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Retrospective study on role of urinary tract infection and urine pH on type of urinary calculi in goats

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ABSTRACT

A retrospective study was conducted on 142 male goats suffering from obstructive urolithiasis to identify common microbes responsible for urinary tract infections and their effect on urinary pH and crystalluria. Culture and isolation of the urine samples were performed in 72 cases, which showed UTI in 69.44% cases with microbes like *Escherichia coli, Alkigenes faecalis* and *Pseudomonas,* to be the most common. The isolated microbes showed high sensitivity for ciprofloxacin and piperacillin. Positive correlation was recorded between urinary pH and urinary tract infection suggesting that urinary tract infection may lead to alkalization of urine. Urine pH affected the type of crystalluria and suggested that alkaline pH of urine may favour struvite calculi formation in goats. It was concluded that urinary tract infection may be an important factor in the formation of urinary stones in goats and should be given due consideration while planning preventive and control measures for urolithiasis in goats.

Key words: Antibiotic sensitivity test, Crystalluria, Goats, Urinary tract infection, Urine pH, Urolithiasis

Role of urinary tract infection in the formation of alkaline urine and subsequent formation of phosphate calculi is well established in canine and laboratory animals. Break in host defenses to urinary tract infection, quickly results in development of cystitis and urinary calculogenesis. *Staphylococcus* infection resulted in development of cystitis and formation of urethral struvite calculi in dogs and cats within 2–8 weeks (Klausner *et al*. 1981). Satoh *et al.* (1984) observed formation of struvite calculi 5 days after introducing *Proteus mirabilis* infection in the urinary bladder of rats. Similarly, inoculation of urinary bladder with *Ureaplasma urealyticum* caused elevation of urinary pH and formation of struvite calculi in rats (Arai *et al*. 1996). Due to this reason Alpay *et al.* (2009) suggested that screening for UTIs is a cost effective tool for early detection of urolithiasis.

The role of urinary tract infection in development of urolithiasis is less established in ruminants. Van Metre (2004) reported that urinary tract infection is not a common concurrent finding in ruminant and porcine urolithiasis. To the authors' knowledge, the role of urinary tract infection in urinary alkalinization and subsequent development of phosphate calculi is not widely studied in goats. The present study was therefore, designed to investigate the presence of bacterial infection in urinary tract of the goats suffering from urolithiasis and its sensitivity to the common antibiotics, and to find interrelationship between urinary tract infection, pH of urine and type of crystalluria.

MATERIALS AND METHODS

The study was conducted on 142 male goats of 2 months to 1 year age, showing the signs of obstructive urolithiasis. The animals were treated surgically to restore the patency of urinary tract either by performing urethral process amputation, pre or post scrotal urethrotomy or tube cystostomy.

Approximately, 2 ml of urine sample was collected aseptically from the urethra or urinary bladder in sterile test tubes at the time of performing surgery for relieving the obstruction. Urine samples from 72 goats were processed for microbial culture and isolation and antibiotic sensitivity test. The 72 urine samples collected were taken from only those animals where tube cystotomy was performed and the sterility of collection of urine samples were not questionable. In rest of the animals where urethral process amputation and post scrotal or prescrotal urethrotomy were the surgical options, the urine samples were not processed for culture isolation and antibiotic sensitivity test owing to the doubt about breach in aseptic procedure for collection of the samples. Urine samples were inoculated in Blood agar and Mac Conkey's Lactose agar. Individual colonies were identified on the basis of colony morphology, Gram's smear and standard biochemical reactions (Quinn *et al*. 1994).

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Antimicrobial sensitivity was performed by Kirby-Bauer disc diffusion method using Mueller-Hinton agar plates (Carter *et al.* 1995). The zone of inhibition was recorded after incubation for 24 h at 37°C. Sensitivity of bacteria was expressed as sensitive and resistant. Microbes having highest sensitivity for an antibiotic were considered positive for that particular antibiotic.

A part of the urine sample was examined immediately after collection for determining the pH by pH strip in all 142 animals. About 5 ml of urine sample was collected in centrifuge tube for microscopic examination to determine nature of the crystals. Both stained (urine sediments stained with methylene blue) and unstained urine sediments were examined by light microscopy first at low power (×100) and then at high power (×450) magnification, for recording the presence and type of urine crystals. Interrelationship between urinary tract infection and pH of urine and also between urinary pH and type of crystal were analysed using χ^2 test (Snedecor and Cochran 1994). For determining interrelationship between UTI and urine pH, 70 samples having both urine pH and results from microbial culture and isolation were taken.

RESULTS AND DISCUSSION

Bacterial infection of the urinary tract is reportedly associated with urolithiasis in canine and cats (Klausner *et al.* 1981). Acar *et al.* (2008) in a retrospective study to determine the possible risk factors in pediatric nephrolithiasis patients, reported a positive correlation between urinary calcium excretion and stone size, recurrent urinary tract infection and hypercalciuria. Urinary citrate excretion showed a negative correlation with recurrent UTI. However, information regarding the role of infection in urolithiasis in goats is lacking. Out of the total 72 urine samples (Fig. 1) subjected to bacterial culture and isolation and antibiotic sensitivity testing, 69.44% (50/72) samples were positive for bacterial infection. *E.coli* was the most frequently isolated organism (16/72; 22.22%) followed by *Alkigenes faecalis* (6/72; 8.33%) and *Pseudemonas aeroginosa* (6/72; 8.33%). Other organisms isolated in small number, included *Proteus* (4/72; 5.56%). *Staphylococcus* (3/72; 4.17%), *Klebsiella* (3/72; 4.17%) and *Streptococcus* (2/72; 2.78%), A total of 30.56% urine samples were sterile. Mostly single organism was isolated but 2 organisms were isolated from 2 samples each. These findings are in corroboration with the observations of Ling *et al.* (1998), Pak *et al*. (1999), Norris *et al*. (2000) and Suarez *et al*. (2002) who reported organisms like *E.coli, Staphylococcus*, *Streptococcus*, *Proteus, Klebsiella* and *Pseudomonas* from the urine samples in dogs. They also reported mixed samples to be of occasional finding.

The sensitivity of different organisms to antibiotics varied greatly. About 25% (4/16) *E.coli* organisms were sensitive to ciprofloxacin, 25% (4/16) were sensitive to enrofloxacin and 18.75% (3/16) were sensitive to piperacillin/tazobactum.

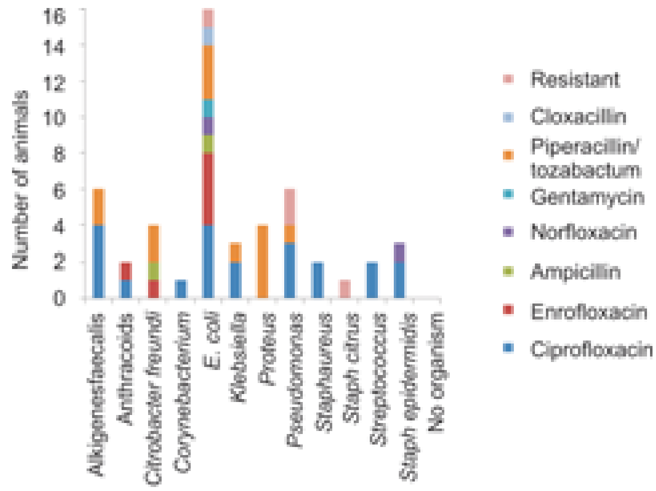

Fig. 1. Relationship between commonly detected microorganisms and most sensitive antibiotics against them.

About 66.67% (4/6) of *Alkigenes faecalis* organisms were sensitive to ciprofloxacin and 33.33% (2/6) were sensitive to piperacillin/tazobactum. All *Proteus* organisms showed high sensitivity to piperacillin/tazobactum. Two (33.33%) *pseudomonas* and 1 (100%) *Staphylococcus citrus* organisms were resistant to all commonly used antibiotics. Among the antibiotics used ciprofloxacin was found most effective (21/72; 29.17%), followed by piperacillin (13/72; 18.05%) and enrofloxacin (6/72; 8.33%). Very few organisms were sensitive to ampicillin (2), norfloxacin (2), gentamicin (1) and cloxacillin (1).

Distribution of the cases of obstructive urolithiasis with and without UTI and various pH ranges is shown in Fig. 2 and 3. Fig. 2 suggested that most of the samples with UTI (45/48; 93.75%) had urinary pH ≥8.0, out of which 12.50% (6/48) animals were having pH of 9.0 or more. Only 3 (6.25%) samples were in the pH in range of 7.0–7.5. On the contrary, 54.55% (12/22) samples without UTI had the pH range of 7.0–7.5, 40.91% (9/22) animals had urine pH from 8.0–8.5 and only 4.51% animals were having urinary pH >9.0.

The distribution of the cases (Fig. 3) suggested that among the samples with urinary pH of 7.0–7.5 very high percentage (80%) of urine samples were sterile and only 20% samples were positive for bacterial infection. The situation was reverse in the pH range of 8.0–8.5, as very high percent (81.25%; 39/48) of urine samples were positive for UTI. In the urinary pH range of 9.0 or above the % of samples positive for infection rose to 85.71% (6/7) as compared to 14.29% sterile samples. Values of χ^2=20.96 (coefficient of contingency=0.55) calculated from the data showed the urinary tract infection had a highly significant (P<0.001) effect on the urine pH.

Urinary tract infection is one of the most important risk factors for magnesium ammonium phosphate, carbonate

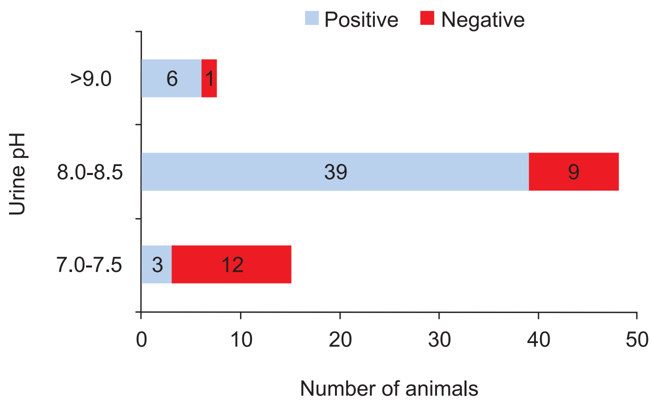

Fig. 2. Distribution of cases of urolithiasis according to urine pH as a function of urinary tract infection.

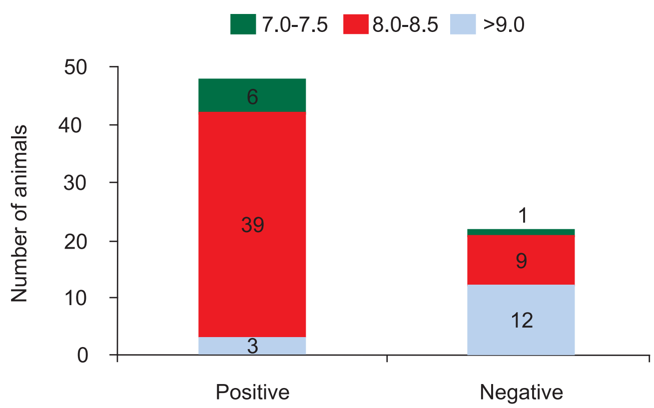

Fig. 3. Distribution of cases of urolithiasis according to presence or absence of urinary tract infection.

apatite and monoammonium urate especially at alkaline urine pH (Miano *et al*. 2007). Presence of urease-producing microorganisms in urine through their urease activity may convert the urea to ammonia, which results in alkalinity of urine. It may subsequently lead to cystitis or pyelitis, a chronic inflammatory condition of urinary bladder or kidneys; characterized by deposits of struvite and carbonate apatite (Griffith 1979, Pak 1999, Suarez *et al.* 2002). Also phosphatase produced by these organisms increases the amount of inorganic phosphates in urine by acting on organic phosphates. Increase in urine pH and hyper-concentration of magnesium, ammonium and phosphate ions may result in the precipitation of struvite crystals and subsequently obstructive urolithiasis (Pak 1999). Considering the importance of urinary tract infection as a risk factor for urolithiasis, Alpay *et al.* (2009) have advocated the importance of screening the patients for UTIs, for early detection of urolithiasis.

Distribution of the cases of obstructive urolithiasis with different types of crystals and various pH ranges is shown in Figs 4 and 5. The interrelationship between the type of crystalluria and urinary pH was studied in 142 samples. A total of 58 (40.84%) animals had urine pH in 7–7.5 range, 73 (51.41%) animals had urinary pH 8–8.5 and 11 (7.75%) animals had a urinary pH of 9.0 or higher. Fig. 4 showed that struvite crystals were observed in 110 cases (77.46%). Struvite crystalluria was recorded in all the pH range, however, 68.18% (75/78) samples with struvite crystalluria had pH equal to or higher than 8.0 and 31.82% (35/110) samples with struvite crystalluria had pH ranging from 7.0–7.5. Contrarily, other types of crystals were recorded mostly in the pH range of 7.0–7.5. Urine samples where no crystalluria was diagnosed also had pH of 7.0–7.5 in majority of the cases (70.00%). Fig. 5 revealed that in the animals having urinary pH range of 7.0–7.5 only 60.34% (35/58) samples were positive for struvite crystals and as the pH increased to 8.0–8.5 the proportion of samples with struvite crystals increased to 90.41% (66/73). In pH range of 9.0 or more, 81.82% (9/11) samples showed the presence of struvite crystals.

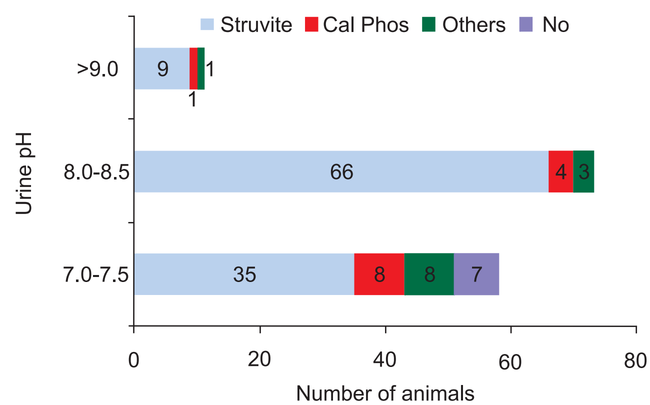

Fig. 4. Distribution of cases of urolithiasis goats according to urine pH as a function of the type of crystalluria.

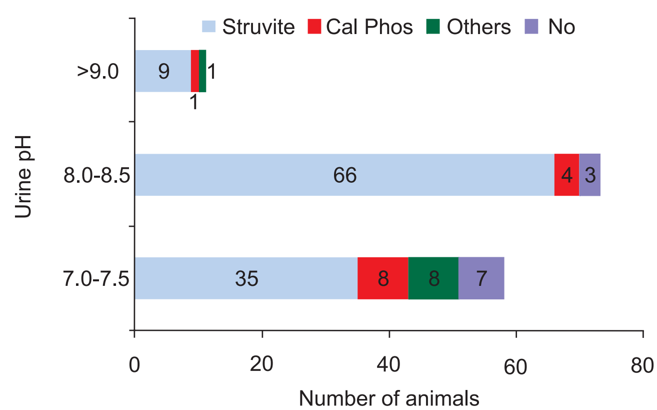

Fig. 5. Distribution of cases of urolithiasis goats according to the type of crystalluria as a function of urine pH.

In the pH range of 7.0–7.5 only 27.58% samples were positive for crystal other than struvite, and 12.07% samples were negative for any crystals. As the pH increased to 8–8.5 the proportion of the samples with other crystal decreased further. In the pH range of 9 or above all the samples were having struvite crystals. Calculated values of c2=17.55 (COC=0.33) showed that the nature of crystalluria was significantly ($P<0.01$) affected by urine pH.

Percentage distribution also indicated struvite crystalluria to be most prevalent at highly alkaline urine pH, whereas other types of crystals were relatively more common at either neutral or slightly alkaline urine pH. This finding was in conformity with the observations of Osborne *et al*. (1986) and Kienzle and Schulknecht (1993), who reported that phosphate crystals were mostly found at alkaline pH of the urine, while cystine, oxalate, leucine, tyrosine and uric acid were, recorded either in acidic or slightly alkaline pH in dogs. High grain rations which was the common practice in these caprines, accompanied by, low water intake, urine tract infection and alkaline urine pH seem to be the major contributing factors for urolithiasis as also reported by McIntosh (1978) and Loretti *et al*. (2003). High phosphourus content in grain ration is being considered to be an important causative factor for precipitation of magnesium ammonium phosphate crystals in the urine (Singh 2005). It was suggested by the present study that crystalluria is dependent upon the urine pH and highly alkaline pH of the urine is one of the most important predisposing factors for struvite lithiasis in goats also.

It was concluded that *E. coli, Alkigenes faecalis, Pseudomonas* were the common organisms in the urine of the goats suffering from obstructive urolithiasis, which were mostly sensitive to ciprofloxacin and piperacillin antibiotics. UTI may lead to alkalinity of urine, which could enhance the chances of struvite urolithiasis in goats by precipitation of the magnesium ammonium phosphate crystals in the urine. Management of urinary tract infection in goats may be therefore, useful in the prevention and treatment of urolithiasis.

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