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

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 Sulphasalicylic 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

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