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# Bacteriological evaluation of urine, bladder mucosal biopsy and uroliths in dogs suffering from urolithiasis

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Bacterial urinary tract infection (UTI) occurs in about 14% of all dogs (Ling 1984) and it frequently occurs in association with urolithiasis. Culture of urine, bladder mucosal biopsy and uroliths helps in isolation of specific bacteria responsible for urinary tract infection, which is commonly associated with urolithiasis.

The study included 9 cases of canine urolithiasis having uroliths at multiple sites in 5 cases, urinary bladder in 3 cases and urethra in 1 case. Either cystotomy or urethrotomy or both were carried out according to the location of calculi. At the time of surgical intervention urine, bladder mucosal biopsies and uroliths were collected aseptically from individual case for bacterial isolation and culture sensitivity test. Mineralysis of uroliths was done as per the standard qualitative method (Varley 1988) to prevent recurrence of urolithiasis. Quantitative urine culture examination was performed according to standard procedure (Quinn *et al.* 1994). For qualitative culture the bladder mucosal biopsy was macerated in 2 ml of brain heart infusion broth and incubated at 37°C for 6–8 h after which subcultures were made on blood agar and MacConkey Lactose agar.

For qualitative culture a urolith greater than 5 mm in diameter was placed in absolute alcohol for 2 h, after which it was washed with sterile saline repeatedly for 4–5 times. It was crushed with a sterile mortar and pestle, sterilized saline was added to crushed urolith and 1 ml of the saline-urolith suspension was added to 4 ml of trypticase soya broth. The broth tubes were incubated at 37°C for 6–8 h, after which subcultures were on blood agar and MacConkey lactose agar.

Antimicrobial sensitivity test for all the above samples was performed by Kirby-Bauer disc diffusion method using Mueller-Hinton agar plates (Carter *et al.* 1995). The concentration of each drug (in μg) per disc was ampicillin (A) 25, amoxyclav (Ac) 30, gentamicin (G) 30, amikacin (Ak) 30, tobramycin (Tb) 30, cephalexin (Cp) 30, cefazolin (Cz) 30, ciprofloxacin (Cf) 30, norfloxacin (Nx) 10, enrofloxacin (Ex) 10, co-trimoxazole (Co) 25, oxytetracycline (O), 30 nitrofurantion (Nf) 100 and nalidixic acid (N) 30. Growth (of 6 to 8 h) of the isolates in brain heart infusion broth was taken. The entire agar surface of Muller-Hinton agar plate was streaked with a sterile cotton swab dipped in the broth and then dried. The antimicrobial discs were placed with centers at least 20 mm apart using aseptic technique and pressed gently with forceps. After incubation at 37°C for 24 h the zone of inhibition was measured and recorded in millimeters. The results interpreted were based on the zone size according to the interpretation chart supplied along with discs by the manufacturer.

In this study, all positive quantitative bacterial cultures from urine collected by cystocentesis yielded a bacterial count>$10^3$ CFU/ml (n=2), >$10^5$ CFU/ml (n=5) and $10^6$ CFU/ml (n=1). Isolation of >$10^2$ CFU/ml in urine sample obtained by cystocentesis is considered to be indicative of UTI (Comer and Ling 1981).

Bacterial UTI was found in 8 cases. In case 2 and 3, *Escherichia coli* was isolated from urine sample. Examination of bladder mucosal biopsy and urolith was not done. *Escherichia coli* was isolated from urethrolith culture. In case 4 and 5, *Staphylococcus intermedius* and *Proteus mirabilis* were isolated respectively in all the 3 sample cultures. In case 6, *Klebsiella pneumoniae* was isolated from urine and biopsy cultures and no growth from urolith culture. In case 7, only urine sample showed *Proteus mirabilis* infection. In case 8 and 9 *Staphylococcus intermedius* and *Escherichia coli* were isolated respectively in urine and biopsy cultures, uroliths of both cases had no growth.

In the present study, 8 out of 9 cases were having metabolic uroliths, which includes CaO, $CaCO_3$, $PO_4$ $CaPO_4$ and uric acid. UTI in these cases might be secondary due to the presence of uroliths and organism isolated were often urease negative. Case 8 had magnesium ammonium phosphate, which was usually due to urease producing organisms. *Staphylococcus aureus* was isolated from the urine culture in this case. Klausner and Osborne (1979) also concluded

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that metabolic urolith might be associated with UTI although not as a cause of these urolith but developed secondary to the presence of uroliths in urinary tract. UTI in all the 9 cases was caused by a single species of pathogen. Ling (1984) also reported that approximately 75% of UTI in dogs was caused by single species of pathogen. In 8 cases, the same bacteria that was isolated from urine, was also isolated either from bladder mucosal biopsy culture alone or from both bladder mucosal biopsy and urolith culture. In one case there were no bacteria isolated in all the three samples, this might be due to host defence mechanism or presurgical antimicrobial therapy, which might have been succeeded in eradicating the infection after urolith formation.

*Excherichia coli* was the most sensitive to amikacin, tobramycin, cefazolin and ciprofloxacin. Keskar *et al.* (1998) found *Escherichia coli* to be 100% sensitive to gentamicin, 20% to ampicillin and 12.5% to oxytetracyclin. Coagulase positive *Staphylococcus* spp was sensitive to ampicillin, amoxyclav, gentamicin, amikacin, tobramycin, cephalexin, cefazolin, ciprofloxacin, norfloxacin, co-trimoxazole and nitrofurantion. *Proteus mirabilis* was the most sensitive to amoxyclav, amikacin, tobramycin, ciprofloxacin, cefazolin, norfloxacin and enrofloxacin. Similar findings were also reported by Rohrich *et al.* (1983). *Klebsiella pneumoniae* was the most sensitive to amikacin, tobramycin, cephalexin, cefazolin, ciprofloxacin and enrofloxacin. The present study implies that sensitivity of tobramycin, amikacin, cefazolin, ciprofloxacin, amoxyclav, cephalexin, norfloxacin and enrofloxacin were suggesting their higher efficacy for the treatment of UTI in canine urolithiasis. Similarly, Singh (2003) reported that the most sensitive antimicrobials were tobramycin, gentamicin, amikacin and cefazolin etc. for treatment of UTI in canines suffering from urolithiasis. Urine samples were again cultured on 20th postoperative day and no growth was found, suggestive of success of antimicrobial therapy. The present study concluded that identification of causative bacteria by comparative bacterial culture examination of urine, bladder mucosal biopsy and uroliths helped in effective planning of post-operative antimicrobial therapy for urinary tract infection and prevention of recurrence of canine urolithiasis.

## SUMMARY

Urolithiasis was studied in 9 dogs in which uroliths were present at multiple site in 5 cases, urinary bladder in 3 cases and urethra in 1 case. Either cystotomy or urethrotomy or both were carried out in the urinary tract. Comparative bacterial culture examination of urine, bladder mucosal biopsy and uroliths collected at the time of sugical interventation revealed *Escherichia coli, Proteus mirabilis, Staphylococcus* spp and *Klebsiella pneumoniae* infection in different cases. Identical bacteria was isolated from urine, bladder mucosal biopsy and urolith cultures. Antimicrobial sensitivity patterns of different bacteria isolated indicated that tobramycin, amikacin, cefazolin and ciprofloxacin were the most sensitive antibiotics. Oxytetracyclin and nalidixic acid were the most resistant antibiotics. Antimicrobial therapy based on CST helped in effective planning of postoperative management in prevention of recurrence of canine urolithiasis.

## REFERENCES

Carter G R, Changappa M M and Roberts A W. 1995. *Essentials of Veterinary Microbiology*. pp 81–94. Williams and Wilkins, Philadephia, USA.

Comer K M and Ling G V. 1981. Results of urinalysis and bacterial culture of canine urine obtained by antepubic cystocentesis, catheterization and the midstream voided methods. *Journal of Veterinary Medical Association* **179**: 891–95.

Keskar D V, Patil V K, Jagadish S, Bhalerao D P and Sharma L K. 1998. Therapeutic management of urinary tract infections in canines. *Indian Veterinary Journal* 75: 713–14.

Klausner J S and Osborne C A. 1979. Urinary tract infection and urolithiasis. *Veterinary Clinic of North America* (*Small Animal Practice*) **9**: 701–11.

Ling G V. 1984. Therapeutic strategies involving antimicrobial treatment of the canine urinary tract. *Journal of Veterinay Medical Association* **185**: 1162–64.

Quinn P J, Carter M E, Markey B K and Carter G R. 1994. *Clinical Veterinary Microbiology*. pp21–66. Mosby Year Book Europe Ltd., London, England.

Rohrich P J, Ling G V, Ruby A L, Jang S S and Johnson D L. 1983. *In vitro* susceptibilities of canine urinary bacteria to selected antimicrobial agents. *Journal of Veterinary Medical Association* **183**: 863–67.

Singh I. 2003. 'A clinical study on the diagnosis and management of bacterial urinary tract infection in canines with urolithiasis'. MVSc. thesis, Punjab Agricultural University Ludhiana, India.

Varley H. 1988. *Practical Clinical Biochemistry*. pp. 717–23. CBSC Publishers and Distributors, Delhi, India.