Comparative analysis of etiological factors of infectious urocystitis of dogs and cats

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Introduction

Diseases of the urinary system are relevant for cats and dogs, because they occur regardless of the age of the animal or the season. Among all pathologies of the urinary system of small animals, urocystitis is the most common. Cats and dogs tend to suffer from urocystitis both at a young and at a geriatric age. It should be noted that at the age of animals, its etiology and degree of spread plays an important role in veterinary science. Identification of pathogens is an important task in veterinary medicine and can help in the early diagnosis and treatment of this disease. This is due to the high prevalence and the ability of urocystitis to cause severe complications, including sepsis and death. Therefore, in order to accurately diagnose and treat urocystitis, it is necessary to identify the etiological factors involved.

It is estimated that the majority of clinical cases of urocystitis involve urinary tract infections caused by bacteria. However, the etiology of urocystitis is complex and can involve a variety of factors. In this study, the authors aim to determine the etiological factors of infectious urocystitis in dogs and cats.

Methods

The study included 82 sick cats and dogs. Urine collected by cystocentesis was subjected to microscopic and urine culture. Sows were used to find the proportion of infectious and non-infectious urocystitis, to identify microbial cells, to determine the species composition of the microflora and its sensitivity to antibiotics.

Results

The results showed that the percentage of animals studied: 67% were dogs and 33% were cats. Microscopy results showed the presence of different shapes and concentrations of bacteria in the urine of the sick animals. The percentage of animals with infection varied depending on the breed and age of the animal. The study also revealed that the most common bacterium in the urine of dogs was Escherichia coli (29.2%), followed by Staphylococcus aureus (12.5%) and Proteus spp. (8.3%). The obtained pure cultures were cultured on the selective Muller-Hinton medium in order to determine their antibiotic resistance.

Conclusion

The study demonstrated that bacterial urocystitis is the most common type of urocystitis in dogs and cats. The etiology of urocystitis is complex and can involve a variety of factors. The study also revealed that the most common bacterium in the urine of dogs was Escherichia coli (29.2%), followed by Staphylococcus aureus (12.5%) and Proteus spp. (8.3%). The obtained pure cultures were cultured on the selective Muller-Hinton medium in order to determine their antibiotic resistance.

Keywords: urine; cystocentesis; microbiological studies; bacteria; antibiotics.

Reference

A wide-spectrum diagnostic approach to each patient provides an opportunity of urine sediment, ultrasound examination (Shulzhenko et al., 2019). Microscopy of urine sediment and sowing it on dense nutrient media made it possible to determine its sterility. The research material was urine, which was collected by cystocentesis in each clinical case. The frequency of occurrence of bacterial urocystitis in comparison with idiopathic ones and the dead animals received broad-spectrum antibiotics, and already in the beginning of the treatment, were performed on animals diagnosed with bacterial urocystitis during their lifetime. According to the owners, at the beginning of the treatment, the dead animals received broad-spectrum antibiotics, and already in the absence of positive dynamics, the doctors performed cystoscopy and microbiological studies of urine. The obtained results became the reason for further research on the identification of the main causative agents of the disease and their sensitivity to antibacterial drugs. For this purpose on the basis of the veterinary clinic “Merlion” of the city of Lviv, microscopic and bacteriological studies of urine of cats and dogs with urocystitis were carried out during the year, where culture was used in the diagnostic process to determine its sterile. The research material was urine, which was collected by cystocentesis in each clinical case. The frequency of occurrence of bacterial urocystitis in comparison with idiopathic ones and the percentage ratio of this pathology in dogs and cats were determined. Microscopy of urine sediment and sowing it on dense nutrient media made it possible to identify and isolate the pathogen that caused bacterial urocystitis in sick animals, as well as the sensitivity of the obtained culture of the pathogen to antibiotics of different groups. Therefore, an important aspect includes: taking a detailed history, conducting biochemical and hematological examinations, the conditions for obtaining isolated colonies were ensured. The cups were placed in a thermostat at a temperature of 37 °C for 20-24 hours (Matuschek et al., 2014).

Cultivation of urinary tract on meat-peptone broth, meat-peptone agar and blood agar.

Preparation of MPB: 20 g of finely chopped horse blood was added to 1 liter of meat-peptone broth and the medium was heated until the agar dissolved. A slightly alkaline reaction was established with a 20% Na2CO3 solution and poured into a column 5 mm high. Tubes with medium were sterilized in an autoclave at 120 °C for 20 minutes.

Preparation of PMA: 1% peptone was added to 100 mL of broth to increase the nutrient content of the medium. For compaction, 2% agar was added. In order to create a slightly acidic pH, 0.5% table salt was added to the medium (the reaction of the resulting medium was from 7.0 to 7.4). After adding agar, the mixture was heated until incomplete solidification. The resulting medium was poured into Petri dishes with a height of 5 mm.

Preparation of blood agar 10% of defibrinated horse blood was added to the meat-peptone agar at the solidification stage when the temperature was below 50 °C. The resulting medium with a pH of 6.8 was poured into Petri dishes. Then it was autoclaved at a temperature of 80 °C for 15 minutes (Shyrokov, 2011).

The resulting pure culture was transplanted onto Muller-Hinton agar in order to determine the sensitivity of the pathogen to antimicrobial agents using the disk diffusion method. When determining sensitivity by the disk diffusion method, a standard inoculum corresponding to 0.5 according to the McFarland standard was used, that is, it contains approximately 1.5×10⁸ colony-forming units per cubic centimeter. The inoculum was applied with a pipette to the surface of the Petri dish in a volume of 1–2 cm³, distributing it evenly, and removing the excess with a pipette. Petri cups were dried at room temperature for 10–15 minutes and discs with antibiotics were placed on the surface of the nutrient medium (Mueller & Hinton, 1941).

In the process of work, discs impregnated with antibiotic solutions produced by LLK “Aspect” Ukraine were used. The content of antibiotics in the discs corresponds to the recommendations of WHO and TU U 24.4-21615987-001:2009. Application of discs was carried out using sterile tweezers, keeping a distance of 15–20 mm from the disc to the edge of the cup. Immediately after the application of the discs, the Petri dishes were placed upside down in a thermostat and incubated at a temperature of 35 °C for 18–24 hours.

Results

Autopsy of the corpses of animals that suffered from bacterial urocystitis during life showed the presence of pathomorphological changes in the bladder and urethra, which were characterized by purulent cystitis and hemorrhagic urethritis (Fig. 1a). It was found that the bladders were enlarged, filled with cloudy urine with serous-purulent exudate. The mucous membrane was swollen, with numerous dotted and spotted hemorrhages (Fig. 1b). The urethra of the dead animals had a thickened wall, a swollen wall, filled with cloudy urine with serous-purulent exudate. The mucous membrane was swollen, with numerous dotted and spotted hemorrhages (Fig. 1b). The urethra of the dead animals had a thickened wall, a swollen

Postmortem examination: a – purulent urocystitis and hemorrhagic urethritis; b – purulent cystitis; c – hemorrhagic exudate in the thickness of the urethra; thickening of the urethral wall

Fig. 1. Postmortem examination: a – purulent urocystitis and hemorrhagic urethritis; b – purulent cystitis; c – hemorrhagic exudate in the thickness of the urethra; thickening of the urethral wall

Fig. 2. Number of animals with urocystitis: — cats; — dogs

Fig. 3. Correlation infectious and non-infectious urocystitis: — microflora growth is present; — there is no growth of microflora

It should be noted that infectious urocystitis in dogs prevailed in females (8 animals out of 12), and in cats in males (9 animals out of 12), which can be explained by the specificity of the pathogenesis of the disease in these types of animals. Female dogs are more prone to the occurrence of infectious urocystitis, since in them the causative agent most often enters the body from the environment through a short, wide urethra. In cats, a favorable factor for infectious urocystitis is that they often suffer from recurrences of urolithiasis, which is characterized by difficult, painful and frequent urination.

Urine freshly collected by cystocentesis was subjected to microscopy. Microscopy of urine sediment in sick animals showed the presence of coccal microflora or rods in the field of view of the microscope. At the same time, the number of bacteria in the studied material was different. In some samples, bacteria were visualized singly, and in order to detect them, it was necessary to view several slides whereas in other samples the entire field of view of the microscope was covered with bacterial cells (Fig. 4). Regardless of whether bacterial microflora was detected in the urine under microscopy, each sample obtained was subjected to bacteriological examination in order to find out the species composition of the microflora and determine its sensitivity to antibiotics. The method of native microscopy makes it possible to detect bacterial cells only under the condition of aseptic sampling of the material for research. However, it is impossible to reliably establish the type of bacteria with this method, and even more so to prescribe one or another type of antibiotic. Its addition is the conduct of bacteriological studies, which directly establish the presence or absence of a specific pathogen and enable the veterinarian to prescribe, according to the results of the antibioticogram, a drug that will be effective against a specific type of bacteria.

Bacteria of the genera Corynebacterium, Enterococcus, Enterobacteriaceae and Staphylococcus, namely Enterococcus spp., were found in urine during bacteriological examination on MPA, MPB and blood agar nutrient media (33.3%), Escherichia coli (29.2%), Corynebacterium urealyticum (12.5%), Staphylococcus spp. (12.5%), Proteus spp. (8.3%), Staphylococcus haemolyticus (4.2%, Fig. 5).

To determine the antibiotic resistance of the obtained cultures, disks with antibiotics of different groups were used, namely: penicillins, cephalosporins, fluoroquinolones, antibiotics of the tetracycline series and aminoglycosides, in particular: azithromycin, amoxicillin, amoxiclav, gentamicin, doxycycline, metronidazole, ofloxacin, furagin, furamag, cefazolin, ceftriaxone and ciprofloxacin. Antibiotics that are widely used in practical veterinary medicine today were chosen for the study. The results of the research showed (Table 1) that the obtained microorganisms are most sensitive to antibiotics of the cephalosporin group: ceftriaxone – 70.8% of the samples, cefazolin – 45.8% and the fluoroquinolone series, in particular ciprofloxacin and ofloxacin, to which 62.5% showed sensitivity of studied samples. From the group of antibiotics of the furazolidone series, the obtained microorganisms were most sensitive to furamag (54.1%).

type of bacteria that caused infectious urocystitis, but also to establish their sensitivity to antibacterial drugs at the stage of early diagnosis (Marques et al., 2016). And if it is impossible to carry out cystocentesis, it is necessary to prescribe antibiotics that will be most effective against the bacteria (Zazhurovski et al., 2019; Buckingham et al., 2023; Dushy et al., 2023; Fares et al., 2023).

Table 1
Antibiotic resistance of the obtained pure cultures (%)

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>sensitive, %</th>
<th>moderately stable, %</th>
<th>resistant, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin</td>
<td>29.2</td>
<td>16.7</td>
<td>54.1</td>
</tr>
<tr>
<td>Amoxiclav</td>
<td>25.0</td>
<td>29.2</td>
<td>45.8</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>30.0</td>
<td>12.5</td>
<td>54.5</td>
</tr>
<tr>
<td>Dicloxacil</td>
<td>50.0</td>
<td>12.5</td>
<td>37.5</td>
</tr>
<tr>
<td>Metronidazole</td>
<td>20.8</td>
<td>12.5</td>
<td>66.7</td>
</tr>
<tr>
<td>Oxytetracin</td>
<td>62.5</td>
<td>4.2</td>
<td>33.3</td>
</tr>
<tr>
<td>Furagin</td>
<td>37.5</td>
<td>16.7</td>
<td>45.8</td>
</tr>
<tr>
<td>Furamag</td>
<td>54.1</td>
<td>8.3</td>
<td>37.6</td>
</tr>
<tr>
<td>Cefazolin</td>
<td>45.8</td>
<td>8.3</td>
<td>45.9</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>70.8</td>
<td>12.5</td>
<td>16.7</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>62.5</td>
<td>8.3</td>
<td>29.2</td>
</tr>
</tbody>
</table>

That is, for the treatment of bacterial urocystitis, antibiotics of a wide spectrum of action are used, and if there are no positive dynamics during therapy, a urine culture should be carried out in order to establish the specific pathogen and determine its sensitivity to antibiotics. But this approach is uninformative, because if antibiotics are previously used, bacteria develop resistance not only to the antibiotic chosen by the doctor, but also to others that are weaker than it in terms of spectrum of action (Selek et al., 2011). It is also necessary to take into account concomitant factors, among which are urocystitis, which can cause the transition from non-infectious to infectious urocystitis (Ishi et al., 2011). These factors include: pyometra in females, ureolithiasis in cats, development of megacolon and diarrhea due to pathologies of the digestive system (Weese et al., 2011). A separate group includes idiopathic urocystitis, which have an unknown pathogenesis. The most common cause is stress, estrus in females and adverse reaction to a number of medications (Wan et al., 2014). There are also publications that indicate that today idiopathic urocystitis is common in small breed dogs and cats, which are the most stress-sensitive (Seward et al., 2008; Barsanti, 2012). Therefore, all the listed factors always create a risk for the occurrence of infectious urocystitis.

It is important not to forget that correct comprehensive diagnosis is the key to effective treatment (Byron, 2019). In the case of bacterial urocystitis, the first and most important stage of diagnosis is the correct collection of material for examination. Only the examination of urine collected by an aseptic method makes it possible to accurately confirm or refute the diagnosis of bacterial urocystitis, since contamination with secondary micro-

**Discussion**

The issue of infectious urocystitis is relevant for small animals, as the disease occurs in both cats and dogs. Urocystitis of infectious and non-infectious origin is characterized by the same clinical manifestation. Typical symptoms of urocystitis are frequent, painful urination with the release of small portions of urine. During the course of the disease, animals become restless and often try to lick the distal part of the urogenital canal, which does not cause any harm to the animal. Therefore, the generalization of the studies conducted on this pathology is mainly based on the results of sowing urine on dense nutrient media after primary therapy, which does not give the desired positive results.

Therefore, as a result of untimely treatment with antibiotics, to which there are insensitive bacteria that cause infectious urocystitis, often irreversible pathological changes occur in the bladder and urethra (Forrest & Dell, 2007). During the long course of bacterial urocystitis, due to the accumulation of purulent exudate, which contains fibrin, the outflow of urine becomes difficult (Halder et al., 2016). In such conditions, uropathogens have the opportunity to multiply in greater numbers and complicate the course of an already existing pathological process (Behzadi, 2020). Purulent cystitis in combination with hemorrhagic urethritis leads to urosepsis (Guliciuc et al., 2021). Therefore, it is important to establish not only the
rolflora is unacceptable (Gordon, 1990; Tanagho & McAninch, 2004). Microscopy of the urine sediment makes it possible to establish the presence of pathological inclusions in the urine. These include: bacteria, struvite or oxalate crystals, mucus and atypical cells (Tanagho & McAninch, 2004). The method of native microscopy makes it possible to detect bacterial cells only under the condition of aseptic sampling of the material for research. However, it is impossible to reliably establish the type of bacteria with this method, and even more so, it is impossible to prescribe one or another type of antibiotic (Weese et al., 2019). Microscopy is only one of the steps in the staged diagnosis of bacterial urocystitis. The next step is to conduct bacteriological studies which directly establish the presence or absence of a specific pathogen and enable the veterinarian to prescribe a drug that will be effective against a specific type of bacteria in accordance with the results of the antibioticogram. Therefore, to generalize the results of the research we obtained, antibiotics were chosen, which today are the most widely used by veterinary specialists all over the world (Listar et al., 2009; Yu et al., 2020). The study made it possible to generalize and display in percentage terms the frequency of infectious urocystitis in comparison with non-infectious urocystitis, to establish the most common bacterial pathogens in urocystitis and the ways of their elimination through the use of antibiotics to which this microflora is most sensitive.

Conclusions
During the course of bacterial urocystitis, the accumulation of serous-purulent and hemorrhagic exudate in the bladder and urethra was noted. Every third animal (33%) with urocystitis had a presence of bacterial growth in cultures of its urine, which was collected aseptically. The growth of a colony of Enterococcus spp. was most often observed in urine cultures (33.3%). The studied microflora shows the greatest sensitivity to fluoroquinolone antibiotics, cephalosporins and nitrofurans.

The authors declare that no conflict of interest exists.

References
Buckingham, M., Sultana, M., Thomas, J. M., & Andrews, V. (2023). Efficacy of antibiotics to which this microflora is most sensitive.


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