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Cultural studies of urine from cystitis dogs

Srikanth Kandula, Satish Kumar Karlapudi and Nagaraj P

Abstract
The present study was carried out on 237 dogs that were presented with the history and signs of various urine abnormalities like polyuria, hematuria or stranguria. These cases were subjected for through clinical examination and the urine was collected and subjected for cultural and antibiotic sensitivity studies. The results were recorded as German shepherd is more susceptible for cystitis during 4 - 8 years and is most commonly associated with *E. coli, Staphylococcus* and *Pseudomonas*. *In vitro* susceptibility revealed Enrofloxacin as highly effective antibiotic in treating such cases.

Keywords: Dogs, Urine culture**,** Enrofloxacin

Introduction
Urinary tract infections associated with bacteria may be ascending or descending. Ascending infection occurs when bacteria from the external body (around the anus) enter the bladder via the urethra and whereas, descending with infections, bacteria may spread through kidneys. Of all the urinary tract infections, bacterial infection of urinary bladder have been estimated to occur in approximately 14% of dogs during their life [11]. Female dogs are at increased risk for UTI due to easier ascension of bacteria through a shorter urethra. Cystitis is the inflammation or infection of the urinary bladder that is characterised by the frequent passage of small amounts of urine, straining to urinate or blood in the urine. Microscopic examination of urine sediment reveals the degree of destruction and urine culture is the gold standard for diagnosis of urinary tract infections. The bacteria most frequently involved in UTIs include *Escherichia coli, Proteus, Staphylococcus, Streptococcus, Klebsiella, Enterobacter* and *Pseudomonas* species [8]. The present investigation was carried out to study the cultural aspects and antibiotic sensitivity of the obtained urine samples from cystitis cases.

Material methods
The present study was carried out on 237 dogs that were presented with the history and signs of chronic recurrent vomiting, weight loss and with various urine abnormalities like polyuria, hematuria or stranguria, to Veterinary Hospital Bhoiguda, College of Veterinary Science, Hyderabad. These cases were subjected for through clinical examination and the urine was collected by catheterization [9]. Urethral catheterization provides a superior sample compared with a free-catch voided sample, but requires more technical skill, especially in female patients. Care was taken during catheterization to prevent contamination from external structures by clipping surrounding hair and cleansing the external genitalia prior to the procedure [17]. At the time of clinical examination the genitalia of the dog was cleaned properly and a spot sample of mid stream urine was collected using a sterile catheter in a sterile test tube and subjected for cultural studies immediately.
The urine samples were collected from the suspected cases and cultured primarily in nutrient broth at 37ºC for 18-24 hr, then sub cultured onto the MacConkey, Mannitol salt agar and EMB agar by streak plate method [2] to observe the colony morphology. The organisms showing characteristic colony, morphology of bacteria were repeatedly subcultured onto selective media until the pure culture with homogenous colonies were obtained. The culture was subjected for antibiotic sensitivity test using antibiotic (Enrofloxacin, Ampicillin, Amoxycillin, Amikacin, Ceftriaxone, Gentamicin and Tetracycline) discs supplied by Himedia, India.

Results and discussion
The present study was carried out in 237 dogs that were presented with the history and signs of chronic recurrent vomiting, weight loss and with different abnormalities of urine like polyuria

hematuria or stranguria, to Veterinary Hospital Bhoiguda, College of Veterinary Science, Hyderabad. These cases were subjected for through clinical examination and the urine was collected by catheterization [9]. Urethral catheterization provides a superior sample compared with a free-catch voided sample, but requires more technical skill, especially in female patients. Care was taken during catheterization to prevent contamination from external structures by clipping surrounding hair and cleansing the external genitalia prior to the procedure [17]. At the time of clinical examination the genitalia of the dog was cleaned properly and a spot sample of mid stream urine was collected using a sterile catheter in a sterile test tube and subjected for cultural studies immediately.

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Results and discussion:

The present study was carried out in 237 dogs that were presented with the history and signs of chronic recurrent vomiting, weight loss and with different abnormalities of urine like polyuria, hematuria or stranguria. When these cases were subjected for through clinical examination and various diagnostic protocols like, urine analysis, urine enzymology, serum chemistry and ultrasonography, 79 dogs were diagnosed for various renal disorders of which, cystitis was recorded as 22.79%, highest among GSD (27.78%) and lowest (5.55%) in Doberman. The common clinical signs recorded were related to the abnormalities associated with urine and GIT such as dysuria, hematuria, stomatitis, halitosis, vomiting, anorexia and weakness, respectively. Whereas, abnormal posture with pale or icteric mucosa, rough hair coat was also recorded among few dogs. Microscopic examination of the urine sediment revealed RBCs, WBCs, pus cells and epithelial cells. Microscopic examination is the most important component of complete routine urine analysis, as the procedure usually helps to identify the presence of cellular debris, crystals and casts. Presence of blood cells and epithelial cells in the present study might be due to inflammation of urinary tract and the presence of more number of these cells may suggest bacterial urinary tract infection [14]. UTIs caused by etiological bacterial agents have been associated with urogenital disease such as cystitis, nephritis, metritis and prostatitis in dogs [19]. Out of 237 dogs that were suspected for urinary tract disorders, ultrasonograpic examination confirmed 18 cases (22.79 %) of cystitis with manifestations of thickened urinary bladder wall with hyperechoeic layers (fig.1) and hyperechoeic bladder contents suggesting cellular debris and sludge (fig.2). Cystitis, that was diagnosed based on urine culture and ultrasonograpic examination was presented as 4.95% [15] and whereas, Crawford and Adams [3] reported the prevalence of cystitis with UTI as 70.58%. The variation in the prevalence of cystitis might be associated with many factors like environmental and unhygienic managemental practices during puerperal stage.

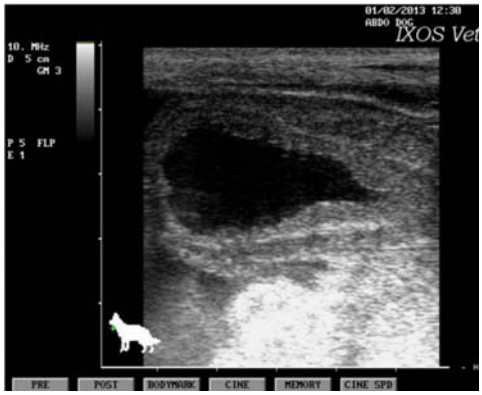

Fig 1: Thickened urinary bladder wall with hyperechoeic layers

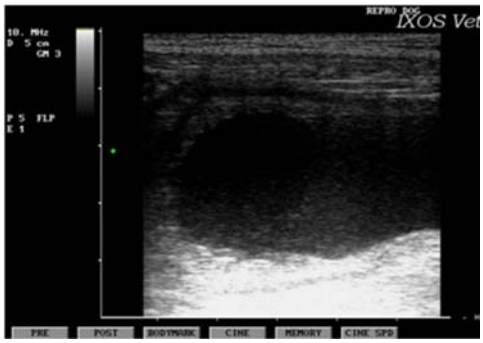

Fig 2: cellular debris and sludge in the urinary bladder

Cultural examination of the urine sample on selective media revealed *E. coli* isolates that were able to produce bright pink colonies on MacConkey agar. MacConkey agar is commonly used to differentiate *E. coli* from other gram-negative pathogens. MacConkey agar, like EMB agar, inhibits the growth of most gram-positive organisms [12]. Lactose-fermenting organisms produce pink colonies [6] and can be differentiated through the level of color change in conjunction with colony morphology and characteristic metallic sheen colonies on the EMB agar and it can be identified with EMB agar based on the occurrence of a green-metallic sheen that appears on the surface of the bacterial colonies [12]. The dyes in EMB agar, eosin Y and methylene blue, are pH indicators and inhibitors of gram-positive bacteria and at an acid pH combine to form a green-metallic sheen [12]. In the present study cultured urine samples were appearing with morphologically significant on selective media after incubation period like greenish black metallic sheen on EMB (fig.3) and pink coloured colonies on Mac Conkey agar (fig. 4), yellow coloured colonies of *Staphylococcus* spp., on Mannitol salt ager of MSA (fig.5), is both selective and differential. It contains 7.5% NaCl, which selects for organisms which are halotolerant. The media also contains mannitol and phenol red, which allows differentiation of organisms based on whether or not they are able to ferment mannitol. If mannitol is fermented, the acidic fermentation products react with the phenol red pH indicator, which changes color from red to yellow [1]. Further, spindle shaped colonies of *Pseudomonas* on nutrient agar turns to pinkish discoloration (fig.6). On nutrient agar, colony of *Pseudomonas* was white in color with smooth edge and convex shape surface. Young colony on nutrient agar were

circular, convex, entire, butyrous, glistening, nearly opaque, and pale yellow with darkened centers. When the colony was observed under sun light, small fluorescent color greenish yellow was appeared around colony growth. *P. aeruginosa* isolates frequently obtained from respiratory and urinary tract secretions, has a mucoid appearance, which is attributed to the production of alginate slime. The smooth and mucoid colonies are presumed to play a role in colonization and virulence. *Pseudomonas aeruginosa* is the third leading cause of hospital-acquired UTIs, accounting for about 12 percent of all infections of this type. The bacterium appears to be among the most adherent of common urinary pathogens to the bladder uroepithelium. As in the case of *E. coli*, urinary tract infection can occur via an ascending or descending route. In addition, *Pseudomonas* can invade the bloodstream from the urinary tract, and this is the source of nearly 40 percent of *Pseudomonas* bacteremias. *P. aeruginosa* produces circular mucoid smooth colonies with emits sweat grape odor in nutrient agar [7] and mixed growth of bacteria on nutrient agar (fig.7).

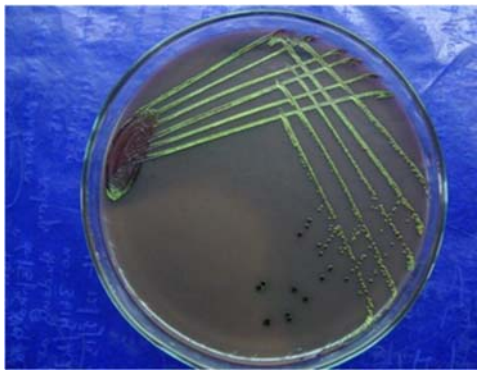

Fig 3: Greenish black metallic sheen on EMB

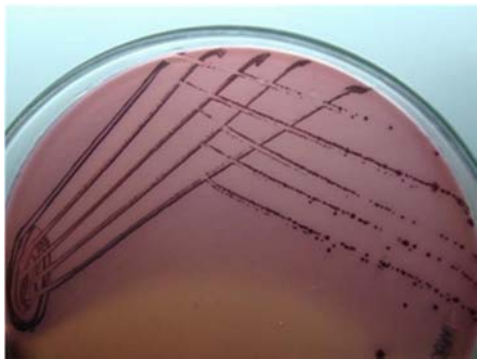

Fig 4: Pink colonies on Mac Conkey agar

Fig 5: *Staphylococcus* spp. on Mannitol Salt Ager

Fig 6: *Pseudomonas* on Nutrient Agar

Fig 7: Mixed growth of bacteria on nutrient agar

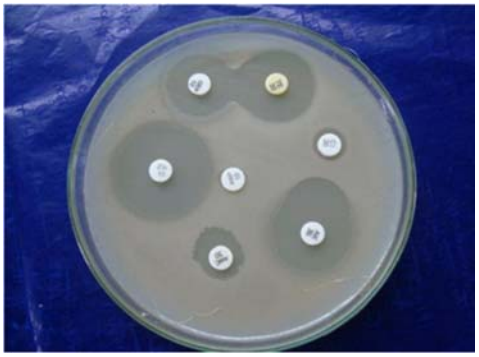

Fig 8: ABST of Urine culture sensitivity for Enrofloxacin

The findings of the present study are in agreement with [18] who reported that *E. coli*, *Proteus*, *Staphylococci*, *Streptococci*, *Enterococus*, *Pseudomonas* were the common pathogens associated with urinary tract infections in dogs. Whereas, Ulrika [16] reported that the *Escherichia coli* was the most frequently isolated pathogen (68%) followed by staphylococci (11%) in most of the canine urinary tract infections. *Staphylococcus aureus* and *Proteus mirabilis* were also found to be among the most common infecting bacteria in UTI cases in dogs [4]. Female dogs are more often affected than male dogs and majority of canine UTIs are caused by a single bacterial species and *E. coli* is singularly most prevalent in canine UTIs [10].

Further, antibiotic sensitivity test revealed maximum sensitivity zone of 2.2cm towards Enrofloxacin followed by Amoxicillin (1.5 cm), Ceftriaxone (0.8cm) and Amikacin (0.4cm), respectively [14] (Fig.8). opined that the antibiotic sensitivity test provides information of suitable antibiotics to which the bacteria is susceptible and reported that *Staphylococcus* and *E. coli* were the common bacteria isolated from urinary tract infections and ceftriaxone & enrofloxacin

were the effective antibiotics. Enroflacin & Amoxicillin were highly effective antibiotics against *E.coli, Staphylococci, Streptococci* organisms that were isolated from urinary tract infections [14]. Enrofloxacin was reported to be highly effective towards otitis and urinary infections in dogs, because concentrations of the drug will be much higher in these sites than the serum levels can be achieved [5]. Under some circumstances therapy may be initiated without knowledge of urine culture results but with empirical choice of antimicrobial agents [13].

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