Prevalence of asymptomatic urinary tract infection in dogs from boarding house around Mafikeng-North West Province-South Africa.

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Abstract: This study aimed to investigate the prevalence of asymptomatic urinary tract infection (AUTI) in dogs from boarding houses and assess the frequency of infection in connection with sex and age. A total of 15 stray dog urine samples were collected aseptically from different boarding house around Mafikeng, of which seven (46.6%) were form males and eight (53.3%) from females respectively. The samples were examined for urinary tract infection (UTI) using physical, microbiological, biochemical, sediment analysis including Gram staining for possible bacterial contamination. Macroscopic examination showed a difference in urine colour with 3 (20%). Among the analysed samples Proteinuria was found to be 26.6%, Bilirubinuria 13.3%,Cristaluria 6%, Cylinduria 6%, white blood cells 46%, red blood cells 47% and epithelial cells 20% respectively. Only one sample had positive gram negative bacilli after Gram stain. These results indicated that AUTI was not a major concern in dogs from shelters in Mafikeng. Clean environment, good diet, fresh water and normal voiding reduce the risk of getting UTI.
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1. Introduction

Urinary system is one of the most important system in the body of an animal as its primary role is to store and eliminate unwanted waste products of metabolism mainly urine, which is important for regulating the body's homeostasis (Bartges, 2007). The system is made up of the kidneys, ureters (tubes that carry urine to the bladder for storage), the urinary bladder, and the urethra that conducts urine outside the body (Smarick et al., 2004). These organs work together to remove metabolic wastes and maintain fluid and electrolyte balance (homeostasis) (Merck, 2010). UTI is defined as the invasion of the normally sterile areas of the urinary tract by pathogenic bacteria with subsequent clinical signs such as stranguria, pollakiuria, dysuria and urinary incontinence, although some animals may have no clinical signs (Batamuzi and Kristensen, 1996; Wang and Chew, 2012). Untreated infection can put animal life at risk by causing serious damage to kidneys and other parts of urinary tract (Seguin et al., 2003). The most commonly isolated causes of UTI in dogs are *Escherichia coli, Staphylococcus*, *Proteus*, *Klebsiella* and *Streptococcus* species. Among them, *E coli* is the bacteria most commonly isolated (Krieger, 2002; Litster et al., 2007). Although male are less affected by this, female dogs are more prone to the infection because they have shorter urethra which makes them more likely to suffer bacterial infections (Merck, 2010). Many reports have shown that UTIs are more common in older female dogs, indicating that age is a major risk factor (Cohn et al., 2003). This infection can affect all parts of urinary tract (lower or upper urinary tract), some cases can be complicated UTI (recurrent or persistent) or uncomplicated UTI (simple). Up to 95% of UTIs in dogs with underlying disease are clinically silent (Seguin et al., 2003). This finding has also been supported by a later study of dogs with recurrent or persistent UTI, where approximately half of the dogs diagnosed with recurrent or persistent UTI were asymptomatic on presentation (Seguin et al., 2003). However, the urinary tract has many important defence mechanisms to avoid infection despite its proximity to the anus and the potential for faecal contamination including normal micturition, mucosal defense barriers, antibacterial properties of urine, specific anatomic structures, and systemic immunocompetence (Osborne and Lees, 1995) In order for bacteria to colonize the urinary tract, there must be some compromise in the host defence mechanism (Brown and Barsanti, 1989). This ability was shown in studies in which *E. coli* was infused once over a period of several weeks into the bladder of healthy dogs. After 5 days, dogs cleared the infection without being given antibiotics within (Seguin et al., 2003). But since many of these infections are asymptomatic, the best way to diagnose is to perform a laboratory test like sediment analysis,

urine culture and biochemical testing (multiple reagent tests). Dog owners around Mafikeng in the North West Province seem to know little about UTI and the impact it can cause on their animal. The sub-clinical type of UTI is even worse since its symptoms are not visible in an infected dog and makes it even harder for the owner to be aware. UTI is a disease that affects dogs of all ages and its condition can rise from better to worse with time. (Bartges, 2007). The objectives of this study were to investigate the prevalence of AUTI in dogs from boarding houses and to assess whether uro-pathogens does exist in urogenital of animal. In addition to evaluate diagnostic accuracy and relative cost effectiveness of dip stick test for nitrite and leucocytes esterase in comparison to laboratory culture.

2. Materials and methods

2.1. Sampling sites

Urine samples were collected from 15 different boarding houses dogs around Mafikeng mainly at the Society for the Prevention of Cruelty to Animals (SPCA). They were of various breeds, sexes (8 females, 7 males) and ages (1-3). All the dogs were looking healthy, strayed and had no form of history. The dogs were handled with care to minimize physical and/or psychologically strain. They were handled by trained personnel.

2.2. Urine collection

Urine samples were obtained using the cystocentesis method without anaesthesia according to the method described by Reine and Langston (2005). The bladder was palpated with one hand, which helps to immobilize it while acquiring the sample. Both lateral and standing approach were used when palpating the bladder and the canine was laid in lateral recumbency for collection. Spirit swab was used to disinfect the area of insertion. Sterile needle was inserted through the abdominal wall into the bladder providing 10 ml of urine (without contaminants from any material in the urethra or the outside air).The urine was emptied in clean sterile glass containers with lid, stored in icepack and send to the laboratory. The samples were allowed to incubate at room temperature prior to analysis.

2.3 Laboratory analysis

2.3.1 Bacterial culturing

A sterile platinum loop that has a 4 mm diameter designed to deliver 0.01 ml was used for quantitative method of plating. A loop full of a well-mixed urine sample was inoculated on MacConkey, Blood, Cled and Potato dextrose agar (Merck, South Africa).plates and incubated at 37^{o}C for 24hrs.

2.3.2 Physical examination of urine samples

Macroscopic observations were used to assess the physical characteristics of urine samples by looking at urine volume, colour and transparency:

2.3.4 Microscopic examination /sediment analysis of urine.

Sediment analysis was used to check both formed and non-formed elements which included cells, casts, crystals, bacteria, fungi, and parasites were performed.

2.3.5 Biochemical/ dipstick analysis of urine

Urine samples were mixed using Krulab urine dipstick intended for veterinary use only. The following parameters were assayed: Specific gravity, using both refract meter and dipstick, pH, leukocytes, nitrites, protein, glucose, ketones, bilirubin, urobilinogen, and blood (Krulab, 2011).

2.3.6 Direct Gram staining

Gram staining was done following the standard laboratory technique (Vasanthakumari, 2009). A known Gram positive cocci and Gram negative bacilli were used as controls. Briefly Smears were fixed by gently passing through the Burnsen flame 3 times and stained with crystal violet stain for 60 seconds and washed with running tap water. After it was stained with iodine for 60 seconds and washed, the smear was decolourized with 95% ethanol and washed with running tap water and then counter stained with dilute Carbol fuscin for 30 seconds and washed with running tap water. The smear was allowed to air dry and examined under high power using oil immersion.

2.3.7 Urine sediment examination

Microscopic examination of the urine sediment is an essential part in the evaluation of renal and urinary tract diseases. Five millilitres of urine was removed from each tube and centrifuged at 10000 rpm for 15minutes. After centrifugation the liquid layer was discarded, the sediment resuspended, and observed under a bright field microscope for the presence of structures that could reflect either a underlying pathological condition, or a normal physiological process (Batamuzi and Kristensen, 1995)

3. Results

Results obtained from this study are summarized in table 1, 2 and 3. A total of 15 dogs urine sample were analysed, 7 samples were obtained from male dogs and 8 from females. Their ages ranged from 1 to 3 years. Urine specific gravity ranged from 1.005 to 1.040. There was no significant difference between the results obtained by dipstick and refractometer (Table 1). The pH ranged from 6-8 with an average of 7. Bilirubinuria was found in two cases one of which was male significance of which is questionable. Traces of protein were found in two samples and the levels were ranging from + (200-500gm/24hrs) to ++ (05-1.5mg/24hrs). None of the tested samples was positive for nitrites, or leucocytes urobilinogen, and glucose suggesting that canines were unlikely to have urinary tract infection. Microscopic results displayed in table 3 showed that out of 15 urine sediments

analyzed there was no abnormality detected (NAD) in 40% of case meaning there was no formed or non-formed elements present in the sample. Of the NAD samples 50% were from female dogs. Crystaluria which is excretion of crystals in the urine and cylinduria which is the presence of renal casts in the urine were found in sample 3 only (Table 3).

Table 1: Biochemical/Dipstick analysis of urine and refractometer to measure the urine samples obtained from dogs in shelters around Mafikeng.

Canine No.	Sex	Age/years	USG	Refractometer	pH	Bil	Pro	Blood
1	M	2	1.005	1.02	8	-	-	-
2	M	2	1.01	1.01	7.5	-	-	-
3	F	3	1.005	1.03	8	-	Trace	-
4	F	2	1.01	1.01	7.5	-	-	-
5	F	2	1.01	1.01	7.5	-	-	-
6	F	1	1.01	1.03	7	-	-	-
7	F	3	1.04	1.04	6	++	++	-
8	F	2	1.03	1.04	6	-	+	-
9	M	1	1.04	1.04	6	-	++	+++
10	F	1	1.04	1.03	8	-	-	-
11	M	1	1.01	1.01	7	-	+	-
12	M	3	1.03	1.03	8	-	-	-
13	M	2	1.01	1.01	6	-	-	-
14	F	1	1.01	1.01	7	-	Trace	-
15	M	3	1.03	1.04	7	+	-	-

USG: Urine specific gravity; Bil: Bilirubin; Pro: Protein.
Normal values: pH: 4.5 to 8.0 (Ave: 6.0), USG – 1.005 – 1.035(Ave: 1.010 - 1.025), Protein: (+) 200 – 500gm/24hrs, (++) 0.5 – 1.5gm/24hrs, (+++) 2 - 5gm/24hrs, Trace – equivalent to 10 - 100gm/hrs.

Macroscopic examination of urine results are shown in table 2 and revealed that the average volume of urine collected was 10 ml. Samples 1, 3, and 11 among the 15 samples collected showed transparency that was consistent with slightly cloudy, and clear respectively. Among these sample, 11 showed a pale yellow to yellow colour which is associated with normal urine while 4 samples showed dark yellow colour associated with concentrated urine or presence of pigment bilirubin.

Sample 3 was the only sample with bacterial contamination among all analyzed urine samples (Table 3). White blood cells which are associated with infection were found in 46% of samples majority of which were male (Table 3). A significant count of white blood cells was found in a female dog sample (sample 7) at WBC 10-12 /HPF (high power field). Red blood cells were also found in 7 out of 15 samples which were analyzed. In additional, transitional epithelial cells were found in only 2 samples (7 and 14) and their count was insignificant (Table 3). Results obtained from the culture showed that only 1 (6.6%) of the 15 urine samples showed bacterial growth (Table2).

Table 2: Macroscopic evaluation of urine obtained from dogs of shelters around Mafikeng.

Canine N^{o}	Sex	Age/years	Volume/ml	Colour	Transparency	Gram staining
1	M	2	10	Pale yellow	Clear	-
2	M	2	10	Yellow	Clear	-
3	F	3	10	Dark yellow	Clear	-
4	F	2	10	Pale yellow	Clear	-
5	F	2	10	Pale yellow	Clear	-
6	F	1	10	Dark yellow	Slightly cloudy	-
7	F	3	10	Dark yellow	Cloudy	+
8	F	2	10	Yellow	Clear	-
9	M	1	13	Dark Yellow	Cloudy	-
10	F	1	10	Yellow	Clear	-
11	M	1	10	Yellow	Clear	-
12	M	3	12	Pale yellow	Clear	-

13	M	2	11	Yellow	Clear	-
14	F	1	10	Dark yellow	Cloudy	-
15	M	3	13	Pale yellow	Clear	-

Table 3: Microscopic examination/ sediment analysis of urine collected from Dogs in Shelter houses around Mafikeng.

Microscopic Evaluation of urine			
Canine N^{o}	Sex	Age/Years	Observations
1	M	2	NAD
2	M	2	RBC 0 to 2./HPF, WBC - 2 to 4 /HPF,
3	F	3	Crystals (Struvite) ++, Granular casts +.
4	F	2	NAD.
5	F	2	RBC 0 to 4 /HPF, WBC 0 to 2./HPF
6	F	1	RBC 0 to 4 /HPF.
7	F	3	WBC 10 to 12/HPF, RBC 0 to 3/ HPF, Epithelial cell 0 to 2 /HPF, Scanty Bacteria
8	F	2	NAD
9	M	1	WBC- 3 to 5 /HPF, RBC – 0 to 1 /HPF.
10	F	1	NAD
11	M	1	WBC – 2 to 4 /HPF
12	M	3	NAD
13	M	2	WBC - 2 to 4 /HPF, RBC 2 to 4 /HPF
14	F	1	RBC- 0-2 /HPF, Epithelial cell 0 to 3/HPF, WB.C 2 to 4 /HPF
15	M	3	NAD

RBC: Red blood cell; WBC: White blood cell; NAD: No abnormalities detected; HPF: High Power Field

4. Discussions

Urinalysis is an important tool in screening, monitoring and disease detection in veterinary practice for conditions as UTIs, defects in organs, systems and metabolic pathways (Bartges et al., 1999; Grauer, 2007). In this study, a total of 15 mixed breeds canines were used to obtain urine sample and the samples were taken from both males and females irrespective of age. Since bladder urine is considered bacteriologically sterile in healthy dogs (Finco et al., 1975; Barsanti et al., 1985) anti pubic cystocentesis was used to obtain urine sample. The procedure was done under aseptic conditions and only sterile materials were used for collection to avoid contamination, as described by Ling (1995) and Gatoria et al. (2006). Aspiration was well done since in 94% of the samples no occult blood was detected (Table 1) in the sample meaning aspiration was stopped prior to withdrawal of needle from the bladder. The biochemical analysis of urines samples (Table 1) revealed that there were no significant differences in pH 6-8. It is known that the ideal pH is alkaline as it diminishes the risk of struvites (Orenstein and Wong, 1999; Merck, 2005). Urine alkalinisation minimizes renal ammonia production; the goal is to achieve a urine pH >7 (Merck, 2005). It is important to note that samples 7, 8, 9, 13, 14 and 15 which had low pH (6 or 7) were the samples having bilirubin, protein and blood (Table 1). The low pH in this samples might had influence on the presence of these elements in urine as acidic pH will favour the damage of bladder tissues and thus release of bilirubin, proteins and red blood cells. It is important to mention that urinary pH might be influenced by several factors such as bacterial contaminated food absorbed by the animal (Merck, 2005) prior to sample collection. Normal urine should contain <5 RBC/field at 400× magnification (Merck, 2005). Increased RBC in urine (haematuria) indicates haemorrhage somewhere in the urogenital system; however, sample collection by cystocentesis or catheterization may induce haemorrhage which might also be the case in this study, In addition, the low pH in samples specifically which contained traces of blood might be explained by collection trauma to the bladder not due to haematuria.

Sine et al. (2003) advocates the use of refractometer to measure urine specific gravity as opposed to dipstick given that refractometer is more sensitive. They argue that the urine dipstick specific gravity is falsely elevated by moderate to high concentration of protein. High protein content of 0.5-1.5 gm/24 hrs (++) in sample 7 and 9 didn't elevate dipstick urine specific gravity (Table 1). Results of these study shows that there was no significant difference between the two methods. It is important to mention that dogs have small amount of protein that pass through the glomerular filter majority of which is resorbed in the renal tubule however the nephron excretes small amount of Tamn Horsfall protein which is excreted in

urine but is not clinically detectable (Mittal et al., 2009). Traces of protein found in 13% of samples could not be attributed to Tamn Horsfall protein; the alkalinic pH of the samples is the probable cause of trace in protein. Significant proteinuria combined with bilirubinuria found in sample 6 and 9 (Table 1) could be attributed to renal disease or intravascular haemolysis due to the presence of occult blood or liver problems (Merck, 2005). However in this study the leucocyte esterase test was negative for all urines tested (Table 1) with sediment WBC of 10-12/HPF which excludes the liver disease problems in dogs from which urine samples were collected. Nitrite test which was based on the reduction of dietary nitrate to nitrite by Gram negative bacteria was also negative in all tested samples (Table 1) which had Gram negative bacilli on direct Gram stain. The test has a low sensitivity and many variables that influence it hence why it should always be interpreted with caution (Sine et al., 2003). No glucose was detected in urine samples analysed. Urinary glucose estimation which was based on the enzymatic reaction involving glucose oxidase and ketones based on the nitroprusside reaction were negative in samples tested indicated that dogs were well fed and had no defective carbohydrate metabolism (Sine et al., 2003). Results obtained from macroscopic evaluation of urine did not had significant value as urine colour might be influenced by the food or other factors such as blood presence in urine or proteins (Merck, 2005). It is usually very important to continue with other analysis as well as microbiological examination to have the correct composition of urine samples. The presence of granular casts in sample 3 (Table 3) or other casts in high numbers could have indicated renal damage, and may have been one of the earliest laboratory abnormalities noted with toxic damage to renal epithelial cells (eg, gentamicin, amphotericin B). The presence of epithelial cells in samples 7 and 14 (Table 3) in female dogs was not of diagnostic value as the levels were very low. However, Transitional epithelial cells are common urine contaminants which are derived from the bladder and proximal urethra, resemble WBC but are larger (Mittal et al., 2009). They have a greater amount of grainy cytoplasm and a round, centrally located nucleus. In a voided urine sample, squamous epithelial cells may be observed. Occasionally, neoplastic transitional cells may be observed in an animal with a transitional cell carcinoma. Neoplastic squamous cells may be observed in an animal with a squamous cell carcinoma (Merck, 2005). Microbiological results obtained from the culture of urine sample were negative for all samples and only sample 7 (Table 4) showed the presence of Gram - bacilli which was obtained after stain. These results are in line with the theory that Urine samples in bladder are sterile and free of bacteria (Merck, 2005). These results also showed those animals were not infected by lower or upper bacterial infection. In addition, the microbiology results explained the other results on urine parameters. The contamination of sample 7 might be explained by an ascending urinary infection (Merck, 2005).

In most studies UTI's have been reported in female dogs more often than male dogs (Ling et al., 2001). This statement supported the results obtained in this study, especially female dog number 7 (Table 4). This dog showed Gram negative bacilli result (Table 2). This may be due to female anatomical structure (Ling, 2000; Ling et al., 2001).

Several studies have associated old age with an increased risk of developing UTI (Yuri et al., 1996; Ling, 2000). This agrees with results from this study which shows that younger dogs less than 3 years were at lower risk of acquiring UTI's as seen in Table 3. However in another study (Seguin et al., 2003) found that UTI's were more likely to develop in younger dogs less than 3years

5. Conclusion

In this study urine samples were collected from dogs in shelters around Mafikeng and analysed for possible AUTI. Results obtained from macroscopic, microscopic and microbiology analysis of all samples revealed that AUTI were not a major problem in canines in shelters around Mafikeng in the North West Province, South Africa. It is important to mention that results obtained in this study would not be applied to all animals as AUTI is known to be prevalent in ageing animals which were not part of the sampled group and that animal sampled in this study were mostly young. In this study, it was also noted that sex and age were not significantly associated with the frequency of AUTI. However, samples from female dogs seem to have more contamination than male samples. Clean environment, good diet, fresh water and normal voiding reduce the risk of getting UTI. The urine from normal animal is devoid of bacteria; although abnormal number of white blood cell and red blood cell was seen in during analysis was due to trauma caused during collection by cystocentesis.

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