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URINALYSIS IN COMPANION ANIMALS

A urinary sediment, microbiology and protein study

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Ph.D. Thesis
Frederiksberg 1996

DEDICATION

I dedicate this thesis to my family, wife: Justina, children: Deogratius, Mectilda, Erick and James. For your patience, perseverance and encouragement.

To my mentor and colleague, Associate Professor Flemming Kristensen. For teaching me to question, make critical analysis of data and facts before reaching conclusions and above all for showing me that as a university lecturer, my obligation is not just to share and apply new knowledge but also must strive to create it.

Acknowledgement

I wish to express my deep gratitude to my supervisor and great teacher, Associate Professor Flemming Kristensen for arousing my interest in the field of companion animal urology/nephrology, provision of valuable suggestions, for constant encouragement and for his enthusiasm about the research work throughout the study period. I am deeply indebted to Associate Professor, Asger Lundorff Jensen, my supervisor for the minor subject, through which some of the techniques and part of the materials for this study was obtained. Most work regarding the minor subject as well as important analyses were undertaken in the Central Laboratory. For this I wish to thank the Head of the central Laboratory Associate Professor Jens Steen Dirch Poulsen for placing the facilities of the Central Laboratory at my disposal. I am indebted to Associate Professor Axel Basse, Department of Pathobiology and Pharmacology for his interest in the project and for the constructive suggestions with regard to histopathology.

I would also like to thank the Head and all members of the Small Animal Hospital for allowing me free access to the facilities, willingness to share their knowledge and skills in companion animal medicine and cooperation amidst their tight working schedules. To them I say, you are wonderful and keep it up. My deep gratitude is extended to Ms Emma Thomsen, Ms Vera S. Østvedt, Ms Rie Sørensen, Mr Bent Rye Nilson and Ms Helen Birkkjær for their excellent technical assistance throughout the study.

I sincerely thank all the dog owners in Denmark and Tanzania; the management Karup Army Base (Karup, Denmark); Officer In charge, Dog section (Morogoro, Tanzania); Director, Vetcare (Dar es salaam, Tanzania) and Private Practitioners in the Copenhagen area (Denmark) for ensuring constant availability of companion animals throughout the study period.

I wish to thank Professor Sven Gade Christensen, then Danida Project Coordinator, Faculty of Veterinary Medicine (FVM), Sokoine University of

Agriculture, Morogoro, Tanzania, for his excellent cooperation during phase II of the study. I also wish to thank Associate Professor Beda Michael kessy, then Dean, FVM; Associate Professor Magishi Nkwabi Mgasa, Head of Department of Veterinary Surgery and Therio-genology, FVM and all members of FVM for the assistance accorded to me during phase II of the study. The technical assistance by Costa Magally and Edson Rugaimukamu, FVM, is gratefully acknowledged.

I also wish to register my sincere gratitude to the Danish International Development Agency (DANIDA) for funding the study. I am thankful to the staff, Danida fellowship Centre (DFC) and to Dr. Kaj Bruhn, Centre for Tropical Agriculture and Environment for skillfully coordinating all matters pertaining to my studies and provision of a conducive working environment. Various issues of "Fellowship Post" for 1993 through 1996 with their ever exciting events of each month, say it all.

Finally but not least, I wish to express my deepest gratitude to my family for an extended tolerance during the entire period of my studies. To my wife Justina, our children Mectilda Kulwa, Deogratius Doto, Erick G'wakami and James Mutahangarwa, I say thank you very much.

Copenhagen, 1996
Emmanuel Kahitwa Batamuzi

PREFACE

This thesis is comprised of two parts, first is a general part which is an overview of the diagnostic methods for the diseases of the urinary system. The purpose of the general part is to bring together contemporary information about a whole range of diagnostic methods for diseases of the urinary system in companion animals.This section has been prepared for students, veterinary technicians and practitioners of veterinary medicine, who have not specialized in urology and nephrology, but whose work requires exposure with respect to available diagnostic techniques.

The six papers listed below forms the second part.

I: Batamuzi, E. K. and F. Kristensen :
Diagnostic importance of urothelial cells of the dog and cat. *Journal of Small Animal Practice* **1995**, 36: 17-21.

II: Batamuzi, E. K., F. Kristensen, A. Basse and S. Dahl: Idiopathic renal hematuria in a dog. *Veterinary Record* **1994**, 135: 603.

III: Batamuzi, E. K. and F. Kristensen: Urinary tract infection: the role of canine transmissible venereal tumour. *Journal of Small Animal Practice* **1996 (In Press)**.

IV: Batamuzi, E. K., F. Kristensen and A. L. Jensen : Analysis of serum proteins in geriatric dogs using agarose electrophoresis. *J. Vet. Med. Series A*. **1996 (In preparation)**.

V: Batamuzi, E. K., F. Kristensen and A. L. Jensen : Subclinical glomerulopathy in selected cases of recurrent pyoderma in dogs. *Veterinary Dermatology* **1996 (In Press)**.

VI: Batamuzi, E. K., F. Kristensen and A. L. Jensen : Composition of protein in urine from dogs with pyoderma. *Veterinary Record* **1996 (Submitted)**.

CONTENTS

LIST OF PROTOCOLS

ABBREVIATIONS

A/G ratio	:	Albumin to globulin ratio
CBC	:	Complete blood count
CHF	:	Congestive heart failure
E.coli	:	*Escherichia coli*
Eg	:	Example giving
et al.	:	And others
Fig.	:	Figure
FUS	:	Feline urological syndrome
GGT	:	Gamma glutamyl transferase
LUT	:	Lower urinary tract
NA	:	Not applicable
NAG	:	N-acetyl-ß-D-glucosaminidase
OR	:	Odds ratio
RBC	:	Red blood cell
Rf	:	Relative mobility
rpm	:	Revolutions per minute
SEM	:	Standard error of the mean
SSA	:	Sulfosalicylic acid
TVT	:	Transmissible venereal tumour
USG	:	Urine specific gravity
UTI	:	Urinary tract infection
WBC	:	White blood cell

DIAGNOSTIC METHODS FOR CONDITIONS OF THE URINARY SYSTEM

Introduction

The urinary tract is among the most important body systems because of its role in a number of physiologic/metabolic functions. However some of these functions place the urinary system at risk of development of diseases. Thus it has been reported that 14% of all dogs develop urinary tract disease sometime during their lives (Rubin, 1990a). Most urinary tract diseases afflicting companion animals have been shown to increase with age, eg among old dogs, renal failure is the second leading cause of non accidental death (Krawiek, 1989). Despite of this high frequency of renal disorders among dogs and cats, not all lesions result in clinical signs (Bush, 1984; Batamuzi et al., 1995; Batamuzi et al., 1996b). This subclinically affected group of companion animals is especially important since it has a potential for developing into chronic renal failure, an irreversible and progressive disease (Polzin et al., 1995).

Urolithiasis, a condition of the entire urinary system, has been reported to affect between 0.4 and 2% of the canine population (Bush, 1984). This condition does also predispose companion animals to other urinary tract ailments, among them urinary tract infection (Nelson and Couto, 1992). Urinary tract infection has a potential for causing serious consequences such as pyelonephritis, kidney scarring, renal failure, infertility, bacteremia and septicemia (Rubin, 1990b).

The diagnosis of diseases of the urinary tract is an extremely important undertaking because of the frequency of their occurrence among companion animals and for the central role that the urinary system plays in body metabolism. The fact that some of the diseases are irreversible, progressive and life threatening, underscores the importance of early diagnosis. In some of the diseases, such as urinary tract infection, clinical signs as for example fever, depression, sublumbar pain and increased frequency

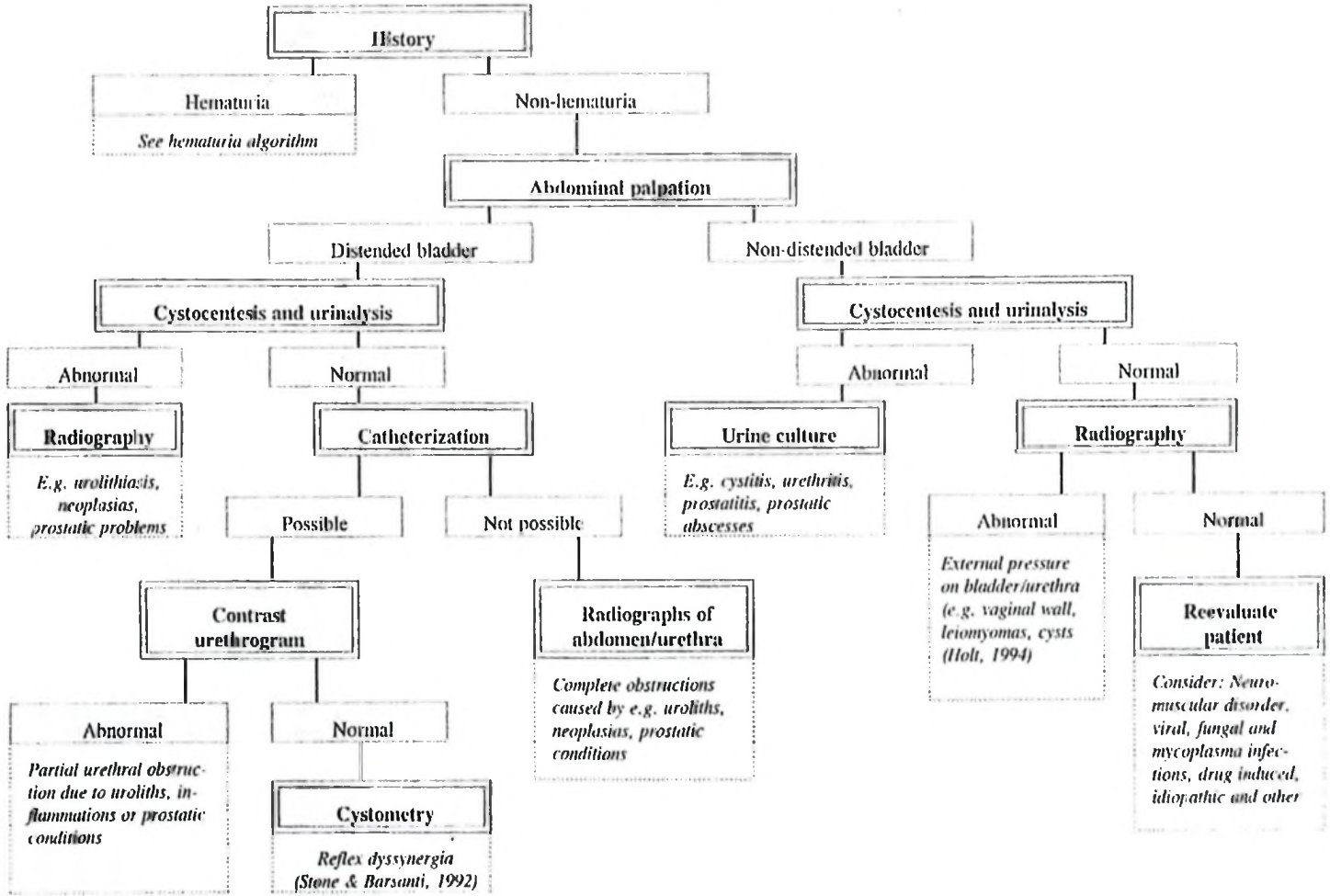
of urination are not commonly seen and thus, their diagnosis can only be reached from laboratory test results (Rubin, 1990b; Shaw, 1990; Batamuzi and Kristensen, 1996).

Companion animals will be suspected to be suffering from urinary tract disease when there is dysuria, hematuria, urinary incontinence, increased frequency of urination/urgency or some combinations of these signs. It is on the basis of the main presenting sign(s) that important decisions about the diagnostic approach will be based (Stone and Barsanti, 1992). Regardless of the presenting sign, good history taking and physical examination are important ingredients for a successful diagnosis (Holt, 1994).

Dysuria which is difficulty urination or straining to urinate essentially indicates that there is an abnormality in the bladder or urethra (Stone and Barsanti, 1992). The main causes of dysuria in companion animals are obstruction, irritation or inflammation of the lower urinary tract (Batamuzi and Kristensen, 1996). Obstruction of the urethra caused by uroliths, neoplasms, space occupying lesions and foreign bodies of the urethra, genital tract as well as the prostate gland is frequently diagnosed in dysuric cases (Wolf, 1988). Cystitis, mainly of bacterial etiology is another frequent cause of dysuria in dogs and to a lesser extent in cats (Stone and Barsanti, 1992). All the above listed causes of dysuria act through physical obstruction of the urethra. Functional obstruction of the bladder neck or urethra does occur and is usually a result of impairment of nerves in the bladder wall or sphincters (Ling, 1995). These nerves, including branches of hypogastric, pudendal and pelvic take part in the voiding phase of micturition (Lees, 1988).

Quite often dysuria is associated with hematuria, mainly because most of its causes are either traumatic or inflammatory in nature (Wolf, 1988). Dysuria may also be associated with bladder distention. Due to these associations, the diagnostic plan for dysuria has to address two main questions: First, is whether dysuria is accompanied by hematuria and secondly, is whether there is a distended or an empty bladder in the dysuric animal under study (Stone and Barsanti, 1992). The diagnostic plan should also take into consideration the potential causes and consequences of dysuria. Thus clinical assessment of a dysuric animal should be combined with laboratory examinations to determine the degree of uremia, hyperkalemia, and acidosis and how they may be correc-ted (Holt, 1990a). Urinalysis on a cystocentesis sample will provide some information regarding the possible cause for the dysuria and may shed light on the consequences of dysuria (Algorithm 1). Urinalysis will provide some indications regarding the possible causes for dysuria such as cystitis, urolithiasis, blood clots and prostatic abscesses (Stamey and Kindrachuk, 1985). Companion animals with dysuria as the main presenting sign should have their caudal abdomen carefully palpated, as a

ASSESSMENT OF PATIENTS WITH DYSURIA (Algorithm 1)



means for distinguishing distended bladder from empty bladder forms of dysuria. In case of a distended bladder form, urethral catheterization will be the first indication. This will bring about relief of dysuria, and it will assist in locating site of obstruction. In some situations catheterization may mislead the clinician in the determination of presence of outflow obstruction leading to false positive or false negative findings (Holt, 1990a). Soft tissue masses in the urethra are easily displaced by the catheter, giving an impression of no obstruction. The os penis may impede smooth catheterization thus leading to pursuit of red herrings. Urinalysis is a necessary test for companion animals with empty bladder dysuria. Depending on the results of urinalysis, urine culture may be useful at this stage since this may give conclusive evidence for the involvement of conditions like cystitis and urolithiasis due to struvite calculi (Chew and DiBartola, 1986). If no infection is detected on urine culture, survey radiography and contrast cystography may be indicated. Where all the above fail to identify the cause for dysuria, exploratory laparotomy and cystotomy may be done (Stone and Barsanti, 1992). However, less invasive diagnostic methods like ultrasonography may be employed in locating the possible cause for dysuria (Lamb, 1990). Ultrasonography is particularly useful for prostatic disorders such as prostatic abscesses and cysts (Holt, 1990b, Dorfman and Barsanti, 1995). Supporting tests such as a complete blood count (CBC) may be used in order to establish whether there is systemic inflammation, a common sequel in animals with urethral obstruction and urinary tract infection (Sodikoff, 1995). Hyperkalemia is serious sequel to urinary obstruction that requires immediate management (Gleadhill, 1994), thus clinical biochemical tests may be required for its early detection. Renal function tests are useful for animals with azotemia.

Companion animals with hematuria will benefit from good history taking which may give indication for the source of hematuria (Holt, 1994). By definition, hematuria refers to the presence of whole blood in the urine (Stone and Barsanti, 1992). However, a significant number of red blood cells may be found in the urine of normal animals (Lusk, 1995). Acceptable numbers of red blood cells per high power magnification in normal animals vary with the methods by which urine is collected (Chew and DiBartola, 1986; Wolf, 1988). Thus for a voided, catheterized and cystocentesis sample respectively, 0 to 8/hpf, 0 to 5/hpf and 0 to 3/hpf are considered normal (DiBartola, 1995). Increase in the number of red blood cells in urine (hematuria) may be caused by hemorrhage due to local trauma to or tumors of the vagina, prepuce, penis or urinary bladder (Stone and Barsanti, 1992). Uroliths in any part along the urinary tract are also implicated in causing trauma and hematuria

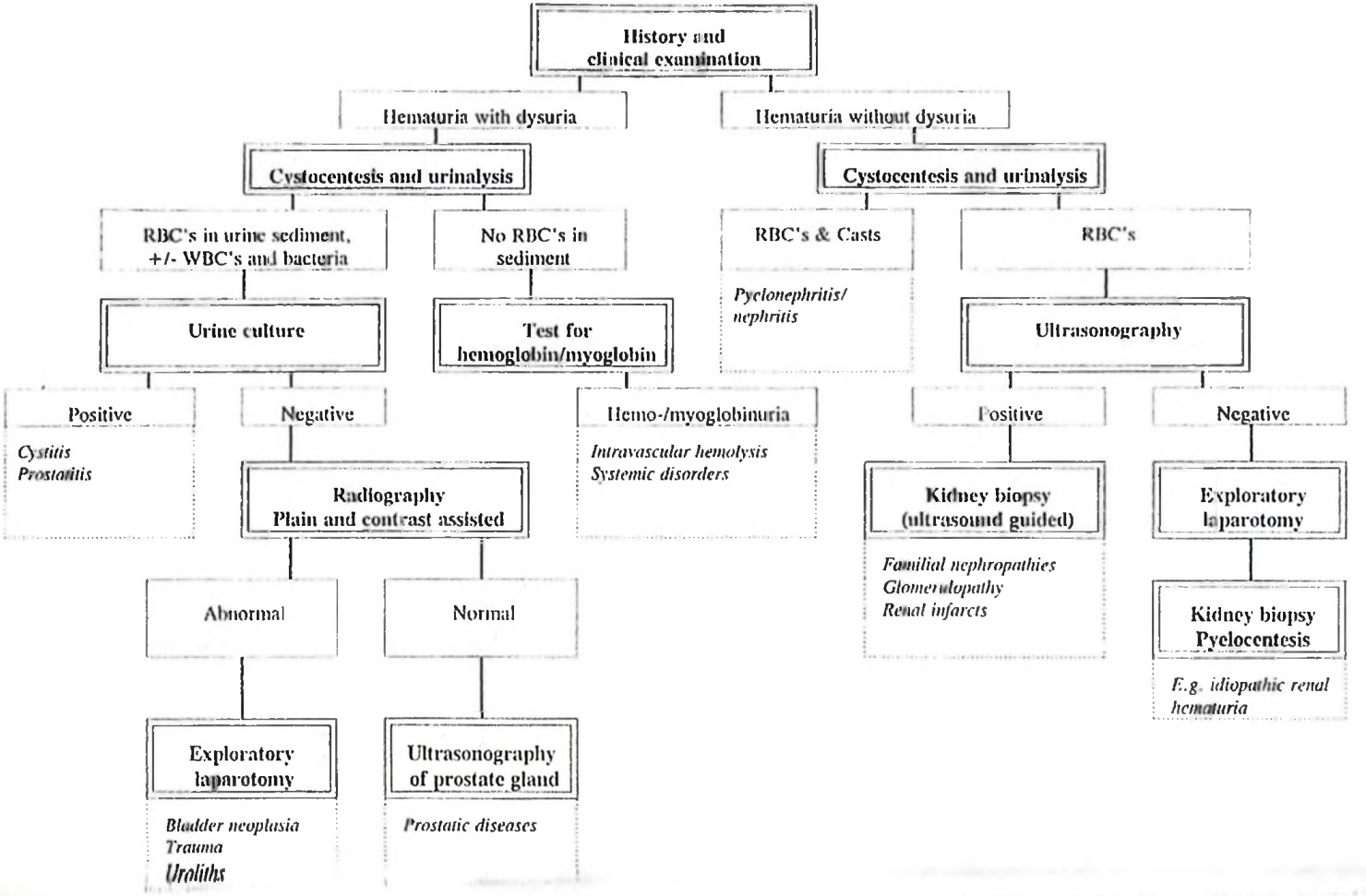
(Stamey and Kindrachuk, 1985). Hemorrhage may also result from bleeding disorders or vascular anomalies as for example in telangiectasis of Welsh corgis (DiBartola, 1995a) or from neoplasia (Metzger et al., 1993). Inflammatory conditions, like urinary tract infection are another potential cause for hematuria (Stone and Barsanti, 1992)). Fortunately, inflammatory conditions also cause significant white blood cell reaction (Stamey and Kindrachuk, 1985). This feature, together with the results of urine culture may prove to be diagnostically important in establishing the cause of hematuria. Idiopathic renal hematuria is sometimes encountered in which the exact cause cannot be established with certainty (Batamuzi et al., 1994).

Any diagnostic plan for a hematuric companion animal has to address the potential causes which range from local trauma, irritants/inflammatory conditions to systemic conditions such as bleeding/coagulation disorders as well as systemic inflammatory conditions and toxemia (Allen, 1989). In algorithms 2-4 the diagnostic plans for the different forms of hematuria are illustrated.

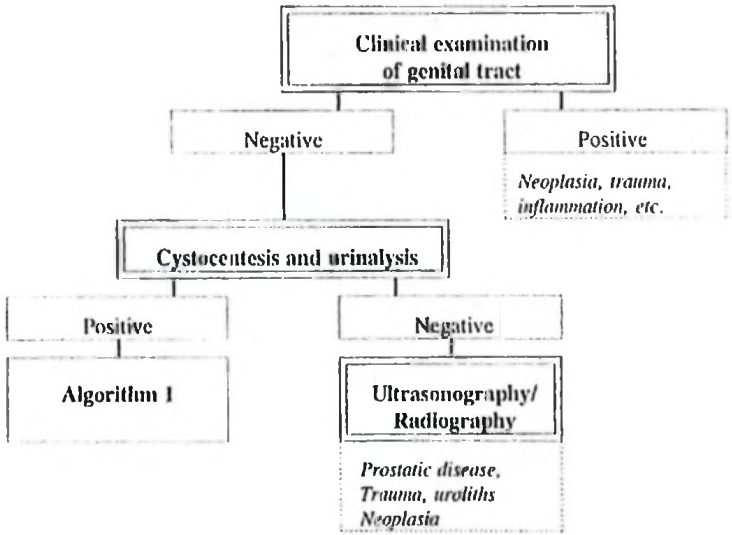
Collection of a voided urine and cystocentesis samples may further help in localizing the source of blood in urine. Blood in the voided and cystocentesis samples is indicative of hemorrhage from the kidneys, ureters, bladder or proximal urethra in females and prostate gland in males (Stone and Barsanti, 1992). Urinalysis for occult blood and urine sediment examination will most likely assist the clinician in reaching a tentative diagnosis (Stamey and Kindrachuk, 1985). Renal functional tests may be of help in associating the problem with the kidney. In some cases other approaches may be necessary before the cause of hematuria is established. A typical example for such a situation is where a radiolucent foreign body in the lower urinary tract is the cause for the persistent hematuria, requiring contrast assisted radiography, exploratory laparotomy and cystotomy (Fooshee et al., 1992). Exploratory laparotomy coupled with collection of urine from pelves by pyelocentesis is sometimes the only way to localize the source of hematuria (Batamuzi et al., 1994).

Urinary incontinence has been defined as lack of voluntary control over the flow of urine from the body (Moreau and Lees, 1995). Urinary incontinence has neurogenic and non neurogenic causes (Holt, 1994). Any condition that disrupts the nervous control of the storage phase of urination and all those conditions that affect the integrity of the effector organs in this case the bladder and urethra may cause urinary incontinence (Moreau and Lees, 1995). Ectopic ureters terminating in wrong places which in effect bypass the normal sphincters are a frequent cause for urinary incontinence (Moreau and Lees, 1995). Urinary incontinence also follows certain surgical procedures which probably lead to acquired sphincter mechanism incompe

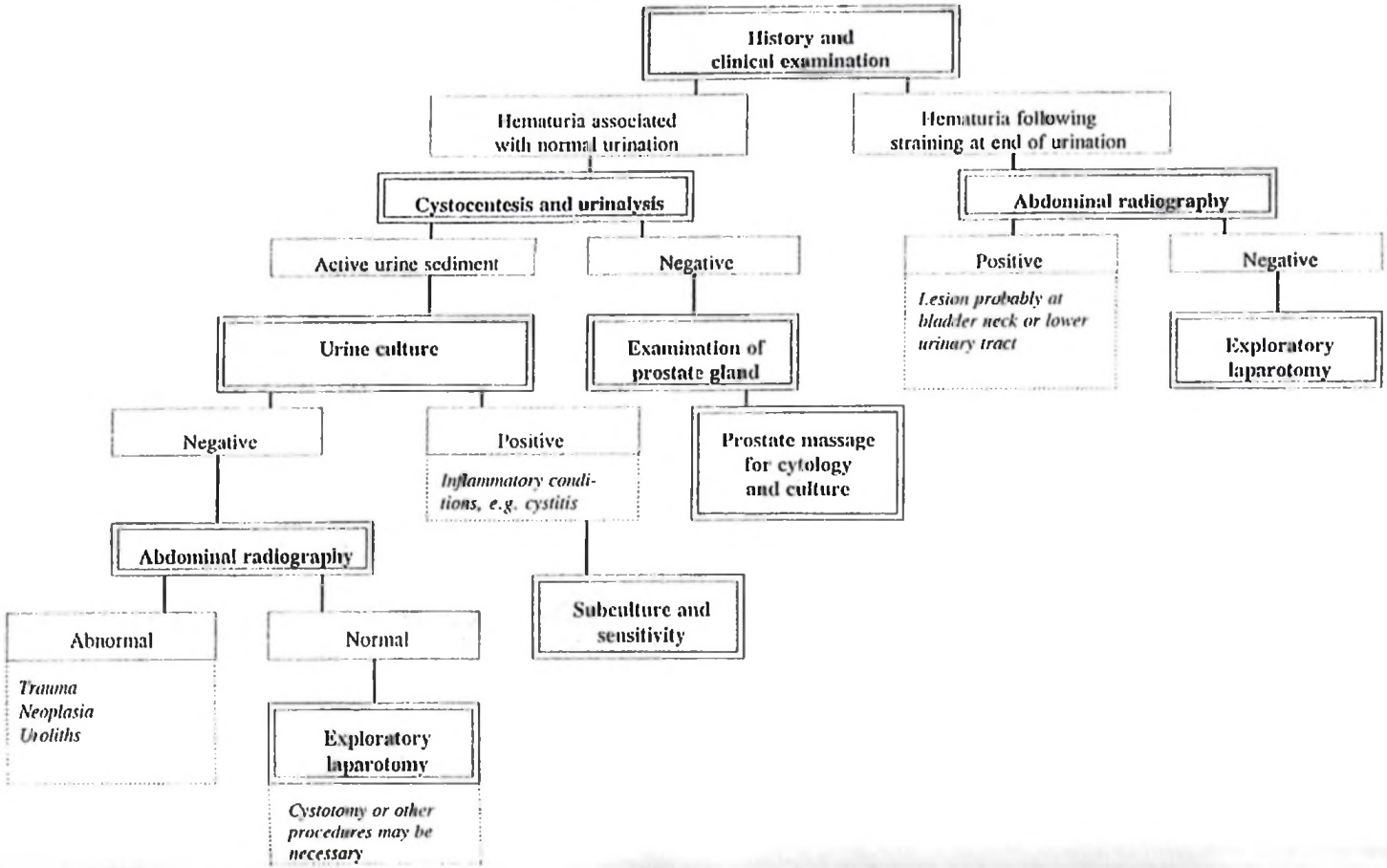
ASSESSMENT OF PATIENTS WITH HEMATURIA THROUGHOUT URINATION (Algorithm 2)



ASSESSMENT OF PATIENTS WITH HEMATURIA INDEPENDENT OF URINATION OR AT THE BEGINNING OF URINATION (Algorithm 3)



ASSESSMENT OF PATIENTS WITH HEMATURIA AT THE END OF URINATION (Algorithm 4)



tence, functional disability or vaginoureteral fistulation (Banks et al., 1991). Vaginoureteral fistulation may be acting through causation of a form of ectopic ureter(s), an acquired disorder.

To formulate a logical and effective approach to urinary incontinence the clinician must understand the anatomy and physiology of the lower urinary tract, consider the impact that normal aging has on the system, and be aware of the theoretical and practical factors that can precipitate incontinence. Careful evaluation of the central nervous system and the sacral reflexes is the key to distinguishing neurogenic from non-neurogenic forms of urinary incontinence (Algorithm 5). Urinalysis and renal function tests must be done in order to determine the severity of the condition and the consequences of obstruction (Moreau and Lees, 1995). Survey radiographs for the abdomen (Pollack, 1992) and cranium as well as other tests like cerebral spinal fluid analysis, electroencephalography, myelography, computed tomography and nuclear scans may also help in the diagnosis of urinary incontinence (Stone and Barsanti, 1992).

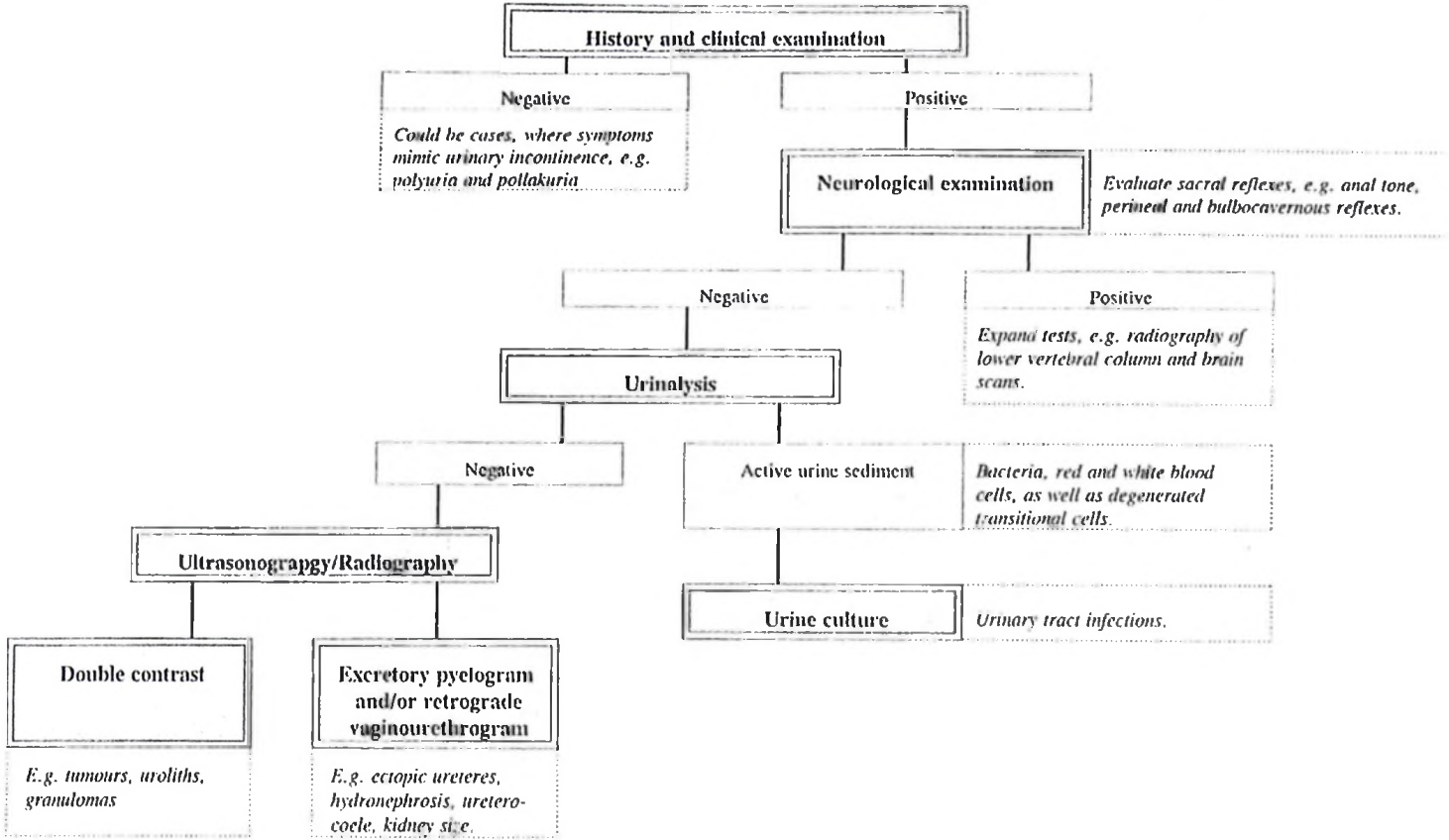
Animals with polyuria/polydipsia need careful evaluation in order to verify the diagnosis. Often companion animal owners confuse polyuria/polydipsia with nocturia and pollakuria (Algorithm 6). Urinalysis, CBC and serum biochemical profiles are necessary at initiation of the evaluation of polyuria/polydipsia in companion animal patients (Meric, 1995). Despite unremarkable urine sediment findings, such animals may benefit from urine culture (Meric, 1995).

Abdominal radiography and ultrasonography are necessary for evaluation of the liver, kidney, uterus and adrenal glands (Lamb, 1990, Pollack, 1992). Those organs are frequently involved in the pathogenesis or affected by polyuria/polydipsia (Stone and Barsanti, 1992).

In some conditions including glomerulonephritis and the initial stage of acute and chronic renal failure, clinical signs may be vague and atypical. However, here again complete urinalysis and protein quantitation tests coupled with renal functional tests may be of value in establishing a diagnosis (Graeur, 1992).

For most conditions of the urinary system proteinuria is a predominant finding. Tests for proteinuria, its quantification and characterization may help clinicians in the diagnosis of such disease conditions (Graeur, 1992). For many of these conditions urinalysis appears to be a necessary test (Table 1 and 2).

ASSESSMENT OF PATIENTS WITH URINARY INCONTINENCE (Algorithm 5)



ASSESSMENT OF PATIENTS WITH POLYURIA/POLYDIPSIA (Algorithm 6)

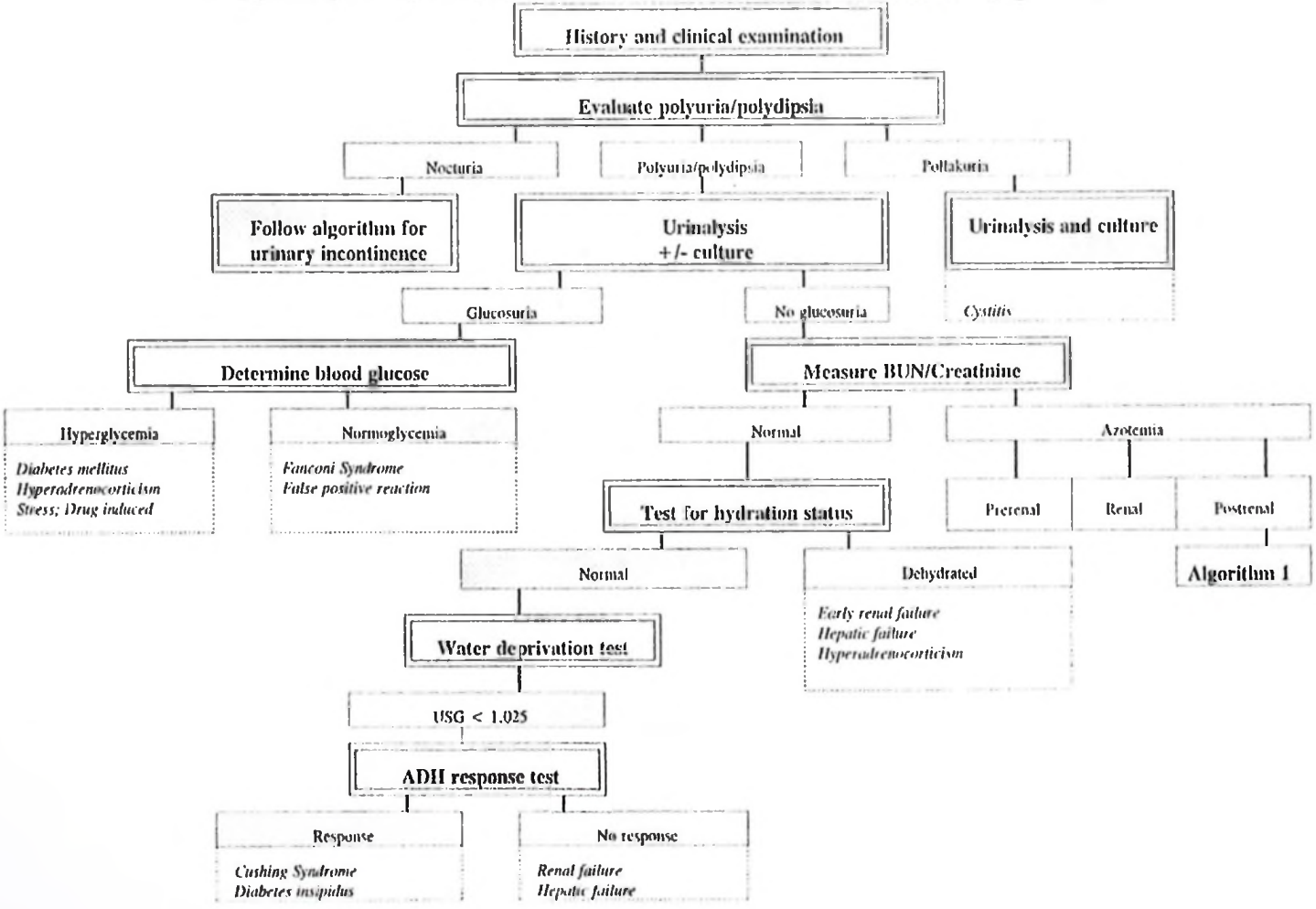


Table 1: *Urinalysis: refractometry and colorimetric analysis*

Disease	pH	USG	Protein	Glucose	Occult blood
Acute renal failure	-	Low	*	-	*
Chronic renal failure$_E$	-	Low	*	-	-
Chronic renal failure$_L$	-	Low	-	-	-
Juvenile renal disease	-	Low	*	*	-
Pyelonephritis (UTI)	-	Low	*	-	*
Glomerulonephritis	-	High	*	-	-
Nephrotoxicosis	-	High	*	*	*
Renal ischemia	-	Low	*	*	-
Post renal uremia$_O$	-	High	*	-	-
Post renal uremia$_{UA}$	-	-	-	-	-
FUS	High	High	*	*	*
Renal thromboembolism	-	-	*	*	*
Fanconi syndrome	Low	Low	-	*	-

Key: *$_{E, L, O}$ and $_{UA}$:* *Early, late, obstructive and uroabdomen.*
**:* *Positive finding.*
-: *Negative (normal) finding.*

Table 2: *Urinalysis: urine sediment and culture*

Disease	WBCs	RBCs	Casts	Crys-tals	Bac-teria
Acute renal failure	*	*	*	-	-
Chronic renal failure$_E$	-	-	*	-	-
Chronic renal failure$_L$	-	-	-	-	-
Juvenile renal disease	-	*	*	-	-
Pyelonephritis (UTI)	*	*	*	-	*
Glomerulonephritis	-	-	-	-	-
Nephrotoxicosis	-	*	*	-	-
Renal ischemia	-	*	*	-	-
Post renal uremia$_O$	*	*	-	-	*
Post renal uremia$_{UA}$	-	-	-	-	-
FUS	-	*	-	*	-
Renal thromboembolism	*	*	*	-	*
Fanconi syndrome	-	-	-	*	-

Key: $_{E, L, O}$ *and* $_{UA}$*:* *Early, late, obstructive and uroabdomen.*
*: *Positive finding.*
-: *Negative (normal) finding.*

URINE SAMPLE COLLECTION AND MANAGEMENT

In order to get meaningful results it is important a prerequisite to collect, preserve and process samples in the best possible way. Concentrated urine obtained after animals have been deprived of water for several hours as is the case for morning urine, tend to give good and reliable results (Ling, 1995). There are three different methods by which urine samples can be collected. The method used in collection of urine will usually have influence on the results to be obtained and the choice is governed in part by the anticipated type of analysis.

Voided urine sample

In this method of sample collection, urine is collected during normal urination or after the animal has been induced to urinate. The advantages of this technique of urine collection are two fold. First, this method is not associated with any risk of complications to the patient and secondly, it can be used by the animal owner/client. A voided sample is indicated for animals suspected to have hematuria since other methods of collection may add red blood cells due to trauma (DiBartola, 1995). However, manual compression which is a form of voided urine sample collection is not suitable for evaluation of hematuric cases as it may cause trauma and vesico-ureteral reflux (Osborne and Stevens, 1981). The voided urine sampling method is not recommended for samples intended to be used for the diagnosis of urinary tract infection for there is a risk of contamination from the urethra and genital tract (Rubin, 1990b).

For more accurate localization of certain forms of urinary tract problems different techniques of voided sample collection have been devised. Thus a two glass

specimen has been used for localization of pyuria much more accurately in m
(Stamey and Kindrachuk, 1985). In this method two specimens are collected, the fir
at initiation of urination and the second after urination has started. The first samp
reflects the status in the urethra, particularly the terminal part which potentially, c
be contaminated. The second glass sample provides the actual situation in the bladd
and the upper urinary tract. In another technique, a three glass specimen, has been use
for localization of hematuria. More still, a four glass specimen has been used for th
localization of urinary tract infection, and it aids in removing the confounding effect o
possible prostatic infection (Lowe and Brendler, 1992).

Catheterized urine sample

Catheterization is done for various reasons in companion animals (McGuire
1991), including the relief of dysuria, and provision of urinary by-pass when there i
urethral injury. It is also used for the drainage of urine when there is bladder paralysis
introducing contrast media into the urinary tract and detection of urinary obstructio
(Holt, 1994). Despite the numerous applications for urethral catheterization, the mos
frequent reason for its use, is to obtain urine samples for laboratory analysis (Osborn
and Stevens, 1981).

Catheterized urine sampling is indicated when samples cannot be obtained by
voiding or cystocentesis (McGuire, 1991). It is also used when a previous voide
sample shows signs of contamination from the distal urethra or genital tract. The
catheterization technique varies between sexes and between dogs and cats. Most dogs
can be catheterized without chemical restraint, but cats usually require sedation o
anaesthesia (Holt, 1994). In tables 3 and 4, protocols for catheterization are explained
Table 5 presents the protocol to be used in female catheterization in the absence of a
vaginal speculum or otoscope. This technique is only possible in large breeds of dogs
(Osborne and Stevens, 1981).

Urethral catheterization is associated with two main problems, both of which
can be overcome by properly following the ideals of the technique in its execution
(Holt, 1994). These are the introduction of organisms into and iatrogenic trauma to the
lower urinary tract. The technique also carries a risk of causing other iatrogenic
conditions like hematuria, hypercellularity and proteinuria (Biertuempfel et al., 1981).
and iatrogenically introduced oil droplets from lubricates used at catheterization.
Catheters bypass all of the local defenses in the urethra (Barsanti et al., 1985). In the
process bacteria from the external environment, hair, skin, genital tract and distal

Table 3: *Urethral catheterization in a male dog.*

Urethral catheterization in a male dog	
PROCEDURE	
A	Select a urinary catheter of appropriate size.
B	Measure the patient for proper placement of catheter into the caudal bladder, noting the distance on catheter.
C	Extrude the penis from prepuce.
D	Wash the tip of penis with surgical scrub, then rinse with saline.
E	Lubricate the tip of catheter with sterile lubricant.
F	Thread the catheter into urethra and advance it towards the bladder until a correct placement is attained or until urine comes out.*
G	Allow the first few drops of urine to come out, then collect required amount of urine.
H	Gently withdraw the catheter when enough urine sample has been collected.
*)	*Note: resistance may be felt at the level of os penis, ischial arch and prostate gland. To overcome this, withdraw the catheter slightly. Then apply gentle pressure to readvance the catheter.*

Table 4: *Urethral catheterization in a female dog*

Urethral catheterization in a female dog	
PROCEDURE	
A	Select the urinary catheter of appropriate size.
B	Position the animal such that hind quarters face the clinician.
C	Assistant supports animal under the abdomen so that urinary bladder is not lowered during catheterization.
D	Wash and rinse vulva and perineum.
E	Measure the patient for proper placement of catheter into caudal bladder, noting the distance on catheter.
F	Insert a sterile vaginal speculum into vagina and part its blades.
G	Lubricate the tip of catheter.
H	Aided by good lighting and opened vaginal lumen, insert catheter into the urethral orifice.
I	Continue advancing the urethral catheter until urine comes out.
J	Gently withdraw catheter when enough urine has been collected.
***)**	*An otoscope may be used for visualization of the urethral papilla thus facilitating threading of the catheter into the urethra.*

Table 5: *Urethral catheterization in a female dog (urethral papilla palpation)*

Urethral catheterization in a female dog
(Urethral papilla palpation technique)

PROCEDURE

A	Select the urinary catheter of appropriate size.
B	Position the animal such that hind quarters face the clinician.
C	Assistant supports animal under the abdomen so that urinary bladder is not lowered during catheterization.
D	Wash and rinse vulva and perineum.
E	Measure the patient for proper placement of catheter into caudal bladder, noting the distance on catheter.
F	Lubricate the tip of the catheter as well as the gloved finger of the free hand.
G	Insert the lubricated finger into the vagina and palpate the vulval papilla on the vaginal floor.
H	Guided by the finger on the urethral papilla, insert the catheter under the finger advancing it towards the urethral papilla and thence into the urethra.
I	Continue advancing the urethral catheter until urine comes out.
J	Gently withdraw catheter when enough urine has been collected.

urethra that contaminate the catheter will be carried into the urinary bladder (Lulich and Osborne, 1995). Also trauma that the catheter may cause to the urothelium predisposes the patient to bacterial infections since it damages normal defense mechanisms (Osborne and Stevens, 1981). Other complications associated with urethral catheterization include urethral or bladder trauma and irritation or laceration (McGuire, 1991).

Most of the problems associated with catheterization can be minimized or even avoided. To reduce the risk of introducing bacteria into the bladder, the procedure should be undertaken aseptically by proper animal restraint, cleaning the tip of penis and the perineum for males and females respectively and handling the catheter within its polythene bag. The use of catheters of right or smaller size and measuring out the catheter on the animal before its placement are reliable controls against trauma and the associated iatrogenic hematuria (Crow and Walshaw, 1987). Introducing the catheter too far, may lead to a perforation of vesica or its following the contour of the bladder wall and then bending backwards to exit into the proximal urethra along itself (Holt, 1994). Urine samples obtained under such circumstances cannot be truly representative for the bladder urine as intended. Even worse, the catheter may form a knot inside the bladder, causing trauma to the bladder and urethra. Such catheters are not retrievable via the urethra. Manual compression of the bladder with the catheter *in situ* or suction on the catheter may result in iatrogenic hematuria and hypercellularity, both of which may lead to misinterpretation of laboratory results (Holt, 1994, Batamuzi and Kristensen, 1995). In view of the risks associated with catheterization, the technique should be used when it is absolutely necessary, and even then instructions for the procedure must be followed to the letter.

Cystocentesis

Cystocentesis has been defined as needle puncture of the urinary bladder for the purpose of removing a variable quantity of urine by aspiration (Yam, 1994). Indications for cystocentesis are the collection of urine for laboratory examination and drainage of urinary tract organs (Wolf, 1988; Yam, 1994; Holt, 1994). Cystocentesis is also used in emergence procedures, such as relief of dysuria in perineal hernias (Holt, 1994). In this case cystocentesis is done through the perineum to drain the bladder before returning it to the abdomen. A fine needle, 22 to 23 gauge, 1 to 1.5 inches should be used for cystocentesis, this reduces the possibility of urine leakage from the bladder puncture site (Holt, 1994; Ling, 1995). In male dogs the best site for the procedure is posterior to the umbilicus, 2-4 cm lateral to the sheath. In bitches, cystocentesis is done on the ventral midline just posterior to the umbilicus. Cysto-

centesis is normally performed on a standing animal or with the animal in lateral recumbency, sedation is not necessary unless the patient is fractious (Table 6). Cystocentesis is easy and safe to perform when the urinary bladder contains sufficient urine for it to be identified by abdominal palpation and to be immobilized against the pelvis/abdominal wall (Figure 1a and b). The technique is indicated for the collection of samples for diagnosis of urinary tract infection and hematuria (Wolf, 1988; Yam, 1994).

Storage and management of urine samples

In order to obtain reliable results from urinalysis, the handling of urine samples before and during analysis is extremely important. Samples should be collected in clean containers. Urine samples intended for culture must be collected and stored in sterile containers (Rubin, 1990b). Regardless of the type of test to be undertaken, good results are obtained if analysis is done as soon as possible (Wolf, 1988).

Analysis of a sample should preferably be done within 15-30 minutes of its collection to avoid the development of changes that will affect interpretation of results (Batamuzi and Kristensen, 1995). Also early performance of urinalysis is important since delays tend to lead to dissolution of casts, changes in chemical composition, growth of contaminating microorganisms and loss of cellular detail (Osborne and Stevens 1981). Generally, changes that occur in urine that has been left standing can significantly alter the results of chemical reagent tests, bacterial cultures and cytological examination of the urine sediment (Rubin, 1990b; Batamuzi and Kristensen, 1995). However, where immediate analysis or processing is not possible samples can be refrigerated for no more than eight hours (Barsanti et al., 1985; Rubin, 1990b). Addition of preservatives such as formaldehyde or toluene may somewhat minimize changes in urine, however, such preservatives may interfere with results of chemical tests which are done as part of a complete urinalysis (Osborne and Stevens, 1981).

Table 6: *Urine collection by cystocentesis*

Urine collection by cystocentesis	
PROCEDURE	
A	Palpate the bladder to determine its size and location.
B	Stabilize/immobilize the bladder against the pelvis.
C	Wipe area of intended needle insertion with alcohol.
D	Insert needle into the abdominal cavity and bladder, such that needle enters the bladder wall at 45° angle to the surface of the bladder (See figure 1).
E	Aspirate urine into the attached syringe as required.
F	If no urine is obtained withdraw the needle completely, replace it and try again*.
***)**	*No more than two attempts should be made as there is a risk of causing trauma to the bladder and/or bowel.*

Figure 1: **(A)** Plain radiograph showing the relationship between the urinary bladder and other internal structures as well as the abdominal wall. Note position of entry of needle into a full bladder. This is the most cranial site for needle penetration into the bladder. **(B)** Cystocentesis. The abdominal wall is punctured at its ventral or ventro-lateral surface, then the needle is directed in the caudo-ventral aspect so that the bladder wall is penetrated obliquely at about 45° angle and near the junction of the bladder and urethra.

PHYSICAL EXAMINATION OF URINE

Introduction

This is usually done as the very first procedure of urinalysis. Color, clarity, odor and specific gravity are the physical parameters evaluated.

Color and clarity are subjective and of questionable value (Fettman, 1987). These tests, however, do not require sophisticated equipment to undertake save for good source of light, a receptacle containing the sample and the clinicians eye, therefore, there is no reason why they should be excluded from a complete urinalysis procedure.

Urine color

Normal urine is light yellow to amber in color, but very dilute urine is almost colorless and concentrated urine is deep yellow (Osborne and Stevens, 1981). Red, orange and brown colors have been reported as being the common abnormal urine colors (Chew and DiBartola, 1986; Lusk, 1995). Occasionally whitish-cream colored urine may be encountered in animals with pyuria and urolithiasis (Rubin, 1990b). A red to brown color suggests hematuria, hemoglobinuria or myoglobinuria (Stone and Barsanti, 1992). A yellow to orange color suggests bilirubinuria (Osborne and Stevens, 1981). Distinction of the different causes for red urine can be done by means of urine dipsticks, urine centrifugation and urine sediment examination (Stone and Barsanti, 1992; Stamey and Kindrachuk, 1985). In instances in which hematuria is the cause for the red urine, occult blood test is positive and red blood cells are seen in the urine sediment. Centrifugation of the urine sample allows red blood cells to settle at the bottom of the tube leaving clear normal colored urine on top as a separate layer where hematuria is involved, whereas in case of hemoglobinuria, a uniformly red color in the urine will not be altered by centrifugation. The distinction of myoglobinuria from

hemoglobinuria may be a bit tricky by the above distinguishing procedures, but it has been reported that myoglobin imparts a more brown color to urine when compared to hemoglobin (Stone and Barsanti, 1992). According to Sodikoff, 1995, for definitive identification of the cause for red urine a protocol has been devised (Table 7).

Urine consistency/clarity.

Normal freshly collected urine, in a clean container is usually clear. Cloudy urine often contains increased cellular elements, crystals, lipid droplets, or micro-organisms. Definitive identification of the cause for turbid urine can be arrived at thorough microscopic examination of the urine sediment prepared from fresh urine. Hypercellularity, inflammatory cells, urine crystals and microorganisms especially bacteria can be detected (Stamey and Kindrachuk, 1985; Lulich and Osborne, 1995). Additionally, a number of qualitative and quantitative tests may be used for the verification and identification of uroliths, if these are the cause for turbid urine (Ruby and Ling, 1986).

Urine odor.

Normal urine has a characteristic odor which varies between sexes and species (Osborne and Stevens, 1981). Abnormal odor may be experienced and is often due to excretion of drugs, urease-producing bacteria such as staphylococcus spp and proteus spp, and ketonuria. Freshly voided urine with ammoniacal odor suggests infection of the urinary tract with urease-producing bacteria (Osborne and Stevens, 1981).

Urine specific gravity.

Urine specific gravity (USG) is a parameter measured by refraction of light using a refractometer. Specific gravity is defined as the weight of a solution compared with an equal volume of distilled water. USG is a measure of the kidney's ability to concentrate and dilute urine and hence an indicator of tubular function (Chew and DiBartola, 1989). USG varies with hydration and water intake and may be altered in certain disease states (Polzin, 1990). Fever, dehydration, diabetes mellitus, vomiting, diarrhoea, and hemorrhage increase USG. On the other hand, chronic renal diseases, diabetes insipidus, hyperadrenocorticism, corticosteroid administration, psychogenic polydipsia, and pyometra decrease USG. Determination of USG aids the clinician during interpretation of other results of urinalysis since most tests are done on relatively

Table 7: *Protocol for distinction of causes for red urine*

Distinction of causes for red urine

PROCEDURE

A	Collect urine in a 5 ml glass or plastic centrifuge tube.
B	Centrifuge the urine sample at (1000 rpm in most centrifuges) for 5 minutes.

With hematuria the supernatant will be clear, but sediment remains red.

C	If supernatant remains red/brown, add 2.5 gms of ammonium sulphate to 5 ml of the supernatant.
D	Mix thoroughly, then centrifuge as before.

With hemoglobinuria the supernatant will become colorless.
If myoglobinuria, however, is responsible for the red color, the supernatant will retain a red/brown color.

small sample size without regard to the rate of formation of urine or total urine volume (Osborne and Stevens, 1981). Also serial evaluations of USG helps in detecting renal functional changes early during the course of primary renal failure or in monitoring functional recovery associated with reversible renal disease. Determination of USG is also used in the localization of azotemia, that is the elevation of urea or creatinine in plasma or serum (Sodikoff, 1995).

DIAGNOSTIC CHEMICAL ANALYSIS OF URINE

Often times various chemical tests aimed at diagnosing urinary and extraurinary tract diseases are undertaken using urine. They range from simple, rapid tests to complicated chemical analyses of urine.

Urine dipsticks (colorimetric chemical assays).

These are colormetric chemical assays normally used as first line diagnostic tests for pH, protein, glucose, ketones, bilirubin, urobilinogen and blood in urine. These are inexpensive tests and they are easy and quick to undertake (Lowe and Brendler, 1992). For good results, the reagent areas on the dipstick must be completely immersed in a fresh uncentrifuged urine specimen and then withdrawn immediately to prevent dissolution of the reagents into urine (Lowe and Brendler, 1992). Protocol for dipstick chemical analysis of urine is detailed in table 8.

(i) Urine pH

Normal urine pH for companion animals is 5.0 to 7.5 (DiBartola, 1995). Generally the body produces an excess of acid metabolites due to the role of the lungs and the kidneys (Gilbert et al., 1992). The lungs regulate acid-base balance by retention or elimination of carbon dioxide, and therefore carbonic acid; whereas the kidneys regulate acid-base balance by excretion of bicarbonate, ammonium ion and phosphates. Variations in pH may be caused by changes in diet and acid base balance in physiological states. However, certain disease situations may lead to changes in pH. Cystitis, lower urinary tract obstruction and alkalosis cause alkaline urine. Whereas any condition causing protein catabolism and acidosis increase acidity of urine (Sodikoff, 1995).

Table 8: *Dipstick urinalysis*

Dipstick urinalysis	
PROCEDURE	
A	Collect urine in a clean 10 ml test tube.
B	Immerse the whole reagent area of the dipstick into uncentrifuged urine for about 10 seconds.
C	Withdraw the dipstick, ensuring its edge is drawn along the rim of the urine container.
D	Hold the dipstick horizontally.
E	Read the color changes comparing them with the color chart at recommended time.
F	Record the results accordingly and properly dispose the used dipsticks.

Therefore, urine pH determination may be important in the diagnosis of several urinary tract problems and can also help in the diagnosis of systemic diseases. In patients suspected to have urinary tract infection with urea-splitting organisms namely *Proteus mirabilis, Klebsiella spp, Pseudomonas spp, Providencia spp* and *Staphylococcus spp,* urine has an alkaline pH (Lowe and Brendler, 1992). Also urine pH determinations help to confirm the diagnosis of renal tubular acidosis, which is an inability for the animal to acidify urine below pH 5.5 despite fasting or ingestion of an acid load (Lowe and Brendler, 1992). Frequent monitoring of urinary pH is necessary to ascertain adequacy of therapy/prognosis.

The formation and persistence of several types of crystals is influenced by pH, thus magnesium ammonium phosphate crystals tend to form in alkaline urine (Osborne et al., 1990). On the other hand uric acid uroliths are usually formed in acidic urine, while ammonium urate crystals are formed in urine with neutral pH (Osborne and Stevens, 1981). In view of the above influence that urine pH has on the formation of uroliths, its determination may aid in diagnostic determination of the type of uroliths present prior to their mineral analysis.

Interpretation of the findings in the urine sediment is subject to the influence of pH, this is because red blood cells, casts and other proteinaceous structures tend to disintegrate in alkaline urine (Osborne and Stevens, 1981). On the whole urine pH provides a reflection of the metabolic state of the body, due to the kidneys' inherent ability to adjust the pH of urine between 4.5 and 8.5 depending on the acid-base status of the body.

(ii) Protein

Normal companion animals excrete very low levels of protein in their urine (DiBartola, 1995). The reason being the capability of the kidney tubules to reabsorb most of the proteins filtered by the glomeruli (Lowe and Brendler, 1992). Most of the protein excreted in the urine is albumin, to which commonly used dipsticks are more sensitive (Lower and Brendler, 1992).

Reagents in Dipstick are tetrabromphenol blue or a combination of tetrachlorophenol and tetrabromosulfophthalein, which turn greenish or bluish in a face of proteinuria.

Persistent proteinuria, despite unremarkable sediment findings in samples (collected by catheterization, cystocentesis) is indicative of glomerular disease (DiBartola, 1995).

(iii) Glucose and ketones

Glycosuria is caused by hyperglycemia, urinary tract hemorrhage or renal tubular defects. Sodikoff (1995) has characterized glycosuria into four main types namely transient, overload, artifactual and pathological glycosuria (Table 9). Pathological glycosuria occurs in metabolic and urinary tract disease (Nelson, 1995). Screening for glucose in urine is often used as a means of identifying animals suffering from diabetes mellitus, renal tubular diseases, chronic renal disease, tubular injury caused by nephrotoxins and familial renal disease (DiBartola, 1995). Diabetes mellitus is characterized by many urologic manifestations such as increased susceptibility to urinary tract infections, hematuria and polyuria (Nelson, 1995). Detection of diabetic cases as well as those named above at an early stage is a key to their management and that of their side effects.

Glucose should not be detected in urine from normal companion animals since whatever is present in the glomerular filtrate is usually reabsorbed in the proximal tubules.

Glycosuria can be caused by any condition causing hyperglycemia to an extent that renal tubular threshold for reabsorption of glucose is exceeded (180mg/dl - dog and 280 mg/dl - cat). Diabetes mellitus is the most common cause. Other causes of glycosuria include hyperadrenocorticism and severe stress with multiple hormonal interactions for example epinephrine, cortisol and adrenocorticotropic hormone. Progressive destruction of renal tubules in nephrotoxicosis, renal ischemia, pyelonephritis, renal calcification, interstitial immune reactions and renal thromboembolism also causes glycosuria (Sodikoff, 1995). Young companion animals are not spared as juvenile renal diseases are reported to have glycosuria among their main features (Koeman et al., 1994). Most dipstick methods use a glucose oxidase colormetric reaction that is specific for glucose (DiBartola, 1995).

Table 9: *Types of glycosuria*

Transient exitement	**Artifactual**	**Overload**	**Pathologic**
Excitement $Corticosteroids_{INJ}$ $Carbohydrates_{INT}$	Urinary hemorrhage	Diabetes mellitus Stress	$Renal_{TD}$

Key: *$_{TD, INJ, INT}$ Tubular defect, injection and intake, respectively.*

Ketones are usually absent from urine and their presence, ketonuria, implies defective carbohydrate, fat and protein metabolism (Chew and DiBartola, 1989). Conditions associated with ketonuria are ketoacidosis and diabetes mellitus. The usual qualitative tests for ketones detect only acetoacetate and acetone. Other causes of ketonuria include starvation, prolonged fasting, persistent fever, non-insulin-mediated hypoglycemia and possibly liver disease or a low carbohydrate diet.

(iv) Occult blood

Occult blood in the urine is caused by hemolysis (hemoglobinuria), strenuous exercise (myoglobinuria) and urinary tract hemorrhage (blood/red blood cells).

The urine dipsticks are used for the detection of intact red blood cells, hemoglobin and myoglobin. The reaction on the dipsticks leads to a diffuse color change as well as patchy color changes seen as colored dots for hemoglobin and intact red blood cells respectively (Lowe and Brendler, 1992). This variation in color change is caused by differences in the amount of hemoglobin in the sample under study (DiBartola, 1995). The chemical reaction leading to the detection of blood or its pigment byproducts is based upon a peroxidase - like activity of hemoglobin and hence the diffuse/field color change observed when there is hemoglobinuria (Lowe and Brendler, 1992). Unfortunately the positive test does not clearly distinguish among hematuria, myoglobinuria or hemoglobinuria (Sodikoff, 1995). Therefore detection and quantitation of red blood cells in urine is best done by microscopic examination of a freshly voided, concentrated urine specimen (Schaeffer and Del Greco, 1992).

Urinary enzymes.

The activity of a number of urinary enzymes can be used in the diagnosis of diseases of the urinary system in companion animals. Other body systems and organs may also benefit from the knowledge regarding the activity of enzymes.

The measurement of urinary enzymatic activity is simple and non invasive and thus of diagnostic value in detection and monitoring of renal disease. Urine alpha amylase enzyme activity can be used in confirming a diagnosis of renal disease. In a study by Corazza et al. (1994), urine alpha amylase activity was found to be positively correlated with proteinuria, and the activity was higher in dogs with renal insufficiency.

Measurement of urine biochemical constituents coupled with urinary enzymes for example N-acetyl-ß-D-glucosaminidase (NAG) and Gammaglutamyl transferase (GGT) activity have been reported to be superior to clinical examination for the detection and monitoring feline immune complex glomerulonephritis (Bishop et al.,

1991). NAG is a lysosomal enzyme which has high activity in the renal proximal convoluted tubules, while GGT is a proximal renal tubular brush border enzyme often used as a marker for renal necrosis (Bishop et al., 1991).

MICROSCOPY IN URINALYSIS

Microscopic examination of the urine sediment is part of a complete urinalysis and is done in order to identify cellular elements, microorganisms, renal tubular casts and crystals (Barlough et al., 1981). Most of these components are essential for the diagnosis of urogenital diseases yet they cannot be detected either through macroscopic examination or by other physicochemical tests. This is exemplified in a study by Fettman (1987) in which it was noted that if physicochemical analyses were to be done without microscopic examination of the urine sediment, it would result in an 11.4% false negative rate for the detection of abnormal urine specimens.

Urine sediment examination is a valuable tool in the interpretation of results from other tests which are also used in the diagnosis of urinary tract diseases. Meaningful interpretation of urine color, specific gravity, turbidity, protein, occult blood and pH test results of routine analyses are dependent on findings from the urine sediment (Osborne and Stevens, 1981). Urine sediment findings can on their own be used in making specific diagnosis say when large numbers of cells, casts or crystals are found and can aid in the diagnosis of systemic diseases (Zinkl, 1995; Batamuzi and Kristensen, 1995). Urine sediment findings suggestive of urinary tract disease have been documented (Batamuzi and Kristensen, 1995). These include hypercellularity, extensive transitional cell degeneration, pyuria, microhematuria, bacteriuria and cylindriuria. Characteristic urine sediment findings are commonly associated with nephritis, urolithiasis, cystitis, pyometra and prostatitis (Batamuzi and Kristensen, 1995). Chronic skin diseases probably acting through immune complex formation and deposition in the glomeruli (DiBartola, 1995; Batamuzi et al., 1995), have also been found to lead to diagnostically important urine sediment findings (Batamuzi and Kristensen, 1995). Thus urine sediment examination findings may help to diagnose urinary tract problems as well as some genital diseases. To obtain good results from microscopic urine sediment examination three important steps have to be followed:

Obtain a clean, fresh specimen; prepare the specimen and examine the specimen under the microscope (Stamey and Kindrachuk, 1985). In tables 10 and 11 the protocols for preparation of the urine sediment smear are presented. Getting the sediment under the coverglass is the most critical stage in the examination of urinary sediment. Much more so when the sediment is small and inapparent at the bottom of the test tube (Stamey and Kindrachuk, 1985). In such a situation, the best procedure is to pour out all the visible supernatant by inverting the centrifuge tube upside down (Batamuzi and Kristensen, 1995), then return the tube to upright position. Then resuspend the sediment in the remaining drop of supernatant by tapping the bottom of the tube against the top of the table. Then pour all the resuspended sediment on the microscope slide (Stamey and Kindrachuk, 1985; Batamuzi and Kristensen, 1995).

Urine sediment findings at low power magnification

Examination of the specimen under low power magnification serves general and specific purposes. Under general purposes, examination at low power magnification allows the clinician to make a quick evaluation of the entire preparation in order to assess the quantity of the sediment and quality of the preparation (Osborne and Stevens, 1981). As for the specific purpose in as far as diagnosis is concerned, examination at low power magnification aids in the detection of those elements which may be present in only a few microscopic fields, such as casts and crystals (Chew and DiBartola, 1986). Under low power, the whole specimen is scanned, but particular attention is paid to the edges of the coverslip, where casts and other elements tend to be concentrated. Table 11 presents common findings at low power magnification.

Casts

Casts are precipitations of Tamm-Horsfall mucoproteins in the lumens of the renal tubules (Lowe and Brendler, 1992). They form in the ascending limb of loop of Henle, the distal tubules and collecting tubules probably due to maximum acidity, high solute concentration and the low flow rate in these areas (Lowe and Brendler, 1992). All casts signify renal disease, such as nephritis, pyelonephritis, amyloidosis, or nephrosis (Table 12).

Casts are therefore of localizing value for the respective kidney diseases (DiBartola, 1995). There are different types of casts (Stamey and Kindrachuk, 1985).

Table 10: *Preparation and examination of unstained urine sediment smear.*

Preparation and examination of unstained urine sediment smear	
PROCEDURE	
A	Centrifuge 5 ml of urine in a conical centrifuge tube at (approx. 1000 rpm) for 5 minutes.
B	Completely decant the supernatant by inverting the tube.
C	Return the tube to upright position and tap it against table top to resuspend the sediment in about 0.02 ml of urine remaining in the tube.
D	Pour the drop of resuspended sediment and tap it onto a glass microscope slide.
E	Then cover it with a cover glass.
F	Examine under the microscope at low power (100 x magnification) and high power (400 or 500 x magnification). A drop of sedistain can be added from the margins of the cover glass for visualization of WBC and casts.

Table 11: *Low power field magnification*

Five objects of diagnostic importance
1. Red blood cell (RBC) casts
2. Cellular-granular casts
3. Oval fat macrophages*
4. Clumps of white blood cells (WBC)
5. Crystals

**) Reported in humans with nephrotic syndrome (Stamey and Kindrachuk, 1985)*

When a cast contains only Tamm-Horsfall proteins it is called a hyaline cast. Casts may contain cells entrapped in them, when they do they are named after the type of cells. On this basis there are cellular casts, red blood cell casts and white blood cell casts (Fig. 2a). Cells entrapped in the cast often undergo degeneration as a result, the appearance and composition of the cast change to course or fine granularity (Fig. 2b). These are called granular casts (Chew and DiBartola, 1986). Further degeneration of the granular casts lead to casts with a homogeneously smooth appearance, the so called waxy casts, (Fig. 2c) (Osborne and Stevens, 1981). Normal urine may have few casts

Table 12: *Common causes of cylindriuria (casts)*

Renal diseases	
Acute renal failure	*(anuric and diuretic renal failure)*
Chronic renal failure	*(latent and maintenance renal failure)*
Pyelonephritis	
Nephrotoxicosis	*(eg by non steroid antiinflammatory drugs)*
Renal ischemia	
Renal thromboembolism	*(embolic nephritis)*
Juvenile renal diseases	
Other diseases	
Acute babesiosis	
Leptospirosis	
Hyperthyroidism	*(primary and pseudo types)*
Vitamin D toxicity	

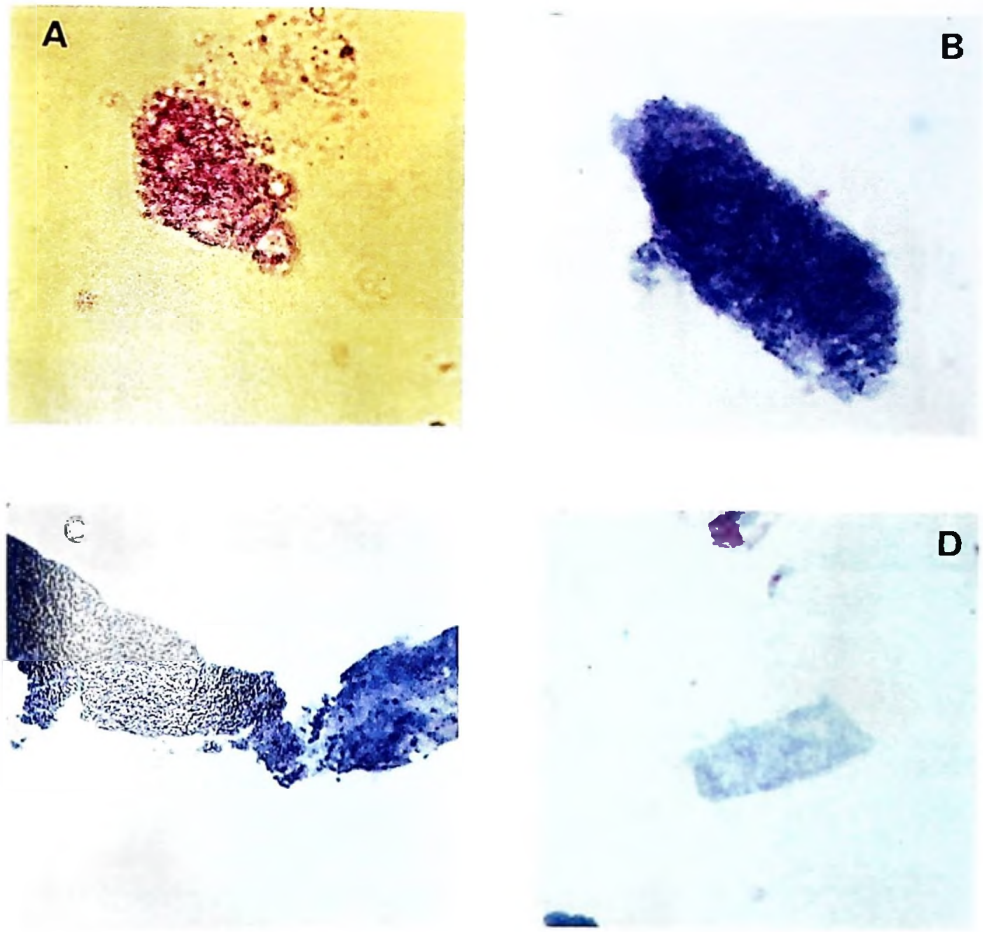


Figure 2: **(A)** Cellular cast. Note distinct matrix borders and well defined cellular contents. Identification of the cellular contents may require staining. **(B)** Granular cast demonstrating course granularity. Granular casts are a result of degeneration of cellular casts caused by oxygen depletion and accumulation of metabolites. **(C)** Waxy cast showing a characteristic homogenous, brittle appearance. The brittleness of this cast is demonstrated by cracks along its lateral margins. Unlike hyaline casts, waxy casts have a dull waxy appearance. **(D)** Pseudocast. Progressive degeneration of urothelial cells in urine progresses from cytoplasmic granulation and fading of nuclear detail to detachment of portions of cytoplasm. These avulsed portions of cytoplasm closely resemble granular casts.

especially of the hyaline type, but no cellular casts should be found in it (Chew and DiBartola, 1986).

Hyaline casts have a transparent appearance because they have no inclusions. These casts are of limited clinical significance (Lowe and Brendler, 1992). However, hyaline casts may be seen in renal diseases associated with severe proteinuria, for example amyloidosis and glomerulonephritis (Chew and DiBartola, 1986).

Red cell casts have a yellow-orange or a yellow-red color when preparations have been made from fresh urine samples (Osborne and Stevens, 1981). They may have a golden brown color if preparations are from urine which have been allowed to stand at room temperature, due to hemoglobin diffusing out of the cells. Red blood cell casts usually occur in association with hemorrhage into the renal tubules (Osborne and Stevens, 1981).

In man, red blood cell casts are associated with glomerular bleeding emanating from glomerulonephritis, for which they are pathognomonic (Lowe and Brendler, 1992).

Cellular casts are casts with sloughed tubular epithelial cells, their presence in urine indicates presence of non-specific renal tubular and nephron damage (Lowe and Brendler, 1992).

For non fresh urine samples, degeneration of urothelial cells *in vitro* may lead to formation of granular, cast like structures (Fig. 2d) (Batamuzi and Kristensen, 1995). That's why it is extremely important to prepare sediment smears from fresh urine samples (Stamey and Kindrachuk, 1985; Batamuzi and Kristensen, 1995).

Another type of casts are white blood cell casts which are composed of white blood cells and Tamm-Horsfall mucoprotein matrix. They occur when the renal tubules and renal interstitial tissues become involved in an inflammatory process. The white blood cell casts like the red cell casts degenerate to form granular casts and thus they are rarely seen in urine sediment preparations.

Crystals

Urinary calculi of different shapes, sizes and types are a common finding in the urine sediment smears from companion animals and often are of little diagnostic significance (DiBartola, 1995).

Urinary calculi are called crystals if they are seen only microscopically and uroliths if they can be visualized macroscopically (Osborne and Clinton, 1986). They can be classified according to their location, shape and chemical composition (Figure 3a-3d). On the basis of location in the urinary tract there are nephroliths, ureteroliths,

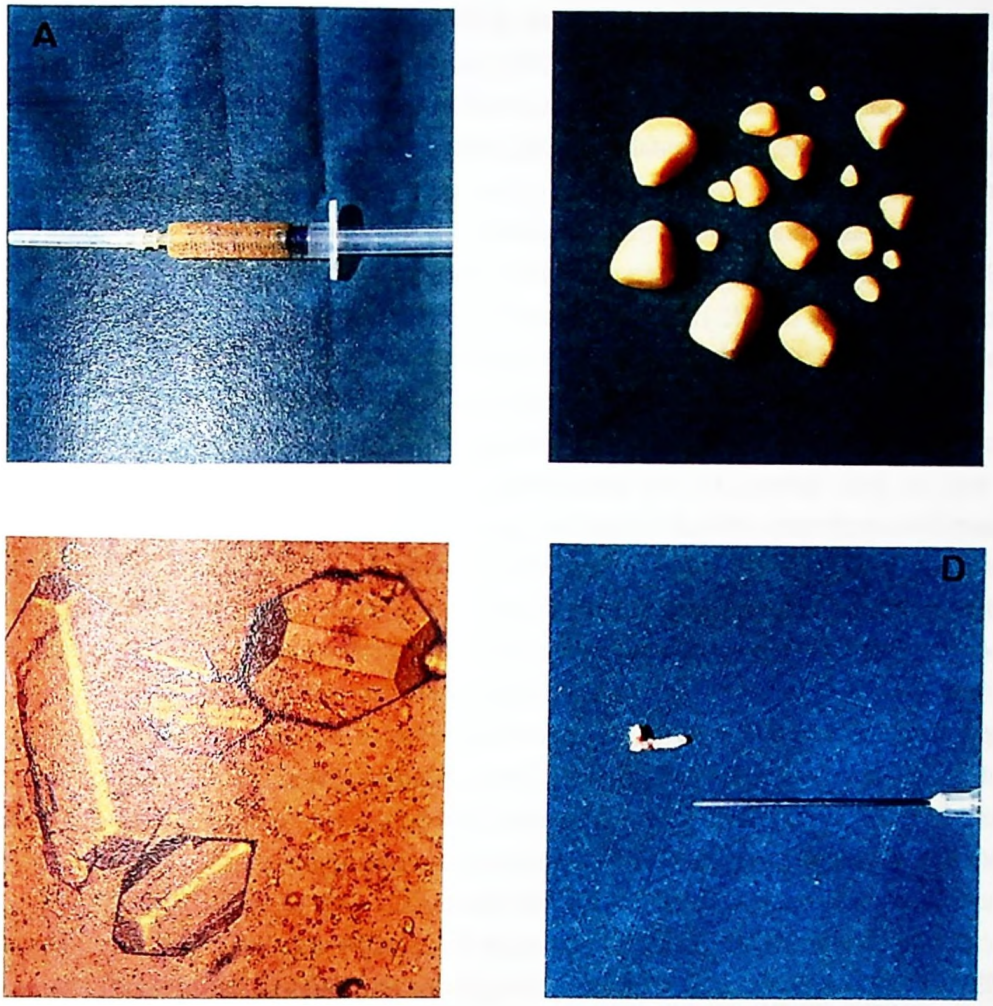


Figure 3: (A) Cloudy urine due to struvite urolithiasis. Note the milky white sediment. Chyle and pus also gives urine a milky white colour, hence the need for confirmation of the cause of the change in consistency of urine by microscopic examination of the urine sediment. **(B)** Struvite uroliths. Note smooth surfaces. **(C)** Photomicrograph of struvite (Magnesium ammonium phosphate) crystals. Typically they are colorless, coffin-like prisms. They have three to six or more sides. **(D)** Struvite urethrolith from a cat. The urethrolith is embedded in paste-like protenaceous material. Radiographs of such urethroliths will show radiolucent areas in the urethra. Note that canine struvite uroliths are normally radiopaque.

urocystoliths and urethroliths if they are in the kidney, ureter, urinary bladder and urethra respectively (Osborne and Clinton, 1986). When classified according to shape there are smooth, pyramidal, laminated, branched, mulberry, jackstone and so on and so forth. Phosphate, calcium oxalate and struvite (magnesium ammonium phosphate) crystals are often found in normal urine, but sometimes they are associated with uroliths (Osborne and Stevens, 1981).

The essential requirement for urolith formation is supersaturation of the urine with a specific mineral or mineral compounds, such that its amount exceeds its solubility in urine (Ling, 1995). However, successful formation of uroliths is subject to a number of factors which facilitate or inhibit their formation.

In dogs, majority of calculi are formed secondary to and as a result of urinary tract infection caused by certain bacterial species (Lulich et al., 1995). In some dogs as well as cats, urinary calculi are usually formed in the wake of abnormalities of absorption or excretion of the minerals in question (Stone and Barsanti, 1992). Struvite calculi are formed when there is supersaturation of urine with magnesium ammonium phosphate. This is usually associated with urinary tract infection caused by urease producing bacteria especially *Staphylococcus* and *Proteus* species and alkaline urine (Lulich et al., 1995). However, dietary, metabolic or familial factors may be responsible for the formation of sterile struvite uroliths. Infection induced struvite urolithiasis has been reported to have breed predisposition, with higher incidence involving schnauzers, dachshunds, poodles, Scottish terriers, beagles, Pekingese and Welsh corgis (Osborne et al., 1986). Sterile struvite uroliths are the commonest form of struvite urolithiasis in the feline species (Stone and Barsanti, 1992).

Calcium oxalate urolithiasis is mainly caused by hypercalciuria (Lulich et al., 1995). Increased calcium, which is thought to be due to faulty absorption or elimination, promotes calcium oxalate crystal formation (Ling, 1995).

Urate urolithiasis is common among Dalmatians owing to their unique metabolism of purines (Lulich et al., 1995). Genetic abnormalities in the metabolism and elimination of end products is thought to be responsible for the higher frequency of urate urolithiasis in Dalmatians (Ling, 1995). Urate urolithiasis is sometimes observed in animals with liver disease or portosystemic shunt (DiBartola, 1995). In such animals, blood bypasses the liver consequently leading to hepatic atrophy and dysfunction (Stone and Barsanti, 1992). Hyperammonemia and hyperuricemia that occurs secondary to liver dysfunction, and subsequent increased concentrations of ammonia and uric acid in urine may explain the occurrence of urate urolithiasis in dogs with portosystemic shunts (Lulich et al., 1995).

Cystine urolithiasis is thought to follow consumption of calculogenic diets but may be caused by genetic abnormalities in absorption or their elimination from the animals body (Hope et al., 1993; Ling, 1995). Cystine crystals are seen in animals with inherited or acquired renal tubular metabolic disorders that interfere with the reabsorption of cystine (Bartges et al., 1994). Thus presence of cystine crystals in urine of companion animals as well as man is abnormal and suggestive of cystinuria (Lowe and Brendler, 1992; DiBartola, 1995).

Urinary calculi have a potential to disrupt normal urinary tract function, as for example when they lead to urinary tract obstruction (Holt, 1994). On this basis their diagnosis, management and prevention assumes importance.

The diagnosis of urolithiasis may be based on the clinical signs, urinalysis, radiography and physical and chemical analysis of the uroliths. Urinalysis and culture are important in the diagnostic workup for urolithiasis.

Microscopic examination of the urine sediment is the key to arriving at a tentative diagnosis thus may help in guiding further diagnoses and therapy (Batamuzi and Kristensen, 1995). In table 13 shapes and other characteristics of common uroliths are presented.

Accurate analysis of the urinary calculi is essential in order to initiate effective management and preventive measures. There are two principal methods of analysis, namely qualitative and quantitative analyses. In the qualitative analysis, small amounts of the crushed urolith are placed in contact with specific chemicals supplied in kits (Oxford Stone Analysis Set. Lancer Division of Sherwood Medical, St. Louis, Missouri). Color changes are produced indicative of certain elements or compounds in the urolith under investigation. Elements/compounds identified in this manner include calcium, magnesium, ammonium, uric acid, cystine (Ruby and Ling, 1986). The qualitative methods, however, tend to be inaccurate due to tendency towards giving high false positive and false negative results (Ruby and Ling, 1986). To obtain reliable results therefore, all uroliths must be analyzed quantitatively (Sorenson and Ling, 1993). Commonly used quantitative methods are optical crystallography, X-ray diffraction and Infrared spectrophotometry (Ruby and Ling, 1986; Lulich et al., 1995).

Findings at high power magnification

As part of urine sediment examination, cytological examination of urine samples can aid in the diagnosis of urinary tract diseases. At high power magnification it is possible to see and differentiate various cell types (Stamey and Kindrachuk, 1985). At high power magnification we are also able to see different types of bacteria and

Table 13: *Appearance of common uroliths*

Type	Microscopic appearance	Radiographic appearance	Radiodensity	Urine pH	Urine culture
Struvite calculi	4-6 sided prisms	Smooth, round/faceted	Radiodense	Alkaline	Positive
Calcium oxalate$_m$	Envelop (octahedral)	Round/spiculated	Radiodense	Acid	Negative
Calcium oxalate$_d$	Spindle shaped	Jackstone shaped	Radiodense	Acid	Negative
Urate calculi	Amorphous shaped	Smooth, round/oval	Radiolucent	Acid	Negative
Calcium phosphate	Amorphous/elongate	Smooth, round/faceted	Radiodense	Alkaline	Negative
Cystine calculi	Flat hexagonal plates	Smooth, round/oval	Radiolucent	Acid	Negative

Key: *$_m$ and $_d$:* *Monohydrate and dihydrate, respectively.*

yeasts such as Candida spp (Fulton and Walker, 1992). These, together with urothelial changes and signs of inflammatory processes in the urinary tract are diagnostically important.

Commonly seen cells are epithelial cells, red blood cells and white blood cells (Table 14). Examination at high power magnification is improved by using different staining techniques.

It is also advisable to dry the smears before staining since dry smears of the sediment provide better cellular detail than wet mounts (Sodikoff, 1995). The use of hemacolor or new methylene blue is quite useful in the identification of different cell types (Batamuzi and Kessy, 1993; Batamuzi and Kristensen, 1995). In Table 15 the protocol for staining urine sediment preparations is presented.

Red blood cells

Depending on the method of urine collection and their number, red blood cells in urine may be a normal finding in urine (Lusk, 1995). It is expected that samples collected by manual bladder compression and by urethral catheterization may have many red blood cells (Osborne and Stevens, 1981). For samples collected by cystocentesis appreciable numbers of red blood cells in urine are a significant finding and constitutes hematuria (Batamuzi et al., 1994). It is advisable at this stage to compare with other findings such as from clinical examination and occult blood on dipsticks. The appearance of red blood cells is variable depending on USG, pH and the presence of bacteria. In fresh urine samples with USG between 1.010 and 1.020, red

Table 14: *High power field (X 400)*

Seven objects of diagnostic importance
1. Bacilli
2. Streptococci
3. Staphylococci
4. Yeasts (e.g Candida spp)
5. Elucidation of casts
6. Types of WBC and RBC*
7. Types and state of urothelial cells

** In man there are epithelial and glomerular/dysmorphic RBC.*

Table 15: *Urine sediment smear preparation and staining for cytological examination.*

Urine sediment smear preparation and staining for cytological examination.

PROCEDURE

A	Centrifuge 5 ml of urine in a conical tip centrifuge tube (at approx. 1000 rpm) for 5 minutes.
B	Completely decant the supernatant by inverting the tube.
C	Return the tube to upright position and tap it against table top to resuspend the sediment in about 0.02 ml of urine remaining in the tube.
D	Pour the drop of resuspended sediment and tap it onto a glass microscope slide.
E	Tilt the slide at 45° to the horizontal plane in order to allow the sediment to spread along the entire length of the slide.
F	Dry the slide with a hair drier.
G	Stain the smear accordingly with hemacolor.
H	Examine under high power magnification for abnormal or neoplastic cells.

blood cells appear yellow and have a uniform round shape (Fig. 4a). When in urine for a long time, they appear colorless as result of loss of hemoglobin to the surrounding medium. Typically, red blood cells are smaller than leukocytes, lack internal structures and are biconcave discs (Lowe and Brendler, 1992). When smears are prepared from concentrated urine, red blood cells are found to be smaller, crenated and distorted in shape sometimes with spiky rim (Fig. 4a). In very dilute urine they become larger, swollen and globular (Osborne and Stevens, 1981). The red blood cells described above are probably of epithelial type reported in man (Stamey and Kindrachuk, 1985). The epithelial type of red blood cells are commonly seen in conditions like bladder tumors, interstitial cystitis, urolithiasis, renal tumors, prostatitis and iatrogenic hematuria caused by urethral catheterization. Typically, epithelial red blood cells are round (biconcave), with uniform hemoglobin distribution. The second type of red blood cells are the ones associated with glomerular disease (Stamey and Kindrachuk, 1985). These have only been reported in man as dysmorphic cells with unevenly distributed hemoglobin (Stamey and Kindrachuk, 1985). However, these are also likely to be present in companion animals (Fig. 4b) (Batamuzi, unpublished data).

White blood cells

Only a few white blood cells are present in the urine of normal companion animals. Large numbers of neutrophils in urine (pyuria) indicate inflammation of the genitourinary tract (Sodikoff, 1995; Batamuzi and Kristensen, 1996). It is advisable to obtain samples by catheterization and cystocentesis in order to localize the inflammation and be able to differentiate pyuria of genital tract origin from that of urinary tract origin (Holt, 1994).

The identification of leukocytes in urine sediment may be indicative of urinary tract injury which may be caused by infection, calculous disease, glomerulopathy or interstitial cystitis (Lowe and Brendler, 1992). The presence of white blood cells does not help to localize the lesion unless white cell casts are present, indicating renal origin (DiBartola, 1995). In general the commonest causes of white blood cells in urine sediment are urinary tract infection and genital contamination, where a voided sample is used (DiBartola, 1995). White blood cells may be found together with red blood cells, bacteria and proteinuria indicating that the inflammatory lesion has been caused or complicated by bacterial infection.

Although infection is the commonest cause for pyuria, non septic causes of inflammation such as urolithiasis and neoplasia could be responsible (Lowe and Brendler, 1992). The appearance of white blood cells in urinary sediment preparations

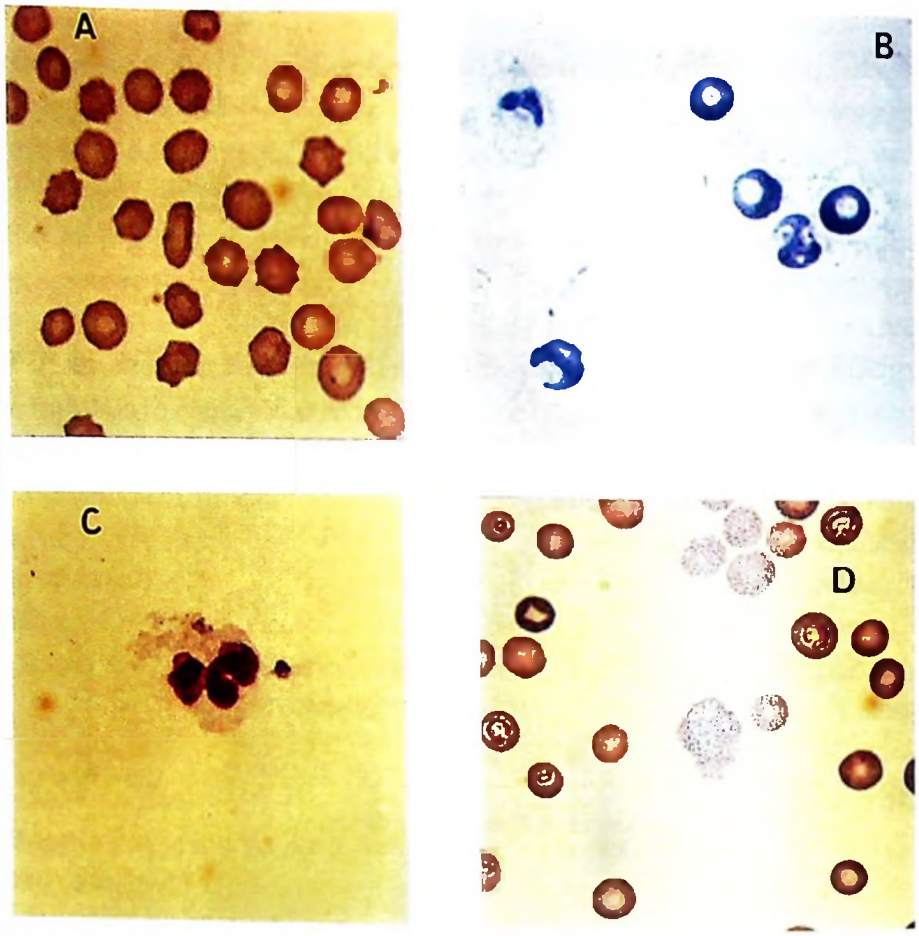


Figure 4: (A) Photomicrograph of urine sediment showing normal and crenated red blood cells from a cat. Note uniform distribution of hemoglobin and lack of biconcave appearance (typical for cat). Red blood cells must be distinguished from yeast and fat globules. Yeasts often have buds while fat globules are double refractile. The crenated red blood cells are normal, as well, but the distortion in shape is caused exposure to concentrated urine. B) Dysmorphic red blood cells from a Lhasa Apso dog with familial nephropathy. (C) Fresh leucocytes. Note the abundant cytoplasm with granules. The segmented nuclei of the polymorphonuclear leucocyte can be seen. Fresh leucocytes are pale with faint nuclei and unstained granules (when sedi-stain or hemacolor stain are used). Fresh leucocytes especially when seen together with degenerated transitional cells and bacteria with or without red blood cells indicate urinary tract injury. (D) Old white blood cells. These dark staining cells are disintegrated leucocytes whose cell membranes cannot inhibit entry of the dye (in the stain). Old leucocytes are normally a result of contamination from the genital tract especially for voided and catheterized samples.

is variable depending on the type of white cells, USG, pH and the presence of toxin producing bacteria (Chew and DiBartola, 1986). In fresh urine samples white blood cells typically appear as spherical granular cells, which are slightly larger than red blood cells but smaller than epithelial cells. According to Osborne and Stevens (1981), white blood cells in urine sediment preparations do not have identifiable nuclei since they tend to disintegrate probably due to the influence of urine. They are small with scant cytoplasm, and nuclei are not clearly distinguishable from the cytoplasm. These cells are commonly seen in vaginal secretions.

In human literature such leukocytes are referred to as old white blood cells since they are not identifiable with a particular cell line (Stamey and Kindrachuk, 1985). The significance of old white blood cells is uncertain. Fresh white blood cells have abundance of cytoplasmic granules (Stamey and Kindrachuk, 1985; Batamuzi, unpublished data). Careful focusing on the nuclei shows that they are polymorphic. Clear distinction of fresh white blood cells from old ones can be done with proper staining (Batamuzi and Kristensen, unpublished data).

Fresh white blood cells repel the sedi stain (Sternheimer Malbin stain), such that they remain pale after staining, their nuclei remain faintly stained and cytoplasmic granules unstained (Fig. 4c). Old white blood cells, probably due to degenerated cell membranes take up the stain excessively so much so that they end up being overstained (Stamey and Kindrachuk, 1985). Unlike the pale staining fresh white blood cells, old white blood cells are dark staining cells (Fig. 4d). The normal urinary tract does not contain fresh white blood cells thus their presence in centrifuged urine sediment obtained from a cystocentesis sample indicates injury somewhere along the urinary tract.

Granularity of the white blood cells' cytoplasm could possibly be caused by nuclear disintegration or presence of phagocytized material, but it is possible that such granules are always present in granulocytes (Osborne and Stevens, 1981).

Epithelial cells

Small numbers of squamous, transitional or renal tubular epithelial cells can be observed in normal urine. Large numbers of normal or atypical epithelial cells indicate renal tubular, distal urinary tract or prostate irritation or neoplasia (Batamuzi and Kristensen, 1995). Specifically it suggests a problem in the renal tubules, bladder or prostate (Sodikoff, 1995).

There are three main types of epithelial cells that may be found in smears prepared from urine sediment (Batamuzi and Kristensen, 1995). They include renal

tubular epithelial cells, transitional cells and squamous cells. Renal tubular epithelial cells are small cells with a large centrally located, spherical nucleus and granular cytoplasm (Osborne and Stevens, 1981). These cells are of diagnostic importance for kidney disease if and only if they are found in casts. After all they are difficult to distinguish from small transitional cells and even white blood cells especially in unstained sediment smear preparations.

The other cell type, the transitional cells, are variable sized cells deriving from the urothelium from the renal pelvis to the urethra (Chew and DiBartola, 1986). The variation in size and probably shape of transitional epithelial cells has been attributed to their depth of origin in the urothelium (Osborne and Stevens, 1981). Transitional epithelial cells may be pear shaped, spindle shaped, caudate or polygonal (Fig. 5a) (Batamuzi and Kristensen, 1995). Typically they have granular cytoplasm.

Multinucleated urothelial cells have been reported to indicate the presence of injury to the urothelium caused by trauma or inflammation, but they are not indicative of malignancy (Fig. 5b) (Rebar, 1987; Zinkl and Feldman, 1989; Batamuzi and Kristensen, 1995). On the other hand malignant transitional cells have altered nuclear size and morphology . Staining of the urine sediment smears with hemacolor can help to identify urotherial tumors (Batamuzi and Kessy, 1993). The protocol for preparation of smears and staining with hemacolor is presented in table 15.

The caudate type of transitional cells are small cells with trailing cytoplasm (Fig. 5c), originally postulated to originate from the renal pelvis and thus thought to be of localizing value (Chew and DiBartola, 1986). It has now been established that caudate transitional cells derive from the urothelium from the renal pelvis to the ureter, including that part of the ureter that is buried within the bladder wall, forming the so called vesicoureteral valve (Batamuzi and Kristensen, 1995). These cells therefore do not qualify to be granted a localizing value status.

Squamous epithelial cells (Figure 5d), are large, polygonal cells with small tight round nucleus and finely granular cytoplasm (Batamuzi and Kristensen, 1995). There are two types of squamous cells, the ordinary type with a small tight nucleus and the one found in the area of the trigone (Batamuzi and Kristensen, 1995). Squamous cells from the trigone have a larger vesicular nucleus when compared to ordinary squamous cells and are distinguishable from transitional cells. The finding of squamous cells in the urine sediment does not necessarily indicate contamination from the urethra or genital tract (Batamuzi and Kristensen, 1995). Squamous cells are of no diagnostic significance and their numbers may increase during estrus (Chew and DiBartola, 1986).

Urothelial cells are often in urine in large numbers in association with inflammation, hyperplasia or trauma, giving a raft like appearance in sediment smear preparations (Fig. 6a). Urothelial cells in urine sediment preparations from animals with urinary tract diseases are numerous and show degenerative changes (Batamuzi and Kristensen, 1995). Degenerated urothelial cells have many intracytoplasmic granules, numerous vacuoles in the cytoplasm and light staining nuclei (Batamuzi and Kristensen, 1995). To appreciate and harness the diagnostic potential of degenerated urotherial cells, it is imperative to have sediment smears prepared from fresh samples. Presence of many transitional cells, together with degenerative changes (Fig. 6b through 6d) and features like pyuria, cylindriuria and microscopic hematuria in fresh samples indicates that there are pathological changes somewhere along the urinary tract (Batamuzi and Kristensen, 1995).

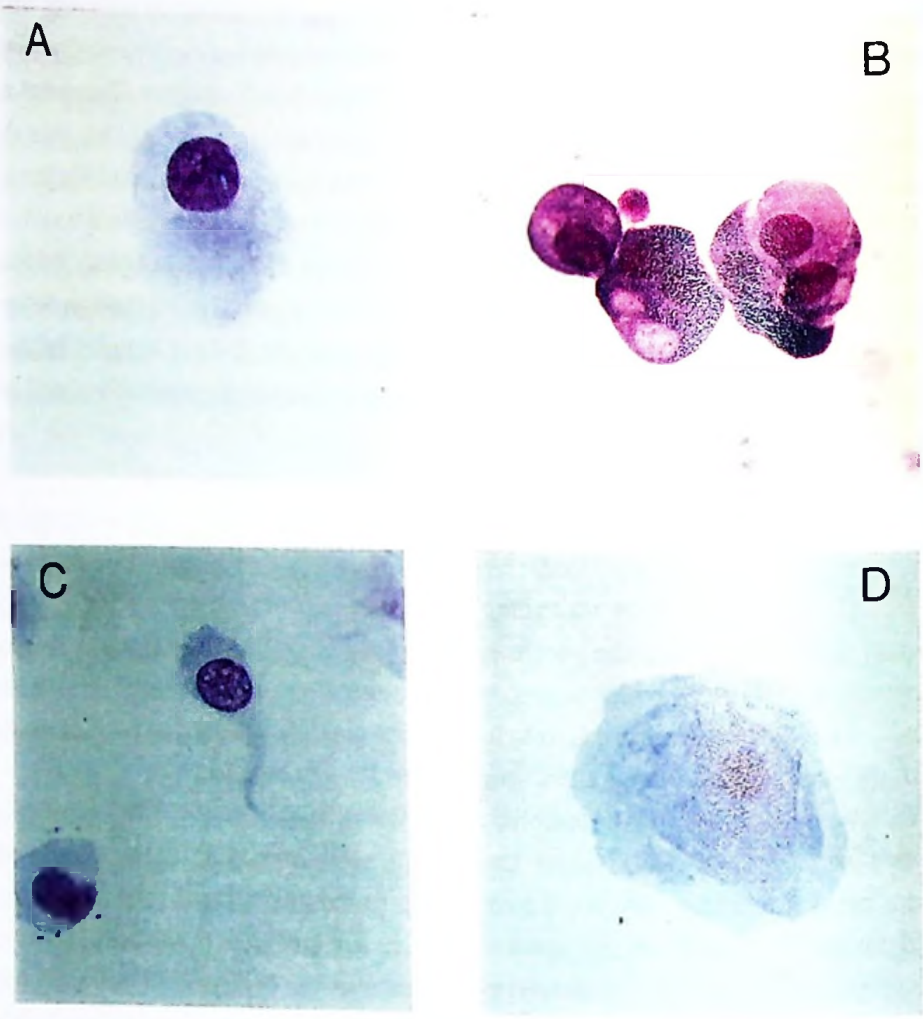


Figure 5: **(A)** Transitional epithelial cell. These cells may be round, oval or pear shaped with oval vesicular nucleus. Note abundant basophilic cytoplasm. **(B)** Multi-nucleated cells. These are common when cells are exfoliated in large numbers. In fact it represents cell overlapping each other giving a false impression that cells are bi- or multinucleated. **(C)** Caudate transitional cells. These are small cells when compared to ordinary transitional cells. Note that these are thin, elongate cells with trailing cytoplasm. As can be clearly seen, the nucleus is located at the broader part of the cell. **(D)** Squamous epithelial cell. These are large cells with a small round nucleus and fine granularity. Cell borders are polyhedral.

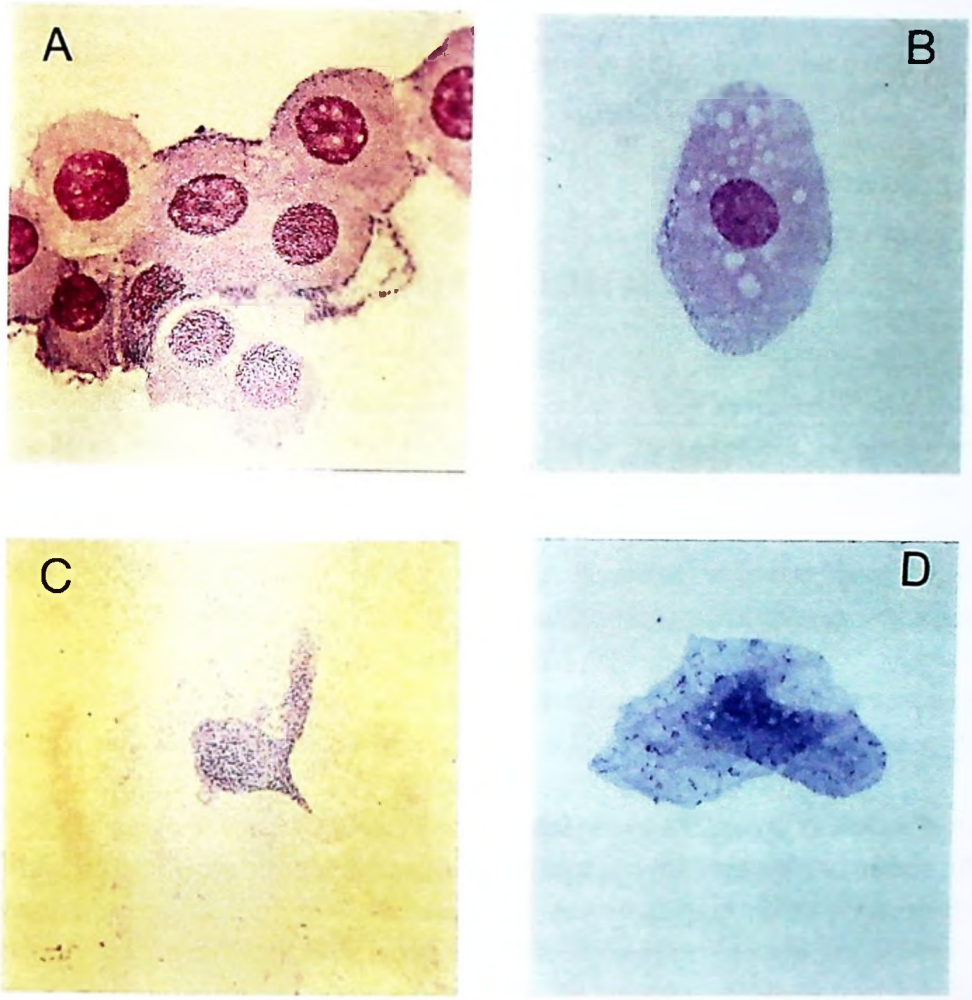


Figure 6: **(A)** A raft of transitional cells. This normally indicates that the cells have been exfoliated in large numbers usually as a result of inflammation, trauma (eg caused by uroliths) or neoplasia. **(B)** Degenerated transitional cells. Note granualation and vacuolation of cytoplasm. **(C)** Degenerated transitional cell. Note detaching portion of cytoplasm indicating an advanced stage in the degeneration process. **(D)** Degenerated squamous cell. Note lack of nucleus, cornification and invasion by bacteria.

URINE MICROBIOLOGY

Urinary tract infection is among the commonly diagnosed problems among companion animals (Ling, 1995; Osborne, 1995). The prevalence of urinary tract infection appears to increase with age (Bush, 1984). As veterinary services improve, the life expectancy of companion animals will definitely increase and so will the prevalence of urinary tract infections. Thus the diagnosis, treatment, morbidity and mortality of urinary tract infection in the geriatric companion animals will assume increasing importance (Batamuzi et al., 1996a).

Urinary tract infection also tends to increase in prevalence with concurrent disease possibly due to increased injury, effects on defence mechanisms and serving as nidus for infection (Schaeffer, 1992; Batamuzi and Kristensen, 1996).

The upper urinary tract, that is the renal pelves and ureters as well as part of the lower urinary tract including the urinary bladder and the proximal and middle parts of the urethra, normally are not inhabited by microorganisms. Thus urine which is aseptically obtained from these areas is sterile. Whenever bacteria are isolated or observed in urine, serves as clear testimony for the presence of urinary tract infection (Rubin, 1990b; Batamuzi and Kristensen, 1996). The prepuce and vagina as well as the terminal urethra in both gender are the usual sources or portals of entry of uropathogens in dogs and cats (Ling, 1995). Most of the uropathogens are of fecal origin and probably attach themselves on epithelia at the portals of entry, colonize them and ascend the urethra until they reach hitherto sterile parts of the urinary tract. The hematogenous, lymphatic spread and direct extension of infection from surrounding areas also serve as sources of infection for the urinary tract albeit at insignificant rates. Success or failure of infection is a function of the invading uropathogens' virulence factors and the hosts natural defense mechanisms (Osborne, 1995). Thus infectious disease is the cumulative result of a microorganism's ability to establish infection and

be able to do so despite the hostile environment and conversely, of the host's ability to resist infection (Kruger and Osborne, 1993). Uropathogens successfully cause disease by inducing cell injury, altering cell functions, suppression of immune response or producing toxic waste byproducts (Shaw, 1990). The host on the other hand fights off the invading uropathogens using different defense mechanisms. Passive washout of uropathogens from the terminal/distal urethra during normal voiding, secretion of immunoglobulins as well as vaginal and preputial mucus coupled with the presence of normal flora, work in unison to prevent uropathogens from reaching sensitive areas of the urinary tract (Kruger and Osborne, 1993).

Apart from the uropathogen's virulence armamentarium, other factors may facilitate infection of the urinary tract. Catheterization of the urethra passively transports microorganisms past the protective barriers at the external urethral orifice to the urinary bladder (Biertuempfel et al., 1981; Barsanti et al., 1985). Trauma of the urothelium as caused by catheters and uroliths, and neoplastic diseases and other space occupying lesions which delay or impede voiding seriously cripple the host's defense mechanism (Stone and Barsanti, 1992; Littman, 1995). Transmissible venereal tumour (TVT), a genital neoplasm, has the vestibulovaginal junction as its predilection site in bitches and is frequently associated with extensive preputial involvement (Batamuzi et al., 1990). In these locations TVT surrounds and may effectively obliterate the external urethral orifice resulting in dysuria thus predisposing the urinary tract to urinary tract infection (Batamuzi and Kristensen, 1996).

Common sequelae following urinary tract infection include struvite urolithiasis, prostatitis, epididymitis, orchitis, acute or chronic renal failure, septicemia and infertility (Rubin, 1990b; Ling, 1995). Peritonitis is another life threatening consequence of urinary tract infection (Hardie, 1989). Most of these conditions help to perpetuate urinary tract infection.

In view of the above consequences of urinary tract infection and its high frequency among companion animals, diagnosis of the same is highly justified.

The diagnosis of urinary tract infection is based on clinical signs, urinalysis and culture. Under certain circumstances, specialized diagnostic techniques may be employed. Unfortunately clinical signs associated with urinary tract infection are non specific as they are also caused by other conditions of the urinary system. Urinalysis and culture therefore remain the only reliable methods in the diagnostic workup for urinary tract infection (Stamey and Kindrachuk, 1985; Osborne, 1995; Batamuzi and Kristensen, 1996).

Urine sampling must be done in such a way as to virtually eliminate the possibility of contamination of the specimen with white blood cells and bacteria from the urethra, vagina or prepuce (Shaw, 1990). Urine samples should be examined immediately or refrigerated within a short time of their collection (Barsanti et al., 1985). Suspicion for urinary tract infection starts with gross appearance of the sample. For cases with urinary tract infection, urine is usually cloudy, hemorrhagic and foul smelling (Rubin, 1990b). White floccules may be seen in the sample and are usually due to pyuria. Care must be taken to distinguish white floccules caused by pyuria from whitish sediment caused by uroliths. Urine allowed to stand for sometime at room temperature may have whitish floccules (Osborne and Stevens, 1981). Such conditions should be considered in the differential diagnosis. A variety of microorganisms may be found in the urine sediment, they include bacteria, yeasts and fungi (Osborne and Stevens, 1981; Kruger and Osborne 1993; Ling 1995). In unstained preparations of the urinary sediment, bacteria are seen as particles showing brownian movement in fresh wet mounts (Stamey and Kindrachuk, 1985). However to appreciate the shapes and relationship to each other, some staining may be required (Stamey and Kindrachuk, 1985). The prevalence of the different uropathogens in companion animals has been reported (Ling, 1995) and is presented in tables 17 and 18.

Table 17: *Prevalence(%) of common uropathogens in dogs which are associated with urinary tract infection in that species.*

Bacterial species	Male	Female
Escherichia coli	42	46
Streptococcus/Enterococcus spp	11	14
Staphylococcus spp	12	12
Proteus spp	6	12
Klebsiella spp	12	8
Pseudomonas spp	3	3
Mycoplasma spp	3	2
Enterobacter spp	3	2
Providencia spp	2	0,3

Modified from Ling, 1995.

Table 18: *Prevalence of uropathogens which commonly cause urinary tract infection in cats.*

Bacterial species	Prevalence (%)
Escherichia coli	52
Staphylococcus spp	19
Streptococcus/Enterococcus	12.5
Klebsiella spp	5
Proteus spp	4
Mycoplasma spp	2
Pasteurella spp	2
Pseudomonas spp	1
Enterobacter spp	1

Modified from Ling, 1995

Bacilli and coccal forms, scattered, in chains or small clumps may be seen in the urine sediment (Stamey and Kindrachuk, 1985). Ancillary urine sediment findings serve as circumstantial evidence for the presence of urinary tract infection. Pyuria and hematuria are good indicators of inflammatory response (Schaeffer, 1992). Large numbers of white blood cells may be indicative of urinary tract infection when they are in well collected urine samples and freshly prepared urinary sediment smears (Batamuzi and Kristensen, 1995). In most cases, the absence of pyuria should cause the diagnosis of urinary tract infection to be questioned until urine culture data are made available. In some situations, however, as for example in animals with diabetes mellitus and hyperadrenocorticism and following administration of immunosuppressive drugs. white blood cell activity is impaired (Ling, 1995). On the other hand, some disease have been reported to stimulate significant white blood cell reaction without urinary tract infection in man (Schaeffer, 1992). A typical example for conditions with pyuria without urinary tract infection is staghorn urolithiasis. There is need therefore to consider such situations when using white blood cells in the active urinary sediment as a criteria for diagnosing urinary tract infection. Hematuria at microscopic or macroscopic level may be an indicator of urinary tract infection. Any injury or irritation to the urinary tract as caused by inflammation or infection as in the case of urinary tract

infection, has hematuria as one of the important signs. Interpretation of the significance of hematuria with regard to urinary tract infection has to be done with care since there are so many causes of hematuria (Stone and Barsanti, 1992; Batamuzi et al., 1994).

To confirm urinary tract infection, culturing of urine samples is required (Ling, 1995). Good results are obtained when samples are plated immediately since left at room temperature bacteria in the sample multiply fast (Osborne and Stevens, 1981). This will inevitably affect quantitative urine culture results interpretation.

Some companion animals with urinary tract infection may have to be radiographed in order to identify those which require other interventions in addition to antimicrobial treatment. In human medicine patients requiring radiography include those in which urinary tract infection is likely to be associated with urinary tract obstruction caused by tumors, uroliths, strictures and those who have had genitourinary surgery (Schaeffer, 1992). In animals not recovering after treatment and those showing recurrence of infection, abnormalities that cause bacterial persistence for example uroliths and prostatic problems (Chew and DiBartola, 1986), should be sought. In such animals radiological evaluation to diagnose a focus of bacterial persistence may be the first step in solving the problem.

PROTEINURIA

Proteinuria is defined as the detection of abnormal quantity of protein in urine (Osborne and Stevens, 1981). Sources for proteinuria are variable and so is the significance of each of the types of proteinuria (Kawai, 1973). Urinary proteins may come from the plasma, the kidney tissue and the lower urinary tract (Table 19). On the basis of the source, proteinuria may be classified as prerenal (also called preglomerular), renal (further classified into glomerular and tubular), or postrenal (Lulich and Osborne, 1990).

Prerenal proteinuria results from systems other than the urogenital tract. In prerenal proteinuria, glomerular permeability is normal but serum proteins of relatively low molecular weight (1500 to 45000 daltons) are excreted into urine when they are increased pathologically in blood. The increase in these proteins may be occasioned by neoplastic disease (myeloma proteins), generalized intravascular hemolysis leading to

Table 19: *Types/sources of proteinuria in companion animals*

Physiologic	Prerenal	Renal	Postrenal
Exercise	Bence Jones	Glomerular	LUT_{ifl}**
Seizures	Hemoglobinuria	Tubular	
Fever	Myoglobinuria	$Parenchymal_{ifl}$	
Stress	$Genital_{ifl}$		
Heat/cold	CHF*		

Note: CFH : Congestive heart failure
LUT : Lower urinary tract.
ifl : Inflammation.

hemoglobinuria and localized or extensive muscular necrosis (myoglobinuria) (Kawai, 1973). Prerenal proteinuria may be further subdivided into functional proteinuria and overload proteinuria (Osborne et al., 1995). Functional proteinuria is frequently referred to as physiological proteinuria in human literature and may be associated with strenuous exercise, stress, fever, seizures, exposure to extremes of temperature and venous congestion in the kidneys (Kawai, 1973; Osborne et al., 1995).

Postrenal proteinuria results from protein loss arising within the urogenital tract but below the level of the kidney. In postrenal proteinuria, protein exudation is commonly the result of inflammatory, neoplastic, ischemic or traumatic diseases such as cystitis, prostatitis, urolithiasis and transitional cell carcinomas (Kawai, 1973; Stone and Barsanti, 1992; Ling, 1995).

Proteinuria of renal origin is either glomerular or tubular. Severe proteinuria in the absence of white blood cells or red blood cells is usually of glomerular origin (Finco, 1989; Grauer, 1992). Capillary walls of the glomeruli act as semipermeable filters that under normal circumstances retain most of the plasma proteins in the vascular compartment (Osborne et al., 1995). The filtration of the individual proteins usually depends on their plasma concentration as well as their size, shape and charge (Finco, 1995; Osborne and Fletcher, 1995). Glomerular proteinuria occurs as a consequence of injury to the glomerulus (resulting in alteration of the integrity of glomerular capillary barriers that normally prevent protein loss) and is predominantly albuminuria (Finco, 1989; Osborne et al., 1995). However, other proteins are also lost albeit at low levels and they include transferrin, immunoglobulin G, various apolipoproteins and antithrombin III (Brown, 1995; Osborne et al., 1995; Batamuzi et al., 1996c). On electrophoresis of urine a characteristic pattern with preponderance of albumin and α_1 globulin, but relatively low levels of β and γ globulins, compared to serum, is obtained (Kawai, 1973). This pattern indicates the presence of selective permeability of the glomerular barrier (Batamuzi et al., 1996c). It is important to note that macromolecules such as α_2 lipoprotein, most large molecular weight immunoglobulins, α_2 macroglobulin and fibrinogen are hardily demonstrated in urine (Kawai, 1973). Primary inflammatory, antiglomerular basement membrane, or neoplastic diseases of the kidneys as well as secondary disorders are the common causes for glomerular injury (Osborne et al., 1995). Secondary glomerular diseases may be caused by drugs, infectious agents, multisystemic diseases, biochemical disturbances and heredofamilial diseases (Biewenga, 1986; DeBoer et al., 1988; Osborne et al., 1995; Batamuzi et al., 1995). Typical examples of the secondary diseases of glomeruli include systemic lupus erythematosus, dirofilariasis, canine pyometra, chronic skin diseases

feline leukemia virus infection, diabetes mellitus and hereditary nephropathies (Biewenga, 1986; Osborne et al., 1995; Batamuzi et al., 1996). Tubular proteinuria occurs as a consequence of normal passage of low molecular weight proteins through the glomerular filter coupled with defective tubular reabsorption (Finco, 1989). Tubular damage caused by toxins is a frequent cause for tubular proteinuria (Mealey and Boothe, 1994). Tubular proteinuria is characterized by excretion of plasma proteins of low molecular weight (1500 to 45000 daltons) as a result of defective reabsorption by the proximal tubules (Kawai, 1973). On electrophoresis the α and β globulins have been reported to be conspicuous while albumin fractions are quite low (Kawai, 1973).

Urine protein evaluation is among the tests that are undertaken as part of complete urinalyses and its interpretation in conjunction with other test results may aid in the detection, localization and diagnosis of the various urinary tract disorders (Sodikoff, 1995). At times urine protein detection may be of extreme importance as an early indicator of disease (Batamuzi et al., 1996b; Batamuzi et al., 1996c). In a recent study, urine protein was reported as the first urine constituent to indicate that there was renal dysfunction (Bishop et al., 1991). When there is a high suspicion for the presence of renal disease, detection of protein in urine is diagnostically important (Krawiek, 1989). Clinical signs associated with mild to moderate urinary protein loss are usually non specific yet proteinuria even at such low levels could be the beginning of glomerular injury and dysfunction (Grauer and DiBartola, 1995). Detection of proteinuria at this stage may help in instituting curative and preventive procedures at as early a stage as possible.

There are several tests that are used for the diagnosis and characterization of proteinuria. For screening purposes, colorimetric (urine dipsticks) and turbidimetric (sulfosalicylic acid) tests are used. Despite their limitations, screening tests are useful due to their simplicity and cost effectiveness. These tests have been recommended for use in selection of urine for quantitative analysis of protein and creatinine to assess proteinuria (Moore et al., 1991).

The colorimetric test consists of bibulous paper impregnated with tetra-bromphenol blue which changes color in the presence of albumin and to a lesser extent in the presence of other proteins (Chemstrip 8, Boehringer Mannheim Diagnostics, D - 68298, Mannheim). The intensity of shades of green is proportional to the concentration of the protein in the urine. Colorimetric tests are sensitive to the concentration as low as 20mg/dl.

The sulfosalicylic acid test (SSA) is equally sensitive for albumin as well as other urinary proteins at levels as low as 5 mg/dl. The SSA test is done on a well

centrifuged supernatant because urothelial cells and inflammatory cells can cause false negative results. The SSA test is undertaken by adding 2.5 ml of supernatant urine to 7.5 ml of 2% sulfosalicylic acid. Any precipitate formed indicates proteinuria (Moore et al., 1991).

The other category of tests for proteinuria are quantitative and unlike the screening tests, they allow for assessment of disease severity, progression and response to treatment (Grauer and DiBartola, 1995). Examples of quantitative tests include Coomassie brilliant blue test and trichloracetic acid ponceaus. The quantitative tests use urine samples collected over a 24 hour period. Collection of urine over a long time is cumbersome, thus quantitative tests using single collection sample have been devised. The urine protein - creatinine ratio has been shown to reflect the quantity of protein excretion over a 24 - hour period and is thus widely used (Grauer et al., 1985; Lulich and Osborne, 1990). The urine protein to creatinine ratio is used to determine the extent of protein loss without the necessity of 24 hour urine collection. A ratio of 0.6 or less is considered normal while ratios above 1 are abnormal and indicate significant urine protein loss (Feldman and Thomas, 1989). The magnitude of proteinuria is roughly correlated with the severity of the underlying glomerular lesion. This makes the urine protein to creatinine ratio a very useful parameter for the assessment of progression of disease and the response to treatment (Grauer et al., 1985).

Accurate localization of the source and therefore type of proteinuria is essential as this will determine the type of management and the prognosis. The colorimetric, turbidimetric as well as urine protein quantification tests can not be used in detection of the source and type of proteinuria.

It has been proposed that determination of the urine protein profile can assist in determining the source of proteinuria (Grauer and DiBartola, 1995; Batamuzi et al., 1996a; Batamuzi et al., 1996c). Urine and serum protein electrophoresis therefore, may help identify the source of proteinuria and establish a prognosis (Biewenga, 1986).

The current widespread use of electrophoretic techniques for the fractionation of protein is commensurate with its reflection of a variety of changes in serum and urine protein patterns in disease as well as certain physiological states (Kawai, 1973). Although just a handful of changes in pattern can be considered characteristic and therefore diagnostic of specific disease syndromes, properly interpreted, these very changes can facilitate the clinician's diagnostic workup. Bence Jones light chains proteins in urine of animals with multiple myeloma can not be well detected by conventional method but by electrophoresis (Finco, 1989).

The principle of the electrophoretic separation of proteins is based on the migration of charged protein particles in an electric field (Westermeier, 1993). The direction and rate of migration of the particles are a function of the type of charge (negative or positive) on the protein, the size of the protein, the intensity of the electric field and the support medium through which the protein particles are induced to migrate (Westermeier, 1993). Agarose gel electrophoresis is capable of resolving urine into several fractions and is therefore termed a high resolution technique (Kristensen and Barsanti, 1977). Agarose gel electrophoresis is for that reason a very useful technique (Batamuzi et al., 1996b; Batamuzi et al., 1996c).

The normal composition of urine protein is 30-40% serum albumin, 30% serum globulin and 40% tissue proteins such as Tamm-Horsfall mucoprotein . This profile can be altered by both physiologic and pathologic conditions (Table 19). Early stages of glomerular damage result in albuminuria and insignificant globulin losses (Fig. 7a and 7b). With advanced glomerular disease, glomerular damage becomes so severe that even globulins are lost in a relatively high proportion (Fig. 8b).

In cases of post renal proteinuria whereby there is an inflammatory process in the lower urinary tract, electrophoretic patterns similar to that of serum will be obtained (Fig. 8a).

Tubular proteinuria is defined as the appearance in urine of the normally filtered proteins as a result of impaired tubular reabsorption. Examples of those proteins are α_2 macroglobulin, immunoglobulin light chains, zinc binding protein and lysosomes. Urine electrophoresis in cases with tubular proteinuria or trace proteinuria (Kawai 1973; Batamuzi et al., 1996c) gives a pattern as shown (Fig. 8c). Such patterns can be obtained when only the interstitium and tubules are affected as is the case with nephrotoxicoses due to lead and mercury poisoning (Schaeffer and Delgreco, 1992). Where both tubular and glomerular damage are present, the distinction between glomerular and tubular proteinuria, may prove to be an unattainable undertaking when urine electrophoresis is the only criteria (Biewenga, 1986).

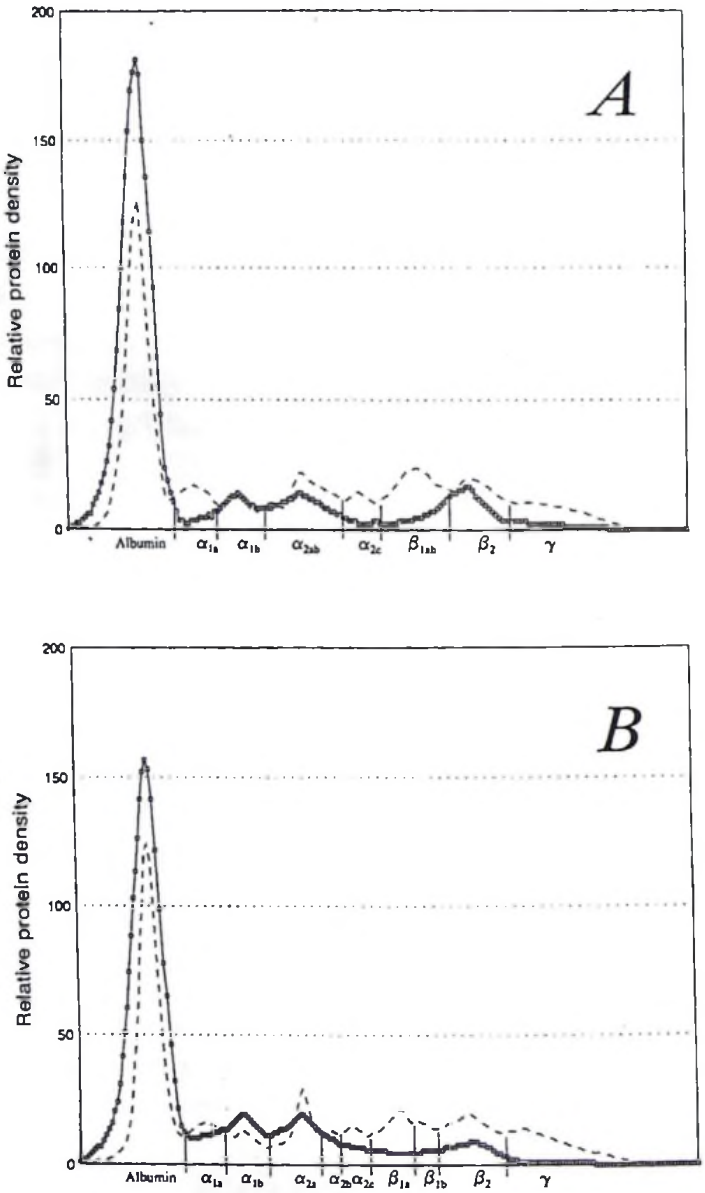


Figure 7: (A) urine protein electrophoresis from a dog with glomerulopathy. Note the marked albumin loss in urine. This usually indicates glomerular proteinuria. **(B)** Urine electrophoretogram from a dog with pyoderma showing glomerular proteinuria pattern

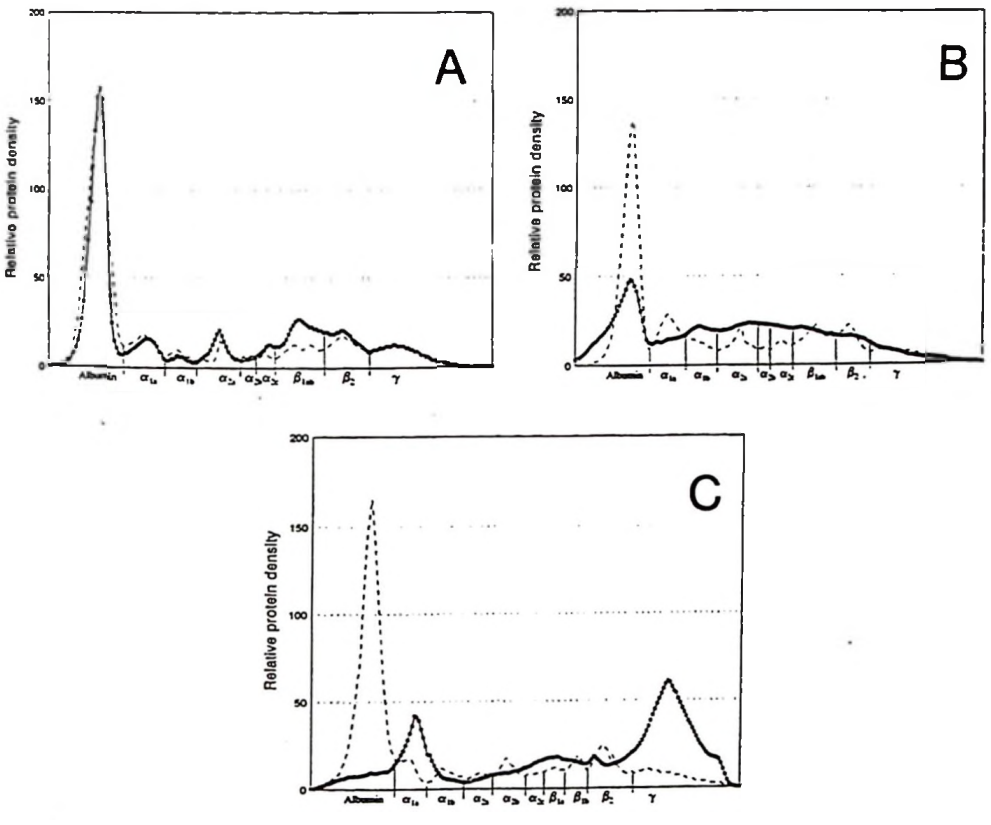


Figure 8: **(A)** Urine protein electrophoresis showing a serum-like proteinuria profile. Note similarity to serum electrophoresis profiles. This pattern is usually obtained when there are inflammatory processes in the lower urinary tract or when there is hematuria. **(B)** Electrophoretogram from a dog with severe glomerulonephropathy lesions. Note losses of both albumin and globulins. **(C)** Urine protein electrophoresis from a dog with trace proteinuria. Note the insignificant albumin losses in urine.

URINALYSIS: FUTURE TRENDS IN SMALL ANIMAL MEDICINE

In companion animal practice, the question of whether renal function is normal or abnormal is of frequent concern to the clientele as it is to the clinician. It would be preferable to obtain an early warning of reduced renal reserve so that measures can be instituted to limit progression of the disease, if possible. Thus the question should be answered fairly quickly and at minimum cost to the owner of the animal. This implies that the evaluation used for that purpose should be simple, risk free and quick to execute. The future of urinalysis as a diagnostic test should be viewed in light of the aforementioned prerequisites.

Urinary sediment:

Urine sediment examination has tremendous potential, properly used the technique may help the clinician to make breakthroughs in present day problem areas. Its high time we reduced or erased from veterinary nomenclature the idiopathic so and so. In man, critical evaluation of red blood cells in the urinary sediment have made it possible to characterize glomerular and nonglomerular diseases with certainty (Stamey and Kindrachuk, 1985). Preliminary studies in this area, indicate that the technique could be applicable in companion animals (Batamuzi, unpublished data). There are two types of red blood cells that may be seen in the urinary sediment. The first type, called epithelial red blood cells, are normally round/biconcave, with uniform hemoglobin distribution. The epithelial red blood cells are seen in conditions like bladder neoplasms, interstitial cystitis, urolithiasis, renal tumors, prostatitis and a variety of iatrogenically initiated hematurias. The second type is the dysmorphic red blood cell (Stamey and Kindrachuk, 1985). These red blood cells are irregularly shaped and lack

uniform hemoglobin distribution. In man, dysmorphic red blood cells have been found to be associated with glomerular diseases (Stamey and Kindrachuk, 1985). At the Royal Veterinary and Agricultural University (RVAU) we have seen similar red blood cells in a case of idiopathic renal hematuria (Batamuzi et al., 1994). There is need to pursue this area further in order to exploit it fully in our diagnostic endeavors.

White blood cells could also be further characterized in order to differentiate different causes of urinary tract inflammatory reactions. Another area requiring attention in future is the diagnostic potential of degenerated urothelial cells as seen in the urine sediment. Up to now, the mechanisms responsible for the alterations in the urothelial cell integrity following prolonged exposure to urine and in disease situations are unknown (Batamuzi and Kristensen, 1995).

Enzymuria:

Renal diseases and nephrotocoses are reflected in changes in enzyme activity in urine but not in serum (Kramer, 1989). The appearance of enzymes of renal origin in urine provides a unique opportunity for detection of renal injury (Greco et al., 1985). Detection of tubular damage or necrosis can be done by analyzing the urinary content of Beta - glutamyl transpeptidase and N-acetyl-ß-D-glucoaminidase (Greco et al., 1985; Stolarek et al., 1989). Other enzymes including urinary amylase, lactic dehydrogenase and aspartate transaminase also show good prospects in this regard (Szczech et al., 1974;, Hardy et al., 1985; Aderka et al., 1988). Further work is needed in order to fully exploit the potential of urinary enzymes in the diagnostic workup for urological/-nephrological problems.

Urinary endocrine metabolites:

Endocrine metabolites found in urine have been used in the diagnosis of diseases affecting other systems/organs. The urinary corticoid/creatinine ratio was used to differentiate dogs having Cushing syndrome from those not having the disease with high sensitivity (Rijnberk et al., 1988). Hyperadrenocorticism is a common endocrine disorder caused as a result of excessive cortisol secretion by the adrenal cortex (Feldman and Mack, 1992). Endocrine metabolites can further be evaluated for their value in the diagnosis of the urogenital and extra-urogenital diseases.

Residues and other indicators of environmental degradation:

Companion animals share the same environment as man and therefore they are subjected to the same potential hazardous influences emanating from the environ-

ment. In this regard drug and other chemical residues could be easily picked from urine of companion animals. Some of these procedures are already in use in the form of tests for various intoxications for example ethylene glycol poisoning as well as poisonings caused by insecticides, rodenticides and heavy metals (Nicholson, 1995). Thus this facility could be exploited further as a way of keeping a finger on the patient's pulse.

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Journal of Small Animal Practice
1995, *36:* 17-21

DIAGNOSTIC IMPORTANCE OF UROTHELIAL CELLS OF THE DOG AND CAT

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Diagnostic importance of urothelial cells of the dog and cat

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Journal of Small Animal Practice (1995) 36, 17-21

ABSTRACT

The cells lining the canine and feline urinary tract and their role in the diagnosis of urological diseases in small animals is assessed. The urothelium was found to consist of transitional epithelial cells ranging from the calyces to the urethra. Caudate cells were found lining the ureter, renal pelvis and the calyces. There was no feature that could be used to distinguish the transitional cells from different parts of the urothelium. Squamous cells were found lining the urinary tract from the trigone to the vagina in females and to the urethra in male animals. Hydropic degeneration in the form of vacuolation of the cytoplasm, granulation and total loss of cytoplasm was one of the urine-induced degenerative changes recorded in the transitional cells. The significance of the degenerative changes in the management of urological problems is discussed.

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INTRODUCTION

Microscopic examination of centrifuged urine sediment is important in the diagnosis of diseases of the urinary system (Barlough and others 1981, Lowe and Brendler 1992). The evaluation of the cellular components of the urine sediment is complicated by the fact that cells may originate from several areas such as the vascular system, the interstitial tissue, the urothelium or the genital tract (Osborne and Stevens 1981, Stamey and Kindrachuk 1985). Furthermore, urine has cytotoxic effects on the exfoliated urothelium and other cells, and this adds to the problem of urine sediment evaluation (Zinkl and Feldman 1989).

Transitional epithelia of different sizes and shapes suggests that they may be originating from different parts of the urothelium (Chew and DiBartola 1986) or that variation in their size may be governed by their depth of origin in the transitional epithelium (Osborne and Stevens 1981). This makes it difficult to use these cells to assess the localisation of a disease.

Working on the human urothelium, Stamey and Kindrachuk (1985) reported the presence of differences in the size and position of intracytoplasmic granules in transitional epithelial cells. As the epithelium advances towards the renal pelvis, the granules of the uroepithelial cells become larger with a greater perinuclear distribution. This finding has contributed immensely to the identification of the site of lesions in the human urinary tract (Lowe and Brendler 1992).

The present study aims to assess the canine and feline urothelium as a means of exploiting its diagnostic potential in urological diseases.

MATERIALS AND METHODS

Animals

Two categories of animals were used in the study. The first consisted of 10 dogs and two cats which were healthy animals brought to the clinic for euthanasia. The dogs were of different breeds and their ages ranged from two to nine years. The cats were domestic shorthairs. The second group consisted of 120 dogs of different age groups and breeds attending The Royal Veterinary and Agricultural University's Small Animal Hospital, for various reasons during the first half of 1993.

Cell samples

The entire urinary tract from a recently euthanased animal from the clinic was isolated

and extirpated from its attachments. The renal vessels were flushed with normal saline to remove red blood cells from the area of the renal calyces and pelvis.

The urinary tract was opened longitudinally, beginning in the renal calyces area up to, and including, the urethra. Epithelial scrapings were then taken from the renal pelvis/calyces, the proximal, mid and distal ureter, urinary bladder at the roof, floor and trigone areas as well as from the urethra. A new scalpel blade was used for the different areas. The scraped material was then freed from the scalpel blade by immersion in 5 ml of saline in a test tube (Stamey and Kindrachuk 1985). Sediment smears were prepared, stained and evaluated as described for the ordinary urine sediment.

Five millilitres of urine was collected by cystocentesis from dogs in the Small Animal Hospital with the animal in a standing position using a sterile syringe and hypodermic needle. The urine sample was centrifuged at 160 *g* for five minutes, after which the supernatant was completely decanted by inverting the centrifuge tube. The sediment at the bottom of the tube was resuspended by the few drops of urine remaining after the procedure. Resuspension and uniform distribution of the sediment in the remaining urine drops was facilitated by vigorously tapping the tube against a table top (Stamey and Kindrachuk 1985). The resuspended sediment was poured on to a microscope slide as completely as possible by tapping the tube on the microscope slide. A urine sediment smear was then prepared by tilting the slide to about 45° to the horizontal – a procedure that allowed the sediment to run freely along the length of the slide. The smear was then air dried and stained with haemacolor (E. Merck, Darmstadt). The stained smears were evaluated under light microscopy using low (×10 objective) and high (×50 oil immersion, objective) magnification.

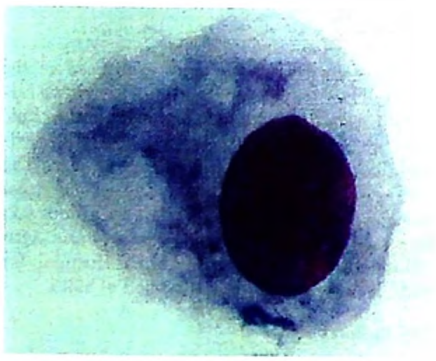

FIG 1. Ordinary transitional epithelial cell. Haemacolor × 125

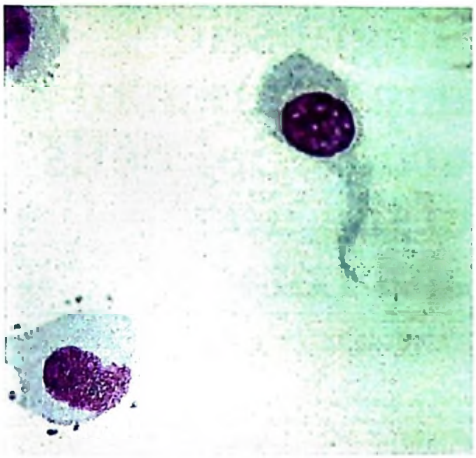

FIG 2. Caudate epithelial cells. Note trailing cytoplasm. Haemacolor × 125

Degenerative changes

Five intact urinary bladders were assessed for degenerative changes. The urinary bladder was freed from its attachments and removed from the pelvic cavity by severing it from the urethra at the level of the prostate. In each case the bladder was emptied as completely as possible by aspirating the urine using a needle and syringe and, following centrifugation, about 40 to 60 ml of urine freed of sediment was reintroduced in to the bladder. The bladder was then clamped at its urethral end, placed in a beaker and incubated at 39°C for 12 hours. About 1 ml of urine was

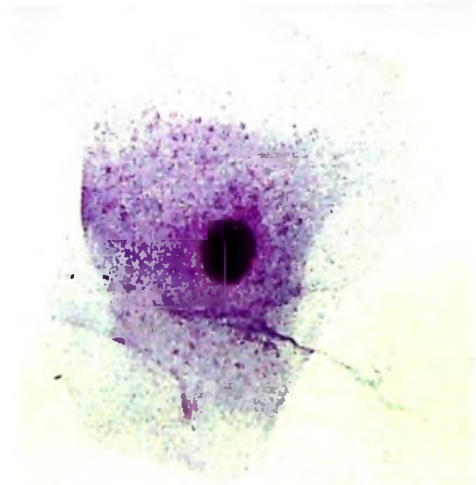

FIG 3. Squamous epithelial cells showing a small nucleus in relation to cytoplasm. Note uniformly staining cytoplasm with occasional but fine granularity. Haemacolor × 125

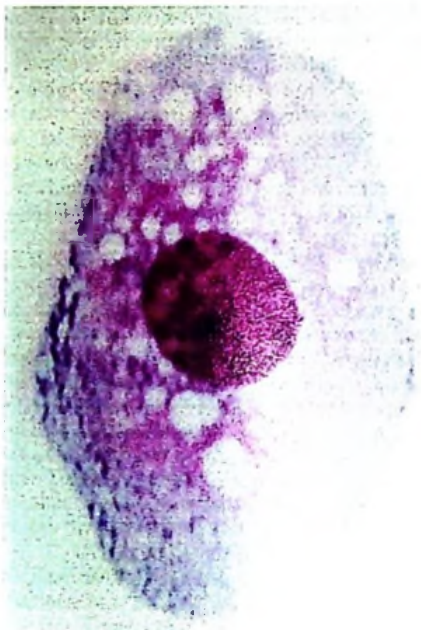

FIG 4. Transitional cell demonstrating cytoplasmic granules and vacuoles. Haemacolor × 125

collected using a needle and syringe every two hours during the incubation period and smears were prepared and examined under the microscope for degenerative changes. Differential counts of normal and degenerated cells were carried out.

RESULTS

Cells from normal urinary tracts

The urothelium of normal urinary tracts was found to have two main cell types, namely transitional and squamous epithelia. The transitional epithelial cells were further subdivided into ordinary transitional cells and caudate transitional cells, the ordinary ones predominating in most areas.

Ordinary transitional epithelial cells. – The ordinary transitional epithelial cells were round, oval or pear-shaped with an oval vesicular nucleus. The nuclei were either eccentrically or centrally located and had one or two small nucleoli. It was not uncommon to find multinucleated and, or, multinucleolated cells. The transitional cells had abundant slightly basophilic, finely granular cytoplasm (Fig 1). There were small and large transitional cells in all the preparations. Ordinary transitional cells were found lining the urethra except the terminal part, bladder, ureter, renal pelvis and calyces.

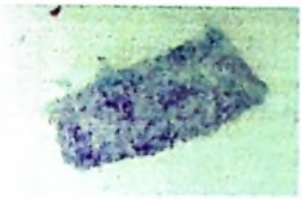

FIG 5. Cast-like cytoplasmic portion of a degenerated cell. Haemacolor × 125

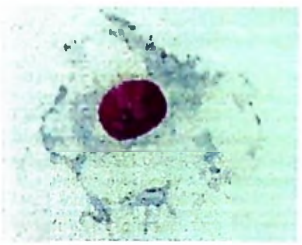

FIG 6. Degenerated transitional cell. Note fenestrated cytoplasm. Haemacolor × 125

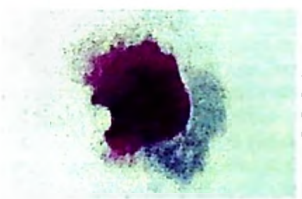

FIG 7. Degenerated transitional cell. Note that only a degenerated nucleus and remnants of cytoplasm are present. Haemacolor × 125

Caudate transitional epithelial cells. – The caudate transitional epithelial cells were small when compared to the ordinary transitional cells (Fig 2). They were thin, elongated cells with trailing cytoplasm. Staining characteristics were the same as ordinary transitional epithelial cells. These cells had round to oval nuclei located at the broader part of the cell. The caudate type of transitional cells were found lining the ureters up to and including the part buried into the bladder wall, the renal pelvis and the calyces but not the bladder and urethra.

Squamous epithelia. – Squamous epithelial cells were found in the trigone, urethra and the vagina. These were large cells with a small round nucleus and fine granularity (Fig 3). The cell borders were polyhedral and were occasionally found folding on themselves. A variant form of squamous epithelial cells was found in the trigone. This type had a vesicular nucleus but other features were similar to the normal squamous epithelial cells.

Degenerative changes

A number of changes were recorded when cells were in contact with urine for a long period.

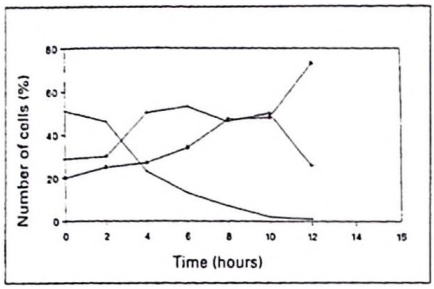

FIG 8. Influence of urine on the integrity of transitional cells. Stage 1 degeneration represents the initial stage of degeneration where there are few granules and vacuolation of cytoplasm. Stage 2 represents significant degenerative changes, with fenestration or total loss of cytoplasm. – Normal cell. + Stage 1 degeneration. * Stage 2 degeneration

Initially, there was an increase in the number and size of cytoplasmic granules as well as numerous vacuoles within the cytoplasm (Fig 4). Large portions of cytoplasm would detach from the rest of the cell and the released portions of the cytoplasm had a tubular shape reminiscent of the fine granular casts (Fig 5). At this stage the cytoplasm was found to be extensively fenestrated (Fig 6). Nuclei without cytoplasmic cover would be seen (Fig 7), or the entire cell would be lost from view. In Fig 8 the progression of degenerative changes for the cells under the influence of urine is presented graphically. These changes were similar in nature and progression for all urothelial cell types.

Clinical cases

Out of the 120 dogs examined, urine sediment findings were suggestive of the presence of urinary tract disease in 26. These findings included the presence of many exfoliated transitional cells and degenerative changes in the form of cytoplasmic vacuolation and granulation of the exfoliated cells. Multinucleated urothelial cells were a common feature in most preparations. Other findings were pyuria, microhaematuria, bacteriuria and cylindriuria. The features of urinary tract disease on the basis of urine sediment examination were found mainly in dogs with urolithiasis, cystitis, pyometra and prostatitis. Chronic cases of pyoderma and atopy were also found to have features of urinary tract disease. These included the presence of fine granular and waxy casts as well as degenerated transitional cells. In one case of diabetes mellitus the authors found neutrophils and bacteria but scanty degenerated transitional cells.

DISCUSSION

In the present study, the urothelium from the distal urethra to the renal calyces was found to have two cell types, namely squamous and transitional epithelial cells. This finding is in agreement with Osborne and Stevens (1981) and Stamey and Kindrachuk (1985). The ordinary transitional epithelial cells from the bladder to the renal calyces in freshly obtained cellular material, had no consistent feature that could be used to differentiate them. Even the cytoplasmic granules, which have been used as a distinguishing feature for these cells in human medicine (Stamey and Kindrachuk 1985), seem not to be present in small animals. These cells therefore cannot be used in the localisation of the site of inflammatory process along the urinary tract.

Caudate cells, a form of transitional cells, were thought to originate from the renal pelvis (Chew and DiBartola 1986) and were supposed to be of localising value for disease processes in that area. In this study, caudate cells were found lining the entire length of the ureter, renal pelvis and renal calyces. Caudate cells, therefore, do not appear to have any localising value for any specific part of the upper urinary tract in the dog and cat.

Squamous epithelial cells have been reported to originate from the genital tract and that their presence in urine sediment was a sign of contamination (Zinkl and Feldman 1989). This work has demonstrated squamous epithelial cells within the bladder on the trigone, a finding also reported in humans (Lowe and Brendler 1992). It is important to note that although the majority of squamous epithelial cells have a small tight nucleus, there exists a small proportion which have larger vesicular nuclei which could be mistaken for large ordinary transitional cells. This could account for the lack of reports of the presence of squamous cells in the urinary bladder. The presence of squamous cells with vesicular nuclei possibly indicates a point of transition from squamous cells in the genitourinary tract to transitional cells in the urinary tract. This type of cell was not demonstrated in other parts of the lower urinary tract.

Urothelial cells in urine sediments of samples from cases with urinary tract disease were numerous and markedly degenerated. Microhaematuria, bacteriuria and cylindriuria were a consistent feature in urine sediments from animals with urinary tract disease. Multinucleated urothelial cells were a common finding in most preparations, especially the ones with traumatic and inflammatory processes of the urinary tract. The presence of multinucleated and multinucleolated cells could easily be taken as an indicator of malignancy. However, these cells have been reported to be relatively common in normal animals and therefore a normal finding (Rebar 1987). Multinucleated cells have also been reported following toxic or traumatic effects on the urothelium (Zinkl and Feldman 1989). These

could possibly be a result of desquamation of cells in large numbers.

A normal urine sediment contains few cells (Chew and DiBartola 1986). In this study, preparations containing many urothelial cells were encountered mainly where there were inflammatory processes in the urinary tract and, or, urolithiasis. In most of these there was an admixture of inflammatory cells, urothelial cells and bacteria. Thus, the finding of many transitional cells in a voided, cystocentesis or catheterised, sample indicates there is a pathological process somewhere along the urinary tract.

The degenerative changes recorded in cells under the influence of urine represent a number of processes. Cytoplasmic vacuolation, a hydropic change, has been reported where there has been a delay in sample processing (Zinkl and Feldman 1989). It has been suggested that the main cause for cytoplasmic vacuolation and halo formation in degenerating cells is sequestration of water in vacuoles by degenerating endoplasmic reticulum (Cheville 1988). With time there is accumulation of granules of protein debris and disorganisation of the cytoplasm as well as clumping of chromatin followed by rupture and total breakdown of the nucleus (Cheville 1988). In this study, degenerative changes of the urothelial cells were found in preparations from actual clinical cases involving the urinary tract and also in situations in which there was a delay in processing and evaluation of the samples. The present observations suggest that, in order to distinguish degenerative changes due to disease from those caused by delayed sample processing, samples have to be processed and examined within half an hour following collection. Casts which are composed of precipitated protein and other substances are usually used as an indicator for pathological changes in the renal tubules (Lobingier and Zinkl 1992). We have also established that 10 hours following collection of urine, urothelial cells degenerate to form pseudocasts. The formation of pseudocasts can easily confuse results since portions of cytoplasm liberated from degenerated cells cannot be distinguished from cylinders of genuine fine granular and cellular casts.

In the present study, a number of processes and changes involving urothelial cells have been recorded in experimental settings and in clinical cases. These processes and changes appear to have a diagnostic significance in the management of small animal urological problems. The degenerative changes observed in the experimental setting whereby cells were suspended in urine, helps to underscore the importance of working on fresh urine samples. A delay in sample processing and examination could lead to false positives where changes like cylindriuria are used in the diagnosis of glomerulonephritis and, or, tubulointerstitial disease. Findings recorded in clinical cases regarding degenerative changes in fresh samples, may be used as additional diagnostic criteria for diseases of the urinary tract. The presence of many transitional cells together with degenerative changes and features like pyuria, cylindriuria and microscopic haematuria, indicates that there is pathology somewhere along the urinary tract. Localisation of the disease will, however, require application of other tests.

The mechanisms responsible for the alterations in the urothelial cell integrity following prolonged exposure to urine and in disease situations need further investigation.

ACKNOWLEDGEMENT

The authors wish to thank Danida for funding the study.

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Veterinary Record
1994, *135:* 603

IDIOPATHIC RENAL HEMATURIA IN A DOG

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Idiopathic renal haematuria in a dog

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Veterinary Record (1994) 135, 603

HAEMATURIA of renal origin is clinically characterised by passing urine containing blood throughout micturition (Polzin and others 1989). The presence of microscopic haematuria with red cell casts in the urine sediment confirms the diagnosis of renal parenchymal haematuria (Kaufman 1989). Idiopathic renal haematuria which is characterised by the presence of gross haematuria in dogs with normal renal function and urine concentrating ability has been reported in five cases (Stone and others 1983, Straw and others 1985). All the reported cases were unilateral and resolved after removal of the affected kidney.

The following report of a labrador retriever with idiopathic renal haematuria illustrates the characteristic features of the disease and represents the first description of a case that did not benefit from unilateral nephrectomy. Following euthanasia and necropsy it was shown to be bilateral.

A two-year-old female labrador was referred to the Royal Veterinary and Agricultural University's small animal hospital with a history of haematuria of two months' duration.

Initially, the abdomen was radiographed, urine cultured and an exploratory laparotomy and a cystotomy carried out without detecting a convincing cause for the persistent haematuria.

Two months later, physical examination followed by ultrasonographic scanning of the abdomen, revealed no abnormalities except haematuria and a small echogenic lesion on the cranial pole of the left kidney. Serum, blood and urine samples, the last by cystocentesis, were obtained. Abnormal results of a complete blood cell count and other biochemical profiles are shown in Table 1.

The remaining parameters were normal at the initial examination. On the second examination the blood and biochemical profiles were within normal ranges.

On unnalysis, a pH of 7·5, 2+ proteinuria and a 3+ occult blood were found. Urine specific gravity was 1·015 by refractometry. Microscopic examination of the urine sediment showed red blood cells too numerous to be quantitated. All other parameters were within the normal range. Urine culture produced no growths.

A second exploratory laparotomy showed no meaningful changes in the urinary bladder and other abdominal structures. The left kidney, however, had an infarct-like lesion on its cranial pole and its ureter was slightly oedematous. Examination of sediment from the left renal pelvis taken as a fine needle aspirate showed microscopic haematuria. The right kidney appeared grossly normal and red blood cells seen in the sediment from it were considered to be iatrogenic.

In view of these observations, a tentative diagnosis of idiopathic haematuria of non-traumatic origin was made. On this basis, all previous reports having described idiopathic renal haematuria as being unilateral, a left nephrectomy was done. This intervention, however, failed to resolve the haematuria and the dog was euthanased one month later. The post mortem examination revealed no gross lesions of diagnostic value. The histological examination of the kidneys showed that bleeding had occurred in the capsule, peripelvic fat tissue and in the lumina of a few cortical cysts. Other kidney findings were a small dysplastic area in the cortex of the left kidney, a few cortical cysts and some mineralisation of the collecting tubules. Staining for haemosiderosis (Perl's stain) showed a positive reaction primarily in areas in which mineralisation had been demonstrated and in only one macrophage in the capsule. There was no reaction for the stain in the pelvic area.

TABLE 1: Blood profile

Parameter		Normal range
Slight decrease in haematocrit	0·35	0·37 – 0·55
Low haemoglobin levels (mmol/litre)	6·70	7·70 – 11·65
Increase in ASAT levels (μKAT/litre)	1·60	0·33 – 0·69

The present case appears to be a further report of idiopathic renal haematuria (Stone and others 1983, Straw and others 1985). Most aspects of this case are in agreement with previous findings but failure to stop haematuria after nephrectomy of the affected kidney, coupled with finding capsular bleeding in both kidneys, appears unusual.

The capsular bleeding that was seen in both kidneys was acute, and tests for the presence of haemosiderosis were negative. These findings, therefore, suggest that the bleeding from the capsule was an incidental finding, and unlikely to explain the pathogenesis of the long standing haematuria in this case. The bleeding in the peripelvic fat tissues was very acute and could be related to surgical manipulation. The dysplastic renal cortical area was chronic and devoid of signs of haemorrhage or inflammatory manifestations. There is, therefore, no evidence to indicate that the dysplastic area had any connection with the pathogenesis of the clinical signs.

Renal tubular mineralisation is not an uncommon finding, and this also fails to explain the process leading to a long standing haematuria.

The erythrocytes in some tubular cysts could possibly explain an association with the haematuria in this case. The presence of erythrocytes in the cysts and in the pelvic lumen could indicate a connection.

All previously reported cases of idiopathic renal haematuria have been in young dogs with ages ranging from two months to two years. The condition may therefore be age-related or congenital.

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Prevalence of salmonellae in dairy herds

SIXTY dairy herds with an average of 584 cows were selected at random from 700 herds in California. Milk samples from each cow were tested by an ELISA for antibodies against Salmonella serogroups B, C1 and D1 antigens, and blood samples were taken from any positive cows and tested in a similar way. Samples for bacteriological culture, including pooled faeces from 20 randomly selected calves, swabs of wet areas, faeces from calf pens and samples of feed components were also taken from each of the 60 farms. Forty-five of the 60 herds had serological evidence that at least one cow had recently been exposed to salmonellae, but only seven had any environmental samples with yielded a positive culture for salmonellae. It was concluded that environmental sampling for salmonellae may underestimate the number of dairy herds with infected cows.

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Journal of Small Animal Practice
in press

URINARY TRACT INFECTION: THE ROLE OF CANINE TRANSMISSIBLE VENEREAL TUMOUR

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ABSTRACT

The objective of this study was to investigate the role of transmissible venereal tumour in the pathogenesis of urinary tract infection in dogs. Eighty six dogs were used for this work. Of these, fifty five were animals with transmissible venereal tumours, and thirty one were used as controls. For each dog used, a thorough clinical examination of the external genitalia was done. For dogs with transmissible venereal tumours, the sites of attachment were recorded. Urine samples were taken by cystocentesis and the external genitalia swabbed. Samples collected were cultured for bacteria using standard methods. The tumours were found located on the prepuce and different parts of the penis for male dogs and in bitches they were found on the vagina, vestibule or the vestibulo-vaginal junction. Dogs with transmissible venereal tumours were found to be at a high risk of having bacteriuria, odds ratio (OR)=7.04. Also obliteration of the urethral orifice by the tumour, possibly leading to urine retention was thought to be the main reason for the high incidence of UTI among dogs with transmissible venereal tumour. Long standing cases of transmissible venereal neoplasia had a higher chance of becoming bacteriuric compared to recent cases (OR=29.60). In conclusion, this study indicates that transmissible venereal tumour may be a predisposing factor for the development of urinary tract infection.

INTRODUCTION

Urinary tract infection (UTI) is defined as the invasion of the normally sterile areas of the urinary tract by pathogenic bacteria, with subsequent causation of clinical signs (Brown and Barsanti 1989). The most commonly isolated causes of UTI in dogs are *Escherichia coli, Staphylococcus spp, Proteus spp, Klebsiella spp* and *Streptococcus spp* (Bush 1984). *E. coli* is the bacteria most commonly isolated. UTI is said to develop when normal host defenses are compromised, thus allowing entrance and proliferation of opportunistic microbes (Lees and Osborne 1979). The most important host defence mechanism is the frequent and complete voiding of urine, which helps mechanically to remove the bacteria from the urinary tract (Senior 1985). Thus urine retention as caused by obstruction, calculi or neoplasia of the urethra is a frequent cause of UTI (Bush 1984). Transmissible venereal tumour (TVT) in bitches, has the vestibulo-vaginal junction as its predilection site (Boscos 1988; Batamuzi and others 1990). The vestibulo-vaginal junction is the anatomical position in which the urethra opens (Ellenport 1975). Owing to this predilection site, TVT in bitches can cause urethral obstruction resulting in dysuria and dribbling of urine. Phimosis, a condition that interferes with voiding of urine, has been reported to occur in male dogs with TVT (Ndiritu 1979). The prepuce forms a complete sheath around the cranial part of the penis (Ellenport 1975). Thus fully established preputial TVT will most likely lead to obstruction of urine outflow.

In view of the threat that TVT poses in the causation of UTI and paucity of information on the same, the present work was designed to investigate its role in the development of UTI.

MATERIALS AND METHODS

Animals:

Dogs with and without TVT were used for this work. These dogs were selected from Sokoine University of Agriculture Veterinary Clinic and from the dog dipping center. They belonged to different owners in Morogoro Municipality and its surroundings. These dogs were of different age groups, both sexes and were either pets, guard dogs or hunting types. Only entire dogs of both sexes for the case as well as the control groups were used. For both categories systematic random sampling was done. In the selection of the case group, every other dog that had TVT was selected. As for the controls, every fifth dog entering the dipping premises was selected for this study. To qualify for selection as control, dogs were subjected to exfoliative cytology in a manner similar to that of Batamuzi and Kessy (1993).

Physical examination:

All dogs used in this study were subjected to a thorough physical examination of the genital system in order to establish the presence or absence of TVT. Also, this examination was aimed at assessing the influence of the sites of attachment of TVT on the development of UTI. The stage of TVT, whether recent or longstanding and the extent of involvement of the external genitalia were also investigated.

For the purposes of this study, recent cases were defined as those with small, solitary and easily accessible tumours. Long standing cases were those with large, multilobulated and multifocal tumours.

Samples:

For each dog in the study, 10ml of urine were collected by cystocentesis using a sterile syringe and needle. Also, the vestibulo-vaginal junction and the preputial cavity for females and males, respectively, were swabbed using a sterile swab. Urine and swabs were cultured within 30 minutes of their collection on blood agar and MacConkey, then incubated at 37°C for 24 hours. Where necessary, subculturing and bacterial species identifying biochemical tests were also undertaken (Cowan 1974).

Urine sediment examination:

Some of the urine collected was used for preparation of the urine sediment and its microscopic examination as described elsewhere (Batamuzi and Kristensen 1995).

Statistical analysis:

Frequencies of UTI for different independent variables were compared using χ^2 statistic. The degree of association between the independent variables and UTI was measured by an odds ratio (OR) as described by Thrusfield (1986). In all calculations used in measuring the strength of association between independent variables and UTI, it was assumed that there were no confounding factors other than sex. Thus the latter was the only variable treated as a confounder for which Mantel Haenszel procedure was employed to remove its effect. The Mantel Haenszel technique is an analytical procedure for controlling the effect of extraneous factors which are commonly referred to as confounders. In this technique data were collected about potential predisposing factors and UTI, data were also collected on the presence or absence of potential confounding variables. Then data were stratified and displayed in a series of 2 x 2 tables, one table for each level of confounding variable. Additionally a summary of the tables was made to display a summary measure of association between the predisposing factors considered and UTI.

RESULTS

Of the eighty six dogs examined, fifty five (33 males and 22 females) had TVT while thirty one (16 males and 15 females) had no TVT.

Sites of attachment:

Of the 37 bitches examined, 22 had TVT, and this was distributed as shown with the numbers of cases in brackets: vagina (6); vestibule (6); vestibulo-vaginal junction (8) and diffuse involvement of the genital tract (2). As for the 49 males, 33 had TVT. The tumour was found on the caudal parts of the penis in 15 cases, middle parts of the penile shaft in 3 cases, prepuce 9 cases, I case on penile tip and diffuse involvement of external genitalia in 5 cases.

Bacteria from the external genitalia:

Table 1 presents the types of bacteria isolated and their frequency in male and female dogs. Bacteria isolated from the external genitalia were *S. intermedius, Pasteurella spp, E. Coli, Streptococcus spp, Proteus spp* and *Bacillus subtilis* in order of decreasing frequency of isolation.

Bacteriuria:

Of the eighty six animals examined, 22 had bacteriuria. *E. Coli, S. internedius* and *Streptococcus spp* were isolated from these bacteriuric cases (Table 2). *E. coli* was the most frequently isolated bacterium. Of the 22 cases of bacteriuria, 19 were from TVT cases, and controls accounted for 3 cases. In all the 3 controls with bacteriuria, *E. coli* was isolated.

There was a strong association between genital TVT and the development of bacteriuria [Odds ratio (OR) = 7.05, χ^2=6.27 and P< 0.05). Even following confounder control to remove the effect of sex, there still remained a strong association between TVT and bacteriuria (Mantel Haenszel OR=7.4, Mantel Haenszel χ^2=6.06, P <0.05). Eighteen of the bacteriuric cases were from dogs in which TVT was found attached to areas surrounding the urethral opening for both sexes. These sites were the prepuce, penile tip and diffuse involvement of external genitalia in males, and the vestibulo - vaginal junction and diffuse involvement of the external genitalia in females. Of the 55 TVT cases, 26 were long standing and 29 were recent. Sixteen long standing cases were bacteriuric compared to only 2 recent cases. Long standing cases had a higher chance of becoming bacteriuric compared to recent cases (OR=29.60, χ^2 yates = 22.05 and P<0.05).

Out of 22 cases of bacteriuria, 7 (30%) had signs of cystitis.

DISCUSSION

The entire urinary tract is at risk of microbial invasion once any of its normally sterile parts becomes colonized with bacteria (Brown and Barsanti 1989).

The main source of infection is the skin and rectal bacteria (Bush 1984). In this study, a number of bacteria which are important in UTI were isolated from external genitalia of dogs with TVT. This could mean that the external genitalia could be another important source of bacteria causing UTI. Studies in man indicate that colonization of the vaginal and urethral mucosae precedes the occurrence of bacteriuria (Bollgren and Winberg 1976). The same mechanisms may be involved in the causation of UTI in dogs. It is a known fact that TVTs easily become traumatized, ulcerate and are frequently secondarily invaded by bacteria (Boscos 1988). Under these circumstances, the environment is made ideal for invasion, colonization and subsequent infection of the urinary tract by such bacterial pathogens.

We managed to isolate the same bacteria from the urinary bladder as from the Vagina. However not all the bacterial species were involved.

In the majority of cases *E. coli* was isolated from the urinary bladder. According to Schaeffer (1992), *E. coli* is endowed with fimbriae, an important facet for adherence on mucosal surfaces. Also some strains of *E. coli* do produce K antigens (Schaeffer 1992). Fimbriae and K antigens could be important in facilitating the pathogenicity of *E. coli* and could explain why it was a frequent isolate from the urinary bladder compared to other bacteria which had an equal chance of reaching it. About 25% of all the dogs examined had bacteriuria and most of these were from cases of TVT. There was also a strong association between TVT and bacteriuria as indicated by a high OR of 7.4, even after eliminating the confounding effect emanating from the sex of the patient. There is thus, a strong indication that dogs with TVT are at a higher risk of contracting UTI compared to those without TVT. However, after considering the effect of location of TVT, it became clear that dogs are at an even higher risk when TVT is located at areas surrounding the urethral opening. Thus male dogs with TVT on the penile tip and prepuce and bitches with the tumour on the vestibulo-vaginal junction, were more likely to have bacteriuria compared to those with the tumour in areas detached from the urethral orifice. The vestibulo-vaginal junction, the natural opening of the urethra, has been reported to be a predilection site for TVT in bitches and this has been found to cause dysuria in affected dogs (Boscos 1988). For male dogs, the caudal parts of the penis, mainly the area behind the bulbus glandis is the site preferred by TVT (Boscos 1988; Batamuzi and others 1990). However, preputial TVT especially in long standing cases is not uncommon (Batamuzi and others 1990). In these cases phimosis and dysuria have been reported (Ndiritu 1979). Thus the location of TVT on these areas could explain the relationship between TVT and bacteriuria.

Long standing cases of TVT were at a very high risk of becoming bacteriuric as indicated by a very high OR (29.60) that was obtained. Long standing cases tend to lead to extensive involvement of the external genitalia in both sexes (Boscos 1988). Under these circumstances, obliteration of the urethral opening, especially in bitches, is likely to occur. Urine retention, a putative causal factor in the pathogenesis of UTI will be facilitated (Lees and Osborne 1979).

Out of 22 cases of bacteriuria, 7 (30%) had signs of cystitis. This appears to suggest that a good proportion of bacteriuric cases have a chance of developing into clinical UTI. Certain factors may be required before clinical disease develops from the bacteriuric cases. It was not the purpose of this study to identify those factors.

In conclusion, this study indicates that TVT may be a risk factor for the development of UTI. However, other factors may be required for initiating clinical UTI from bacteriuric cases of TVT. Thus more studies are necessary to establish the role of other factors in UTI causation.

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Table 1: *Bacteria isolated from the genital mucosa.*

	Number of isolations			
	Control		TVT cases	
Bacteria Spp	*Male*	*Female*	*Male*	*Female*
Staphylococcus intermedius	4	3	11	10
Pasteurella multocida	4	2	4	3
Escherichia coli	2	2	4	5
ß-hemolytic Streptococcus	1	3	5	0
Bacillus subtilis	0	2	2	1
Proteus Spp	0	2	2	2
Pasteurella hemolytica	1	0	0	1
α-hemolytic Streptococcus	0	0	0	2

Table 2: *Bacteria isolated from urine of dogs with and without TVT*

	Number of isolations			
	Control		TVT cases	
Bacteria spp	*Male*	*Female*	*Male*	*Female*
Escherichia coli	1	2	9	5
ß-hemolytic Streptococcus	0	0	1	0
Staphylococcus intermedius	0	0	1	3

Journal of Veterinary Medicine, Series A
in preparation, 1996

ANALYSIS OF SERUM PROTEINS IN MIDDLE AGED TO OLD DOGS USING AGAROSE ELECTROPHORESIS

With one table and four figures

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Summary

The electrophoretic pattern of 37 serum samples from clinically healthy middle aged to old dogs was evaluated using agarose as supporting matrix. Three electrophoretic patterns were identified after densitometric scanning. The two predominant patterns were found to be one with an increase in α_2-globulin and another with increases in both ß- and γ-globulins. These patterns appear to suggest that because of ageing and/or accumulated injury geriatric dogs will have high levels of polyclonal γ-globulinemia or acute phase reactants.

Introduction

It is well established that geriatric dogs have high susceptibility to a variety of diseases some of which are potentially life threatening (BUSH, 1993). The detection of these disorders at their early stage of development may improve the chances of success in their treatment.

Electrophoretic separation of serum proteins is among the less invasive diagnostic tests which may be used in the detection of diseases that affect geriatric patients. The procedure has been used for a number of diseases and thus proven to work in real life situations (VAN DEN BROEK, 1992). However, the value of serum electrophoresis in the study of diseases of geriatric dogs is only valid when sets of base-line values have been established. Thus the objective of this work was to determine base-line information for agarose gel electrophoresis of serum proteins of clinically healthy geriatric dogs.

Materials and methods

Animals and sample collection:

Samples of blood were taken from 37 clinically healthy geriatric dogs. The average age of dogs used for this work was 9.7 years (range 6.5 - 15 years) and the breeds were German shepherd, Spaniel, Labrador, Cairn terrier, Fox terrier, Dachshund, Pekingese, Great dane, Irish setter, Golden retriever and a number of mixed breed types. Of these, 26 were male dogs and 11 were female. As a control 18 clinically healthy young dogs, with a mean age of 3.2 years (range 1.5 - 5.5 years) were used. According to the history, none of the dogs had suffered from any illness during the past 2 months. A second control group consisted of 25 diseased middle aged to old dogs attended at the Small Animal Hospital of The Royal Veterinary and Agricultural University. Dogs in the second control group had an average age of 9.6 years (range 6.5 - 15 years). The blood samples obtained from the cephalic vein were allowed to clot at room temperature before the serum was separated by centrifugation. The serum samples were dispensed into Eppendorff tubes, labelled and stored at -20°C pending electrophoresis.

Electrophoresis

Electrophoresis of serum was carried out according to the procedure described by the manufacturer (Beckman Instruments, Inc. - application manual). Of each serum sample 0.5 μl was applied into a preformed, numbered sample wells on the agarose film. The maximum capacity for each film was ten samples. The films were electrophoresed for 25 minutes at 100 volts with barbital buffer solution (pH 8.6, 0.05 ionic strength). After electrophoresis the films were fixed in an acetic acid - methanol solution for 3 minutes, dried completely then stained with paragon blue stain for 3 minutes. The films were then destained with acetic acid, fixed in acetic acid - methanol solution and destained for a second time. After this the films were completely dried and scanned at 600 nm in a Beckman Appraise™ Densitometer. From the densito-metric traces Rf values (relative mobility) of the serum protein fractions were determined as a basis for identification of the protein fractions (BARSANTI et al., 1977; KRISTENSEN and BARSANTI, 1977).

Results

In all geriatric dog sera analyzed, albumin, α_1, α_2, β_1, β_2 and γ globulin fractions were clearly resolved. In addition α_1, α_2 and β_1 showed distinct subdivisions. Further more, α_2-globulin fraction of almost all dogs could be divided into 3 subfractions.

Three main patterns were identified after densitometric scanning and quantification of the fractionated proteins (Table 1). The first pattern, detected in 9 dogs (24%), had proportions of the proteins not different from the ones for young dogs (Figures 1a & b). The second pattern had increases in the ß and the γ regions and was demonstrated in 19 dogs (51%), (Figure 1c). The third pattern was the one which displayed obvious increases in α_2-globulin and reduction in γ globulin (Figure 1d), it was found in 9 dogs (24%). The three patterns clearly correlated to the age of the dogs. Hence, pattern 1 was detected in dogs having a mean age of 8.6 years, pattern 2 in dogs with a mean age of 9.2 years, and the third pattern being observed in dogs with a mean age of 11.4 years.

Discussion

Electrophoretic techniques utilizing agarose as a supporting matrix have been used by several workers (BARSANTI et al., 1977, KEAY, 1982, STRASSER et al., 1993). In the present study, findings of the protein bands are in agreement with those of the workers sighted above and shows that the technique is being applied in a consistent manner. In the α_2 region however, most workers reported two subfractions (BARSANTI et al., 1977; KEAY, 1982). Unlike them we demonstrated three subfractions in the α_2-globulin fraction. The observed changes in the α_2 region could be due to improvement in the technique or could represent an age-related phenomenon.

In this study three major electrophoretic patterns were obtained (Table 1 and Figures 1a through d). In the first pattern proportions of the protein fractions were almost similar to those of the young dog control group. In this pattern, the albumin content was generally higher compared to the other patterns. This finding is consistent with the reports by other workers who have reported that albumin tends to decrease with advancing age (ROCHMAN, 1988; STRASSER et al., 1993). As the dogs became older, the protein compositions apparently changed leading to the second pattern (i.e. increase in the ß- and γ-globulin fractions) developed. Thus in a large proportion of sera from geriatric dogs used for this study there were significant increases in the ß-globulins either alone or together with γ-globulins (Figure 1c). This result is in agreement with the findings of BARSANTI et al., (1977) who reported that increases in $ß_2$- and γ-globulins were tending to correlate significantly with increasing age. Increases in ß-globulin have been associated with a number of diseases including parasitic diseases, liver disease, dermatological conditions and renal conditions (KAWAI, 1973). Geriatric dogs are known to be common victims of such diseases, albeit at subclinical levels (BUSH, 1993). Similarly, the increases in γ-globulins has been associated with diseases like acute and chronic liver diseases, chronic infections, malignancies and autoimmune diseases all of which also afflict geriatrics with higher frequency. However, it is worthy noting that the high levels of ß and γ globulins indicates that the animals with this pattern have ability to mount sufficient immunity to diseases. Then, in the oldest dogs the third pattern (i.e increase in the α_2-globulin as well as decrease in γ-globulin fraction) was obtained. These findings appear to suggest the possibility of insufficiency of the immune system in very old animals as evidenced by absence of immunoglobulins in the latter group. This would help to explain the well known tendency (BUSH, 1993) for geriatric animals to have multiple disorders, limited functional reserve and decreased ability to respond to illness. With advancing age the

immune system becomes increasingly less well regulated, probably due to a decrease in T cell systems (MOSIER, 1981) and a progressive decline in serum T_4 has been reported to be an indicator for reduced immunological competence in old age (GONZALEZ and QUADRI, 1988). This breakdown in the immune system could be typified by an increase in the acute phase proteins as demonstrated in sera from old dogs (Figures 1c). Our observation, that majority of serum samples from these dogs displayed electrophoretic patterns with high proportions of α_2-globulin fraction is in agreement with the results in a recent study by STRASSER et al., (1993). This region is known to be a region for the migration of some of the acute phase reactants such as haptoglobin (HARVEY and WEST, 1987). Working on diabetic dogs, VAN DEN BROEK (1992) did record significant increases in α_2-globulins and went on to suggest that the increase possibly reflected an increased susceptibility of the animals in question to infection. A similar explanation would appear logical for the increases of the same in some geriatric dogs.

Some of the alterations of the laboratory and other physiological values are already known to have significant clinical implications; for the ones reported in the present study, clinical implications are only suspect but of great potential importance. As pointed out by ECKERSAL and CONNER (1988) the assaying of the proteins gives an indication that tissue damage has occurred at the time when there are no clinical signs of the processes in question. For geriatric dogs such tests could particularly be useful in as far as they are likely to ensure longer and better quality life when such conditions are detected and managed earlier.

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Table 1: ***Relative protein values (%) for the different serum electrophoretic patterns (Mean ± standard error of a mean [SEM]).***

	Electrophoretic protein patterns				
	Young	**Old_{γ}**	**$Old_{\beta/\gamma}$**	**Old_{α}**	**Old_{D}**
No. of animals	18	9	19	9	25
Mean age (yrs)	3.2	8.6	9.3	11.5	9.6
Albumin	53.3±0.64	54.2±0.13	43.7±0.14	44.6±4.80	41.0±0.45
α_1-globulin	10.9±0.56	11.4±0.06	10.9±0.14	10.6±0.14	12.5±0.72
α_2-globulin	8.9±0.64	9.6±0.32	10.3±0.14	15.7±0.05	18.0±0.32
β_1-globulin	8.2±0.12	8.1±0.80	9.9±1.70	11.1±0.04	9.6±0.10
β_2-giobulin	8.7±0.09	8.2±0.06	11.4±0.50	9.8±0.07	10.4±0.08
γ-globulin	10.3 ±0.70	8.6±0.09	13.7±2.70	7.5±0.04	8.4±0.80

Key: The data are expressed as average ±SEM

Young : Average pattern for clinically healthy young dogs

Old_{γ} : Average pattern for old dogs with pattern similar to young dogs

$Old_{\beta/\gamma}$: Average pattern for old dogs with high levels of β- and γ-globulins

Old_{α} : Average pattern for old dogs with high levels of α and β globulins

Old_{D} : Average pattern for old diseased dogs

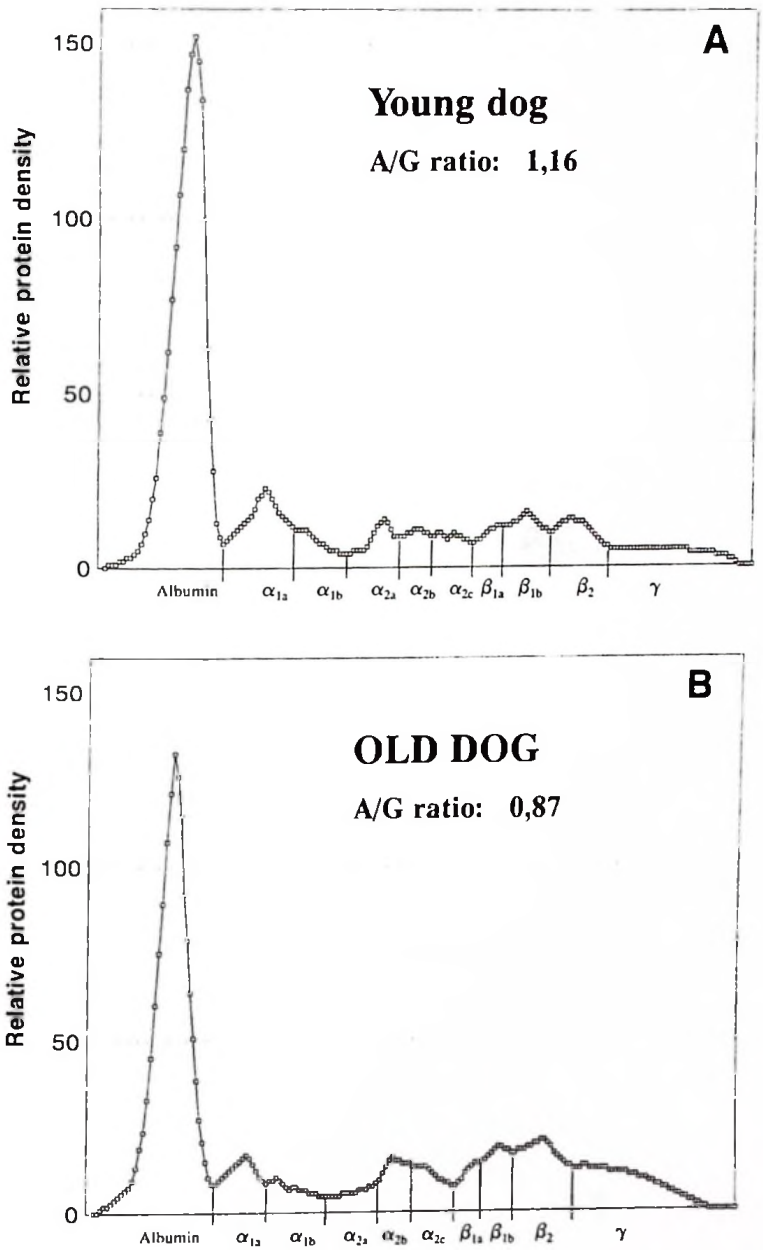

Figure 1a+b: Serum electrophoretograms from young **(a)** and geriatric dogs **(b)**, showing similar patterns. α, ß and γ represent alpha, beta and gamma globulins, respectively.

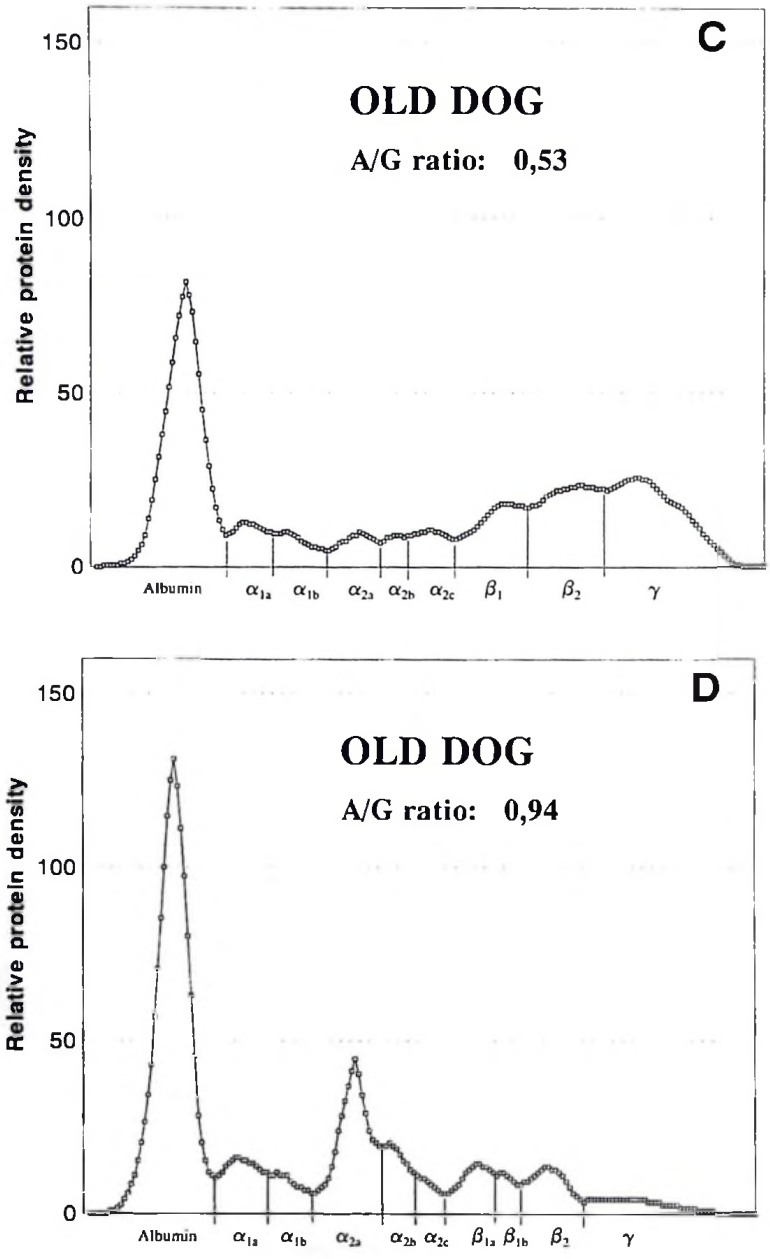

Figure 1c+d: Serum electrophoretograms from geriatric dogs. Note the high proportions of ß and γ globulins in some dogs **(c)** and increase in proportion of α_2 globulin fraction in others **(d)**. α, ß and γ represents alpha, beta and gamma globulins, respectively.

Veterinary Dermatology
in press

Subclinical glomerulopathy in selected cases of recurrent pyoderma in dogs.

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Abstract

Proteinuria especially albuminuria is a hallmark for glomerular disease. Glomerulopathy has been reported to be a sequel to chronic skin infections. Essentially, deposition of immune complexes in the glomerular capillaries is the main inciting cause for glomerular injury. Although immune complexes have been detected in blood from dogs with pyoderma, little is known about their fate and consequences. We therefore, screened dogs with recurrent pyoderma for proteinuria and went on to characterize the urinary proteins in order to establish whether the dogs in question had glomerulopathy at subclinical level.

Thirty nine dogs with recurrent pyoderma (21 with superficial and 18 with deep pyoderma) were used as a study group, and 25 clinically healthy dogs, 10 dogs with glomerulopathy and 151 diseased dogs were used as control groups. Urine was collected from all dogs used for this work. Urine was used for the detection of proteinuria and for agarose electrophoresis. Fifteen (38%) dogs with pyoderma had proteinuria, of these 11 had albuminuria. This was 5.3 times higher than that of diseased control group ($p < 0.05$). It was also found that compared to the diseased control group, dogs with recurrent pyoderma had been diseased for 27 months on average before developing albuminuria ($p < 0.05$). Therefore, it can be concluded that dogs with recurrent pyoderma appear to be predisposed to glomerulopathy. Thus, it is proposed that a thorough examination of patients with recurrent pyoderma includes a complete urinalysis.

Key words: Skin; Dog; Glomerulopathy; Recurrent pyoderma; Albuminuria; Proteinuria; Electrophoresis

INTRODUCTION

The skin produces a variety of microbial nutrients, including sebaceous gland secretions and thus, supports a microbial ecosystem on its surface (1, 2-3). If the epidermal integrity is disturbed, however, infections with *Staphylococcus intermedius* most likely will occur (4). A host will in this situation respond with an inflammation, and if the infection is significant, an immune response will be launched. Production of antigen-specific antibodies takes place and thus, inactivation and elimination of offending microorganisms promoted.

This process is vital for maintenance of health as long the infection has a temporary nature. On the other hand, if the epidermal integrity is persistently disturbed and the infection becomes more permanent, the immune response system may - apart from its protective role - lead to an allergic (type III) reaction, due to a constant presence of antigens (5). This situation does occur e.g. in patients with secondary seborrheic dermatitis or idiopathic seborrhea, in that surface integrity of the skin is persistently disturbed under such circumstances (6). Here a specific antibody response to constantly present antigens may lead to immune complex formations that are present locally and/or find their way to the blood stream. Indeed, DeBoer and others (7) found that circulating immune complex concentrations were significantly increased in dogs with recurrent pyoderma. Furthermore, they made the same observations in dogs with generalized demodicosis, provided the condition was complicated with pyoderma.

Based on such findings, it seems logical to expect that in some patients with recurrent pyoderma, the consequence of circulating immune complexes, e.g. the development of glomerulopathy, takes place. Therefore, this study was initiated to determine whether signs of subclinical glomerulopathy could be found in dogs with recurrent pyoderma.

MATERIALS AND METHODS

Animals

Twenty five clinically healthy dogs were used as control dogs. They all served on a danish airforce base and had no record of disease.

Ten dogs with histologically verified glomerulopathy, according to (8), but without dermatological lesions were chosen as a control group for evaluation of urine analyses.

In the selection of the study group, 190 dogs referred for various reasons to the Small Animal Hospital of the Royal Veterinary and Agricultural University, Copenhagen, were examined for signs of recurrent pyoderma. This was done through history taking and recognition of characteristic lesions. Dogs with other infectious skin diseases, e.g. parasitic and mycotic diseases, and dogs with lower urinary tract diseases were excluded from this study. None of the patients had been treated with steroids 3 weeks prior to hospitalization. In dogs with pyoderma, primary and secondary diagnoses (see table 1) were established according to Muller, et al.(9) and to Hill and Moriello (10).

Sample collection and urinalyses

From each dog 10 ml of urine was collected by cystocentesis. Immediately after collection, refractometry for urine specific gravity was performed and colorimetric urinalysis done, using dipsticks (Chemistrips 8 Boehringer Mannheim Diagnostics, D-68298 Mannheim). The colorimetric method for identification of patients with proteinuria was chosen because of its simplicity, wide application by veterinarians and proven comparability of performance with quantitative methods (11-12). Then urine was centrifuged at 300 g for 5 minutes and supernatants separated from the sediments. The sediment was prepared and examined, as described elsewhere (13). Urine specific gravity, pH and urine sediment examination were undertaken in order to identify proteinuria due to inflammatory reactions of the lower urinary tract (such dogs were excluded from the study, as previously mentioned). If the dipstick gave a 2+ or more reaction (only dogs with 2+ or more were included in the study), the remaining supernatant was poured into a Nanosep Urine Concentrator (Intercep Filtration Systems, Berkshire, UK) for its concentration; usually 200 times. The cut off molecular weight for the concentrators was 10000 daltons. A concentrated urine sample was then harvested and dispensed into labelled eppendorff plastic tubes and stored at -20°C until electrophoresis was done, usually within two weeks.

Also blood samples for serum were collected from all the animals. Separated serum samples were stored in eppendorff tubes at -20°C until electrophoresis was done.

Urine electrophoresis

Five microlitres of concentrated urine were applied to Beckman Paragon SPE Agarose gels (Beckman Instruments, Brea, California) and allowed to diffuse in for 5 minutes. Electrophoresis was carried out at 100 V for 25 minutes in barbital buffer, pH 8.6 and 0.05 ionic strength. Staining and destaining was carried out according to the manufacturer's instructions. After complete drying of the gels, densitometric quantitation of the fractionated proteins was done using a 600 nm Beckman Appraise™ densitometer (Beckman Instruments, Brea, California). Then the fractionated protein bands were identified by comparing to serum electrophoretic profiles obtained from the same animals and on the basis of established criteria albumin/globulin ratios were calculated (14).

Statistical methods

Data were subjected to statistical analysis by using the Statistical Analysis System (SAS) version 6.03 software package (SAS Institute, Cary, NC, USA). Differences between control and pyoderma groups with respect to duration of disease as well as albuminuria were analyzed via the χ^2 statistic. Differences were considered significant at $p < 0.05$ level.

RESULTS

As mentioned previously, dogs with pyoderma were included if:

1) Pyoderma was present at the time of hospitalization, and the referring veterinarian could confirm that the patient had recurrent pyoderma.

2) Based on urinalysis, no signs of lower urinary tract diseases were present.

3) The patients were free of other infectious skin diseases (mycotic and parasitic diseases).

Accordingly, 39 out of 190 patients were selected. As shown in Table 1, 21 dogs had superficial and 18 deep pyoderma. Six dogs with keratinization defects had idiopathic seborrhea, and finally, the group of patients with German Shepherd Deep

Pyoderma was selected according to the criteria, described in the literature (9), irrespective of findings being suggestive of underlying primary diseases, such as flea allergy (15) or genetic disorder (16).

Following the clinical examination, a urine sample was taken from each dog. As seen in Table 2, 52 dogs, gave a positive reaction for proteins in urine. Thus, all dogs with glomerulopathy and 10 out of 18 dogs with deep pyoderma (56%) had proteinuria. Previously, the urine sediment had been examined to assure that no signs of inflammation of the lower urinary tract were present (a total of 5 dogs have been excluded from this study due to cystitis and/or prostatitis). Based on agarose electrophoresis and calculation of albumin/globulin (A/G) ratio, patients showing albuminuria were identified (Fig. 1 and Table 2). As seen 11 out of 39 dogs with pyoderma had albuminuria. This was an incidence 5.3 times higher than that of the diseased control group ($p < 0.05$). As expected all dogs with glomerulopathy showed albuminuria. As a criteria for characterizing the proteinuria as albuminuria, an A/G ratio > 1.2 (17) was used. This ratio, however, was chosen somewhat arbitrarily, but seemed logical according to Fig. 1.

It has been reported by DeBoer and others (7) that dogs with recurrent pyoderma have an increased concentration of circulating immune complexes. If this observation was of relevance for the findings presented here, it should be expected that the duration of recurrent pyoderma would influence the incidence of albuminuria. Thus, it could be expected that dogs with recurrent pyoderma and albuminuria has a history of pyoderma over a longer period of time as compared to dogs with pyoderma, but without albuminuria. As seen in Fig. 2, indeed this was the case. Based on owners and referring veterinarians' information, dogs with recurrent pyoderma, but without albuminuria had been diseased 11 months in average, whereas dogs with recurrent pyoderma and albuminuria had been diseased for 27 months in average ($p < 0.05$).

Finally, it is noticeable that among the 15 patients with pyoderma and proteinuria, 4 dogs showed exclusively globulinuria. Although this number is low, it might prove to be an indication that all 4 had been diseased for at least 18 months and in average 41 months.

DISCUSSION

The detection of proteinuria in a well collected and analyzed urine sample in dogs always raises the question of renal disease (8, 18) and the finding of albuminuria

must be considered as a strong indication for the glomeruli being involved (19-21) as it was found in all 10 patients with confirmed glomerulopathy (table 2).

Accordingly, the data presented here imply that the kidneys are affected in 56% of the dogs with deep pyoderma. If this observation is related to the findings of De Boer et al. (7), they can best be explained by the recurrent pyoderma causing an increase in circulating immune complexes and with glomerulopathy as a consequence. This interpretation is further supported by the observation that the incidence of albuminuria is higher in dogs with deep pyoderma as compared to dogs with superficial pyoderma.

With regard to categorizing patients with proteinuria, the distinction between albuminuria and globulinuria was somewhat arbitrary. As seen in Fig. 1, however, the two types of proteinuria were clearly separable, based on the A/G ratios (17) but it seems reasonable to assume that the two types of proteinuria are aspects of the same pathophysiological process. In how far the globulinuria is a result of kidney lesions at a later stage (glomerular damage complicated with tubular damage?), remains to be shown. Only 4 patients with recurrent pyoderma and globulinuria were found, and they can hardly be considered to be more than an indication.

Duration of the skin problems prior to hospitalization was significantly different between dogs without and with albuminuria (11 and 27 months, respectively, $p < 0.05$), and proteinuria was only seen in patients, if the duration of recurrent pyoderma had exceeded 9 months. Again, this is a finding compatible with the concept that antigens need to be present over a period of time before circulating immune complex formations can be expected and causes glomerular damage.

At this stage, it should be emphasized that all dogs included in this study were referred patients, and thus, they hardly reflected the composition of dermatological cases as seen in a primary clinic. One of the consequences is very likely an above average duration of disease before clinical examination. This phenomenon might especially influence the data shown in Fig. 2. Also, it should be stressed that some of the primary diseases might have had a direct effect on the proteinuric condition (22). After all, kidney biopsies were not available and thus, attempts to identify involved antigens could not be made. On the other hand, no difference could be seen between groups with different primary skin diseases.

None of the dermatological patients showed clinical signs of kidney lesions. They were exclusively found as a consequence of the working hypothesis in this study, and yet, more than half of the dogs with deep pyoderma showed proteinuria. This probably indicates the presence of subclinical glomerulopathy, perhaps in the form of

minimal change glomerulopathy in recurrent cases of pyoderma (23). Therefore, it can be proposed that a thorough examination of patients with recurrent pyoderma includes a complete urinalysis. On the other hand, it will require further investigations to delineate, whether the finding of proteinuria, in particular albuminuria will require special therapeutic measures apart from application of suitable diets and an intensified effort to solve the underlying skin problem and treat accordingly.

ACKNOWLEDGEMENT

We would like to thank the Danish International Development Agency (DANIDA) for funding the study.

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Table 1: *Primary and secondary skin diseases in patients with recurrent pyoderma.*

Primary diseases	No.	Seborrhea	No.	No. of patients with pyoderma	
				Superficial	Deep
Endocrine disorder	8	Present	6	6	0
		Absent	2	0	2
Allergic dermatitis	21	Present	18	7	11
		Absent	3	3	0
Keratinization defects	6	Present	6	5	1
		Absent	0	0	0
German shepherd deep pyoderma	4	Present	3	0	3
		Absent	1	0	1
Total	39			21	18

Table 2: *Dogs with a positive dipstick reaction (equal to/more than 2+) for proteinuria.*

Dog category	No. of dogs tested	No. of proteinuric dogs		No. of albuminuric dogs*	
Healthy control	25	0	(0%)	0	(0%)
Diseased control	151	27	(18%)	8	(30%)
Glomerulopathy	10	10	(100%)	10	(100%)
Superficial pyoderma	21	5	(24%)	3	(60%)
Deep pyoderma	18	10	(56%)	8	(80%)
Total	225	52		29	

*) Albumin/Globulin ratio > 1.20 (17)

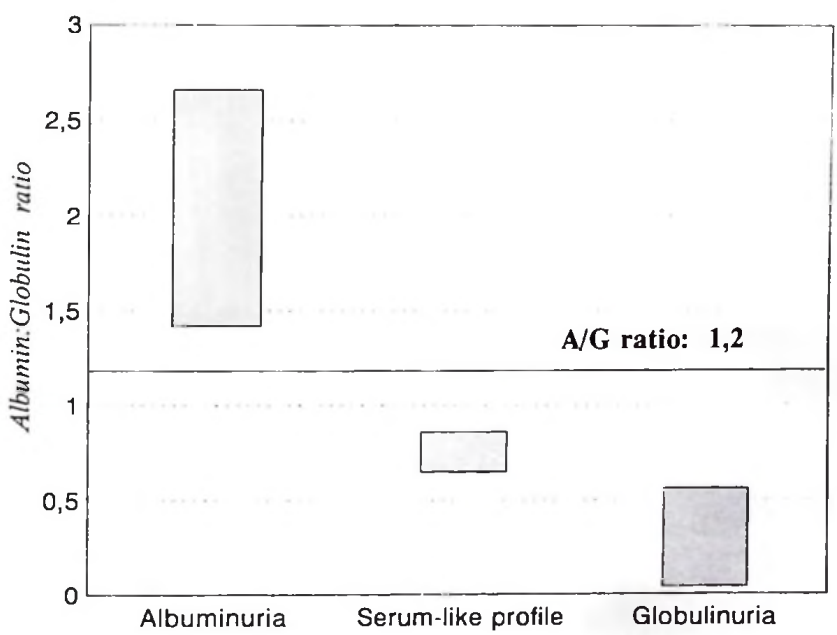

Figure 1: Albumin/Globulin (A/G) ratios for the albuminuria, serum-like proteinuria and globulinuria. The bars represent the maximum and minimum A/G ratios.

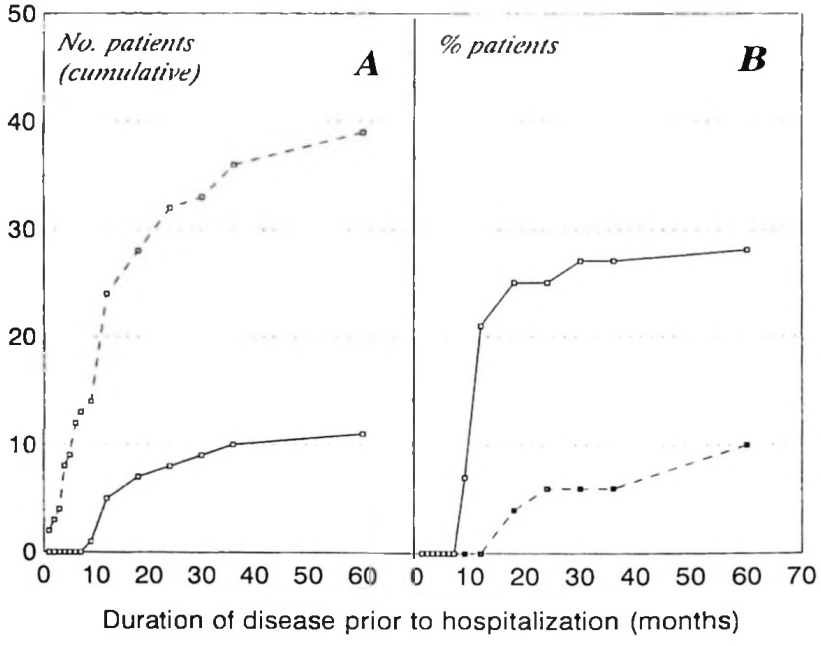

Figure 2: Relationship between duration (in months) of disease prior to hospitalization and development of proteinuria.
(A) Cumulative number of patients. Dotted line represents cases with recurrent pyoderma. Solid line represents animals with recurrent pyoderma and albuminuria. **(B)** Percentage of patients. Solid line represents the proportion of dogs with albuminuria among dogs with recurrent pyoderma. Dotted line represents the proportion of dogs with globulinuria and serum-like proteinuria among dogs with recurrent pyoderma.

Veterinary Record
submitted, 1996

COMPOSITION OF PROTEIN IN URINE FROM DOGS WITH PYODERMA

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Abstract

Urine from proteinuric dogs with and without pyoderma was evaluated for its composition with regard to protein fractions. Fifteen dogs with pyoderma (5 with superficial and 10 with deep pyoderma) and proteinuria were used as a study group. The control groups were formed by ten dogs with glomerulopathy and twenty seven proteinuric dogs with diseases other than pyoderma or urinary tract problems. Agarose gel electrophoresis was used for fractionation of proteins in urine. Three types of electrophoretograms were obtained. These were the albuminuria, globulinuria and the serum-like profile patterns. An albuminuria pattern was found in 30% of the proteinuric dogs in the diseased control group, 60% of the proteinuric dogs with superficial pyoderma, 80% of the proteinuric dogs with deep pyoderma and in 100% of the dogs with glomerulopathy. The albuminuria pattern (average A/G ratio 1.98 and 0.10 SEM), though predominantly with albumin, was also characterized by α_{1b}-, α_{2a}- and $ß_2$-globulin peaks in all 29 dogs showing this pattern. This was therefore thought to indicate that albuminuria (glomerular proteinuria) was a result of glomerular damage and inflammation (α_{1b}-, α_{2a}- and ß -globulin) as the latter were considered to be acute phase proteins. The serum-like profile pattern (average A/G ratio 0.72 and 0.01 SEM) was demonstrated in 13% of the proteinuric dogs examined and had all the protein fractions normally present when serum electrophoresis is undertaken. This pattern was considered to be a variant form of the albuminuria pattern, probably indicating advanced glomerular lesions and inflammation. The globulinuria pattern (average A/G ratio 0.33 and SEM 0.08) was significantly different from the other two in that it was characterized by a low albumin peak and the presence of globulin losses not clearly distinguishable from each other because of their confluency and absence of individual peaks. This could indicate severe glomerulotubular lesions and degradation of certain protein fractions. It could also be a result of increased secretion of tissue and other proteins by damaged tubules. It was concluded that glomerular damage leads to glomerular proteinuria characterized by albumin in high proportion as well as α_1b, α_2a and $ß_2$ globulins in lower but significantly diagnostic proportions.

Introduction

The qualitative detection of protein in urine always raises the question of possible renal disease (Osborne and Stevens 1981). Protein in urine can originate from prerenal, renal and postrenal causes (Grauer and others 1985). Prerenal proteinuria is caused by filtration in large quantities of low molecular weight proteins following their

increased plasma concentration (Schaeffer and Del Greco 1992). Prerenal proteinuria, however is rare in companion animals (Koeman and others 1994). Postrenal proteinuria is usually caused by inflammatory lesions of the lower urinary tract (Koeman and others 1994), and is often accompanied by characteristic urine sediment findings typical for inflammatory conditions such as epithelial red blood cells, white blood cells, bacteria and degenerated urotherial cells (Batamuzi and Kristensen 1995). Renal proteinuria is a clinical manifestation of glomerular as well as tubular dysfunction (Lulich and Osborne 1990). Tubular proteinuria normally results from tubular impairment leading to decreased reabsorption of low molecular weight proteins normally filtered in low quantities by the glomeruli (Schaeffer and Del Greco 1992). A typical example of tubular proteinuria is the one seen in Fanconi syndrome in man and certain breeds of dogs, such as the basenji. Glomerular proteinuria, the most common type of proteinuria, is usually caused by increased glomerular capillary permeability to proteins, especially albumin (Grauer and DiBartola 1995). Glomerular proteinuria may be due to any primary glomerular disease, however, glomerulopathy associated with systemic illness is by far the commonest cause in the dog (Lulich and Osborne 1990). Circulating antigen - antibody complexes deposited or trapped in the glomerular capillaries as a result of systemic disease is known to cause glomerular injury and consequently increased glomerular capillary permeability to protein (Grauer and DiBartola 1995). A number of diseases have been implicated as being involved in the pathogenesis of immune complex formation including dirofilariasis and other parasitic infections, diabetes mellitus, hypothyroidism, arthropathies, nephropathies, mycoses, systemic and discoid lupus erythematosus, cutaneous vasculitis and bacterial pyodermas (Biewenga 1986, DeBoer and others 1989). In the study by DeBoer and others (1989) pyoderma was reported to be associated with high concentration of circulating immune complexes. The circulating immune complexes, if deposited or trapped in the glomerulus in mild antigen excess, may lead to glomerular damage upon activation of complement and attraction of inflammatory cells (Grauer and DiBartola 1995). Identification and characterization of proteinuria in dogs with pyoderma may therefore provide valuable insight into the possible sequelae to this fairly common syndrome in companion animal practice. In view of the above observation, this study was designed to characterize the urinary proteins detected in dogs with pyoderma.

Materials and methods

Animals

Fifty two proteinuric dogs with and without pyoderma were randomly selected from a total of 225 dogs examined for various dermatologic and urinary tract diseases at the Small Animal Hospital, Royal Veterinary and Agricultural University, Copenhagen, Denmark. The study group was formed by 15 dogs with pyoderma, of which 5 had superficial pyoderma and 10 with deep pyoderma. The 15 dogs were actually drawn from 39 dogs with pyoderma. As for the control groups, two categories of dogs were used. In the first, 10 dogs histologically fullfilling the description of glomerulopathy (Grauer and DiBartola 1995) were selected. In the second category, 27 dogs suffering from diseases other than pyoderma and urinary tract diseases were used.

Sample collection and urinalysis

From each of the dogs in the study as well as the control groups, 10 ml of urine was collected by cystocentesis. Immediately after collection, refractometry for urine specific gravity and colorimetric urinalysis was done using dipsticks (Chemistrips 8 Boehringer Mannheim diagnostics, D-68298, Mannheim). Then urine was centrifuged at 300 g for 5 minutes and supernatant separated from the sediment. The urine sediment was prepared and examined as described elsewhere (Batamuzi and Kristensen 1995). Urine specific gravity, pH and urine sediment examination were undertaken in order to rule-out potential causes for false positives/negatives and proteinuria due to inflammatory reactions of the lower urinary tract. The remaining supernatant was poured into a Nanosep urine concentrator (Intercep Filtration Systems, Berkshire, UK) for its concentration, usually 200 times. These concentrators had a reported cut off molecular weight of 10,000 Daltons. A concentrated urine sample was then harvested and dispensed into labelled eppendorff plastic tubes and stored at - 20°C until electrophoresis was done, usually within two weeks. Also blood samples for serum were collected from all the animals in the study and control groups. Separated serum samples were stored in eppendorff tubes at - 20°C until electrophoresis was done.

Urine electrophoresis

Five microlitres of concentrated urine were applied to Beckman Paragon SPE Agarose gels (Beckmann Instruments, Brea, California) and allowed to diffuse into the gel for 5 minutes. Electrophoresis was carried out at 100 V for 25 minutes in barbital buffer, pH 8.6 and 0.05 ionic strength. Staining and destaining was carried out

according to the manufacturer`s instructions. After complete drying of the gels, densitometric quantitation of the fractionated proteins was done using a 600 nm Beckman Appraise™ densitometer (Beckmann Instruments, Brea, California). Then the fractionated protein bands were identified by comparing to serum electrophoretic profiles obtained from the same animals and on the basis of previously established criteria (Kristensen and Barsanti 1977).

Statistics

Results are expressed as mean changes ± SEM. Chi-square test and where necessary Fisher's exact test were used to determine significance of diferrences between the test results and controls. A p-value of 0.05 or less was considered significant.

Results

In comparison to other diseases, pyoderma cases were found to be frequently proteinuric ($p < 0.05$) (Table 1). Three electrophoretic patterns, namely albuminuria, globulinuria and serum-like proteinuria profile types were found, and they were distributed to the different study groups as shown in Table 2. Of 27 proteinuric dogs in the diseased control group, 8 (30%), 6 (22.2%) and 13 (48.1%) had albuminuria, serum-like proteinuria and globulinuria respectively. Among the 5 proteinuric dogs with superficial pyoderma, 3 (60%) had albuminuria, 2(40%) had globulinuria and none showed a serum-like proteinuria profile. Out of 10 dogs with proteinuria in the deep pyoderma group, 8 (80%) displayed an albuminuria profile while serum-like proteinuria and globulinuria profiles were evident in 1 (10%) dog each. All the dogs in the glomerulopathy group gave an albuminuria profile.

The albuminuria pattern had an average Albumin to Globulin (A/G) ratio of 1.98 with a standard error of a mean (SEM) of 0.10 (Table 3). In this pattern (Figures 1a & b) four clearly identifiable protein peaks were consistent with albumin being prominent (albumin) and three others in the globulin area. Upon calculation of Relative mobility (Rf) values, the three peaks were identified as being α_{1b}-, α_{2a}- and $ß_2$-globulins (Table 4). The albuminuria pattern was found in 29 (56%) of the 52 proteinuric dogs studied.

The urine electrophoretic pattern with a serum-like proteinuria profile (Fig. 1c) was detected in 7 (13%) of the 52 proteinuric dogs. This pattern had an A/G ratio

of 0.72 and 0.01 SEM. The serum-like profile pattern commonly had eight peaks namely albumin, α_{1a}, α_{1b}, α_{2a}, α_{2b}, β_1, β_2 and γ.

The globulinuria pattern had an average A/G ratio of 0.33 and a 0.08 SEM. As can be seen in Fig. 1d, the globulinuria pattern had only two clearly identifiable protein peaks (albumin and α_{1a}), the other protein peaks were almost confluent forming one mound (Table 5). The globulinuria pattern was detected in 16 (31%) of the 52 dogs evaluated. Compared to serum electrophoretograms from the same animals, the globulinuria pattern had proportionately low levels of albumin and higher levels of globulins, hence the low A/G ratio. Although our cut off proteinuria value was 2+ on dipsticks, lower levels of proteinuria (Trace and +) were also evaluated in order to gain an insight into their electrophoretic separation characteristics. The low level proteinuria gave an electrophoretic profile as shown in fig. 1e and was distinct from the preceding patterns.

Discussion

Pyoderma cases were found to be predisposed to proteinuria. This finding is in agreement with the findings of other workers with regard to chronic skin diseases (Biewenga and others 1982). Proteinuria in this case may be a consequence of immune complex deposition in the glomerular capillaries.

Results of this study indicate that whereas dogs with clinical features of glomerular disease have one urine electrophoretic pattern, pyoderma and other diseases tend to give three patterns (Table 2). The reason for these differences could be that different mechanisms are involved. Pathologic renal proteinuria is reported to result from increased transport across glomeruli, leakage of protein from damaged tubular cells, impaired tubular reabsorption or parenchymal inflammation (Chew and DiBartola 1989). Thus there is a large number of pathomechanisms that ultimately lead to proteinuria in different disease situations. Studies on these pathomechanisms in relation to the type(s) of proteinuria caused will probably have important clinical application, and may in the process provide the reason for the differences in urine electrophoretic patterns.

Glomerular proteinuria exhibits a characteristic pattern on electrophoresis, and it is called albuminuria because the greater part is made up of albumin (Kawai 1973, Lulich and Osborne 1990, Beetham and Cattell 1993, Grauer and DiBartola

1995). Thus proteinuria, especially albuminuria is a hallmark for glomerular disease (Lulich and Osborne 1990, Brown 1995). Compared to serum, urine electrophoresis in man has been reported to show increases in albumin and α_1-globulin, and decrease in levels of α_2- and γ-globulin fractions for patients with glomerular proteinurias (Kawai 1973). Working on samples also obtained from man, Ehrich and others (1984) detected increased levels in albumin and β_2-microglobulin in subjects with physiological proteinuria, a form of glomerular proteinuria. In the present work, the albuminuria pattern was characterized by high levels of albumin, and significant levels of α_{1b}-, α_{2a}- and β_2-globulin fractions. These findings for the albuminuria pattern, which is in fact a glomerular proteinuria, are therefore in agreement with the findings in man. In a study by Biewenga and others (1982), glomerular proteinuria was found to be associated with significant losses in urine of albumin as well as high and low molecular weight proteins. Possibly high and low molecular weight proteins in that study were similar to the globulins detected in the present work. In a recent study (Codner and others 1992) in dogs with experimentally induced ehrlichiosis, urine electrophoresis done during peak proteinuria, revealed that albumin was the main protein lost in urine (A/G ratio, around 1.2). Other proteins (globulins) were however, also detected in urine from the dogs in question. The dogs also had hypoalbuminemia (Codner and others 1992) and this further supported the presence of glomerular lesions and that albumin and α_1-globulin are important components of glomerular proteinuria (Codner and Maslin 1992). Also, Brown (1995) pointed out, that glomerular proteinuria is characterized by albumin, transferrin, immunoglobulin G (IgG), apolipoproteins and antithrombin III. Thus albumin is a major component but not the sole component that is important diagnostically for glomerular proteinuria.

It is a well established fact that albuminuria as detected in glomerular proteinuria is a result of glomerular damage (Grauer and DiBartola 1995). However, the pathomechanisms and/or sources for the three globulins (α_{1b}, α_{2a} and β_2) that has been consistently demonstrated in the dogs with recurrent pyoderma and in histologically verified cases of glomerulopathy, in the present study are not documented. There are a number of possibilities which could help in explaining this phenomenon. One possibility is that these, like albumin may be filtered into urine as a result of glomerular damage. Another likely mechanism may be associated with the development of an inflammatory reaction in the glomerular micro-environment. With this, local accumulation of inflammatory cells and subsequently acute phase proteins will follow. This being the case, a kind of glomerular overload proteinuria involving acute phase proteins will develop. The globulins detected in this study showed the same migratory

patterns as acute phase reactants, which are known to increase under inflammatory conditions (Kawai 1973, Eckersal and Conner 1988). Thus the distinct pattern that was seen in all the 29 dogs with albuminuria and absence of the other serum protein appear to support this hypothesis. It has been found that dogs with reccurent pyoderma require a minimum of 27 months to develop albuminuria, and an even higher period was needed before globulinuria was detected (Batamuzi and others [to be published elsewhere]). The serum-like profile detected in about 13 % of the proteinuric dogs could represent those cases with advanced, severe glomerular pathologic changes (Lulich and Osborne 1990). In this case the macromolecules like α_2-lipoprotein, IgM-immunoglobulins, α_2-macroglobulin and fibrinogen are able to leak through the filtration barrier of the glomeruli. It could however, be a sequel to inflammatory insults in the glomeruli themselves, adjacent tubules or in the contiguous interstitium. Haemorrhage within the glomeruli say as a result of advanced glomerular lesions may be responsible for the serum-like profile (Stone and Barsanti 1992). Glomerular bleeding is reported on frequent basis in glomerular diseases in dogs and cats (Bishop and others 1991, Stone and Barsanti 1992) and microscopic hematuria is detected upon examination of the urinary sediment in man and in animals (Stamey and Kindrachuk 1985, Batamuzi and others 1994). However, in this study those features were not demonstrated in that hematuria was not detected.

As for those cases (31%) with a globulinuria pattern, the presence of relatively low levels of albumin indicates minimal albumin loss in urine of the dogs in question. This pattern was also characterized by lack of distinct protein peaks probably suggesting that families of proteins rather than intact serum proteins are involved (Fig 1d). These families of proteins are probably formed by degradation of serum proteins and/or by secretion of additional proteins by tubules, primed by the pathologic processes in the glomeruli. Degradation of some serum proteins, eg fibrinogen, has been detected in urine of dogs under experimental settings (Codner and others 1992). There is a possibility for the same occurring in real life situations with most if not all of the serum proteins.

Proteinuria at low levels (Trace and +) appear to give characteristic electrophoretic patterns distinct from albuminuria (Kawai 1973) (Fig. 1e), which clearly indicate that the latter is not a physiological process that is being enforced under pathophysiological conditions. In view of this observation, it is warranted to suggest that the source of the degraded proteins, hypothesized to be responsible for the globulinuria pattern observed in this study, to be serum. Another possible mechanism behind the globulinuria pattern could be severe glomerular leakage coupled with

defective tubular reabsorption of low molecular weight proteins (> 10,000 daltons). But if this was true it would be expected that distinct peaks would be formed or at least a tubular proteinuria pattern would be seen (Lulich and Osborne 1990). It has been reported that tubular damage leads to prominent α and ß globulin fractions while albumin remains completely small (Kawai 1973). In man, tubular proteinuria is characterized by $ß_2$-microglobulin, lysozyme, α-acid glycoprotein in addition to amino acids (Osborne and others 1995). Tubular proteinuria is a result of defective reabsorption as in Fanconi syndrome, but may be due to tubular overload as in cases of myoglobulinuria (muscle necrosis), hemoglobinuria (intravascular hemolysis or paraproteinemias). It is however possible that globulinuria represents severer glomerular lesions complicated by other pathomechanisms.

In conclusion, this study indicates that recurrent pyoderma and other diseases lead to 3 proteinuria patterns whilst glomerulopathy gives only one pattern. Glomerular proteinuria is characterized by selective losses of albumin and other globulins, α_{1b}-globulin, $\alpha 2_a$-globulin and $ß_2$-globulin. A firm confirmation of the nature and identity of the three globulins that were consistently found accompanying albumin would require more studies.

Acknowledgements

The present study was supported by the Danish International Development Agency (Danida). We thank Mrs Emma Thomsen and Mrs Vera S. Østvedt for excellent technical assistance.

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Table 1: *Comparison of dogs with pyoderma to those with other diseases for their predisposition to proteinuria*

	Pyoderma	Other diseases	Total
Proteinuria	15 (7%)	37 (16%)	52 (23%)
No proteinuria	24 (11%)	149 (66%)	173 (77%)
Total	39 (17%)	186 (83%)	225 (100%)

$p < 0.05$, RR= 2.079 (Fisher's Exact test)

Table 2: *Urine electrophoresis: types of proteinuria for the study and control groups*

Dog category	Albuminuria	Serum-like proteinuria	Globulinuria
Diseased control	8 (30%)*	6	13
Superficial pyoderma	3 (60%)*	0	2
Deep pyoderma	8 (80%)*	1	1
Glomerulopathy	10 (100%)*	0	0

*) *Percentage of dogs with albuminuria (glomerular proteinuria) among all the proteinuric dogs in the respective category.*

Table 3: *Albumin:Globulin (A/G) Ratios of urine and serum from dogs with proteinuria.*

Types of proteinuria	No. of dogs	A/G Ratios (± SEM)	
		Urine	Serum
Albuminuria	29	1.98 ± 0.10*	1.03 ± 0.03**
Serum-like proteinuria	7	0.72 ± 0.01*	1.10 ± 0.04**
Globulinuria	16	0.33 ± 0.08*	1.05 ± 0.04**

* *Urine A/G ratios of the different types of proteinuria are significantly different (χ^2 test; $p < 0.05$) between the 3 types of proteinuria.*

** *Serum A/G ratios are not different (χ^2 test; $p > 0.05$) between the 3 types of proteinuria.*

Table 4: *Major protein fractions in urine from dogs with albuminuria (glomerular proteinuria).*

Protein Fraction	% Total protein (Average ± SEM)
Albumin	67.4 ± 0.9
α_{1b}-globulin	8.6 ± 0.4
α_{2a}-globulin	8.7 ± 0.4
β_2-globulin	7.8 ± 0.3

* *In average 7.5 % of total protein belonged to globulin fractions other than those listed.*

Table 5: *Protein fractions for the different urine electrophoretic patterns.*

Pattern	Protein fraction							
	Alb	α_{1a}	α_{1b}	α_{2a}	α_{2b}	β_1	β_2	γ
Albuminuria	P	.	P	P	.	.	P	.
Serum-like proteinuria	P	P	P	P	P	P	P	P
Globulinuria	P	P	1	1	1	1	1	1
Trace proteinuria	.	P	.	P	.	P	P	P

Key :
Alb : *Albumin*
. : *Protein fraction absent*
l : *Protein fraction present, but lacks distinct peak*
P : *Protein fraction present*

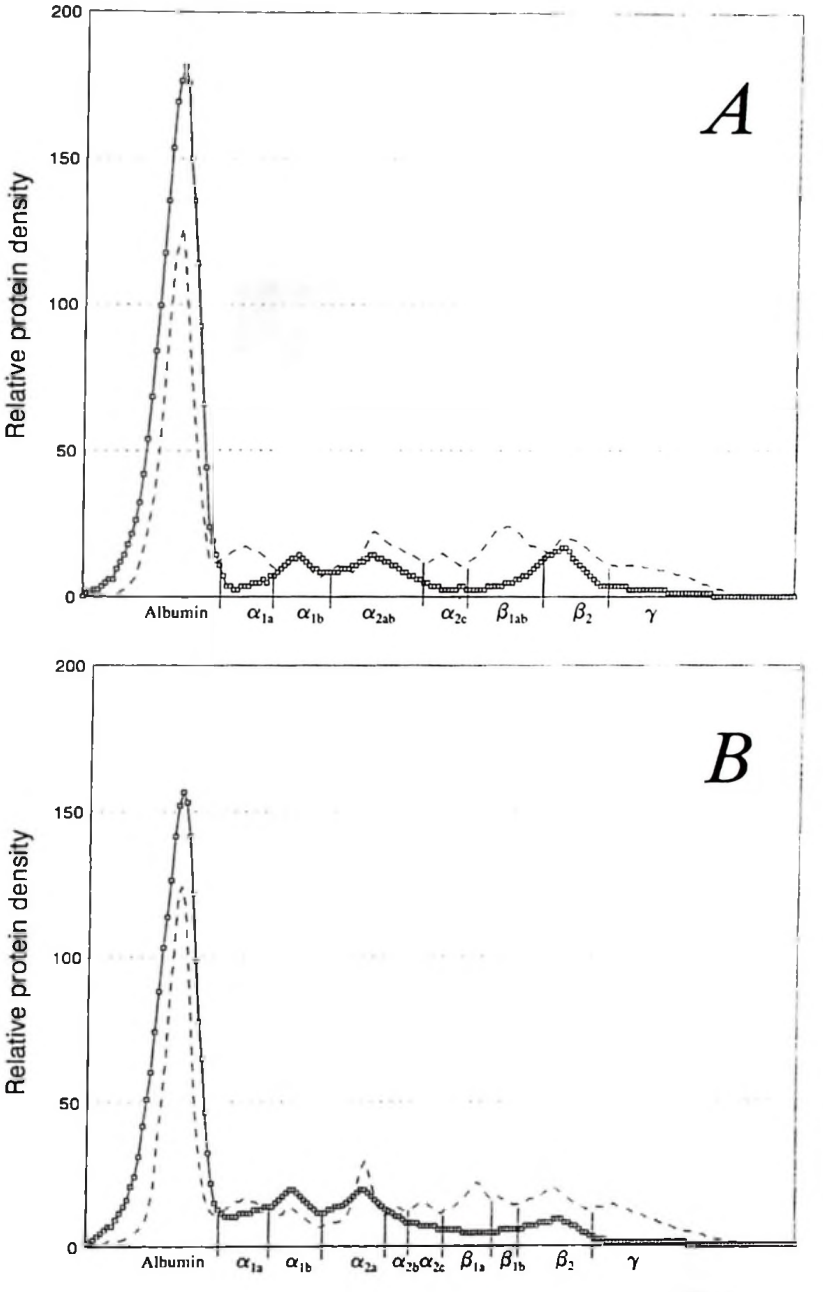

Figure 1: **(A)** Urine protein electrophoresis (solid line) from a dog with glomerulopathy showing an albuminuria pattern (A/G ratio 1.98 $\pm$ 0.10). α, ß and γ represents alpha, beta and gamma globulins. The serum electrophoretogram from the same dog is shown (dotted line). **(B)** Urine (solid line) and serum (dotted line) protein electrophoretogram from a dog with recurrent pyoderma showing albuminuria (A/G ratio 1.98 $\pm$ 0.10).

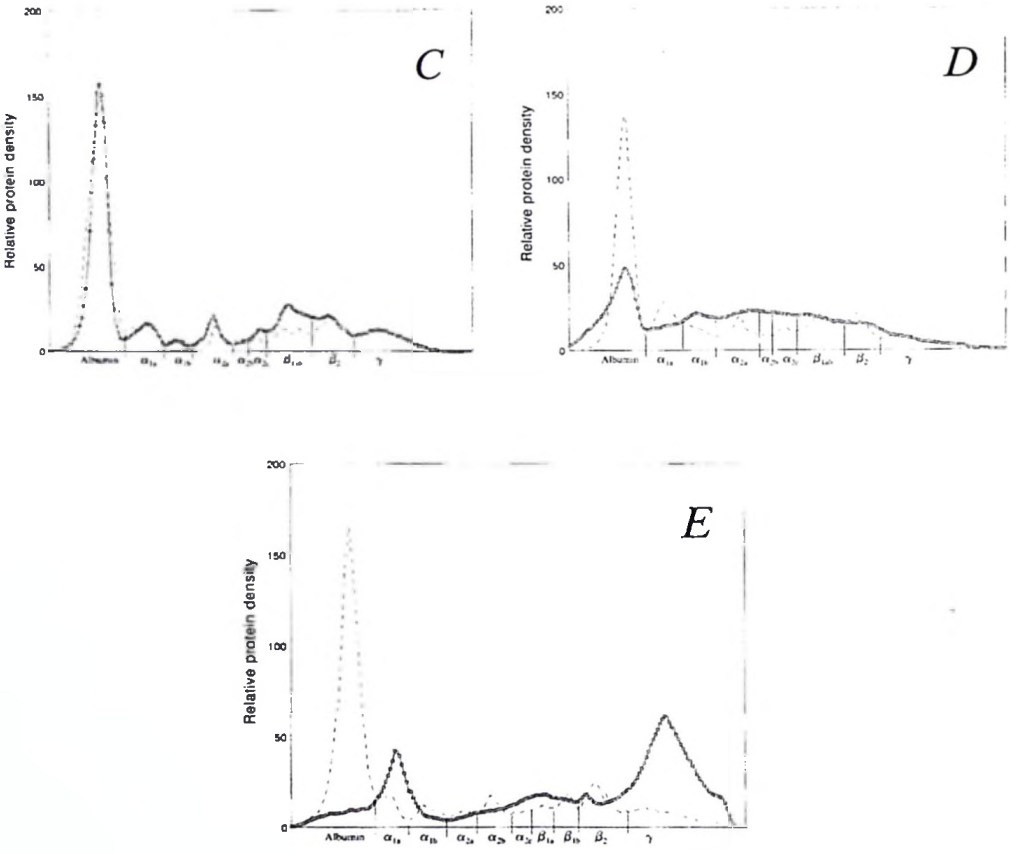

Figure 1 (continued): **(C)** Urine protein electrophoretogram (solid line) showing a serum-like proteinuria profile (A/G ratio 0.72 $\pm$ 0.01). Note resemblance to that of serum (dotted line) from the same animal. **(D)** Urine electrophoretogram (solid line) showing a globulinuria pattern (A/G ratio 0.33 $\pm$ 0.08). Note lack of identifiable peaks for the urine electrophoretogram as compared to that of serum (dotted line). **(E)** Urine electrophoretogram from a dog with trace proteinuria (solid line). The dotted line represents serum electrophoretogram from the same dog. Note that this profile is quite different from the albuminuria pattern (A and B). α, ß and γ represents alpha, beta and gamma globulins.

SUMMARY

Diseases of the urinary system in companion animals tend to develop insidiously, showing atypical clinical features but with time they may become progressive and life threatening as is the case with chronic renal failure. Recognition of these diseases at a reasonably early stage in their development is a key to their successful management. A number of predisposing factors for the diseases of the urinary tract have been documented. Knowledge of these and their effects is another important approach toward tangible management of diseases of the urinary system. Considerable work has been done in the determination of predisposing factors and in the diagnosis of the diseases of the urinary system. However a lot remains to be done in determination of those diagnostic approaches that are simple to carry out and most important are cost effective. Furthermore, basic studies regarding the diseases of the urinary tract for companion animals have been done only on those diseases and predisposing factors of importance to temperate breeds of dogs and no information exists about the same in the local breeds of dogs in the tropics. The purpose of the present study was to investigate the diagnostic potential of various components of the urinary sediment and in particular the urothelial cells, as well as the value of urinary protein fractionation in the overall diagnostic workup for the diseases of the urinary system (part I of the study). As a parallel to part I of the study, the role of transmissible venereal tumour in the development of urinary tract infection was investigated (part II of the study).

In part Ia of the study, 12 clinically healthy companion animals (10 dogs and 2 cats) brought to the clinic for euthanasia were used for studying the characteristics of urothelial cells. The entire urinary tract was extirpated, renal vessels flushed with saline and then the urinary tract was opened longitudinally. Epithelial scrapings were then taken from the renal pelvis/calyces, the proximal, mid and distal ureter, urinary bladder at the roof, floor and trigone areas as well as from the urethra. Scraped material was

then freed from the scalpel blade into saline, from which sediment smears were prepared, stained and evaluated under the microscope. The effects of urine on the integrity of the cells was also evaluated when cells were allowed to stand in urine for a prolonged period of time. Also 120 dogs with different diseases were used. From these urine was collected by cystocentesis, centrifuged, sediment smears prepared and examined as before. The urothelium was found to consist of transitional epithelial cells ranging from the calyces to the urethra. Caudate cells were found lining the ureter, renal pelvis and calyces. There was no feature that could be used to distinguish the transitional cells from different parts of the urothelium. Squamous epithelial cells were found lining the urinary tract from the trigone to the vagina in females and to the urethra in male animals. Hydropic degeneration in the form of vacuolation of the cytoplasm, granulation and total loss of the cytoplasm was one of the urine-induced degenerative changes recorded in the transitional cells. The same degenerative changes were seen in clinical cases with inflammatory or irritational conditions. A case of idiopathic renal hematuria was encountered during examination of the clinical cases mentioned above. For this case, urine collection methods and diagnostic methods beyond the scope of this study, namely laparotomy facilitated pyelocentesis, ultrasonography and special histopathogical section staining techniques had to be employed in pursuit of the source and cause for hematuria. Urinary sediment, Ultrasonography and histopathological findings helped to establish the source of hematuria and that unlike the ones reported before, it was bilateral and did not resolve after unilateral nephrectomy.

In part Ib of the study, 39 dogs with recurrent pyoderma (21 with superficial and 18 with deep pyoderma) were evaluated for signs of proteinuria. Twenty five clinically healthy dogs, 10 dogs with glomerulopathy and 151 diseased dogs were used as control. From each of these 10 ml of urine were collected then tested for proteinuria using colorimetric methods. Urine was then centrifuged, supernatant harvested and concentrated 200 times and then electrophoresed. In electrophoresis, 5 microlitres of concentrated urine were applied to Beckman Paragon SPE Agarose gels and allowed to diffuse in for 5 minutes. Electrophoresis was carried out at 100 V for 25 minutes in barbital buffer, pH 8.6 and 0.05 ionic strength. Afterwards fractionated proteins were quantified using a 600 nm Beckman Appraise densitometer. Fifteen dogs with pyoderma (38%) had proteinuria, of these 11 had albuminuria. This was 5.3 times higher than that of diseased control group ($p < 0.05$). It was also found that compared to the diseased control group, dogs with recurrent pyoderma had been diseased for 27 months on average before developing albuminuria ($p < 0.05$).

In part Ic of the study, urine from proteinuric dogs with and without pyoderma was evaluated for its composition with regard to protein fractions (a total of 52 dogs were used). Upon fractionation of protein in urine using agarose gel electrophoresis, three types of electrophoretograms were obtained namely, albuminuria, serum-like proteinuria and globulinuria patterns. An albuminuria pattern was found in 30% of the proteinuric dogs in the diseased control group, 60% of the proteinuric dogs with superficial pyoderma, 80% of the proteinuric dogs with deep pyoderma and in all dogs with glomerulonephritis. The albuminuria pattern (Average A/G ratio ± SEM [1.98 ± 0.10]) though predominantly with albumin, was also characterized by α_{1b}, α_{2a} and β_2 globulin peaks in 29 dogs showing this pattern. This was therefore thought to indicate that glomerular proteinuria was a result of glomerular damage (albuminuria) and inflammation (α_{1b}, α_{2a} and β_2 globulins) as the latter are considered to be acute phase proteins. The serum-like proteinuria pattern (average A/G ratio ± SEM [0.72 ± 0.01]) was demonstrated in 13% of the proteinuric dogs examined and had all the protein fractions normally present when serum electrophoresis is undertaken. This pattern was considered to be a variant form of the albuminuria pattern, probably indicating advanced glomerular lesions and inflammation. The globulinuria pattern (average A/G ratio ± SEM [0.33 ± 0.08]) was significantly different from the other two, in that it was characterized by a low albumin peak and the presence of globulin losses not clearly distinguishable from each other as they had confluent peaks. This could be more a reflection of degradation of certain protein fractions coupled with increased tubular secretion of proteins than severity of glomerular lesions or both due to unidentified mechanisms. Correct identification of protein fractions in urine of dogs with diseases under investigation and proper interpretation of the patterns named above often require knowledge of the Rf values. However, definitive identification of proteins excreted in urine in health and disease, and the magnitude of the problem as well as the consequences, requires comparison of urine electrophoresis to serum electrophoresis results. In view of the aforementioned prerequisites and for comparative purposes serum protein electrophoresis was undertaken. Samples were obtained from 37 clinically healthy dogs as study material. For control, serum samples were taken from 25 geriatric dogs with different diseases and 18 clinically healthy dogs. Following electrophoresis and densitometric scanning of the proteins in serum, Rf value were calculated and used for the identification of protein fractions in corresponding urine samples.

In part II of the study 86 dogs (55 with transmissible venereal tumour and 31 controls) were used to investigate the role of transmissible venereal tumour in the

pathogenesis of urinary tract infection for dogs in Tanzania. For each dog used, a thorough clinical examination of the external genitalia was done. For dogs with transmissible venereal tumour, the sites of attachment were recorded. Urine samples were taken by cystocentesis and the external genitalia swabbed. samples collected were cultured for bacteria using standard methods. The tumours were found located on the prepuce and different parts of the penis in male dogs and in bitches they were found in the vagina, vestibule or the vestibulo-vaginal junction. Dogs with transmissible venereal tumour were found to be at a high risk of having bacteriuria, odds ratio (OR) = 7.04. Also obliteration of the urethral orifice by the tumour, possibly leading to urine retention was thought to be the main reason for the high incidence of urinary tract infection among dogs with transmissible venereal tumour. Long standing cases of transmissible venereal tumour had a higher chance of becoming bacteriuric compared to recent cases (OR = 29.60).

CONCLUSIONS

The results from the current investigations leads to the following conclusions:

* The simple microscopic study of the centrifuged urinary sediment is a reliable, cost effective diagnostic method for diseases of the urinary tract. Degenerative changes of the urothelial cells caused by urine may be of diagnostic importance.

* Urinary sediment examination can be used in directing advanced diagnostic approaches in obtaining definitive diagnoses and prognosis. In the process the diagnostic potential of microscopic urinary sediment is strengthened.

* Screening of dogs for proteinuria is useful in detection of early cases of renal disease as caused by diseases likely to predispose dogs to renal failure.

* Recurrent pyoderma may predispose dogs to glomerulopathy. It is therefore proposed that a thorough examination of patients with recurrent pyoderma includes a complete urinalysis.

* Recurrent pyoderma and other systemic diseases lead to three different proteinuria patterns while glomerulonephritis gives only one pattern on urine electrophoresis.

* Glomerular proteinuria is characterized by selective losses of albumin and other globulins, α_{1b}-globulin, α_{2a}-globulin and β_2-globulin.

* Transmissible venereal tumour may be a predisposing factor for the development of urinary tract infection.

PERSPECTIVES

Further research into the characteristics of cellular elements (especially urothelial cells and different types of blood cells), considering the effects imposed on the cells in question by urine, in health and disease, the effect of predisposing factors coupled with the use of advanced urinalysis techniques such as urine protein electrophoresis should eventually lead to a deeper understanding of nephrologic and urologic conditions, especially, present day idiopathic conditions of companion animals. It will also lead to the development of improved diagnostic methods in this important field of companion animal medicine.

SAMMENFATNING

Sygdomme i urinvejene hos mindre husdyr har en tendens til at udvikle sig snigende og kan under omstændigheder frembyde atypiske kliniske symptomer, men som problemerne udvikler sig, vil de i stigende grad blive progressive, for til slut at optræde som en livstruende tilstand, for eksempel i form af kronisk nyresvigt. Identifikationen af disse lidelser på et tidligt tidspunkt er derfor en forudsætning for terapeutisk succes. I dag kendes adskillige prædisponerende faktorer for urinvejslidelser, og kendskab til disse og deres effekt på urinvejene er af stor betydning for den terapeutisk håndtering af sådanne lidelser.

Vurderingen af de prædisponerende faktorer og diagnosticeringen af urinvejenes sygdomme har været underkastet intense studier i de senere år, men mange spørgsmål kan fortsat rejses. Simple og omkostningsgunstige diagnostiske protokoller kan udvikles, og mange grundliggende studier har kun været gennemført hos almindelige racer med henblik på de enkelte lidelser og prædisponerende faktorer, medens meget begrænset viden foreligger, hvad angår lokale hunderacer i f.eks. de tropiske lande.

Formålet med det foreliggende studium har været at undersøge det diagnostiske potentiale af urinsedimentets forskellige komponenter, specielt epithelcellerne, samt værdien af uroproteinerne som et diagnostisk element i patientvurderingen, hvor lidelser i urinvejene måtte foreligge (første del af studiet). Parallelt hermed blev den potentielle rolle for transmissible venereal tumour i udviklingen af urinvejsinfektioner undersøgt (anden del af studiet).

I studiets første del (a) blev 12 klinisk raske mindre husdyr (10 hunde og 2 katte), som aflivedes på Hospitalet for Mindre Husdyr, inkluderet med hensyn på at vurdere epithelcellernes morfologiske karakteristika. Urinvejene blev udtaget og ved hjælp af arteria renalis blev nyrernes blodkar gennemskyllet med fysiologisk saltvand for at fjerne intravaskulært placerede blodlegemer. Efterfølgende blev urinvejene spaltet

longitudinelt. Epithelskrab blev udtaget fra de renale pelvis/calyces, den proximale, midterste og distale part af ureter, vesicas loft og bund samt trigonum, såvel som fra urethra og ydre genitalier. Det herved indhentede materiale blev opslemmet i fysiologisk saltvand og cytologiske præparater fremstillet, farvet og mikroskoperet. Effekten af urin på cellernes integritet blev ligeledes vurderet, idet celler blev opslemmet i urin og cytologisk vurderet efter forskellige inkubationsperioder. Herudover blev 120 hunde med forskellige lidelser inddraget i studiet. Fra disse blev der opsamlet urin ved hjælpe af cystocentesis for cytologiske undersøgelser.

De mest almindelige celler var overgangsepithelceller. De fandtes i områderne strækkende sig fra calyces i nyrerne og til urethra. De såkaldte caudate cells fandtes primært i ureter, nyrebækken og calyces. Der var tilsyneladende ingen morfologiske karakteristika, som kunne anvendes til at nærmere placere overgangsepithelceller i de forskellige områder af urotheliet.Pladeepithelceller fandtes i trigonum, urethra og hos tæver vagina.Hydropisk degeneration i form af cytoplasmatiske vacuoler, granulering og komplet tab af cytoplasma var nogle af de registrerede urin-inducerede degenerative forandringer, som blev fundet hos overgangsepithelcellerne. De samme degenerative forandringer fandtes i urinsedimentpræparater fra patienter med irritative eller inflammatoriske forandringer i de nedre urinveje.

I forbindelse med de ovenstående studier, blev et tilfælde af idiopatisk renal hæmaturi identificeret. I dette tilfælde blev urinopsamlings- og diagnostiske metoder anvendt, som lå uden for selve studiet, nemlig laparotomi, pyelocentesis, ultralydsscanninger og specialfarvede snit for histopatologi. På det grundlag blev etableret, at i modsætning til tidligere rapport var dette et tilfælde af bilateral hæmaturi, som naturligt nok ikke kunne respondere på unilateral nefrektomi.

I studiets første del (b), undersøgtes 39 hunde med recidiverende pyodermi (21 med superficiel og 18 med profund pyodermi) med henblik på tilstedeværelsen af proteinuri. Femogtyve klinisk raske hunde, 10 hunde med histopatologisk bekræftet glomerulonefritis and 151 hunde med andre lidelser blev anvendt som kontrol. Fra hver hund blev 10 ml urin udtaget og kolorimetrisk testet for proteinuri. Efterfølgende blev urinen centrifugeret, supernatanten isoleret, koncentreret 100-200 gange og analyseret ved hjælp af agarose elektroforese. Til gennemførelse af elektroforese undersøgelsen blev 5 mikroliter af koncentreret urin placeret på en Beckman Paragon SPE Agarose gel og tilladt at diffundere i 5 minutter. Selve elektroforese proceduren blev udført ved 100 volts spænding i 25 minutter i barbiturat buffer (pH 8.6). Efter farvning af præparatet, blev de separerede proteiner kvantiteret ved hjælp af et 600 nm Beckman Appraise densitometer.

Femten hunde med pyodermi (38%) havde proteinuri, hvoraf de 11 havde albuminuri. Det var 5.3 gange højere end hos de 151 kontrol hunde med andre lidelser ($p < 0.05$). Ligeledes blev det fundet, at patienterne med recidiverende pyodermi havde haft deres hudproblemer i mindst 9 måneder, før albuminuri kunne påvises, og i 18 måneder før globulinuri sås.

I studiets første del (c) blev urin fra hunde med proteinuri, med eller uden recidiverende pyodermi analyseret med hensyn til proteinsammensætningen (52 hunde blev undersøgt). Baseret på den elektroforetiske fraktionering, blev 3 typer af elektroforetogrammer identificeret, nemlig albuminuri, serum-lignende proteinuri og globulinuri. Albuminuri-typen blev fundet i 30% af patienterne med proteinuri i sygdoms-kontrol gruppen, 60% af patienter med superficiel pyodermi og proteinuri, 80% af patienter med profund pyodermi og proteinuri og endeligt hos samtlige hunde med glomerulonefritis. Albuminuri typen (gennemsnitlig albumin/globulin ratio ± SEM [1,98 ± 0,10]) kunne karakteriseres ikke alene ved det dominerende indhold af albumin, men også den konstante tilstedeværelse af α_{1b}-, α_{2a}- og β_2-globuliner. Dette karakteristiske fund blev opfattet som et udtryk for ændret filtrationskapacitet (albuminuri) og inflammatoriske skader (α_{1b}-, α_{2a}- og β_2-globuliner), idet de nævnte globulin- grupper indeholder de såkaldte akut fase proteiner.

Den serum-lignende proteinuri-type (gennemsnitlig albumin/globulin ratio ± SEM [0,72 ± 0,01]) sås hos 13% af patienterne med proteinuri. I disse tilfælde kunne de i serum eksisterende protein fraktioner ses. Denne type af proteinuri skønnedes at være en variant af den førnævnte albuminuri-type, formodentligt med den forskel, at typen repræsenterer et mere fremskredent stadium.

Globulinuri-typen (gennemsnitlig albumin/globulin ratio ± SEM [0,33 ± 0,08]) fremviste et mønster, som var markant forskellig fra de øvrige to, idet albumin var tilstede i lave mængder, samt at de forskellige globulin bånd kun var svært erkendelige. Det opfattedes som et udtryk for, at enkelte eller mange af globulin fraktionerne (og albumin?) undergik en nedbrydning, mulig-vis samtidigt med de tubulære skader og derigennem en patologisk sekretion.

Den korrekte identifikation af protein fraktionerne i urin og serum hos hunde, der undersøges som her, kræver en præcis identifikation af de enkelte proteiner. Hertil anvendes de såkaldte Rf-værdier. For at sikre, at den fornødne tekniske præcision forelå, blev en indledende undersøgelse gennemført. Hertil blev serumprøver fra 37 klinisk raske gamle hunde, 25 geriatriske patienter og 18 klinisk raske unge hunde anvendt. Rf værdier blev beregnet og anvendt for identifikation af de forskellige protein fraktioner. Udover de markante ændringer, som blev fundet hos gamle hunde, og i

særdelehed hos de geriatriske patienter, kunne det vises, at brugen af Rf værdierne var en pålidelig metode til identifikation af individuelle protein fraktioner.

I studiets anden del blev 86 tanzanianske hunde, hvoraf de 55 havde transmissible venereal tumours (TVT), undersøgt for bakterielt betinget cystitis. Udover en grundig klinisk undersøgelse af de eksterne genitalier, blev placerin-gen af neoplasierne registreret. Sterile prøver fra de externe genitalier samt urinprøver (ved hjælp af cystocentesis) blev udtaget. Prøver dyrkedes med henblik på at identificere tilstedeværende bakterier. Der blev fundet, at hunde med TVT i vagina, vestibulum, den vestibulo-vaginale overgang, præputium og penis havde en stærkt forøget risiko for at udvikle bakteriuri (odds ratio = 7.04). Det vurderedes ligeledes, at obstruktion af den urethrale orificium var hovedårsagen til den høje incidence af urinvejsinfektioner hos hunde med (TVT). Hos hunde, der havde haft TVT over en længere periode, var risikoen for at udvikle bakteriuri stærkt forøget (OR = 29.60).

KONKLUSIONER

Resultaterne fra dette studium fører til følgende konklusioner:

* Mikroskopiske undersøgelser af urinsediment er en pålidelig og billig diagnostisk metode for urinvejslidelser, og hvor vurderingen af de degenerative forandringer i urothelial cellerne kan være af stor betydning.

* Undersøgelser af urin sediment kan anvendes som hjælpemiddel til at udvælge udvidede diagnostiske metoder.

* Screening af hunde for proteinuri er en anvendelig teknik for tidlig identifikation af patienter med kliniske/subkliniske nyreskader.

* Recidiverende pyodermi prædisponerer for glomerulopati hos hund. Det er derfor anbefalelsesværdigt, at undersøgelsen af sådanne hunde også omfatter en komplet urinanalyse.

* I henhold til de foreliggende undersøgelser kan recidiverende pyodermi og andre systemiske sygdomme udløse dannelsen af én af 3 forskellige typer af proteinuri, hvorimod glomerulonefritis kun giver én type proteinuri, nemlig albuminuri.

* Glomerulær proteinuri eller albuminuri er karakteriseret ved et selektivt tab af albumin og de 3 globuliner: α_{1b}-, α_{2a}- and β_2-globuliner.

* Transmissible venereal tumour er en prædisponerende faktor for udviklingen af infektioner i urinvejene.

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