INFECTIONS OF THE URINARY TRACT OF BACTERIAL ORIGIN IN DOGS AND CATS

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ABSTRACT

The diseases of the urinary tract in small animal medicine, especially in dogs and cats, occur frequently with different etiologies. The most common cause of urinary tract infections (UTIs) are bacteria which enter upwards through the urethral opening. UTIs occur in hosts with compromised defence mechanisms in which the virulent microbes can adhere, multiply and persist in the urinary tract. In addition to bacteria, also viruses or fungi may infect the urinary tracts. Bacterial infection can develop in the upper (kidneys and ureters) or lower (bladder, urethra) urinary tract and are manifested by various clinical signs such as: frequent or difficult and painful urination, presence of blood in the urine, or its foul odour. The symptoms in sick animals are often accompanied by fever, discomfort, and loss of appetite. The UTIs in dogs and cats are caused by both Gram-negative (e.g. *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella* spp., *Proteus* spp.) and Gram-positive (e.g. *Staphylococcus* spp., *Streptococcus* spp., and *Enterococ-*

cus spp.) bacteria. The properly performed aseptic sampling of the urine is one of the most important steps in bacteriological diagnostics. In this study, 105 urine samples were collected from dogs and cats. The cultivation, microscopy and biochemical examinations were used for species identification. Finally, the bacteriological examination included also determining the susceptibility of pathogens against antibiotics by the disc-diffusion method. The results were processed and expressed as a percentage according to identified pathogens, animal species, sex, breed and age. The uropathogens were diagnosed mainly in males aged 7—10 years. The most commonly identified pathogens were *Escherichia coli*, *Streptococcus* spp. and *Staphylococcus* spp. Tests of bacterial susceptibility to antibiotics showed that enrofloxacin, cefotaxime, and cephalosporins were the most effective against uropathogens.

Key words: bacterial pathogens; cats; dogs; urinary infections; urinary tract

INTRODUCTION

Urinary tract infections (UTIs) in the veterinary literature are used to describe both bacterial cystitis and subclinical bacteriuria. They are caused mainly by bacterial pathogens, but urinary disorders correlate very often with the occurrence and the development of urinary bladder stones, insufficient protection of the urogenital tract (UGT) against external influences due to immunosuppression, tumours and others. Also, viruses, fungi or parasites can cause UTIs. The age, gender, immunity, dietetic principles or trauma are important predisposing factors contributing to urinary tract disorders in small animals. Persistent or recurrent UTIs can involve refractory bacterial isolates and may also prove difficult to resolve using conventional antimicrobial therapy [8].

From the anatomical point of view, it is necessary to mention that the urethra in the females of dogs and cats is shorter and rather wide in comparison to males and this is the reason why urethritis develops more often in males than in females. The urethra in tomcats is particularly very narrow and long which is a frequent cause of their urinary disorders [6].

Another risk factor that is significant in the development of UTIs is the age of the animals. UTIs are more common in older female dogs. The mean age at diagnosis regardless of sex is approximately 7–8 years [16].

Dogs and cats suffering from urinary tract problems show symptoms such as dysuria, even anuria, stranguries, enlarged abdomen or haematuria. During urination the animals can adopt a hunched posture which indicates painfulness. The symptoms may suggest inflammatory processes in both lower and upper parts of the urinary tract that may be induced by various factors whether primary infectious agents or secondary issues.

Composition, concentration and the volume of eliminated urine directly affect the occurrence of uroinfections. Pathological processes in the body leading to the loss of fluids and electrolytes result in excessive multiplication of uropathogens [5]. Physiologically, urine must not contain blood, proteins, glucose or microorganisms. It should be sterile without any contamination. The presence of any of the above-mentioned components indicates pathological status. Collection of urine samples and their examination is an important part of basic clinical examinations.

Frequently, the problems associated with urination

in dogs and cats are related to the composition of feed consumed in the long term by these animals. The feed with high levels of proteins that exceeds the requirements of animals results in increased synthesis of urea that increases the burden on kidneys. Excessive intake of animal fat aggravates the course of chronic failure of kidneys which is manifested by increased concentration of lipids and cholesterol in animal blood. Feed deficient in fibres suppresses the progress of microbial fermentation in the large intestine. This results in reduced elimination of urea in the faeces and, on the contrary, increased excretion via urine that again puts considerable load on the kidneys. In this way, the renal insufficiency is exacerbated [17].

A diet, age or hormone imbalance can influence the creation of urinary stones. The urinary stones (uroliths) are defined as the solid crystalline masses and the most common urinary stones in cats and dogs are struvite and calcium oxalate (Fig. 1).

The phosphate (magnesium-ammonium-phosphate or struvite) stones form at alkaline pH and thus the intake of nutrients, particularly those of plant origin that increase pH, stimulates formation of such stones that are a conglomeration of bacteria, crystals and protein matrix. Deficient production of the active form of vitamin D by kidneys results in hyperphosphataemia, demineralization of bones and subsequent deposition of crystals in the renal parenchyma. The crystals irritate the tissue and lead to inflammatory processes. This type of stones occurs most frequently in dogs. Struvite stones usually develop in dogs due to a

Fig. 1. Types of urinary stones

Source: <https://allhealthpost.com/crystals-in-urine/>

UTI with urease-producing bacteria while in cats, they are typical as sterile stones [15]. These bacteria create alkaline urine, the ideal environment for struvite to form in.

Acidic pH predisposes for formation of oxalate stones. Calcium oxalate is the most common type of stone found in the upper urinary tract of both species of cats and dogs [7]. Feeding the acidifying compounds, hypercalcemia and increased intake of vitamins A and C result in the formation of oxalate stones that are typical of cats, particularly tomcats.

The generally reduced intake of water increases the concentration of urine and contributes to increased occurrence of stones in the urinary tract. The uncontroversial recommendation for preventing the recurrence of calcium oxalate stones is to increase the urine volume. Dogs that produce more concentrated urine and that urinate less frequently might be predisposed to calcium oxalate formation [14].

The samples of urine for bacteriological examination can be collected in several ways (spontaneous miction, catheterization, cystocentesis). The optimum method is cystocentesis which prevents undesirable contamination of urine during sampling. The aim of the bacteriological examination of urine is the identification of the agent of infection and the determination of suitable therapy based on the determination of the susceptibility of the infectious agent to antibiotics. The pathogens that occur most frequently in the urinary tract are microorganisms of the family *Enterobacteriaceae* (*E. coli*, *Proteus* spp., *Klebsiella* spp.). However, even the presence of Gram-positive bacteria, such as staphylococci, streptococci or enterococci in the urinary system is also frequent [8].

Currently, due to excessive and frequently imprudent use of antibiotics, resistance to them increases and represents a serious issue not only in veterinary but also in human medicine. The resistance of enterobacteria, particularly of multiresistant strains of *Escherichia coli* and *Klebsiella pneumoniae*, the frequent causative agents of uroinfections in animals and humans, has become a worldwide problem. The multiresistant strains are those that are resistant to the effective antimicrobials of at least 3 different groups [9].

The aim of this study was to identify bacteria that most frequently induce the inflammation of the urinary tract and to provide information about the percentage of occurrence of these pathogens in dogs and cats. Urine samples were taken *lege artis* from dogs and cats that showed clin-

ical signs of urinary problems. The disc-diffusion method was used to determine the susceptibility or resistance of the isolated bacteria to clinically related antibiotics. The obtaining of an antibiogram is an important step in the struggle against increased antibiotic resistance. The study also included the evaluation of the data with respect to the most affected individuals according to animal species, gender, age and breed.

MATERIALS AND METHODS

Samples of urine must be collected into sterile vessels, preferably in the morning. Urine can be obtained during spontaneous micturition, by manual compression of the urinary bladder, catheterization or cystocentesis [19]. In this study, 105 samples were collected into sterile vessels mainly during spontaneous micturition of the animal patients. All procedures involving animals followed the guidelines stated in the Guide for the Care and Use of Animals (Protocol No. 3323/16-221/3) which was approved by the State Veterinary and Food Administration of the Slovak Republic and by Ethics Commission of the University of Veterinary Medicine and Pharmacy in Košice, Slovakia. Respective aliquots (100 µl volume) of undiluted samples were inoculated onto the surface of solid nutrient media and cultivated at 37 °C for 24 hours under aerobic conditions. The following nutrient media were used: blood agar, Endo agar, CLED agar, Bile-esculin agar (BEA), Baird-Parker agar (BPA) and Mueller-Hinton agar (HiMedia, India). The blood agar was used for the detection of haemolytic activity of potential pathogens such as *E. coli*, staphylococci, and streptococci. Endo agar and CLED agar were used as selective media for Gram-negative bacteria of the *Enterobacteriaceae* family and Mueller-Hinton agar was used for the determination of the susceptibility to antibiotics. Also, 2 diagnostic selective media were used, Bile-esculin agar for the enterococci identification and Baird-Parker agar for the identification and confirmation of coagulase-positive staphylococci, particularly *S. aureus* and *S. pseudintermedius*.

The solitary colonies were subjected to Gram staining, catalase and oxidase testing and some isolates were also subjected to the extended biochemical examination that included the proof of sugar fermentation and other relevant tests. Commercially available test as EnteroTest 24,

StaphyTest 24, StreptoTest 24 (Lachema, Brno, Czech Republic) were used and the results obtained after biochemical examinations were evaluated by means of software TNW ProAuto 7.0. The susceptibility of the isolates to antibiotics was determined by the disc-diffusion method based on the measurement of the inhibition zones on Mueller-Hinton agar (HiMedia, India) according to the Kirby-Bauer's method and the results were interpreted according to EUCAST. For the determination of antibacterial effectiveness, the following antibiotic discs were used: enrofloxacin (ENR), 5 µg/disc; ciprofloxacin (CIP), 5 µg/disc; amoxicillin with clavulanic acid (AMC), 30 µg/disc; cephalotin (CLT) 30 µg/disc; cephalozin (CZL), 30 µg/disc; cefuroxime (CXM), 30 µg/disc; cefoxitin (FOX), 30 µg/disc; cefotaxime (CTX), 30 µg/disc; and trimethoprim-sulfamethoxazole (COT), 25 µg/disc (Thermo Fisher Scientific, UK).

The results were processed and reported as the percentage of positive samples (finding/confirmation of bacterial infectious agent) to negative samples, including the species, gender, age and breed of animals from which the samples originated. In the case of samples in which the agent of infection was confirmed, we differentiated between the mono-infections (1 agent) and mixed infections/co-infections (several agents) in the sample.

RESULTS AND DISCUSSION

Dogs and cats that are kept in households and are not regularly taken out outside are exposed to higher risk of the development of UTIs. Regular and complete emptying of the urinary bladder at least four times per day acts as a preventive measure. The first important step for the correct diagnosis of UTIs is the aseptic urine sampling. The processing of freshly produced urine is suggested for microbiological cultures. The cystocentesis approach is recommended as the procedure that ensures the most reliable result of microbiological examination as the sample will not become contaminated during sampling by impurities and bacteria that may be present in the efferent urinary passages. This method is a safe and simple way of obtaining the samples but requires some expertise [2]. On the other hand, even unskilled persons can collect the urine samples (like the animal's owner), but the collecting of samples via spontaneous micturition can provide dubious results. It is strongly

advised that the urine sample be analysed within 60 min of the collection as it is an unpredictably unstable biological fluid. Therefore, it is recommended that urine samples be examined as soon as possible following collection [12].

In our study, the total number of the collected and examined samples was 105; 83 originated from dogs and 22 from cats. Most of the samples were collected via spontaneous micturition. Not considering the species of the patients, the results showed that 54 samples were positive for the presence of bacteria and 51 were negative. Bacterial infections of the urinary tract were detected mostly in dogs (45 of the 54 were positive samples). Only 9 samples originating from cats were positive. Our results indicated that dogs suffer more frequently from bacterial infections of the urinary system (83 % vs. 17 %) (Fig. 2). The results of this study may have been influenced by the method of sampling (spontaneous micturition). Also, the diets recommended by veterinarians are part of the prevention of the disease as they ensure the correct concentration and composition of urine and thus prevent urolithiasis.

Bacterial infections of the urinary tract were detected in 15 bitches and 28 male dogs and in 2 female cats and 7 tomcats. This indicated that males of both species were affected more frequently by UTIs than the females.

The age of most patients with the detected bacterial UTIs ranged from 10 months to 15 years. Bacterial infections were most frequent in individuals 7–10 years old. The age of the animals and the percentage of positive findings are summarised in Fig. 3.

Fig. 2. Positive results for the presence of bacteria in the urine samples of the dogs and cats

Fig. 3. Age distribution of the dogs and cats with bacterial UTIs

The breeds of all of the animals were not reported. However, of the positive samples with known breed ap-purtenance, most originated from the breed Chihuahua (6 of 26 samples). Other affected breeds were: Dachshund (3×), Yorkshire Terrier (2×), Bichon (2×), Cocker Spaniel (2×), Shar-Pei, Beagle, Poodle, French Bulldog, Schnauzer, Maltese dog, Vizsla, German Shepherd and mongrels.

The species, age and gender of the patients, particularly the respective anatomic differences in the UGT between males and females may constitute important predispo-sitions for the development of urinary tract infections. In dogs, UTIs were diagnosed in 2—3 % of the animals and was more frequent in females than in males; particularly affecting the older dogs. In cats, the positivity was lower than 1 % and the infections were more frequent in older in-dividuals, at the age of 10 or more years [3]. However, tom-cats suffered more frequently from urogenital infections than the females due to the anatomical structure of the ure-thra that is narrow and long. This results in stagnation of urine caused even by minimal obstruction, and the devel-opment of conditions that support propagation of micro-organisms [13]. Sometimes more care and attention may be devoted to dogs with respect to their hygiene habits and protection against diseases in comparison with cats, but one cannot accept this as an explanation why less bacterial

infections should occur in dogs. On the contrary, this can result in more frequent diagnosis of urogenital infections by veterinarians. The percentage evaluation of our results showed that UTIs affected dogs more frequently than cats. Our examinations showed that 28 male dogs were affected. A lower prevalence was detected in cats and in this species the males were affected more (7 males, 2 females). With re-gard to the age of the animals with positive results, the posi-tivity was observed in various age categories. The majority of positive samples originated from animals 7—10 years old which confirms the assumption that urinary tract in-fections occur more frequently in older individuals.

The presence of urinary stones is closely related to the development of secondary infections. This is considered with regard to the occurrence of UTIs in various breeds of dogs, as the small breeds are more susceptible to forma-tion of urinary stones. Thus, predisposition to this problem is expected in Yorkshire Terrier, Dachshund, Chihuahua, Miniature Schnauzer, Shi-tzu, Poodle, Bichon, but also in Dalmatian; although the latter belongs to larger breeds. The occurrence of stones in cats involves breeds such as Burma, Himalayan and Persian cats [10]. In our study, the highest number of positive samples originated from the Chihuahua breed (6 cases). A higher percentage of posi-tive samples were found in Dachshund and Yorkshire Ter-

Fig. 4. Percentage proportion of the most frequently detected bacterial agents of the urinary tract.

rier breeds. One can suppose that secondary UTIs may be a consequence of the presence of urinary stones, although this has not been confirmed.

Physiologically, no microorganisms occur in the kidneys or the urinary passages. However, bacteria may be introduced to the urinary tract by blood or ascend there from the efferent ways. Generally, the most common causative agents of canine UTIs are *E. coli* bacteria [1].

Our examination of urine samples showed that in our case, the UTIs were most frequently caused by *E. coli* bacteria (haemolytic and non-haemolytic together—35.5 %) and *Streptococcus* spp. (28.1 %). The representatives of the genus *Staphylococcus* rated third (23.4 %). Streptococci and staphylococci were most abundant in the group of Gram-positive bacteria; altogether 51.5 % of the samples were positive for its presence. Other bacteria like *Serratia* spp., *Pseudomonas* spp. or *Bacillus* spp. were detected at very low counts (up to 2 %). The percentage proportion of the most frequently detected bacteria in animal patients are presented in Fig. 4.

When investigating bacterial diseases, we often encounter infections induced by several species of microorganisms. They are referred to as mixed infections or the so-called co-infections. According to our culture investigations, the proportion of mixed infections was lower (26 %) than that of mono-infections (74 %). Co-infections were confirmed in 13 samples from dogs and only 1 sample from cats. The proportions of individual bacterial pathogens in the co-infections varied. Mixed infections were rel-

atively frequently caused by staphylococci and streptococci (28 % of all mixed infections). The second more frequent combinations of bacteria were *E. coli* and staphylococci (14 %) and *E. coli* and streptococci (14 %). Mono-infections were detected in 8 samples from cats and 32 samples from dogs.

The microbial disease agents were tested for their susceptibility to antibiotics (ATBs) using the disc-diffusion method. The antibiotics were selected on the basis of recommendation of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and also on the basis of information about drugs used in small animal practice in patients suffering from UTIs. For the therapeutic purposes recommended were those antimicrobials that ensured the biggest inhibition zone diameters in the tests of bacterial susceptibility. Bacterial colonies isolated from one sample were tested for several ATBs in order to allow the veterinarian to select the best therapeutical approach.

To alleviate the patient's signs, amoxicillin-clavulanic acid (AMC) (11–15 mg.kg⁻¹ p.o. every 8 h) or trimethoprim-sulphonamide (15 mg.kg⁻¹ p.o. every 12 h) was administered as an initial treatment until obtaining results of the tests on antibiotic resistance. The recommended length of treatment is 1–2 weeks [18].

Our determinations of the susceptibility of colonies to individual types of antibiotics showed that the most effective were enrofloxacin (ENR) and cefotaxime (CTX) that most effectively inhibited the growth of *E. coli*. Cephazolin (CZL) inhibited particularly Gram-positive bacteria. The

aim of our study was to determine the causative agent of UTI and its susceptibility to antibiotics. However, the attending veterinarian is responsible for the selection of the drugs and their dosages.

E. coli was susceptible to enrofloxacin and cefotaxime in 33 % of the cases, *Staphylococcus* spp. to cephalosin in 26 %, *Streptococcus* spp. to cefotaxime and cephalosin in 24 %, *Klebsiella* to enrofloxacin in 50 % and *Pasteurella* spp. to enrofloxacin in 75 %. Additional antibiotics subjected to the disc-diffusion testing were ciprofloxacin, cefuroxim, amoxicillin, cotrimoxazole, cephalotin and cefoxitin that were also effective against bacteria but less frequently than the antibiotics mentioned above. During the years 2002–2007, agents of UTI in dogs were tested at the Western College of Veterinary Medicine in Canada. The agents most frequently isolated from positive samples were *E. coli*, *Staphylococcus intermedius*, *Enterococcus* spp. and *Proteus* spp. Increasing resistance was detected in *E. coli*. In general, the most frequent UTIs inducing bacterial agents were *E. coli* [1]. The examination of urine samples in our study also showed that the UTIs were most frequently caused by *E. coli* bacteria (haemolytic and non-haemolytic strains 36 %) and *Streptococcus* spp. (28.1 %). The third most frequently identified pathogens were the members of the genus *Staphylococcus* (23.4 %). Also, other studies have confirmed the high antibiotic resistance of *E. coli* isolates from dogs presented with UTIs with high levels of resistance to penicillin-G (99 %), clindamycin (100 %), tylosine (95 %), and cephalotin (84 %). On the other hand, there were relatively low levels of resistance to enrofloxacin (16 %) and orbifloxacin (21 %) [11].

Drugs pass through the blood to kidneys, to the primary urine and are then excreted by efferent passages and in this way, they eliminate the microorganisms. Thus, it is necessary to ensure sufficient concentrations of the ATBs in the urine, kidneys and urinary bladder. When speaking about sufficient concentrations, we use the term MIC (minimum inhibitory concentration) which is the lowest concentration of an antibiotic needed for elimination of pathogens [4]. In order to alleviate the patient's symptoms, the initial treatment involves the use of amoxicillin potentiated by clavulanic acid (11–15 mg.kg⁻¹ p. o. every 8 h) or trimethoprim-sulphonamide (15 mg.kg⁻¹ p. o. every 12 h), administered in the indicated cases even before obtaining laboratory results about the susceptibility of bacteria to different ATBs. The recommended length of treatment is

1–2 weeks with the absence of side effects; the length of administration must be observed [18]. The relapse of UTIs is prevented by observation of the length of administration of drugs despite the disappearance of signs. Our determinations of susceptibility of bacteria to individual ATBs showed the highest effectiveness of enrofloxacin and cefotaxime that inhibited most effectively the growth of *E. coli*. Cephalosin inhibited particularly Gram-positive bacteria.

CONCLUSIONS

Our results allowed us to conclude that infections of the urinary tract affected more frequently males than females and this was observed for both dogs and cats. The highest number of positive samples (presence of bacteria) originated from the dogs. However, we should mention that the total number of samples obtained from dogs was higher than that from cats and thus we should consider the fact that the owners of cats, particularly those kept outside are not able to detect the UTIs problems as readily as dog owners. Thus, one should realise that this could contribute to some distortion of our results. The age range associated with the highest prevalence of UTIs was 7–10 years. The samples investigated in our study originated from animals of various breeds but included mostly small breeds of dogs, such as Chihuahua, Dachshund or Yorkshire Terrier. The most frequent agents of urinary infections were *E. coli*, *Streptococcus* spp. and *Staphylococcus* spp. According to our testing, the antibiotic enrofloxacin was the most effective against all isolated pathogens which correlated with the literary data that confirmed this antibiotic was one of the most frequently used drugs to treat urinary infections in a small animal clinical practice.

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