per cent of dogs which was in accordance with Biegen *et al.* (2013) and Biasibetti *et al.* (2019) who recorded irregularity of bladder wall in dogs affected with LUTI.

Prostate enlargement in the present study was noticed in 8.57 per cent of dogs which was in accordance with Robotti and Lanfranchi (2013) who recorded enlargement of prostate in dogs affected with LUTI.

5.3.5 Isolation and identification of bacteria

Among the 35 samples subjected for isolation and identification in 33 cases bacterial isolates were identified and remaining 2 samples were found negative for bacterial growth. Out of 33 samples affected with bacterial lower urinary tract infections 33.33 per cent were positive for *E.coli*, 21.21 per cent were positive for *Staphylococcus* spp, 18.18 per cent were positive for *Streptococcus* spp, 12.12 per cent were positive for *Pseudomonas* spp, 9.10 per cent were positive for mixed infection by *E.coli*+*Staphylococcus* spp remaining 6.06 per cent were positive for *Klebsiella* spp. The cultural findings in the present study were in accordance with Ling *et al.* (2001), Cetin *et al.* (2003), Papini *et al.* (2006), Smee *et al.* (2013_a), Punia *et al.* (2018) and Fumeo *et al.*

(2019) who isolated and identified *E.coli* as a single bacterial isolate and major uropathogen in urine of dogs affected with lower urinary tract infections.

5.4 ANTIBIOTIC SUSCEPTIBILITY TESTING

In the present study out of 33 samples susceptibility to amikacin was noticed in all 33 samples (100%), whereas susceptibility to gentamicin was noticed in 31 samples (93.94%), 30 samples (90.91%) to ceftriaxone + tazobactam, 27 samples (81.82%) to amoxicillin sodium + sulbactam and nitrofurantoin each, 26 samples (78.79%) to trimethoprim-sulphamethaxazole and 25 samples (75.76%) to ciprofloxacin. The findings in the present study was in accordance with McGovern *et al.* (2019) who isolated *E.coli* as a major pathogen and found 90.2 per cent sensitivity to amikacin.

In the present study 93.94 per cent sensitivity was found to gentamicin which was in accordance with Oxenford *et al.* (2006) who identified *E.coli* as a major pathogen and found 100 per cent sensitivity to gentamycin and Ball *et al.* (2008) who identified *E.coli* as one of the major uropathogens and found 93.3 per cent sensitivity to gentamicin.

In the present study 90.91 per cent sensitivity was found to ceftriaxone+ tazobactam which was in accordance with Punia *et al.* (2018) who recorded *E.coli* as one of the major uropathogens and found 88.88 per cent sensitivity to ceftriaxone+ tazobactam.

Amoxicillin sodium + sulbactam in the present study was found to be 81.82 per cent sensitive to the isolated bacterial uropathogens which was in accordance with Ball *et al.* (2008) and Punia *et al.* (2018) who recorded sensitivity of 89.1 per cent and 76 per cent respectively against *E.coli* in their study.

In the present study 81.82 per cent sensitivity was recorded with nitrofurantoin which was in accordance with Cetin *et al.* (2003) who recorded a sensitivity of 58.82 per cent to bacterial uropathogens in dogs affected with lower UTI.

Trimethoprim-sulphamethaxazole in the present study was found to be 78.79 per cent sensitive to the isolated bacterial uropathogens which was in accordance with Ball *et al.* (2008) and Wong *et al.* (2015) who recorded a sensitivity of 86.7 per cent and 85.7 respectively.

In the present study ciprofloxacin was found to be 75.76 per cent sensitive to bacterial uropathogens which was not in accordance with that of Macotpet *et al.* (2009) who found 100 per cent sensitive to isolated bacterial uropathogens in their study.

5.5 UNDERLYING OR ASSOCIATED FACTORS

In the present study prostate enlargement was seen in 3 dogs (8.57%) which was in accordance with Dinesh *et al.* (2015) and Somu *et al.* (2015) who recorded prostate enlargement as an underlying cause in development of recurrent lower urinary tract infections.

Long term use of glucocorticoids was noticed in 2 dogs (5.71%) affected with lower urinary tract infections in the present study. This was in accordance with Torres *et al.* (2005), Gaeta *et al.* (2009), Smee *et al.* (2013_a) and Smee *et al.* (2013_b) who observed long term use of glucocorticoids as an underlying cause in dogs affected with lower urinary tract infections.

In the present study cystoliths were seen in 2 dogs (5.71%) and urethroliths in 1 dog (2.86 %) which was in agreement with Weese *et al.* (2011), Sravanthi *et al.* (2014)

and Dinesh *et al.* (2015) who recorded urocystoliths as underlying factor in development of lower urinary tract infection in dogs.

5.6 HAEMATOLOGY

A significant decrease in mean haemoglobin value was observed in the present study in dogs affected with lower urinary tract infections when compared to apparently healthy dogs which was attributed to decreased appetite, loss of blood due to damage of mucosal epithelial barrier and inflammation causing leakage of RBC into urinary space which was in agreement with that of Ukwueze (2015), Das *et al.* (2017), Merkel *et al.* (2017) and Liebelt and Pigott (2019) who recorded a significant decrease in the mean values of Hb in dogs affected with LUTI.

A significant decrease in mean PCV value was observed in the present study in dogs affected with lower urinary tract infections when compared to apparently healthy dogs which can be attributed to loss of blood due to damage of mucosal epithelial barrier and inflammation resulting in leakage of RBC into urinary space. This finding is in agreement with Merkel *et al.* (2017), Roopali *et al.* (2018) and Yogeshpriya *et al.* (2018) who recorded a significant decrease in the mean values of PCV when compared to healthy control group of dogs.

A significant increase in the mean total leucocyte count was recorded in dogs affected with lower urinary tract infections when compared to apparently healthy dogs which could be due to tissue damage of mucosal epithelium caused by inflammation leading to the release of bacterial toxins which is in accordance with Cetin *et al.* (2003), Roopali *et al.* (2018), Yogeshpriya *et al.* (2018) and Biasibetti *et al.* (2019) who recorded an increase in total leucocyte count in dogs affected with lower urinary tract infections.

There was a significant increase in the mean neutrophil count in dogs affected with lower urinary tract infections when compared to healthy dogs. These findings are in agreement with Cetin *et al.* (2003), Roopali *et al.* (2018), Merkel *et al.* (2017) and Yogeshpriya *et al.* (2018). The reason for increase in neutrophil count could be attributed to cell injury and variable extent of stress induced by bacterial organisms in the urinary tract as well as a sign of manifestation of body defense mechanism against bacterial infection resulting in release of more neutrophils into the blood stream. A significant decrease in the mean values of lymphocytes was recorded when compared to apparently healthy dogs was in accordance with Das *et al.* (2017), Merkel *et al.* (2017), Roopali *et al.* (2018) and Yogeshpriya *et al.* (2018). The decrease in lymphocyte count might be due to stressful stimuli caused by bacteria that occurs in lower urinary tract infections. A nonsignificant difference in the mean values of monocytes and eosinophils were recorded when compared to apparently healthy dogs which was in accordance with Roopali *et al.* (2018) and Yogeshpriya *et al.* (2018) who recorded non significant decrease in mean values of eosinophils and monocytes of dogs affected with LUTI.

5.7 SERUM BIOCHEMISTRY

There was a significant decrease in the mean values of total protein and albumin when compared to apparently healthy control group which could be attributed to the loss of protein and albumin through urine. Decreased production and increased loss of albumin during inflammation due to bacterial actions which causes increased vascular permeability and passage of albumin into interstitial space was responsible for hypoalbuminemia in dogs affected with lower urinary tract infections (Throop *et al.*, 2013 and Fransson *et al.*, 2007). This was in accordance with Das *et al.* (2017) and Roopali *et al.* (2018) who recorded significant decrease in mean values of total protein, Squires (1994), Andonova *et al.* (2010), Roopali *et al.* (2018) and Yogeshpriya *et al.* (2018) who

recorded significant decrease in mean values of albumin in dogs affected with lower urinary tract infections.

The present study revealed a significant increase in the mean total serum creatinine levels when compared to apparently healthy control dogs. This was in agreement with Blanco and Bartges (2001), Cetin *et al.* (2003), Jasim (2012), Biegen *et al.* (2013), Dinesh *et al.* (2015), Das *et al.* (2017), Focak *et al.* (2017), Roopali *et al.* (2018), Yogeshpriya *et al.* (2018) and Grimes *et al.* (2019) who recorded a significant increase in mean serum creatinine levels due to the ascending infection to kidneys from lower urinary tract which causes muscle metabolism and elevation in serum creatinine levels.

The present study revealed a significant increase in the mean total serum urea nitrogen levels (BUN) when compared to apparently healthy control dogs. This was in agreement with Cetin *et al.* (2003), Jasim (2012), Biegen *et al.* (2013), Dinesh *et al.* (2015), Roopali *et al.* (2018), Yogeshpriya *et al.* (2018) and Grimes *et al.* (2019) which might be due to feeding of high protein diet which causes elevation in serum urea nitrogen levels in the affected dogs.

5.8 THERAPEUTIC TRIAL

Therapeutic trial were undertaken up in 24 dogs affected with lower urinary tract infections after allocating the dogs randomly into three groups with eight in each group. Group I dogs were treated with amoxicillin sodium + sulbactam, Group II were treated with ceftriaxone + tazobactam and dogs of Group III were treated with amikacin. Antibiotic therapy was continued for a period of one week beyond the point of clinical recovery. Response to therapy was assessed based on the resolution of exhibited clinical signs in the dogs under study which includes fever, haematuria, inappetence/anorexia,

pollakiuria, oliguria, urinary incontinence, stranguria, vomiting, pyuria, polyuria, anuria, polydipsia, dribbling of urine; recovery pathway; negative bacterial growth of urine after therapy and improvement in haemato biochemical values. All the dogs were monitored for a period of two months after the completion of therapy for the presence of recurrence of infection.

5.8.1 Group I

Dogs in this group were treated with amoxicillin sodium + sulbactam at the dose rate of 20 mg/kg body weight twice daily I/M. Six dogs showed improvement by 7th day with remission of clinical signs like fever, haematuria, pollakiuria, oliguria and complete recovery by 14th day by subsidence of clinical signs, normal urinalysis findings and negative bacterial culture after therapy. The remaining two dogs in this group showed improvement by 14th day with remission of clinical signs like fever, inappetence, pollakiuria, urinary incontinence, anuria, polydipsia, dribbling of urine and complete recovery by 21st day with, normal urinalysis findings, negative bacterial culture after therapy. Among two dogs in this group which recovered by 21st day, one dog was diagnosed to be affected with prostate enlargement and the other with cystolithiasis as the underlying factor. The reason for more time taken for recovery in these two dogs could be attributed to prolonged time taken for the cure of underlying factors viz., lower UTI developed due to prostate enlargement and cystolithiasis. Based on the recovery pathway in dogs affected with lower urinary tract infections in this group the average number of days taken for recovery was 15.75 ± 1.07 .

Amoxicillin exerts its mechanism of action by adhering to the bacterial penicillin binding proteins thus inhibiting the bacterial cell wall synthesis. Sulbactam has weak

antibacterial activity and does not affect the mechanism of action of amoxicillin. However, it has a beta lactam ring and is structurally similar to penicillins and cephalosporins thus binds irreversibly with certain betalactamases and prevents them from inactivating amoxicillin which enhances its bactericidal activity (Riviere and Papich 2018).

There was a significant decrease in the mean values of haemoglobin and packed cell volume on the day of presentation which on treatment increased significantly. The pre therapeutic mean value of total leucocyte count was significantly higher which upon treatment was found to be significantly decreased. The mean values of neutrophils decreased significantly after therapy when compared with the mean values before therapy. There was a significant increase in the lymphocyte count and a nonsignificant decrease in monocytes and eosinophils after therapy when compared with pre therapeutic values.

The mean value of serum total protein and albumin was found to be significantly lower which significantly increased after therapy. There was a significant decrease in the mean value of serum creatinine and urea after therapy when compared with the values on the day of presentation.

Thus based on the improvement in the resolution of clinical signs, negative bacteriological culture after therapy, recovery in all the dogs and improvement in haemato biochemical changes i.e., a significant increase in haemoglobin, packed cell volume & lymphocytes; significant decrease in total leucocyte count, neutrophils; non significant decrease in eosinophils and monocytes; significant decrease in serum urea and creatinine levels and significant increase in total protein and albumin value it can be concluded that Amoxicillin sulbactam is efficacious in the treatment of lower urinary tract infections in

dogs. Cotard *et al.* (1995), Westropp *et al.* (2012) reported that amoxicillin sodium + sulbactam is efficacious in the treatment of urinary tract infections in dogs.

In the dogs of this group recurrence was not observed up to a period of two months after the clinical cure.

5.8.2 Group II

Dogs in this group were treated with ceftriaxone + tazobactam at the dose rate of 20 mg/kg body weight once daily I/M. Five dogs showed improvement in subsidence of clinical signs like fever, vomiting, inappetence, stranguria by 7th day and complete recovery with remission of all clinical signs normal urinalysis findings and negative bacterial growth by 14th day. Two dogs in this group showed response to therapy with subsidence of all clinical signs like haematuria, inappetence, pollakiuria, urinary incontinence, anuria and negative bacterial growth by 7th day indicating complete recovery with normal urinalysis findings after therapy. Remaining one dog showed improvement in subsidence of clinical signs by 14th day and complete recovery with remission of all clinical signs like stranguria, vomiting, pyuria, polyuria and normal urinalysis findings with negative bacterial growth in urine by 21st day of therapy. One dog which recovered by 21st day and two dogs which recovered by 14th day in this group was diagnosed with cystolithiasis, prostate enlargement and urolithiasis as an underlying cause in the development of lower urinary tract infection. The reason for more time taken to recovery in one dog could be attributed to prolonged time taken for the cure of underlying factor viz., cystitis due to prostate enlargement, urolithiasis and cystolithiasis. Based on the recovery pathway in dogs affected with lower urinary tract infections in this group the average number of days taken for recovery was 13.13 ± 1.48 .

Ceftriaxone tazobactam exerts its mechanism of action by interfering with the biosynthesis of the peptidoglycan component of the bacterial cell wall by binding to and inactivating penicillin-binding proteins (PBPs). Tazobactam is a penicillanic acid sulfone derivative with β -lactamase inhibitory properties. It enhances the activity of β -lactam antibacterials against β -lactamase-producing bacteria (Riviere and Papich 2018).

There was a significant increase in the mean values of Hb and PCV in this group of dogs after therapy when compared with pre therapeutic values. The mean value of total leucocyte count showed a significant decrease after treatment when compared to values before therapy. A significant decrease in the mean value of neutrophils, significant increase in lymphocytes, nonsignificant increase in monocytes and eosinophils were recorded after therapy when compared with pre therapeutic values.

A significant increase in the levels of total protein and albumin were noticed after treatment when compared with pre therapeutic values. The mean value of serum creatinine and serum urea concentrations levels showed a significant increase on the day of presentation which after therapy decreased significantly when compared with the mean value before therapy.

Thus based on the improvement in the resolution of clinical signs, negative bacteriological culture after therapy, recovery in all the dogs and improvement in haemato biochemical parameters i.e., a significant increase in haemoglobin, packed cell volume and lymphocytes; significant decrease in total leucocyte count, neutrophils; non significant increase in monocytes and eosinophils; significant decrease in serum urea and creatinine levels and significant increase in total protein and albumin value it can be concluded that ceftriaxone in combination with tazobactam is efficacious in the treatment of lower urinary tract infections in dogs. Thirunavukkarasu *et al.* (2010) and Punia *et al.*

(2018) reported that ceftriaxone tazobactam @ 20 mg/kg once daily and 20mg/kg twice daily respectively is effective in the treatment of urinary tract infections in dogs.

In the dogs of this group recurrence was not observed up to a period of two months after the clinical cure.

5.8.3 Group III

Dogs in this group were treated with amikacin at the dose rate of 18 mg/kg body weight once daily I/M. Five dogs in this group showed improvement in subsidence of clinical signs like fever, haematuria, normal urinalysis findings and negative bacterial growth by 7th day of therapy. Remaining three dogs showed improvement in subsidence of clinical signs like haematuria, inappetence, oliguria, urinary incontinence, pyuria, polyuria by 7th day of therapy but urinary culture revealed positive bacterial growth on 7th day. The dogs were reexamined on 14th day of therapy which revealed complete remission of clinical signs, normal urinalysis findings and negative bacterial growth by 14th day of therapy. Three dogs in this group which recovered by 14th day of therapy were diagnosed with prostate enlargement and long term use of glucocorticoids as an underlying cause in development of lower urinary tract infection. The reason for more time taken to recovery in these three dogs could be attributed to prolonged time taken for the cure of underlying factors viz., cystitis due to prostate enlargement and long term use of glucocorticoids. Based on the recovery pathway in dogs affected with lower urinary tract infections in this group the average number of days taken for recovery was 9.62 ± 1.19 .

Amikacin exerts its mechanism of action through disruption of the bacterial cell wall by irreversible binding to the 30S ribosomal subunit and interferes with mRNA binding, tRNA acceptor sites resulting in inhibition of bacterial protein synthesis which

there by inhibits bacterial growth by production of non-functional or toxic peptides indicating the bactericidal property of amikacin (Riviere and Papich 2018).

A significant decrease in the mean values of Hb and PCV were recorded on the day of presentation which upon treatment was found to be significantly increased. The mean value of TLC was found to be significantly decreased after therapy when compared with pre therapeutic values. Post therapeutically a significant decrease in the mean value of neutrophils, significant increase in lymphocytes, nonsignificant decrease in monocytes and eosinophils were recorded when compared with pre therapeutic mean values.

A significant increase in the levels of total protein and albumin were noticed after treatment when compared with pre therapeutic values. The mean value of serum creatinine and serum urea concentrations levels showed a significant increase on the day of presentation which after therapy decreased significantly when compared with the mean value before therapy.

Thus based on the improvement in the resolution of clinical signs, negative bacteriological culture of urine, recovery in all dogs in this group and improvement of haemato biochemical findings i.e. a significant increase in haemoglobin, packed cell volume and lymphocytes; significant decrease in total leucocyte count, neutrophils; non significant decrease in the values of eosinophils and monocytes; significant increase in total protein, albumin and significant decrease in serum urea and creatinine levels it can be concluded that amikacin is efficacious in the treatment of lower urinary tract infections in dogs. Torres and Cooke (2014) reported amikacin as an effective antibiotic in treatment of lower urinary tract infections.

In the dogs of this group recurrence was not observed up to a period of two months after the clinical cure.

Therapeutic efficacy

In all the dogs of Group I, Group II and Group III taken up for therapeutic trial complete recovery was seen in all the dogs and no recurrence was noticed even after two months of therapy .

From the above findings it can be concluded that all the three antibiotics i.e., amoxicillin + sulbactam, ceftriaxone + tazobactam and amikacin were efficacious in the treatment of lower urinary tract infections in dogs. Based on recovery pathway by subsidence of clinical signs, urinalysis findings and bacteriological culture after therapy, amikacin is more efficacious when compared to ceftriaxone + tazobactam followed by amoxicillin sodium + sulbactam in the treatment of bacterial lower urinary tract infections.

CHAPTER VI

SUMMARY

The study entitled “Clinico - diagnostic and therapeutic studies on lower urinary tract infections in dogs” was carried out in dogs presented to the Veterinary Clinical Complex, NTR College of Veterinary Science, Gannavaram during the study period of January 2020 to December 2020 to record the occurrence of lower urinary tract infections in dogs, to study the clinical symptomatology, haemato-biochemical findings, antibiotic sensitivity pattern and to assess the therapeutic regimen in dogs affected with bacterial lower urinary tract infections.

Out of 2930 dogs presented to the small animal medical ward 246 (8.39%) dogs exhibited the signs of urinary tract infections which accounted for 8.39 per cent of total number of cases. Of the 246 cases 82 (33.33%) showed clinical signs suggestive of lower urinary tract infections. Occurrence of lower urinary tract infections was analyzed in 35 dogs taken up for the detailed study.

The highest occurrence of lower urinary tract infections was recorded in dogs of the age group 0-4 years (45.71%), Labrador Retriever breed of dogs (28.57%) and female dogs (57.14%).

The clinical signs exhibited by dogs affected with lower urinary tract infections include fever; haematuria; inappetence/anorexia; pollakiuria; oliguria; urinary incontinence; stranguria and vomiting; pyuria, polyuria; anuria; polydipsia and dribbling of urine .

Radiographic examination revealed 8.57 per cent of dogs with prostate enlargement, urethroliths was noticed in 2.85 per cent of dogs, cystoliths in 5.71 per cent of dogs, distension of bladder and distension of bladder with a strip of gas in lumen in

34.28 per cent each and remaining 14.29 per cent of dogs revealed increased radioopacity of the bladder wall in dogs affected with lower urinary tract infections.

Ultrasonographic findings in dogs affected with lower urinary tract infections revealed 45.71 per cent of dogs had thickened bladder wall ($> 2\text{mm}$), 5.71 per cent of dogs with hyperechoic calculi in lumen and neck of bladder with acoustic shadow, 31.44 per cent of dogs with sludge in lumen of bladder, 8.57 per cent of dogs each with irregularity of bladder mucosa with hyperechoic bladder wall and prostate enlargement.

Isolation and identification of bacteria from thirty five samples of dogs affected with lower urinary tract infections revealed that 33 samples were positive for bacteriological growth while in 2 samples no bacterial growth is seen. Out of 33 positive samples *E.coli* (33.33%) was identified as major uropathogen followed by *Staphylococcus* spp (21.21%), *Streptococcus* spp (18.18%), *Pseudomonas* spp (12.12%), mixed infection of *E.coli* and *Staphylococcus* spp (9.10%) and least identified was *Klebsiella* spp (6.06%).

Antimicrobial susceptibility test of 33 samples were revealed susceptibility to amikacin in all 33 samples (100%), whereas susceptibility to gentamicin was noticed in 31 samples (93.94%), 30 samples (90.91%) to ceftriaxone + tazobactam, 27 samples (81.82%) to amoxicillin sodium + sulbactam and nitrofurantoin each, 26 samples (78.79%) to trimethoprim-sulphamethaxazole and 25 samples (75.76%) to ciprofloxacin.

Among 35 dogs that were affected with lower urinary tract infections underlying factors were noticed in 8 dogs which include prostate enlargement in 3 dogs (8.57%), long term use of glucocorticoids as well as cystoliths in 2 dogs (5.71%) each and urethroliths in 1 dog (2.86%).

Haematology revealed a significant decrease in haemoglobin (11.96 ± 0.40 g/dl), packed cell volume ($33.00 \pm 0.89\%$) and lymphocytes ($18.58 \pm 1.01\%$) [$P < 0.01$]; significant increase in total leucocyte count ($11.62 \pm 0.26 \times 10^3/\mu\text{l}$) and neutrophils ($80.04 \pm 1.04\%$) [$p < 0.01$]; non significant decrease in monocytes ($0.83 \pm 1.16\%$) and eosinophils ($0.63 \pm 0.18\%$) in dogs affected with lower urinary tract infections when compared with apparently healthy control group of dogs. Serum biochemistry revealed decrease in total protein level (7.04 ± 0.07 g/dl) and albumin levels (2.76 ± 0.07) [$P < 0.01$]; increase in blood urea nitrogen (34.76 ± 0.07 mg/dl) and creatinine (1.70 ± 0.07 mg/dl) [$P < 0.01$] in dogs affected with lower urinary tract infections when compared with healthy control group of dogs.

Therapeutic trial was taken up in 24 dogs affected with lower urinary tract infections by dividing them randomly into three groups with 8 dogs each for evaluation of efficacy of three different antibiotics. Antibiotic therapy in all the dogs was continued for one week beyond the clinical cure. Underlying factors were treated accordingly. All the dogs were monitored at weekly intervals for the resolution of clinical signs, urinalysis findings and bacteriological culture examination. All the dogs were monitored for a period of 2 months after the therapeutic trial for the presence of any recurrence of infection.

Dogs in the Group I were treated with amoxicillin sodium + sulbactam at the dose rate of 20 mg/kg body weight twice daily I/M. Six dogs showed complete recovery by 14th day by subsidence of clinical signs, normal urinalysis findings and negative bacterial culture. Remaining two dogs in this group showed complete recovery by 21st day with, normal urinalysis findings, negative bacterial culture. Two dogs in this group were diagnosed to be affected with prostate enlargement and the other with cystolithiasis as the underlying factor. Based on the recovery pathway in dogs affected with lower urinary

tract infections in this group the average number of days taken for recovery was 15.75 ± 1.07 . In the dogs of this group recurrence was not observed up to a period of two months after the clinical cure.

After treatment haematological examination revealed a significant increase in the values of Hb, PCV and lymphocytes; a significant decrease in TLC, neutrophils; a nonsignificant decrease in monocytes and eosinophils when compared with pre therapeutic values. Serum biochemistry revealed a significant decrease in mean blood urea nitrogen and serum creatinine values, significant increase in total protein and albumin levels when compared with pre therapeutic values.

Dogs in the Group II were treated with ceftriaxone + tazobactam at the dose rate of 20 mg/kg body weight once daily I/M. Five dogs showed complete recovery with remission of all clinical signs, normal urinalysis findings and negative bacterial growth by 14th day. Two dogs in this group showed response to therapy by 7th day indicating complete recovery with normal urinalysis findings and negative bacterial culture. Remaining one dog showed complete recovery with remission of all clinical signs, normal urinalysis findings and negative bacterial growth by 21st day of therapy. One dog in this group was diagnosed with cystolithiasis as an underlying cause and two dogs in this group were diagnosed with prostate hypertrophy and urolithiasis as underlying cause in the development of lower urinary tract infection. In the dogs of this group recurrence was not observed up to a period of two months after the clinical cure.

A significant increase in the values of Hb, PCV and lymphocytes; a significant decrease in TLC, neutrophils; a nonsignificant decrease in monocytes and eosinophils were recorded after therapy when compared with values before therapy. Serum biochemistry revealed a significant decrease in mean blood urea nitrogen and serum

creatinine values, significant increase in total protein and albumin levels were recorded after treatment when compared with pre therapeutic values.

Dogs in the Group III were treated with amikacin at the dose rate of 18 mg/kg body weight once daily I/M. Five dogs in this group showed normal urinalysis findings and negative bacterial growth by 7th day of therapy. Remaining three dogs showed response to therapy by 14th day which revealed complete remission of clinical signs, normal urinalysis findings and negative bacterial growth by 14th day of therapy. Three dogs in this group were diagnosed with prostate enlargement and long term use of glucocorticoids as an underlying cause in development of lower urinary tract infection. Based on the recovery pathway in dogs affected with lower urinary tract infections in this group the average number of days taken for recovery was 9.62 ± 1.19 . In the dogs of this group recurrence was not observed up to a period of two months after the clinical cure.

After therapy a significant increase in the values of Hb, PCV and lymphocytes; a significant decrease in TLC and neutrophils; a nonsignificant decrease in monocytes and eosinophils were recorded when compared with pre therapeutic values. A significant decrease in blood urea nitrogen and serum creatinine values; significant increase in total protein and albumin were recorded after treatment when compared with pre therapeutic values.

From the above study the following conclusions are drawn:

1. The occurrence of lower urinary tract infections recorded was 33.33 per cent in the present study.
2. Highest occurrence was noticed in the age group of 0-4 years, Labrador Retriever breed of dogs and spayed females.
3. Most common observed clinical signs in dogs affected with lower urinary tract infections were fever, haematuria, inappetence/anorexia, pollakiuria, oliguria, urinary incontinence, stranguria and vomiting.
4. Preliminary diagnosis of bacterial LUTI was done by urinalysis and confirmative diagnosis was done by isolation and identification of bacteria revealing *E.coli* as a major uropathogen and *Klebsiella* spp as the least common uropathogen identified in the present area under study.
5. Antibiotic susceptibility test revealed that 100 percent of samples were susceptible to amikacin and least susceptible to ciprofloxacin (75.76%).
6. Haemato biochemical examination in dogs affected with bacterial LUTI revealed significant increase in TLC, neutrophils, blood urea nitrogen and serum creatinine while a significant decrease in haemoglobin, packed cell volume, lymphocytes, total protein and albumin was noticed.
7. All the three antibiotics viz., amoxicillin sodium + sulbactam, ceftriaxone + tazobactam and amikacin used in the present study were effective in the treatment of lower urinary tract infections in dogs.
8. Based on recovery pathway by subsidence of clinical signs, urinalysis findings and bacteriological culture after therapy, amikacin is more efficacious when compared to ceftriaxone + tazobactam followed by amoxicillin sodium + sulbactam in the treatment of bacterial lower urinary tract infections.

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APPENDIX - A

CASE SHEET

I) Hospital record

Case no :

Date of admission :

II) Owner information

Name of owner :

Address :

Phone number :

III) Signalament

Species :

Sex :

Breed :

Age :

B.wt :

IV) Anamnesis

Chief complaint:

Chief complaint	Present	Absent
Fever		
Vomiting		
Inappetence / anorexia		
Stranguria		
Urinary incontinence		
Haematuria		
Dysuria		
Oliguria		
Polyuria		
Pyuria		
Anuria		
Pollakiuria		
Polydipsia		

Type of urination : Free flow/ Dribbling

When did problem start :

Duration of problem :

Any abnormal odour from urine : Yes/No

Any previous history of Urinary tract infection : Yes / No

Any previous treatment that has been taken up : Yes/No

Appearance of animal : Active/Dull/Recumbent

Appetite : Normal/Reduced/Absent

History of deworming : Regular/Irregular

History of vaccination : DHPPi+L₂ - Yes/No

Rabies - Yes/No

Feeding habits : Homemade / commercial

Water intake : Normal / Reduced /Absent/Increased

Reproductive status : Spayed/ Neutered/Intact

V) Clinical examination

Temperature : RR :

CMM : HR :

Pulse

Abdominal palpation :

Samples to be collected : whole blood, Serum, Urine

Radiography : calculi / size of bladder / continuity of bladder wall

Ultrasonography : calculi / size of bladder / thickness of bladder wall /sludge

VI) Laboratory examination

Urinalysis:

Parameter	Before therapy	After therapy
Colour		
Specific gravity		
PH		
Glucose		
Proteins		
Ketones		
Leucocytes		
Microscopic Examination		

Hematology:

Parameter	Before therapy	After therapy
Hemoglobin (g/dL)		
Packed cell Volume (%)		
Total Leucocyte count ($\times 10^3/\mu\text{l}$)		
Differential Leucocyte count		
Neutrophils (%)		
Lymphocytes (%)		
Monocytes (%)		
Eosinophils (%)		
Basophils(%)		

Serum biochemistry:

Parameter	Before therapy	After therapy
Total protein (g/dl)		
Albumin (g/dl)		
BUN (mg/dl)		
Creatinine (mg/dl)		

Isolation and identification of bacteria:

Gram's staining

Catalase test

Oxidase test

IMViC test

Culture on specific agar

Antibiotic sensitivity test:

ABST disc	Enterobacteriaceae (<i>E.coli</i> , <i>Pseudomonas</i> spp, <i>Klebsiella</i> spp)			<i>Staphylococcus</i> spp			<i>Streptococcus</i> spp			Test mm	Result
	S	I	R	S	I	R	S	I	R		
CIT	23	20-22	19	21	14-20	13	27	25-26	24		
AMS	19	-	19	19	-	20	19	-	20		
GEN	17	14-16	14	18	-	18	18	-	18		
CIP	26	24-25	24	15	16-20	21	15	16-20	21		
NTT	17	15-16	14	17	15-16	14	17	15-16	14		
COT	14	11-13	11	17	14-16	14	18	15-17	15		
AK	18	15-17	15	18	16-17	16	18	16-17	16		

CIT - Ceftriaxone + tazobactam

GEN - Gentamicin

AMS - Amoxicillin sodium + sulbactam

CIP – Ciprofloxacin

COT - Trimethoprim-sulphamethaxazole

NTT – Nitrofurantoin

AK - Amikacin

Diagnosis :**Treatment :**

Resolution of clinical signs

Clinical sign	0th day	7th day	14th day	21st day
Fever				
Vomiting				
Inappetence/anorexia				
Stranguria				
Urinary incontinence				
Hematuria				
Dysuria				
Oliguria				
Pollakiuria				
Pyuria				
Anuria				
Polydipsia				
Polyuria				

Recurrence of clinical signs:

APPENDIX- B

ESTIMATION OF SERUM TOTAL PROTEIN

Method: Biuret Method, End point assay

Principle:

The peptide bonds of proteins react with cupric II ions in alkaline solution to form blue violet complex, (biuret reaction). Each copper ion complexing with 5 or 6 peptide bonds. Tartrate is added as a stabilizer whilst iodine is added to prevent auto reduction of the alkaline copper complex. The colour formed is proportional to the protein concentration and is measured at 546 nm.

Reagents:

Reagent 1 (Total protein Reagent) : Copper II sulphate 19 m mol/L
Sodium hydroxide 600 m mol/L
Sodium- Potassium tartrate 43 m mol/L
Potassium iodide 30 m mol/L

Reagent 2 (Total protein Standard) : 6.0 g/dl.

Procedure:

Pipette into tube	Blank	Standard	Test
Protein reagent	1000 µl	1000 µl	1000 µl
Distilled water	20 µl	--	--
Standard	--	20 µl	--
Test	--	--	20 µl

The contents of test tube were mixed well and incubated for 10 minutes at 37⁰ C and then absorbance was read against blank at 546 nm on semiautomatic analyzer.

APPENDIX- C

ESTIMATION OF SERUM ALBUMIN

Method: Bromo cresol green (BCG) Dye Method, End point assay

Principle:

Albumin binds with Bromocresol green (BCG) at p^H 4.2 causing a shift in absorbance of the yellow BCG dye. The blue-green colour formed is proportional to the concentration of albumin present, when measured photometrically between 580-630 nm with maximum absorbance at 625 nm.

Reagents:

Reagent 1 (Albumin Reagent) : Succinate buffer, $pH\ 4.2 \pm$ – 50 m mol/l.

Bromocresol green – 0.08 m mol/L

Reagent 2 (Albumin Standard) : 3.6 g/dl

Procedure: A sterile test tube was taken

Pipette into tube	Blank	Standard	Test
Albumin reagent	1000 μ l	1000 μ l	1000 μ l
Distilled water	10 μ l	--	--
Standard	--	10 μ l	--
Test	--	--	10 μ l

The contents of the test tube were mixed well and incubated for 1 minutes at room temperature and then absorbance was read against reagent blank at 630 nm on semiautomatic analyzer.

APPENDIX- D

ESTIMATION OF BLOOD UREA NITROGEN

Method: GLDH (Glutamate dehydrogenase) - UREASE, Initial rate assay

Principle:

The principle of urea assay kit is urea is converted into glutamate in a twostep reaction with the help of enzymes urease and glutamate dehydrogenase. The rate of decrease in absorbance is monitored at 340 nm and is directly proportional to urea concentration in the sample.

Reagents:

Reagent 1 (Urea reagent) : α - Ketoglutarate 7.5 m mol/L
NADH – 0.32 m mol/L
Urease $\geq$ 8.00 IU/L
GLDH $\geq$ 1.00 IU/L
ADP - 1.2 m mol/L
Tris Buffer p^H 7.9 – 100 m mol/L
Urea standard (Bun) : 50 mg/dl (23.4 mg/dl)

Procedure: A sterile test tube was taken

Pipette into tube	Blank	Standard	Test
Urea reagent	1000 μ l	1000 μ l	1000 μ l
Distilled water	20 μ l	--	--
Standard	--	20 μ l	--
Test	--	--	20 μ l

The contents of the test tube were mixed well at room temperature and then absorbance was read against reagent blank at 340 nm on semiautomatic analyzer.

APPENDIX- E

ESTIMATION OF SERUM CREATININE

Method: Jaffe's Method, Initial rate assay

Principle:

The principle of creatinine assay kit is creatinine reacts with alkaline picrate to produce an orange-yellow colour (Jaffe's reaction). Specificity of the assay has been improved by the introduction of an initial rate method. The absorbance of the orange-yellow colour formed is directly proportional to creatinine concentration and is measured photometrically at 500-520 nm.

Reagents:

Reagent 1 (Picric acid reagent) : Picric acid - 25.8 m mol/L

Reagent 2 (Sodium Hydroxide reagent) : Sodium Hydroxide – 95 m mol/L

Creatinine standard : 2 mg/dl (0.166 m mol/L)

Procedure: A sterile test tube was taken

Pipette into tube marked	Blank	Standard	Test
Creatinine Reagent	1000 µl	1000 µl	1000 µl
Distilled water	100 µl	--	--
Standard	--	100 µl	--
Test	--	--	100 µl

The contents of the test tube were mixed well at room temperature and then Initial absorbance was read 20 seconds after mixing and final absorbance is read 80 seconds after mixing against reagent blank at 505 nm on semiautomatic analyser.