A Study on Bacteriological Analysis of Urine, Urinary Bladder Mucosa and Uroliths in Obstructive Canine Urolithiasis in 37 Cases

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ABSTRACT

The study was conducted on 37 canine clinical cases suffering from obstructive urolithiasis. Qualitative bacteriological isolation was carried out from the urine collected by cystocentesis; uroliths and urinary bladder mucosal biopsies were collected during cystotomy. Out of the 37 clinical cases, 26 (70.3%) of dogs, had at least one sample (urine, urolith nidus or bladder mucosa) positive for bacterial infection. *Staphylococcus intermedius* was the most common isolate in urine, urolith nidus or bladder mucosa. The second most common isolate was *Escherichia coli* in urine and urolith nidus while *Corynebacterium urealyticum and E. coli* were present in the bladder mucosa. Out of 26 dogs only 4 dogs (15.4%) had identical bacteria cultured from all samples. In 5 dogs (19.2%), although the urine culture was negative, urolith nidus and urinary bladder mucosal biopsy cultures were positive. In conclusion, often bacterial isolates from urine, urolith nidus and bladder mucosa vary. Urolith nidus and bladder mucosa samples were found to be of greater diagnostic value in order to identify the type of urinary tract bacterial infection.

Key words: Bacteriology; Bladder Mucosal Biopsy; Canine; Cystotomy; Urolithiasis; Urolith Composition.

INTRODUCTION

Urolithiasis is the formation of mineral sediments in any part of the urinary tract, which are usually composed of one or more mineral types, deposited due to oversaturation of poorly soluble crystalloids in urine (1). Factors that could initiate or aggravate the urolith formation could be urinary pH, diet, urinary tract infection, abnormalities of metabolism that could be anatomical or functional including breed predisposition, age and sex (2). Most frequently implicated bacteria in UTIs such as *Escherichia coli*, *Proteus*, *Staphylococcus*, *Streptococcus*, *Klebsiella*, *Enterobacter* and *Pseudomonas* species, which may or may not be associated with urolithiasis (3).

The mineral composition of uroliths varies with the bacterial urinary tract infection (UTI). Bacterial UTI, especially caused by urease-producing bacteria such as *Staphylococcus intermedius* and Proteus species are often an important predisposing factor in the initiation, growth and recurrence of struvite uroliths (magnesium ammonium phosphate); whereas, secondary bacterial UTI occurs in patients with metabolic uroliths (calcium oxalate, cystine, urate or silica) (2). Bacteria contained within uroliths probably represent those present at the time of the initiation of urolith formation (4). It has been hypothesised that the type of bacteria present at the time of initiation of urolith may be different from bacteria present during the growth of urolith. Therefore, the present study was planned to compare the

microbiological analysis during nidus formation and growth of uroliths.

MATERIALS AND METHODS

This study included 37 clinical cases of dogs (34 male and 3 female) with a mean ± standard error, age of 6.15 ± 0.62 years (range 2-13 years) presented for obstructive urolithiasis to the Department of Veterinary Surgery and Radiology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, India. The majority of the affected dogs were Pugs (n=18) followed by Pomeranian and mixed breed (3 each), Beagle and German shepherd (2 each) and 1 each of Bull Terrier, Cocker Spaniel, Lhasa Apso and Daschund.

Qualitative bacterial isolation was carried out from urine samples collected by cystocentesis, bladder mucosal biopsy and nidus of uroliths collected during cystotomy. In all 37 dogs, cystotomy was performed under general anaesthesia with premedication of a combination of butorphanol at 0.2 mg/kg (Butodol, Neon Labs Pvt., Mumbai, India), acepromazine @ 0.05 mg/kg (Vet One, MWI, Animal Health, Boise, Idaho, USA) and atropine sulphate @ 0.04 mg/kg (Tropine, Neon Labs Pvt., Mumbai, India), intramuscularly. Induction of anaesthesia was carried out with propofol @ 4 mg/kg body weight, intravenously (Neorof, Neon Labs Pvt, Mumbai, India) and maintained on 1.5 to 2.5% isoflurane (Isoflurane USP, Raman and Weil Pvt., Ltd., Mumbai, India) and oxygen combination. Uroliths from 13 dogs subjected to composition analysis using FTIR (Fourier Transform Infrared) Spectrometer using the 'Perkin Elmer Spectrum Bx-2' (5).

Sample collection procedure

A urinen sample was collected by cystocentesis either prior to surgery by abdominal cystocentesis or intraoperatively using a 24 gauge needle. Incisional biopsy from the mucosal edges of bladder wall was collected during cystotomy in a sterile container. Urocystoliths retrieved during cystotomy were collected in a sterile container. All samples were stored at 4°C immediately in a refrigerator until bacterial isolation.

Bacteriological analysis of samples

Qualitative bacteriological cultures were carried out for urine, bladder mucosal biopsy and nidus of uroliths to identify the bacteria. For urine samples, centrifugation of 5 ml urine in a sterilised tube was carried out. Supernatant was poured-off and loopfuls of sediments were inoculated to blood agar and if required on selective media such as MacConkey's agar plate, Mannitol salt agar, Edward's medium, Eosin methylene blue agar plate (*HiMedia Laboratories* Pvt., Ltd., Mumbai, Maharashtra, India) and incubated at 37°C for 24 hours. Following incubation, individual colonies were identified on the basis of morphology, Gram staining and standard biochemical reactions such as catalase test, coagulase test, acid production from different sugars, CAMP test, urease test IMViC (Indole, Methyl red, Voges-Proskauer's and citrate utilisation) tests, nitrate reduction test, growth on triple sugar iron agar and sulphide indole motility medium was performed (6).

For bladder mucosal biopsy, macerated samples were directly inoculated on blood agar media which was prepared by the researchers themself as per the procedure described earlier (6). Uroliths were immersed in absolute alcohol for two hours and thereafter washed with normal saline and then ground down in autoclaved pestle and mortar. Using sterilised inoculation, the loop nidus part of urolith was inoculated on blood agar plates as described earlier (6). All the procedures were performed in laminar flow hood within the area of flame sterilised region, taking sterile precautions.

RESULTS

Various types of bacteria isolated from the urine, bladder mucosal biopsy and urolith nidi are shown in Table 1. Bacterial isolates from the urine culture were present in 54.1% of cases with *Staphylococcus intermedius* being the most common followed by *Escherichia coli,* whereas, 37.8% cases showed positive isolates from urinary bladder mucosa with *Staphylococcus intermedius* being the most common followed by *Corynebacterium urealyticum* and *Escherichia coli.* The 46.0% cases from urolith nidus showed positive isolates with *Staphylococcus intermedius* as the most common followed by *Escherichia coli*. In seventeen dogs, no bacterial isolates were observed in urine (46.0%). In six (16.2%) out of these seventeen dogs, bacterial isolates were obtained either from bladder mucosal biopsy and/or the urolith nidi.

The detailed results of bacteria isolated from various samples are shown in Table 2. In this study out of 37 samples, 26 (70.3%) cases had at least one sample (urine, urolith nidus or bladder mucosal biopsy) positive for bacteria. Out of which only 4 (15.4%) cases (S. No. 3, 7, 10, 11) were found with

Table 1: Relative occurrence of bacteria in the culture of urine, urolith nidi and bladder mucosal biopsies

Bacteria isolated from	Urine (n=37)		Urolith nidus (n=37)		Bladder mucosal biopsy (n=37)		Total (N=111)	
	No.	%	No.	%	No.	%	No.	%
Staphylococcus intermedius	12	32.43	8	21.62	5	13.51	25	22.52
Escherichia coli	3	8.11	3	8.11	3	8.11	9	8.11
Corynebacterium urealyticum	1	2.70	2	5.41	3	8.11	6	5.41
Bacillus species	1	2.70	2	5.41	0	0	3	2.70
Pseudomonas species	1	2.70	0	0	0	0	1	0.90
Streptococcus species	1	2.70	1	2.70	2	5.41	4	3.60
Proteus mirabilis	0	0	1	2.70	0	0	1	0.90
Mixed	1	2.70	0	0	1	2.70	2	1.80
No Growth	17	45.95	20	54.05	23	62.16	60	54.05

an identical bacterium cultured from each samples. Out of these, two, (5.4%) cases had *Staphylococcus intermedius* and in one case (2.7%), had *Corynebacterium urealyticum* and *Streptococcus* species. In six cases (23.1%) identical bacteria were cultured from the urine and bladder mucosal biopsy (S. No. 4, 6, 8, 12, 13, 14). Out of these, two (S. No. 4, 13) had differing isolates from the urolith nidus in which one case had *Staphylococcus intermedius* isolated from urine and urinary bladder mucosal biopsy with *Bacillus* species from a urolith. However in another case *Escherichia coli* was isolated from urine and urinary bladder with *Bacillus* species from the urolith, and in the remaining 4 cases no bacterium was isolated from urolith nidi.

On comparing culture results of urolith nidi and urine, four cases (15.4%) had *Staphylococcus intermedius* (S. No. 1, 2, 5, 9). Out of these, two were negative on culture of bladder mucosal biopsy while remaining two cases had a different isolate, identified as *Corynebacterium urealyticum* in one case and *Streptococcus* species in other. Only one case (2.7%) had a similar bacterial (*Escherichia coli*) isolate from the bladder mucosal biopsy and the urolith nidus (S. No. 21).

There were nine cases in which bacterial infection was found in every sample (urine=4; urolith nidus=4 and bladder mucosal biopsy=1). *Staphylococcus intermedius* was isolated in two cases (5.4%) from urine and in two cases from uroliths; *Escherichia coli* was isolated in one case (2.7%) each from the urine and urolith nidus; *Corynebacterium urealyticum* was isolated in one case (2.7%) each from the urolith and urinary bladder mucosal biopsy and also *Pseudomonas* species from one case (2.7%) from urine.

In fourteen cases out of 26 (53.9%), the bacteria isolated from urine obtained by cystocentesis, the same bacteria was also isolated from the bladder mucosal biopsy (23.1%), urolith (15.4%) and from both the bladder mucosal biopsy and uroliths (15.4%).

In four cases (S. No. 22, 23, 25, 26) in which urine and bladder mucosal biopsy culture were found negative, urolith nidus culture was positive for bacteria: (*Staphylococcus intermedius* in 2 cases; *Corynebacterium urealyticum* in 1 case and *Escherichia coli* in 1 case). In two cases (S. No. 15, 16), the bacteria isolated from urine and urolith nidi were different from bladder mucosal biopsy where no bacteria could be isolated.

DISCUSSION

The present study investigated various samples to prove the hypothesis that the environment during urolith initiation may be different from those present during its growth. For this, urolith nidus can provide important information about the environment at the time of initiation of urolith, whereas urine and bladder mucosal biopsy can provide prevailing environment for growth of urolith.

In the present study the urine, urolith nidi and bladder mucosal biopsy cultures were positive in 54.1%, 46.0% and 37.8% and *Staphylococcus intermedius* was the most common isolated bacteria followed by *Escherichia coli*. Perry *et al.* (2013) also reported similar results with *Staphylococcus* sp. being most common isolate (7). This implies that in the present study, coagulase-positive Staphylococcus species followed by *Escherichia coli* were found to be the most common bacteria

Table 2: Detailed information of bacteriological isolation from the urine, urolith and bladder mucosal biopsy

Sample No.	Urine	Urolith nidus	Bladder mucosal biopsy
1	*Staphylococcus intermedius*	*Staphylococcus intermedius*	*Corynebacterium urealyticum*
2	*Staphylococcus intermedius*	*Stapylococcus intermedius*	*Streptococcus* species
3	*Staphylococcus intermedius*	*Staphylococcus intermedius*	*Staphylococcus intermedius*
4	*Staphylococcus intermedius*	*Bacillus* species	*Staphylococcus intermedius*
5	*Staphylococcus intermedius*	*Staphylococcus intermedius*	*No growth*
6	*Staphylococcus intermedius and Corynebacterium urealyticum*	*No growth*	*Staphylococcus intermedius and Corynebacterium urealyticum*
7	*Corynebacterium urealyticum*	*Corynebacterium urealyticum*	*Corynebacterium urealyticum*
8	*Staphylococcus intermedius*	*No growth*	*Staphylococcus intermedius*
9	*Staphylococcus intermedius*	*Staphylococcus intermedius*	*No growth*
10	*Strephtococcus* species	*Streptococcus* species	*Streptococcus* species
11	*Staphylococcus intermedius*	*Staphylococcus intermedius*	*Staphylococcus intermedius*
12	*Escherichia coli*	*No growth*	*Escherichia coli*
13	*Escherichia coli*	*Bacillus* species	*Escherichia coli*
14	*Staphylococcus intermedius*	*No growth*	*Staphylococcus intermedius*
15	*Staphylococcus intermedius*	*Proteus mirabilis*	*No growth*
16	*Bacillus* species	*Escherichia coli*	*No growth*
17	*Escherichia coli*	*No growth*	*No growth*
18	*Staphylococcus intermedius*	*No growth*	*No growth*
19	*Staphylococcus intermedius*	*No growth*	*No growth*
20	*Pseudomonas* species	*No growth*	*No growth*
21	*No growth*	*Escherichia coli*	*Escherichia coli*
22	*No growth*	*Corynebacterium urealyticum*	*No growth*
23	*No growth*	*Staphylococcus intermedius*	*No growth*
24	*No growth*	*No growth*	*Corynebacterium urealyticum*
25	*No growth*	*Staphylococcus intermedius*	*No growth*
26	*No growth*	*Escherichia coli*	*No growth*
27-37	*No growth*	*No growth*	*No growth*

causing UTIs. Similar findings have previously been reported in dogs (8, 9, 10, 3) and in bovines (11).

In the present study, the distribution of isolated organisms was similar to some extent to the distribution commonly listed in the literature, with *Escherichia coli* and coagulase-positive *Staphylococcus* species being most common bacteria. Ling (1984) reported that approximately 75% of UTIs in dogs were caused by a single species of pathogen (12), 18% by two species and 6% by three species. Similarly, in the present study, in 19 (51.4%) cases the urinary tract infection urolith nidi were infected by only one species of bacteria and in 7 (18.92%) cases had two species of bacteria. It has been reported that urine sample collected under aseptic condition showing any bacterial growth should be considered significant (13, 14).

In dogs (n=4, 15.4%), identical bacteria were isolated from all three samples. Infection by urease producing bacteria was reported as an important predisposing factor in the initiation, growth and recurrence of struvite (magnesium ammonium phosphate) uroliths (2, 10). Ruby and Ling (1986) (15) isolated predominantly coagulase-positive *Staphylococcus* (47%) followed by *Streptococcus* (10%) and *Proteus mirabilis* (3%) from the struvite uroliths in dogs. *Staphylococcus intermedius* and *Proteus* species (potent urease

producers) were commonly isolated from the dogs with infection-induced struvite uroliths (2). Bacteria harboured inside the uroliths might not always be the same species as those present in the urine (4). However, in the present study, four (15.4%) dogs revealed identical bacteria from the urine, bladder mucosal biopsy and urolith nidus, however in another four (15.4%) dogs, similar bacteria were isolated only from the urine and bladder mucosal biopsy but not from the urolith nidus. Thus there may be a possibility that infection occurred at the same time or a different time of initiation and growth of the urolith.

In dogs (n=4, 15.4%), bacteria were isolated from the urolith nidus only, but not from the urine and bladder mucosal biopsy. The bacterial species isolated from inside the uroliths were positive for *Staphylococcus intermedius*, *Corynebacterium urealyticum* and *Escherichia coli*. However, in contrast to present study, Klausner and Osborne (1979) reported that cultures of the internal aspect of such metabolic uroliths are usually sterile (10). However, calcium oxalate and other sterile stones in the urinary tract might become infective with urease-producing or non-urease-producing bacteria during the UTI (16, 17). Radostits *et al.* (2000) also reported that a nidus may favour the deposition of crystals around itself to form a urolith, and this nidus might constitute a group of desquamate epithelial cells or necrotic tissue that maybe occasionally develop as a result of a local infection in the urinary tract (18). Bacteria contained within the uroliths probably represented those present at the time the urolith formation (4). These bacteria might embed within the urolith, thereby becoming invulnerable to antibiotics for treatment (19). Ling (1984) also isolated coagulase positive Staphylococcus from 14% of the non-struvite uroliths (12).

In present study *Corynebacterium urealyticum* was isolated from bladder mucosal biopsy in one dog with no growth from urine and urolith. Tissue obtained from urinary tract (such as bladder wall or renal biopsy) aseptically showing bacterial growth would be indicative of UTI, regardless of the number of bacteria present (13, 14). Similarly, Hamaide *et al.* (1998) also cultured *Staphylococcus intermedius* from the bladder mucosal biopsy of two dogs with negative on urine and urolith cultures (14).

In the present study, negative culture were observed in eleven dogs suggesting that dietary and/or metabolic factors might have been involved in the formation of these sterile uroliths, as reported by Osborne *et al.* (1999) (2). Host defence mechanisms or presurgical antimicrobial therapy could be the possible reason for negative urine culture in dogs (8, 11).

CONCLUSION

From the present study it was concluded that often bacterial isolates from urolith nidus, urine and bladder mucosa are diverse. *Staphylococcus intermedius* is the most common bacteria associated with urinary tract infection.

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