

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

**ASSESSMENT OF THE EFFECT OF BLOOD CONTAMINATION ON
THE URINARY PROTEIN TO CREATININE RATIO IN THE DOG.**

**A dissertation presented in partial fulfilment of the requirements for
the degree of**

Master of Veterinary Studies

In

Veterinary Pathology

**at Massey University, College of Science, Turitea, Palmerston North
New Zealand**

Eloise Katherine Puia Jillings

2007

ABSTRACT

The urine protein to creatinine ratio (UPCR) is a reliable method to assess the total urinary protein loss in the dog from a single urine sample. Interpretation of the urine protein to creatinine ratio has been difficult in the presence of haematuria in the sample and previously the presence of blood in the urine has negated the use or interpretation of the UPCR. In 2 previous studies blood has been added to the urine sample of a single dog to aid interpretation of the UPCR in the presence of blood contamination. In this study blood contamination of urine samples in 21 dogs was assessed to develop guidelines for interpretation of the UPCR in the face of haemorrhage. Blood was added to the urine from the same dog to make samples with blood contamination levels ranging from 0 to 5%. Urine dipstick analysis, microscopic examination and a UPCR was performed on all samples. The current recommended cut off level for UPCR for normal dogs is <0.5 . Results greater than 1.0 are considered abnormal, results greater than 2.0 suggests glomerular disease, and UPCR results between 0.5 and 1.0 are questionable. The results of the present study suggest that when urine is visibly red, haemorrhage may be considered as a differential for a UPCR up to 2.0. The practice of attributing proteinuria in non discoloured (yellow) urine samples with microscopic haemorrhage to the blood present should be discontinued, as microscopic haemorrhage that does not result in a visible change in colour of the urine sample from yellow will not substantially increase the UPCR. As such, the UPCR level in yellow urine even in the presence of microscopic haematuria can be considered valid.

ACKNOWLEDGEMENTS

There are many people who have contributed towards the completion of my research.

Firstly, I would like to acknowledge my husband Bevan for his encouragement throughout this and all that I do. To my wonderful parents, Peter and Marina, your continuing support and influence throughout my life has been pivotal in creating who I have become. Arohanui ki a koutou kātoa.

I would like to thank my supervisor Keith Thompson for his guidance and leadership. You are a great example both personally and professionally to those of us who have the opportunity to work with you. Many thanks also to Richard Squires, for sparking my interest in proteinuria, and to Sandra Forsyth for her collegiality and support.

I am especially grateful to the staff of New Zealand Veterinary Pathology for allowing me to use their equipment, and putting up with me in their workspace. Additionally, I would like to thank Alasdair Noble for his statistical assistance, without which I would have been lost.

My gratitude goes to the veterinarians who submitted larger than normal samples for me to assess, and for all the samples from volunteer dogs belonging to the staff and students of IVABS and the Massey University Veterinary Teaching Hospital. This research would not have been possible without you all.

I am grateful to the Foundation for Research, Science and Technology for their financial support through the Tūāpapa Pūtaiao Māori Fellowship, and particularly Christine Romanes for her support and patience.

Ki a koe te rangatira a Nick Roskruge, he mihi aroha ki a koe mo tō manaakitanga, tiakitanga hoki māku. Tēnā koe e hoa.

The whakatauki (proverb) below encapsulates my appreciation for everyone who has contributed in some way to the completion of my research.

Ko koutou ki tēnā, ko ahau ki tēnei kīwai o te kete, ka oti te mahi.

With you all on that, and with me on this handle of the basket, the work is done.

TABLE OF CONTENTS

Chapter 1: Proteinuria in the Dog – A Review of Aetiology	
Clinical Signs and Diagnosis	1
1.0 Introduction	1
2.0 Renal Anatomy and Function	2
2.1 Renal Vasculature.....	2
2.2 Glomerular Structure and Function.....	2
2.3 Tubular Structure and Function.....	5
2.4 The Interstitium.....	5
3.0 Causes of Proteinuria	6
3.1 Pre-Renal Proteinuria.....	6
3.2 Renal proteinuria.....	7
3.2.1 Functional Renal Proteinuria.....	7
3.2.2 Pathological Renal Proteinuria.....	8
3.2.2.1 Tubular Proteinuria.....	8
3.2.2.2 Interstitial Proteinuria.....	8
3.2.2.3 Glomerular Proteinuria.....	8
3.2.2.3.1 Glomerulonephritis.....	10
3.2.2.3.2 Amyloidosis.....	13
3.3 Post-Renal Proteinuria.....	15
4.0 Clinical Manifestations of Glomerular Disease	18
4.1 Signalment.....	18
4.2 Clinical Signs.....	19
5.0 Detection and Diagnosis of Proteinuria	20
5.1 Urine Chemistry Dipsticks.....	20
5.2 Non-Dipstick Urinary Protein Assessment.....	22
5.2.1 Sulphosalicylic Acid Turbidity (SSA).....	22
5.2.2 Trichloroacetic Acid (TCA).....	22
5.2.3 Trichloroacetic Acid-Ponceau S (TCA-PS).....	23
5.2.4 Coomassie Brilliant Blue (CBB).....	23
5.2.5 Benzethonium Chloride.....	23
5.3 24 hour urine sample collection for protein assessment.....	24
5.4 Urinary Protein to Creatinine Ratio (UPCR).....	26
5.4.1 UPCR Values.....	28
5.4.2 Interpretation of the Urine Protein to Creatinine Ratio.....	29
5.4.3 Prognostic Use of the UPCR.....	30
5.4.4 Monitoring Progress with the UPCR.....	30
5.4.5 Differentiation of Causes of Glomerular Disease with the UPCR.....	31
5.5 Microalbuminuria.....	31
5.6 Renal Biopsy.....	36
6.0 References	38

Chapter 2: Assessment of the effect of blood contamination on the urinary protein to creatinine ratio.....	46
1.0 Abstract.....	46
2.0 Introduction.....	47
3.0 Materials and Methods.....	49
4.0 Results.....	51
5.0 Discussion.....	55
6.0 References.....	58
Appendices.....	61
Appendix A: Raw data for colour scoring of urine.....	62
Appendix B: Raw data for urinary protein.....	63
Appendix C: Raw data for urinary creatinine.....	64
Appendix D: Raw data for urinary protein to creatinine ratio.....	65
Appendix E: Massey university animal ethics approval.....	66

LIST OF FIGURES

Figure 1.1	Cut surface of a canine kidney with amyloidosis.....	14
Figure 1.2	Cut surface of a canine kidney with amyloidosis after exposure to Lugols Iodine.....	15
Figure 2.1	Scatter plot of colour score versus level of blood contamination of the urine sample, with background colouring to match the colour of each urine sample.....	51

LIST OF TABLES

Table 1.1	Causes of Canine Glomerular Disease.....	9
Table 1.2	24 hour urinary protein loss in mg/kg for healthy and proteinuric dogs.....	25
Table 1.3	Trial results for UPCr in healthy and proteinuric dogs.....	28
Table 1.4	Recommendations for the normal, equivocal and abnormal range of UPCr in dogs.....	29
Table 2.1	The number of individuals (n=13) that scored each urine sample by colour.....	51
Table 2.2	Colour, urine protein to creatinine ratios, and urinary dipstick blood results for all urine samples.....	52
Table 2.3	The number of samples, and percentage of total samples (n=15) with urine protein to creatinine ratios that exceed varying cut-off limits as the level of blood contamination increases, when the UPCr of the initial sample is 0.5.....	53

CHAPTER 1

PROTEINURIA IN THE DOG – A REVIEW OF AETIOLOGY, CLINICAL SIGNS AND DIAGNOSIS

1.0 Introduction

In the dog proteinuria can be due to relatively benign causes, through to more serious and life threatening glomerular disease. Persistent proteinuria is an indication to the veterinary clinician of the need for further investigation of the kidney. The unique anatomy and physiology of the kidney is central to the mechanism of glomerular proteinuria.

The kidney is a complex, multi-functional organ that not only regulates the body's concentration of water and electrolytes, but also excretes the waste products of metabolism, filters, excretes and reabsorbs protein, and functions as an endocrine organ secreting hormones such as erythropoietin, renin and prostaglandins (Stockholm and Scott 2002; Alpers 2005; Newman et al 2007).

Approximately 1000-2000 litres of blood perfuse the kidneys of a large dog each day, with 200-300 litres of that volume filtered through the renal glomeruli. From this filtrate, the kidneys reabsorb fluids, electrolytes, protein and other molecules until eventually only 1-2 litres of urine are excreted (Dyce et al 2002).

2.0 Renal Anatomy and Function

The canine kidney is a bean shaped organ enclosed within a fibrous capsule, and found in the retroperitoneum, either adjacent to or ventral to the lumbar vertebrae (Dyce et al 2002; Newman et al 2007). The parenchyma of the kidneys can be functionally and visibly divided into the outer cortex and the inner medulla. Most mammalian kidneys develop as a collection of pyramid-shaped lobes with the cortex forming the base. While pigs and cattle retain the multi-pyramidal developmental structure into maturity, the pyramids within the canine kidney fuse, retaining limited evidence of their developmental structure (Newman et al 2007).

The functional unit of the kidney is the nephron, composed of a glomerulus and tubule. In addition to the nephron the kidney includes several other essential structures, and as such it is useful to consider the kidney as the sum of its 4 major components: the blood vessels, glomeruli, tubules and interstitium (Dyce et al 2002; Ross et al 2003; Alpers 2005; Newman et al 2007).

2.1 Renal Vasculature

The renal artery branches directly off the aorta and is the only vascular supply to the kidneys (Dyce et al 2002; Alpers 2005; Newman et al 2007). It enters at the hilus and divides to form interlobar arteries that course between what would have been the lobes of the kidney developmentally. The interlobar arteries further divide into arcuate arteries that traverse the corticomedullary junction. Once in the cortex these arcuate arteries divide into interlobular arteries that further separate into afferent arterioles. These arterioles enter the glomerular tuft where they divide into numerous glomerular capillaries, supported by the surrounding mesangium. The capillary loops coalesce upon exiting the glomerular tuft to form the efferent arterioles. These arterioles form the peritubular capillary network, which continue into the interlobular veins, arcuate veins, interlobar veins and finally to the renal vein in a system mirroring that of the arteries.

2.2 Glomerular Structure and Function

The glomerulus has a unique, complex structure that allows for high permeability to water and small ions, while providing a barrier to other molecules. It consists of a complex, highly branched network of capillaries enclosed in a double layer of

epithelium (the visceral and parietal epithelium) supported by a glycoprotein matrix, or mesangium. The visceral epithelium is contiguous with the capillary wall and is separated from the endothelial cells by a basement membrane. The epithelial layers and basement membrane comprise Bowman's capsule, while the space within the epithelial layers is referred to as Bowman's space (Jergens 1987; Ross et al 2003; Newman et al 2007).

Endothelial cells in glomerular tufts have larger fenestrations than those found in most other tissues and often lack the connecting diaphragm (Ross et al 2003; Alpers 2005). Underlying the fenestrated endothelial cells is the basement membrane, which is composed largely of type IV collagen and can be separated into 3 layers. The lamina densa is the thick electron-dense central layer, while the two peripheral electron-lucent layers are the lamina rara externa and the lamina rara interna (Ross et al 2003; Alpers 2005; Newman et al 2007).

Adjacent to the basement membrane is the visceral epithelium, composed of modified epithelial cells referred to as podocytes (Ross et al 2003). Podocytes have numerous foot processes or pedicels, which interdigitate with those of other podocytes, and are fixed to the lamina rara externa of the basement membrane. The spaces between the pedicels, called filtration slits, are covered with a thin membrane composed of nephrin (Ross et al 2003; Alpers 2005; Newman et al 2007). In contrast to the visceral epithelial layer, the parietal epithelium of Bowman's capsule is simple and attenuated (Ross et al 2003; Alpers 2005).

Associated with the glomerular capillary endothelium and the basement membrane are mesangial cells, which produce the collagenous mesangial matrix that provides the structural support framework of the glomerular tuft (Ross et al 2003; Alpers 2005; Newman et al 2007).

The fluid that is filtered from the plasma through the glomerulus is called the plasma ultrafiltrate or glomerular filtrate. In summary, this filtrate has to pass through the three layers of the glomerular wall, namely the fenestrated capillary endothelium, the glomerular basement membrane and the filtration slits formed by the visceral epithelial podocytes. It then accumulates in Bowman's space before entering the proximal tubule via the urinary pole.

Although many small proteins are filtered through the glomerulus, there is usually little to no protein in the urine of healthy animals due to reabsorption of protein in the renal tubules. The dog is an exception to this and may have small amounts of urinary protein in the absence of urinary tract disease (Barsanti and Finco 1979). This protein is usually composed of albumin and smaller plasma proteins filtered by the glomeruli, as well as protein added in tubules (Tamm-Horsfall proteins) (Barsanti and Finco 1979; Stockholm and Scott 2002).

Protein filtration through the glomerular capillaries depends on several factors, including: protein size, charge, structural conformation and haemodynamic factors, such as glomerular filtration rate and transcapillary hydrostatic pressure (Deen et al 1980; Lulich and Osborne 1990; Alpers 2005). The filtration of protein macromolecules through the glomerular capillary walls decreases as the size of the molecule increases (Lulich and Osborne 1990; Alpers 2005). Once proteins reach or exceed the size of albumin (approximately 70kDa) they are larger than the filtration slits in the visceral epithelium and their filtration is largely prevented in the healthy kidney (Alpers 2005).

The charge of each protein molecule will also influence its filtration through the glomerular wall (Deen et al 1980; Deen and Satvat 1981; Alpers 2005). Negatively charged acidic glycoproteins (sialoproteins) associated with the endothelial cells and podocytes in the glomerular capillary wall contribute to its overall anionic charge. This impedes the passage of anionic molecules, while promoting the passage of cationic molecules (DiBartola et al 1980a; Deen and Satvat 1981; Alpers 2005; Newman et al 2007). Given the same size, the passage of a polyanionic molecule through the glomerular wall will be more restricted than a neutral macromolecule, which will in turn be more restricted than a polycationic molecule (Deen et al 1980).

As the glomerular filtration rate or the transcapillary hydrostatic pressure increases, the proportion of plasma protein filtered through the glomerulus decreases for neutral or anionic macromolecules, but increases for cationic molecules (Deen and Satvat 1981). Changes in haemodynamic parameters affect the passage of more highly charged molecules, whether anionic or cationic, to a larger extent than neutral macromolecules (Deen and Satvat 1981).

Proteinuria in glomerular disease can be due to either structural damage of the glomerulus allowing filtration of larger sized protein molecules, or loss of the negatively charged glycoproteins (Deen and Satvat 1981).

2.3 Tubular Structure and Function

Renal tubules concentrate the glomerular filtrate and modify its electrolyte and protein content. The proximal tubule has a high capacity for protein reabsorption (Christensen and Gburek 2004). In addition to protein reabsorption the proximal tubule also reabsorbs water, electrolytes (sodium, chloride and potassium), glucose and bicarbonate (Alpers 2005; Newman et al 2007). The thick segment of the proximal tubule is composed of interdigitating simple, cuboidal, epithelial cells that have a microvillus brush border on their luminal surface. At the base of these microvilli, the cell membrane invaginates to form pinocytotic vesicles which coalesce with each other to form larger vesicles. These vesicles in conjunction with the cytoplasmic lysosomes endocytose and remove protein from the plasma filtrate (Ross et al 2003; Newman et al 2007).

The thick proximal tubule continues into the thinner loop of Henle, composed of a simple squamous epithelium (Ross et al 2003). As the filtrate passes through the descending loop of Henle it becomes more concentrated due to the passive movement of water into the interstitium. After the descending Loop of Henle, the filtrate passes through the ascending loop of Henle where its osmolality progressively becomes hypotonic due to the active reabsorption of sodium and chloride (Stockholm and Scott 2002; Newman et al 2007).

In the distal tubule and collecting tubule/duct the filtrate becomes hypertonic by resorption of water, due to the interstitial concentration gradient and the effects of anti-diuretic hormone (ADH) (Stockholm and Scott 2002; Newman et al 2007).

2.4 The Interstitium

The interstitium is a connective tissue network containing nerves, blood and lymphatic vessels, that supports the nephrons and collecting ducts. The solute level in the medullary interstitium helps maintain the counter current system essential to concentration of the plasma filtrate (Newman et al 2007).

3.0 Causes of Proteinuria

In this review the causes of proteinuria are divided into 3 major categories – pre-renal, renal and post-renal with further subdivision of each. This concurs with the categorisation of proteinuria in the 2004 American College of Veterinary Internal Medicine (ACVIM) consensus statement for the assessment and management of proteinuria in dogs and cats, and other authors (DiBartola et al 1980a; White et al 1984; Grauer et al 1985; Stockholm and Scott 2002; Lees et al 2005). A slight variation, categorising the causes of proteinuria as pre-glomerular, glomerular and post glomerular has been adopted by some authors (Lulich and Osborne 1990; Brunker 2005).

3.1 Pre-Renal Proteinuria

This category is also referred to as overflow, tubular overload or pre-glomerular proteinuria (Lulich and Osborne 1990; Stockholm and Scott 2002). Pre-renal proteinuria occurs as a result of increased plasma protein concentration and subsequently increased filtration of small protein molecules through the glomeruli (Christensen and Gburek 2004). When present in high concentration, these protein molecules exceed the resorptive capacity of the renal tubules, with the excess remaining in the urinary filtrate, detected as proteinuria. Examples of protein molecules that may cause pre-renal proteinuria include haemoglobin (released during intravascular haemolysis), myoglobin (due to marked muscle damage), and light chain immunoglobulin (i.e. Bence-Jones proteins in multiple myeloma) (DiBartola et al 1980a; Lulich and Osborne 1990; Stockholm and Scott 2002; Christensen and Gburek 2004; Lees et al 2005). With the exception of Bence-Jones proteinuria, most incidences of pre-renal proteinuria are mild and transient (Brunker 2005; Lees et al 2005).

3.2 Renal proteinuria

Renal proteinuria can be due to functional or pathological causes (Lees et al 2005).

3.2.1 Functional Renal Proteinuria

Functional renal proteinuria occurs due to altered renal physiology during states of increased sympathetic nervous system activity (i.e. fever, strenuous exercise, stress and extremes of temperature) and some other temporary phenomena such as seizures or venous congestion (DiBartola et al 1980a; Lulich and Osborne 1990; Lees et al 2005). In functional renal proteinuria there are no renal lesions so the proteinuria resolves with removal of the initiating cause. Usually the proteinuria is low grade and consists primarily of albumin (DiBartola et al 1980a; Lulich and Osborne 1990; Lees et al 2005).

In a study of the effects of exercise on the urinary albumin excretion in 26 dogs it was reported that the proportion of dogs that might be expected to develop microalbuminuria in response to exercise was 0-15% (Gary et al 2004).

Microalbuminuria is defined as the presence of albumin in the urine in quantities greater than normal, but below the limit of detection of standard urine dipstick assays (<30mg/dl or 0.3g/l) (Whittemore et al 2006; Grauer 2007), or as a urine albumin concentration between 1.0 and 30mg/dl (Vaden et al 2001; Grauer et al 2002; Pressler et al 2003; Radecki et al 2003; Whittemore et al 2003; Grauer 2005a; Grauer 2005c, 2005b; Whittemore et al 2006; Grauer 2007). In this report all dogs were classified as being either negative or positive for microalbuminuria before and after exercise (Gary et al 2004). All the dogs that were negative for microalbuminuria before exercise remained negative after exercise. Also, all dogs that were positive for microalbuminuria after exercise were already positive before exercise and did not show an increase in albumin concentration in response to exercise. As such, the conclusion that up to 15% of dogs might develop microalbuminuria due to exercise appears questionable.

In one study 9 (19%) of 47 dogs were diagnosed with febrile proteinuria, which subsequently resolved during hospitalisation (Biewenga and Gruys 1986). Six (66.7%) of the 9 dogs with febrile proteinuria had arthralgia, and histological evidence of glomerular lesions were found in 7 of these 9 dogs. Whether the transient proteinuria in these dogs was due to the fever or due to non-renal infection inducing secondary

renal changes was not clear. The significance and cause of transient proteinuria observed in febrile dogs is not clear (Biewenga and Gruys 1986).

3.2.2 Pathological Renal Proteinuria

Pathological renal proteinuria is the most concerning cause of proteinuria. It can be due to structural or functional lesions within the tubules, interstitium, or most commonly the glomeruli (DiBartola et al 1980a; Grauer et al 1985; Lees et al 2005).

3.2.2.1 Tubular Proteinuria

Tubular proteinuria usually occurs as a result of tubular lesions that impair recovery of normally filtered plasma proteins (DiBartola et al 1980a). These proteins are usually low (DiBartola et al 1980a) to occasionally medium molecular weight (e.g. albumin) (Lees et al 2005). There are congenital causes of tubular proteinuria, but it is more commonly seen with acute renal disease (e.g. hypoxia, toxicoses) (Stockholm and Scott 2002).

Tubular proteinuria occurs less commonly due to Tamm Horsfall proteinuria or secondary to tubular necrosis (DiBartola et al 1980a). Tamm Horsfall proteins are mucoproteins secreted by the tubule. They are soluble above pH 7 and insoluble below pH7 and form the major component of hyaline casts (Stockholm and Scott 2002).

3.2.2.2 Interstitial Proteinuria

Interstitial proteinuria is caused by inflammatory lesions or disease of the interstitium. Disease states such as acute interstitial nephritis result in exudation of proteins from peritubular capillaries into the urinary space (DiBartola et al 1980a; Lees et al 2005). Interstitial proteinuria is likely to be an uncommon cause of significant proteinuria in the dog.

3.2.2.3 Glomerular Proteinuria

Glomerular proteinuria occurs as a result of glomerular lesions affecting the filtration barrier, and decreasing the selective permeability of the glomerular capillaries (DiBartola et al 1980a; Grauer et al 1985; Stockholm and Scott 2002; Lees et al 2005).

These lesions can be either structural, or due to loss of the negative charge of the capillaries (Deen and Satvat 1981; Lulich and Osborne 1990). As a result, proteins that were previously too large or too negatively charged to pass through the glomerular membrane become filtered in increasing amounts. Albumin is one of the first abnormally filtered proteins to appear, and is usually the primary component of urinary protein due to glomerular disease. As the disease progresses larger molecular weight proteins may also be filtered (DiBartola et al 1980a; Jergens 1987; Grant and Forrester 2001b; Stockholm and Scott 2002; Grauer 2005c).

Table 1.1 Causes of Canine Glomerular Disease (Grant and Forrester 2001b; Brunker 2005; Grauer 2005c; Vaden 2005; Maxie and Newman 2007; Newman et al 2007)

General	Examples	General	Examples
Infectious	Babesiosis	Drugs	Glucocorticoid excess
	Bacterial Endocarditis		Trimethoprim-Sulpha
	Bartonellosis	Endocrine	Hyperadrenocorticism
	Blastomycosis		Diabetes Mellitus*
	Borreliosis	Hereditary	Beagle
	Brucellosis		Bernese Mountain Dog
	CAV-1 Infection		Dalmation
	Chronic infection (Pyoderma, gingivitis)		Doberman Pinscher
	Dirofilariasis		English Cocker Spaniel
	Ehrlichiosis		English Foxhound
	Leishmaniasis		Golden Retriever
	Lyme Disease		Greyhound
	Pancreatitis (chronic)		Newfoundland
	Prostatitis		Rottweiler
	Pyelonephritis (Bacterial)		Samoyed
	Pyometra		Shar-pei
	Rocky Mtn. Spotted Fever		Soft Coated Wheaten terrier
	Septicaemia		Standard Poodle
	Trypanosomiasis		Neoplastic
	SLE	Leukaemia	
IMHA, IMTP	Lymphoma		
Inflammatory bowel disease	Transitional cell carcinoma		
Immune-Mediated	IMPA	Other	Systemic Hypertension

* Questionable or uncertain causes.

CAV = Canine adenovirus SLE = Systemic lupus erythematosus. IMHA = immune mediated haemolytic anaemia. IMTP = immune mediated thrombocytopenia. IMPA = immune mediated poly-arthritis.

Glomerulonephropathy is a significant problem due to its possible progression to chronic renal disease, and the capacity of proteinuria to further damage glomeruli and tubules (Grauer 2005c; Vaden 2005) .

The most common glomerular diseases causing proteinuria are glomerulonephritis and amyloidosis, with glomerulonephritis occurring more commonly (Lulich and Osborne 1990; Cook and Cowgill 1996; Grauer 2005c). Other rare causes of glomerular disease include structural abnormalities (e.g. Type IV collagen defects in Samoyeds) and haemodynamic abnormalities (Grauer 2005c).

3.2.2.3.1 Glomerulonephritis

While originally reported to be rare (Murray et al 1971; Kurtz et al 1972), glomerulonephritis is now recognised with increasing frequency (Mullerpeddinghaus and Trautwein 1977a; Maxie and Newman 2007), presumably due to a greater awareness and improved diagnostic methods. Although reported in a wide age range (from <1-15 years) it is more common in dogs over 5 years of age (Mullerpeddinghaus and Trautwein 1977a; DiBartola et al 1980b).

Glomerulonephritis is almost synonymous with immune complex glomerulonephritis, but can occasionally be seen with chemical toxicity (Newman et al 2007). Immune complex glomerulonephritis may be caused by glomerular deposition of preformed immune complexes, or immune complex formation *in situ* (DiBartola et al 1980b; Jergens 1987; Grant and Forrester 2001b; Grauer 2005c; Newman et al 2007).

In situ glomerular complex formation may occur as a result of circulating antibodies reacting to endogenous glomerular membrane antigens (auto-immune), or non-glomerular antigens that become attached to the glomerular wall (DiBartola et al 1980b; Center et al 1987; Jergens 1987; Cook and Cowgill 1996; Grant and Forrester 2001b; Grauer 2005c; Maxie and Newman 2007; Newman et al 2007). While auto-immune glomerulonephritis has been well documented in humans and reported experimentally in dogs, natural occurrence in the dog has not been reported. Deposition of non-glomerular antigens into the glomerular wall, with subsequent immune complex formation has been reported in association with *Dirofilaria immitis* infection in the dog (Center et al 1987; Grauer 2005c).

On immunofluorescence, the glomerular deposits of *in situ* immune complex formation have a linear appearance, while immune complex deposition glomerulonephritis tends to produce granular deposits (Center et al 1987; Jergens 1987; Grauer 2005c; Maxie and Newman 2007).

Immune complex deposition due to preformed antibody-antigen complexes occurs when there are similar numbers of antigen and antibody molecules present, or when there is a mild antigen excess. This results in the formation of small antigen-antibody complexes, which are deposited or trapped in the glomerulus (DiBartola et al 1980b; Abbas 2005; Grauer 2005c; Maxie and Newman 2007; Newman et al 2007). When there is a large antigen excess, the complexes formed can only bind complement to a limited degree, and do not significantly damage the glomeruli. When a large antibody excess occurs the complexes are relatively harmless as they tend to be large and insoluble, and are readily phagocytosed by cells of the tissue macrophage system (Jergens 1987; Abbas 2005; Grauer 2005c; Newman et al 2007).

Grossly, kidneys with glomerulonephritis are often slightly swollen, and normal to pale tan in colour. On cut surface, the glomeruli may appear as multiple red pin points (Newman et al 2007). There are many recognised causes of preformed glomerular complex deposition glomerulonephritis, as shown in Table 1.1, but up to 50% of canine cases are idiopathic (DiBartola et al 1980b; Jergens 1987; Grant and Forrester 2001b; Grauer 2005c). Concurrent disease was only identified in 35 of 61 dogs (57%) with glomerulonephritis in one study (Cook and Cowgill 1996).

A variety of classification schemes for canine glomerulonephritis have been suggested, which are usually based on human classification schemes (Murray and Wright 1974; Mullerpeddinghaus and Trautwein 1977b; DiBartola et al 1980b; Jergens 1987; Cook and Cowgill 1996; Grant and Forrester 2001b; Maxie and Newman 2007; Newman et al 2007). In human beings the commonly accepted classification system for glomerular disease is that of the World Health Organisation (WHO) "Collaborating Centre for the Histological Classification of Renal Diseases". In one study of 115 dogs with glomerular disease it was reported that the WHO criteria could be applied to canine glomerulopathy, (Vilafranca et al 1994) however, this has not been widely accepted. Currently, the more commonly accepted classification system for canine glomerulonephritis includes the following categories: membranous, proliferative, membranoproliferative and glomerulosclerosis (Jergens 1987; Grant and Forrester 2001b; Vaden 2005; Maxie and Newman 2007; Newman et al 2007).

Membranous glomerulonephritis is characterised by diffuse thickening of the glomerular basement membrane, while proliferative glomerulonephritis has increased cellularity due to proliferation of mesangial cells, glomerular endothelial and epithelial cells, and an influx of neutrophils and monocytes (Jergens 1987; Grant and Forrester 2001b; Vaden 2005; Maxie and Newman 2007; Newman et al 2007).

Membranoproliferative glomerulonephritis has a combination of the changes seen in membranous and proliferative glomerulonephritis. In glomerulosclerosis, the glomeruli become shrunken, hyalinized and hypocellular due to increased fibrosis and loss of capillaries (Grant and Forrester 2001b; Maxie and Newman 2007; Newman et al 2007). Some authors include glomerulosclerosis as a separate, fourth category of glomerulonephritis, but it can also be considered an end stage of glomerulonephritis, or any other end stage renal disease characterised by significant nephron loss. As such, it is probably better considered as a progression of the three main forms of glomerulonephritis rather than a separate entity (Newman et al 2007).

The mechanism of damage in immune-mediated glomerulonephritis involves a complex and intertwined series of events that often self-perpetuate. Antibody- antigen (Ab-Ag) complex deposition in the glomerular wall leads to complement activation, platelet aggregation and activation, and basement membrane damage. Components of complement can directly damage the basement membrane (C5b-C9), as well as further activate platelets, increase vascular permeability (C3a and C5a), and attract neutrophils and monocytes (C5a) (Abbas 2005; Newman et al 2007). These leukocytes attempt to phagocytose the Ab-Ag complexes, and in doing so release pro-inflammatory substances, lysosomal enzymes, free radicals and arachidonic acid metabolites that further damage the basement membrane (Jergens 1987; Abbas 2005; Maxie and Newman 2007). Various leukocytes also release platelet activating factor (PAF), which causes further platelet activation.

When damaged, the subendothelial collagen of the glomerular basement membrane is exposed, leading to activation of Hagemann factor, further platelet activation and release of vasoactive amines. Activated Hagemann factor then causes release of kinins and, along with platelets, activates the coagulation cascade. This results in increased conversion of fibrinogen to fibrin, which predisposes to thrombosis and subsequent ischaemic necrosis (Jergens 1987; Abbas 2005; Newman et al 2007). Kinins, vasoactive amines and the anaphylatoxins C3a and C5a all lead to increased

vascular permeability, which then predisposes to further complex deposition, perpetuating the cycle.

However, Ab-Ag complexes can also be present in glomeruli without causing glomerulonephritis, leading some authors to dispute their primary role in the mechanism of glomerulonephritis. It has been suggested that immunological damage may be secondary to primary damage by other causes e.g. hydrocarbons (Maxie and Newman 2007).

3.2.2.3.2 Amyloidosis

Amyloidosis is not a specific disease but rather a group of diseases characterised by the pathological, extracellular deposition of proteinaceous fibrils (DiBartola and Benson 1989). With increased deposition, amyloid progressively causes ischaemia and pressure necrosis of surrounding cells (Maxie and Newman 2007).

Approximately 95% of amyloid is composed of non-branching, 7-10nm fibrils arranged in a β -pleated sheet conformation. The remaining 5% consists of serum amyloid P component, glycosaminoglycans and proteoglycans (DiBartola and Benson 1989; Abbas 2005; Maxie and Newman 2007; Newman et al 2007). Amyloid persists in tissue as the β -pleated sheet conformation is insoluble and resistant to proteolysis (DiBartola and Benson 1989; Abbas 2005; Maxie and Newman 2007).

The two main forms of amyloidosis in domestic animals are immunocytic (primary) and reactive systemic (secondary). Primary amyloid is derived from AL protein, which is composed of immunoglobulin light chains and is most commonly associated with plasma cell dyscrasias (DiBartola and Benson 1989; Abbas 2005; Maxie and Newman 2007; Newman et al 2007). Primary amyloidosis is rare in the dog (Brunker 2005). Secondary amyloid is not associated with immunoglobulin, but is composed of amyloid fibril protein (AA) derived from serum amyloid A, an acute phase reactant protein produced by the liver (DiBartola and Benson 1989; Abbas 2005; Maxie and Newman 2007; Newman et al 2007). Secondary or AA type amyloid is most commonly associated with chronic disease, and while most canine amyloid cases are idiopathic they seem to be of this type (Newman et al 2007). Canine amyloidosis most commonly affects the kidney, and in most breeds, with the exception of the Shar pei, is usually deposited initially in the mesangium and subendothelium of the glomerulus (Slauson et al 1970; Abbas 2005; Maxie and Newman 2007; Newman et al 2007). In severe

amyloid deposition glomeruli are often obliterated, appearing as lowly cellular, eosinophilic, homogeneous spheres (Slauson et al 1970; Newman et al 2007).

Grossly, the kidney with amyloidosis is normal to slightly enlarged, firm and paler tan than usual, with fine granularity to the cortical surface due to the amyloid laden-glomeruli as shown in Figure 1.1. Applying iodine to the kidney will cause a brown discolouration of the glomeruli as shown in Figure 1.2. Upon subsequent exposure to dilute sulphuric acid the brown discolouration of the glomeruli becomes purple (Slauson et al 1970; Maxie and Newman 2007; Newman et al 2007). In 44 dogs with amyloidosis, gross renal lesions were similar bilaterally (Slauson et al 1970).

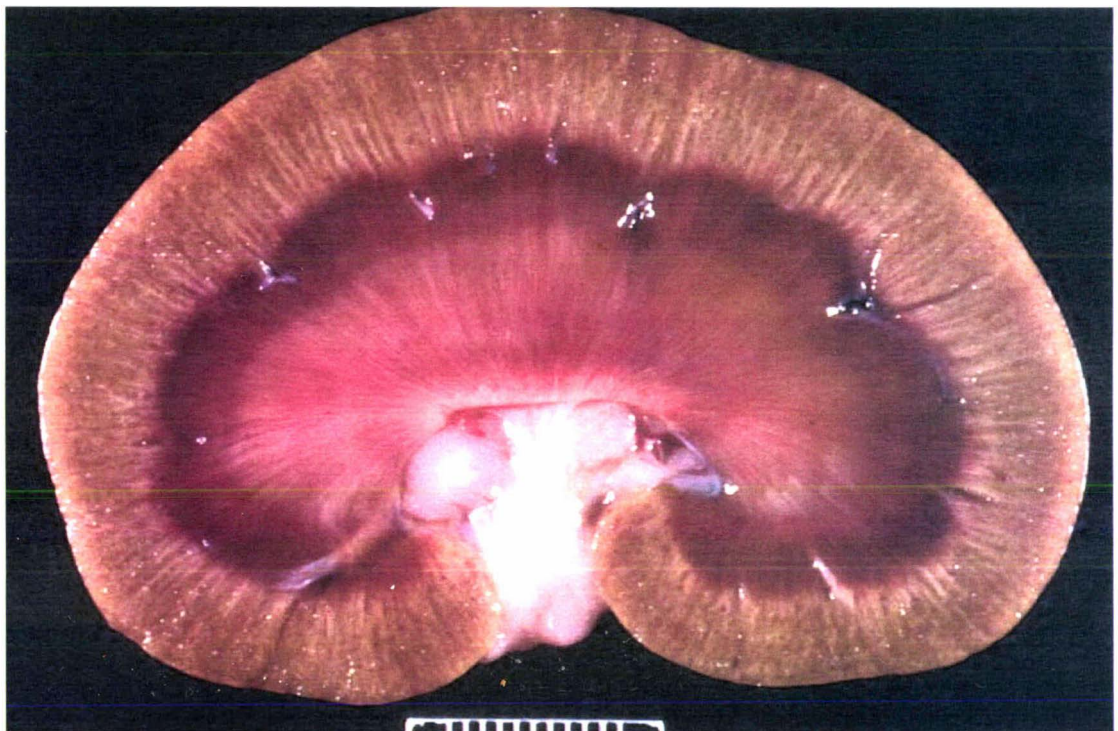


Figure 1.1 – Cut surface of a canine kidney with amyloidosis. Non-Stained. Courtesy of R.A. Fairley.

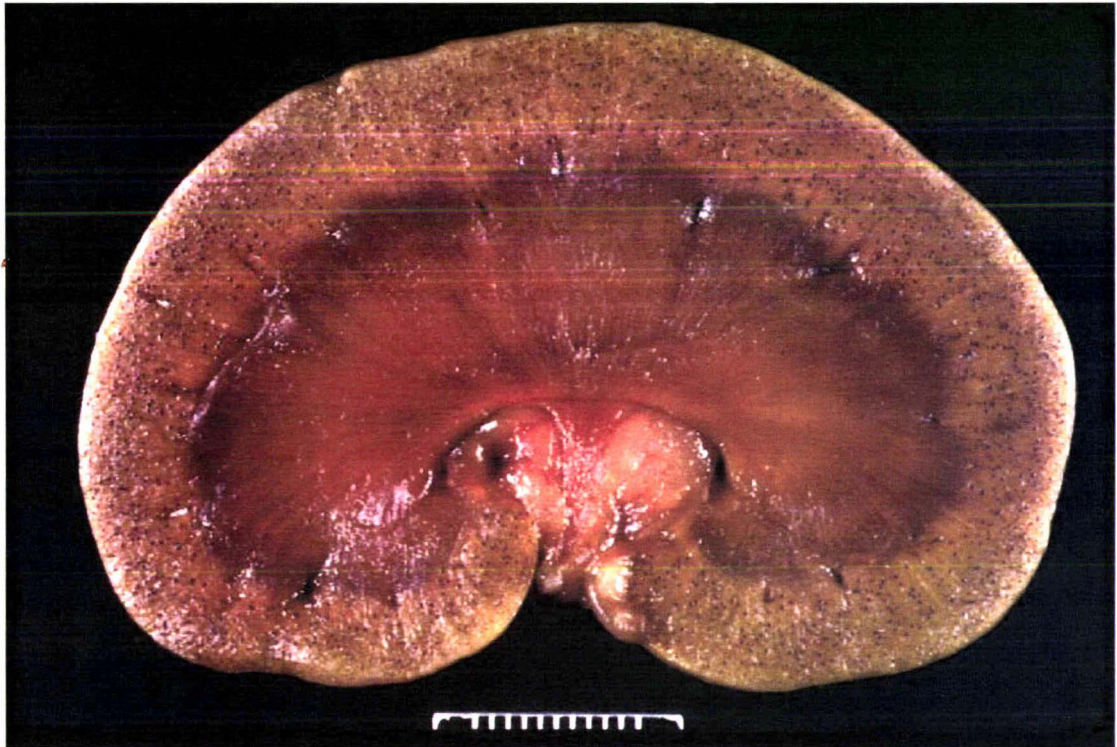


Figure 1.2 – Cut surface of a canine kidney with amyloidosis after exposure to Lugols iodine. Courtesy of R.A. Fairley.

In sections stained with haematoxylin and eosin, amyloid has an amorphous, homogeneous, eosinophilic, hyaline appearance, similar to that of other tissue deposits (DiBartola and Benson 1989) and is mildly bi-refringent under polarised light. When stained with Congo red and viewed under polarised light, the β -pleated sheet conformation of amyloid gives a distinctive green bi-refringence (DiBartola and Benson 1989; Abbas 2005; Maxie and Newman 2007). This helps to distinguish amyloid from other tissue deposits, which stain pink to red with Congo red. In reactive amyloidosis, the distinctive congo red staining can be decolourised by potassium permanganate oxidation, which can be helpful in distinguishing it from other forms of amyloidosis (DiBartola and Benson 1989).

3.3 Post-Renal Proteinuria

In post-renal proteinuria, plasma or tissue proteins are added to the urinary filtrate after it exits the renal pelvis. These proteins may originate from normal genital tract secretions, or neoplastic, haemorrhagic or exudative lesions of the urogenital tract (DiBartola et al 1980a; Lulich and Osborne 1990; Stockholm and Scott 2002; Lees et al 2005).

While post-renal proteinuria is reported to be the most common form of proteinuria, it is usually of limited magnitude and does not cause hypoalbuminaemia (Stockholm and Scott 2002). Of the possible causes of post renal proteinuria, urinary tract inflammation and haemorrhage occur most commonly (Vaden et al 2004).

Many authors have reported that in the absence of evidence of inflammation, infection or haemorrhage in the urinary sediment, persistent proteinuria is the hall mark of glomerular disease (Center et al 1985; McCaw et al 1985; Center et al 1987; Lulich and Osborne 1990; Brunker 2005; Grauer 2005c, 2005b; Maxie and Newman 2007). In several studies on canine glomerular disease, dogs with proteinuria were excluded from the study if they had evidence of haematuria, pyuria or inflammation (Barsanti and Finco 1979; Center et al 1985; Grauer et al 1985; Center et al 1987). As such, there are only limited reports on the interpretation of protein levels in urine samples with evidence of concurrent haemorrhage, infection or inflammation despite their common occurrence (Bagley et al 1991).

The presence of either whole blood (haemorrhage) or haemoglobin in a urine sample can be due to impaired haemostasis, intravascular haemolysis, vasculitis, infection, trauma, neoplasia, necrosis or diagnostic manipulations (Jansen and Lumsden 1985; Stockholm and Scott 2002). When a urine sample is positive for both protein and blood/haemoglobin, it is often unclear whether the protein present is due to renal proteinuria, or post renal proteinuria due to focal haemorrhage (Jansen and Lumsden 1985). In the past urine samples that are positive on dipstick for both protein and blood have often had the proteinuria attributed to the blood present (i.e. post renal proteinuria).

In a study of 14 hospitalised dogs, a urine sample was collected by catheterisation from each dog during the day, and a second urine sample was collected at night (McCaw et al 1985). It was noted that the second sample often contained erythrocytes, presumably due to trauma from the first catheterisation. However, no significant difference in the protein level was found between the first and second samples, despite the increased number of erythrocytes in the urine (McCaw et al 1985).

In a review of proteinuria in people it was reported that while gross haematuria may cause proteinuria on dipstick urinalysis, microscopic haematuria will not (Carroll and Temte 2000). In a study to determine the sensitivity of urine protein dipstick tests to haemoglobin, or haemoglobin and plasma protein, it was found that the haemoglobin

(blood) detection pads were 50 times more sensitive than the protein detection pads at indicating the presence of haemoglobin (Jansen and Lumsden 1985). It was reported that if the protein content of a urine sample was due solely to the haemoglobin or whole blood content, and the positive haemoglobin test pad reaction was lower than the maximum for that strip, it would not cause a concurrent positive reaction on the protein detection pad. This suggests that a positive protein dipstick result, with a less than maximal positive blood detection pad result must be due to protein in excess of that associated with haemoglobin whether from lysed erythrocytes or intact erythrocytes in whole blood (Jansen and Lumsden 1985). It was further reported that a protein level of greater than 1 g/l in a non-pigmented urine exceeded that which could be due to haemoglobin and plasma protein due to haemorrhage, and reflects true proteinuria.

An additional study assessed the effect of urine sample contamination with blood, on the urinary albumin and total protein concentrations in a single dog. The authors found that haematuria did not cause the urinary albumin to increase above 1mg/dl (0.01g/l) until a visible colour change (i.e. gross haematuria) occurred (Vaden et al 2004). It was concluded that haematuria should only be considered as a differential diagnosis for albuminuria when the urine is red (Vaden et al 2004).

Post-renal proteinuria due to inflammation occurs from exudation of plasma protein through vessel walls into the urogenital tract (Stockholm and Scott 2002). In a study of the effect of urinary tract inflammation on urinary albumin and total protein concentrations in 70 canine urine samples, it was reported the majority of samples with pyuria (defined as >5 leukocytes/HPF) did not have increased urinary albumin concentration, and had a normal urinary protein to creatinine ratio (Vaden et al 2004). However, in another study, *E. coli* cystitis was induced in 5 beagles to assess the effect on the urinary protein to creatinine ratio (Bagley et al 1991). It was found that the urinary protein to creatinine ratio of each dog increased significantly but variably post-infection, ranging from 1.5 to 40.8 (7 out of 10 results were less than 15.0) and that the magnitude of increase of the UPCR did not correlate with the magnitude of the pyuria as assessed by the number of leukocytes per high power field. It was concluded that substantial and confusing proteinuria develops in dogs with lower urinary tract inflammation (Bagley et al 1991). Historically, in urine samples with evidence of haemorrhage or inflammation, pre-renal and renal causes of proteinuria were often excluded with the proteinuria attributed to post-renal causes. However, recent work suggests that this may not be appropriate (Jansen and Lumsden 1985; Bagley et al 1991; Vaden et al 2004).

4.0 Clinical Manifestations of Glomerular Disease

4.1 Signalment

Protein-losing glomerulopathy primarily affects middle aged to older dogs, with most cases occurring in animals over 4-6 years of age (Osborne et al 1968; Slauson et al 1970; Murray and Wright 1974; Mullerpeddinghaus and Trautwein 1977b; DiBartola et al 1980b; Jergens 1987; DiBartola et al 1989; Cook and Cowgill 1996). Exceptions to this occur in hereditary glomerular diseases where manifestations of clinical signs may occur in younger animals than in sporadic glomerular disease.

There is some disagreement in the literature as to whether a breed disposition exists for glomerulopathy. In a study of 51 dogs with proteinuria (Biewenga and Gruys 1986) and 2 studies of dogs with amyloidosis (Osborne et al 1968; Slauson et al 1970) no breed disposition was reported. However in another study of 59 dogs with amyloidosis, it was reported that beagles, collies and walkers hounds had an increased risk of developing amyloidosis, while german shepherds and cross breed dogs had a decreased risk (DiBartola et al 1989).

There is similar discord as to whether a gender disposition exists for glomerulopathy. In the same 59 dogs it was reported that females were more commonly affected with amyloidosis than males (DiBartola et al 1989), and in a study of 31 dogs with amyloidosis 19 were female and 12 were male (Cook and Cowgill 1996). Conversely, in a study of 44 dogs with amyloidosis no sex disposition was noted (Slauson et al 1970).

In 2 reports of 106 dogs (Cook and Cowgill 1996) and 41 dogs with glomerulonephritis (Center et al 1987) no clear gender disposition was reported.

4.2 Clinical Signs

In cases of mild to moderate proteinuria the clinical signs are often limited, non-specific and not necessarily related to renal dysfunction (DiBartola et al 1980b; Jergens 1987). Several authors have reported that weight loss and lethargy are the most commonly reported clinical signs in dogs with glomerular disease (DiBartola et al 1980b; Grauer 2005c), while other possible clinical signs include anorexia, vomiting, ascites, polyuria and polydipsia (DiBartola et al 1989; Cook and Cowgill 1996). Where proteinuria occurs secondarily to an underlying disease process (i.e. infectious, inflammatory or neoplastic disease) the clinical signs often reflect the primary problem, or when present, hypoproteinaemia (Jergens 1987; Grant and Forrester 2001b; Grauer 2005c).

As the glomerular damage becomes progressively worse, renal failure may occur. The clinical signs then reflect those of renal failure and may include: polyuria, polydipsia, anorexia, vomiting, diarrhoea and possibly non-regenerative anaemia (Jergens 1987; Cook and Cowgill 1996; Grant and Forrester 2001b; Bruner 2005).

Thromboembolism, associated with low ATIII activity, is a relatively commonly reported complication of glomerular disease, which has been associated with high mortality (Slauson et al 1970; DiBartola et al 1989; Cook and Cowgill 1996).

Nephrotic syndrome is a manifestation of severe glomerular disease characterised by proteinuria, hypoproteinaemia/hypoalbuminaemia, and hypercholesterolaemia, with oedema and ascites possible clinically (Slauson et al 1970; Center et al 1987; Relford and Leed 1996). Systemic hypertension and hypercoagulability are relatively frequent complications, and clinical signs of their effects may also be noted (Cook and Cowgill 1996; Grauer 2005c).

Nephrotic syndrome appears to be an uncommon finding in dogs with glomerular disease (DiBartola et al 1989; Cook and Cowgill 1996). Only 6 (15%) of 41 dogs with glomerulonephritis, 3 (14%) of 21 dogs with non specified glomerular disease, and 3 (7%) of 44 dogs with renal amyloidosis had all the hallmarks of nephrotic syndrome (Slauson et al 1970; DiBartola et al 1980b; Center et al 1987).

5.0 Detection and Diagnosis of Proteinuria

A small amount of protein may be present in normal dog urine (Barsanti and Finco 1979; DiBartola et al 1980a). The hall mark of glomerular disease is persistent, significant proteinuria in the absence of evidence of inflammation or haematuria in the urine sediment (Center et al 1985; Center et al 1987; Brunker 2005). As many cases of significant proteinuria are not accompanied by hypoalbuminaemia, a complete urinalysis (physical examination, chemistry, sediment and specific gravity) should be included in all patient diagnostic plans (Cook and Cowgill 1996).

Caution must be exercised in the interpretation of urinalysis results because the various chemical tests used to measure the amount of protein in urine samples differ in their sensitivity and specificity. Also, the urine specific gravity or concentration of the urine sample affects the relative concentration of protein present, which will influence the test results and their interpretation (DiBartola et al 1980a).

There is some disagreement in the literature as to whether urine protein concentration is affected by gender or method of urine sample collection. In two studies it was reported that the urine protein concentration in an individual animal was not significantly affected by method of collection (Barsanti and Finco 1979) or sex (Biewenga and Gruys 1986). In a study of 159 dogs there was no statistical difference in protein levels from male and female dogs when the urine was collected by cystocentesis or catheterisation (Barsanti and Finco 1979). However, there was a statistically significant difference between the two sexes when the urine was from a voided sample. A second study also noted this difference, but it was not statistically significant (Grauer et al 1985). This mild difference suggests that the increased protein in the male dog was due to urethral or preputial origin (Barsanti and Finco 1979; Grauer et al 1985).

5.1 Urine Chemistry Dipsticks

Semi-quantitative urine chemistry dipsticks are commonly used to screen for proteinuria in canine patients due to their convenience, rapidity of results, ease of use, and low cost (James et al 1978; White et al 1984; Center et al 1985). Due to the limitations of urine dipsticks, it has been recommended that positive protein results should be confirmed and/or quantified by another method of protein assessment.

The reagent test pad on the urine dipstick contains tetrabromophenol blue, a colorimetric pH indicator. As negatively charged proteins bind to the test pad they cause a change in colour that corresponds to the concentration of the protein (DiBartola et al 1980a; Moore et al 1991; Stockholm and Scott 2002). Urinary dipsticks are more sensitive to albumin than globulin and Bence-Jones proteins, and as such are better indicators of urine albumin than urine total protein (James et al 1978; Barsanti and Finco 1979; DiBartola et al 1980a; Stockholm and Scott 2002).

Artefactually low urinary dipstick protein levels have been reported to occur in very dilute or acidic urine samples, and in urine samples with low albumin to total protein ratios (James et al 1978; DiBartola et al 1980a). However, in a study of 151 canine and feline urine samples no false negative protein dipstick results were found when compared to the sulphosalicylic acid precipitation method (Moore et al 1991). The authors of that study concluded that the dipstick method of protein assessment was an adequate screening test for determining whether a urine sample had an increased protein level requiring further quantification.

It is generally accepted that alkaline urine can cause artefactually elevated protein results on urinary dipsticks (Barsanti and Finco 1979; DiBartola et al 1980a; Stockholm and Scott 2002). As such it is important to confirm all positive results with an alternative assessment method, such as the sulphosalicylic acid precipitation method (Stockholm and Scott 2002). Artefactually elevated urinary dipstick protein results have also been reported with concentrated urine samples, with gross haematuria, and in samples containing mucus, semen or white blood cells (Carroll and Temte 2000; Stockholm and Scott 2002).

An important source of error of protein assessment by urinary dipsticks is the large potential for variation between different technicians (James et al 1978). Several companies offer brand specific automated urinary dipstick readers to combat this type of error.

Urinary dipsticks have a reported sensitivity in the literature of 20mg/dl (0.2g/l) (DiBartola et al 1980a), however the sensitivity varies by brand, and ranges from 15mg/dl (0.15g/l) to 30mg/dl (0.30g/l).

5.2 Non-Dipstick Urinary Protein Assessment

There are numerous biochemical methods for the assessment of protein and comments on some of the different methods are below.

5.2.1 Sulphosalicylic Acid Turbidity (SSA)

In the sulphosalicylic acid turbidity test the denaturation of protein with acid forms a precipitate, which increases turbidity of the solution. The turbidity level can then be compared to standard solutions to give a qualitative 1+ to 4+ assessment (Osborne et al 1995). Alternate SSA methods use spectrophotometry to allow more quantitative results (Stockholm and Scott 2002).

Like many of the protein assessment methods, the SSA method detects albumin better than globulin and as such can be affected by decreased albumin: globulin ratios. Unlike urinary dipsticks the SSA method can also detect Tamm-Horsfall and Bence Jones proteins (Osborne et al 1995; Stockholm and Scott 2002; Brunner 2005)

Artefactually increased urinary protein results have been reported in urine samples contaminated with radiographic contrast media, or in patients that have had large doses of penicillin, tolbutamide, sulfisoxazole, and tolmetin sodium (DiBartola et al 1980a; Osborne et al 1995).

Artefactually decreased protein measurements may be seen in highly buffered alkaline urine (Osborne et al 1995; Stockholm and Scott 2002).

5.2.2 Trichloroacetic Acid (TCA)

Like the SSA method the TCA test is based on assessment of the increase in solution turbidity due to the precipitate formed from the denaturation of protein with acid. In comparison to the SSA method the TCA test is less affected by the albumin: globulin ratio, but is temperature dependant and must be performed within a narrow range of 20-25°C (Stockholm and Scott 2002).

5.2.3 Trichloroacetic Acid-Ponceau S (TCA-PS)

A modified TCA process involves the precipitation of protein, removal of the supernatant and spectrophotometric colour assessment (DiBartola et al 1980a). Falsely high values can be seen if the supernatant is not all removed, and falsely low values may be seen if any of the precipitate is lost during decanting (Barsanti and Finco 1979).

The advantages of the TCA-PS method is that the test is relatively simple to perform and only small sample amounts are needed. The test is not dependant on temperature and is not affected by the albumin to globulin ratio (Barsanti and Finco 1979; DiBartola et al 1980a; Dilena et al 1983). The main disadvantage is the requirement for spectrophotometry (DiBartola et al 1980a). This test has a sensitivity of 2mg/dl (0.02g/l).

5.2.4 Coomassie Brilliant Blue (CBB)

The CBB method uses colorimetric assessment whereby the amount of dye that binds to the amine groups of amino acids is proportional to the quantity of protein present (Stockholm and Scott 2002). The advantage of the CBB method is that it is simple to perform, will detect Bence Jones proteins and is only minimally affected by the albumin to globulin ratio (Barsanti and Finco 1979; Stockholm and Scott 2002). The main disadvantage is that the CBB method requires spectrophotometry (Barsanti and Finco 1979). In one report the CBB method was noted to be sensitive and precise (Barsanti and Finco 1979) however, another author reported that the CBB method had a narrow range of linearity and poor precision (Dilena et al 1983).

The CBB and TCA-PS methods of urinary protein measurement were compared in a study of 145 random urine samples (Barsanti and Finco 1979). It was found that the two methods were the most comparable at low protein concentrations, and as the concentration increased the difference between the two methods protein results increased, with the TCA-PS method giving lower results (Barsanti and Finco 1979).

5.2.5 Benzethonium Chloride

In urinary protein assessment by the benzethonium chloride method, the sample is incubated in an alkaline solution containing EDTA, which denatures the protein and

eliminates interference from magnesium ions. Benzethonium Chloride reacts with protein to produce a turbidity that is proportional to the amount of protein present. An advantage of this method is that it detects albumin and globulin similarly, and does not stain plastic or glassware (Stockholm and Scott 2002).

5.3 24 hour urine sample collection for protein assessment

In the renal tubules water is reabsorbed under the influence of anti-diuretic hormone and the interstitial concentration gradient (Stockholm and Scott 2002; Newman et al 2007). Reabsorption of water alters the concentration of the urine, as reflected by the urine specific gravity and urine volume (Grauer et al 1985). The concentration of protein in a single urine sample is also altered by the amount of water reabsorbed in the tubules, so with increasing urine concentration, the protein level may artefactually increase. As such, urinary protein assessment on a single urine sample is invalid as the protein level may fluctuate with urine concentration. Prior to the urinary protein to creatinine ratio, the 24 hour urine collection was the only way to minimise error due to fluctuations in urine specific gravity, and thereby accurately assess daily urinary protein loss (DiBartola et al 1980a; White et al 1984; Grauer et al 1985; McCaw et al 1985). When performed properly the 24 hour urine collection method has historically been the gold standard to quantitate the daily urinary protein excretion in the dog (White et al 1984; Grauer et al 1985; McCaw et al 1985).

To perform a 24 hour urine collection the dog is catheterised to remove all urine. The dog is then weighed and placed in a metabolic cage, and all urine over the next 24 hour period is collected. At the end of the 24 hour period the dog is re-catheterised to collect any remaining urine. An alternative to the metabolic cage is an in-dwelling urinary catheter. The total urine collected is then pooled together, and the protein is measured on an aliquot of the pooled urine. This is multiplied by the total volume of urine produced over 24 hours to calculate the 24 hour urinary protein loss (DiBartola et al 1980a).

While being the most accurate way to collect a urine sample to assess daily protein loss, disadvantages of the 24 hour collection method include the requirement for a metabolic cage, which are uncommon outside of research facilities, or multiple/indwelling catheterisation, which increases the risk of urinary tract infection. There is also the risk of specimen contamination, inadvertent loss of some of the

specimen, and a significant time delay to quantifying the protein loss and making a diagnosis (White et al 1984; Grauer et al 1985; McCaw et al 1985). Even with proper sampling facilities 24 hour urine sample collection can be difficult, time consuming and expensive, and as such is often impractical or unfeasible in the clinical setting (Barsanti and Finco 1979; Grauer et al 1985; McCaw et al 1985; Grauer 2005c).

Several studies have been done to assess the protein content in healthy and abnormal dogs as shown in Table 1.2.

Table 1.2 - 24 hour urinary protein loss in mg/kg for healthy and proteinuric dogs.

Author	State	# Dogs	Protein mg/kg	Method
(White et al 1984)	Healthy	8	1.9 – 11.7	TCA-PS
(Center et al 1985)	Healthy	19	0.2 – 7.7	TCA-PS
(Grauer et al 1985)	Healthy	16	0.6 - 5.1	CBB
(Biewenga et al 1982)	Healthy	29	2 - 22	TCA-PS
(White et al 1984)	Proteinuric	10	32.2 – 271.1	TCA-PS
(Center et al 1985)	Proteinuric	38	7.5 – 533.7	TCA-PS
(Grauer et al 1985)	Proteinuric	14	12.2 – 287.5	CBB
(DiBartola et al 1980b)	Proteinuric	21	31.95 – 959.6	TCA/ TCA-PS
(Biewenga and Gruys 1986)	Proteinuric	13	47 - 897	TCA-PS

It has been suggested that normal dogs should have 24 hour protein loss of less than 400mg/day, or less than 30mg/kg/day (Barsanti and Finco 1979).

From these studies some trends relating the level of proteinuria and cause of glomerular disease emerged. It was reported that dogs with amyloidosis have a greater level of proteinuria than dogs with glomerulonephritis (DiBartola et al 1980b; Center et al 1985; Center et al 1987; Cook and Cowgill 1996). Of dogs with glomerulonephritis, those with membranous glomerulonephritis were reported to have greater daily protein losses in comparison to the other forms of glomerulonephritis (Biewenga and Gruys 1986; Center et al 1987). While the magnitude of proteinuria was found to be correlated more with the nature of the glomerular lesion than with the stage of renal disease (DiBartola et al 1980b), due to the considerable overlap in the level of proteinuria between dogs with amyloidosis and glomerulonephritis the absolute magnitude of protein loss in mg/kg/day in an individual dog can not be used to diagnose the nature of the lesion.

5.4 Urinary Protein to Creatinine Ratio (UPCR)

The variability in urine volume and urine concentration has previously necessitated 24 hour urine collection in order to accurately measure urinary protein. By relating urinary protein to urinary creatinine concentration, variations in urine volume and specific gravity are negated (Center et al 1985; Grauer et al 1985).

Plasma creatinine concentration is proportional to total muscle mass, and its production remains relatively constant on a daily basis (Carroll and Temte 2000). Creatinine is freely filtered from the plasma through the glomerulus, and is neither reabsorbed nor significantly secreted by the tubules (Finco 1995). As such the concentration of creatinine in the glomerular filtrate is the same as the plasma creatinine concentration (Finco 1995). If urinary creatinine clearance is reduced then the urinary protein loss through the glomerulus should be similarly affected, as creatinine is more readily filtered through the glomerulus (Stockholm and Scott 2002). If the glomeruli are damaged and more protein enters the urine, the rate of protein excretion will be increased in comparison to creatinine excretion. When renal function is stable, glomerular filtration and tubular concentration mechanisms affect protein and creatinine similarly (Lulich and Osborne 1990).

The urinary protein to creatinine ratio (UPCR) has no units, and is calculated by dividing the urinary protein concentration (mg/dl) by the urinary creatinine concentration (mg/dl). In the International System of Units (SI) the formula is:

$$\text{UPCR} = \frac{(\text{urinary protein g/l})(100)}{(\text{urinary creatinine mmol/l}) / (1000) / (0.0884)}$$

$$\text{UPCR} = \frac{(\text{urinary protein g/l})(8840)}{(\text{urinary creatinine mmol/l})}$$

In the 1980's the urinary protein to creatinine ratio was validated for the quantification of urinary protein in human beings on single voided urine samples (Ginsberg et al 1983; Schwab et al 1987). In a study of 101 ambulatory and hospitalised human patients, the UPCR was highly correlated with 24 hour urine collection protein assessment in both genders, independent of renal function, age or body size (Schwab et al 1987). Further, the UPCR was reported not to be inaccurate or unsuitable for any level of renal dysfunction, degree of proteinuria or specific disease. It was reported that the UPCR from a single voided urine sample in human patients, is a convenient,

inexpensive, accurate, and reliable estimate of total proteinuria (Schwab et al 1987). In a further study of 46 human beings it was suggested that a single urine sample UPCr may even be more accurate than the 24 hour collection, due to possible errors with 24 hour urine collection (Ginsberg et al 1983).

Several studies were done in the 1980's to assess the validity of the UPCr in canine patients. In a study of 10 beagles, the UPCr from 24 hour pooled urine samples correlated better with 24 hour protein loss than did protein concentration alone (Barsanti and Finco 1979). A second study of 19 healthy dogs and 38 dogs with protein losing nephropathy reported a positive correlation between the UPCr from a 24 hour pooled urinary sample, and the urinary protein loss in 24 hours per kg of body weight (Center et al 1985).

In two studies of 18 and 30 dogs, highly significant correlations ($P < 0.0001$ and $P < 0.01$ respectively) between the UPCr on a single urine sample and the 24 hour urinary protein loss per kg of bodyweight were reported (White et al 1984; Grauer et al 1985). It was further reported that the type and severity of renal dysfunction did not prevent significant correlation between UPCr and the 24 hour protein loss per kg (White et al 1984). From these studies it was concluded that the UPCr from a 24 hour pooled urine sample or a single urine sample was an accurate measurement of 24 hour protein loss in the dog (Barsanti and Finco 1979; White et al 1984; Center et al 1985; Grauer et al 1985).

Changes in the rate of urinary protein excretion in man have been observed with time of day, activity level, and body position (orthostatic proteinuria) (White et al 1984; Grauer et al 1985; Jergens et al 1987; Carroll and Temte 2000). Postural proteinuria has not been documented in the dog (DiBartola et al 1980a).

A study of 10 healthy dogs reported no significant difference in the UPCr of each dog when fasting compared to the UPCr when they were fed (Jergens et al 1987). The UPCr was also reported to be unaffected by the sex of the dog (McCaw et al 1985), or collection time (McCaw et al 1985; Jergens et al 1987).

Urine collected by either cystocentesis or midstream voiding are suitable for measurement of the urinary protein to creatinine ratio (Grauer et al 1985; Lulich and Osborne 1990; Bagley et al 1991). An additional study noted no significant difference

in protein concentration between cystocentesis, voided or catheterised samples (Barsanti and Finco 1979).

Overall, the UPCr from a random, single urinary specimen accurately reflects the 24 hour protein loss in the dog, and is a rapid, dependable and sensitive technique for the quantitative estimation of proteinuria over a broad range of daily protein excretions (White et al 1984; Grauer et al 1985; Lulich and Osborne 1990).

5.4.1 UPCr Values

Several studies have reported UPCr results in groups of healthy and proteinuric adult dogs as shown in Table 1.3 below. Additionally, in a study of 45 newborn Rottweiler puppies it was noted that although a UPCr of up to 2.0 was reported to be physiologically normal in young puppies, in this study much higher UPCr (up to 10.0) were found in very young puppies (0-3 days) that all progressed to become normal, healthy dogs (Schafer-Somi et al 2005).

Table 1.3 – Trial results for UPCr in healthy and proteinuric dogs.

Author	Sample	State	# Dogs	UPCr	Method
(Center et al 1985)	24 hour	Healthy	19	0.01 – 0.38	TCA-PS
(White et al 1984)	single	Healthy	8	0.08 – 0.54	TCA-PS
(Grauer et al 1985)	Single	Healthy	16	0.02 – 0.14	CBB
(McCaw et al 1985)	Single	Healthy	14	0.0 – 0.31	CBB
(Jergens et al 1987)	single	Healthy	10	0.09 -1.02	CBB
(Center et al 1985)	24 hour	PLN*	38	0.47 – 46.65	TCA-PS
(McCaw et al 1985)	24 hour	Proteinuric	8	2.33 – 17.31	CBB
(Grauer et al 1985)	Single	Proteinuric	14	0.48 – 15.0	CBB
(McCaw et al 1985)	Single	Proteinuric	5	2.0 – 18.36	CBB
(White et al 1984)	single	Proteinuric	10	1.09 – 8.63	TCA-PS

*PLN = protein losing nephropathy

From these trials authors recommended cut off levels for the normal, equivocal, and abnormal range for the UPCr in dogs were suggested as shown below in Table 1.4.

Initially UPCr values less than 1.0 were considered to be normal for dogs, however recently this has been reduced to less than 0.5, and may reduce further in the future (Lulich and Osborne 1990; Stockholm and Scott 2002; Gary et al 2004; Lees et al 2005; Grauer 2007). UPCr values of 0.5 to 1.0 are considered equivocal. UPCr

values greater than 1.0 are considered abnormal, and UPCr measurements greater than 2.0 are suggestive of glomerular disease (Lees et al 2005; Grauer 2007).

Table 1.4 – Recommendations for the normal, equivocal and abnormal range of UPCr in dogs.

Author	Normal	Equivocal	Abnormal
(Center et al 1985)	<0.4	0.4 – 2.0	>2.0
(McCaw et al 1985)	<1.0	1.0 – 2.0	>2.0
(Grauer et al 1985)	<0.2	0.2 – 1.0	>1.0
(Lulich and Osborne 1990)	<0.5	0.5 – 1.0	>1.0

5.4.2 Interpretation of the Urine Protein to Creatinine Ratio

Use of the UPCr to quantify urinary protein loss is based on the premises that glomerular filtration rate is stable, that urinary protein loss is constant throughout the day, and that the glomerular filtration and tubular concentration of urine affect protein and creatinine similarly (White et al 1984; Lulich and Osborne 1990). In acute renal failure, rapidly changing renal function may affect the reliability of the UPCr (White et al 1984).

In the absence of evidence of inflammation, infection or haemorrhage in the urinary sediment, persistent proteinuria is the hall mark of glomerular disease (Center et al 1985; McCaw et al 1985; Center et al 1987; Lulich and Osborne 1990; Brunker 2005; Grauer 2005c, 2005b; Maxie and Newman 2007). Interpretation of the UPCr should always be done in conjunction with a complete urinalysis, as the results of many studies on UPCr can not be extrapolated to include dogs with haematuria or pyuria (Grauer et al 1985). Proteinuria can often be found in urine samples that have cytologic evidence of inflammation/infection or haemorrhage. In these cases the validity of the UPCr is then questionable due to the inability of the UPCr to differentiate between renal and post-renal proteinuria (Bagley et al 1991)

In a study of the effect of iatrogenic blood contamination on the UPCr in a single dog, a urine sample was serially contaminated with blood (PCV 42%) to concentrations of 10 to 75% (Bagley et al 1991). The UPCr ranged from 1.53 in the 10% sample, to 30.6 in the 75% sample, and the magnitude of haematuria did not correlate with the magnitude of increase of UPCr. As the 10% and all subsequent samples were visibly red, the authors concluded that gross blood contamination of a urine sample increases

the urine protein to creatinine ratio. It was further suggested that a small amount of blood contamination in the urine may not significantly affect the diagnostic usefulness of the urine protein to creatinine ratio, but that the extent to which the UPCr is affected by haemorrhage was unknown (Bagley et al 1991).

A later similar study designed to assess the effect of blood on the UPCr serially contaminated a single canine urine sample with blood (PCV 55%) to 1:25,600 (0.0039%) to 1:400 (0.25%) levels (Vaden et al 2004). The two urine samples with the highest level of blood contamination (0.125% and 0.250%) were reported as red, while still having a UPCr less than 0.3 (Vaden et al 2004). It was concluded in this report that haematuria should only be considered as a differential diagnosis as a cause of proteinuria as assessed by the UPCr if the urine is red in colour.

While it was suggested in one study, that if both protein concentration and UPCr are used together to detect renal disease it may be able to replace the more labour intensive task of microscopic urinalysis (Fettman 1989), it has not become generally accepted.

5.4.3 Prognostic Use of the UPCr

In a recent prospective cohort study of 45 dogs with chronic renal failure the association of the magnitude of initial UPCr with risk of development of uraemic crisis and death was examined (Jacob et al 2005). From this study it was reported that dogs with chronic renal failure with a UPCr greater than 1.0 had a significantly greater risk of development of uraemic crisis, and death. These authors concluded that the relative risk of development of uraemic crisis and death in dogs with UPCr greater than 1.0 was three times greater than dogs with UPCr less than 1.0. The relative risk of dogs with a UPCr greater than 3.0 for development of uraemic crisis and death was significantly greater than compared to the group of dogs with UPCr greater than 1.0, and for each increase in UPCr of 1 unit the relative risk of adverse outcomes increased by 1.5 times (Jacob et al 2005).

5.4.4 Monitoring Progress with the UPCr

Serial measurement of the UPCr is often used to monitor changes in disease severity and response to treatment. In the 2004 ACVIM Forum Consensus Statement for the Assessment and Management of Proteinuria in Dogs and Cats it was suggested from

observations that the UPCr would need to change by at least 40% (especially in the lower range) to conclude with a high level of confidence that the magnitude of proteinuria had actually changed (Lees et al 2005). In an abstract in the 2005 ACVIM abstracts section of the Journal of Veterinary Internal Medicine (JVIM), authors reported findings from serial UPCr assessments on 45 female X linked nephropathy (XLHN) carrier dogs (Nabity et al 2005). The data suggested that serial UPCr in individual dogs can differ up to 50% before it can be concluded that the magnitude of proteinuria had actually changed. These authors published a further abstract in the 2007 ACVIM abstracts section of JVIM based on serial UPCr testing in 48 female XLHN dogs. It was reported that to show a significant difference in serial values of the UPCr, the UPCr must change by at least 80% for low values (near 0.5), whereas at high values (near 12) the UPCr must change by at least 35% (Nabity et al 2007). It was suggested that these guidelines for female dogs with XLHN may be helpful for the interpretation of serial UPCr monitoring in dogs with other glomerulonephropathies.

5.4.5 Differentiation of Causes of Glomerular Disease with the UPCr

In a study of the 24 hour UPCr in 38 dogs with protein losing nephropathy, dogs with amyloidosis had higher UPCr than did dogs with glomerulonephritis (median UPCr for amyloidosis (n=6) was 22.50 compared to 5.73 for glomerulonephritis (n= 26) (Center et al 1985). In an additional study of 137 dogs with protein losing nephropathy, dogs with amyloidosis had significantly higher UPCr than dogs with glomerulonephritis (Cook and Cowgill 1996). While it has been accepted that very high UPCr is more common with amyloidosis, the absolute magnitude of the UPCr is not sufficient to differentiate between amyloidosis and immune mediated glomerulonephritis, as there can be considerable overlap in the UPCr values seen with each. Absolute differentiation between causes of glomerular proteinuria requires renal biopsy.

5.5 Microalbuminuria

Microalbuminuria is defined as the presence of albumin in the urine in quantities greater than normal, but below the limit of detection of standard semi-quantitative urine protein-screening methodology (i.e. urinary dipstick assays) (Grauer 2005b; Whittemore et al 2006; Grauer 2007). The upper limit of this range is defined as the lower limit of detection of urinary protein by urine dipstick assays, which the previously mentioned authors have reported as <30mg/dl (0.3g/l). However, the product

manufacturer information for two brands of dipsticks (Bayer Healthcare, Leverkusen, Germany and Roche Diagnostics, Basel, Switzerland) report their lower limit of detection as being 15mg/dl to 25mg/dl.

While the upper limit of the range for microalbuminuria can be fairly clearly defined, the lower limit is less easily determined, as it depends on the level of urinary protein defined as normal (Grauer 2007). In 2001 it was noted that the normal range of urinary albumin in dogs had not been established (Pressler et al 2001). Subsequently, there has not been published data to establish the normal range of urinary albumin in dogs, yet the lower limit for the microalbuminuria range is regularly quoted as 1mg/dl (0.01g/l), and the range of microalbuminuria defined as 1.0 to 30mg/dl (0.01 to 0.30 g/l) (Vaden et al 2001; Grauer et al 2002; Pressler et al 2003; Radecki et al 2003; Whittemore et al 2003; Grauer 2005b, 2005c; Whittemore et al 2006; Grauer 2007). The value for normal urinary albumin of less than 1 mg/dl (0.01g/l) was defined based on the log mean plus 2 standard deviations of populations of apparently healthy dogs (Grauer 2007). The data on which this statement is based is not published, and appears to be determined from data from the Heska Corporation (Fort Collins, Colorado, USA) as reported in an information article sponsored by the Heska Corporation that was originally found on their website, but as of July 2007 was no longer there.

In a study of 145 normal dogs it was reported that a small amount of total protein (not albumin alone) may be present in normal dog, with results ranging from 4 to 64mg/dl (benzethonium chloride method), and 4 to 95mg/dl (TCA-PS method) (Barsanti and Finco 1979). It was further suggested that a urinary total protein concentration of <65mg/dl (0.65 g/l) should be considered normal at any specific gravity regardless of the method being used. More recently authors have reported that the level of protein regarded to be normal has decreased (Grauer 2005b, 2007), however no full text published data was found to support this.

Microalbuminuria has been shown in humans to be useful in detecting early renal disease in hypertensive, diabetic, and non diabetic patients and underlying inflammatory or systemic diseases associated with glomerulopathy (Grauer 2005b; Whittemore et al 2006; Grauer 2007). This has led to investigation as to whether the detection of microalbuminuria would be similarly useful in canine patients.

The E.R.D.-HealthScreen® Canine Urine Test is a semi-quantitative, in-clinic, immunoassay that has been developed by the Heska Corporation for the detection of microalbuminuria in dogs. The test is reported to be highly sensitive, specific and simple to use. As reported on the company website the E.R.D.-HealthScreen® Canine Urine Test is intended to be used as a test to determine whether more comprehensive evaluation for early renal damage is indicated.

There is little peer reviewed published literature on the use and interpretation of canine microalbuminuria. Abstracts of 11 articles associated with urinary albumin testing and microalbuminuria were published in the 2001 to 2003 Journal of Veterinary Internal Medicine (JVIM) abstracts section, but only two have been subsequently published in full. It is perhaps worth noting that employees of the Heska Corporation are listed as authors in at least 8 of these 11 abstracts. Much of the literature available references conclusions from these abstracts, in the absence of full text articles in print. As such, some of the conclusions drawn have yet to be peer reviewed, despite their wide dissemination in the literature.

An abstract in the 2002 ACVIM Abstracts section of JVIM reported findings from serial, biweekly urine sample collections from 36 males with XLHN and 10 normal littermates, while they were 8 to 30 weeks of age (Lees et al 2002). It was reported that persistent albuminuria was observed 0 to 16 weeks (median 4 weeks) before the onset of overt proteinuria in these dogs. From these results, it was suggested that screening for albuminuria could be an effective method for early detection of other progressive renal diseases involving glomerular damage in dogs. This abstract has been quoted by Grauer (2005b, 2007) in the literature to suggest that microalbuminuria is a good indicator of early renal disease in dogs, often without the mean of 4 weeks noted. It has been suggested that the ability to diagnose renal disease “earlier” may allow clinicians earlier and more appropriate intervention to possibly slow the progression to renal failure. The clinical relevance of this is highly questionable as it would be very uncommon to test asymptomatic canine patients as frequently as every 4 weeks with any test. Most dogs are presented to their veterinarian annually (or less frequently), and as such the ability to detect microalbuminuria in a population of dogs with hereditary glomerular disease, a median of 4 weeks earlier than overt proteinuria, would not seem clinically relevant to the diagnosis or enhanced management of renal disease in the general canine population.

Another abstract in the 2002 ACVIM Abstracts section of JVIM reported findings from 12 dogs that were experimentally infected with *Dirofilaria immitis* L3 larvae, in which all dogs developed microalbuminuria (Grauer et al 2002). It was further reported that the onset of microalbuminuria preceded the onset of overt proteinuria by unspecified durations of time. This abstract has also been referenced to support the use of microalbuminuria as a good indicator of early renal disease in dogs by Grauer (2005b, 2007).

The third of the three abstracts often referenced to support the claim that microalbuminuria is a good indicator of early renal disease in dogs is found in the 2003 ACVIM Abstracts section of JVIM. In this article, 17 dogs (9 pure bred soft coated wheaten terriers and 8 SCWT x beagles) had multiple urine samples assessed for microalbuminuria (Vaden et al 2001). Thirteen (76%) of the 17 dogs had microalbuminuria in one or more samples. Only 3 of the 13 dogs developed subsequent overt proteinuria (UPCR >0.5). Of these 3 dogs, 2 developed overt proteinuria at the same time as onset of microalbuminuria, while the third dog developed microalbuminuria one year before proteinuria.

In a recent review article, it was reported that microalbuminuria seems to be a good indicator of early renal disease in dogs, particularly in those diseases that involve the glomerulus (Grauer 2007). As previously mentioned all three references quoted to support this statement were all from abstracts only which have not been published as full text articles. The author also reports that due to the high sensitivity of the microalbuminuria assay, benign, or physiologic proteinuria may cause some positive microalbuminuria results. In that scenario the proteinuria should be transient and subsequent microalbuminuria assays should be negative (Grauer 2007).

In one of the full text studies published, 408 dogs that were negative for urinary protein by dipstick assessment were assessed for microalbuminuria (Whittemore et al 2006). Statistically significant associations between dogs with positive results on the quantitative urinary albumin test for microalbuminuria, and the presence of azotaemia, neoplasia and non-specified "other" diseases was reported. On the basis of these results the authors support the use of testing for microalbuminuria in geriatric patients even when the urinary dipstick for protein is negative, to increase the likelihood of detecting occult disease (Whittemore et al 2006).

While the clinical importance of low grade proteinuria remains unknown (Whittemore et al 2006), currently the published uses of microalbuminuria testing in canine patients are suggested as follows (Whittemore et al 2006; Grauer 2007).

- To confirm positive dipstick test results (Whittemore et al 2006).
- To detect protein loss below the limits of detection of urine dipsticks (Whittemore et al 2006).
- When conventional screening tests for proteinuria:
 - Produce equivocal or conflicting results or false positives are suspected
 - Are negative in apparently healthy older dogs and a more sensitive screening test is desired,
 - Are negative in apparently healthy young dogs with a familial risk for developing proteinuric renal disease and a more sensitive screening test is desired (Grauer 2007).
- When a previous microalbuminuria test result(s) was positive and monitoring for persistence or progression of the microalbuminuria is desired (Grauer 2007).
- To test for occult disease in geriatric patient in association with other routine geriatric screening tests (Whittemore et al 2006).

In the product information FAQ section of the Heska Website for the E.R.D.-HealthScreen® Urine Test the questions and answers below are given.

Why is microalbuminuria a sensitive indicator of a dog's or cat's health status?

The kidneys filter the entire blood volume of the dog or cat every 30 minutes. As a result, they are continually exposed to a myriad of potentially damaging substances, infectious agents, or conditions (e.g., antigen-antibody complexes, toxins, bacteria, hypertension). Various disease processes may damage nephrons, resulting in leakage of albumin into the urine.

Examples of these disease processes in dogs include the following:

- Inflammatory diseases (e.g., dental disease, pyoderma, immune-mediated diseases, inflammatory bowel disease)
- Infectious diseases (e.g., heartworm, ehrlichiosis, Lyme)
- Metabolic diseases (e.g., diabetes mellitus, hyperadrenocorticism, hypertension)
- Neoplasia

Persistent microalbuminuria suggests the presence of either an underlying disease process causing early renal damage or lower urinary tract disease (LUTD). Detection of microalbuminuria during a routine health examination provides veterinarians with a new tool to discover many common canine and feline diseases that are subclinical.

Will all animals testing positive with the E.R.D.-HealthScreen® Urine Test develop end-stage renal disease?

The prevalence of microalbuminuria in dogs and cats exceeds the reported occurrence of end-stage renal disease. Thus, the majority of microalbuminuric dogs or cats will not progress to develop end-stage renal disease. Microalbuminuria, especially when increasing in magnitude over time, is a risk factor for the development of end-stage renal disease. While all persistently microalbuminuric dogs and cats are "at risk" of developing end-stage renal disease, most will not due to tremendous renal reserve capacity. Increased monitoring of "at risk" dogs and cats will allow for earlier identification of individual animals that progress to end-stage renal disease.

As such on careful review of the available literature, it would appear that some of the published conclusions on the use and interpretation of microalbuminuria appear tenuous, while others may be valid. However, without the data and full text articles on which these conclusions are based they are difficult to evaluate.

5.6 Renal Biopsy

Renal biopsy for histological examination is the definitive method of diagnosis of glomerular disease, and differentiation between glomerulonephritis and amyloidosis. Possible reasons to perform a renal biopsy include: persistent proteinuria (Grauer 2005c), if all other diagnostics fail to reveal the cause of the proteinuria (Grant and Forrester 2001a), if proteinuria persists despite treatment (Grant and Forrester 2001a), to estimate the severity of renal lesions (Vaden et al 2005), and to differentiate between amyloidosis and glomerulonephritis (Slauson et al 1970; Grant and Forrester 2001a). In a retrospective study of the medical records of 283 dogs that had renal biopsies, proteinuria was found to be the most common reason a renal biopsy was performed (Vaden et al 2005).

Cortical biopsies are recommended to maximise the number of glomeruli in the sample as well as decrease the likelihood of haemorrhage, which is more commonly seen with medullary biopsy (Grant and Forrester 2001a; Brunker 2005; Grauer 2005c). Renal biopsies can be obtained percutaneously via needle, or surgically (wedge or needle). From the medical records of dogs with renal biopsies it was concluded that surgical wedge biopsies obtained under general anaesthesia were of a higher quality, more likely to only contain renal cortex, and more likely to give good results than needle biopsies (Vaden et al 2005). However, needle biopsies were performed approximately three times more than wedge biopsies (Vaden et al 2005).

Histological diagnosis of glomerular disease may require one or more of several appropriate stains including: haematoxylin and eosin (H&E), periodic acid-Schiff (PAS), Jones methenamine silver stains, and stains specific for amyloid (Congo red) (Center et al 1987).

Many clinicians are reluctant to perform renal biopsy in the diagnostic evaluation of their patients. Only 13.4% of dogs with renal biopsies performed had associated complications. The most common complication was severe haemorrhage, while hydronephrosis and death were both very uncommon (Vaden et al 2005). Overall renal biopsy was reported to be a relatively safe procedure (Vaden et al 2005).

6.0 References

- Abbas AK.** Diseases of Immunity. In: Kumar V, Abbas AK, Fausto N (eds). *Robbins and Cotran Pathologic Basis of Disease*. Pp 210-5,58-68. Elsevier Saunders, Philadelphia, PA, 2005
- Alpers CE.** The Kidney. In: Kumar V, Abbas AK, Fausto N (eds). *Robbins and Cotran Pathologic Basis of Disease*. Pp 956-9. Elsevier Saunders, Philadelphia, PA, 2005
- Bagley RS, Center SA, Lewis RM, Shin S, Dougherty SA, Randolph JF, Erb H.** The Effect of Experimental Cystitis and Iatrogenic Blood Contamination on the Urine Protein Creatinine Ratio in the Dog. *Journal of Veterinary Internal Medicine* 5, 66-70, 1991
- Barsanti JA, Finco DR.** Protein-Concentration in Urine of Normal Dogs. *American Journal of Veterinary Research* 40, 1583-8, 1979
- Biewenga WJ, Gruys E, Hendriks HJ.** Urinary Protein Loss in the Dog - Nephrological Study of 29 Dogs without Signs of Renal-Disease. *Research in Veterinary Science* 33, 366-74, 1982
- Biewenga WJ, Gruys E.** Proteinuria in the Dog - a Clinicopathological Study in 51 Proteinuric Dogs. *Research in Veterinary Science* 41, 257-64, 1986
- Brunker J.** Protein-losing nephropathy. *Compendium on Continuing Education for the Practicing Veterinarian* 27, 686-+, 2005
- Carroll MF, Temte JL.** Proteinuria in adults: A diagnostic approach. *American Family Physician* 62, 1333-40, 2000
- Center SA, Wilkinson E, Smith CA, Erb H, Lewis RM.** 24-Hour Urine Protein Creatinine Ratio in Dogs with Protein-Losing Nephropathies. *Journal of the American Veterinary Medical Association* 187, 820-4, 1985
- Center SA, Smith CA, Wilkinson E, Erb HN, Lewis RM.** Clinicopathological, Renal Immunofluorescent, and Light Microscopic Features of Glomerulonephritis in

the Dog - 41 Cases (1975-1985). *Journal of the American Veterinary Medical Association* 190, 81-90, 1987

Christensen EI, Gburek J. Protein reabsorption in renal proximal tubule-function and dysfunction in kidney pathophysiology. *Pediatric Nephrology* 19, 714-21, 2004

Cook AK, Cowgill LD. Clinical and pathological features of protein-losing glomerular disease in the dog: A review of 137 cases (1985-1992). *Journal of the American Animal Hospital Association* 32, 313-22, 1996

Deen WM, Satvat B, Jamieson JM. Theoretical-Model for Glomerular-Filtration of Charged Solutes. *American Journal of Physiology* 238, F126-F39, 1980

Deen WM, Satvat B. Determinants of the Glomerular-Filtration of Proteins. *American Journal of Physiology* 241, F162-F70, 1981

DiBartola SP, Chew DJ, Jacobs G. Quantitative Urinalysis Including 24-Hour Protein Excretion in the Dog. *Journal of the American Animal Hospital Association* 16, 537-46, 1980a

DiBartola SP, Spaulding GL, Chew DJ, Lewis RM. Urinary Protein Excretion and Immunopathologic Findings in Dogs with Glomerular-Disease. *Journal of the American Veterinary Medical Association* 177, 73-7, 1980b

DiBartola SP, Benson MD. The Pathogenesis of Reactive Systemic Amyloidosis. *Journal of Veterinary Internal Medicine* 3, 31-41, 1989

DiBartola SP, Tarr MJ, Parker AT, Powers JD, Pultz JA. Clinicopathologic Findings in Dogs with Renal Amyloidosis - 59 Cases (1976-1986). *Journal of the American Veterinary Medical Association* 195, 358-64, 1989

Dilena BA, Penberthy LA, Fraser CG. 6 Methods for Determining Urinary Protein Compared. *Clinical Chemistry* 29, 553-7, 1983

Dyce KM, Sack WO, C.J.G. W. Textbook of Veterinary Anatomy, Third edn. W.B.Saunders Company, Philadelphia, Pennsylvania, 2002

- Fettman MJ.** Comparison of Urinary Protein-Concentration and Protein Creatinine Ratio Vs Routine Microscopy in Urinalysis of Dogs - 500 Cases (1987-1988). *Journal of the American Veterinary Medical Association* 195, 972-6, 1989
- Finco DR.** Evaluation of Renal Functions. In: Osborne CA, Finco DR (eds). *Canine and Feline Nephrology and Urology*. Pp 216-29. Williams & Wilkins, Media, PA, 1995
- Gary AT, Cohn LA, Kerl ME, Jensen WA.** The effects of exercise on urinary albumin excretion in dogs. *Journal of Veterinary Internal Medicine* 18, 52-5, 2004
- Ginsberg JM, Chang BS, Maltarese RA, Garelia S.** Use of single voided urine samples to estimate quantitative proteinuria. *New England Journal of Medicine* 309, 1543 - 6, 1983
- Grant DC, Forrester SD.** Glomerulonephritis in dogs and cats: Diagnosis and treatment. *Compendium on Continuing Education for the Practicing Veterinarian* 23, 798-805, 2001a
- Grant DC, Forrester SD.** Glomerulonephritis in dogs and cats: Glomerular function, pathophysiology and clinical signs. *Compendium on Continuing Education for the Practicing Veterinarian* 23, 739-+, 2001b
- Grauer GE.** Proteinuria. In: *Textbook of Veterinary Internal Medicine*. Pp 114-6. Elsevier Saunders, St. Louis, Missouri, 2005a
- Grauer GF, Thomas CB, Eicker SW.** Estimation of Quantitative Proteinuria in the Dog, Using the Urine Protein-to-Creatinine Ratio from a Random, Voided Sample. *American Journal of Veterinary Research* 46, 2116-9, 1985
- Grauer GF, Oberhauser EB, Basaraba RJ, Lappin MR, Simpson D, Jensen WA.** Development of microalbuminuria in dogs with heartworm disease (abstr). *Journal of Veterinary Internal Medicine* 16, 352, 2002
- Grauer GF.** Early detection of renal damage and disease in dogs and cats. *Veterinary Clinics of North America-Small Animal Practice* 35, 581-+, 2005b

- Grauer GF.** Canine glomerulonephritis: new thoughts on proteinuria and treatment. *Journal of Small Animal Practice* 46, 469-78, 2005c
- Grauer GF.** Measurement, interpretation, and implications of proteinuria and albuminuria. *Veterinary Clinics of North America-Small Animal Practice* 37, 283-95, 2007
- Jacob F, Polzin DJ, Osborne CA, Neaton JD, Kirk CA, Allen TA, Swanson LL.** Evaluation of the association between initial proteinuria and morbidity rate or death in dogs with naturally occurring chronic renal failure. *Javma-Journal of the American Veterinary Medical Association* 226, 393-400, 2005
- James GP, Bee DE, Fuller JB.** Proteinuria - Accuracy and Precision of Laboratory Diagnosis by Dip-Stick Analysis. *Clinical Chemistry* 24, 1934-9, 1978
- Jansen BS, Lumsden JH.** Sensitivity of Routine Tests for Urine Protein to Hemoglobin. *Canadian Veterinary Journal-Revue Veterinaire Canadienne* 26, 221-3, 1985
- Jergens AE.** Glomerulonephritis in Dogs and Cats. *Compendium on Continuing Education for the Practicing Veterinarian* 9, 903-&, 1987
- Jergens AE, McCaw DL, Hewett JE.** Effects of Collection Time and Food-Consumption on the Urine Protein Creatinine Ratio in the Dog. *American Journal of Veterinary Research* 48, 1106-9, 1987
- Kurtz JM, Lee JC, Slauson DO, Russell SW, Schechte.Rd.** Naturally Occurring Canine Glomerulonephritis. *American Journal of Pathology* 67, 471-&, 1972
- Lees GE, Jensen WA, Simpson D, Kashtan CE.** Persistent albuminuria precedes onset of overt proteinuria in male dogs with X-linked hereditary nephropathy (abstr). *Journal of Veterinary Internal Medicine* 16, 1, 2002
- Lees GE, Brown SA, Elliott J, Grauer GE, Vaden SL.** Assessment and management of proteinuria in dogs and cats: 2004 ACVIM forum consensus statement (small animal). *Journal of Veterinary Internal Medicine* 19, 377-85, 2005

- Lulich JP, Osborne CA.** Interpretation of Urine Protein-Creatinine Ratios in Dogs with Glomerular and Nonglomerular Disorders. *Compendium on Continuing Education for the Practicing Veterinarian* 12, 59-8, 1990
- Maxie MG, Newman SJ.** The Urinary System. In: Maxie MG (ed) *Jubb, Kennedy and Palmer's Pathology of Domestic Animals*. Pp 425-522. Elsevier Ltd., 2007
- McCaw DL, Knapp DW, Hewett JE.** Effect of Collection Time and Exercise Restriction on the Prediction of Urine Protein Excretion, Using Urine Protein Creatinine Ratio in Dogs. *American Journal of Veterinary Research* 46, 1665-9, 1985
- Moore FM, Brum SL, Brown L.** Urine protein determination in dogs and cats: comparison of dipstick and sulfasalicylic acid procedures. *Veterinary Clinical Pathology* 20, 1991
- Mullerpeddinghaus R, Trautwein G.** Spontaneous Glomerulonephritis in Dogs .2. Correlation of Glomerulonephritis with Age, Chronic Interstitial Nephritis and Extrarenal Lesions. *Veterinary Pathology* 14, 121-7, 1977a
- Mullerpeddinghaus R, Trautwein G.** Spontaneous Glomerulonephritis in Dogs .1. Classification and Immunopathology. *Veterinary Pathology* 14, 1-13, 1977b
- Murray M, Pirie HM, Thompson H, Jarrett WFH, Wiseman A.** Glomerulonephritis in a Dog - Histological and Electron Microscopical Study. *Research in Veterinary Science* 12, 493-8, 1971
- Murray M, Wright NG.** Morphologic Study of Canine Glomerulonephritis. *Laboratory Investigation* 30, 213-21, 1974
- Nabity MB, Lees GE, Boggess MM, Kashtan CE.** Day-to-day variation of urine protein: Creatinine ratio in female dogs with stable glomerular proteinuria caused by x-linked hereditary nephropathy (XLHN). *Journal of Veterinary Internal Medicine* 19, 432-, 2005
- Nabity MB, Boggess MM, Kashtan CE, Lees GE.** Day-to-day variation of the urine protein: Creatinine ratio in female dogs with stable glomerular proteinuria

caused by X-linked hereditary nephropathy (abstr). *Journal of Veterinary Internal Medicine* 21, 425-30, 2007

Newman SJ, Confer AW, Panciera RJ. Pathologic Basis of Veterinary Disease. In: McGavin MD, Zachary JF (eds). *Pathologic Basis of Veterinary Disease*. Pp 613-91. Mosby Elsevier, St Louis 2007

Osborne CA, Johnson KH, Perman V, Schall WD. Renal Amyloidosis in Dog. *Journal of the American Veterinary Medical Association* 153, 669-&, 1968

Osborne CA, Stevens JB, Lulich JP, Ulrich LK, K.A. B, Koehler LA, Swanson LL. A Clinicians Analysis of Urinalysis. In: Osborne CA, Finco DR (eds). *Canine and Feline Nephrology and Urology*. Pp 136-205. Williams & Wilkins, Media, PA, 1995

Pressler B, Vaden S, Jensen WA, Simpson D. Prevalence of Microalbuminuria in dogs evaluated at a referral veterinary hospital (abstr). *Journal of Veterinary Internal Medicine* 15, 300, 2001

Pressler B, Proulx DA, Williams LE, Jensen WA, Vaden SL. Urine albumin concentration is increased in dogs with lymphoma or osteosarcoma (abstr). *Journal of Veterinary Internal Medicine* 17, 404, 2003

Radecki SV, Donnelly R, Jensen WA, Stinchcomb DT. Effect of age and breed on the prevalence of microalbuminuria in dogs (abstr). *Journal of Veterinary Internal Medicine* 17, 406, 2003

Relford RL, Leed GE. Nephrotic syndrome in dogs: Diagnosis and treatment. *Compendium on Continuing Education for the Practicing Veterinarian* 18, 279-&, 1996

Ross MH, Kaye GI, Pawlina W. Urinary System. In: *Histology: A text and atlas*. Pp 602-41. Lippincott Williams and Wilkins, Baltimore, Maryland, 2003

Schafer-Somi S, Bar-Schadler S, Aurich JE. Proteinuria and immunoglobulinuria in neonatal dogs. *Veterinary Record* 157, 378-82, 2005

- Schwab SJ, Christensen RL, Dougherty K, Klahr S.** Quantitation of Proteinuria by the Use of Protein-to-Creatinine Ratios in Single Urine Samples. *Archives of Internal Medicine* 147, 943-4, 1987
- Slauson DO, Gribble DH, Russell SW.** A Clinicopathological Study of Renal Amyloidosis in Dogs. *Journal of Comparative Pathology* 80, 335-&, 1970
- Stockholm SL, Scott MA.** Urinary System. In: *Fundamentals of Veterinary Clinical Pathology*. Pp 277-336. Iowa State Press, Ames, Iowa, 2002
- Vaden S.** Glomerular Diseases. In: *Textbook of Veterinary Internal Medicine*. Pp 1786 - 800. Elsevier Saunders, St Louis, 2005
- Vaden SL, Jensen WA, Longhofer S, Simpson D.** Longitudinal study of microalbuminuria in soft-coated wheaten terriers (abstr). *Journal of Veterinary Internal Medicine* 15, 300, 2001
- Vaden SL, Pressler BM, Lappin MR, Jensen WA.** Effects of urinary tract inflammation and sample blood contamination on urine albumin and total protein concentrations in canine urine samples. *Veterinary Clinical Pathology* 33, 14-9, 2004
- Vaden SL, Levine JE, Lees GE, Groman RP, Grauer GE, Forrester SD.** Renal biopsy: A retrospective study of methods and complications in 283 dogs and 65 cats. *Journal of Veterinary Internal Medicine* 19, 794-801, 2005
- Vilafranca M, Wohlsein P, Trautwein G, Leopoldtemmler B, Nolte I.** Histological and Immunohistological Classification of Canine Glomerular-Disease. *Journal of Veterinary Medicine Series a-Zentralblatt Fur Veterinarmedizin Reihe a-Physiology Pathology Clinical Medicine* 41, 599-610, 1994
- White JV, Olivier NB, Reimann K, Johnson C.** Use of Protein-to-Creatinine Ratio in a Single Urine Specimen for Quantitative Estimation of Canine Proteinuria. *Journal of the American Veterinary Medical Association* 185, 882-5, 1984
- Whittemore JC, Jensen WA, Prause L, Radecki SV, Gill VL, Lappin MR.** Comparison of microalbuminuria, urine proteindipstick, and urine protein

creatinine ratio results in clinically ill dogs (abstr). *Journal of Veterinary Internal Medicine* 17, 437, 2003

Whittemore JC, Gill VL, Jensen WA, Radecki SV, Lappin MR. Evaluation of the association between microalbuminuria and the urine albumin-creatinine ratio and systemic disease in dogs. *Javma-Journal of the American Veterinary Medical Association* 229, 958-63, 2006

CHAPTER 2

ASSESSMENT OF THE EFFECT OF BLOOD CONTAMINATION ON THE URINARY PROTEIN TO CREATININE RATIO IN THE DOG.

1.0 Abstract

The urine protein to creatinine ratio (UPCR) is a reliable method to assess the total urinary protein loss in the dog from a single urine sample. Interpretation of the urine protein to creatinine ratio has been difficult in the presence of haematuria in the sample and previously the presence of blood in the urine has negated the use or interpretation of the UPCR. In 2 previous studies blood has been added to the urine sample of a single dog to aid interpretation of the UPCR in the presence of blood contamination. In this study blood contamination of urine samples in 21 dogs was assessed to develop guidelines for interpretation of the UPCR in the face of haemorrhage. Blood was added to the urine from the same dog to make samples with blood contamination levels ranging from 0 to 5%. Urine dipstick analysis, microscopic examination and a UPCR was performed on all samples. The current recommended cut off level for UPCR for normal dogs is <0.5 . Results greater than 1.0 are considered abnormal, results greater than 2.0 suggests glomerular disease, and UPCR results between 0.5 and 1.0 are questionable. The results of the present study suggest that when urine is visibly red, haemorrhage may be considered as a differential for a UPCR up to 2.0. The practice of attributing proteinuria in non discoloured (yellow) urine samples with microscopic haemorrhage to the blood present should be discontinued, as microscopic haemorrhage that does not result in a visible change in colour of the urine sample from yellow will not substantially increase the UPCR. As such, the UPCR level in yellow urine even in the presence of microscopic haematuria can be considered valid.

2.0 Introduction

Proteinuria in the dog, can be due to relatively benign causes, through to more serious and life threatening glomerular diseases. Persistent proteinuria is an indication to the veterinary clinician of the need for further investigation of the kidney. The causes of proteinuria can be classified as pre-renal, renal and post renal (DiBartola et al 1980; White et al 1984; Grauer et al 1985; Stockholm and Scott 2002; Lees et al 2005).

Pre-renal proteinuria occurs as a result of increased plasma protein concentration, and subsequently increased filtration of small protein molecules through the glomeruli. When present in high concentration these proteins exceed the resorptive capacity of the renal tubules, with the excess remaining in the urinary filtrate (DiBartola et al 1980; Lulich and Osborne 1990; Stockholm and Scott 2002; Lees et al 2005).

Renal proteinuria can be functional or pathological in the dog. The former is usually transient, while the latter may be terminal. The most common and significant causes of pathological renal proteinuria are glomerular disease due to glomerulonephritis or amyloidosis (Lulich and Osborne 1990; Cook and Cowgill 1996).

In post renal proteinuria proteins are added to the urine filtrate after it enters the renal pelvis. These proteins originate from normal genital tract secretions, or neoplastic, haemorrhagic or exudative lesions of the urogenital tract (DiBartola et al 1980; Lulich and Osborne 1990; Stockholm and Scott 2002; Lees et al 2005). While post-renal proteinuria is reported to be the most common form of proteinuria, it is usually of limited magnitude and does not cause hypoalbuminaemia (Stockholm and Scott 2002). Of the possible causes of post renal proteinuria, urinary tract inflammation and haemorrhage are considered to be occur commonly (Vaden et al 2004).

The urine protein to creatinine ratio is a reliable method of the assessment of total urinary protein loss in the dog from a single urine sample (White et al 1984; Grauer et al 1985; McCaw et al 1985; Jergens et al 1987). Increased UPCr ratios may be seen with any cause or transient or persistent proteinuria. Initially UPCr values less than 1.0 were considered to be normal for dogs, however recently this has been reduced to less than 0.5, with suggestions that it may reduce further in the future (Lulich and Osborne 1990; Stockholm and Scott 2002; Gary et al 2004; Lees et al 2005; Grauer 2007). UPCr values between 0.5 to 1.0 are considered questionable, while UPCr

values greater than 1.0 are considered abnormal, and UPCr measurements greater than 2.0 are usually due to glomerular disease (Lees et al 2005).

In the absence of evidence of inflammation, infection or haemorrhage in the urinary sediment, persistent proteinuria is the hall mark of glomerular disease (Center et al 1985; McCaw et al 1985; Center et al 1987; Lulich and Osborne 1990; Brunker 2005; Grauer 2005a, 2005b; Maxie and Newman 2007). Interpretation of the UPCr should always be done in conjunction with a complete urinalysis as the results of many studies on UPCr can not be extrapolated to include dogs with haematuria (Grauer et al 1985). Proteinuria can often be found in urine samples that have cytologic evidence of haemorrhage or positive urinary test pad results for blood or haemoglobin (Bagley et al 1991). In this situation, it is often unclear whether the protein present is due to renal proteinuria, or post renal proteinuria (i.e. due to focal haemorrhage) as the UPCr can not distinguish between renal and post-renal proteinuria (Jansen and Lumsden 1985; Bagley et al 1991). Previously samples with evidence of haematuria frequently had pre-renal and renal causes of proteinuria ruled out, with the proteinuria attributed to the haematuria (Vaden et al 2005).

Two previous studies investigated the effect of blood contamination on the UPCr in a single urine sample with somewhat conflicting results. The purpose of this study was to serially contaminate urine samples from a sample population of dogs to: a) assess the consistency with which several observers could determine urine colour b) assess the effect of blood contamination on the UPCr ratio over a range of dogs, and c) determine whether a relationship between colour of the urine sample and the UPCr can be determined to allow assessment of the UPCr in macroscopically haematuric samples.

3.0 Materials and Methods

Experiment A

Urine and whole blood (EDTA) was collected from a fit and healthy 18 month old, male castrated Dalmation. The urine sample was serially contaminated with blood from the same dog to form urine samples with final blood contamination percentages of 0.0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.3, 1.4, 1.6, 1.8, 2.0, 3.0, and 5.0%. The samples were then shuffled in random order, and the colour of each tube was assessed independently by 4 veterinary pathologists, 4 veterinary laboratory technicians, and 5 veterinary nurses. The people assessing the urine samples had the option of the following colours to label each urine sample; yellow, peach, orange, orange/red, and red. The colours were assigned the following numerical values; 1 – yellow, 2 – peach, 3 – orange, 4 - orange red, 5 – red. The average and median colour assessment for each sample was calculated. Due to the nature of the data and the sample size it was considered that graphical representation of the data was more appropriate than statistical evaluation.

Experiment B

Urine, serum and whole blood (EDTA) samples were obtained from 10 routine canine submissions to a commercial veterinary laboratory (New Zealand Veterinary Pathology) and from 11 staff or student-owned dogs from the Massey University Veterinary Teaching hospital, Palmerston North, New Zealand. No attempt was made to pre-select dogs on sex, age, breed, or the absence or presence of disease.

Whole blood and serum were collected from each dog and assessed for a complete blood count (CBC) (Advia 120, Bayer Healthcare, Leverkusen, Germany) and serum biochemistry profile (Hitachi 911, Roche Diagnostics, Basel, Switzerland). To mimic the effect of haematuria on the UPCr, the urine sample from each dog was serially contaminated with whole blood (from the same dog) to make samples with blood contamination levels ranging from 0 % to 5% of the total urine volume.

A complete urinalysis was performed on all urine samples, which included microscopic sediment examination, urine specific gravity by refractometer (Reichert Inc. Depew, NY, USA)), and biochemistry using two brands of standard urine dipsticks (Multistix 9, Bayer Healthcare and Combur 10 Test UX, Roche Diagnostics), read on brand specific automated urine dipstick analysers.

Urinary protein and creatinine were assessed on all urine samples. The urinary protein concentration was assessed by a benzethonium chloride assay (Urinary/CSF protein method, Roche Diagnostics)(Luxton et al 1989), while the urinary creatinine concentration was assessed by the alkaline picrate method (Creatinine Method, Roche Diagnostics)(Bartels et al 1972). A UPCR of <0.5 was considered normal, $0.5 - 1.0$ was considered questionable and UPCR of >1.0 were considered abnormal.

The colour of each urine sample was recorded by a single observer as yellow, peach, orange, orange/red, or red. The colours were assigned a score from 1 to 5 corresponding to yellow (1), peach (2) orange (3), and orange/red (4) and red (5). The median and average colour score was calculated to give the overall urine colour for each urine contamination level.

For the purposes of this article, macroscopic haemorrhage is defined as urine samples in which any appreciable colour change from yellow is observed, while gross haemorrhage refers to visibly red urine samples.

Due to the nature of the data and the sample size it was considered that graphical representation of the data was more appropriate than statistical evaluation.

4.0 Results

Experiment A

The colour scores for each urine sample are shown in Figure 2.1. The only possible score whole integers of 1 to 5. Where different observers gave the same colour score to a sample their scores were staggered around the whole integer number for clarity.

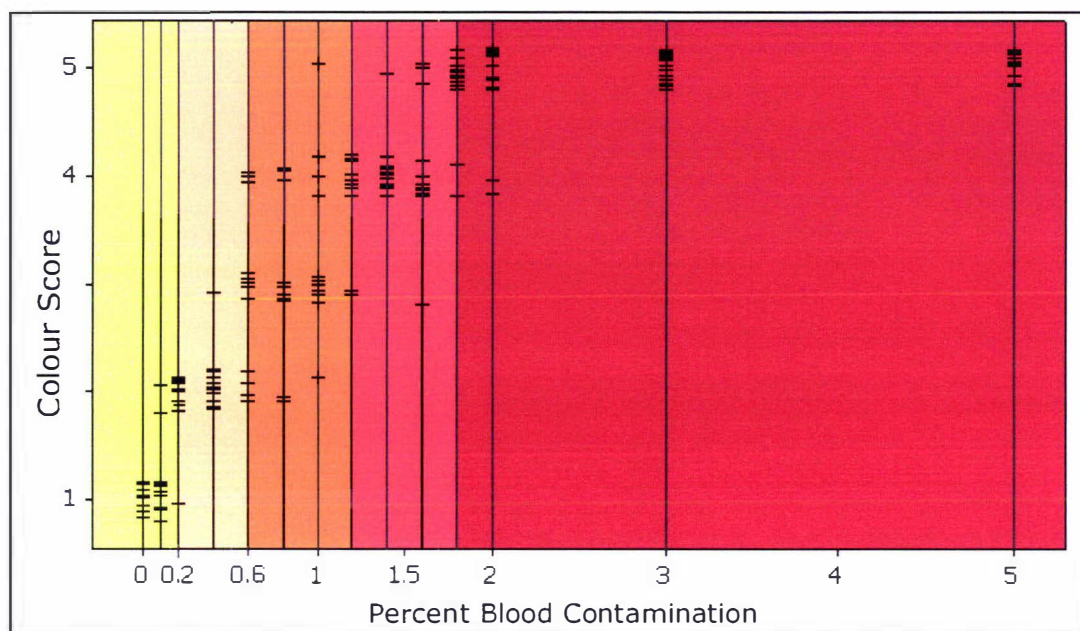


Figure 2.1 – Scatterplot of colour score versus level of blood contamination of the urine sample, with background colouring to match the colour of each urine sample.

The scores for the urine colours were perfectly consistent for the 0%, 3% and 5% blood contamination samples, with all 13 assessors giving the same colour score to those samples (Table 2.1). For 10 of the 14 urine samples (excluding 0.6%, 0.8%, 1.0% and 1.6%) all assessors chose one of 2 colours for the urine samples, with 11 or more of the 13 assessors choosing the same urine colour as each other (Table 2.1).

Table 2.1 –The number of individuals (n=13) that scored each urine sample by colour.

Percent Blood contamination (%)	0	0.1	0.2	0.4	0.6	0.8	1	1.2	1.4	1.6	1.8	2	3	5
Colour	The number of individuals who scored each urine sample as the colours show below.													
Yellow	1	13	11	1	0	0	0	0	0	0	0	0	0	0
Peach	2	0	2	12	11	5	2	1	0	0	0	0	0	0
Orange	3	0	0	0	2	5	8	8	2	0	1	0	0	0
Orange/Red	4	0	0	0	0	3	3	3	11	12	9	2	2	0
Red	5	0	0	0	0	0	0	1	0	1	3	11	11	13
Median Colour Score	1.0	1.0	2.0	2.0	3.0	3.0	3.0	4.0	4.0	4.0	5.0	5.0	5.0	5.0
Average Colour Score	1.0	1.2	1.9	2.2	2.8	3.1	3.3	3.8	4.1	4.2	4.8	4.8	5.0	5.0

Experiment B

The colour, UPCR results and urinary dipstick blood results for all the levels of blood contamination are shown in Table 2.2. Urine dipstick blood results increased from negative to the maximum (Roche 4+, Bayer 3+), when the percentage of blood contamination rose from 0 to 0.1%, and remained maximal for all subsequent levels of blood contamination.

Table 2.2 – Colour, urine protein to creatinine ratios, and urinary dipstick blood results for all urine samples.

Percentage blood contamination	0.0%	0.1%	0.2%	0.4%	0.6%	0.8%	1.0%	1.2%	1.4%	1.6%	1.8%	2.0%	3.0%	5.0%
Blood Dipstick Roche	0	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
Blood Dipstick Bayer	0	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+
Average Colour score	1.0	1.0	1.0	1.4	2.0	2.7	3.0	3.6	3.9	4.1	4.6	5.0	5.0	5.0
Colour	Y	Y	Y	**	P	O	O	**	OR	OR	**	R	R	R
Animal ID	Urine Protein to Creatinine Ratio													
1	0.09	0.13	0.14	0.17	0.19	0.23	0.27	0.26	0.29	0.31	0.35	0.38	0.88	1.06
2	0.08	0.10	0.12	0.16	0.19	0.23	0.27	0.26	0.31	0.33	0.36	0.40	0.59	0.88
3	0.06	0.10	0.13	0.17	0.20	0.22	0.24	0.27	0.31	0.34	0.37	0.46	0.47	0.72
4	0.08	0.11	0.12	0.16	0.19	0.22	0.26	0.29	0.34	0.37	0.42	0.48	0.69	0.96
5	0.11	0.17	0.19	0.22	0.25	0.29	0.32	0.36	0.39	0.43	0.45	0.50	0.73	1.12
6	0.17	0.20	0.22	0.27	0.30	0.36	0.38	0.41	0.45	0.50	0.54	0.60	0.87	1.50
7	0.07	0.12	0.15	0.21	0.26	0.32	0.36	0.43	0.50	0.53	0.55	0.65	0.99	1.31
8	0.23	0.23	0.25	0.29	0.35	0.38	0.42	0.46	0.48	0.58	0.62	0.70	0.83	1.32
9	0.08	0.12	0.16	0.22	0.28	0.35	0.42	0.46	0.54	0.62	0.66	0.83	1.31	1.70
10	0.11	0.14	0.20	0.24	0.31	0.34	0.37	0.46	0.59	0.56	0.72	0.81	1.04	1.75
11	0.07	0.12	0.18	0.23	0.31	0.37	0.45	0.51	0.60	0.67	0.78	0.89	1.53	1.93
12	0.08	0.15	0.20	0.29	0.35	0.42	0.50	0.59	0.57	0.64	0.72	0.79	1.02	1.37
13	0.20	0.23	0.26	0.34	0.41	0.48	0.57	0.65	0.75	0.85	0.96	1.07	1.31	2.20
14	0.07	0.14	0.21	0.34	0.45	0.55	0.69	0.76	0.79	0.97	1.10	1.42	2.75	4.32
15	0.35	0.42	0.46	0.53	0.60	0.69	0.74	0.83	0.87	1.00	1.08	1.17	1.67	3.20
16	0.70	0.74	0.79	0.85	0.90	1.00	1.04	1.05	1.16	1.20	1.26	1.41	1.89	2.89
17	0.83	0.83	0.90	1.01	1.08	1.16	1.24	1.32	1.41	1.49	1.58	1.67	2.11	3.34
18	0.66	0.85	1.05	1.58	1.97	2.45	3.05	3.58	4.09	4.76	5.32	6.30	7.12	14.72
19	4.57	4.76	4.93	5.03	5.21	5.49	5.51	5.78	6.20	6.19	6.45	6.89	8.47	12.12
20	4.77	5.00	5.26	5.32	5.58	6.06	6.25	6.64	7.02	6.84	7.71	8.17	10.40	8.39
21	6.15	6.53	7.02	7.16	7.86	8.83	9.23	9.37	10.66	13.21	14.42	12.98	19.43	35.43

** The colour has been left white for those contamination levels in which the overall average colour is not within 0.3 of a whole colour score integer, i.e. the 0.4% blood sample has an average urine score of 1.4, and so has been left white to show it is in between yellow and peach.

Key for Interpretation of UPCR in Table 1	
	UPCR values <0.5
	UPCR values 0.5 - 1.00
	UPCR values 1.01 - 2.00
	UPCR values >2.00

As the percentage of blood contamination in the samples increased, the UPCR level also increased. As shown in column A of Table 2.3, 100% of the samples with an initial UPCR less than 0.5 still had a UPCR less than 0.5 if the urine colour remained yellow (i.e. 0.2% blood contamination or less).

When the blood contamination of the sample caused discolouration of the urine sample from peach to orange-red, (i.e. macroscopic, but not gross haemorrhage) the UPCR of some urine samples increased above 0.5. In this colour range no samples exceeded the abnormal UPCR cut off level of greater than 1.0 (Table 2.2). When the urine colour was marginally discoloured to peach, only 1 (6.7%) of 15 dogs with an initial UPCR less than 0.5 had a UPCR which exceeded 0.50 (UPCR = 0.53) (Table 2.3). When the urine samples were orange (0.8% and 1% blood contamination), up to 4 dogs (26.7%) had UPCR greater than or equal to 0.5 (range of 0.50 to 0.74). As the sample became orange-red, the UPCR of 8 dogs (53%) with an initial UPCR less than 0.5, rose to 0.5 or greater (Table 2.3). However, none of these 8 dogs had a UPCR which exceeded the abnormal UPCR cut off of greater than 1.0.

Table 2.3 – The number of samples, and percentage of total samples (n=15) with urine protein to creatinine ratios that exceed varying cut-off limits as the level of blood contamination increases, when the UPCR of the initial sample is 0.5.

The number and percentage of samples with a UPCR ratio of "X" when the UPCR of the initial (uncontaminated) urine sample is less than 0.5. n=15						
Sample Contamination Level (%)	A		B		C	
	X is 0.5 or greater		X is >1.0		X is >2.0	
	Count	Percentage	Count	Percentage	Count	Percentage
0.0	0	0.0	0	0.0	0	0.0
0.1	0	0.0	0	0.0	0	0.0
0.2	0	0.0	0	0.0	0	0.0
0.4	1	6.7	0	0.0	0	0.0
0.6	1	6.7	0	0.0	0	0.0
0.8	2	13.3	0	0.0	0	0.0
1.0	4	26.7	0	0.0	0	0.0
1.2	5	33.3	0	0.0	0	0.0
1.4	8	53.3	0	0.0	0	0.0
1.6	10	66.7	0	0.0	0	0.0
1.8	10	66.7	2	13.3	0	0.0
2.0	11	73.3	3	20.0	0	0.0
3.0	15	100.0	7	46.7	1	6.7
5.0	15	100.0	12	80.0	3	20.0

Of the 15 dogs with an initial UPCr less than 0.5, the UPCr did not increase above 1.0 in any samples until the sample was between orange-red to red in colour (Table 2.3 – Column B).

The UPCr of samples that were grossly haemorrhagic or visibly red (2% to 5% blood contamination) ranged from 0.38 to 4.32 for samples with an initial UPCr less than 0.5, 1.41 to 14.72 for samples with an initial UPCr of 0.5 to 0.99, and 6.89 to 35.43 for samples with an initial UPCr of greater than 1.0 (Table 2.2). Four (26.7%) of the 15 dogs with an initial UPCr less than 0.5 still had UPCr less than 0.5 in the 2% blood contamination urine sample, which appeared red (Table 2.2).

From column C of Table 2.3 it is shown that none of the 15 samples with an initial UPCr less than 0.5 developed a UPCr greater than 2.0 until the blood contamination percentage was 3%, which correlates to a visibly red sample with no hint of orange remaining (Table 2.3 – Column C). At 3% blood contamination, 14 dogs (93.3%) had a UPCr less than 2.75.

5.0 Discussion

From experiment A, all individuals could consistently determine whether a sample was yellow (or non-discoloured), or red due to gross haemorrhage. While there was some mild variation in ability to determine the colours in between, the majority (11 of 13 observers) chose the same colour in 10 of the 14 dilutions. The dilutions where there was the most discord were 0.6%, 0.8%, 1.0% and 1.6% blood contamination. The 0.6% to 1.0% blood contamination samples corresponded to those samples that by the average colour score were orange. The 1.6% blood contamination level corresponded to an average colour score of 4.2, or orange-red (4). This is not completely unexpected as the difference between an orange-red sample to one that is only red with no hint of orange remaining is not as obvious as the change between two other colours (i.e. yellow to peach). Overall, it would appear that an individual should be able to determine urine colour fairly consistently.

In experiment B, the urine dipstick blood results in the uncontaminated samples were generally negative. With the smallest level of blood contamination in this study (0.1%) the urine dipstick blood results increased to the maximum detectable by both brands of urinary dipsticks used (Roche 4+, Bayer 3+). As all of the contaminated samples had maximal levels of blood present by the urinary dipstick detection pad, the dipstick did not allow for differentiation of the amount of blood present between the contaminated samples. This is in contrast to that reported in serial contaminations of a single dog urine with its own blood (PCV 55%) to levels from 1:25,600 (0.0039%) to 1:400 (0.25%) (Vaden et al 2004). In that study, the urinary dipstick blood test pad result for samples with 0.125% to 0.25% blood contamination, was 1+ as evaluated on a Roche Diagnostics dipstick (Vaden et al 2004).

The protein haemoglobin can be detected on urine dipsticks by both the protein and haemoglobin/blood detection test pads, whether the haemoglobin is within intact washed erythrocytes, in whole blood in association with plasma proteins, or in the form of lysed erythrocytes (Jansen and Lumsden 1985). The haemoglobin/blood detection strips on urinary dipsticks are highly sensitive for the detection of haemoglobin in a urine sample, and were found to be fifty times more sensitive at detecting haemoglobin than the protein test pads on urinary dipsticks (Jansen and Lumsden 1985). As such it is not unexpected that the urinary dipstick test pad for blood did not allow differentiation between the different levels of blood contamination.

The UPCR does not substantially increase in urine with microscopic haematuria (i.e. RBC in sediment and/or positive results on the urinary dipstick blood test pad) if the urine sample remains yellow in colour. These findings concur with results of a previous study in which a single dog urine sample was contaminated with its own blood (Vaden et al 2004). The samples in which the urine appeared yellow (0.0 to 0.016%) all had a UPCR less than 0.5. In a study of 14 hospitalised dogs, a urine sample was collected by catheterisation from each dog during the day, and a second urine sample was collected at night (McCaw et al 1985). It was noted that the second sample often contained erythrocytes, presumably due to trauma from the first catheterisation. However, no significant difference in the protein level was found between the first and second samples, despite the increased number of erythrocytes in the urine (McCaw et al 1985). The results of the present study and that of Vaden et al (2004) suggest that as long as the sample is yellow, even though there may be microscopic haematuria, the level of proteinuria as detected by the UPCR will not be substantially elevated due to the level of blood present.

As the percentage of blood contamination in the samples became macroscopic and the colour of the urine was no longer clearly yellow (0.4% blood) to orange (0.8 to 1.0% blood), one to four (26.7%) of 15 dogs with an initial UPCR less than 0.5 developed UPCR ranging from 0.5 to 0.74. The results of this study suggest that macroscopic haemorrhage can increase the UPCR of a urine sample with a normal UPCR ratio of less than 0.5, above the normal limit, in approximately 25% of dogs. This is in contrast to that reported in serial contaminations of a single dog urine with its own blood (Vaden et al 2004). In that report, even in samples that were observed as being red the UPCR levels remained below the cut off level. However, the colours of the blood contaminated urine samples in that study contrasted markedly with those in the present study. In that study, a sample with a blood contamination level of 0.03% was observed to be pink (i.e. macroscopic haemorrhage) while the highest level of contamination (0.25%) was noted as red. In the present study, samples were noted as being yellow up to and including a blood contamination level of 0.2% blood contamination, and were not noted as red until there was 2% blood contamination or greater. The colour notations in the present study concur with those seen in the assessment of colour in a single urine sample serially contaminated with increasing levels of blood by 13 observers (see Chapter 2).

For dogs with an initial UPCR <0.5, their UPCR did not increase above 1.0 in any samples until the sample colour exceeded orange/red. This suggests that if a urine

sample is orange-red and has a UPCR equal to or less than 1.0, it is likely that the uncontaminated sample would have a UPCR less than 0.5.

None of the 15 samples with an initial UPCR less than 0.5 developed a UPCR of 2.0 or greater until the blood contamination percentage was 3% or greater, which correlates to a visibly red sample, with no hint of orange remaining. This suggests that if the UPCR is <2.0 in a sample that is visibly red it is highly likely that the original uncontaminated samples had a UPCR less than 0.5.

The UPCR of samples that were grossly red (2% to 5% blood contamination) ranged from 0.38 to 4.32 for samples with an initial UPCR less than 0.5. This concurs with results of an earlier study in which a single urine sample was contaminated with blood (PCV 42%) to make up 10-75% of the total sample volume. Microscopically all the samples had very large numbers of erythrocytes (too numerous to count per high power field), and were visibly red. The UPCR of the samples ranged from 1.5 to 30.6, leading the authors to conclude that gross blood contamination of a urine sample increases the UPCR (Bagley et al 1991). However, contrasting results were obtained from a similar study in which a single dog urine sample was contaminated with its own blood (PCV 55%) to levels from 1:25,600 (0.0039%) to 1:400 (0.25%) (Vaden et al 2004). The two urine samples in that study with the highest level of blood contamination (0.125% and 0.250%) were reported as visibly red in colour while still having a UPCR less than 0.3, which would be considered normal. As previously noted the colour observations for similar levels of blood contamination differed markedly between that study and the present study. This study confirms earlier suggestions by Bagley et al (1991) that when the blood contamination of a urine sample causes red discolouration, it may cause the UPCR to be elevated above the normal range (i.e. less than 0.5).

The results of the present study suggest that when urine is visibly orange-red, haemorrhage may be considered as a differential for a UPCR up to 1.0. The practice of attributing proteinuria in non discoloured (yellow) urine samples with microscopic haemorrhage to the blood present should be discontinued, as microscopic haemorrhage that does not result in a visible change in colour of the urine sample from yellow will not substantially increase the UPCR. As such, the UPCR level in yellow urine, even in the presence of microscopic haematuria, can be considered valid.

6.0 References

- Bagley RS, Center SA, Lewis RM, Shin S, Dougherty SA, Randolph JF, Erb H.** The Effect of Experimental Cystitis and Iatrogenic Blood Contamination on the Urine Protein Creatinine Ratio in the Dog. *Journal of Veterinary Internal Medicine* 5, 66-70, 1991
- Bartels H, Bohmer M, Heierli C.** Serum creatinine determination without protein precipitation. *Clinica Chimica Acta* 37, 1972
- Brunker J.** Protein-losing nephropathy. *Compendium on Continuing Education for the Practicing Veterinarian* 27, 686-+, 2005
- Center SA, Wilkinson E, Smith CA, Erb H, Lewis RM.** 24-Hour Urine Protein Creatinine Ratio in Dogs with Protein-Losing Nephropathies. *Journal of the American Veterinary Medical Association* 187, 820-4, 1985
- Center SA, Smith CA, Wilkinson E, Erb HN, Lewis RM.** Clinicopathological, Renal Immunofluorescent, and Light Microscopic Features of Glomerulonephritis in the Dog - 41 Cases (1975-1985). *Journal of the American Veterinary Medical Association* 190, 81-90, 1987
- Cook AK, Cowgill LD.** Clinical and pathological features of protein-losing glomerular disease in the dog: A review of 137 cases (1985-1992). *Journal of the American Animal Hospital Association* 32, 313-22, 1996
- DiBartola SP, Chew DJ, Jacobs G.** Quantitative Urinalysis Including 24-Hour Protein Excretion in the Dog. *Journal of the American Animal Hospital Association* 16, 537-46, 1980
- Gary AT, Cohn LA, Kerl ME, Jensen WA.** The effects of exercise on urinary albumin excretion in dogs. *Journal of Veterinary Internal Medicine* 18, 52-5, 2004
- Grauer GF, Thomas CB, Eicker SW.** Estimation of Quantitative Proteinuria in the Dog, Using the Urine Protein-to-Creatinine Ratio from a Random, Voided Sample. *American Journal of Veterinary Research* 46, 2116-9, 1985

- Grauer GF.** Canine glomerulonephritis: new thoughts on proteinuria and treatment. *Journal of Small Animal Practice* 46, 469-78, 2005a
- Grauer GF.** Early detection of renal damage and disease in dogs and cats. *Veterinary Clinics of North America-Small Animal Practice* 35, 581-+, 2005b
- Grauer GF.** Measurement, interpretation, and implications of proteinuria and albuminuria. *Veterinary Clinics of North America-Small Animal Practice* 37, 283-95, 2007
- Jansen BS, Lumsden JH.** Sensitivity of Routine Tests for Urine Protein to Hemoglobin. *Canadian Veterinary Journal-Revue Veterinaire Canadienne* 26, 221-3, 1985
- Jergens AE, McCaw DL, Hewett JE.** Effects of Collection Time and Food-Consumption on the Urine Protein Creatinine Ratio in the Dog. *American Journal of Veterinary Research* 48, 1106-9, 1987
- Lees GE, Brown SA, Elliott J, Grauer GE, Vaden SL.** Assessment and management of proteinuria in dogs and cats: 2004 ACVIM forum consensus statement (small animal). *Journal of Veterinary Internal Medicine* 19, 377-85, 2005
- Lulich JP, Osborne CA.** Interpretation of Urine Protein-Creatinine Ratios in Dogs with Glomerular and Nonglomerular Disorders. *Compendium on Continuing Education for the Practicing Veterinarian* 12, 59-8, 1990
- Luxton RW, Patel P, Keir G, Thompson EJ.** A micro-method for measuring total protein in cerebrospinal-fluid by using benzethonium chloride in microtiter plate wells. *Clinical Chemistry* 35 (8), 1731-4, 1989
- Maxie MG, Newman SJ.** The Urinary System. In: Maxie MG (ed) *Jubb, Kennedy and Palmer's Pathology of Domestic Animals*. Pp 425-522. Elsevier Ltd., 2007
- McCaw DL, Knapp DW, Hewett JE.** Effect of Collection Time and Exercise Restriction on the Prediction of Urine Protein Excretion, Using Urine Protein Creatinine Ratio in Dogs. *American Journal of Veterinary Research* 46, 1665-9, 1985

Stockholm SL, Scott MA. Urinary System. In: *Fundamentals of Veterinary Clinical Pathology*. Pp 277-336. Iowa State Press, Ames, Iowa, 2002

Vaden SL, Pressler BM, Lappin MR, Jensen WA. Effects of urinary tract inflammation and sample blood contamination on urine albumin and total protein concentrations in canine urine samples. *Veterinary Clinical Pathology* 33, 14-9, 2004

Vaden SL, Levine JE, Lees GE, Groman RP, Grauer GE, Forrester SD. Renal biopsy: A retrospective study of methods and complications in 283 dogs and 65 cats. *Journal of Veterinary Internal Medicine* 19, 794-801, 2005

White JV, Olivier NB, Reimann K, Johnson C. Use of Protein-to-Creatinine Ratio in a Single Urine Specimen for Quantitative Estimation of Canine Proteinuria. *Journal of the American Veterinary Medical Association* 185, 882-5, 1984

APPENDICES

APPENDIX A: RAW DATA FOR COLOUR SCORING OF URINE

Percent Blood Contamination (%)	0.0%	0.1%	0.2%	0.4%	0.6%	0.8%	1.0%	1.2%	1.4%	1.6%	1.8%	2.0%	3.0%	5.0%
OBSERVER	COLOUR SCORE GIVEN													
Pathologist 1	1	1	2	2	2	3	3	4	4	4	5	5	5	5
Pathologist 2	1	1	2	2	2	3	3	4	4	4	4	5	5	5
Pathologist 3	1	2	2	2	4	3	3	3	4	4	5	5	5	5
Pathologist 4	1	1	2	2	2	4	3	4	4	4	5	5	5	5
Technician 1	1	1	2	2	2	4	5	3	4	3	4	5	5	5
Technician 2	1	1	2	2	3	3	3	4	4	5	5	5	5	5
Technician 3	1	1	1	3	2	2	2	4	5	4	5	4	5	5
Technician 4	1	2	2	3	3	3	4	4	4	5	5	5	5	5
Veterinary Nurse 1	1	1	2	2	4	3	3	4	4	4	5	4	5	5
Veterinary Nurse 2	1	1	2	2	4	4	4	4	4	4	5	5	5	5
Veterinary Nurse 3	1	1	2	2	3	2	3	4	4	4	5	5	5	5
Veterinary Nurse 4	1	1	2	2	3	3	3	4	4	5	5	5	5	5
Veterinary Nurse 5	1	1	2	2	3	3	4	4	4	4	5	5	5	5
Median	1	1	2	2	3	3	3	4	4	4	5	5	5	5
Average	1.0	1.2	1.9	2.2	2.8	3.1	3.3	3.8	4.1	4.2	4.8	4.8	5.0	5.0
Std. Deviation	0.0	0.4	0.3	0.4	0.8	0.6	0.8	0.4	0.3	0.6	0.4	0.4	0.0	0.0
Colour	Y	Y	P	P	O	O	O	OR	OR	OR	R	R	R	R

APPENDIX B: RAW DATA FOR URINARY PROTEIN

Percent Blood Contamination (%)	0.0%	0.1%	0.2%	0.4%	0.6%	0.8%	1.0%	1.2%	1.4%	1.6%	1.8%	2.0%	3.0%	5.0%
Animal ID	Urinary Protein													
1	0.28	0.41	0.42	0.51	0.57	0.70	0.82	0.77	0.85	0.93	1.05	1.14	2.53	3.08
2	0.19	0.24	0.29	0.37	0.45	0.53	0.61	0.62	0.70	0.76	0.83	0.91	1.32	1.96
3	0.20	0.33	0.45	0.58	0.68	0.76	0.84	0.92	1.03	1.14	1.27	1.55	1.55	2.38
4	0.23	0.30	0.35	0.43	0.52	0.61	0.71	0.81	0.94	1.03	1.17	1.36	1.87	2.61
5	0.27	0.38	0.43	0.50	0.57	0.63	0.71	0.78	0.85	0.93	0.97	1.09	1.56	2.35
6	0.31	0.35	0.40	0.48	0.55	0.64	0.68	0.74	0.81	0.88	0.99	1.08	1.56	2.67
7	0.10	0.17	0.21	0.30	0.37	0.44	0.52	0.61	0.69	0.73	0.78	0.91	1.32	1.79
8	0.55	0.61	0.67	0.80	0.94	1.04	1.13	1.30	1.33	1.56	1.64	1.86	2.16	3.41
9	0.10	0.15	0.19	0.27	0.34	0.41	0.49	0.55	0.64	0.74	0.77	0.98	1.55	1.89
10	0.19	0.24	0.34	0.41	0.52	0.57	0.63	0.78	0.96	0.91	1.18	1.35	1.69	2.79
11	0.07	0.13	0.18	0.25	0.33	0.40	0.49	0.56	0.66	0.75	0.85	0.97	1.64	2.02
12	0.12	0.22	0.29	0.42	0.50	0.60	0.72	0.83	0.82	0.92	1.03	1.11	1.44	1.90
13	0.23	0.27	0.31	0.39	0.48	0.55	0.66	0.76	0.87	0.99	1.11	1.26	1.48	2.44
14	0.04	0.08	0.12	0.20	0.26	0.33	0.41	0.47	0.48	0.57	0.66	0.83	1.59	2.37
15	0.38	0.45	0.49	0.56	0.63	0.71	0.76	0.86	0.92	1.00	1.09	1.21	1.71	3.19
16	0.94	0.98	1.03	1.08	1.16	1.25	1.30	1.35	1.43	1.50	1.62	1.80	2.35	3.58
17	0.75	0.79	0.84	0.91	0.96	1.05	1.12	1.19	1.27	1.32	1.42	1.50	1.89	2.87
18	0.14	0.18	0.22	0.32	0.41	0.51	0.61	0.70	0.81	0.95	1.08	1.31	1.44	2.90
19	1.71	1.76	1.77	1.84	1.92	2.00	2.05	2.11	2.24	2.24	2.32	2.51	3.03	4.25
20	1.85	1.93	1.95	2.05	2.15	2.30	2.41	2.51	2.68	2.59	2.91	3.10	3.89	3.17
21	0.64	0.74	0.77	0.82	0.89	0.98	1.03	1.05	1.22	1.49	1.60	1.49	2.09	3.90

APPENDIX C: RAW DATA FOR URINARY CREATININE

Percent Blood Contamination (%)	0.0%	0.1%	0.2%	0.4%	0.6%	0.8%	1.0%	1.2%	1.4%	1.6%	1.8%	2.0%	3.0%	5.0%
Animal ID	Urinary Creatinine													
1	26987	27294	26958	26955	27055	26786	26583	25857	26277	26701	26480	26509	25509	25746
2	20028	20464	20700	20640	20539	20247	19902	20707	20047	20368	20307	20223	19808	19609
3	30724	30545	30461	30061	29473	30417	30586	30377	29621	29604	30278	29723	29233	29376
4	24832	25045	25109	24445	24500	24621	24463	24468	24796	24475	24496	24825	23800	23977
5	20891	20049	20112	19962	19949	19192	19762	19229	19478	19111	19079	19159	18830	18620
6	16272	15814	15832	15816	16061	15797	15921	16046	15929	15623	16289	15999	15861	15716
7	12651	12562	12453	12495	12657	12304	12621	12465	12139	12248	12635	12379	11796	12111
8	21541	23933	23874	24106	23640	23975	23660	25156	24374	23630	23394	23406	23101	22851
9	10753	10683	10507	10645	10568	10470	10429	10605	10471	10511	10383	10473	10422	9834
10	15311	15342	14791	15151	15041	14661	14983	14863	14499	14432	14448	14684	14392	14078
11	9373	9756	8969	9640	9514	9557	9543	9615	9690	9836	9636	9670	9482	9243
12	12855	12741	12647	12760	12694	12780	12746	12352	12634	12753	12572	12354	12463	12238
13	10154	10566	10401	10227	10279	10211	10148	10268	10273	10264	10209	10442	9977	9795
14	4821	5130	5090	5209	5115	5266	5286	5487	5343	5221	5301	5156	5119	4854
15	9607	9442	9489	9403	9320	9141	9033	9176	9298	8863	8888	9117	9068	8825
16	11817	11760	11539	11229	11348	11050	11023	11326	10877	11058	11339	11258	11013	10937
17	7994	8384	8278	7994	7850	8019	7971	7994	7981	7831	7952	7946	7903	7602
18	1872	1866	1860	1796	1844	1837	1768	1727	1752	1765	1795	1839	1787	1742
19	3308	3266	3175	3231	3259	3223	3287	3225	3192	3199	3182	3222	3164	3100
20	3429	3409	3276	3407	3409	3355	3408	3344	3374	3349	3335	3353	3307	3342
21	920	1002	970	1013	1001	981	987	991	1012	997	981	1015	951	973

APPENDIX D: RAW DATA FOR URINARY PROTEIN TO CREATININE RATIO

Percent Blood Contamination (%)	0.0%	0.1%	0.2%	0.4%	0.6%	0.8%	1.0%	1.2%	1.4%	1.6%	1.8%	2.0%	3.0%	5.0%
Animal ID	Urinary Protein to Creatinine Ratio													
1	0.09	0.13	0.14	0.17	0.19	0.23	0.27	0.26	0.29	0.31	0.35	0.38	0.88	1.06
2	0.08	0.10	0.12	0.16	0.19	0.23	0.27	0.26	0.31	0.33	0.36	0.40	0.59	0.88
3	0.06	0.10	0.13	0.17	0.20	0.22	0.24	0.27	0.31	0.34	0.37	0.46	0.47	0.72
4	0.08	0.11	0.12	0.16	0.19	0.22	0.26	0.29	0.34	0.37	0.42	0.48	0.69	0.96
5	0.11	0.17	0.19	0.22	0.25	0.29	0.32	0.36	0.39	0.43	0.45	0.50	0.73	1.12
6	0.17	0.20	0.22	0.27	0.30	0.36	0.38	0.41	0.45	0.50	0.54	0.60	0.87	1.50
7	0.07	0.12	0.15	0.21	0.26	0.32	0.36	0.43	0.50	0.53	0.55	0.65	0.99	1.31
8	0.23	0.23	0.25	0.29	0.35	0.38	0.42	0.46	0.48	0.58	0.62	0.70	0.83	1.32
9	0.08	0.12	0.16	0.22	0.28	0.35	0.42	0.46	0.54	0.62	0.66	0.83	1.31	1.70
10	0.11	0.14	0.20	0.24	0.31	0.34	0.37	0.46	0.59	0.56	0.72	0.81	1.04	1.75
11	0.07	0.12	0.18	0.23	0.31	0.37	0.45	0.51	0.60	0.67	0.78	0.89	1.53	1.93
12	0.08	0.15	0.20	0.29	0.35	0.42	0.50	0.59	0.57	0.64	0.72	0.79	1.02	1.37
13	0.20	0.23	0.26	0.34	0.41	0.48	0.57	0.65	0.75	0.85	0.96	1.07	1.31	2.20
14	0.07	0.14	0.21	0.34	0.45	0.55	0.69	0.76	0.79	0.97	1.10	1.42	2.75	4.32
15	0.35	0.42	0.46	0.53	0.60	0.69	0.74	0.83	0.87	1.00	1.08	1.17	1.67	3.20
16	0.70	0.74	0.79	0.85	0.90	1.00	1.04	1.05	1.16	1.20	1.26	1.41	1.89	2.89
17	0.83	0.83	0.90	1.01	1.08	1.16	1.24	1.32	1.41	1.49	1.58	1.67	2.11	3.34
18	0.66	0.85	1.05	1.58	1.97	2.45	3.05	3.58	4.09	4.76	5.32	6.30	7.12	14.72
19	4.57	4.76	4.93	5.03	5.21	5.49	5.51	5.78	6.20	6.19	6.45	6.89	8.47	12.12
20	4.77	5.00	5.26	5.32	5.58	6.06	6.25	6.64	7.02	6.84	7.71	8.17	10.40	8.39
21	6.15	6.53	7.02	7.16	7.86	8.83	9.23	9.37	10.66	13.21	14.42	12.98	19.43	35.43

APPENDIX E: MASSEY UNIVERSITY ANIMAL ETHICS APPROVAL

AEC/9 (Amended 09/05)

Massey | 1932
Animal Ethics Committee

To: Secretary
 Animal Ethics Committee
 Room 2.02, Old Main Building
 Turitea, Palmerston North

Please send this original (1) application plus fourteen (14) copies
 Application due one week prior to the meeting

APPLICATION FOR APPROVAL OF PROPOSED EXPERIMENTAL/TEACHING PROCEDURES USING LIVE ANIMALS

1. CHIEF APPLICANT: (staff member only)

(a) Name: Keith Thompson
 Qualifications: BVSc PhD DipACVP
 Position: Professor
 Inst/Sch/Dept: IVABS

2. OTHER APPLICANTS: (see Code Section 5.1 for those who should be listed)

(a) Name: Eloise Jillings
 Qualifications: BVSc MACVSc
 Position: Masters Candidate

+

3. DETAILS OF PROJECT:

(a) Title: Assessing the effect of blood contamination on the urine protein to creatinine ratio
 (b) Commercial sensitivity status: Yes
 No

OFFICE USE ONLY

Copy for: _____ Date sent: 4-5-06
 Applicant: _____
 Date Received: _____ Head of Institute/Department: _____
 Office: _____ ✓
 Protocol No. 06/43
 Decision: **MASSEY UNIVERSITY ANIMAL ETHICS COMMITTEE APPROVED**
 Date: 21-4-06 *P.R. Watson*