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The correlation of pH on *Escherichia coli* Growth and crystalluria formation in canine urinary tract infections

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Abstract
Urinary tract infections (UTIs) in dogs are due to multifactorial reasons and mainly by due to bacterial infections. Persistent or recurrent UTIs result in spending many costs on treating the dogs. The present study was carried out with the objective to detect the predominant types of crystals in *E. coli* causing UTIs in canines and in correlation with its pH range. Overall 18, samples were collected from canines, which showed symptoms specific to UTIs and were presented to VCC, Rajiv Gandhi Institute of Veterinary Education and Research (RIVER), Puducherry. The urine samples were subjected to microscopic examination, bacterial culture, and pH analysis. The predominant crystals were calcium oxalate, triple phosphate, and abnormal crystals like ammonium biureate crystals, and the most predominant bacteria from UTIs were identified as *E. coli* (12, 75%) and *Klebsiella* spp.+ *E. coli* (3, 18.75%). The pH range in these urine samples was found to be between 4-7. Therefore, the present study showed the predominant type of crystals in canine urine with a higher incidence of *E. coli* infection in canines of the Puducherry region.

Keywords: Crystalluria, pH, *E. coli*, urinary tract infections

Introduction

Urinary tract infections (UTIs) are common in dogs. Studies have shown 14% of all dogs will develop at least one UTI during their lifetime [10]. The number of bacteria and their pathogenicity, as well as the dog's overall health, including the structural function of the genitourinary tract and systemic immunity, are important variables in the development of UTIs. The Majority of UTIs are brought on by bacteria, but other causative organisms including fungi, mycoplasmas, and parasites are responsible for certain cases [15]. The clinical signs noticed in UTIs include dysuria, vomit, dark-colored feces, and pollakuria. Persistent or recurrent UTIs result in spending many costs on treating the dogs. The gold standard test for diagnosing canine urinary tract infections is bacterial culture, which allows veterinarians to make better treatment decisions [16].

The innate defense mechanisms of the canine with the physio-chemical properties of the urine protect against bacterial invasion. Specifically, low pH and high concentration in urine are two specific probable pathways for the antibacterial characteristics found in urine [15]. Incompatible data has been found in the literature about the influence of pH and urine concentration on bacterial growth in urine, considering the fact that many have hypothesized these antimicrobial properties but they have not been verified. Even the urine pH is important for finding urine sedimentation, urinary crystals are described to help in the identification of underlying abnormalities responsible for various UTIs including uroliths [2]. Urate, calcium oxalate, and struvite are some of the most typical forms of urine crystals [5].

The information on different types of crystals in *E. coli* causing UTIs in canines was not much in India. Therefore, the present study was taken to detect the predominant types of crystal in canines with UTIs in correlation with their pH range.

Materials and Methods

Collection of urine samples

A total of 18 dogs presented to the VCC, RIVER, with the signs specific to UTIs such as vomition, anorexia, dysuria and dark-colored feces. 10ml of urine samples were collected in a sterile container by catheterization and processed immediately for laboratory diagnosis.

Urine pH analysis

"The urine samples were numbered consecutively and pH strips were soaked in 5ml of urine. According to the color change, the pH value was noted" [4].

Microscopic examination of urine

The urine samples were subjected to microscopic examination after centrifugation at 3000 rpm for 5 minutes. Discard the supernatant and a drop of urine sediment was placed onto a glass slide with the coverslip. The microscopic examination was done at low power (10x) and high power (40x) objectives for the presence of different types of crystals [8].

Urine for bacterial isolation and identification

The urine sediment was streaked onto a MacConkey agar and EMB agar and incubated aerobically at 37 °C for 24-48 hours. Based on culture characteristics, microscopic examinations using gram staining and biochemical tests (catalase test, oxidase test, methyl red, indole test, citrate test, Voges-Proskauer test, and triple sugar iron), the bacteria were identified as described by Ling [10].

Results

Age-wise distribution

Among the 18 samples, the maximum number of cases was found in the age group of 8 to 10 years (61.1%) followed by 11 to 15 years (27.7%) in table 1.

Table 1: Age-wise collection of urine samples

Age of the dogs	Number of dogs	Percentage (%)
4-6	2	11.1
7-10	11	61.1
11-13	5	27.7

pH analysis

The pH range of urine samples from UTIs was found to be between the pH range of 4 to 7.9. Most of the urine samples are having pH of 5 to 5.9 (50%). pH values of the urine samples were given in Table 2.

Table 2: Determination of urine pH samples

pH range	Number of samples	Percentage (%)
4.0-4.9	2	11.1
5.0-5.9	9	50
6.0-6.9	4	22.2
7.0-7.9	3	16.6

Urine crystal determination

In the present study, calcium oxalate was recorded as high followed by triple phosphate. Bilirubin crystals, brown or yellow-brown spherical entities with erratic protrusions that made up ammonium urate (or biurate) crystals were called "thorn apples". Calcium oxalates dehydrate crystals had the usual colorless squares with intersecting lines connecting their corners (Fig.2). Various types of crystals found in this study, both normal and abnormal, are in Table 3.

Table 3: Determination of urinary crystals

Normal crystals	Abnormal crystals
Calcium oxalate	Ammonium biureate crystals
Triple phosphate	Bilirubin crystals
Tyrosine	

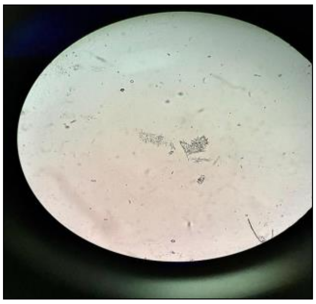


Fig 1: Ammonium biureate crystals

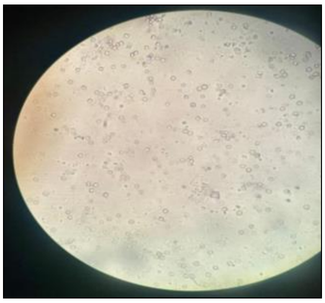


Fig 2: Calcium oxalate

Bacteriological examination

A positive bacterial culture was obtained from 16 samples. Of these samples, 1 sample yielded 3 different bacterial isolates, 3 samples yielded 2 species, and the remaining samples yielded a single bacterial species. The prevalence of bacterial species differed between cases of co-infection and pathogens isolated in pure culture (table 3). *E. coli* was the most common periodic pathogen, followed by Enterococcus spp, and Klebsiella spp. *E. coli* was isolated in 75% of all the cases (Fig 4).

Table 4: Bacteria isolated from dogs with recurrent UTI

Species	Number of urine samples	Percent of recurrent infections (%)
E. coli	12	75
Klebsiella spp+ *E. coli*	3	18.75
Enterococcus spp+ *E. coli*+ *klebsiella* spp	1	6.25
Total	16	100

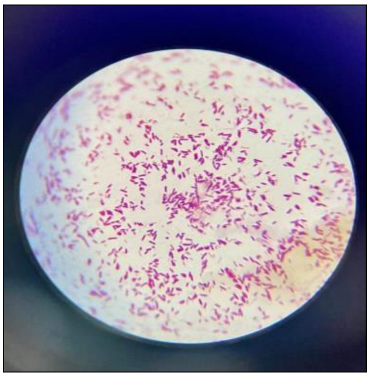
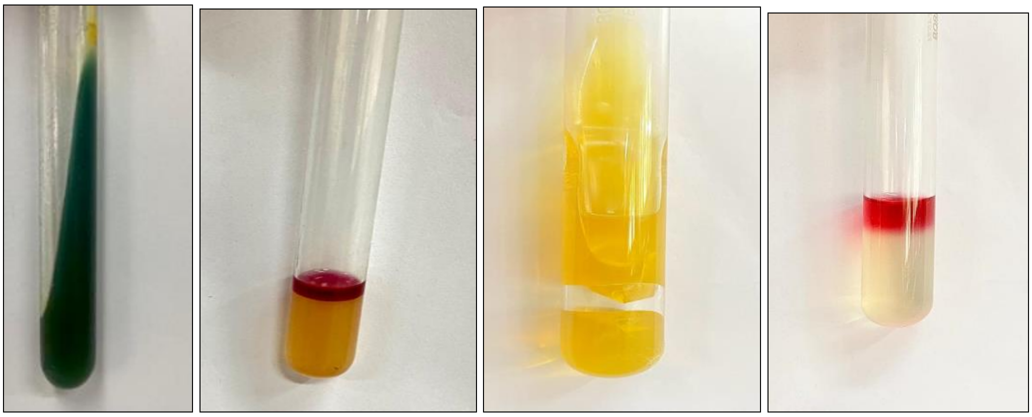


Fig 3: Microscopic examination of gram-negative stained colony gram- negative bacilli under oil immersion objective (100x).



Biochemal test for identification of *E. coli*

Fig 4: A- Citrate test, B- Indole test, C- Triple sugar iron, D- Methyl red

Discussion

While it is important for the interpretation of urine chemistry and sediment results, urine pH is a crucial aspect of the full urinalysis [15]. Urinary tract infection is the most common infectious disease in dogs and is diagnosed by routine laboratory evaluation including urinalysis and urine culture [6]. pH measurement of the urine is closely related to the growth of bacteria with crystal formation. The urine pH range of the current study (5-5.9) correlates with the crystal formation at its maximum cases. Pathogenesis of normal crystals may turn abnormal in specific clinical circumstances, resulting in a variety of chronic to acute diseases. Types of crystals present are based on canine urine pH. A similar observation with a pH range of 5 to 7 was studied by Callens [3]. A reasonable correlation was obtained from Osborne's [11] study, where the inhibition index and correlation factor are proportional to the crystal growth rate at different pH.

In most circumstances, a single species is responsible for UTI. *E. coli* grows faster in acidic urine compared to alkaline urine. Our collected sample results are consistent with studies of impaired growth *in vitro* at pH <5.5 and >7.6 [7]. The findings indicate a significant connection between urine pH, crystal formation, and *in vitro E. coli* growth. “Another finding in the present study was the difference in the distribution of bacterial species isolated from the urine. *E. coli* was the most common isolate in all groups, which is consistent with other results by Seguin” [14]. However, the proportion of organisms isolated in the present study shows *Enterococcus* spp and *Klebsiella* spp other than *E. coli*, which are relevant to Bubenik [1].

It's common to detect urine crystals when examining urine under a microscope. Several factors can impact crystal formation, and crystals may or may not be harmful depending on their presence [9]. Often, There's no clinical significance for crystals in the urine. Normal dog urine usually contains significant amounts of triple phosphate and calcium oxalate. “Calcium oxalate, uric acid, and amorphous urate crystals are often present in acidic urine”. Ammonium biureate crystals are always abnormal and are found in dogs that are having liver problems. By providing a vitamin-rich diet, the canine urine should be alkalized or acidified in order to avoid crystalluria, depending on the type of crystal Queau [12].

Conclusion

However, urinalysis characteristics are still widely utilized to direct the empirical treatment of UTIs. Through this investigation, To understand the apparent pathophysiology of renal function, the authors found that cross-examination of urine samples was required, starting with pH and progressing through bacterial isolation and crystal formation. Likewise, we suggest additional study into how urine concentration and pH influence the in vivo growth of *E. coli*. At the same time, it is concluded that urinalysis is of high significance for the early diagnosis of renal function damage, enabling us to treat early, thereby improving the recovery rate of patients with renal disease.

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