PATHOMORPHOLOGICAL STUDIES ON RENAL FAILURE IN DOGS

SANJAY C. MELINAMANI

DEPARTMENT OF VETERINARY PATHOLOGY
VETERINARY COLLEGE, BANGALORE
KARNATAKA VETERINARY, ANIMAL AND FISHERIES SCIENCES UNIVERSITY, BIDAR

SEPTEMBER, 2010

PATHOMORPHOLOGICAL STUDIES ON RENAL FAILURE IN DOGS

Thesis submitted to the

KARNATAKA VETERINARY, ANIMAL AND FISHERIES SCIENCES UNIVERSITY, BIDAR

In partial fulfillment of the requirements
for the award of the degree of

Master of Veterinary Science

In

VETERINARY PATHOLOGY

By

SANJAY C. MELINAMANI

DEPARTMENT OF VETERINARY PATHOLOGY
VETERINARY COLLEGE, BANGALORE
KARNATAKA VETERINARY, ANIMAL AND FISHERIES SCIENCES UNIVERSITY, BIDAR

SEPTEMBER, 2010

KARNATAKA VETERINARY ANIMAL AND FISHERIES SCIENCES UNIVERSITY, BIDAR

DEPARTMENT OF VETERINARY PATHOLOGY
VETERINARY COLLEGE, HEBBAL, BANGALORE

CERTIFICATE

This is to certify that the thesis entitled *"**Pathomorphological studies on renal failure in dogs"*** submitted by **Mr. SANJAY C. MELINAMANI, I. D. No. MVHK 811** in partial fulfillment of the requirements for the award of degree of **MASTER OF VETERINARY SCIENCE** in **VETERINARY PATHOLOGY** of the **Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar** is a record of bonafide research work carried out by him during the period of his study in this University under my guidance and supervision and the thesis has not previously formed the basis for the award of any degree, diploma, associate ship, fellowship or other similar titles.

Bangalore
September, 2010

(Dr. M.L. SATYANARAYANA)
Major Advisor

Approved by:

Chairman: _____
(Dr. M.L. SATYANARAYANA)

Members: **1.** _____
(Dr. SUGUNA RAO)

2. _____
(Dr. S. YATHIRAJ)

3. _____
(Dr. M. NARAYANA BHAT)

4. _____
(Dr. L. RANGANATH)

Affectionately Dedicated
To
My Beloved Parents
and Teachers

ACKNOWLEDGEMENT

I take this opportunity as my privilege and honour to express my deep sense of appreciation, gratitude and wholehearted indebtedness to ***Dr. M. L. Satyanarayana****, Professor and Head, Department of Pathology and Chairman of my Advisory Committee for his constant supervision, support and encouragement. Gratitude is offered not only for his excellence as a teacher but also his gentle, persistent and wise mentoring.*

I was fortunate to have advice, constructive suggestions, untiring help, friendly behaviour and constant interest in my work throughout this study from ***Dr. Suguna Rao,*** *Professor, Department of Pathology, as my Advisory Committee member.*

I am really grateful to ***Dr. S. Yathiraj****, Dean, Veterinary College, Bangalore, for his continuing encouragement, moral support and valuable suggestions as a member of advisory committee.*

I owe my special thanks to ***Dr. M. Narayana Bhat*** *Professor and Head, Department of Medicine Bangalore, for his constant help, advice and support to carry out my research work.*

I feel honored to express my deep sense of gratitude to ***Dr. L. Ranganath****, Professor and Head, Department of Surgery and Radiology, Bangalore, for the constructive criticism, suggestions and expert advice during his study.*

I am extremely thankful to ***Dr. H. D. Narayanaswamy****, Professor, Department of Pathology for his constant encouragement, help rendered during my study.*

I take this opportunity to extend my deep sense of gratitude to ***Mr Narayana Gowda,*** *Senior Lab technician,* ***Nadira madam,*** *lab technician and non teaching staff members of the department for their kind help and cooperation during the course of my research work.*

I take this opportunity to thank ***Mr. Nagaraj, Obleshppa, Narayanappa*** *and* ***Lakshmi Bai*** *for their timely help.*

I remain ever thankful to ***Dr. Byregowda****, Joint Director, IAH & VB, Bangalore for his help rendered during my study. I am also thankful to* ***Dr. Balamurugan****, Senior Scientist, Leptospira Laboratory, Project Directorate Animal Disease Monitoring and surveillance,*

Bangalore for providing facilities for carrying PCR during my research work. Also I thank ***Dr. Gangadhar*** *for helping me conducting work in the same lab.*

Dr. Anjan kumar *and* ***Dr. Vishwanath*** *(Contract Teachers) of the department have helped me open heartedly in all means and I am really thankful to them.*

Dr, M. Shetter, Dr. Azeemulla, Dr, Shankar, Dr. S. Uma, Dr. Muniyellappa and Dr. Ravikumar *my seniors (Ph. D. scholars), were a lot to me during the research work. Their helping hands really took me up. I am really thankful to all the support given by them.*

I owe special thanks to my fellow M.V.Sc. Colleague ***Drs. Sanjeev, Naveen, Akshata, Anitha*** *and* ***Noorali*** *Seniors* ***Drs. Srikanth, Mallikarjun, Dhanush, Roopadevi,*** *and* ***Sarvesh*** *Juniors* ***Drs. Praveen, Vijayalaxmi, Pragathi*** *and* ***Mudasir*** *for their cordial help and co-operation.*

Drs. Kumar, Mahesh, Mutturaj, Vinod, Sudhir, Vittal, Ashish, Ramesh, Amol, Jagdeesh, Sudarshan, Mallikarjun, Ramu, Sailo, Anil, Vijay, Shivappa, Lohith, Vishwanath *and* ***Manjunath*** *the batch-mates and neighbours and friends in the VCH, made the life in Bangalore a memorable one.*

It's my bound duty to thank Drs. ***Jayachandra, Manjunath, Shivanand Prithviraj, Vedmurthy, Venkanagouda, Sannakki, Prashant, Shekhar, Madhu, Vinay and Sunil for*** *their suport and encouragement.*

I admire and thank to my roommate ***Dr. Sanjeevkumar*** *for his needful help, love and affection.*

Finally and foremost, without the encouragement, help and support provided by ***my parents,*** *and brother* ***Raju*** *this thesis would remain the idea it has always been rather than the reality it has become. To all, I am most grateful.*

Finally, I thank the ***Almighty*** *with whose heavenly driving force and blessings made this investigation possible.*

Bangalore

September 2010 ***(Sanjay C. Melinamani)***

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

PLATE No.	TITLE
1	Photograph of kidney from a dog with renal failure to show slightly enlarged and congested appearance
2	Photograph of kidney from a dog with renal failure to show granular cortical surface and pitted appearance
3	Photograph of kidney from a dog with renal failure to show pale and pitted cortex with adherent capsule and shrunken size
4	Photograph of kidney from a dog with renal failure to show multiple necrotic foci and small cysts distributed throughout the kidney
5	Photograph of kidney from a dog with renal failure (leptospirosis) to show pale to icteric enlarged kidney with variable sized necrotic foci
6	Photograph of kidney from a dog with renal failure to show unilateral cyst containing watery like fluid extending upto medulla
7	Photograph of kidney from a dog with renal failure to show granular cortical surface and pitted and congested appearance
8	Photograph of kidney from a dog with renal failure to show reduced cortical area with mild congestion
9	Photomicrograph of kidney from a dog with renal failure to show severely congested vessel in the medullary region H& E X 100
10	Photomicrograph of kidney from a dog with renal failure to show severe congestion and haemorrhage involving tubules and glomeruli with mild infiltration of inflammatory cells in interstitium H & E X 100
11	Photomicrograph of kidney from a dog with renal failure to show enlarged and congested glomeruli, inter tubular haemorrhage with mild degree of connective tissue proliferation H & E X 200
12	Photomicrograph of kidney from a dog with renal failure to show congestion, haemorrhage, degeneration & necrotic changes in the tubule & glomeruli H & E X 200
13	Photomicrograph of kidney from a dog with renal failure to show congestion of intertubular vessels and glomeruli, granular to vacuolar degeneration and protein droplets inside the tubules H& E X 100

14	Photomicrograph of kidney from a dog with renal failure to show granular to vacuolar degeneration in the tubules with eosinophilic homogeneous material in tubular lumen H & E X 200
15	Photomicrograph of kidney from a dog with renal failure to show congestion, tubular degeneration, tubular necrosis and fatty change H & E X 100
16	Photomicrograph of kidney from a dog with renal failure to show tubular coagulative of necrosis H & E X 200
17	Photomicrograph of kidney from a dog with renal failure to show glomerular congestion and coagulative type of necrosis of tubules H & E X 100
18	Photomicrograph of kidney from a dog with renal failure to show thickening of Bowman's basement membrane and presence of protein droplets in the Bowman's space, also note tubular degeneration, desquamation & presence of hyaline material in the lumen H & E X 200
19	Photomicrograph of kidney from a dog with renal failure to show distended tubules and glomeruli with protein rich eosinophilic fluid, flattened lining epithelium and reduction in glomerular tuft H & E X 40
20	Photomicrograph of kidney from a dog with renal failure to show cystic dilatation of tubules lined by flattened epithelium, hyalinsed fibrous connective tissue and congested cystic tubules with hyaline casts H & E X 200
21	Photomicrograph of kidney from a dog with renal failure to show cystic dilated tubules H & E X 200
22	Photomicrograph of kidney from a dog with renal failure to show pure population of plasma cell and mild connective tissue proliferation H & E X 200
23	Photomicrograph of kidney from a dog with renal failure to show lymphoid aggregation on the necrotic area along with necrotic and cystic tubules H & E X 200

24	Photomicrograph of kidney from a dog with renal failure to show severe degree of interstitial fibrosis with hyalinization and protein rich fluid filled tubules H & E X 100
25	Photomicrograph of kidney from a dog with renal failure to show calcification in the interstitial space H & E X 100
26	Photomicrograph of kidney from a dog with renal failure to show acute tubulo-intestitial nephritis characterized by severe aggregation of lymphocytes in the interstitial space, intact basement membrane, mild proliferation of connective tissue with hyaline casts H& E X 100
27	Photomicrograph of kidney from a dog with renal failure to show massive infiltration of inflammatory cells with fibrous connective proliferation in the interstitium and proliferative tubules H& E X 100
28	Photomicrograph of kidney from a dog with renal failure to show severe degree of fibrosis with infiltration of mononuclear cells H& E X 100
29	Photomicrograph of kidney from a dog with renal failure to show cyst lined by cuboidal cells, tubular necrosis and adjacent pressure atrophy of renal structure H & E X 40
30	Photomicrograph of kidney from a dog with renal failure to show glomeruli with thickened basement membrane and bowman capsule and also segmental attachment of glomerular tuft H & E X 200
31	Photomicrograph of kidney from a dog with renal failure to show glomerulus with absence of tuft and thickening of Bowman’s capsule H & E X 400
32	Photomicrograph of kidney from a dog with renal failure to show glomeruli containing protein droplets accumulation in Bowman’s space and coagulative necrosis of tubules H & E X 400
33	Photomicrograph of kidney from a dog with renal failure to show extracapillary proliferation of parietal cell forming half moon shaped structure (crescents) within the glomerulus H & E X 200

46	Photograph of liver from a dog with renal failure to show multi focal whitish necrotic foci and roundening of liver lobes in leptospirosis
47	Photomicrograph of liver from a dog with renal failure to show foci of granular to highly swollen hepatocytes and fat droplets H & E X 100
48	Photomicrograph of liver from a dog with renal failure to show moderate degree of congestion, mild degree of connective tissue proliferation and area of bile duct proliferation and formation of cystic spaces H & E X 100
49	Photomicrograph of liver from a dog with renal failure to show moderate degree of congestion, vacuolar degeneration of hepatocytes and multifocal nodular aggregates of mononuclear cells H & E X 100
50	Photomicrograph of liver from a dog with renal failure to show periportal infiltration of inflammatory cells surrounded by atropic hepatic cords and cells undergoing apoptosis H & E X 400
51	Photomicrograph of liver from a dog with renal failure to show severe congestion involving sinusoids and vessels, vacuolar degeneration of centrilobular hepatocytes and necrosis H & E X 100
52	Photomicrograph of liver from a dog with renal failure to show severe congestion, periportal fibrotic change with infiltration of inflammatory cells and mild degree of biliary cirrhosis H & E X 100
53	Photomicrograph of liver from a dog with renal failure to show cirrhotic changes with edematous connective tissue, infiltration of inflammatory cell and hemosiderosis H & E X 200
54	Photomicrograph of liver from a dog with renal failure to show hemosiderin laden macrophages in the periportal area and infiltration of inflammatory cells H & E X 200
55	Photomicrograph of liver from a dog with renal failure to show congestion and mild fibrous tissue proliferation in the portal triad region H & E X 100

56	Photomicrograph of liver from a dog with renal failure to show severe congestion, granular to vacuolar degeneration and hemosiderin pigment H & E X 200
57	Photomicrograph of liver from a dog with renal failure to show congestion, granular to vacuolar degeneration with hemosiderin pigments H & E X 100
58	Photomicrograph of liver from a dog with renal failure to show congestion focal area of fatty change with haemosiderin pigments H & E X 100
59	Photomicrograph of liver from a dog with renal failure to show multiple vacuolar degeneration with congestion H & E X 40
60	Photomicrograph of liver from a dog with renal failure to show venous congestion , perivascular fibrosis and hemosiderosis H & E X 100

LIST OF ABBREVIATIONS

bp	base pairs
BUN	Blood Urea Nitrogen
DFM	Dark Field Microscopy
DW	Distilled Water
DNA	Deoxyribo Nucleic Acid
EDTA	Ethylene Diamine Tetra Acetic acid
FAT	Fluorescent Antibody Test
H & E	Haematoxylin & Eosin
Hb	Haemoglobin
mg/dL	Milligram per deci Litre
MCH	Mean Corpuscular Haemoglobin
MCV	Mean Corpuscular Volume
MCHC	Mean Corpuscular Haemoglobin Concentration
NBF	Neutral Buffered Formalin
PBS	Phosphate Buffer Saline
PCV	Packed Cell Volume
PCR	Polymerase Chain Reaction
rpm	revolutions per minute
SC	Serum Creatinine
SE	Standard Error

TEC	Total Erythrocyte Count
TLC	Total Leukocyte Count
%	Per cent
µ	Microns
^{0}C	Degree Celsius
Viz	Namely

INTRODUCTION

I. INTRODUCTION

Among the companion animals, dogs have the credit of being man's oldest friend. Both man and dogs have been mutually benefited by this relationship. Man provided dogs with food and shelter; in return they served him with loyality, friendliness and faithfulness. This relationship between them has grown so intimate over the years that man is very much concerned about the well being of this companion and willing to take all possible steps to reduce their distress and suffering.

Kidney is a complex organ and regulates essential homeostatic functions such as balance of body water, acid base status, electrolyte production, modification and degradation of hormones, excretion of metabolic and nitrogenous waste products, conservation of nutrients and gluconeogenesis. Although, the kidney constitutes less than one per cent of the body weight, they receive 25 per cent of the total cardiac output, thus rendering them susceptible to a great amount blood borne toxicants.

The number of diseases affecting the kidneys of dogs have increased considerably over the years with acquired diseases being more common than congenital defects. In spite of advancement in preventive and trauma medicine, organ failures have become common causes of death in recent years (Defrancesco, 2002). Renal failures are important clinical problems encountered in dogs and are frequent causes of illness and death.

Chronic kidney disease is one of the leading problems that cause considerable morbidity and mortality in dogs. Majority of the old dogs suffer some degree of kidney damage thus it is one of the major causes of death in older animals. The underlying cause

of the disease might have occurred previously and remained unknown in most cases. The prevalence of renal disease is high in dogs and cats especially in aged population (Polzin *et al.,* 2000). A recent study of general canine population indicated that more than 50 per cent of dogs die or euthanized due to renal failure before 10 years of age and constitutes third most common cause of death in dogs in that age interval (Bonnet *et al*., 1997).

Renal diseases are often found to be associated with glomerulus, tubules, interstitial tissue or vessels. Some renal diseases may be associated with dysfunction such as nephrogenic diabetes mellitus or biochemical abnormalities (cystinurea) without detectable morphological abnormalities. Leptospira has been identified as one of the important etiological factor in chronic kidney diseases. Due to reserve capacity of the kidneys, early renal disease may have only few consequences to the animal. Renal disease may progress, persist, or advance leading to renal failure.

Renal function tests such as Serum creatinine, Blood urea nitrogen are indices of glomerular filtration, since the excretion of creatinine occurs almost entirely by glomerular filtration. The level of BUN and Serum creatinine start to rise only after 75 per cent of nephrons are damaged (Polzin *et al.,* 2000). Unfortunately, many diseases are not detected until they become generalized leading to serious impairment of renal function. End stage renal failure is associated with a high rate of fatality and cost of therapy.

A systematic study of diseases affecting this organ has not received adequate attention. Kidney being a vital organ performs great variety of metabolic functions than any other organ in the body. Often, it becomes the target in the course of systemic

infections and toxaemias. Although, a few workers in various parts of the country have approached this problem, a systematic study on renal pathology was lacking, hence the present study was undertaken with following objectives.

1. To record the epidemiological information on the occurrence of renal failure cases among dogs presented to Veterinary College Hospital.

2. To record certain hematological and biochemical parameters among renal failure cases in dogs to ascertain the severity of the condition.

3. To study the gross and histological lesions in kidney and liver.

4. To identify the involvement of leptospira organisms based on dark field microscopy and silver stain.

REVIEW OF LITERATURE

II. REVIEW OF LITERATURE

Perusal of literature has shown several reports of work being carried out on pathomorphological studies on renal failure in dogs. The available literature on historical back ground, epidemiological study, clinical pathology, gross pathology and histopathology of renal failure cases among dogs have been reviewed as follows.

2.1 Historical background

Renal failure has been diagnosed in dogs since 1930s but was called by the term "nephritis". Nephritis had been defined as "functional interference with the blood flow through the glomeruli, causing glomerulonephritis. This damage affects all the glomeruli of both the kidneys" (Volhard, 1936).

Bloom (1939) classified renal disease based on histological examination and concluded that interstitial nephritis (now known as renal failure) as the most common renal inflammatory disease in dogs and that of glomerulonephritis extremely rare.

Garner *et al*. (1968) described the concept of renal biopsy for the diagnosis of renal disease during 1960s. The author described surgical method of renal biopsy in cattle.

Cook and Cowgill (1996) opined that canine glomerular diseases were associated with the presence of immune complex of glomerular capillary wall.

Lefebvre and Toutain (2004) reported that regardless of etiology of the underlying renal disease, glomerulosclerosis and interstitial fibrosis are key pathologic findings in

end stage of renal failure. Common underlying physiopathologic mechanisms include glomerular capillary hypertension and enhanced passage of plasma proteins across the glomerular capillary barrier. Activation of RAAS (Renin angiotensin aldosterone system) and consequently increased production of angiotensin II play a major role in the progression of hypertension and renal fibrosis.

2.2 Epidemiological study

2.2.1 Incidence

Langaham and Hallman (1941) in a study of nephritis in domestic animals reported that out of 236 cases examined 22 were from canines out of which only two had glomerulonephritis.

Rouse and Lewis (1975) reported glomerulonephritis in 21 out of 71 stray dogs examined. Using light and immunofluorescent microscopy, they found proliferative glomerulonephritis in 26 per cent of adult dogs and 47 per cent of aged dogs, but none in dogs less than one year old. They also found that 5 per cent of the adult dogs and 13 per cent of the aged dogs had membranous glomerulopathy.

McKenna and Carpenter (1980) reported polycystic kidney disease in 3 related Cairn terriers and observed enlarged and reniform kidneys containing multiple fusiform or cylindrical cysts lined by cuboidal epithelium radiating through cortex and medulla.

Cook and Catell (1985) reported that 52 per cent glomerular disease and 48 per cent non glomerular disease out of 111 cases of chronic renal disease examined in dogs.

Jaenke and Allen (1986) reported membranous nephropathy in 29 per cent of 46 proteinuric dogs whereas Holt *et al*. (1987) reported idiopathic renal haemorrhages in 9 dogs.

Vishwanathan (1988) reported 9 cases of membranous glomerulonephritis, 35 membrano proliferative glomerulonephritis, 12 proliferative glomerulonephritis, 13 glomerulosclerosis and 141 tubulo-intestitial nephritis among 221 canine nephritis cases studied.

DiBartola *et al.* (1989) studied histological lesions in 21 dogs with glomerular disease. Of these, 11 cases showed morphologic evidence of glomerulonephritis and 39 exhibited renal amyloidosis.

Familial kidney disease was reported in English cocker Spaniels (Lees *et al.*,1998), Doberman Pinscher (Picut and Lewis, 1987), Rottweilers (Cook *et al.,* 1993), Alaskan Malamutes (Villafranca and Ferrer, 1994), Bernese Mountain dogs (Minkus *et al.,* 1994), Beagles (Rha *et al.*, 2000), Samoyed dogs (Rawdon, 2001).

Burrows *et al*. (1994) reported polycystic kidney disease in 8 related bull terriers.

Cook and Cowgill (1996) reported amyloidosis in 23 per cent of protein losing nephropathies in dogs.

Birnbaum *et al.* (1998) reported moderate to severe tubulo-intestitial nephritis in 36 dogs which had leptospirosis.

2.2.1.1 Age

Scott *et al*. (1985); Dru and Catharina (1994) and Gregory and India (1995) reported advanced age of more than 7 years as one of the risk factor for acute renal failure.

Slauson *et al*. (1970) reported mean age of dogs with chronic renal failure as 9.2 years with the range of 1-15 years.

Among 50 cases of glomerulonephritis, the age of affected dogs was above six years in 36 dogs and less than one year in 5 dogs (Lewis, 1976).

Wright and Nash (1983) reported that no breed or sex predisposition in dogs with glomerulonephritis.

Among 46 proteinuric dogs, Jaenke and Allen (1986) reported that the age of dogs with membranous nephropathy ranged from 1-14 years with median age of 6.5 years.

Cook and Catell (1985) found that no age or sex difference between those having glomerular lesions and non glomerular lesions in 111 cases of canine chronic renal disease.

In a survey of 170 canine patients with chronic renal failure, the mean age of diagnosis was 7 years. In another study of 119 dogs, the mean age of diagnosis was 6.5 years. Based on data submitted from 1983-1992 to the Veterinary Medical Data Base at Purdue University, 18 per cent of dogs with renal failure were less than 4 years old, 17

per cent of dogs were between 4 and 7 years, 20 per cent of dogs were between ages of 7 and 10 and 45 per cent of dogs were older than 10 years of age (David *et al.,* 1995).

2.2.1.2 Sex

Wright and Nash (1983) reported that no breed or sex predisposition in dogs with glomerulonephritis. Cook and Catell (1985) found no age or sex difference between those having glomerular lesions and non glomerular lesions in 111 cases of canine chronic renal disease.

Behrend *et al*. (1996) who reported that the occurrence of renal failure was slightly higher in the males when compared to females in 29 hospital acquired acute renal failure cases in dogs.

Birnbaum *et al*. (1998) reported male dogs were more affected than females. Mrudula *et al.* (2005) studied 60 canine nephritis cases and observed 60 per cent of the affected animals were males & 40 per cent were females.

2.2.1.3 Breed

Jaenke and Allen (1986) studied 46 proteinuric dogs comprising of eight different breeds and observed highest incidence of renal disorders in Doberman Pinscher and Miniature Schauzer.

Vishwanathan (1988) reported that nondescript animals showed a higher incidence (56.68 per cent) out of 221 dogs with nephritis studied. Among pure breeds, Alsatian recorded a higher incidence followed by Labrador, Doberman and Pomeranian.

Birnbaum *et al*. (1998) observed 36 animals with leptospirosis out of which nine animals were of mixed breed, three Labrador Retrievers, two German Shepherds, two miniature Schnauzers, two Boxers and the remaining 18 dogs belonged to 16 different breeds.

2.3 Clinical signs

Important clinical signs noticed in renal amyloidosis were weight loss, weakness, lethargy, anorexia, depression and dehydration. Several cases had polydypsia, polyuria and anaemia. Some cases were complicated by cyanosis, dyspnoea and pulmonary thrombosis (Slauson *et al.*, 1970; Dibartola *et al.*, 1989; Bowles and Moisier, 1992).

Mc Ewan (1971) described the clinical signs in acute renal failure. In the mild type, the clinical signs observed were vague malaise, reduced appetite, excessive thirst and polyuria. In severe cases the signs included dullness, complete anorexia, vomition, helitosis and oral ulceration. The clinical signs associated with chronic renal failure were dullness anorexia, loss of weight, polydypsia, polyuria, hypertension, anaemia and oseodystrophiafibrosa. The advanced cases also showed emesis, helitosis, and oral ulceration.

Neel and Grindem (2000) indicated that patients with renal failure or renal disease often presented with nonspecific signs such as anorexia, weight loss, lethargy and depression but certain signs such as polydypsia, polyuria and oral ulcers were highly suggestive of renal disorder.

Cook *et al.* (1993) observed diarrhoea, vomition, anorexia and lethargy in renal dysfunction associated with pyometra.

Stone *et al.* (1988) reported diarrhoea, vomition, anorexia and lethargy in renal dysfunction associated with pyometra

Birnbaum *et al.* (1998) described the most common presenting complaints in dogs with leptospirosis viz., anorexia, lethargy, depression, polyuria, polydypsia and vomition. Other complaints included weight loss, weakness, diarrhoea, stranguria, alopecia, haematochezia, haematuria, malena and dyspnoea.

Vaden (2000) opined that polyuria, polydypsia in a dog with chronic renal failure may be overlooked by the owner and the disease may only be noticed when symptoms such as anorexia and vomition develops. Differentiation can be challenging in dogs with chronic renal failure but have acute deterioration of their condition called acute on chronic renal failure.

Haller (2002) reported that polyuria and polydypsia were the first clinical signs of the renal disease in dogs. Symptoms of reduced appetite, weight loss, vomition, weakness and poor condition of hair coat were common. He also observed pallor of the mucous membrane and ulcerations in the oral cavity in advanced cases of renal disease. He opined that signs of hypertension such as retinal edema and detachment, hemorrhage or vascular tortuosity might be found on fundic examination. He also indicated that in early stages of renal disease, the clinical signs may be mild nonspecific or totally absent. Thus making laboratory evaluation of blood and urine samples mandatory for diagnosis.

Meyer (2004) reported dullness, poor appetite, weight loss, vomition, diarrhea, polyuria, polydipsia, poor hair coat, anaemia, stomatitis and bad breath as common

clinical signs and these clinical signs in chronic renal failure were non specific for chronic renal failure.

2.4 Haematology

Slauson *et al.* (1970) observed moderate anaemia in 15 (47 per cent) out of 32 cases of renal amyloidosis.

McEwan (1971) indicated that there was definite information on the correlation of anaemia with uremia in chronic renal failure and also found 57 per cent of the animals to have anaemia with chronic renal failure.

Wright *et al.* (1976) reported that only the haematocrit showed a significant decrease in chronic interstitial nephritis when compared to chronic glomerulonephritis.

Holt *et al.* (1987) reported reductions in packed cell volume (PCV), haemoglobin level and reduced blood cell counts in dogs with idiopathic renal haemorrhage.

Robinson *et al*. (1989) observed PCV, haemoglobin and TLC values to be 35 per cent, 130g/L and 17,208 cells /µl respectively in Bull Terrier dogs with chronic renal failure.

McCaw *et al.* (1989) reported that the renal failure accompanied by normocytic normochromic anaemia was more likely to be chronic than acute. Leucocytosis indicated an inflammatory lesion.

Cowgill, 1992; Polzin *et al.,* 2000; opined that renal failure accompanied neutrophilia with left shift, suggested pyelonephritis. Hypoproliferative anaemia was a

consistent feature of chronic renal failure in dogs. The pathogenesis of this anaemia was multifactorial but erythropoietic failure of bone marrow secondary to moderate renal production of erythropoietin was regarded as the primary cause.

Forrester and Brandt (1994) observed leukocytosis may occur in patients with nephritis and bacterial endocarditis. It was also observed in dogs with ethylene glycol toxicosis and stress.

Polzin *et al*. (1995) stated that progressive hypo proliferative anaemia was characteristic of dogs with moderate to advanced chronic renal failure. Anaemia in patients with chronic renal failure was multifactorial. There are experimental and clinical evidences for the supporting roles of short red cell life span, nutritional abnormalities erythropoietic inhibitors, and blood loss and erythropoietin deficiency.

Birnbaum *et al.* (1998) reported mild nonregenerative anaemia in 33 per cent of 36 dogs with leptospirosis. Leukocytosis characterized by mature neutrophilia was observed in 31 per cent of dogs. Only two per cent showed a left shift and one toxic change.

Hurley (1998) reported that in acute renal failure a complete blood count may reveal a regenerative, blood loss anaemia, thrombocytopenia, a stress or inflammatory leukogram.

Vaden *et al.* (1998) stated that though non regenerative anaemia is associated with chronic renal failure, in his study he found 32 per cent of dogs with acute renal failure to have anaemia.

Kraje (2002) indicated that acute renal failure might reveal anaemia of normal haematocrit and leukocytosis. Anaemia might be initially marked by dehydration and was not evident until fluid therapy was initiated. The anaemia might have been caused by gastrointestinal ulceration and haemorrhage or rarely haemolytic anemic syndrome.

2.5. Serum biochemistry

2.5.1. Blood urea nitrogen

Anderson and Edney (1969) indicated that high protein diets could cause clinically important increase in BUN concentration and thus an 18 hour fasting had been recommended to eliminate the influence of diet on BUN. Any process that can induce protein catabolism could result in increased BUN which included haemorrhage into the small bowel, fever, burns, corticosteroid administration, starvation, infection and tetracycline administration (Kopple and Coburn, 1974; Coles, 1974; Stockholm and Scott, 2002).

Robinson and Gopinath (1974) stated that renal clearance of creatinine has been used for many years in the measurement of glomerular filtration rate. Creatinine is an endogenous end product of muscle metabolism and provides a measure of glomerular filtration rate.

Finco and Duncan (1976) reported that there was no difference between dogs and cats with regard to elevation of serum creatinine in renal and post renal azotemia. The mean BUN values reported were 80 mg/dL, 140 mg/dL and 194mg/dL in pre-renal and

post-renal azotaemia respectively. Prerenal causes rarely elevate BUN levels above 120mg/dL (Cowgill, 1983).

Vishwanathan (1988) found that the BUN values in dogs with nephritis varied from 10.3 to 200mg/dL with a mean of 48.12 mg/dL. The BUN value of more than 30 mg/dL was observed in 72 per cent of 221 dogs studied.

English *et al*. (1980) and Finco *et al*. (1995) reported that an increase in blood urea nitrogen value outside the normal range in renal failure case.

Haller (2002) stated that the main problem using BUN and creatinine values in diagnosis was that their levels in blood become evident only after 75 per cent of the nephrons are non functional. They only provide a crude estimate of the glomerular filtration rate and cannot be used for early detection of renal damage.

2.5.2 Serum Creatinine

Osborne *et al*. (1972) opined that a marked elevation in serum creatinine concentration indicated severe functional organic impairment of nephron function, it was not significantly greater than BUN concentration indicating that the degree of reversibility or irreversibility of the underlying disease process, since it did not establish a specific diagnosis.

Gabrisch (1973) reported that creatinine was more reliable diagnostic tool compared to serum urea. He established normal values of creatinine as 0.71±0.37 mg/100ml in healthy dogs, he stated that the prognosis based on the creatinine values of 2-3mg/100ml be treated cautiously, 3-4 doubtful and over 4 as unfavorable.

Coles (1974) stated that creatinine concentration unlike that of urea was not influenced by diet. However increased blood creatinine concentration has been reported in people after eating cooked meat, presumably because of partial conversion of creatin to creatinine during cooking.

Finco and Duncan, 1976; English *et al.* 1980; Finco *et al*. (1995) opined that both BUN and serum creatinine were relatively insensitive in detecting renal dysfunction. On the basis of the mechanism of renal excretion of the two compounds creatinine was a better indicator of glomerular filtration rate as it was affected by few nonrenal parameters.

Jacobson *et al.,* 1979; Watson *et al.,* 1981, indicated that serum creatinine value varied from 0 to 10 mg/dL with an average of 1.46 mg/dL. Serum creatinine values of 2 and above were recorded in 34 percent of dogs with nephritis.

Evans (1987) studied post prandial changes in plasma creatinine and stated that creatine is converted to creatinine by heat treatment during food processing, so plasma creatinine might be expected to increase following higher levels of dietary creatinine. In his study he noticed that there was an increase of plasma creatinine after a pelleted diet feeding, but the changes were much smaller than that of BUN.

2.6 Dark Field Microscopy and PCR

Leptospires in cultures, body fluids or tissue suspensions are identified based on their typical morphology and motility under dark field illumination.

Doherty (1966) examined urine by dark field microscopy and inferred that this method was superior to guinea pig inoculation technique.

Ellis *et al.* (1982) described that the dark field microscopy was not a useful method for demonstrating leptospires in serum and urine.

Smith *et al*. (1994) reviewed the laboratory techniques and their use in diagnosis of bovine leptospirosis caused by serovar hardjo. They reported that FAT was more sensitive than dark field microscopy and technique of culturing was difficult and took more than three months to be achieved. They mentioned that DNA probes and PCR were more sensitive, specific and quick to perform and could be used on fluid and tissue samples.

Brown *et al*. (1995) evaluated PCR for the early detection of leptospires in clinical samples from patients with acute leptospirosis. Blood and urine samples from 71 patients were examined by PCR, culture and serology of which PCR detected 44 cases (62 per cent) and culturing 34 (48 per cent) cases. They reported that the PCR detected leptospires in 13 patients before the development of antibodies and concluded that PCR was rapid, sensitive and specific means of diagnosing the leptospiral infection, especially during the first few days of the disease.

Marien *et al*. (1995) studied 200 patients with various clinical syndromes compatible with leptospirosis, PCR was compared with culture and MAT for diagnosis. They reported that the PCR was an efficient tool for early diagnosis of leptospirosis

during the first 10 days of disease, especially when the clinical expression of the disease was confusing.

Zamora *et al*. (1995) compared four microscopic techniques for the diagnosis of leptospirosis in wild rodents. They examined kidneys from 93 wild rodents by leviditi's silver stain, DFM in wet smears, immunofluorescence and immunoperoxidase techniques using pooled antiserum against serovar *hardjo* and *pomona.* They reported that Leviditi's technique detected highest number of positive samples (67.5 per cent) and DFM the lowest (32.5 per cent).

Venkatesh (1997) compared the PCR and culturing methods of diagnosis for leptospirosis and concluded that the DNA based methods were more sensitive than the culturing and hence they are better for early diagnosis of leptospirosis.

Birnbaum *et al*. (1998) studied 36 dogs with confirmed leptospirosis diagnosed at the New York State College of Veterinary Medicine from 1980 to 1995 and clinical, serological and pathological findings were recorded to characterise the epidemiology of this disease in upstate New York. Titres were directed predominantly against serovars *grippotyphosa* and/or *pomona* in 31 of 34 dogs. Convalescent titres were measured for 53 per cent of dogs. The most common clinical presentation documented was acute renal failure in 22 of 36 dogs. They reported from study that *Leptospira pomona* and *grippotyphosa* are important pathogens capable of causing severe renal and hepatic injury in dogs.

In a prospective case study, Kenneth (2003) studied 132 dogs with clinical signs suggestive of leptospirosis and 13 healthy dogs and evaluated the use of PCR assay on urine samples for diagnosis of leptospirosis in dogs. PCR was found positive in 8 dogs that had conventional confirmation where as it was positive in 16 dogs that had not conventional confirmation.

2.7 Pathology

2.7.1 Gross pathology of kidney

Murray *et al.* (1971) reported that the animals with glomerulonephritis, showed pale and swollen kidney and exhibited prominent bulging surface.

Wright *et al.* (1976) reported kidneys of normal size, pale with a finely irregular surface, firm in consistency with pale granular cortex and easy stripping of capsule in chronic glomerulonephritis. The kidneys of reduced in size, grayish in color, irregular in outline, firm to cut and with irregular narrowing cortices and varying degrees of cyst formation ranging from isolated cortical and medullary cysts to widespread cystic bulging of medulla were reported in chronic interstitial nephritis.

Slauson and Lewis (1979) reported that in acute cases, the cortex was grossly enlarged and the cut surface was bulged on removal of the capsule. In subacute to chronic glomerulonephritis cases, the kidneys were uniformly affected with slight increase in firmness, a finely granular or rough sub capsular cortex and variable cortical pallor. Only in the more chronic cases with tubular atrophy, they noticed visible changes in the width of cortical zone.

McKenna and Carpenter (1980) reported polycystic kidney disease in 3 related Cairn terriers and observed enlarged and reiniform kidneys containing multiple fusiform or cylindrical cysts lined by cuboidal epithelium radiating through cortex and medulla.

Bowler and Mosier (1992) found that kidneys from dogs with renal amyloidosis were pale, tan with irregular pitted surface and fine granular cortices.

Birbaum *et al*. (1998) noticed generalized discrete yellow to pale to discoloration and areas of haemorrhages on renal surface, diffuse petechial haemorrhages in lungs and gastric mucosa, multifocal lingual haemorrhages and uremic pleuritis in dog with leptospirosis.

2.7.2 Histopathology of kidney

Murray *et al*. (1971) reported enlarged glomeruli and moderate hypercellularity thickened mesangial stalks of some lobule, thickened capillary loops, patent capillary lumen, and distinct lobular accentuation of glomerular tuft.

Slauson *et al*. (1970) and Bowles and Mosier, (1992); reported homogeneous, eosinophilic, amorphous deposits which were occasionally somewhat blurred or smudgy. Lesions in renal tubules were usually light in comparison with glomerular changes. Interstitium contained scattered groups of lymphocytes and plasma cells.

Krohn *et al.* (1971**)** found interstitial nephritis in 28 per cent of dogs autopsied in Helsinki. Glomerular disease was noted in only 4 per cent of these dogs, but the investigators that speculated glomerular disease might have played an important role in the pathogenesis of the tubular disease.

Wright *et al.* (1976) observed glomerular scarring varying in severity from mesangial expansion with segmental obliteration of capillaries to complete glomerular obsolescence, segmental areas of mesangial proliferatation in occasional tufts, capsular adhesions. Further, they observed completely obsolescent tufts consisted of a shrunken glomerulus and excessive mesangial matrix and mild interstitial fibrosis of cortex and medulla, tubular regeneration and in every case a few isolated pockets of lymphocytes and plasma cells in the interstitium were also noticed.

Muller-Peddinghaus and Trautwein (1977) observed different types of diffuse glomerulonephritis in morphological study of 103 dogs and stated that membranous and membranoproliferative glomerulonephritis were more common in middle-aged and older animals and glomerulonephritis largely occurred independently of interstitial nephritis. They noticed 71 per cent incidence of interstitial nephritis and glomerulonephritis in severe interstitial damage as well as in slight damage and also opined that mesangial changes were more predominant in end-stage kidneys with severe fibrosis.

Slauson and Lewis (1979) reported that the lesions of canine glomerulonephritis varied from acute to chronic, membraneous to sclerosis, proliferative to hyalinising. They described canine glomerulonephritis as a mixed membranoproliferative lesion with thickening of peripheral capillary loops accompanied by irregular cellular proliferation and focal increase in mesangial matrix. Further, they opined that purely membranous lesion was less common in dogs but could occur as chronic sclerosing forms with abundant tubular atrophy, interstitial fibrosis, chronic glomerular lesions including scarring, concentric periglomerular fibrosis and total glomerular obsolescence.

Spangler *et al*. (1980) reported necrosis of proximal tubular epithelium, individual cell necrosis characterized by homogeneous eosinophilic cytoplasm with pyknosis or karyorrhexis in gentamicin toxicity and many dilated tubules containing amorphous granular casts or necrotic debris. Spectrum of changes from normal to hyaline droplet degenerations and lack of brush border from individual cell necrosis to segmental necrosis of entire tubule were observed in contiguous areas of renal cortex.

Spencer and Wright (1981) described the glomerular lesions in chronic interstitial nephritis. In every section a range of glomerular morphology from normal to complete obsolescence were noticed and opined that degree of glomerular damage was related to the degree of interstitial fibrosis.

In a prospective survey, Dugald *et al*. (1986) studied 76 dogs with renal disease out of one hundred and eleven dogs identified as canine chronic renal failure and reported forty cases (52per cent) with glomerular (GN) and 36 (48per cent) non-glomerular (NGN) disease. Chronic liver lesions as extra renal lesions were noticed in two cases out of 40 animals with glomerulonephritis.

Jaenke and Allen (1986) grouped fourteen cases of membranous nephropathy into different stages which reflected the progression of this syndrome and in general demonstrated the relationship between its duration and the histological appearance of the glomerular lesions as well as the clinical manifestations.

Center *et al.* (1987) stated that condition known to be associated with naturally developing glomerulonephropathies in dog included pyometra, neoplasia, systemic lupus

erythematosis, dirofiariasis, canine adeno virus – 1, diabetes mellitus and pancreatitis. They also suggested in their study that a high prevalence of glucocorticoid excess (34 per cent), chronic inflammatory skin disease (27 per cent) and systemic lupus erythematosis (97 per cent) were observed as clinical problems concurrent with glomerulonephritis.

Koeman *et al*. (1987) found thickening of Bowman's capsular membrane and hyperplasia/hypertrophy of parietal epithelial cells in 27 of 51 protein uric dogs. Based on the histological changes in the glomerular tuft, dogs were divided into groups having mesangioproliferative, membranoproliferative and membranous glomerulonephritis and amyloidosis.

Koeman *et al.* (1987) reported dilatation of tubules accompanied by cast formation in 28 proteinuric dogs. The casts localized in distal tubules lined by flattened epithelium, focal or diffused infiltration with mononuclear cells, areas with an increase of intertubular collagen were noticed.

Chacko (1988) studied histological lesions in 120 kidneys of 60 animals. Of these tubular, tubulo-intestitial, glomerular lesions were recorded in 68, 45 and 100 kidneys respectively and reported higher prevalence of glomerulonephritis.

Vishwanathan (1988) noticed tubulo-intestitial diseases with prominent inflammatory and degenerative changes in 141 out of 221 dogs and the pathology varied from acute focal or generalized interstitial nephritis with infiltration of lymphocytes, plasma cells and edema to chronic focal or generalized fibroplasias. Tubules showing degenerative and necrotic changes with focal atrophy of tubules and affection to

glomeruli in all cases with a variable degree and showing typical picture of membranous change and glomerulosclerosis were observed.

Robinson *et al*. (1989) observed grossly kidneys of two-thirds normal size, tough in consistency, with a pale cortex and a finely nodular capsular surface in 15 Bull terrier dogs with chronic renal failure. Histologically, marked loss of nephrons, diffuse interstitial fibrosis, focal dense radial fibrosis in the renal medulla. Further, he noticed widespread tubular dilation with focal mineralization of tubular epithelium and adjacent basement membranes, shrunken segmental fibrotic glomeruli and extremely dilated Bowman's spaces.

Brown (1995) divided glomerular disease in dogs and cats, on the basis of changes in glomerular structures into proliferative (mesangial) glomerulonephritis, membranous glomerulonephritis, minimal change disease, glomerulosclerosis and amyloidosis.

Adamus *et al*., (1997) identified lesions in the liver of leptospirosis and observed firm, tan colored and mottled liver and severe chronic hepatitis to mild hepatocellular vacoulation with lymphocytic aggregates on gross and microscopic examination respectively. Special stains revealed spirochetes within the bile canaliculi and lesions were not observed in kidney.

Birnbaum *et al.* (1998) reported that canine leptospirosis revealed moderate to severe, primarily lymphoplasmacytic and neutrophilic tubule interstitial nephritis.

Short *et al. (*1999) performed a cortical wedge biopsy on 42 South African National Defence Force (SANDF) dogs from around the country for histopathological examination and noticed 9 normal kidneys, 50 per cent mesangioproliferative glomerulonephritis and the other conditions detected were hemosiderosis (47 per cent of animals), focal nephrosis (2.4 per cent), membranoproliferative glomerulonephritis (2.4 per cent), focal interstitial nephritis (4.7per cent) and acute tubular nephrosis (4.7 per cent).

Haller (2002) reported that histological evaluation of renal tissue helped in the diagnosis of renal disease. Unfortunately in many cases of chronic renal disease, fibrosis of renal tissue is found independently of the cause of the disease. Therefore renal biopsy should be considered only when alterations in patient management could be expected establishing an exact diagnosis. The main indication for renal biopsy is differentiation between glomerulonephritis and amyloidosis in cases of severe proteinuria.

Shilpa Rani (2003) reported inflammatory and degenerative changes more prominent in canine renal failure cases studied.

Shelley *et al.* (2004) studied 9 dogs that had consumed aflatoxin contaminated commercial dog food. Enlarged, pale yellow livers were noted macroscopically at necropsy in the dogs with sub acute hepatopathy and cirrhosis was noted in the dog with chronic hepatopathy. Hepatic lipidosis, portal fibroplasia, and biliary hyperplasia were observed in sub acute toxic hepatopathy in the 8 symptomatic animals. Marked lobular atrophy, bridging portal fibrosis, and regenerative hepatocellular nodules were observed in the dogs with chronic hepatopathy.

Grauer (2005) opined that chronic renal disease could be caused by diseases and/or disorders that affect any portion of the nephron, including its blood supply and supporting interstitium. Futher, he opined early detection of chronic renal disease, before the onset of renal azotemia and chronic renal failure could facilitate appropriate intervention that stabilizes renal function or at least slows its progressive decline.

Mrudula *et al*. (2005) studied 56 canine nephritis cases and observed sub acute and chronic type of nephritis. Further they attributed sub acute and chronic type of nephritis to failure to diagnose the disease. The observed lesions were thickened capillary basement membrane, glomerular hemorrhage, partial to complete loss of tufts, epithelial crescents in subacute glomerulonephritis.

2.7.3 Hepato-renal pathology

Ramirez *et al.,* 1978 reported that experimental infectious canine hepatitis produced an acute interstitial nephritis in 74.8 per cent of the dogs The lesions consisted of large foci of lymphocytes, plasma cells in the interstitium with tubular epithelial cell necrosis polymorphonuclear cell infiltration and opined that interstitial nephritis was associated with acute hepatitis.

Adamus *et al*. (1997) identified lesions in the liver of leptospirosis and observed firm, tan colored and mottled liver and severe chronic hepatitis to mild hepatocellular vacoulation with lymphocytic aggregates on gross and microscopic examination respectively Special stains revealed spirochetes within the bile canaliculi and lesions were not observed in kidney.

Vegad and Katiyar (1998) classified hepatic lesions as random, zonal and massive types. Random hepatocellular degeneration was characterized by single cell necrosis where as, zonal necrosis was in centrilobular, midzonal or periportal areas and massive necrosis involved complete necrosis of entire lobule.

Johnson (2000) reported the occurrence of portal fibrosis in dog with chronic hepatitis. Periacinar fibrosis was seen as chronic passive congestion secondary to right sided heart failure or toxins.

Greenlee *et al.* (2004) carried out clinical and pathologic comparison of acute leptospirosis in dogs caused by two strains of *Leptospira kirschneri* serovar *grippotyphosa* and observed interstitial nephritis, renal tubular degeneration and necrosis, pulmonary hemorrhage, hepatic edema and perivasculitis in infected dogs.

Katherine *et al*. (2006) studied histological lesions of aflatoxicosis in dogs suspected for contamination with commercial foods and reported marked cytoplasmic vacuolar degeneration consistent with accumulation of hepatocellular lipids in all 8 dogs. Portal fibrosis was identified in 7 dogs, and 7 dogs had biliaryhyperplasia. Hepatocellular cholestasis was detected in all 8 dogs. Inflammation with primarily lymphocytes was identified in the portal triads of 7 dogs, and scattered hepatocytes necrosis was identified in 4 dogs. Lesions of lesser identified were sinusoidal extramedullary hematopoiesis in 4 dogs, nodular hyperplasia in 2 dogs, and arterialization of central veins in 3 dogs.

Richard and Goldstein (2010) opined that leptospira organisms persist and multiply in the renal tubular epithelial cells causing acute nephritis and fatality,

inappropriate treatment may lead to chronic interstitial nephritis and a persistent carrier state. Further, he stated that liver was characterized centrilobular necrosis, bile canaliculi and duct occlusion leading to icterus in *L. Icterohaemorrhagiae* infection.

MATERIALS AND METHODS

III. MATERIALS AND METHODS

The materials utilized and the methods followed in the present study were described under the following headings.

3.1 Materials

3.1.1 Clinical Cases

The dogs presented to Veterinary College Hospital, Hebbal, Animal shelter houses and those referred from other Government Hospitals and private practitioners in Bangalore, suspected to have renal failure and those that were apparently normal based on the history and clinical signs were considered for the present study.

3.1.2 Blood

Blood samples from confirmed cases of renal failure were collected by cephalic or saphenous venepuncture in clean and dry glass EDTA vials fitted with rubber stopper.

3.1.3 Urine

The genetalia of the dog was cleaned properly and midstream urine was collected using a sterile catheter and test tube.

3.1.4 Laboratory Materials

3.1.4.1 Glasswares

Glass vials of 5 ml capacity, test tubes and beakers were cleaned, washed and sterilized by hot air oven and stored till use. Thomas pipettes, PCV tubes, micro

haematocrit capillary tubes, (sodium heparinized length 75MM+0.5 MM, Top Tech biomedicals) and haemocytometer were used for the present study.

3.1.4.2 Anticoagulant

Ethylene Diamine Tetra Acetic acid (EDTA) was used as an anticoagulant at the rate of 0.5 mg/ ml for collection of blood for the routine hematological procedures.

3.1.4.3 Reagents

RBC and WBC diluting fluids were procured from M/s Prince Chemicals, Bangalore. Creatinine and Blood urea nitrogen kits were procured from Span diagnostics limited Bangalore.

3.1.5.3 Instruments

1. ARTOS Biochemical analyser was used for analysis of various biochemical parameters.

2. Pipettes-Micropipette, Kasablnka digital variable micropipette was used in this study.

3. Neubaeur slide- bright line, American optical corporation. Buffalo. N. Y 14215 USA, Microscope, Refrigerated centrifuge, Centrifuge (Remi instruments), WBC and RBC diluting pipettes, water bath and refrigerator were used to process, examine or store the samples.

3.2 Methods

3.2.1 Diagnosis of renal failure cases

The dog population that attended Veterinary College Hospital from January 2009 to December 2009 were considered for epidemiological study. A total of 130 dogs with renal failure were selected and epidemiological details pertaining to age, sex and breed were collected. A total of 40 renal failure dogs were selected from the cases presented to the Veterinary College Hospital, with one or more signs of renal failure and were subjected to estimation of serum creatinine. Such of those dogs with serum creatinine value more than 4 mg/dl were diagnosed as renal failure were included in the study and subjected to clinical and histopathological study.

3.2.2 Blood

Blood samples were collected from dogs suspected of renal failure. The blood samples were collected in duplicates, 2 ml of blood was centrifuged, plasma separated and stored as aliquots at 4˚C for biochemical estimation. The other 2 ml was used for various hematological examinations, immediately after collection.

3.2.3 Haematology

3.2.3.1 Total leukocyte count

The total leukocyte counts were enumerated by Neubaeur slide method as described by Schalm *et al.* (1975).

3.2.3.2 Haemoglobin

The hemoglobin level was determined by Sahli's Comparator method as described by Schalm *et al.* (1975).

3.2.3.3 Packed cell volume

The packed cell volume was determined by microhaematocrit method as described by Schalm *et al.* (1975).

3.2.3.4 Total erythrocyte count

The total erythrocyte count was enumerated by Neaubaeur slide method as described by Schalm *et al.* (1975).

3.2.4 Blood chemistry

Blood urea nitrogen levels and serum creatinine levels were estimated by ARTOS biochemical analyzer (M/S Swemed Diagnostic, Bangalore) using respective diagnostic kits as per manufacturer's instruction.

3.2.5 Urine

Dark field microscopy of urine sediment:

Midstream urine (30 ml) was collected from clinical cases of renal failure preferably after one week of the onset of clinical signs, in equal quantity of sterile PBS. The samples were centrifuged at 15000 rpm for 20 minutes. The pellet was suspended in one ml of PBS and subjected to dark field microscopy. In the present study, a total of 40

urine samples from dogs with renal failure were screened (immediately after collection) for leptospira by dark field microscopy (BX 50 Olympus microscope).

3.2.6 Gross Pathology

Detailed post mortem examination was conducted on all the cases submitted to department of pathology for postmortem examination and gross lesions, if any were recorded.

3.2.7 Histopathology

Kidney and Liver tissue pieces were collected in neutral buffered formalin.They were routinely processed and embedded in paraffin. Tissue sections of 5µ thickness were cut and stained by H & E as per the method described by Luna (1968). The sections were under 10, 20 and 40 X of BX 50 Olympus microscope for recording the histopathological changes. The lesions of interest were photographed. Criteria for classification of histological lesions was based on the severity as mild, moderate and severe.

3.2.8 Silver staining method for identification of leptospira

Some of the sections retained were subsequently stained by silver staining method for leptospira organisms. Silver staining procedure for leptospirosis (Warthin & Starry Method for spirochetes described by Bancroft and Stevens, 1996) was carried out on the prepared sections as per the following procedure.

1. Section: formalin fixed and paraffin embedded.

2. **Solutions:**

a. pH 3.6 acetate buffer

Sodium acetate	: 4.1g
Acetic acid	: 6.25ml
DW	: 500ml

b. One per cent silver nitrate in pH-3.6 acetate buffer.

c. Developer solution: 0.3 g of hydroquinone was added to 10 ml of pH 3.6 buffer. 1ml of this solution was added 15 ml of warmed 5 per cent Scotch glue or gelatine and mixed and kept at 40^{o}C. 3 ml of a 2 per cent silver nitrate in pH 3.6 buffer was taken and kept at 55^{o}C. The two solutions were mixed immediately before use.

Method:

1. Tissue sections were deparaffinised and rehydrated to distilled water.
2. The hydrated sections were celloidinised in 0.5 per cent celloidin and excess of celloidin was drained off and further the celloidin was hardened in water for 1min.
3. The slides were impregnated in preheated (55-60^{o}C) silver solution (b) for 90-105min.
4. In the mean time the developer solution was prepared and preheated in a water bath.

5. Slides were treated with developer solution (c) for 3.5 min at 55^{o}C (immediately, the sections turn golden-brown). The developer was poured off and rinsed in tap water for several minutes at 55-60^{o}C and then in buffer at room temperature.
6. The slides were toned in 0.2 per cent gold chloride solution.
7. Sections were then dehydrated in graded alcohols, cleared in xylene and coverslipped with DPX.

3.2.9 Detection of Leptospires by PCR in clinical samples obtained from dogs with renal failure

A total of 35 (17 urine samples, 11 serum samples, 7 blood samples) samples were tested by PCR using the oligonucleotide primers. The work was carried out at Leptospira laboratory at Project Directorate on Animal Disease Monitoring and Surveillance, Hebbal, Bangalore.

3.2.9.1 DNA purification from clinical sample

Extraction of DNA was performed using Qiagen QIAamp DNA mini kit (Qiagen, Catalogue no.51306) using the blood and fluid protocol recommended by the manufacturer. The extracted DNA (50 µl volume) was kept at -20^{0}C until further use.

Materials

- QIAamp Mini spin column
- QIAGEN Protease (or proteinase K) stored at -20^{0}C
- Buffer AL (stored at room temperature, 15–25°C)
- Ethanol (96–100 per cent)

- Buffer AW1 (wash buffer stored at room temperature, 15–25°C).
- Buffer AW2 (wash buffer stored at room temperature, 15–25°C).
- Buffer AE (Elution Buffer stored at room temperature, 15–25°C).

Procedure

- Clinical sample (400 µl) *viz* serum, blood, urine was added into the microcentrifuge tube and 20 µl of QIAGEN proteinase K was pipetted into the bottom of a 1.5 ml microcentrifuge tube.
- Buffer AL (80 µl) was added to the sample and mixed by pulse-vortexing for 15 s. and the sample containing protease and Buffer AL was incubated at 56°C for 20min.
- Centrifugation of 1.5 ml microcentrifuge tube was done to remove the drops from the inside of the lid.
- Buffer AL (200 µl) was added to the sample and mixed by pulse-vortexing for 15 s and incubated at 70^{o} c for 10 min.
- Centrifugation of 1.5 ml microcentrifuge tube was done to remove the drops from the inside of the lid.
- Ethanol (200µl of 96–100 per cent) was added to the sample and mixed again by pulse-vortexing for 15s. After mixing, centrifugation was done to remove the drops from the inside of the lid.
- The above mixture was added to the QIAamp mini spin column (in a 2 ml collection tube) without wetting the rim. Cap was closed and centrifuged at 13000

rpm for 1-2 min. Later, the QIAamp Mini spin column was placed in a clean 2 ml collection tube and the tube containing the filtrate was discarded.

- The QIAamp Mini spin column was opened carefully and 500 µl Buffer AW1 was added without wetting the rim. Cap was closed and centrifuged at 13000 rpm for 1-2 min, later QIAamp mini spin column was placed in a clean 2 ml collection tube and the collection tube containing the filtrate was discarded.
- Buffer AW2 (500 µl) was added to the QIAamp mini spin column without wetting the rim. Cap was closed and centrifuged at full speed (14,000 rpm) for 3 min.
- The collection tube containing the filtrate was the QIAamp mini spin was placed in a clean 1.5 ml microcentrifuge tube.
- 50 µl of AE buffer was added to the column and incubated at room temperature for 3 to 5 minutes and further centrifuged at 13000rpm for 2 minutes. Eluted DNA was stored at -20^0c.

3.2.9.2 Polymerase Chain Reaction

Leptospira genus specific PCR based on the 16rs RNA gene using the primer set

Lepto1= 5’ GGCGGCGCGTCTTAAACATG 3’

Lepto2= 5’ TTCCCCC CATTGAAGCAAGATT 3’

was carried out as per the method described by Marien *et al*. (1995) which amplified the 331 bp amplicon from all the leptospira. Ten µl of DNA extracted from serum, urine and blood was taken and PCR reaction was carried out in a total of 25 µl reaction mixture in a thermal cycler.

Composition of reaction mixture

DNA	10 µl
Primer Lepto 1(10 pm)	1 µl
Primer Lepto2 (10pm)	1ul
PCR master mix	12.5 ml
Nucleus free water	0.5 ml

3.2.9.3 Analytical agarose gel electrophoresis

DNA amplified by PCR was subjected to agarose gel electrophoresis according the standard procedure described by Sambrook *et al.* (1989). Eighty mg agarose (1.2 per cent) in 1.5 ml 0.5 x TBE buffer was dissolved, melted and allowed to cool to 50^{o} C. To this ethidium bromide (final concentration 0.5 µg/ ml) was added and mixed thoroughly. Then the mixture was poured into a gel casting tray fitted with acrylic comb and allowed to solidify. Once the gel was formed a few ml of TBE was added near the comb, which was later removed carefully and the gel was immersed in electrophoresis tank containing 0.5 x TBE buffer. 6 x DNA loading buffer was mixed with DNA samples and loaded into the wells. Electrophoresis was carried out at 5 volts/cm until the thickening dye (Bromo phenol blue) passed out, the bands were illuminised under UV illumination and photographed. The size of bands separated was calculated from the slandered DNA molecular weight markers.

3.2.10 Statistical analysis

The data generated from different parameters of study were subjected to t-test using Graph Pad Prism version 5 for windows.

RESULTS

IV RESULTS

In the present study, total of 130 renal failure cases in dog were recorded at the Veterinary College Hospital, Bangalore, during the year 2009. Epidemiological details were collected from the renal failure cases. Clinico-pathological study was carried out in forty cases of renal failure cases. Out of 40 cases, 27 were from the Veterinary College Hospital and 13 cases were referred from other organisations. Gross and histopathological examinations were carried out on these cases and the results of same have been presented under respective headings.

4.1 Epidemiological study

4.1.1 Agewise occurrence

The different age groups showing occurrence of renal failure among dogs have been presented in Table 1 and Fig. 1. The occurrence of renal failure was found to be 18.46 per cent in the age group of eight to nine years. Highest occurrence of renal failure (23.84 per cent) was recorded in the age group of ten to eleven years and lowest occurrence of 6.10 per cent in four to five years age group. The age of 130 renal failure cases ranged from 1.5 to 15 years with a mean age of 8.45 years.

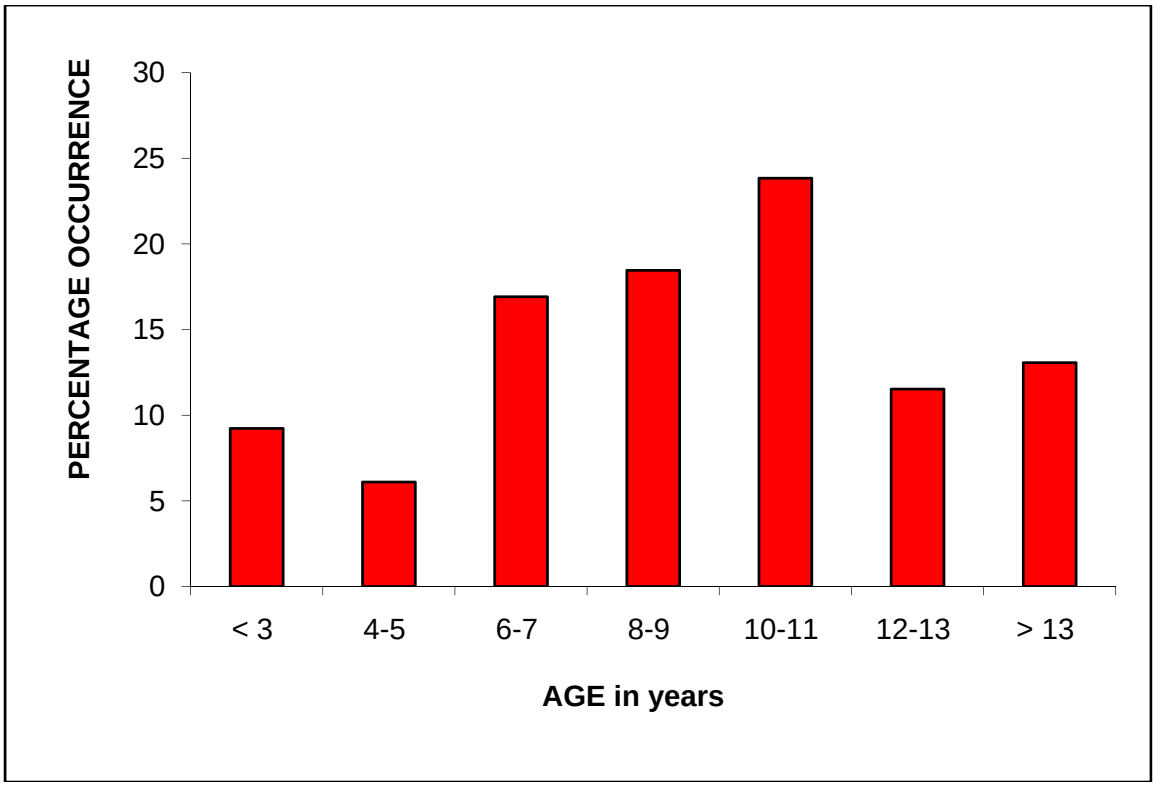
4.1.2 Genderwise occurrence

Out of 130 cases of renal failure observed in the present study 80 were male and 50 were female which constituted 61.53 and 38.46 per cent respectively.

Table 1. Agewise distribution of renal failure in dog

Age groups	No. of animals	Percentage
Below 3 years	13	09.23
4-5 years	08	06.10
6-7 years	22	16.92
8-9 years	24	18.46
10-11 years	31	23.84
12-13 years	15	11.53
Above 13 years	17	13.07
Total	130	100

Fig. 1. Age- wise distribution of renal failure in dog



4.1.3 Breedwise occurrence

The different breeds of dog that were affected with renal failure included German shepherd (25/130), Pomeranians (23/130), Labrador Retrievers (20/130) in the decreasing order. The occurrence of renal failure among different breeds and their per cent occurrence has been presented in the Table 2 and Fig. 2. The most commonly presented breeds were German shepherd and Pomeranian. The other breeds in order of decreasing frequency were Labrador Retrievers, Nondescript, Crossbreeds, Great Dane, Dalmatian Daschund and Boxer. It was observed that maximum occurrence of renal failure was observed in German shepherd (19.23 per cent) followed by Pomeranian (17.69 per cent). Labrador Retrievers and Nondescripts were next frequently affected breeds showing 15.38 per cent and 13.84 per cent breed occurrence respectively. Great Dane, Dalmatian, Daschund and Boxer showed occurrence of less than seven per cent occurrence.

4.2 Clinicopathological study

4.2.1 Clinical Signs

The important clinical signs observed in renal failure cases in the present study have been presented in Table 3 and Fig. 2. The predominant clinical signs were loss of appetite, vomiting, lethargy, polyuria, polydypsia, diarrhoea and pale conjunctival mucous membranes. The other lesions included oral ulcers, halitosis, and loss of weight, oliguria, recumbency, melena, haemetemesis, lumbar pain and convulsions.

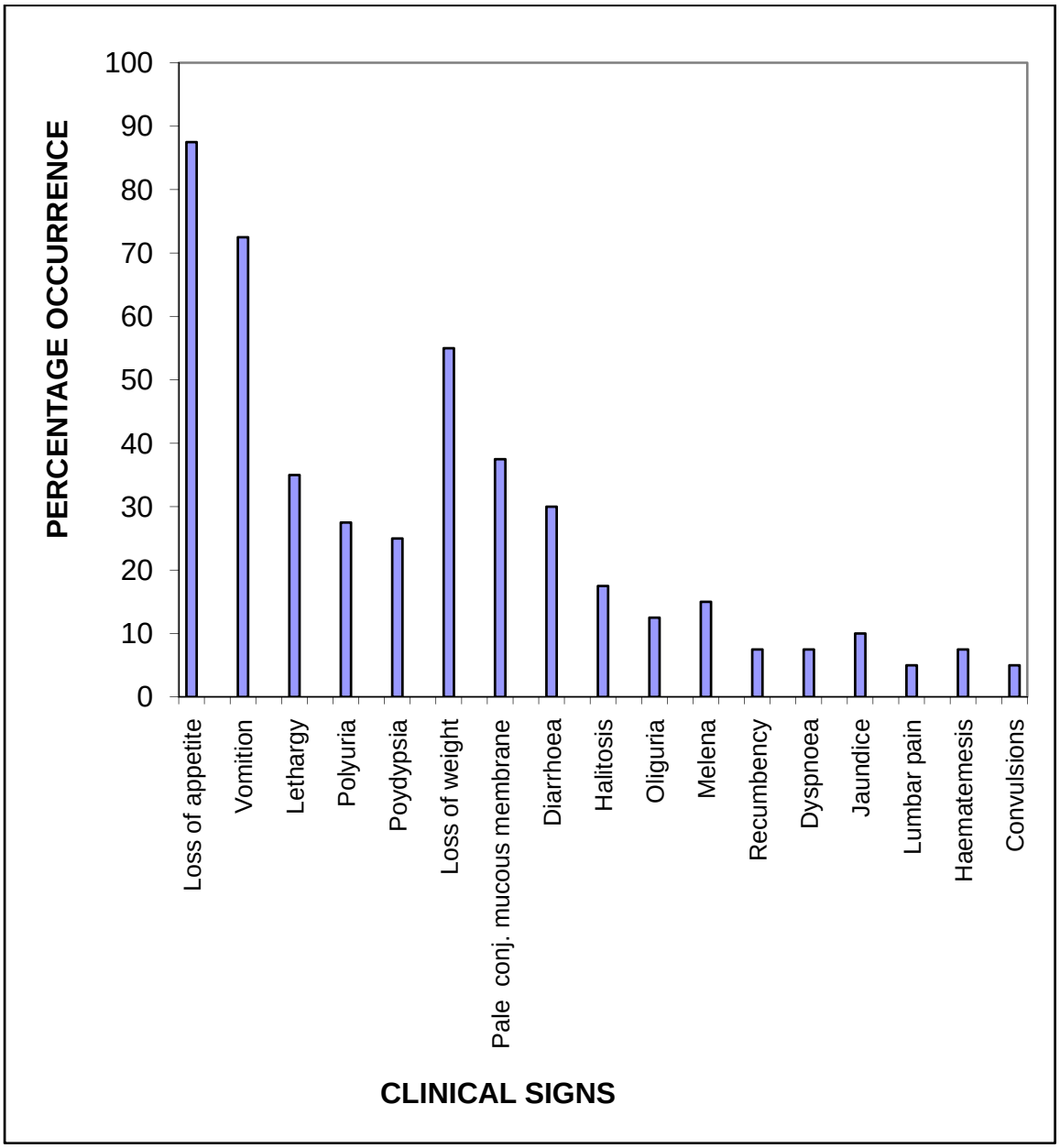
Table 2. Gender and Breedwise distribution of renal failure in dogs

Breed	Male		Female		Total	
	No	Percent	No	Percent	No	Percent
German Shepherd	17	21.25	8	16	25	19.23
Pomeranian	12	15.00	11	22	23	17.69
Labrador Retrievers	10	12.50	10	20	20	15.38
Non-descript	14	17.50	04	08	18	13.84
Crossbred	05	06.25	03	06	08	06.15
Great Dane	02	02.50	05	10	07	05.38
Daschund	05	06.25	01	02	06	04.61
Dalmatian	04	05.00	02	04	06	04.61
Golden Retriever	03	03.75	01	02	04	03.07
Doberman	02	02.50	02	04	04	03.07
Boxer	02	02.50	01	02	03	02.30
Bull terrier	01	01.25	01	02	02	01.53
Weimaraner	01	01.25	00	00	01	00.76
Mudhol	02	02.50	00	00	02	01.53
Collie	00	00.00	01	02	01	00.76
Total	80		50		130	

Table 3. Salient clinical signs observed in dogs with renal failure

Clinical signs	No. of animals	Percentage
Loss of appetite	35	87.50
Vomition	29	72.50
Lethargy	14	35.00
Polyuria	11	27.50
Poydypsia	10	25.00
Loss of weight	22	55.00
Pale conjunctival mucous membrane	15	37.50
Diarrhoea	12	30.00
Halitosis	07	17.5
Oliguria	05	12.50
Melena	06	15.00
Recumbency	03	07.50
Dyspnoea	03	07.50
Jaundice	04	10.00
Lumbar pain	02	5.00
Haematemesis	03	07.50
Convulsions	02	05.00

Fig. 2. Salient clinical signs observed in dogs with renal failure



4.2.2 Haematology

The mean (± S.E.) values of different haematological parameters in apparently healthy and renal failure cases recorded in the present study are presented in Table 4 and Fig 3. There was a significant decrease (P< 0.05) in the values of haemoglobin between the values of apparently healthy (14.66 ± 0.37) and renal failure cases (10.03 ± 0.50). There was a significant decrease (P< 0.05) in the values of PCV between the values of apparently healthy (46.50 ± 0.87) and renal failure cases (32.10 ± 1.15). There was a significant decrease in the (P< 0.05) in the values of TEC between the values of apparently healthy (06.43 ± 0.20) and renal failure cases (04.66± 0.16).

There was a significant increase (P> 0.05) in the values of TLC between apparently healthy (06.57 ± 0.36) and renal failure cases (17.28 ± 1.51).

There was a significant decrease (P<0.05) in the MCV values. However, MCH and MCHC values were similar when compared between apparently healthy and renal failure cases.

4.2.3 Serum biochemistry

4.2.3.1 Blood Urea Nitrogen

The mean (± S.E) BUN values in apparently healthy and renal failure cases were presented in Table 5 and Fig 4. There was significantly (P> 0.05) higher BUN value in renal failure cases (113.0 ± 10.16) as compared to their healthy counterparts (19.10 ± 03.63) in the present study.

Table 4. Mean ± S.E. values for haematological parameters in healthy animals and dogs with renal failure

Parameters	Apparently healthy Animals (n=8)	Renal failure cases (n=40)
Hb (g/dl)	14.66 ± 0.37	10.03 ± 0.50**
PCV(per cent)	46.50 ± 0.87	32.10 ± 1.15 **
TEC (x 10^6/µl)	06.43 ± 0.20	04.66 ± 0.16 **
MCV(fl)	76.95 ± 0.87	67.23 ± 1.98*
MCH(pg)	24.29 ± 0.59	21.34 ± 0.67^{NS}
MCHC(g/dl)	32.83 ± 0.94	30.80 ± 0.79^{NS}
TLC(x 10^3/µl)	06.57 ± 0.36	17.28 ± 1.51**

NS- Non significant

*- Significant

**- Highly significant

Fig. 3. Mean ± S.E. values for haematological parameters in healthy animals and dogs with renal failure

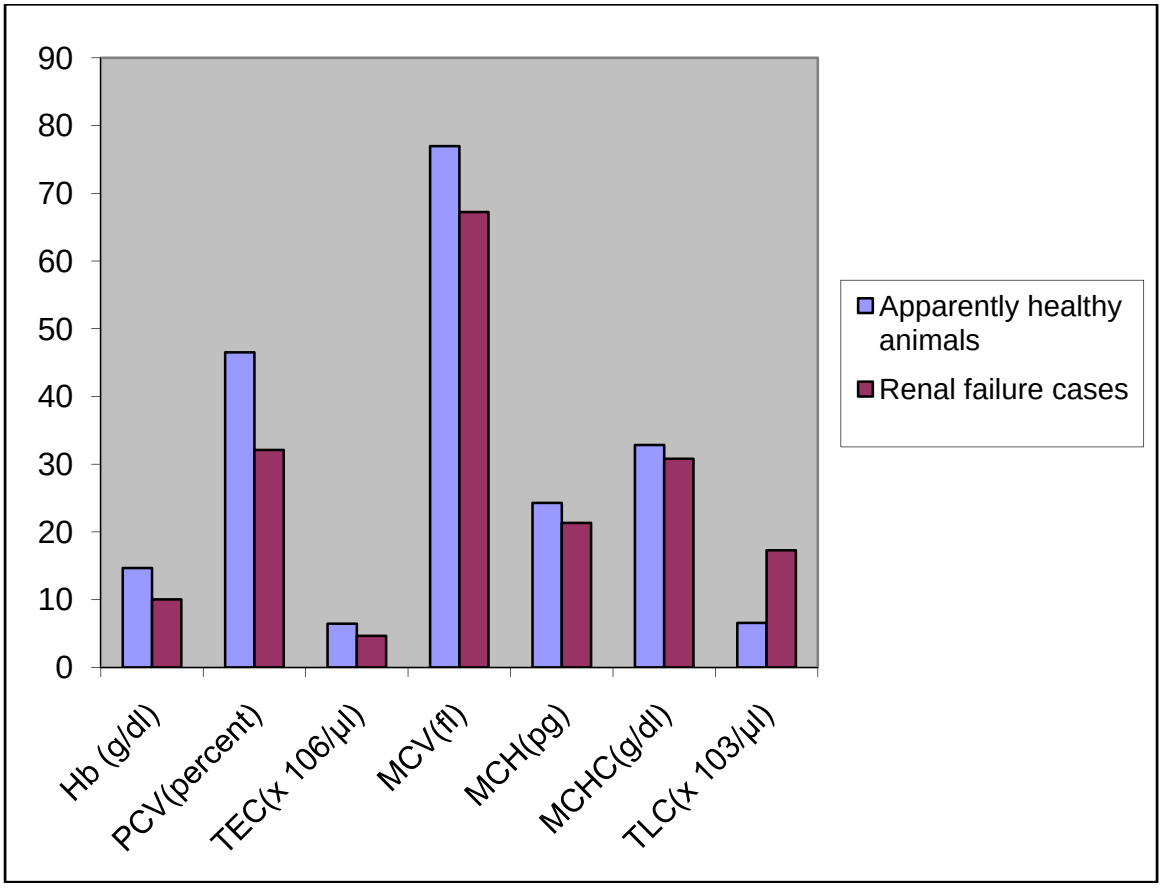
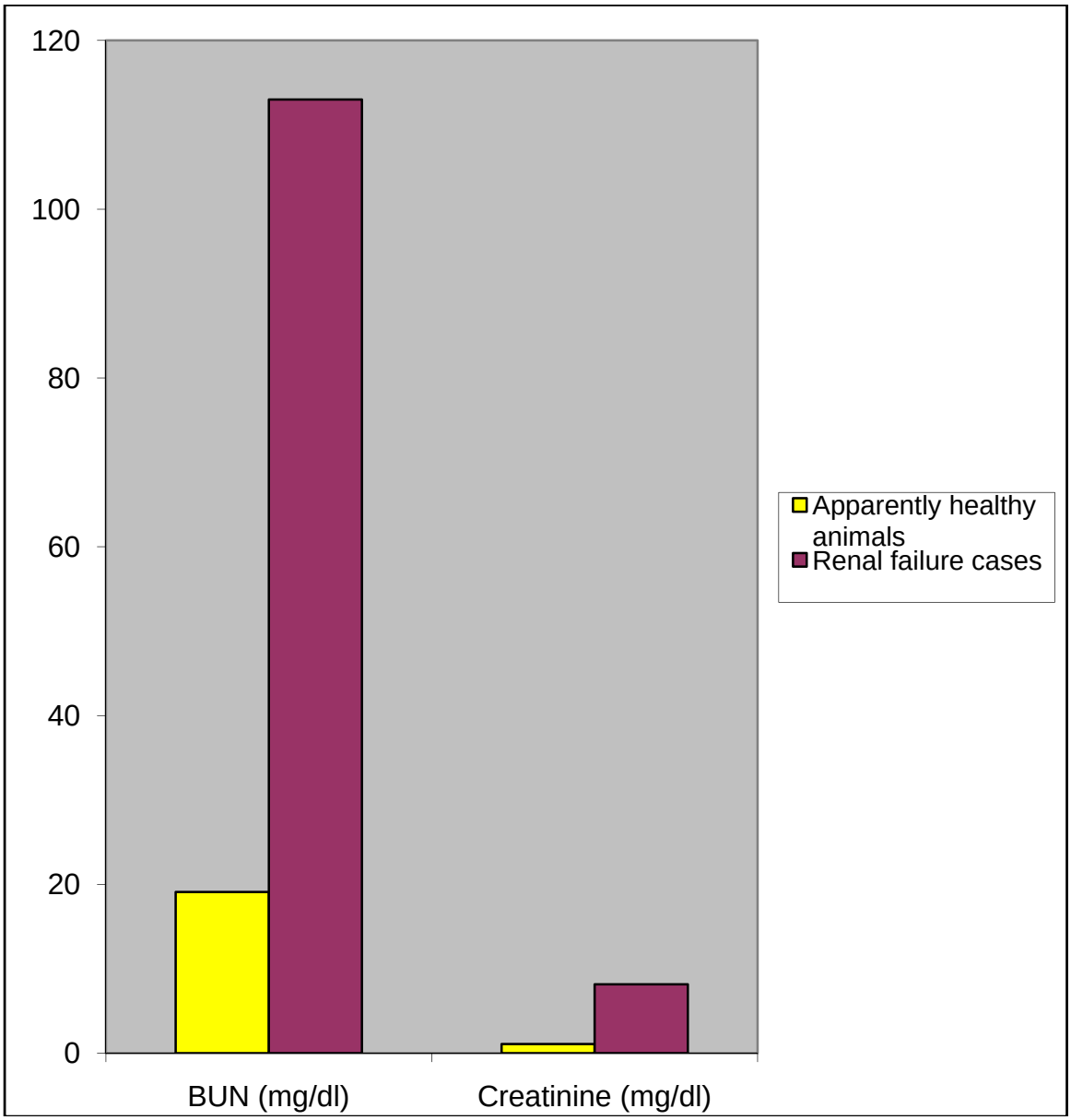


Table 5. Mean ± S.E. values of BUN and Serum creatinine in apparently healthy animals and dogs with renal failure

	Apparently healthy animals (n=8)	**Renal failure cases (n=40)**
BUN (mg/dl)	19.10 ± 03.63	113.0 ± 10.16
Serum creatinine (mg/dl)	01.08 ± 00.20	08.16 ± 00.70

Fig. 4. Mean± SE BUN and Serum creatinine values in apparently healthy animals and dogs with renal failure



4.2.3.2 Serum creatinine

The mean ± S.E. serum creatinine values in apparently healthy and renal failure cases are given in Table 5 and Fig 4. There was a significant (P> 0.05) increase in the values of serum creatinine between apparently healthy animals (01.08±0.20) and renal failure cases (8.16 ± 0.70) in the present study.

4.2.4 Dark Field Microscopy

In the present study, a total of 40 urine samples from dogs with renal failure were screened for leptospira by dark field microscopy out of which, 3 samples were found positive for leptospira.

4.2.5 Detection of leptospira by PCR in renal failure cases.

A total of the 35 samples were collected from 20 dogs with renal failure cases and were subjected to PCR. Among the 11 serum samples tested 3 were positive, of 7 blood samples tested 3 were positive and of 17 urine samples tested 3 were positive for leptospira organism in the present study.

4.3 Pathology

Various gross and histopathological changes of kidney and liver observed in 40 renal failure cases in dogs were systematically recorded and presented under the respective headings.

4.3.1 Gross pathology of kidney

Gross lesions observed in kidneys of 40 selected cases of renal failure in dogs have been presented in the Table 6. The lesions included enlarged kidney (Plate 1) in 8, reduced and contracted kidney (Plate 2 & 7) in 26. They were congested (Plate 1 & 7) in 12 cases, pale in 12, icteric (Plate 5) in 7 and apparently normal in 9 cases. Pinhead sized necrotic spots were visible (Plate 4 & 5) in 9 cases where as large areas of necrosis were observed in 5 cases. The capsule was adherent to the parenchyma (Plate 3) in 21 cases where as in 14 cases, they were easily peeled off. The renal surface showed pitted appearance (Plate 3) in 21 cases. Single to multiple cysts ranging from small size to large size were seen in 8 cases (Plate 4 & 6). Cortical surface was granular and pitted in appearance (Plate 2) in 11 cases. The kidneys were firm in consistency in 22 cases and smooth in 11 cases. Reduced corticomedullary junction was observed in 9 cases (Plate 8).

4.3.2 Histopathology of kidney

Various histopathological lesions observed in the kidneys of dogs with renal failure are described under the heading as vascular changes, degenerative changes, necrotic changes, inflammatory changes and hyperplastic changes.

4.3.2.1 Tubular changes

The nature of the tubular changes observed in the present study comprised of congestion, haemorrhages and varying degrees of cell swelling ranging from granular to vacuolar degenerative changes. In addition, fatty changes, presence of proteinacious material in the tubular lumen, protein droplets in the renal tubular cells, hyaline casts,

necrosis and desquamative changes of the tubular epithelium were also noticed. Tubular lesions were observed in 38 out of 40 kidneys examined.

4.3.2 Histopathology of kidney

Various histopathological lesions observed in the kidneys of dogs with renal failure are described under the headings as vascular changes, degenerative changes, necrotic changes, inflammatory changes and hyperplastic changes.

4.3.2.1 Tubular changes

The nature of the tubular changes observed in the present study comprised of congestion, haemorrhages and varying degrees of cell swelling ranging from granular to vacuolar degenerative changes. In addition, fatty changes, presence of proteinacious material in the tubular lumen, protein droplets in the renal tubular cells, hyaline casts, necrosis and desquamative changes of the tubular epithelium were also noticed. Tubular lesions were observed in 38 out of 40 kidneys examined.

Vascular changes

Congestion

Congestion was characterized by distension of blood vessels stuffed with erythrocytes (Plate 9, 10 and 11) and opening of new vessels was also observed. Based on the severity of this lesion an arbitrary classification of mild, moderate and severe lesion was applied. Congestion was observed in 17 of 40 cases examined. It was mild in 10, moderate in 4, severe in 3 cases. Congestion was consistently associated with degenerative or inflammatory conditions of the kidney.

Haemorrhages

Haemorrhages were featured by extravasation of blood into tissue spaces. Based on the extent of extravasation, they were classified as focal, multifocal and diffuse. They were focal in 6, multifocal in 5 and diffuse 2 instances (Plate 10, 11 and 12).

Degenerative changes

The cell swelling was characterized by swollen tubular epithelial cells with granular to vacuolated cytoplasm (Plate 13 & 15). These changes were classified as mild, moderate and severe vacuolar degenerative changes depending on the severity. Mild change was characterized by presence of vacuolar degeneration in the stray tubules. On other hand, moderate change was featured by presence of many small areas of vacuolar degeneration in many tubules. Severe change was characterized by presence of uniform vacuolar degeneration in the entire tubules. In the present study, vacuolar degeneration was observed in 31 kidneys. It was mild in 6, moderate in 10 and severe in 15 cases.

Fatty changes

Fatty changes involving the proximal convoluted tubular epithelium featured by presence of fat globules and it was observed in 3 kidneys. These fat droplets were of varying size (Plate 15).

Necrosis

Necrosis of the tubular epithelium was confined particularly to proximal convoluted tubules to start with and it was characterized by varying degrees of

degenerative nuclear changes. Desquamation and coalescence of the necrosed epithelium was also evident in tubules. This lesion was observed in 21 cases (Plate 16 and 17)**.**

Proteinacious material or hyaline casts

Proteinacious material or hyaline casts in the tubules appeared as light staining homogeneous eosinophilic substance in H & E stained sections (Plate 14, 18, 19 & 24). Large number of tubules in cortex and medulla were filled with eosinophilic proteinacious material and were lined by flattened tubular epithelial cells. This lesion was observed in 12 cases.

Protein droplets

Protein droplets were seen as numerous homogeneous eosinophilic droplets of varying size in the cytoplasm of tubular cells and tubular lumina in H& E sections (Plate 18). This was observed as a consistent feature in 9 cases out of 40 cases examined in the present study.

Tubular dilatations

Cystic dilatation of renal tubules was observed in 17 cases. It was characterized by distended tubules with flattened lining epithelial cells (Plate 20 and 21). Occasional tubules were lined with regenerating epithelium characterized by highly basophilic cells (Plate 36 & 42). Occasionally, some tubules showed hypertrophy and others hyperplastic with tall cuboidal cells (Plate 35).

4.3.2.2 Interstitial changes

Interstitial changes were featured by varying degrees of congestion, haemorrhages, necrosis of the interstitial tissue and mononuclear cell infiltration in the intertubular spaces with connective tissue proliferation. Mineralization and pigmentation were the additional changes recorded in the present study.

Congestion

Congestion was characterized by distension of blood vessels stuffed with erythrocytes (Plate 9, 10 and 11) and opening of new vessels was also observed. Based on the severity of this lesion an arbitrary classification of mild, moderate and severe lesion was applied. Congestion was observed in 15 of 40 cases examined. It was mild in 8, moderate in 4 and severe in 3 cases. Congestion was consistently associated with degenerative or inflammatory conditions of the kidney.

Haemorrhages

Haemorrhages were featured by extravasation of blood into tissue spaces. Based on the extent of extravasation, they were classified as focal, multifocal and diffuse. They were focal in 4, multifocal in 5 and diffuse in 2 instances (Plate 10 and 12).

Necrosis

Necrosis of interstitium consisted of focal to multifocal areas of tissue destruction with presence of nuclear debris and infiltration with mononuclear cells. These changes were noticed in 7 cases out of 40 cases examined in the present study (Plate 23).

Inflammatory cells

The infiltrating mononuclear cells consisted of pure plasmacytic populations in one case (Plate 22). Mixed populations of lymphocytes, plasma cells and macrophages were observed in 32 cases (Plate 23) out of 40 cases examined.

Depending on the duration and type of inflammatory cells involved, tubulo-interstitial nephritis was classified as acute, subacute and chronic types.

Acute interstitial nephritis

It was characterized by moderate to severe degree of congestion of glomerular capillaries and interstitial vessels. There was tubular degeneration with intact basement membrane. Tubular epithelium showed degenerative and necrotic changes. There were scattered areas of inflammatory aggregates (Plate 27) predominantly of mononuclear cells in the interstitium as well as lumen. The tubules were filled with hyaline casts (Plate 26). Some of the tubules showed the proliferative change with neutrophils.

Subacute interstitial nephritis

It was characterized by moderate to severe degree of congestion of glomerular capillaries and interstitial vessels with coagulative type of necrosis. The tubules were filled with hyaline casts (Plate 14 & 24) in medullary region and cortex. Tubules also showed mild degree of cystic dilatation. Mild to moderate degree of connective tissue proliferation with infiltration of mononuclear cells was also evident. The interstium showed fibrosis and hyalinization of connective tissue (Plate 28). Periglomerular fibosis was present in occasional cases.

Chronic interstitial nephritis

Chronic interstitial nephritis was featured by extensive proliferation of fibrous connective tissue with massive infiltration of mononuclear cells comprising of lymphocytes, plasma cell and macrophages through out the interstitium of the kidney. Thickening of basement membrane and peritubular fibrosis (Plate 28) was observed in a few cases. Tubules showed cystic dilatation with flattening of tubular epithelium and presence of coagulated eosinophilic material in the lumen. Interstitium showed hyalinised connective tissue (Plate 24), focal areas of calcification and hemosiderin pigments. Occasional tubules showed proliferative changes. Some of the tubules also showed granular to vacuolar degeneration of lining epithelium.

Glomeruli showed thickening of basement membrane with varying degrees of atrophy of tufts. In some glomeruli, the tuft was replaced by homogeneos eosinophilic material. Periglomerular fibosis was also present. Some glomeruli were completely obliterated. (Plate 41)

Connective tissue proliferation

Interstitial connective tissue proliferation was classified as mild, moderate and severe changes based on the severity and this was observed in as many as 33 kidneys. It was mild in 7, moderate in 10 and severe in 15 cases (Plate 24 & 28) in the present study.

Pigmentation

Hemosiderin pigments were found in the tubular epithelial cell or in the cytoplasm of the macrophages in the interstitium. They appeared as brownish granular deposits in H & E sections. It was noticed in 10 cases.

Mineralization

It was characterized by presence of deposits of calcium in Tubulo-interstitial space as intensely basophilic amorphous material either in the lumen of the tubules or interstitium. It was observed in 15 cases (Plate 25).

Cysts

Multiple cysts were seen on the surface and within the substance of the kidneys. Grossly, the cysts were seen on the surface of the kidney as spherically elevated areas. The size of the cysts varied from two to eight mm in diameter and apparently looked transparent containing clear watery fluid. Cysts were seen involving both cortex and medulla. Microscopically, the cysts showed capsule made comprising of connective tissue with inflammatory cells and lined by flattened epithelium on the inner aspect. Numerous glomeruli were atrophied surrounding the connective tissue capsule. The lumens of the cysts were either empty or contained faintly staining eosinophilic material (Plate 29). The renal cysts were observed in three instances as unilateral lesions involving left kidney in two cases and right kidney in one case.

4.3.2.3 Glomerular changes

In the present study, kidneys from canine renal failure cases were examined for evidence of glomerular changes. It was observed that changes attributable to glomerular nephritis were present in 7 cases out of 40 kidneys examined.

In instances of glomerulonephritis, certain histological changes which were not characteristic of any particular subtype were also noticed. These lesions were considered as associated lesions.

Congestion

Congestion was characterized by distension of swollen glomerular endothelial cells and capillaries. It was mild in 7cases, moderate 5 cases and severe in 4 cases.

Leukocytic infiltration

Leukocytic infiltration featured by segmental infiltration of lymphoid cells, or thus giving a hyper cellular appearance to the glomeruli in H &E sections (Plate 40 & 43). This was observed in 3 cases. (Plate 11 & 17)

Capsular adhesions

Capsular adhesions occurred as segmental or global attachments of the glomerular tuft to Bowman's capsule (Plate 30 & 37). This lesion was clearly visible in H & E stained sections. This was observed in 15 cases. The associated lesions were described under following headings;

Capsular thickening or periglomerulitis

Capsular thickening or periglomerulitis seen as or global or segmental fibosis of Bowman's capsule in five cases (Plate 31).

Proteinacious material

Proteinacious material in Bowman's space stained faintly eosinophilic in H& E sections. In some cases there was cystic dilation of Bowman's space with presence of larger amounts of proteinacious material as well as cellular debris leading to atrophy of the glomerular tuft (Plate 32).

Crescents

Crescents were seen as extra capillary proliferation of parietal epithelial cells often forming a half moon shaped structure at the urinary pole (Plate 33). They were distinguished from periglomerular fibrosis by their cellular character and lack of connective tissue elements.

Reduced Bowman's space

Reduced Bowman's space was featured by reduced or complete absence of the glomeruli or glomerular tuft. This change was seen involving few to many glomeruli (Plate 41).

Cystic glomeruli

Cystic glomeruli were featured by increase in the glomerular space coupled with pressure atrophic changes on the glomeruli (Plate 34). Again this lesion was noticed involving either occasional or more than few glomeruli.

The glomerulonephritis was classified into 4 major categories namely:

1. Membranoproliferative glomerulonephritis
2. Membranous glomerulonephritis
3. Mesangial sclerotic glomerulonephritis
4. Membranoproliferative glomerulonephritis with sclerosis

1. Membranous glomerulonephritis

Membranous glomerulonephritis was recorded in 2 cases. Membranous glomerulonephritis was characterized by changes predominantly restricted to the basement membrane (Plate 39& 41). The glomerular basement membrane was thickened. The other lesions observable were congestion in 2; leucocytic infiltration in 2; capsular adhesions in 2; periglomerulitis in 2; demonstrability of protein in Bowman's space in 3; crescents in 1; mesangial sclerosis in 1; reduced Bowman's space in 3.

2. Membranoproliferative glomerulonephritis.

Membranoproliferative glomerulonephritis was recorded in 2 cases showing morphological changes of glomerulonephritis. Lesion was either segmental or global. Membranoproliferative glomerulonephritis was characterized by thickening of glomerular basement membrane and mesangial proliferation (Plate 38) in the absence of mesangial sclerosis. In instances of membranoproliferative glomerulonephritis, the associated lesions observed were: Glomerular congestion(1), capsular adhesions (2) periglomerulitis (1) protein in Bowman's space (1), Reduced Bowman's space (1) and cystic glomeruli (2).

3. Mesangial sclerotic glomerulonephritis

Mesangial sclerotic glomerulonephritis was seen in only two cases and characterized by an increase in mesangial matrix (Plate 41 & 39) in the absence of membranous and mesangial proliferative changes. In these instances, mesangial cells were often masked and not clearly visible due to increased matrix. These lesions occurred in segmental and global forms in H and E sections. There was an increase in

homogeneous faintly eosinophilic material (Plate 43) in intercapillary areas and other findings were congestion, capsular adhesions, capsular thickening and protein in tubules.

4. Membranoproliferative glomerulonephritis with sclerosis

Membranoproliferative glomerulonephritis with sclerosis was seen in one case and characterized by changes in all three major components, namely thickening of basement membrane, mesangial cell proliferation and mesangial sclerosis (Plate 37) and the other findings were congestion, capsular adhesions, capsular thickening and protein in tubules.

In all the cases of glomerulonephritis, tubules showed degenerative and necrotic changes. Interstium revealed mild to moderate infiltration of mononuclear cells in all the cases.

4.3.3. Gross pathology of liver

Grossly, the lesions observed in the liver consisted of congestion, haemorrhages, fatty change, necrotic foci, nodular growths and cyst. Congestion was observed in 13 cases (Plate 44). The livers were slightly enlarged and dark in appearance with cut surface revealing free oozing of blood. Haemorrhages were recorded in 7 cases and it was characterized by petechial (Plate 45) or ecchymotic type on the surface. Fatty changes were noticed in three cases and grossly, liver were enlarged, pale yellow to creamy in colour and soft in consistency.

Necrosis was noticed in 8 cases and grossly two types of necrosis were evident with greyish whitish focal necrotic foci (Plate 46). observed through out the parenchyma

in 5 cases and large patchy irregular necrotic area involving the liver parenchyma in 3 cases (Plate 46). Grossly, the lesions were multifocal and appeared as grayish white patches. These areas of necrosis were seen to be surrounded by zones of varying degrees of hyperemia. In addition, there were also haemorrhages in them.

Cysts

Cyst was noticed in one case. It was single located in the substance of liver and contained straw colored fluid in one case. Microscopically, the cysts showed capsule made up of fibrous connective tissue with inflammatory cells and lined by flattened epithelium on the inner aspect.

4.3.4 Histopathological changes of liver

The histopathological changes observed in the liver of renal failure cases in the present study included congestion, haemorrhages, cell swelling, vacuolar degeneration, fatty changes, necrosis, connective tissue proliferation, periportal fibrosis, cirrhosis, bile duct proliferation, and lymphocytic infiltration, periportal infiltration, multifocal nodular aggregation and hemosiderosis (Plate 53, 54, 56, 57 & 58).

4.3.4.1 Congestion

The changes were predominant in central veins and sinusoidal spaces and appeared distended with erythrocytes and resultant compression of adjoining hepatic cords (Plate 51, 55 & 57). Hepatocytes in these centrilobular areas were less conspicuous. The kupffer cells in the neighboring areas showed brownish yellow pigment in their cytoplasm (Plate 54). The hepatocytes in the periphery showed atrophy, gradations of

degeneration and fatty changes (Plate 58). Based on the severity, congestion was classified as mild, moderate and severe changes. A mild degree was characterized by slight distension of occasional vessels which were stuffed with blood. A moderate degree of congestion was included when distension of many vessels and sinusoids of more than few hepatic lobules were noticed and severe lesion was featured by distension of vessels and sinusoid of entire hepatic lobule.

In the present study congestion was observed as mild degree in 4 cases, moderate in 12 cases and severe in 20 cases.

4.3.4.2 Haemorrhages

In the present study, haemorrhages were recorded in 6 cases. Haemorrhages were characterized by disruption of vessels with extravasations of erythrocytes (Plate 51). Based on the severity of the lesion, they were classified as multifocal and diffuse changes. More than four to five areas of extravasations of blood in any lobe of liver was regarded as multifocal haemorrhages, while extensive haemorrhages were characterized by involvement of part or entire lobe with considerable loss of architecture. Among the 6 cases, haemorrhages were multifocal in 3 and extensive in 3 cases.

4.3.4.3 Degenerative changes

The cell swelling was characterized by swollen hepatocytes with either granular basophilic or vacuolated cytoplasm. These changes were classified as mild, moderate and severe vacuolar degenerative changes depending on the severity. Mild change was characterized by presence of vacuolar degenerations in the stray hepatic lobules. On the

other hand, moderate change was featured by presence of many small areas of vacuolar degenerations (Plate 59) in many lobules. Severe change was characterized by presence of uniform vacuolar degenerations of the entire lobe of liver. In the present study, vacuolar degenerations were observed in 27 livers. It was mild in 6, moderate in 16 and severe in 5 cases (Plate 59).

4.3.4.4 Fatty changes

Histologically, fatty change was characterized by presence of clear fat vacuoles of varying sizes. When the fat droplets were large, the nucleus was pushed to a side giving signet cell appearance. These changes were classified as either mild or moderate fatty changes depending on the severity. Mild fatty change was characterized by presence of occasional fat droplets in the stray hepatic lobules. On other hand, moderate fatty change was featured by presence of small droplets of fat in many lobules. In the present study, fatty changes were observed in 5 livers. It was mild multifocal fatty change in 3 and moderate multifocal in 2 cases (Plate 47 and 58).

4.3.4. 5. Necrotic changes

Necrosis was recorded in 4 cases. These changes appeared confined to focal areas within the hepatic lobule. The foci of necrosis tended coalesce and become confluent in two cases. Infiltration of mononuclear cell (Plate 49) was also evident. Changes of periportal fibrosis (Plate 52 with collections of mononuclear cells in the portal tracts was noticed with biliary hyperplasia (Plate 50).

4.3.4.6 Inflammatory changes

In the present study, inflammatory changes were noticed in 7 cases. Histologically cirrhosis was characterized by proliferation of fibrous connective tissue around the portal area and the bile duct with infiltration of inflammatory cells. Multifocal nodular aggregation of mononuclear cells was noticed in 2 cases (Plate 49). Further, fibrous connective tissue was seen extending surrounding parenchyma causing pressure atrophic changes in the adjacent hepatic cords. Mild cirrhosis comprising of fibrous tissue proliferation in occasional portal area with a few inflammatory cells was observed in two cases (Plate 52 and 53). Moderate cirrhosis was featured by fibrous connective tissue proliferation involving many portal areas.

4.3.4.7 Hyperplastic changes

Bile duct hyperplasia was noticed in 3 cases in the present study. Histologically, the hyperplasia was characterized by proliferation of biliary epithelium with formation of new bile ducts. The proliferating biliary epithelium had round or oval nuclei and stained more intensely compared to hepatic parenchyma (Plate 48 & 52).

4.3.4.8 Fibrosed bile ducts

Fibrosed bile duct was noticed in 3 cases and microscopically, it was featured by marked increase in the collagen tissue around bile ducts.

4.3.4..9 Portal lymphocytic infiltration

Lesions of portal lymphocytic infiltration was noticed in 3 cases. It was featured by accumulation of lymphoid cells in the portal area (Plate 52).

Pathology of liver in leptospirosis.

The various gross and histopathological changes were noticed in seven renal failure cases due to leptospirosis. Grossly, liver appeared normal to enlarged in size with petechial haemorrhages. Histopathologically, the liver revealed mild to moderate degree of congestion and haemorrhage, vacuolar degeneration of hepatocytes, multifocal aggregation of mononuclear cells (Plate 49) periportal infiltration of inflammatory cells (Plate 50) and hemosiderin pigments. This was observed in two cases. In the remaining cases, hepatic lesions ranged from those of chronic hepatitis to mild to moderate vacuolar degenerations of hepatocytes, occasional scattered lymphocytic aggregates and occasional bile stasis.

Table 6. Gross pathology of kidney in renal failure in dogs

	Gross lesions	No. of cases (n=40)	Percentage
Size	Normal in appearance	06	15.00
	Enlarged	08	20.00
	Reduced and contracted	26	65.00
Color	Normal	09	07.50
	Dark	12	15.00
	Pale	12	60.00
	Icteric	07	17.50
Consistency	Firm	21	72.50
	Smooth	11	27.50
Surface	Granularity and pitted appearance	21	52.50
	Capsular adhesions	21	62.50
	Necrotic foci	15	40.00
	Congestion	12	35.00
	Haemorrhagic	09	22.50
	Cysts	08	20.00
	Reduced cortico-medullary junction	11	22.50

Table 7. Glomerular lesions observed in renal failure in dogs

Histopathological Lesions	No of cases (n=40)	Percentage
Congestion	17	42.50
Capsular adhesions	15	37.50
Reduced Bowman's Space	13	32.50
Cystic glomeruli	12	30.00
Capsular thickening	11	27.5
Proteinacious droplets	04	10.00
Crescents	02	05.00
Leukocytic infiltration:	03	07.50
Periglomerular fibrosis	04	10.00
Periglomerular infiltration	06	15.00
Mineralization	02	05.00
Pigmentation	02	05.00

Table 8. Tubulo-intestitial lesions observed in renal failure in dogs

Histological lesions	No. of cases (n=40)	Percentage
Congestion	14	35.00
Tubular haemorrhage	17	42.50
Tubular degenerations	33	82.50
Tubular necrosis	21	52.50
Fibrosis	30	75.00
Inflammatory cells	32	80.00
Cystic tubules	17	42.50
Protein droplets	08	20.00
Fatty change	03	05.00
Proteinacious material	12	30.00
Regenerating tubules	07	45.00
Hyaline casts	16	40.00
Tissue debris	05	12.50
Pigmentation	05	12.50
Mineralisation	09	22.50

Table 9. Gross lesions of liver in dogs with renal failure

Lesions	No. of cases (n=40)	Percentage
No abnormality detected	10	25.00
Congestion	13	32.50
Haemorrhages	07	17.50
Necrotic foci	06	15.00
Nodular growths	02	05.00
Cysts	02	05.00

Table 10. Histopathological lesions of liver in dogs with renal failure

Lesions	No. of cases (n=40)	Percentage
Congestion	36	90.00
Haemorrhages	15	37.50
Degenerative changes	27	67.50
Fatty changes	10	25.00
Necrotic changes	08	20.00
Inflammatory changes	07	17.50
Hyperplastic changes	04	10.00
Fibrosed bile ducts	04	10.00
Portal lymphocytic infiltration	04	10.00

DISCUSSION

V DISCUSSION

Considerably, more number of dogs with uremic signs are commonly encountered by canine practitioners. As the clinical signs observed in renal failure patients are non-specific and vague, they are not identified at early stage. In view of this, the epidemiological details were collected from dogs that were referred to Veterinary College Hospital Bangalore. Clinicopathological study was carried out in forty cases suspected for renal failure in dogs. The results of present study are discussed under the headings epidemiological study, clinical pathology, gross and histopathology for better understanding.

5.1 Epidemiological study

5.1.1 Agewise occurrence

In the present investigation, renal failure among dogs was observed in the age group of 1.5 to 15 years of age with maximum occurrence observed in the age group of 10 to 11 years (23.84 percent). The occurrence of renal failure among dogs was found to be more common in more than 5 years of age. Slauson *et al.* (1970) reported an average age of onset of renal failure as 9.2 years, a finding similar to that of present investigation. However, renal failure can occur at any age ranging from 1 year to 14 years (Slauson *et al*., 1970, David *et al*., 1995). In the present study, the average age of patients with renal failure was more than 8 years which agreed with the findings of Polzin *et al.* (1995) who reported that renal failure cases were commonly considered as a disease of older animals. Meyer (2004) has observed that chronic renal failure occurred in dog and cats breed at any age, but the older animals were more frequently affected than younger ones. Similar

observations have also been made by Janke and Allen (1986) who reported the age of dogs affected with glomerulonephritis as 6.5 years. Mrudula *et al.,* 2005 reported 7.8 years as the susceptible age for renal failure in 60 cases of canine nephritis studied. From the findings of the present investigation and observations of previous workers, it could be inferred that renal failure was a disease of aged animals.

5.1.2 Breedwise occurrence

In the present study, the maximum occurrence of renal failure was noticed in German shepherd (19.23%), followed by Pomeranian (17.69%) breed of dogs. Although, the occurrence of renal failure appear to be more common in some breeds than in others. The pattern of occurrence can be attributed to the popularity of certain breeds like the Labrador, Spitz and German shepherd in this geographical location rather than any breed predisposition. In another study, Cook and Catell 1985, found no age or sex difference between those having glomerular lesions and nonglomerular lesions in 111 cases of canine chronic renal disease patients. Further, it could also be attributed to wide range of kidney diseases of a proven, or suspected, familial or hereditary nature in certain breeds like Terriers, Lhasa apso, Shih tzu, Standard poodle, Dobermann Pinscher, Cocker Spaniel and German shepherd as reported by Hood *et al.* (1990).

5.1.3 Genderwise occurrence

Out of the 130 cases with renal failure recorded in the present study, 61.53 percent were males and 38.46 percent were females which agreed with the findings of Behrend *et al*. (1996) who reported that the occurrence of renal failure was slightly

higher in the males when compared to females. This could be attributed to the fact that the male dogs are more preferred as pets than female dogs.

5.2 Clinicopathological Study

5.2.1 Clinical Signs

A total of 40 cases of renal failure were chosen to study clinicopathological features. The predominant clinical signs noticed were loss of appetite, vomition, lethargy, polyuria, polydypsia, diarrhoea, pale conjunctival mucous membranes, loss of weight and oliguria. Some animals exhibited recumbency, melena, oral ulcers, halitosis, lumbar pain and convulsions. These findings were similar to the findings of earlier workers (Stone *et al*., 1988; Robinson *et al.,* 1989; Rubin (1997); Shilpa Rani (2003). Neel and Grindem (2000) who opined that even though most of the clinical signs were non-specific, certain signs such as polydypsia, polyuria and oral ulcers were highly suggestive of renal failure.

5.2.2 Haematology

The mean TLC, hemoglobin, PCV and TEC values were 17.28 ± 1.51X10^3 cells/ µl, 10.03 ± 0.50 g %, 32.10 ± 1.15 % and 4.6 ± 0.16 X 10^6 cell/µl respectively in the present study. It is evident that there was leukocytosis in renal failure cases. Increased white blood cell count generally indicated the presence of inflammation, which may either have an infectious or a non-infectious cause. In patients with renal failure, leukocytosis could be often due to inflammation in one or more organ system. When leukocytosis is caused by inflammation within the urinary system, the site of inflammation generally is in parenchyma rather than in the excretory pathway (Osborne

et al., 1995; Hurley (1998). Leukocytosis may occur in patients with nephritis and bacterial endocarditis (Forrester and Brandt, 1994).

In the present study, there was a significant decrease in the hemoglobin, PCV and TEC values in renal failure cases when compared to that of control animals indicating anemia. These findings are similar to those reported by Robinson *et al.* (1989). Anemia is a feature in chronic renal failure due to either reduced erythropoietin secretion, McEwen (1971), Polzin *et al.* (1995) or decreased RBC life span, uremic inhibitors of erythropoiesis and external blood loss (Osborne *et al.*, 1995). Anemia could also be seen in acute renal failure due to blood loss in haemorrage or concurrent infection like leptospirosis (Forrester and Brandt, 1994; Vaden *et al*., 1998; Shilpa Rani (2003).

5.2.2.1 Erythrocyte indices

Erythrocyte indices in the present study showed decrease in the MCV value and this could be attributed to activity of bone marrow associated with acute haemorrhage or hemolysis (Benjamin, 1985). Decreased MCV in association with a normal MCHC is indicative of decreased size of the erythrocyte in response to the bone marrow stimulation (Coles, 1974). Similar observations were also made by Shivakumar (2001) and Shilpa Rani (2003).

5.2.3 Biochemistry

The serum creatinine and BUN values were employed in the present study for diagnosis of renal failure cases among dogs. It may be pertinent to mention here that serum creatinine are formed in muscle from spontaneous, non-enzymatic degradation of

phosphocreatine in muscle and is freely filtered by the glomerulus and appears in the same concentration in plasma. Creatinine is neither reabsorbed nor secreted by the tubules of the kidneys (Finco *et al.,* 1995) and hence the rise in plasma creatinine levels is a sure indicator of kidney dysfunction and decline of glomerular filtration rate i.e., after the damage had occurred (Finco and Duncan, 1976 and Haller, 2002).

Finco *et al.*(1995), Lefebrve and Toutain (2005) reported that BUN was synthesized in the liver from ammonium bicarbonate and urea excreted exclusively by the kidney. Its values in blood are influenced by protein catabolism, increased protein intake and also urine flow rate. Behrend *et al.* (1996) had taken serum creatinine value of 2.5 mg /dl as azotemia and Gabrisch (1973)) have considered the dogs to have renal failure if they had a serum creatinine concentration 4.5mg/dl.

In the present study the plasma BUN and creatinine values in this group were 19.10 ± 3.63 mg/dl and 8.161 ± 0.70 mg/dl respectively which is much higher than the control group and indicated renal damage. These findings were similar to the observations made by Finco and Duncan, 1976; Evans, (1987); Haller (2002) and Shilpa Rani (2003).

5.2.4 Dark field microscopy and PCR

Dark Field Microscopy is a simple method requiring least facilities, which can be used as preliminary test for direct observation of the leptospires based on their characteristic morphology and motility. In the present study, a total of 40 urine samples from dogs with renal failure were screened for leptospires by DFM. Three samples were found positive. Ellis (1982) reported that this technique was not useful for diagnosis as

there were many cell debris, artifacts that may resemble intact or partially intact leptospires. Bolin *et al.* (1989) reported that DFM was insensitive and require a skilled observer to differentiate leptospires from artifacts. Smith *et.al.* (1994) also opined that DFM requires a skilled microscopist for detection of leptospires but cannot reveal the serovar involved.

It was observed that DFM was not useful in samples with low level contamination, as detection of leptospira will be very difficult. Also, few DFM negative samples were positive by PCR when tested for confirmation. Hence, failure to detect the organism in direct microscopic examination does not rule out the absence of leptospires in samples and diagnosis based only on DFM cannot be taken as confirmatory. These observations should always be followed by further specific confirmatory tests like isolation of organisms and PCR.

A total of the 35 samples were collected from renal failure cases and were subjected to PCR. Among the 11 serum samples examined 3 were positive, of 7 blood samples examined 3 were positive and of 17 urine samples examined 3 were positive for leptospira organism. These observations were in agreement with Brown *et al.,* (1995) who reported higher detection by PCR (62%) than bacteriological culturing (48%) and Venkatesha (1997) reported that PCR detected 80.90% of samples while culturing detected only 64.04% samples.

The probable reason for higher detection by PCR in comparison to the culture could be that PCR detects DNA from both viable and nonviable organisms while culturing has an absolute requirement of viable organisms in the samples. Bolin *et al.*

(1989) reported that culturing was the least sensitive technique for detection of leptospires when compared PCR. The principal value of PCR lies in the ability to obtain a definitive diagnosis during early stage of illness solutions. Treatment may be effective even before antibodies are detectable. In the present study, 7 renal failure cases suspected for leptospirosis were positive by PCR. This indicated that leptospires remain associated with canine renal failure cases and continuously excreted in urine. Similar observations were made were obtained by Brown *et al.*(1995), Birnbaum *et al*.(1998), Kenneth *et al*.((2003) and Venkatesh (1997) who reported higher sensitivity of PCR for detection of leptospires in early infection.

5.3 Pathology of kidney

5.3.1 Gross pathology of kidney

Grossly, the lesions observed in kidney of 40 selected cases of renal failure in dogs included normal size in 6, enlarged in 8, reduced and contracted size in 26. They were congested in 12, pale in 12, and icteric in 7 cases. Pinhead sized necrotic spots were visible in 9 cases where as large areas of necrosis were observed in 5 cases.The capsule was adherent to the parenchyma in 21 cases. They were easily peeled off. The renal cortical surface showed pitted appearance in 21 cases. Single to multiple cysts ranging from 2mm to 8mm were seen in 8 cases. Kidneys were firm in consistency in 21 cases and smooth in 11 cases. Reduced corticomedullary junction was observed in 9 cases. Similar observations have been reported by Murray *et al*., 1971, Wright *et al*., 1976 Slauson and Lewis (1979), Mrudula *et al.* (2005). McKenna and Carpenter (1980) observed enlarged and uniform kidneys containing multiple fusiform or cylindrical cysts

lined by cuboidal epithelium radiating through cortex and medulla. The higher occurrence of reduced and contracted, pale, pitted appearance of kidneys was attributed to diffuse fibrosis and older age of renal failure cases (Vegad and Katiyar, 1998).

5.3.2 Histopathology of kidney

5.3.2.1 Tubulo-interstitial changes

Tubulo-interstitial lesions were noticed 82.5 percent of cases. Tubulo-interstitial lesion comprised of cell swelling ranging from granular to vacuolar degenerative changes, fatty changes, presence of proteinacious material in the tubular lumen, protein droplets in the renal tubular cells, hyaline casts, cystic distension of renal tubule and necrosis and desquamative changes of the tubular epithelium. In addition, occasional tubules lined with regenerating epithelium characterized by highly basophilic cells was also noticed.

In interstitium the changes observed were varying degree of mononuclear cell infiltration in the intertubular space and connective tissue proliferation. Mineralisaton, pigmentation and necrosis of the interstitial tissue were the additional microscopic changes recorded in instances of tubulointerstial nephritis.

Tubulo-interstitial nephritis was noticed 82.5 percent of cases. Tubulointerstitial nephritis has been recorded in canines by Slauson *et al*. (1970), Koeman *et al.* (1987), Spangler *et al*, (1980) and Bowles and Mosier (1992). Shilpa Rani (2003) reported that inflammatory and degenerative changes were more prominent in two renal failure cases studied. Mrudula *et al.* (2005) reported 87.92 per cent occurrence of tubulointerstial

nephritis out of 56 canine nephritis cases studied. Shirota and Fugiwara (1982) noticed focal and diffuse interstitial nephritis with tubular degenerative changes and generalised proliferative glomerulopathy. Birnbaum *et al.* (1998) reported that canine leptospirosis revealed moderate to severe, primarily lymphoplasmacytic and neutrophilic interstitial nephritis. `

A wide range of prevalence of tubule-interstitial nephritis has been reported by many earlier workers in dogs. The reason for such wide variation in the frequency of occurrence of tubulo-interstitial nephritis could probably be due to variation in the age of the subjects studied, prevalence of local disease problems and the different pet practices adopted. This assumption is fortified by the observations in the present study, the majority of the animals belonged to older aged group. Further, a change in local managemental practices may also have contributed for higher occurrence of tubulointerstitial nephritis in the present investigation.

5.3.2.2 Glomerular changes

In the present study, associated glomerular lesions were observed in 37 kidneys (92.5%) out of 40 kidneys screened.

Glomerulonephritis

Glomerulonephritis was featured by focal to diffuse thickening of the capillary basement membrane, reduction in glomerular cellularity, crescent formation, capsular adhesions, congestion, hypercellularity, reduced Bowman's space and cystic glomeruli. Wright *et al*. (1976) observed glomeruloscelerosis, obliteration with thickening of

Bowman's capsule, periglomerular fibrosis, completely atrophied tuft and shrunken glomerulus. Similar observations were made in the present study. In addition, secondary changes in the interstitium and tubules were also recorded. Glomerulonephritis was observed in 7 (17.4 per cent) of 40 renal failure cases. Low prevalence of glomerular lesions has been reported by Bloom (1939), Rouse and Lewis (1975) and Mrudula *et al.* (2005). On the contrary, higher prevalence of glomerular lesions was recorded by Biewenga *et al*., 1982 and Cook and Catell (1985).

In the present investigation, glomerulonephritis has been divided into four major categories based on histological changes observed viz., membranoproliferative glomerulonephritis, membranous glomerulonephritis, mesangialsclerotic glomerulonephritis, membranoproliferative glomerulonephritis with sclerosis. However, glomerulonephritis has been discussed under six categories namely; membranous with and without spikes, membranoproliferative either with mesangial sclerosis or with mesangial proliferation, mesangial proliterativetive and mesangial sclerotic forms by Lewis (1976), Muller-peddinghaus and Trautwein (1977) and Chacko *et al.* (1989).

From the perusal of the earlier reports it could be noticed that the glomerulonephritis has been systematically classified in canines. Koeman *et al*. (1987) reported glomerulonephritis based on the histological changes in the glomerular tuft in proteinuric dogs as mesangioproliferative, membranoproliferative and membraneous glomerulonephritis and amyloidosis. In the present study, an attempt has been made to classify different types of glomerulonephritis based on histological criteria and only 4 types of glomerulonephritis were found. Similar form of glomerular disease have been

reported in the canines by Murray *et al*. (1971), Lewis (1976), Muller-Pedding Trautwein (1977), Chacko *et al.*(1988) and Short *et al. (*1999)

5.4 Liver pathology

In the present study, 40 livers were examined out of which only 31 revealed gross lesions of various types. The gross lesion observed included congestion, hemorrhages, fatty changes and necrotic foci. Histopathological examination revealed various types of hepatic lesions like congestion, haemorrhage, degenerations, fatty changes, necosis, chronic hepatitis, bile duct proliferation and fibrosis. A systematic histopathological examination was necessary to categorize the hepatic lesions in a proper way and to find out the involvement of liver lesions as primary or secondary lesion.

5.4.1 Histopathology

5.4.1.1 Vascular changes

Congestion was recorded in majority of cases. They were mild in 4 cases, moderate in 12 and severe degree in 20 cases. In the present study, the cases of congestion may be regarded as manifestation of early inflammatory or toxic conditions. These findings were in accordance with the lesions observed in the livers due to inflammatory process (Smith and Jones, 1957). In addition to the above, haemosiderin pigments were also noticed in the areas of congestion and this could be either due to toxic or infectious origin.

In the present study, hemorrhages were observed in 6 cases. The haemorrhages were featured by disruption of blood vessel with extravasation of erythrocytes into the

parenchyma and this lesion could be due to infectious agent or toxins produced in uremic state. Viewed in the light of these observation haemorrhages observed in the present study could be attributed toxic agents.

5.4.1.2 Degenerative changes

The cell swelling was charactererized by swollen hepatocytes with either granular basophilic or vacuolated cytoplasm. Mild and moderate degree of cell swelling was characterized by presence of vacuolar degeneration in the stray hepatic lobules and small areas of vacuolar degeneration in many lobules respectively. On other hand, severe change was characterized by presence of uniform vacuolar degeneration of the entire lobe of liver. It was mild in 6, moderate in 16 and severe in 5 cases. Similar observations have been reported by Shastri (1998). Insults such as hypoxia, damage by wide range of toxins and overload by bile pigments may all produce hydropic degeneration and also seen in well nourished animals that recently fasted.

In the present study, fatty changes were observed in 10 livers. It was mild multifocal fatty change in 3 and moderate multifocal in 2 cases. Fatty changes could be resulted from hypoxia, toxaemia, deficiency of cobalt or Vitamin B12 and excessive energy in the diet. Similar observations were made by Jubb and Peter (1989), Shelley *et al.* (2004) and Katherine *et al.* (2006).

5.4.1.3 Necrotic changes

In the present study, hepatic necrosis was observed in 4 cases. The changes appeared confined to focal areas within the hepatic lobule. These foci of necrosis tended to coalesce and become confluent in two cases. Infiltration of of mononuclear cell was

also evident. Periportal fibrosis with collections of mononuclear cells in the portal area was noticed with biliary hyperplasia. They were attributable to toxic and other infectious causes. Similar observations were made by Jubb and Peter (1989) and Shelley *et al.* (2004).

5.4.1.4 Inflammatory changes

In the present study chronic hepatitis was noticed in 7 cases. Histologically, cirrhosis was characterized by proliferation of fibrous connective tissue around the portal area and the bile duct with infiltration of inflammatory cells. Multifocal nodular aggregation of mononuclears was noticed in 2 cases. Further, fibrous connective tissue was seen extending surrounding parenchyma causing pressure atropic changes in the adjacent hepatic cords. The changes in the chronic hepatitis might be due to toxic substances acting for longer duration. An analysis of the findings of present study and observation of earlier workers; Smith and Jones (1957), Shastri (1998) and Vegad and Katiyar (1998) suggested the influence of toxic and/or infectious causes.

5.4.1.5 Hyperplastic changes

Bile duct hyperplasia was noticed in 3 cases in the present study. Fibrosed bile duct was noticed in 3 cases and microscopically it was featured by marked increase in the the collagenous tissue around the bile ducts. It is a characteristic reaction of the liver to particular type of insult and an attempt to regenerate parenchyma when the parenchymal cells themselves have lost this capacity. From the above observations, it could be concluded that lesions of bile duct proliferation and fibrosis were the feature of toxicosis. Similar observations were made by Jubb and Kennedy (1989) and Shelley *et al.* (2004).

5.4.1.6 Liver pathology in leptospirosis

Grossly, liver appeared normal to enlarged in size, mottled with varying degrees of haemorrhages. Histopathologically, the liver revealed mild to moderate degree of congestion and haemorrhage, vacuolar degeneration of hepatocytes, multifocal aggregation of mononuclear cells, periportal infiltration of inflammatory cells and haemosiderin pigments. This was observed in two cases. In the remaining cases, hepatic lesions ranged from those of chronic hepatitis to mild to moderate vacuolar degeneration of hepatocytes and occasional scattered lymphocytic aggregates. Similar observations were made by Ramirez *et al.,* (1978), Adamus *et al.* (1997) and Richard (2010). It was opined that liver showed characteristic microscopic changes in acute phase of the illness and no lesions are found in liver in chronic renal failure patients. This could be due to localisation of leptospira in kidney and continuous excretion through urine.

Conclusion

In the present study, occurrence of renal failure was found to be more common among aged dogs. German shepherd (19.23 percent) and Pomeranian (17.69 percent) breeds of dog. Leukocytosis and anemia were a consistent feature in all the cases. BUN and serum creatinine were consistently high in all the cases studied. Leptospirosis was associated with renal failure in 7 cases out of 40 cases studied. Tubulo interstitial lesion was the most common type of lesion seen in the renal failure cases.

SUMMARY

VI SUMMARY

The dogs suspected for renal failure that attended the Veterinary College Hospital, Hebbal from January 2009 to December 2009 were considered for epidemiological study. A total of 130 dogs with renal failure were selected and epidemiological details pertaining to age, sex and breed were collected. The mean age of affected animals was found to be 8.45 years. Sixty one percent were male and 39 percent were females. German shepherd (19.23 percent) and Pomeranian (17.69 percent) showed a higher incidence of renal failure.

A total of 40 renal failure dog were selected from the cases presented to the Veterinary College Hospital, with one or more signs of renal failure and were subjected to estimation of serum creatinine and BUN values Such of those dogs with serum creatinine value more than 4 mg/dL were diagnosed as renal failure were included in the study and subjected to clinical and histopathological study.

Clinical signs observed in dogs with renal failure in the present study were loss of appetite in 35 dogs (87.5 per cent), vomition in 29 (72.50 per cent), diarrhoea in 12(30 per cent), lethargy14 (35 per cent), pale conjunctival mucus membrane in 15(37.5 per cent), oliguria in 5 (12.5 per cent), polyuria in 11(27.5 per cent), polydypsia in 10 (25 per cent) and oral ulcers in 4 (25 per cent) cases.

Majority of the animals were anaemic. There was a significant decrease in the packed cell volume, hemoglobin and total erythrocyte count in cases of renal failure compared to apparently healthy animals. Erythrocyte indices showed no significant

difference between the two groups revealing normocytic to hypochromic anaemia. There was significant increase in the total leucocyte count of renal failure cases compared to apparently healthy animals.

There was highly significant increase in the values of BUN and plasma creatinine in renal failure cases.

Grossly, majority of the kidneys were of small in size, firm in consistency, pale in color, shrunken and pitted with granular appearance. Capsule was adherent in many cases.

Histologically, Tubulo-interstitial disease was noticed 82.5 percent of cases. Tubulo-interstitial lesion was featured by cell swelling ranging from granular to vacuolar degenerative changes, fatty changes, protein droplets in the renal tubular cells, hyaline casts, cystic distension of renal tubules, necrosis and desquamative changes of the tubular epithelium. In addition, tubules occasionally lined with regenerating epithelium characterized by highly basophilic cells was also noticed. In interstitium, the changes observed were varying degree of mononuclear cell infiltration in the inter-tubular space and connective tissue proliferation. Mineralization, pigmentation and necrosis of the interstitial tissue were the additional microscopic changes recorded in some cases of tubulo-interstitial nephritis. Tubular lesions were observed in 38 out of 40 cases examined. Interstitial lesions were observed in 33 out of 40 cases examined.

Glomerulonephritis was noticed in 17.5 percent of cases and associated lesions were observed 33 out of 40 kidneys and glomerulonephritis was featured by focal to

diffuse thickening of the capillary basement membrane, reduction in glomerular cellularity, crescent formation, capsular adhesions, congestion, hypercellularity, reduced Bowman's Space, cystic glomeruli and protein droplets in Bowman’s space.

The gross examination of liver in the present study revealed changes like congestion, hemorrhages, fatty changes and necrotic foci while histopathological examination revealed congestion, hemorrhages, degeneration, fatty change, chronic hepatitis, bile duct proliferation and fibrosis.

Leptospirosis was identified in 7 renal failure cases using PCR and silver staining technique.

BIBLIOGRAPHY

VII BIBLIOGRAPHY

ANDERSON, R.S. and EDNEY, A.T., 1969. Protein intake and blood urea in the dog. *Vet. Rec*., **84**: 348-349

ADAMUS, C., BUGGIN, D. M., IZEMBART, A., PIERRE, S.C., GUIGAND, L., MASSON, M.T., ANDERFONTAINE, G. and WYERS, M. 1997. Chronic hepatitis associated with leptospiral infection in vaccinated beagles. *J. Comp. Pathol.,* **117**: 311-328

BEHREND, E.N., GRAUER, G.F., MANI, I., GROMAN, R.P., SALMAN, M.D. and GRECO, D.S., 1996. Hospital acquired acute renal failure in dogs: 29 cases (1983-1992). *J. Am. Vet. Med. Assoc*., **208**: 537-541

BANCROFT, J.D. and STEVENS, A., 1996. Theory and Practice of Histological Techniques. 4th *Edn*. Churchill Livingstone, London

BENJAMIN, M.M., 1985. Outline of Veterinary Clinical Pathology. 3rd *Edn***.** Kalyani Publishers, New Delhi, *pp.* 175-212

BIEWENGA, W.J., GRUYS, E. and HENDRIKS, H. J., 1982. Urinary protein loss in the dog. Nephrological study of 29 dogs without signs of renal disease. *Res. Vet. Sci*., **33**: 366-374

BIRNBAUM, N., BARR, S.C., CENTER, S.A., SCHERMERHORN, T., RANDOLPH, J.F., and SIMPSON, K.W., 1998. Naturally acquired leptospirosis in 36 dogs. Serological and clinicopathological features. *J. Small. Anim. Pract*., **35**: 231-236

BLOOM, F., 1939. Classification and pathology of renal disease in the dog. Comparison with nephritis in man. *Archs. Path.,* **28**: 236 - 254

BOLIN, C. A., ZUERNER, R.L. and TRUEBA, G., 1989. Comparision of three techniques to detect *L. Interrogans* serovar hardajo type, hardjobovis in bovine urine. *Am. J. Vet. Res.*, **50**: 1001-1003

BONNET, B., EGENVALL, A., OLSON, P. and HEDNAMMAR, A., 1997. Mortality in insured sweedish dogs: Rates and causes of death in various breeds. *Vet. Rec*., **141**: 40-44

BOWLES, M.H. and MOSIER, D.A., 1992. Renal amyloidosis in a family of beagles. *J. Am. Vet. Med. Assoc*., **201**: 569-574.

BROWN, P.D., GRAVEKAMP, C., CARRINGTON, D.G., VAN DE KAMP, H., HARTSKEERL, R.A., EDWARDS, C.N., REVERARD, C.O., TERPSTRA, W. J. and LEVETT, P.N., 1995. Evaluation of polymerase reaction for early diagnosis of leptospirosis. *J. Med. Microbiol*., **43**: 110-114

BROWN, S.A. 1995. Primary Diseases of glomeruli. In: Canine and Feline nephrology and Urology. OSBORNE, C. A. and FINCO, D. R. (*edn*) Williams and Wilkins, Philadelphia. *pp*. 368-385

CENTER, S. A., WILLKINSON, E., SMITH, C.A., 1987. 24 hour urine protein/creatinine ratio in dogs with protein losing nephropathy.*J.Am.Vet.Med. Assoc*., **187**: 820-824.

CHACKO, W.G., 1988. Morphological pathology of canine renal disorders with special reference to glomerular disease. Thesis submitted to University of Agricultural Sciences, Bangalore

COLES, E.H., 1974. Kidney function. In: Veterinary clinical pathology. W.B. Saunders Company. *pp*. 228

COOK H.T. and CATELL, V., 1985. Perspective survey of canine chronic renal disease and prevalence and type of canine glomerulonephritis. *J. Pathol.*, **146**: 274. Abstract

COOK, S.M., DEAN, D.F., GOLDEN, D.L., WILKINSON, J.E. and MEANS T.L., 1993. Renal failure attributable to atropic glomerulopathy in four related Rottweilers. *J. Am. Vet. Med. Assoc*., **202:** 107-109

COOK A.K. and COWGILL, L.D., 1996. Clinical and pathological features of protein losing glomerular disease in the dog: A review of 137 cases (1985-1992). *J. Am. Anim. Hosp. Assoc*., **32**: 313-322

COWGILL, L.D. 1983. Diseases of the kidneys. In: Text Book of Veterinary Internal Medicine. ETTINGER, S.J. (Ed). W.B. Saunders company, Philadelphia. *pp*. 1793-1879

COWGILL, L.D. 1992. Pathophysiology and management of anaemia in chronic renal progressive renal failure. *Sem. Vet. Med. Sur. (small Animal)*. **7**: 175-182

DAVID, J.P., CARL, A.O., JOSEPH, W.B., KATHERINE, M.J. and JULIE, A.C., 1995. Chronic renal failure. In: Small Animal internal medicine. Ed. STEPHAN, J. E. and FELDMAN, E. C., Vol II. W. B. SAUNDERS, Philadelphia., *pp*. 1734-1759

DEFRANCESCO, T.C., 2002. Advanced discussions in the diagnosis of heart failure. www.waltham.com

DE SCHEPPER, J. COCK, I. D. and CAPIAU, E. 1989. Urinary gamma glutamyl transferase and the degree of renal dysfunction in 75 bitches with pyometra. *Res. Vet. Sci.*, **46**: 396-400

DIBARTOLA, S.P., TARR, M.G., PARKER, A.T., POWER, J.D., PUTZ, J.A., 1989. Clinicopathological findings in dogs with renal amyloidosis: 59 cases (1976-1986). *J. Am. Vet. Med. Assoc*., 358-364

DI BARTOLA., 2000, In : *small animal internal medicine*. ED ETTINGER, S. J and FELDMAN, E C., Vol II. W. B. SAUNDERS Co., Philadelphia

DOHERTY, P.C., 1966. Comparision of direct microscopic examination and guineapig inoculation techniques for demonstrating leptospires in bovine urine. *Aus. Vet. J*., **42**: 466-467

DRU, F.S. and CATHARINA, S.B., 1994. The diagnostic approach to the patient with acute renal failure. Symposium on acute renal failure. *Vet. Med*. 212-218

DUGALD, F. M., TERENCE, C., ADRIAN, P. S., and VICTORIA, C., 1986. Canine chronic renal disease, prevalence and types of glomerulonephritis in the dog. *Kid. Int*., **29:** 1144-151

ELLIS, W. A., O'BREIN, J. J., NEIL, S. D. FERGUSION, H.W. and HANNA, J., 1982. Bovine leptospirosis: Microbiological and serological findings in aborted foetuses. *Vet. Rec.,* **110**: 147-150

ENGLISH, P. B., FILIPPICH, L. J. and THOMPSON, H. L., 1980. Clinical assessment of renal function in the dog with a reduction in nephron number. *Aust.Vet. J.,* **56:** 305-312

EVANS, G. O., 1987. Post prandial changes in canine plasma creatinine. *J. Smal. Anim. Pract.,* **28:** 311-315

FAINE, S., 1982. Guidelines for the control of leptospirosis. Edr., Dept, of Microbiology, Monash University, Melbourne, Australia

FINCO, D. R. and DUNCAN, R. J., 1976. Evaluation of blood urea nitrogen and serum creatinine concentrations as indicators of renal dysfunction. A study of 111 cases and a review of related literature. *J Am Vet Med Assoc*., **168:** 593-601

FINCO, D.R. BROWN, S.A., VADEN, S.L. and FERGUSON. 1995. Relationship between plasma creatinine concentraton and glomerular filtration rate in dog. *J. Vet. Pharmacol. Therap*., **18**: 418-421

FORRESTER, D.S. and BRANDT, K.S. 1994. The diagnostic approach to the patient with acute renal failure. *Vet. Med., pp.* 212-218

GARBRISCH, K. 1973. Serum urea and serum creatinine as indicator of kidney function in the dog. *Vet. Bull.,* **18** (5):133-135. Abst. No.5843

GARNER, H.E., THERMON, J.C. and ROMACK, F.E. 1968. Biopsy: surgical method for obtaining large samples of liver, kidney and muscles from cattle. *Am. J. Vet. Res.*, **29:** 2407-2409

GRAUER, G.F. 2005. Early detection of renal damage and disease in dogs and cats. *Vet. Clin. North. Am. Small. Anim. Pract*., **35**:581-96

GREENLEE, J.J., BOLIN, C.A., CHEVILLE, N.F. and ANDREASEN, C.B., 2004. Clinical and pathologic comparison of acute leptospirosis in dogs caused by two strains of Leptospira kirschneri serovar grippotyphosa. *Am. J. Vet. Res*., **65**: 1100-1107

GREGORY, F.G. and INDIA, F.L., 1995. Acute renal failure in: small animal internal medicine. Ed. Stephan, J.E. and Feldman, E.C., Vol-II. W.B. Saunders. Co., Philadelphia. *pp* 1720-1760

HALLER, M. 2002. Assessment of renal function in cats and dogs. Waltham focus, **12:** (2):10-14

HOLT, P.E., LUCKE, V.M. and PEARSON. H., 1987. Idiopathic renal haemorrhage in the dog. *J. Am. Anim. Pract.,* **28**: 253-263

HOOD, J.C., ROBINSON, W.F., HAXLABLE, C.R., BRADLEY, J.S., SNLHERLAND, R.J. and THOMAS, M.A.B.1990. Hereditary nephritis in the bullterrier; evidence for inheritance by an autosomal dominant gene., *Vet. Rec*., **126**:456-459

HOOD, J.C., HUXTABLE, C., NAITO, I., SMITH, C., SINCLAIR, R. and SAVIGE, J.A., 2002. Novel model of autosomal dominant Alport syndrome in Dalmatian dogs. *Nephrol. Dial. Transplant* ., **17**:2094-2098

HURLEY, K. 1998. Acute renal failure. *Waltham Focus* Special edition on urinary tract.

JERAJ, K., OSBORNE, C.A. and STEVENS, J.B., 1982. Evaluation of renal biopsy in 197 dogs and cats. *J. Am. Vet. Med. Assoc*., **181**(4)**:** 367-369

JACOBSON, F.K., CHRISTENSEN, C.K. and MOGENSEN. C.E., 1979. Marked postprandial increase in serum concentration after meals containing cooked meat. *Dan. Med. Bull.,* **26:** 43

JAENKE, R.S. and ALLEN. T.A., 1986. Membranous nephropathy in the dog. *Vet. Pathol.,* **23**: 718-733

JERAJ, K.P., VERNIER, D., POLZIN, J.K., KLAUSNER, C.A., OSBORNE, J.B., STEVENS and A.F. MICHAEL., 1984. Idiopathic immune complex glomerulonephritis in dogs with multisystem involvement. *Am. J. Vet. Res*., **45:** 1699-1705

JOHNSON, S.E., 2000. Chronic hepatic disorders. In: ETTINGER, S.J. AND FELDMAN, E.C. (EDS). Textbook of Veterinary internal medicine. *Edn*. 5th W.B. Saunders Company, Philadelphia, *pp*. 1299-1325

JUBB, K.V.F., PETER, C.K., 1989. Pathology of domestic animals. Vol II *Edn* 4th. Academic press, *pp* 447-510

KATHERINE, A.S., JOANNE, R.S., SHELLEY, J.N., LESLIE, B.N., CLAUDIA, A.K., 2006. Aflatoxicosis in dogs and dealing with suspected contaminated commercial foods. *JAVMA*, **228** (11): 1686-1691

KENNETH, R. H., YVETTE, M. R. and JENNIFER, T. S., 2003. Clinical application of a Polymerase chain reaction assay for diagnosis of leptospirosis in dogs. *J. Am. Vet. Assoc*, **222 (**9): 1224-1229

KOEMAN, J.P., BEIWENGA, W. J. And GRUYS, E., 1987. Proteinuria in dogs: a pathomorphological study of 52 proteinuric dogs. *Res. Vet. Sci.,* **43:** 376-378

KOPPLE, J.D., and COBURN. J.W., 1974. Evaluation of chronic uraemia. Importance of serum urea nitrogen, serum creatinine and their ratio. *J. Am. Med. Assoc*., 227: 41-44. Cited by Finco and Duncan, 1976

KROHN, K., MERO, M., OKSANEN, A. and SANDHOLM, M., (1971). Immunologic observations in canine interstitial nephritis. *Anim. J. Path.,* **65**: 157-172.

KRAJE, A.C., 2002. Helping patients that have acute renal failure. *Vet. Med.,* **96:** 461-474

LANGAHAM, R.F. and HALLMAN, E.T., 1941. The incidence of glomerulonephritis in domesticated animals. *J. Am. Vet. Med. Assoc.,* **98**: 471-475

LEES, G. E., HELMAN, R.G., MILICAMP, N.J., HUNTER, J. F. and FREY, M. S., 1998. Early diagnosis of familial nephropathy in English Cocker Spaniels. *J. Am. Anim. Hosp. Assoc.* **34**: 189-195

LEFEBVRE, H.P. and TOUTAIN. P.L., 2004. Angiotensin converting enzyme inhibitors in the therapy of renal diseases. *Ind. J. Vet. Pharmacol. Thera.* **27**: 265-281

LEFEBVRE, H.P. and TOUTAIN, P. L. 2005. Angiotensin converting enzyme inhibitors in the therapy of renal diseases. *Ind. J. Vet. Pharmaco.* and *Thera*. **25:** 265-281

LEWIS, R.J., 1976. Canine glomerulonephritis. Results from microscopic evaluation of fifty cases. *Can. Vet. J.,* **17:** 171-176

LUNA, G., 1968. Manual of histologic Staining Methods of the Armed Forces Institute of Pathology, 3rd *Edn*. McGraw-Hill Book Company, New York

McCAW, D.L., FLEMING, E.J. and MIKICUIK, M.G., 1989. Selecting the right diagnostic test for renal disease. *Vet. Med*., **84:** 266-272

MACDOUGALL, D. F., COOK, T., STEWARD, A. P., CATTELL, V., 1986. Canine chronic renal disease: Prevalence and types of glomerulonephritis in the dog. *Kid. Int*.. **29**: 1144-1151

McEWAN, E.D., 1971. The clinical diagnosis of renal disease in the dog. *J. Small. Anim. Pract*., **12:** 543-553

MCKENNA, S.C. and CARPENTER, J.L., 1980. Polycystic disease of the kidney and liver in the Cairn terrier. *Vet. Pathol*., **17**: 436-442

MERIEN, F., BARANTON, G.A. and PEROLAT, P., 1995. Comparision of Polymerase chain reaction with microagglutianation test and culture for diagnosis of leptospirosis. *J. Infect. Dis*., **172**: 281-285

MEYER, H.P., 2004. The treatment of chronic renal failure in the dog and cat. In: *WSAVA., FECAVA., HVMS World Congress, Sci. Proc., pp* 522- 528

MINKUS, G., BREUER, W., WANKE, R., REUSCH, C., LEUTERER, G., BREM, G. and HERMANS, W., 1994. Familial nephropathy in Bernease Mountain dogs. *Vet. Pathol*., **31:** 421-428

MRUDULA, V., TITUS GEORGE, V., BALACHANDRAN, C. and MURALI MANOHAR, 2005. Haematological, urinalysis and urinary enzyme alterations in canine nephritis. *Ind. Vet. J.*, **82:** 826-829

MRUDULA, V., TITUS GEORGE, V., BALACHANDRAN, C. and MURALI MANOHAR., 2005. Bacteriological and histopathological study of canine nephritis on clinical samples. *J. Anim. Vet. Adv.*, **4** (12): 954-958

MULLER-PEDDINGHAUS, R. and TRAUTWEIN, G., 1977. Spontaneous glomerulonephritis in dogs. II. Correlation of glomerulonephritis with age, chronic interstitial nephritis and extra renal lesions. *Vet. Pathol*., **14**: 121-127

MURRAY, M., PIRIE, M., THOMPSON, H. and JARROT, W. F. H., 1971. Glomerulonephritis in dog. A histological and electron microscopical study. *Res. Vet. Sci.*, **12**: 493-495

NEEL, J.A. and GRINDEM, C.B., 2000. Understanding and evaluating renal function. *Vet. Med*., **95**:555-565

OSBORNE, C.A., LOW, D.G. and FINCO, D.R., 1972. Canine and Feline Urology. W.B. Saunders Company, Philadelphia

OSBORNE, C. A. and R. L. VERNIER. 1973. Glomerulonephritisin the dog and cat. A comparative review. *J. Am. Anim. Hosp. Assoc.*, **9:** 101-127

OSBORNE, C.A. and FINCO, D.R., 1995. *Canine and Feline Nephrol. and Urol*., Williams and Wilpins, Baltimore

PICUT, G.A. and LEWIS. R.M., 1987. Comparative Pathology of Canine Hereditary Neuropathies: An Interpretive Review. *Vet. Res. Comm.* **11**: 561-581

POLZIN, D.J., OSBORNE, C.A., BARTGES, J.W., JAMES, K.M. and CHURCHILL, J.A., 1995. Chronic renal failure in: Small animal internal medicine. Edn. STEPHEN, J.E. and FELDMAN, E.C., Vol-II. W.B.Saunders. Co., Philadelphia. *pp*. 1734-1759

POLZIN, D.J., OSBORNE, C.A., JACOB, F. and ROSS, S., 2000. Chronic renal failure. In: Text book of Veterinary Internal medicine - Disease of dog and cat ETTINGER, S.J. and E.C.FELDMAN (EDS). Vol II. W. B. Saunders Company, Philadelhia,, *pp*: 1634-1662

RAMIREZ, G., JAMES, B. S., EDWARD, T. Z. and FARHAD, M., 1978. Acute interstitial nephritis associated with chronic active hepatitis. *South. Med. J.* **76**: 391-394

RAWDON, T.G., 2001. Juvenile nephropathy in a Samoyed bitch. *J. Small. Animal. Pract.,* **42**: 235-238

RHA, J., M.N. LABATO, L.A.ROSS, E. BREITSCHWERDT and J. ALROY. 2000. Familial glomerulopathy in a litter of Beagles. *J. Am. Vet. Med. Assoc.,* **216**: 46-50

ROUSE, B.T and R.J. LEWIS. 1975. Canine glomerulonephritis: Prevalence in dogs submitted at random for euthanasia *Can. J. Comp. Med.,* **39**: 365-370

RICHARD, E. GOLDSTEIN, 2010. Canine Leptospirosis and Its Challenges. Uronephrology.WASAVA *congress*

ROBINSON, M. and GOPINATH, C., 1974. A comparative histochemical study of some oxidative and hydrolytic enzymes in the kidneys of domestic food animals, laboratory animals and fowl. *Res. Vet. Sci*., **16**: 355-369

ROBINSON, W.F., SHAW., S.E., STANLEY, B., HUXTABLE, C.R., WATSON, A.D., FRIEND, S.E. and MITTEN, R., 1989. Chronic renal disease in bull terriers. *Vet Pathol*., **45**: 467-474

RUBIN, S.I., 1997, Chronic renal failure and its management and nephrolithiasis. *Vet. Clin. North. Am*., **27: (6)** 1331-1334

SASTRY, G.A. 1998 Veterinary Pathology 6th *Edn*. CBS publishers and distributors, Delhi

SAMBROOK, J., FRITSCH, E.F. and MANIATIS, T., 1989. Molecular cloning: A laboratory manual *Edn* 2nd N.Y., cold spring harbor laboratory press.

SCHALM, O.W., JAIN, N.C. and CAROLL, E.J. 1975, Veterinary hematology *Edn* 3rd. Lea and Febiger. Philadelhia

SCOTT, A.B., JEANNE, A.B. and WAYNE, A.C., 1985. Gentamicin associated acute renal failure in the dog. *J. Am. Vet. Med. Assoc*. **186**: 686-690

SHELLEY, J.N., JOANNE, R., SMITH, K.A., STENSKE, L.B., JOHN, R., DUNLAP, P.M. and CLAUDIA A.K., 2004. Aflatoxicosis in nine dogs after exposure to contaminated commercial dog food

SHORT, R.P., LOBETTI, R.G., NESBIT, J.W., 1999. Renal pathology in working dogs in the South African National Defence Force. *J. S. Afr. Vet. Assoc.,* **70:**158-60

SHILPA RANI., 2003. In: Studies on urinary enzymes in renal failure in dogs, *M.V.Sc*. Thesis submitted to university of Agricultural sciences, Bangalore

SHIROTA, K., TAKAHASHI, R. and FUJIWARA, K., 1982. Canine interstitial nephritis with special reference to glomerular lesions and filariasis. *J. Vet. Sci.,* **41**: 119-129

SHIVAKUMAR, M., 2001. Identification and management of acute renal failure in dogs, *M.V.Sc*. Thesis submitted to university of Agricultural sciences, Bangalore

SLAUSON, D.O., GRIBBLE, D.H. and RUSSEL, S.W., 1970. Clinicopathological study of renal amyloidosis in dogs. *J.Comp. Pathol*., **80**: 335-343

SLAUSON, D.O. AND LEWIS, R.M., 1979. Comparative Pathology of glomerulonephritis in animals. *Vet. Pathol*., **16**: 135-164

SMITH, C.R., KETTERER, M.R. and CORNEY, B.G., 1994. A review of laboratory techniques and their use in diagnosis of L. Interrogans serovar hardjo infection in cattle. *Aus. Vet. J.*, **71**: 290-294

SMITH, H.A. and JONES, T.C., 1957. Text book of veterinary pathology *Edn* 1st. Lea and Febiger philadelpgia

SPANGLER, W.L., ADLEMEN, R.D., CONZELMEN, G.M. and ISHIZAKI, G., 1980. Gentamicin nephrotoxicity in the dog: Sequential light and electron microscopy. *Vet. Pathol.,* **17**: 206-217

SPENCER, A.J. and WRIGHT, N.G., 1981. Glomerular lesions in chronic interstitial nephritis in the dog: Histological and ultra structural features. *J. Comp*. *Pathol*., **91**: 393-408

STOCKHAM, S. L. and SCOTT, M.A., 2002. Fundamentals of Veterinary Clinical Pathology. Iowa State Press, London, *pp* 279-336

STONE, E.A., LITTMAN, M.P., ROBERRSON, J.L. and BOVEE, K.C., 1988. Renal dysfunction in dogs with pyometra. *J. Am. Vet. Med. Assoc*., **193**: 457-464

VADEN, S.L., GOOKIN, J., TROGDON, M., LEVINE, C.E., and COWGILL, L.D., 1998. Use of carbamylated hemoglobin concentration to differentiate acute from chronic renal failure in dogs. *Am. J. Vet. Res*., **58** (11): 1193-1196

VADEN, S.L., 2000. Differentiation of acute from chronic renal failure. In: Kirks current veterinary therapy XII. Small animal practice. Ed. BONAGURA, J.D. and ROBERT, W.K. W.B. Saunders. Co. Philadelphia. *pp* 856-858

VEGAD, J.L. and KATIYAR, A.K., 1998. Veterinary systemic pathology, *Edn*. 1st International book distributors, Lucknow

VENKATESH, M.D., 1997. Molecular characterization of Leptospiral serovars. Ph.D. Thesis, Tamil Nadu Veterinary and Animal Sciences University, Chennai

VILAFRANCA, M. and FERRER, L. 1994. Juvenile nephropathy in Alaskan Malamute Litermates. *Vet. Pathol*., **31**: 375-377

VISHWANATHAN, S., 1988. Studies on canine nephropathy. Ph. D. Thesis Submitted to Tamil Nadu Agricultural University, Coimbatore

VOLHARD, F., 1936. Nephrosis 3rd International congress of comparative pathologists. 325-394

WATSON, D.J., CHURCH, D.B. and FAIRURN, A.J., 1981. Postprandial changes in plasma urea and creatinine concentration in dogs. *Am. J. Vet. Res*., **42:** 1878-1880

WRIGHT, N.G., FISHER, E.W., MORRISON, W.I., THOMSON, W.B. and NASH, A.S., 1976. Chronic renal failure in dogs: A comparative clinical and morphological study of chronic glomerulonephritis and chronic interstitial nephritis. *Vet. Rec.*, **98**: 288- 293

WRIGHT, N.G. and NASH, A.S., 1983. Glomerulonephritis in dog and cat. *Irish. Vet. J*., **37**: 4-8

ZAMORA, J., REIDEMAN, S., CABEZA, X. and VEGA, S., 1995. Comparision of four microscopic techniques for the DNA of leptospirosis in wild rodents in rural area of Valdivia, Revista Latine Americana de Microbiologica., **37**: 267-272

ABSTRACT

VIII ABSTRACT

The dogs presented to Veterinary College Hospital Hebbal and animal shelter houses in Bangalore suspected to have renal failure based on the history and clinical signs were considered for the present study. Epidemiological details were collected from 130 renal failure cases during the year 2009. Clinical signs, biochemical alterations, gross and histopathological lesions were recorded in 40 cases. The involvement of leptospira was identified by dark field microscopy, PCR and silver staining.

The mean age of dogs with renal failure was 8.45 years. The salient clinical signs observed included loss of appetite, vomition, diarrhoea, lethargy, polyuria, polydipsia and decrease in haemoglobin, PCV, TEC and increase in TLC, Creatinine and BUN values in dogs with renal failures as compared to healthy animals.

Grossly, majority of kidneys were of small in size, firm in consistency, pale in colour, shrunken and pitted with granular appearance. Liver revealed changes like congestion, haemorrhages, fatty changes and necrotic foci.

Histopathologically, tubulo-interstitial nephritis was recorded in 33 cases and it was featured by granular to vacuolar degeneration, hyaline casts, and protein droplets in the renal tubules, necrosis and desquamative changes of tubular epithelium and varying degrees of mononuclear cell infiltration and connective tissue proliferation.

Glomerulonephritis was recorded in 7 cases and it was featured by thickening of capillary basement membrane, hypercellularity, mesangial sclerosis and cystic glomeruli. Liver revealed congestion, vacuolar degeneration in majority of the cases. Leptospirosis was identified in 7 renal failure cases using PCR and silver staining.

Plate 1. Photograph of kidney from a dog with renal failure to show slightly enlarged and congested appearance

Plate 2. Photograph of kidney from a dog with renal failure to show granular cortical surface and pitted appearance

Plate 3. Photograph of kidney from a dog with renal failure to show pale and pitted cortex with adherent capsule and shrunken size

Plate 4. Photograph of kidney from a dog with renal failure to show multiple necrotic foci and small cysts distributed throughout the kidney

Plate 5. Photograph of kidney from a dog with renal failure (leptospirosis) to show pale to Icteric, enlarged kidney with variable sized necrotic foci

Plate 6. Photograph of kidney from a dog with renal failure to show unilateral cyst containing watery like fluid extending upto medulla

PLATE 1

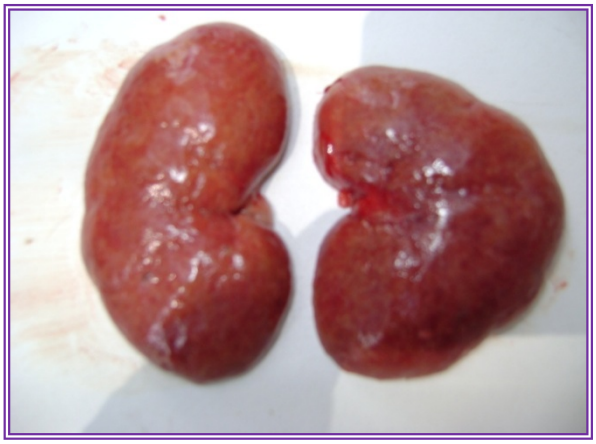


PLATE 2

PLATE 3

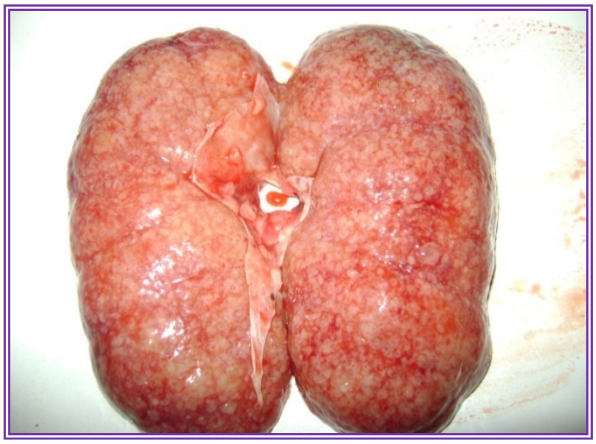


PLATE 4

PLATE 5

PLATE 6

Plate 7. Photograph of kidney from a dog with renal failure to show granular cortical surface and pitted and congested appearance

Plate 8. Photograph of kidney from a dog with renal failure to show reduced cortical area with mild congestion

Plate 9. Photomicrograph of kidney from a dog with renal failure to show severely congested vessel in the medullary region

H & E X 100

Plate 10. Photomicrograph of kidney from a dog with renal failure to show severe congestion and haemorrhage involving tubules and glomeruli with mild infiltration of inflammatory cells in interstitium

H & E X 100

Plate 11. Photomicrograph of kidney from a dog with renal failure to show enlarged and congested glomeruli, inter tubular haemorrhage with mild degree of connective tissue proliferation

H & E X 200

Plate 12. Photomicrograph of kidney from a dog with renal failure to show congestion, haemorrhage, degeneration & necrotic changes in the tubule & glomeruli

H & E X 200

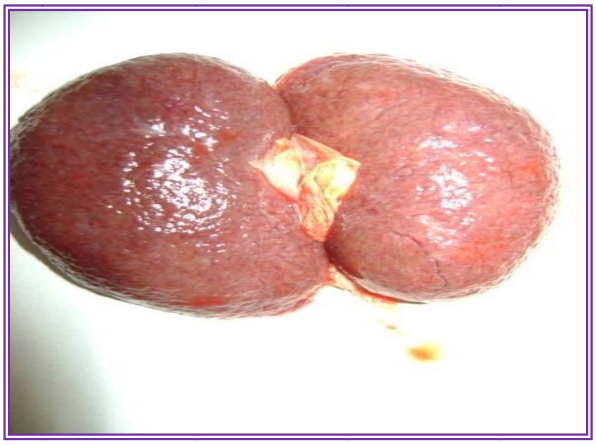


PLATE 7

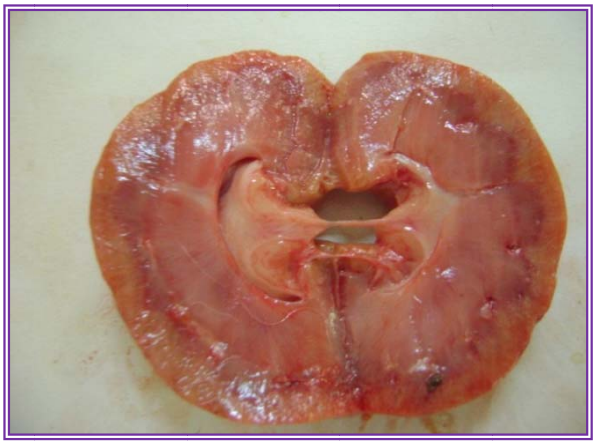


PLATE 8

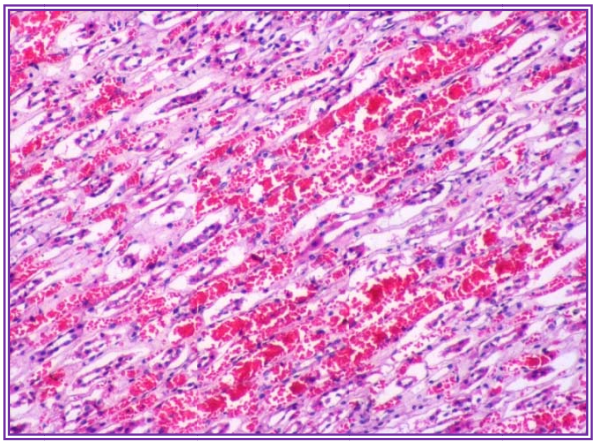


PLATE 9

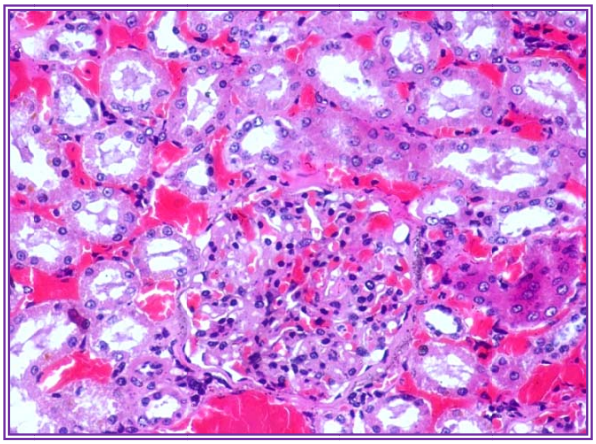


PLATE 10

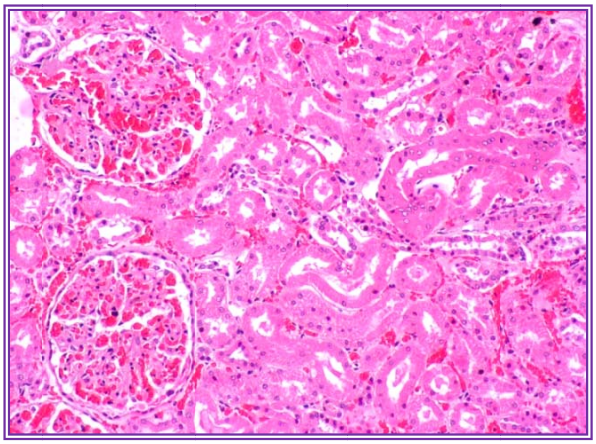


PLATE 11

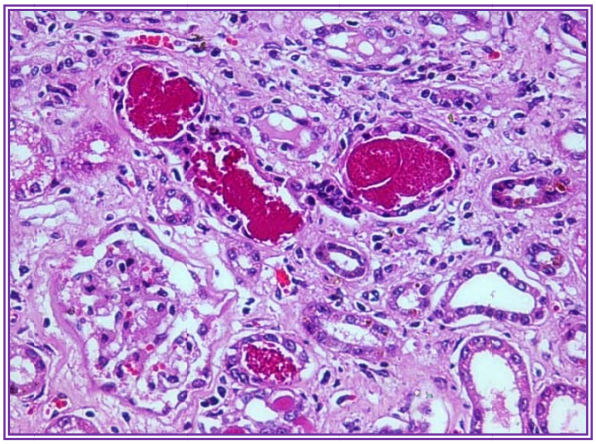


PLATE 12

Plate 13. Photomicrograph of kidney from a dog with renal failure to show congestion of intertubular vessels and glomeruli, granular to vacuolar degeneration and protein droplets inside the tubules

H & E X 100

Plate 14. Photomicrograph of kidney from a dog with renal failure to show granular to vacuolar degeneration in the tubules with eosinophilic homogeneous material in the tubular lumen

H & E X 200

Plate 15. Photomicrograph of kidney from a dog with renal failure to show congestion, tubular degeneration, tubular necrosis and fatty change

H & E X 100

Plate 16. Photomicrograph of kidney from a dog with renal failure to show coagulative type of necrosis.

H & E X 200

Plate 17. Photomicrograph of kidney from a dog with renal failure to show glomerular hypermia and coagulative type of necrosis of tubules.

H & E X 100

Plate 18. Photomicrograph of kidney from a dog with renal failure to show thickening of Bowman's basement membrane and presence of protein droplets in the Bowman's space; also note tubular degeneration, desquamation & presence of hyaline material in the lumen

H & E X 200

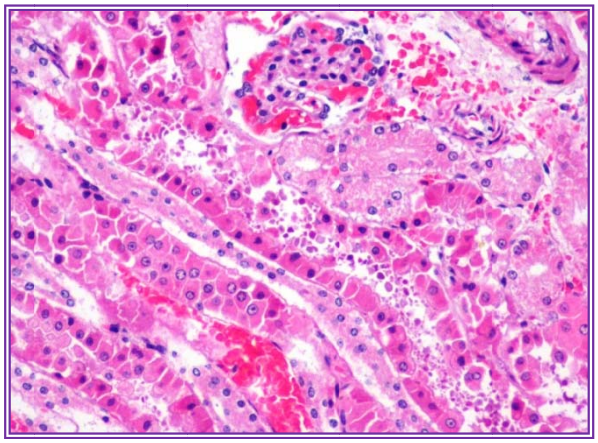


PLATE 13

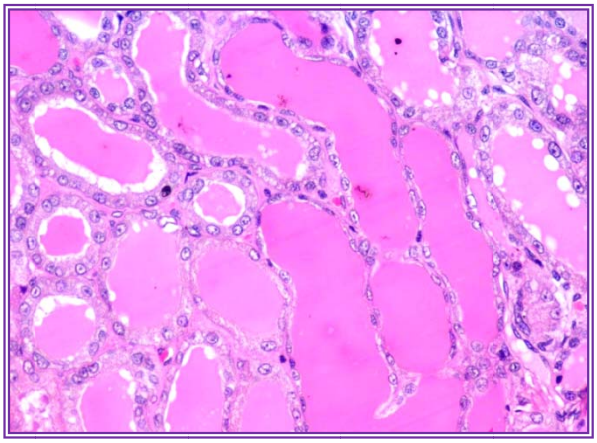


PLATE 14

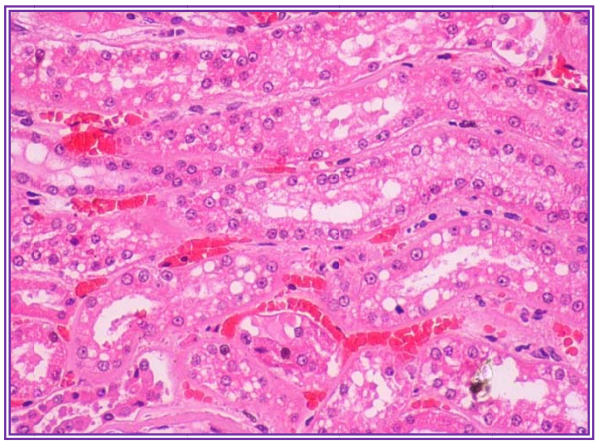


PLATE 15

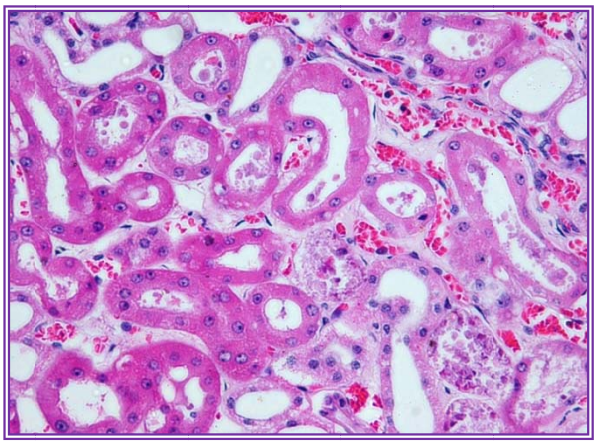


PLATE 16

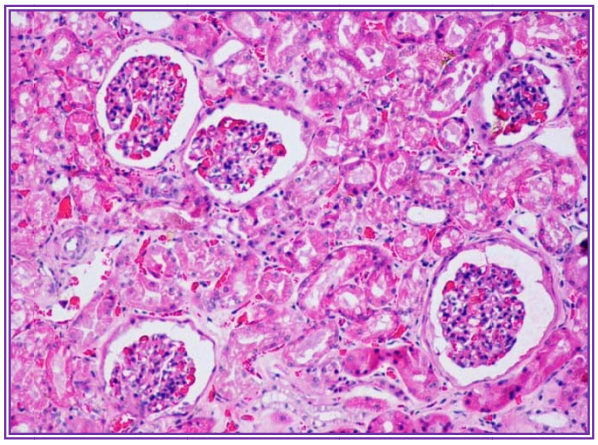


PLATE 17

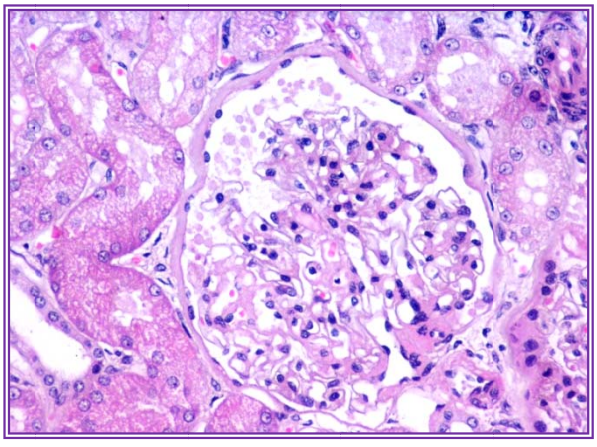


PLATE 18

Plate 19. Photomicrograph of kidney from a dog with renal failure to show distended tubules and glomeruli with protein rich eosinophilic fluid, flattened lining epithelium and reduction in glomerular tuft

H & E X 40

Plate 20. Photomicrograph of kidney from a dog with renal failure to show cystic dilatation of tubules lined by flattened epithelium, hyalinsed fibrous connective tissue and congested cystic tubules with hyaline casts

H & E X 200

Plate 21. Photomicrograph of kidney from a dog with renal failure to show cystic dilated tubules

H & E X 200

Plate 22. Photomicrograph of kidney from a dog with renal failure to show pure population of plasma cell and mild connective tissue proliferation

H & E X 200

Plate 23. Photomicrograph of kidney from a dog with renal failure to show lymphoid aggregation on the necrotic area, along with necrotic and cystic tubules

H & E X 200

Plate 24. Photomicrograph of kidney from a dog with renal failure to show severe degree of interstitial fibrosis with hyalinization and protein rich fluid filled tubules

H & E X 100

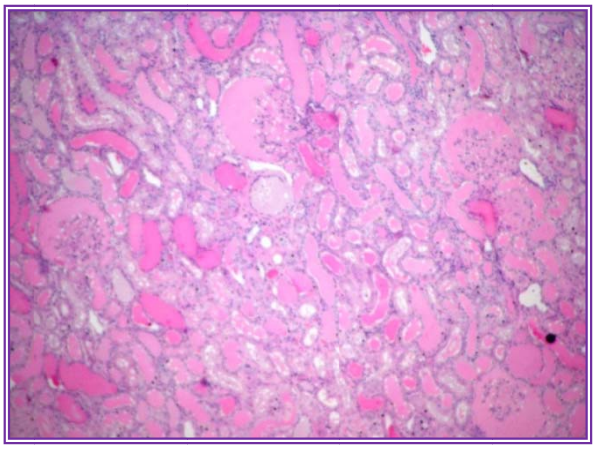


PLATE 19

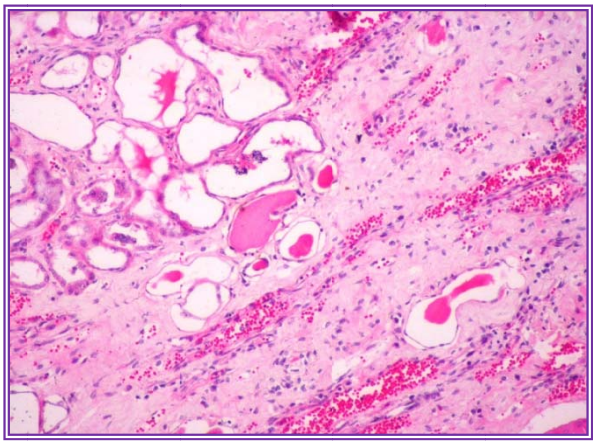


PLATE 20

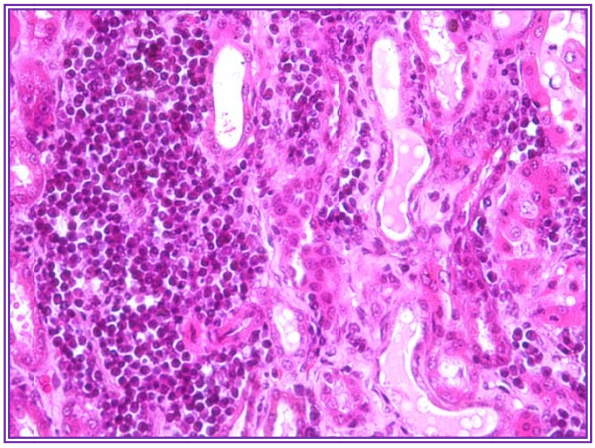


PLATE 21

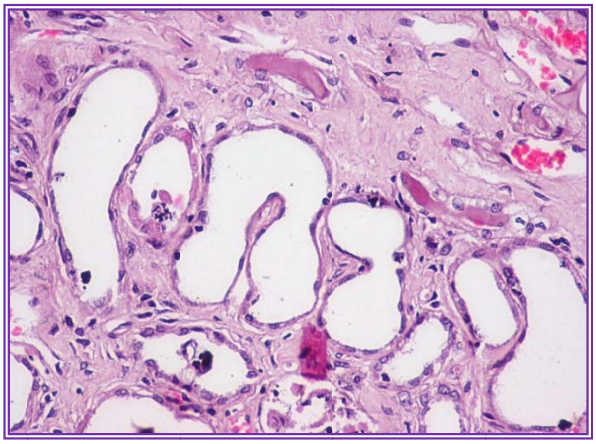


PLATE 22

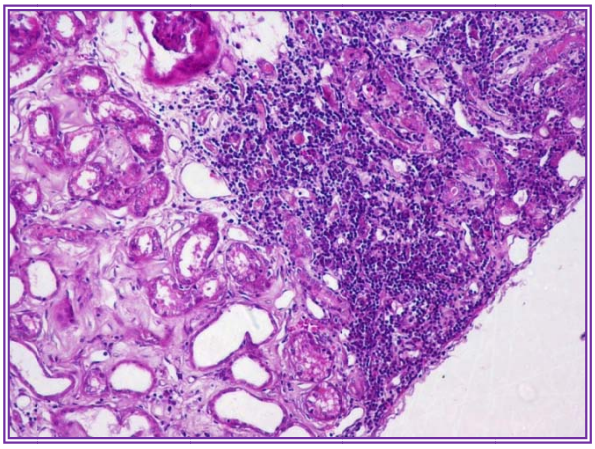


PLATE 23

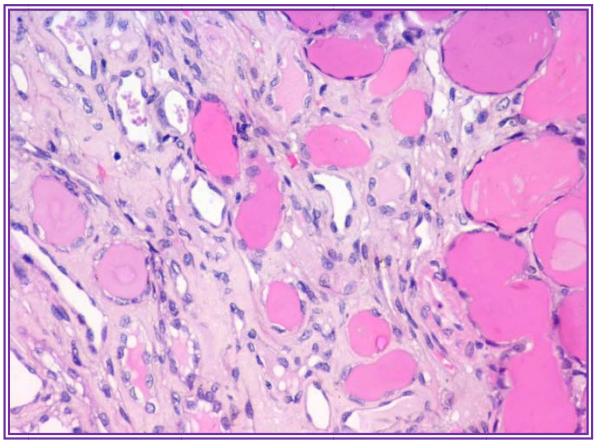


PLATE 24

Plate 25. Photomicrograph of kidney from a dog with renal failure to show calcification in the interstitial space

H & E X 100

Plate 26. Photomicrograph of kidney from a dog with renal failure to show acute tubulo-intestitial nephritis characterized by severe aggregation of lymphocytes in the interstitial space, intact basement membrane, mild proliferation of connective tissue with hyaline casts

H & E X 100

Plate 27. Photomicrograph of kidney from a dog with renal failure to show massive infiltration of inflammatory cells with fibrous connective proliferation in the interstitium and proliferative tubules

H & E X 100

Plate 28. Photomicrograph of kidney from a dog with renal failure to show severe degree of fibrosis with infiltration of mononuclear cells

H & E X 100

Plate 29. Photomicrograph of kidney from a dog with renal failure to show cyst lined by cuboidal cells, tubular necrosis and adjacent pressure atrophy of renal structure

H & E X 40

Plate 30. Photomicrograph of kidney from a dog with renal failure to show glomeruli with thickened basement membrane and bowman capsule and also segmental attachment of glomerular tuft

H & E X 200

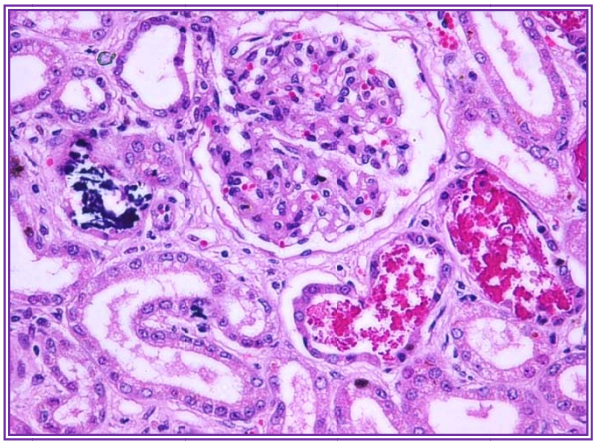


PLATE 25

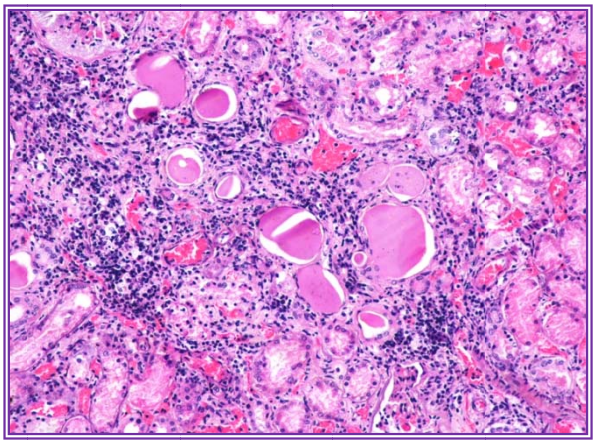


PLATE 26

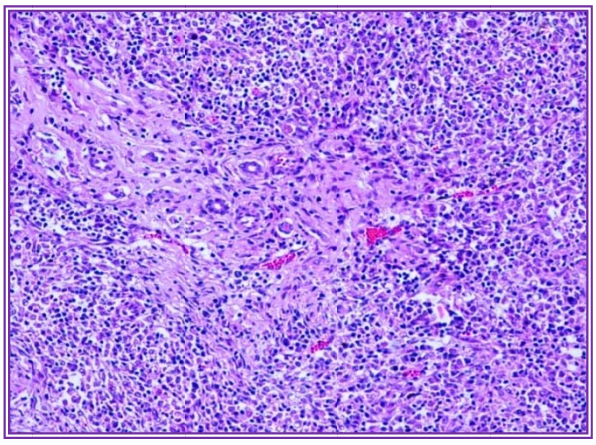


PLATE 27

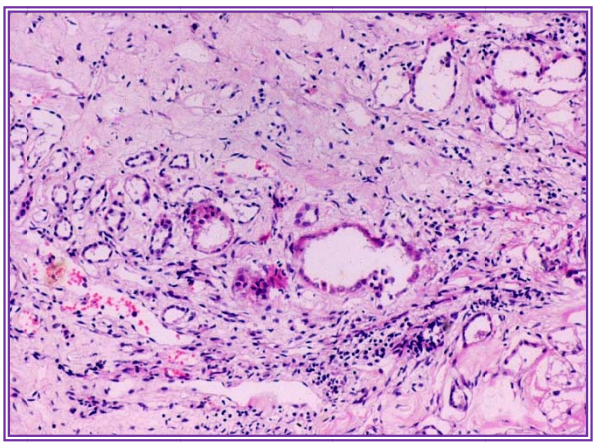


PLATE 28

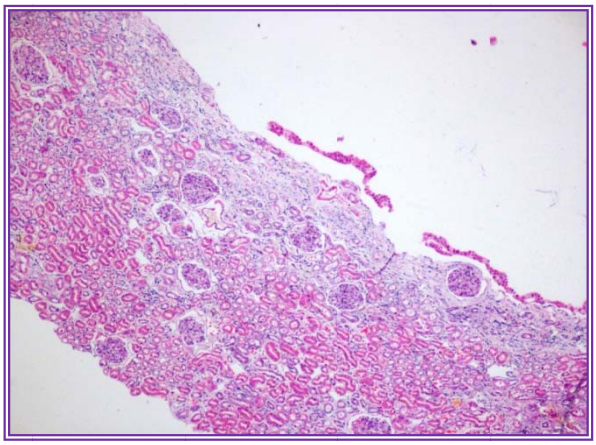


PLATE 29

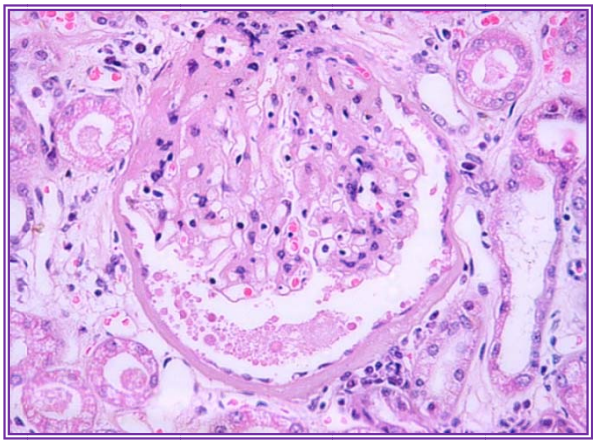


PLATE 30

Plate 31. Photomicrograph of kidney from a dog with renal failure to show glomerulus with absence of tuft and thickening of Bowman's capsule

H & E X 400

Plate 32. Photomicrograph of kidney from a dog with renal failure to show glomeruli containing protein droplets accumulation in Bowman's space and coagulative necrosis of tubules

H & E X 400

Plate 33. Photomicrograph of kidney from a dog with renal failure to show extracapillary proliferation of parietal cell forming half moon shaped structure (crescents) within the glomerulus

H & E X 200

Plate 34. Photomicrograph of kidney from a dog with renal failure to show multiple cystic glomeruli containing eosinophilic debris and mononuclear cell infiltration in the interstitium.

H & E X 100

Plate 35. Photomicrograph of kidney from a dog with renal failure to show hypertrophied and hyperplastic tubules with tall cuboidal cells.

H & E X 200

Plate 36. Photomicrograph of kidney from a dog with renal failure to show tubules with proliferative and regenerative change and neutrophils inside the lumen.

H & E X 200

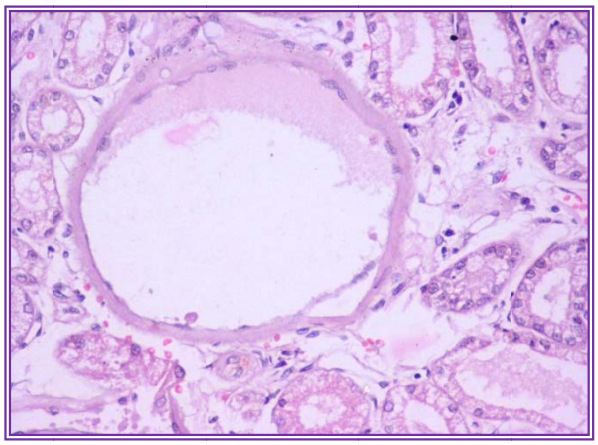


PLATE 31

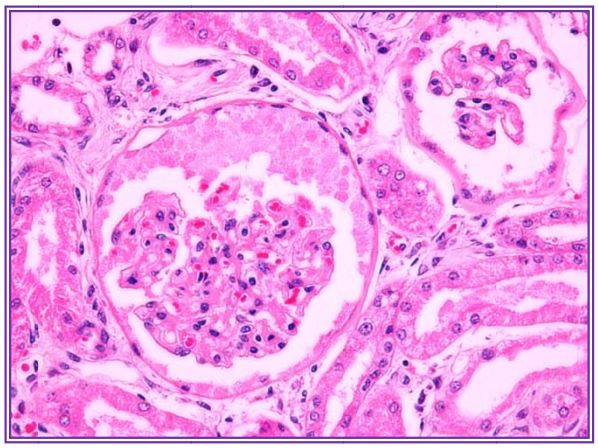


PLATE 32

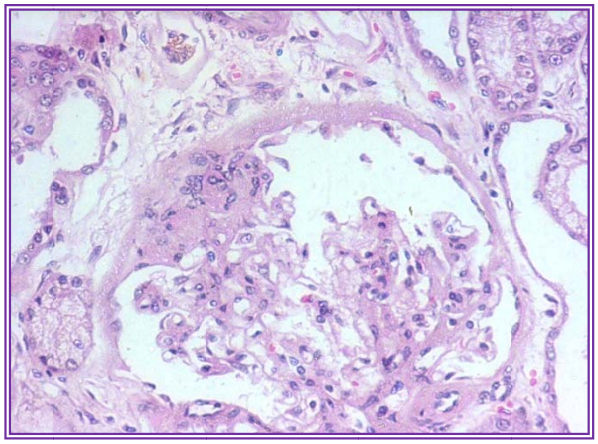


PLATE 33

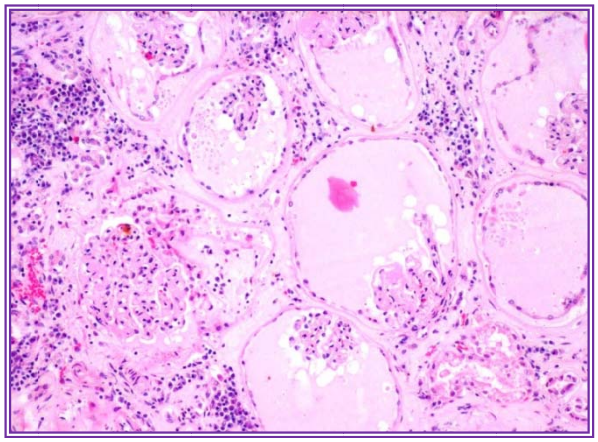


PLATE 34

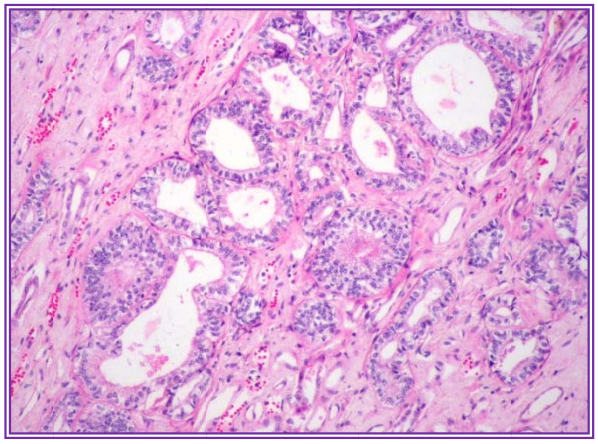


PLATE 35

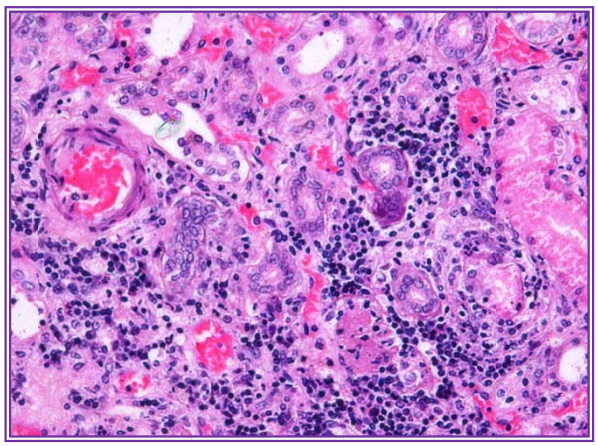


PLATE 36

Plate 37. Photomicrograph of kidney from a dog with renal failure to show severe peri glomerular fibosis with basement membrane thickening and nodular capsular adhesions

H & E X 400

Plate 38. Photomicrograph of kidney from a dog with renal failure to show membranous thickening with mesangial proliferation

H & E X 400

Plate 39. Photomicrograph of kidney from a dog with renal failure to show sclerotic glomeruli with mesangial thickening and periglomerular fibrosis

H & E X 200

Plate 40. Photomicrograph of kidney from a dog with renal failure to show periglomerular infiltration

H & E X 200

Plate 41. Photomicrograph of kidney from a dog with renal failure to show sclerosed glomeruli with obliteration

H & E X 200

Plate 42. Photomicrograph of kidney from a dog with renal failure to show regenerative tubules and young cells

H & E X 200

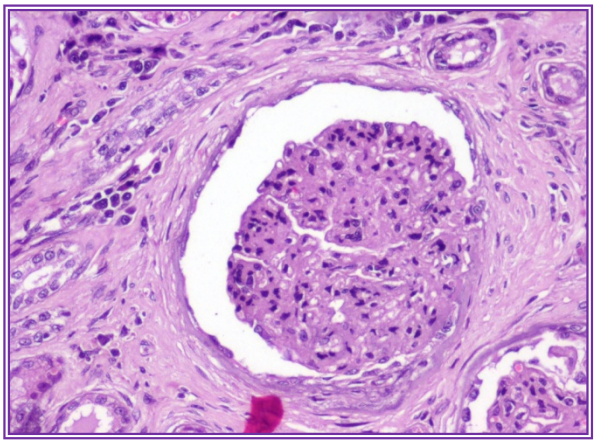


PLATE 37

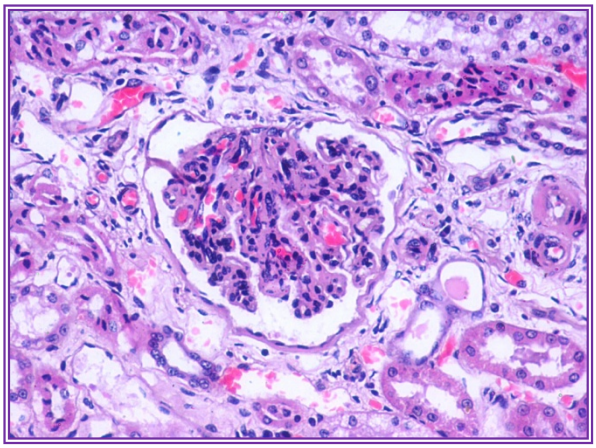


PLATE 38

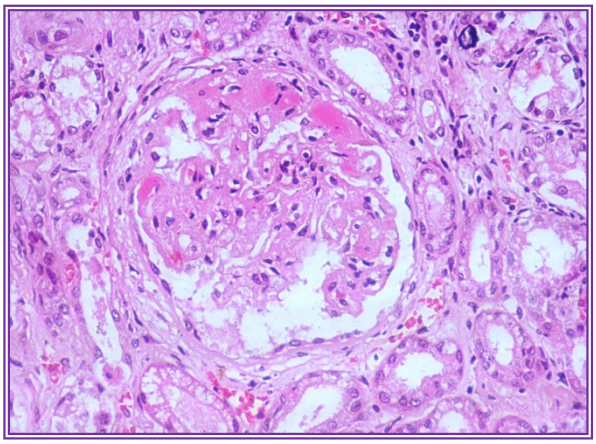


PLATE 39

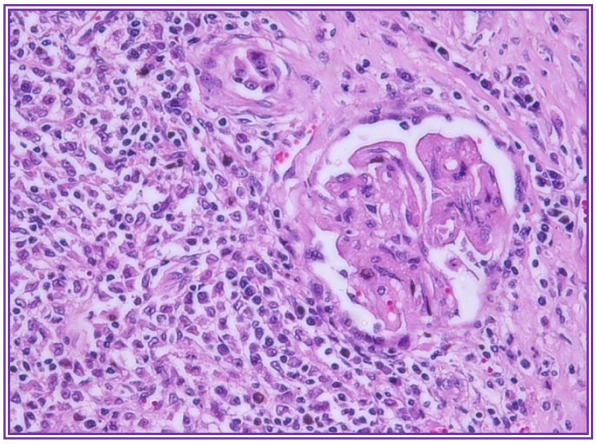


PLATE 40

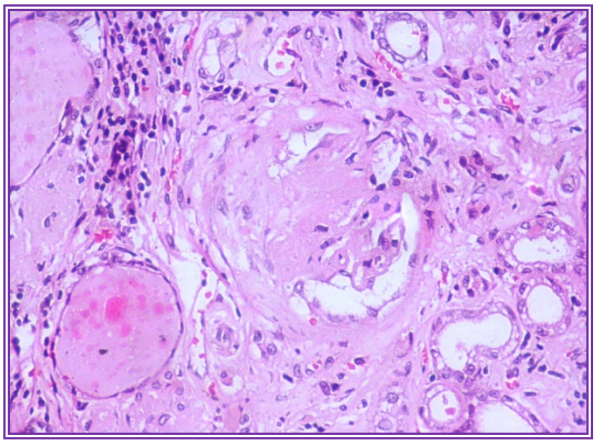


PLATE 41

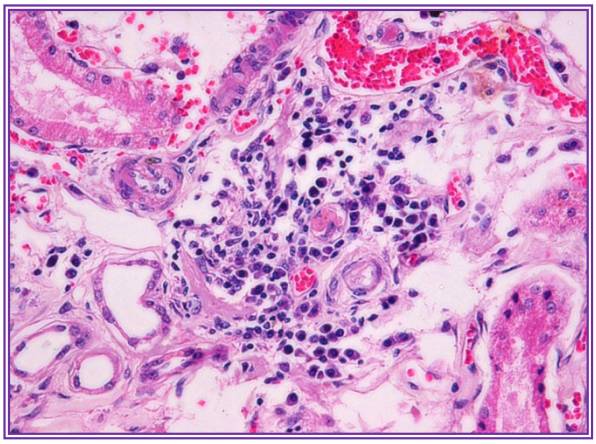


PLATE 42

Plate 43. Photomicrograph of kidney from a dog with renal failure to show leukocytic infiltration and highly eosinophilic material inside the tuft

H & E X 400

Plate 44. Photograph of liver from a dog with renal failure to show coagulation and reduction in size

Plate 45. Photograph of liver from a dog with renal failure to show diffused multifocal petechial haemorrhages with pale to mottling appearance

Plate 46. Photograph of liver from a dog with renal failure (leptospirosis) to show multi focal, grayish whitish necrotic foci and roundening of liver lobes

Plate 47. Photomicrograph of liver from a dog with renal failure to show foci of granular to highly swollen hepatocytes and fat droplets

H & E X 100

Plate 48. Photomicrograph of liver from a dog with renal failure to show moderate degree of congestion, mild degree of connective tissue proliferation and area of bile duct proliferation and formation of cystic spaces

H & E X 100

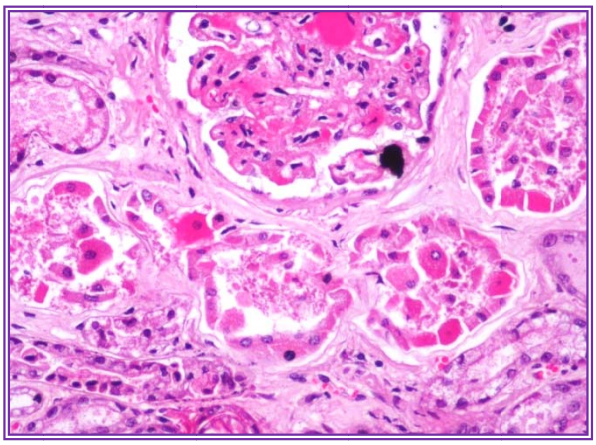


PLATE 43

PLATE 44

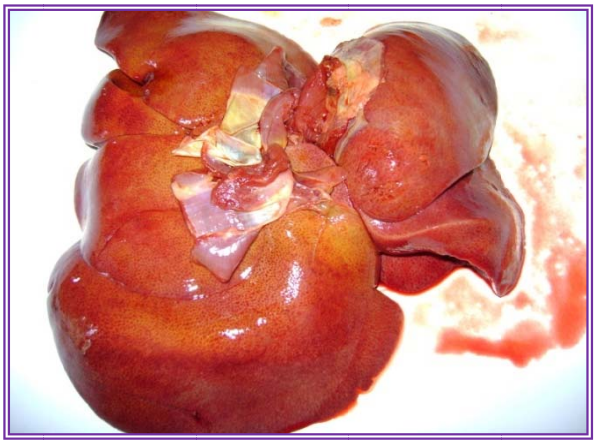


PLATE 45

PLATE 46

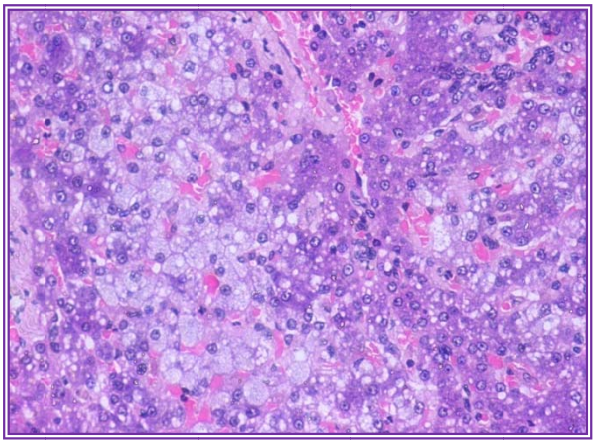


PLATE 47

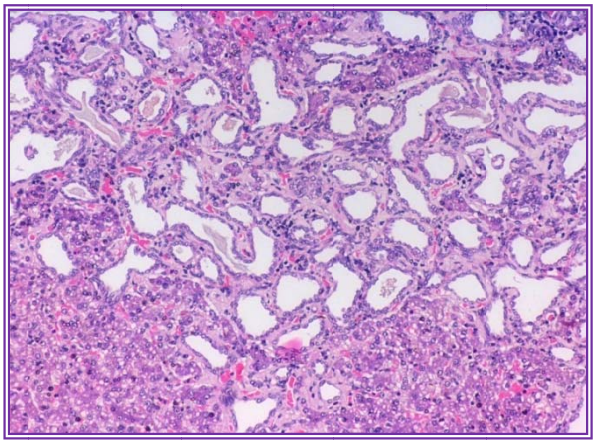


PLATE 48

Plate 49. Photomicrograph of liver from a dog with renal failure to show moderate degree of congestion, vacuolar degeneration of hepatocytes and multifocal nodular aggregates of mononuclear cells

H & E X 100

Plate 50. Photomicrograph of liver from a dog with renal failure to show periportal infiltration of inflammatory cells surrounded by atropic hepatic cords and cells undergoing apoptosis

H & E X 400

Plate 51. Photomicrograph of liver from a dog with renal failure to show severe congestion involving sinusoids and vessels, vacuolar degeneration of centrilobular hepatocytes and necrosis

H & E X 100

Plate 52. Photomicrograph of liver from a dog with renal failure to show severe congestion, periportal fibrotic change with infiltration of inflammatory cells and mild degree of biliary cirrhosis

H & E X 100

Plate 53. Photomicrograph of liver from a dog with renal failure to show cirrhotic changes with edematous connective tissue, infiltration of inflammatory cell and hemosiderosis

H & E X 200

Plate 54. Photomicrograph of liver from a dog with renal failure to show hemosiderin laden macrophages in the periportal area and infiltration of inflammatory cells

H & E X 200

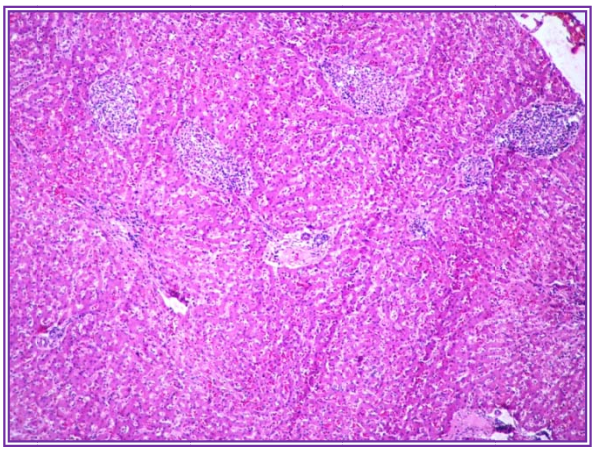


PLATE 49

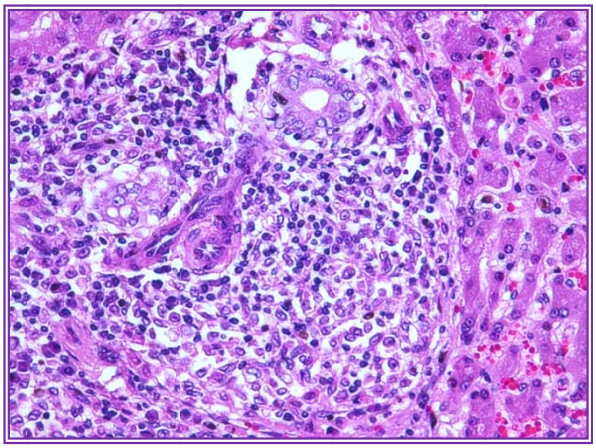


PLATE 50

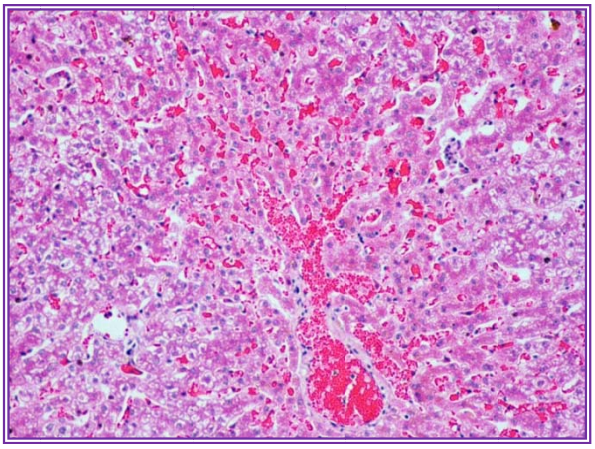


PLATE 51

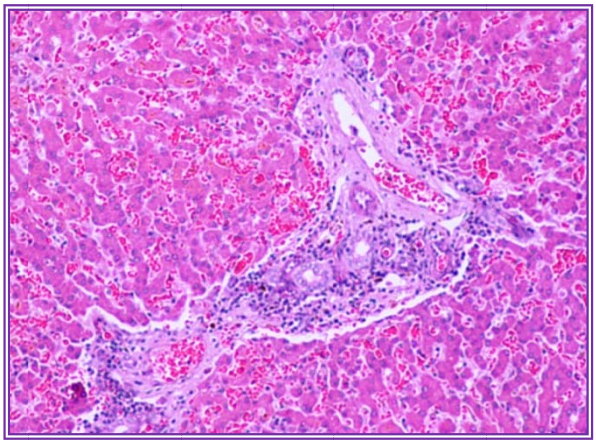


PLATE 52

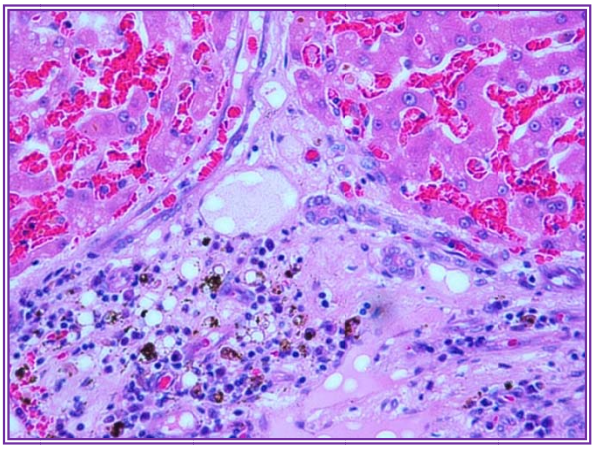


PLATE 53

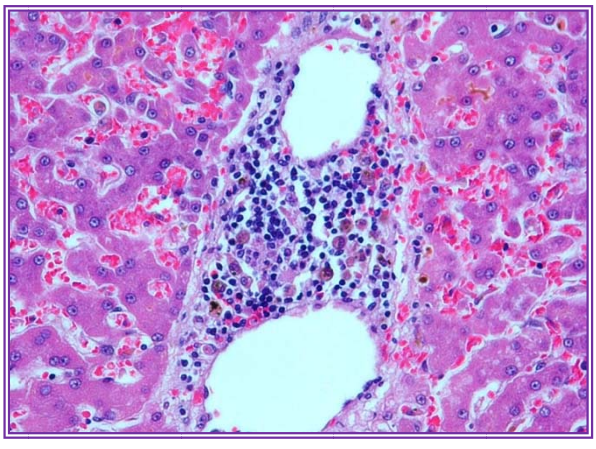


PLATE 54

Plate 55. Photomicrograph of liver from a dog with renal failure to show congestion and mild fibrous tissue proliferation in the portal triad region

H & E X 100

Plate 56. Photomicrograph of liver from a dog with renal failure to show severe congestion, granular to vacuolar degeneration and hemosiderin pigment

H & E X 200

Plate 57. Photomicrograph of liver from a dog with renal failure to show congestion, granular to vacuolar degeneration with hemosiderin pigments

H & E X 100

Plate 58. Photomicrograph of liver from a dog with renal failure to show congestion focal area of fatty change with haemosiderin pigments

H & E X 100

Plate 59. Photomicrograph of liver from a dog with renal failure to show multiple vacuolar degeneration with congestion

H & E X 40

Plate 60. Photomicrograph of liver from a dog with renal failure to show venous congestion, perivascular fibrosis and hemosiderosis,

H & E X 100

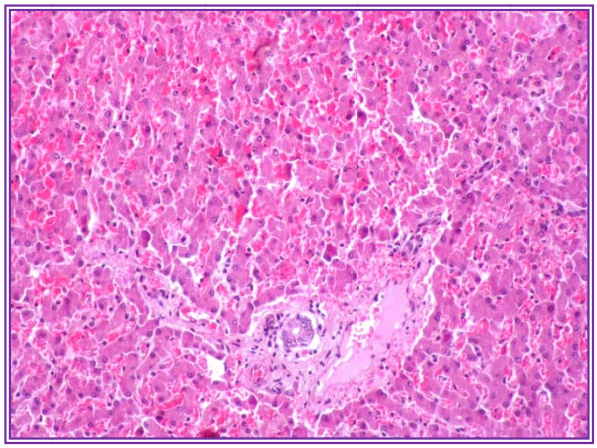


PLATE 55

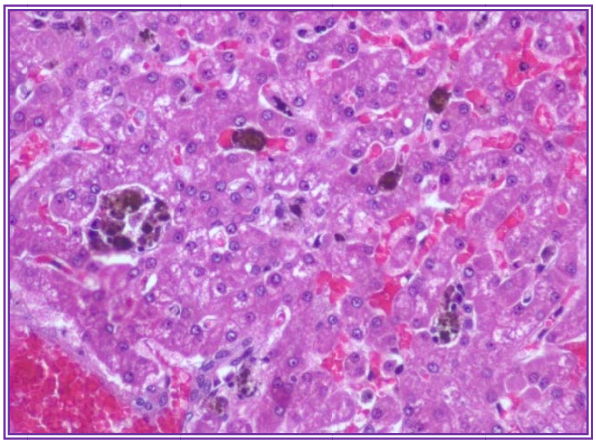


PLATE 56

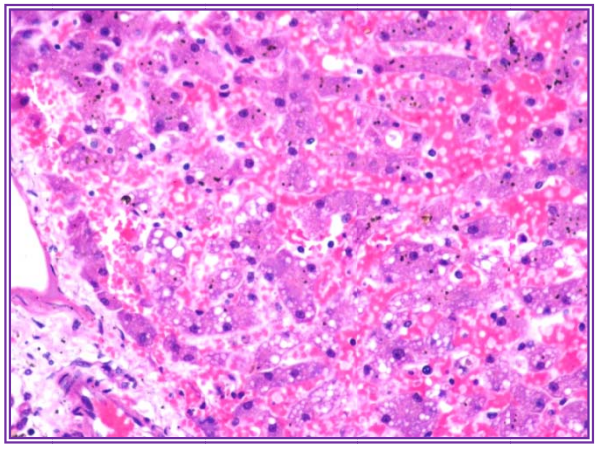


PLATE 57

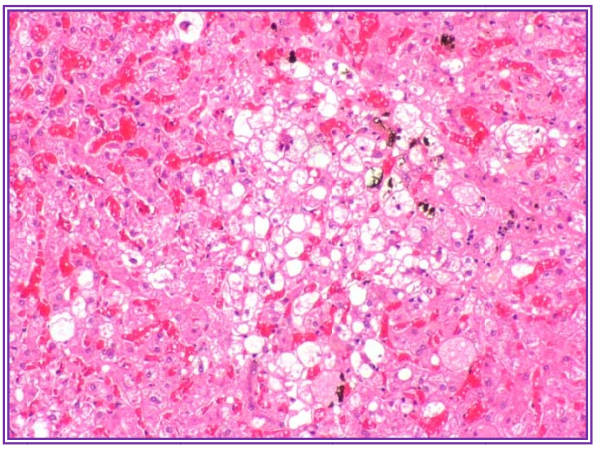


PLATE 58

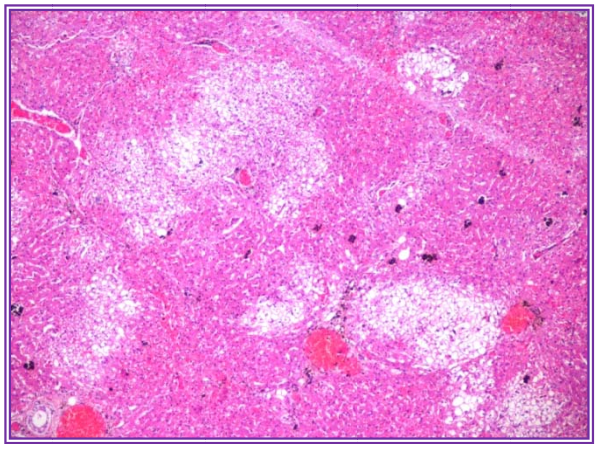


PLATE 59

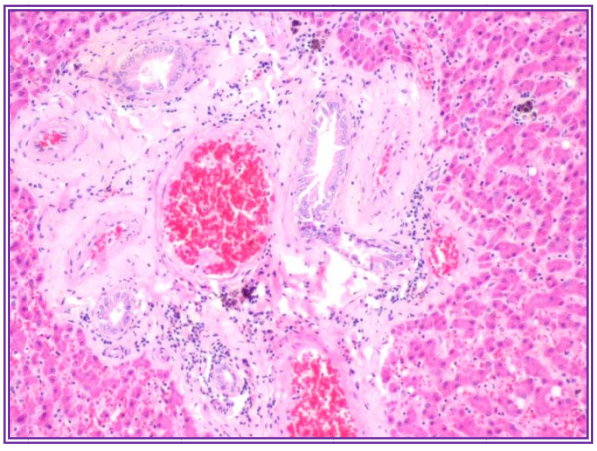


PLATE 60