

ASSESSMENT AND COMPARISON OF URINARY PROTEIN: CREATININE RATIOS OF HEALTHY DOMESTIC DOGS AND WORKING DOGS IN KENNELS DIVISION IN SRI LANKA POLICE FOR EARLY DETECTION OF RENAL DISEASE

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Summary: Healthy dogs usually excrete small amounts of protein in urine. Persistently high proteinuria is usually a marker of kidney disease. The urinary protein: creatinine ratio (UPC) is used in quantifying urine protein excretion in order to diagnose kidney diseases, guiding recommendations for monitoring and treatment of kidney diseases and evaluation of prognosis. However, use of UPC ratio has not been well established/ used to diagnose renal diseases in dogs in Sri Lanka. Therefore, for the first time in Sri Lanka, we used this method to detect UPC ratio of healthy dogs (n=51). Once we established the UPC ratios of healthy dogs, UPC ratios were measured in working dogs (n=45) in Kennels Division of Sri Lanka Police, since renal diseases are a common cause of mortality in these dogs. In control group, the mean UPC ratio was 0.06 ± 0.05 . This was similar to the UPC ratio of tracking group among working dogs. Two out of 22 dogs used for explosive duties had UPC ratio within the borderline proteinuric stage (0.2-0.5) according to the staging system implemented by the International Renal Interest Society (IRIS). In the narcotic group 2 out of 12 dogs were borderline proteinuric and one dog was proteinuric. In this study, we have successfully established the measurement of UPC ratio to detect proteinuria in Sri Lankan dogs and have identified dogs that are susceptible to renal diseases.

INTRODUCTION

Proteinuria is one of the risk factors of renal diseases. In addition to this, proteinuria is used as a predictor of end organ damage (Barnas *et al.*, 1997). Early and accurate detection of persistent renal proteinuria is of high importance to reduce mortality in both dogs and cats due to renal damage (Littman, 2011; Wehner *et al.*, 2008). By detecting acute renal disease early in its course, appropriate intervention can be made to arrest or at least to attenuate renal damage and the development of acute renal failure. Similarly, by detecting chronic renal disease before the onset of renal azotemia and chronic renal failure, appropriate intervention can be made to stabilize renal function or to slow its progression (Jacob *et al.*, 2005). Once a complete diagnosis is made, therapeutic and management plans can be arranged to manage the disease. The urine protein creatinine (UPC) is the gold standard tests to quantify and monitor proteinuria in dogs. UPC ratio detects persistent proteinuria and aid in making clinical decisions and monitoring response to therapy. UPC values recommended by American College of Veterinary Internal Medicine (ACVIM) (Lees *et al.*, 2005) and International Renal Interest Society (IRIS) for azotemic and non azotemic dogs provide guidance in monitoring, diagnosing, and treating dogs and cats with renal disease (Brown *et al.*, 2013; [http://www.iris-](http://www.iris-kidney.com)

[kidney.com](http://www.iris-kidney.com)). We have observed higher mortality rates in working dogs in Police and armed forces in Sri Lanka, due to renal diseases. Therefore, the primary goal of this study was to establish the reference for UPC ratio for dogs in Sri Lanka, and to compare UPC ratios of working dogs in Sri Lanka Police to detect the dogs with possible renal diseases early.

MATERIALS & METHODS

Design and study population

Fifty one client-owned, apparently healthy dogs presented to the Veterinary Teaching Hospital (VTH), University of Peradeniya, identified as reference group, were used to establish the UPC ratio. These dogs were included in the study only after signed consent had been received. Their average age was 2.8 years (range 1-10 years). All the working dogs (n=45) except three puppies (< 6 months) and six dogs attached to Presidential Security Division of Sri Lanka Police were used in this study. Their average age was 4.3 years (range 2-8 years). This group comprised of dogs assigned for explosive detection, narcotic detection and tracking duties. Dogs were enrolled for this study using the following inclusion criteria: (1) more than six months of age, any breed, gender, size and (2) were either healthy animals, or working dogs with or without underlying disease/s that could induce proteinuria.

A single random mid-stream sample of 10 ml urine (Monroe *et al.*, 1989) was collected aseptically into sterile tubes from each animal by catheterization and transported in ice immediately after collection. Urine samples from animals with active sediment were excluded from the study. An inactive urine sediment for this study was defined as <5 WBC/ hpf, <20 RBC/ hpf, and no visible bacteria (Duffy *et al.*, 2015). Humanrol quality control sera (Human GmbH-Max-Plank-Ring, Germany) were used to check the experimental procedures.

Blood samples

Venous blood sample (2.5 ml each) was collected into EDTA tubes from cephalic vein. Plasma was separated within 30 minutes from collection. Hematologic and plasma analytes were determined within 12 hours with standard methods. Samples were analyzed for Full Blood Count, Blood Urea Nitrogen (BUN), plasma creatinine, total plasma protein and albumin according to the standard diagnostic laboratory procedures established at the VTH. Blood smears were prepared and checked to exclude tick-borne parasitic infections.

Urinalysis and UPC measurement

Urine samples were used to estimate specific gravity, protein and creatinine concentrations, and subjected to microscopic sediment evaluations, within 12 hours of collection (Rosi *et al.*, 2012). Samples were assessed visually for evidence of debris, or gross discoloration (Vaden *et al.*, 2004). Urine specific gravity was measured using a refractometer. Multistix Uripath strips were used according to the manufacturer's guidelines to detect glucose, bilirubin, ketones, specific gravity, occult blood, pH, protein, nitrite and leucocytes in urine by assessing the colour change at recommended times after immersing the dipstick in the sample of urine. Urine sediment, 0.5 ml, harvested by centrifuging 5 ml of urine sample at 2000 rpm for 5 minute was evaluated microscopically at low power (10x) for epithelial cells, casts and at high power (40x) for leukocytes, erythrocytes, bacteria and crystals.

Urine protein concentration

Total protein concentration (mg/dl) in urine was measured in the supernatant obtained after centrifugation by Pyrogallol red- molybdate method (Johnson *et al.*, 1999) on a semi-automated biochemistry analyzer (Erba Mannheim, ERBA Diagnostics, North Miami Avenue Miami, U.S.A). In this method pyrogallol red/molybdate formed a red complex in the presence of proteins and the colour was directly proportional to the protein concentration.

Urine creatinine concentration

Urine creatinine concentrations (mg/dl) were measured using RANDOX Monza reagent with the semi automated chemical analyzer using a modified Jaffe method (Bartels, 1972). In this colorimetric method creatinine in alkaline solutions reacts with picric acid and forms a coloured

complex which is proportional to the creatinine concentration.

Blood Urea Nitrogen concentration

Blood urea nitrogen concentration (mg/dl) was measured using urease-Berthelot method using RANDOX reagent with the semi automated chemical analyzer. In this colorimetric method ammonia which is produced by urea in serum in the presence of urease is measured photometrically.

Albumin concentrations in plasma

Albumin concentrations (mg/dl) in plasma were measured using RANDOX Monza reagent with the same automated chemical analyzer. In this colorimetric method the measurement of serum albumin is based on its quantitative binding to the indicator bromocresol green.

Classification

IRIS staging classification was used to classify UPC ratios <0.2 as nonproteinuric, =0.2 and <0.5 as borderline proteinuric and =0.5 as proteinuric. Clinical response category based on the ACVIM consensus statement (Lees *et al.*, 2005) was also assessed with the serum creatinine concentrations. For nonazotemic category, "no action" was taken for dogs with UPC <0.5, monitoring for kidney function was advised for those who had UPC =0.5 and further investigations to assess the kidney function were recommended for dogs with UPC =1 and <2 and medical intervention for UPC with =2. Azotemic dogs with UPC <0.5 was considered as "nonintervention" category and UPC =0.5 was considered as "intervention" category for management of the condition.

Health records of Working dogs in Kennels division of Sri Lanka Police

In a retrospective study, carried out in parallel to this study, we collected data of working dogs in the police for past two years.

Statistical Analysis

Data are expressed as mean±SD, and 95% CIs were calculated. All analyses were performed with MINITAB® Release 14.1 software. Results obtained for control group and working dogs were compared by using the two sample *t* test and ANOVA. The Regression test was used to assess a possible relationship between UPC ratio and other factors. Values of $p < 0.05$ were considered significant.

RESULTS

Population

Urine samples were obtained from 51 client-owned dogs. Samples from all of these dogs were included in the study, since they had inactive urine sediment. Number of dogs in the kennels division of Sri Lanka Police had variable degrees of active urine sediment and the samples which were compatible with our inclusion criteria were included in the study (Figure 1).

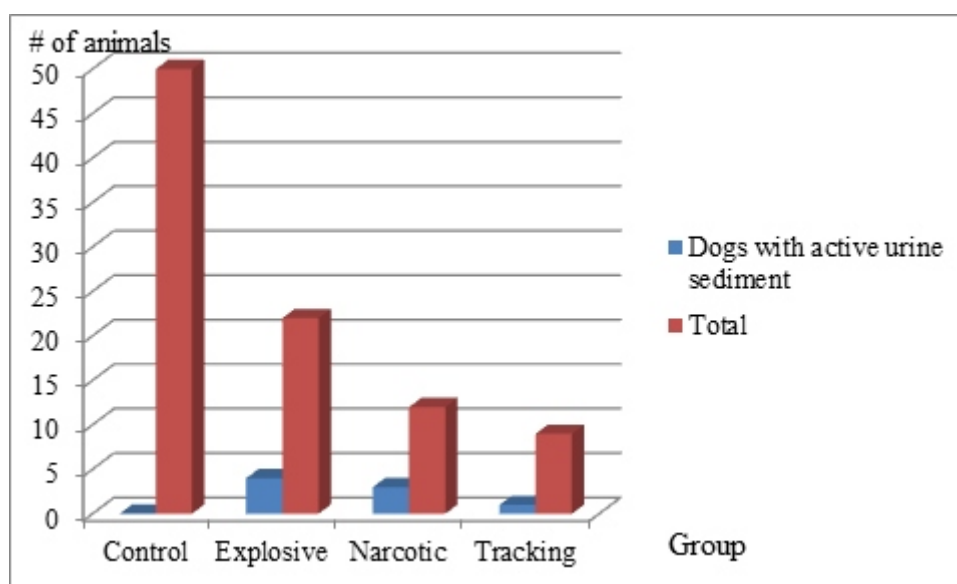


Figure 1. Presence of active urine sediment among different groups of dogs in the study population

Table 1: Blood urea nitrogen (BUN), plasma concentrations of creatinine, total proteins (TP), albumin (Alb), urine specific gravity (USG), urine pH and urine protein creatinine ratio (UPC) of samples collected from healthy dogs

BUN	Creatinine (mg/dl)	TP (g/dl)	Albumin (g/dl)	USG	pH	UPC
11.46±6.44	1.09±0.29	5.85±1.08	2.47±0.38	1.02±0.01	6.44±0.69	0.06±0.05

BUN- Blood urea nitrogen; TP- Total protein; USG- Urine specific gravity; UPC- urine protein: creatine ratio

Urinalysis of control group

Average UPC ratio of 51 healthy animals in the control group was estimated to be 0.06 ± 0.05 (Table 1).

Results obtained using health records of working dogs in kennels division of Sri Lanka Police showed that twenty one deaths have been reported and necropsies performed at the Division of Pathology of the Department of Pathobiology, and Division of the Diagnostic Pathology of the VTH, Faculty of Veterinary Medicine and Animal Science, University of Peradeniya. Necropsy reports indicated that the highest number of deaths was due to renal failure (33.3%). In addition to this, haemoparasitism (24.7%), heart failure (19%), multi organ dysfunction (9.5%), toxicosis (9.5%) and lymphosarcoma (4%) were associated with the deaths of working dogs in police.

Results of UPC ratios in control group and working dogs

Mean and standard deviation of UPC ratio in healthy dogs was 0.06 ± 0.05 ($n=51$). Mean and standard deviation of UPC ratio in dogs in explosive ($n=22$), narcotic ($n=12$)

and tracking ($n=9$) duties were 0.09 ± 0.09 , 0.15 ± 0.26 and 0.05 ± 0.06 respectively. One way ANOVA-revealed a significant difference between mean UPC values in control group and working dogs ($p < 0.05$, $R-Sq = 9.91\%$). Two sample t- tests were used to compare UPC values between groups. Results showed that there were significant differences ($p < 0.05$) between UPC ratios in dogs in the control group vs explosive and narcotic groups. There was no significant difference ($p > 0.05$) in UPC value of dogs in the control group vs tracking group.

One of the key objectives of this study was to screen the renal diseases of working dogs in the kennels division of Sri Lanka Police. Therefore, rather than comparing the mean UPC ratios between groups, we compared UPC ratios of individual animals in the study population with the IRIS standards recommended by ACVMA (Figure 2). In the narcotic group, one animal was proteinuric but nonazotemic. According to the ACVIM consensus statement this animal was subjected for monitoring of kidney function. Two animals in the narcotic and two in the explosive groups were borderline proteinuric hence no action was taken (<http://www.iris-kidney.com/>) (Table 2).

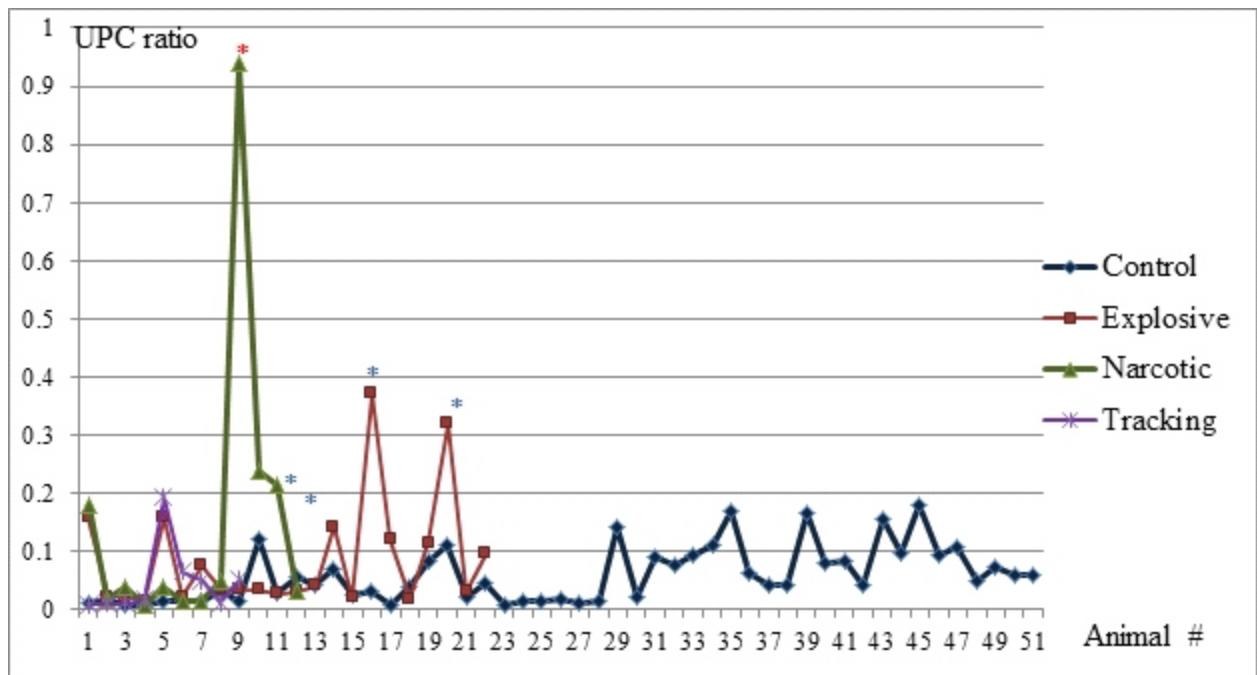


Figure 2: UPC ratios in control group and different categories of working dogs in Sri Lanka Police. In the narcotic group, one animal was proteinuric (marked with a red asterisk). Two animals in the narcotic and two in the explosive groups were borderline proteinuric (marked with blue asterisks)

Table 2: Matching the stage of proteinuria, blood urea nitrogen (BUN), plasma creatinine, Urine specific gravity (USG), Urine pH, urine protein (U. protein) in working dogs that were borderline proteinuric, according to the ACVIM consensus statement

Group	BUN	P. creatinine (mg/dl)	USG	Urine pH	U. Protein(mg/dl)	UPC ratio
Narcotic (n=3)	17.68 ± 5.8	1.31 ± 0.23	1.03 ± 0.01	6.66 ± 1.15	533.33 ± 404.2	0.46 ± 0.41
Explosive (n=2)	16.29 ± 2.59	1.42 ± 0.21	1.02 ± 0.01	7 ± 1.41	100	0.35 ± 0.03

DISCUSSION

An increase in urinary protein excretion is a widely accepted tool in the detection, diagnosis, and management of renal disease (Price *et al.*, 2005). The UPC ratio is the gold standard test to quantify proteinuria in dogs (Harley and Langston, 2012). However, in Sri Lanka there had been no evidence of using this test to stage renal diseases in dogs. Therefore, in this study we were able to successfully assess and establish UPC ratios in a cohort of healthy dogs in Sri Lanka. All the animals sampled in the control group were nonproteinuric and had no sign of active urine sediment. The differences in UPC values among the animals in the control group would have been due to the time of the day of urine samples collected as one report suggested that the first morning specimens are preferred to detect protein excretion (Xin *et al.*, 2004).

However, there are several reports supporting the idea that use of random urine samples for the measurements of UPC ratios work accurate and more practical (Newman *et al.*, 2000; Price *et al.*, 2005).

Study of necropsy records (from 2012-2014), of working dogs in Kennels division of Sri Lanka Police supports the fact that renal failure is the major cause for deaths. Bringing up a working dog to their working capacity from the time of importation is quite an expensive task. Therefore, detecting renal function at early age is of high importance for successful monitoring and therapeutic interventions. In our survey, we have found that even young working dogs (age 2-5 years) have died of renal disease. Chronic renal failure has been the cause of death (~71%) in many of these animals diagnosed with renal disease. Although, the cases of renal failure are presented towards the latter part of their illness, proper

screening of kidney function in dogs using UPC ratio would significantly reduce the mortality. It is not just the animals with kidney disease that should be screened for proteinuria. According to the author's experience, working dogs in the Sri Lanka police are very prone to have conditions or diseases such as drug reactions, hyperadrenocorticism, acute renal failure, viral disease, immune mediated diseases, tick-borne diseases, exogenous steroid use, and urogenital diseases that may contribute to proteinuria. This is further supported by report of Harley and Langston (2012). UPC ratios of working dogs are affected by number of other factors such as dietary protein content, exercise and hyperthermia (Schaefer *et al.*, 2007; Mustafa *et al.*, 2011). Previous studies reported that cage confined animals had significantly higher UPC ratios compared to unconfined animals, and stress in these animals would have contributed for the difference (McCaw *et al.*, 1985).

When we compared UPC values of dogs in the tracking group and control groups, there was no significant difference. This could possibly be due to the fact that they are used for minor duties such as tracking crimes where the chances for inhalation/ ingestion of nephrotoxic agents are low.

There was one animal in the explosive group who was proteinuric, but nonazotemic. According to the ACVIM consensus statement this animal was subjected to monitoring of kidney function. Two animals in the narcotic and two in the explosive groups were borderline proteinuric hence no action was taken. Therefore, advantage of the method we established in the Diagnostic laboratory of the VTH, would be to routinely screen dogs for renal diseases. Nine months old puppies in the explosive group are trained to sniff explosives such as Tri NitroToluene (TNT), Research Department Explosive (RDX/ Cyclonite), Dynamite, plastic explosive, Pentaerythrite Tetranitrate (PETN), NH_4NO_3 , Detcord, electrical detonator, non-electrical detonator every day for four hours in the morning and one hour in evening which continues throughout their life time and this chronic exposure makes them prone to renal injury.

In the narcotic group, one animal was proteinuric but nonazotemic and two animals were borderline proteinuric. This could possibly be due to the fact that they are trained to sniff narcotics such as heroin, marijuana, cocaine, toddy, ganja and arrack. It has been reported from humans who abuse narcotics are more prone to be affected with renal failure resulting from chronic glomerulonephritis and hypertensive nephrosclerosis (Xiqian *et al.*, 2015).

In summary, this study assessed and established UPC ratios in a cohort of healthy dogs in Sri Lanka. These values were in agreement with the UPC ratios reported in IRIS staging system. The degree of difference in UPC values in working dogs of the Sri Lanka police warrants attention of the authorities and clinicians regarding clinical decisions about diagnostic, monitoring, or therapeutic plans. Nevertheless, screening of these high risks groups of working dogs for their kidney function and also use of UPC measurement in other dogs as a screening

test would assist clinicians in detecting possible kidney diseases in advance.

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