

# Effectiveness of Hypochlorous Acid in Cats with Bacterial Cystitis

## Bakteriyel Sistitli Kedilerde Hipokloröz Asidin Etkinliği

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### ABSTRACT

This study was aimed to reveal the efficacy of hypochlorous acid in cats with bacterial cystitis. The animal material of the study consisted of 14 cats of different races, 1-8 years of age, of different sexes, diagnosed with bacterial cystitis as a result of clinical and urine examinations and bacterial urine culture, who applied to Afyon Kocatepe University Veterinary Health Application and Research Centre Internal Diseases Clinic with complaints of lower urinary system disease and diagnosed with bacterial cystitis as a result of the examinations. After the diagnosis of bacterial cystitis, the cats were divided into 2 groups: fluid therapy + enrofloxacin + vitamin C + nitrofurantoin (group I, n = 7) and fluid therapy + enrofloxacin + hypochlorous acid (group II, n = 7). Both groups were treated for 7 days. It was observed that clinical recovery was faster according to the clinical scoring table created in the cats in group II, and bacterial growth in the urine was eliminated more quickly and effectively as a result of urine cultures. As a result, it was concluded that hypochlorous acid is effective in the clinical improvement and elimination of bacteriuria in cats with bacterial cystitis.

**Keywords:** Cat, cystitis, FIC, hypochlorous acid

### Öz

Bu çalışmada bakteriyel sistitli kedilerde hipokloröz asit etkinliğinin ortaya koyulması amaçlandı. Çalışmanın hayvan materyalini Afyon Kocatepe Üniversitesi Veteriner Sağlık Uygulama ve Araştırma Merkezi İç Hastalıkları Kliniğine getirilen ve yapılan muayeneler sonucunda bakteriyel sistit tanısı konan 14 kedi oluşturdu. Bakteriyel sistit tanısı konulduktan sonra kediler sıvı tedavisi+enrofloksasin+vitamin C+nitrofurantoin (grup I, n=7) ve sıvı tedavisi+enrofloksasin+hipokloröz asit (grup II, n=7) olmak üzere iki gruba ayrıldı. Her iki gruba 7 günlük tedavi uygulandı. Grup II'deki kedilerde oluşturulan klinik skorlama tablosuna göre klinik iyileşmenin daha hızlı olduğu ve idrar kültürleri sonucunda idrarda bakteriyel üremenin daha hızlı ve etkili ortadan kaldırıldığı gözlemlendi. Sonuç olarak hipokloröz asidin bakteriyel sistitli kedilerde klinik iyileşme ve bakteriyürinin giderilmesinde etkili olduğu kanaatine varıldı.

**Anahtar Kelimer:** Kedi, sistit, FIC, hipokloröz asit

### INTRODUCTION

The occurrence of urinary tract infections (UTIs) is multifactorial, due to interactions between the virulence of a microorganism and the anatomical, environmental, and immunological competence of the host. Microorganisms that play a role in UTIs are generally uropathogenic bacteria originating from the enteric flora and ascending from the distal urogenital system to the proximal urethra and urinary bladder.<sup>1,2,3</sup> Urinary tract infections caused by parasitic, fungal, and viral infections constitute less than 1% of all urinary system infections.<sup>4</sup> Bacterial infection rate in cats with lower urinary tract disease symptoms such as pollakiuria, hematuria, strangury, periuria, and urethral obstruction varies between 2% and 19%.<sup>5</sup> Although most UTIs (85%) are caused by a single bacterial pathogen, 2 different species can be isolated in 13% of cats.<sup>6</sup> Infections with more than 1 bacterial species are associated with indwelling urinary catheters and other comorbidities.<sup>7</sup> The most common pathogen isolated from cat urine is *Escherichia coli*, which is present in 39%-59% of positive cultures. Other microorganisms reported to be frequently isolated are *Streptococcus* spp. (2%-19%), *Enterococcus faecalis* (5%-27%) and *Staphylococcus felis* (17%-20%).<sup>8,9,10,11</sup> Clinical signs of lower UTIs include pollakiuria, hematuria, periuria, dysuria,

and strangury. These findings are not specific and can be seen in every case characterized as idiopathic cystitis. The diagnosis of UTIs can be made based on clinical findings, urinalysis findings, and quantitative bacterial culture results. It is very difficult to distinguish cats with bacterial cystitis from cats with idiopathic cystitis and those with clinical or subclinical bacteriuria because the clinical findings and urinalysis results might be the same. Positive urine cultures are indispensable for a reliable diagnosis, especially in cats with other clinical signs of lower urinary tract disease.<sup>4,12</sup> Third- and fourth-generation cephalosporins and fluoroquinolones are overused in cats with lower urinary tract disease. Therefore, UTIs contribute to the development of antimicrobial resistance.<sup>4,13,14,15,16</sup>

Hypochlorous acid is an endogenous agent in all mammals and is effective against a wide variety of microorganisms. Neutrophils, eosinophils, mononuclear phagocytes, and B-lymphocytes produce hypochlorous acid in response to injury and infection through mitochondrial membrane-associated enzymes.<sup>17,18</sup> Hypochlorous acid selectively binds to the unsaturated lipid layer and subsequently disrupts cellular integrity.<sup>19</sup> Hypochlorous acid is used in many fields, such as ophthalmology, dentistry, wound care, hand sanitation, and dermatology in both human and veterinary medicine.<sup>20</sup> Hypochlorous acid shows antimicrobial activity at various times depending on the dilution rate. Undiluted 100% solutions neutralize viruses and bacteria within 1 minute, 25% solutions within 15 minutes, and 50% and 75% solutions within 5 minutes.<sup>21</sup> Hypochlorous acid irrigation has been successfully used in the treatment of neurogenic lower urinary tract dysfunctions and recurrent UTI in humans.<sup>22</sup>

In this study, it was aimed to compare the effectiveness of hypochlorous acid with the effect of nitrofurantoin and to reveal its effectiveness in cats with bacterial cystitis.

## MATERIALS AND METHODS

### Animal Material

Ethical committee approval was received from the Ethics Committee of Afyon Kocatepe University (Date: 24.02.2020, Number: 49533702/220). The animal material of the study consisted of 14 cats of different races, 1-8 years of age, of different sexes, diagnosed with bacterial cystitis as a result of clinical and urine examinations and bacterial urine culture, who applied to Afyon Kocatepe University Veterinary Health Application and Research Centre Internal Diseases Clinic with complaints of lower urinary system disease. The cats were enumerated, and the physical examination findings, laboratory findings, and urinalysis results were recorded in the study.

### Study Groups

14 cats in the study were divided into two groups randomly.

#### Group I

The cats in the first group were treated with standard bacterial cystitis treatment. To do this, a 0.9% NaCl solution was calculated clinically according to the patient's dehydration status, then enrofloxacin (Baytril-K 5%®) at a dose of 5 mg/kg every 24 hours, vitamin C (Vitce®) at a dose of 20 mg/kg every 24 hours intravenously, and a 5 mg/kg dose of nitrofurantoin (Pyeloseptyl®) was administered orally.

#### Group II

For cats in the second group, 0.9% NaCl solution calculated clinically according to the patient's dehydration status, enrofloxacin

(Baytril-K 5%®) intravenously at a dose of 5 mg/kg every 24 hours, and a 20% hypochlorous acid solution diluted with 0.9% NaCl solution (Crystalin 200 ppm®) was administered intravesical through a urinary system catheter.

### Sample Collection and Evaluation

Two milliliters of blood samples was taken from each cat in both study groups on the zeroth, third, and seventh days of the treatment into tubes containing ethylenediaminetetraacetic acid by using a 21-gauge injector tip through the vena cephalic antebrachia. White blood cell, red blood cell, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, hematocrit, and hemoglobin measurements of blood samples were recorded using a fully automatic hemogram device (HumaCount 80TS, Vet Mode, Germany).

Five milliliters of urine samples was taken from cats by cystocentesis in both study groups, and semi-quantitative urobilinogen, glucose, ketone bodies, specific gravity, blood, pH, nitrite, leukocytes, protein, and creatinine were measured immediately (Uranotest 11C®). Urine samples taken by cystocentesis were centrifuged at 1500 rpm for 5 minutes (NF 200R, Nüve, Türkiye), and urine sediment examination was performed under the microscope (Olympus CX41®).

Urine samples taken by cystocentesis from cats in both study groups were kept for bacterial culture under the conditions specified by the laboratory and sent to a private laboratory.

### Clinical Scoring

A clinical scoring table was made and numbered from 0 to 5 according to ultrasonographic imaging and the semiquantitative and sediment examination findings of the urine samples (Table 1).

### Statistical Analysis

In the study, cats brought to Afyon Kocatepe University Veterinary Health and Application Centre Internal Diseases Clinic between April 16, 2020, and May 07, 2021, and diagnosed with bacterial cystitis as a result of the examinations were examined and the growth values in the hematological and bacterial urine cultures were separated into group I and group II, and whether the growth values in the hematological and bacterial urine cultures of the groups showed a significant difference compared to the 0, 3, and 7 days was tested with the Kruskal-Wallis test at the 5% significance level. Statistical Package for the Social Sciences Statistics version 25.0 software (IBM Corp.; Armonk, NY, USA) was used in the analysis of the data compiled in the study.

## RESULTS

In this study, the effects of standard treatment and hypochlorous acid on bacterial cystitis were evaluated with clinical, hematological, clinical scoring, and urinalysis examinations, and the findings were presented after applying the experimental models created for both groups.

Table 1. Clinical Scoring

| Clinical Score | Findings  |
|----------------|---|
| 0              | Normal  |
| 1              | Mild inflammation, clear appearance   |
| 2              | Thickening on bladder wall, mild inflammation   |
| 3              | Inflammation, presence of crystals, presence of leukocytes  |
| 4              | Smelly urine, urination so often, presence of leukocytes, increase in pH                            |
| 5              | Hematuria, presence of leukocytes, epithelial rash, crystal formation, smelly urine, increase in pH |

### Clinical Examination Findings

The zeroth day was determined as the starting time of the treatment in both groups. It was decided which group of cats could potentially be included in the study, and the treatment was applied to that group. Three of the cats in the group I have fully recovered. Four of the cats in group I have shown clinical improvement in their symptoms. Decreasing the number of leukocytes, normalization in pH, hematuria, bad smell, and a decrease in blur were spotted after analyzing the urine in all the cats in group I. Four of the cats in group II have fully recovered. Three of the cats in group II have shown clinical improvement in their symptoms. Decreasing the number of leukocytes, normalization in pH, hematuria, bad smell, and decrease in blur were spotted after analyzing the urine in all the cats in group II.

### Hematological Findings

There was a significant improvement in leukocyte, neutrophil, hematocrit, and platelet values on day 7 compared to day 0 in both groups ( $P < .05$ ). There were no significant differences in hematological findings between day 0 and day 3, and day 3 and day 7 in both groups ( $P > .05$ ).

### Clinical Scoring Findings

The zeroth scores of 4 of the cats in group I were determined as 5, and the day 0 scores of 3 of them were determined as 4. In group I, the third-day scores were determined as 4 in 2 of the cats whose zeroth-day scores were 5, and third-day scores in the remaining 2. In group I, the seventh day score was determined as 2 in 2 of the cats whose zeroth-day scores were 5, 1 in the seventh-day score in 1, and 0 in the seventh-day score in the remaining one. In group I, the third-day score was determined as 3 in 2 of the cats with a zeroth-day score of 4, while the third-day score was determined as 2 in the remaining one. In group I, the seventh-day scores were determined as 0 in 2 of the cats with a zeroth-day score of 4, while the seventh-day score was determined as 1 in the remaining one (Table 2).

The zeroth-day scores of 4 of the cats in group II were determined to be 5, and the zeroth scores of 3 of them were determined to be 4. In group II, the third-day score was 3 in 2 of the cats whose zeroth-day scores were 5, and the third day's score was 4 in the remaining 2. In group II, the seventh-day score was 0 in 2 of the cats whose zeroth-day scores were 5, 1 of them seventh-day score in 1, and score was 2 in the remaining one on seventh day. In group II, the third-day score was determined to be 3 in 1 of the cats with a zeroth-day score of 4, while the third-day score was determined to be 2 in the remaining 2. In group II, the seventh-day scores were determined to be 0 in all of the cats whose zeroth-day scores were 4 (Table 3).

### Urine Analysis Findings

Improvement in urine color was detected in both groups, and a statistically significant difference was found between day 0 and

Table 2. Clinical Scoring Findings of Cats in Group I

| Number | Zeroth Day | Third Day | Seventh Day |
|--------|------------|-----------|-------------|
| 1      | 5          | 4         | 2           |
| 2      | 5          | 3         | 1           |
| 3      | 4          | 2         | 0           |
| 4      | 5          | 4         | 2           |
| 5      | 4          | 3         | 0           |
| 6      | 5          | 3         | 0           |
| 7      | 4          | 3         | 1           |

Table 3. Clinical Scoring Findings of Cats in Group II

| Number | Zeroth Day | Third Day | Seventh Day |
|--------|------------|-----------|-------------|
| 1      | 5          | 3         | 0           |
| 2      | 4          | 3         | 0           |
| 3      | 4          | 2         | 0           |
| 4      | 5          | 4         | 1           |
| 5      | 4          | 2         | 0           |
| 6      | 5          | 4         | 2           |
| 7      | 5          | 3         | 0           |

day 7 in both groups ( $P < .05$ ). In the semiquantitative analysis of urine, no significant difference was found in pH change in both groups ( $P > .05$ ). There was no significant difference found in group I in terms of improvement in protein-creatinine ratio ( $P > .05$ ). A significant difference was found in group II in terms of improvement in protein-creatinine ratio ( $P < .05$ ).

Only *E. coli* growth was detected in 4 (29%) of 14 cats, 2 cats from both groups, on day 0 after the bacterial cultures were made from urine samples taken by cystocentesis from cats in the study. Mixed growing of *E. coli*, *Staphylococcus* spp., *Streptococcus* spp., and *Proteus* spp. were found in 8 (57%) of 14 cats, 2 cats from both groups, on the zeroth day. *Streptococcus* spp. and *Proteus* spp. growth was seen in 1 cat in group I on zeroth day. Growth of *Staphylococcus* spp. was detected in 1 cat in group II on zeroth day. A decrease in the bacterial species grown in bacterial cultures was determined in 3 of the cats in group I, while this number was determined to be 5 in the cats in group II on the third day of the treatment. No bacterial growth was found in 3 of the cats in group I, while *E. coli* in 2 cats, *Streptococcus* spp. and *Proteus* spp. and in a cat *Staphylococcus* spp. reproduction was determined in bacterial cultures made from urine samples taken by cystocentesis on the seventh day of the treatment. No bacterial growth was detected in 4 cats in group II, while *E. coli* in 2 cats and *Staphylococcus* spp. in 1 cat was seen after the bacterial cultures made from urine samples taken by cystocentesis (Table 4 and Table 5). There was a decrease between day 0 and day 7 in terms of bacterial growth in both groups, and a statistically

Table 4. The Results of Bacterial Urine Cultures in Cats in Group I

| Number | Zeroth Day     | Third Day                  | Seventh Day                |
|--------|----------------|----------------------------|----------------------------|
| 1      | Mixed growth   | <i>E. coli</i>             | <i>E. coli</i>             |
| 2      | Mixed growth   | Mixed growth               | Mixed growth               |
| 3      | <i>E. coli</i> | No growth                  | No growth                  |
| 4      | Mixed growth   | <i>Staphylococcus</i> spp. | <i>Staphylococcus</i> spp. |
| 5      | Mixed growth   | Mixed growth               | No growth                  |
| 6      | Mixed growth   | Mixed growth               | <i>E. coli</i>             |
| 7      | <i>E. coli</i> | <i>E. coli</i>             | No growth                  |

*E. coli*, *Escherichia coli*; spp, types

Table 5. The Results of Bacterial Urine Cultures in Cats in Group II

| Number | Zeroth Day                 | Third Day                  | Seventh Day                |
|--------|----------------------------|----------------------------|----------------------------|
| 1      | <i>E. coli</i>             | <i>E. coli</i>             | No growth                  |
| 2      | Mixed growth               | <i>Staphylococcus</i> spp. | No growth                  |
| 3      | Mixed growth               | <i>E. coli</i>             | <i>E. coli</i>             |
| 4      | <i>E. coli</i>             | <i>E. coli</i>             | No growth                  |
| 5      | <i>Staphylococcus</i> spp. | No growth                  | No growth                  |
| 6      | Mixed growth               | <i>E. coli</i>             | <i>E. coli</i>             |
| 7      | Mixed growth               | <i>Staphylococcus</i> spp. | <i>Staphylococcus</i> spp. |

*E. coli*, *Escherichia coli*; spp, types

significant difference was found between day 0 and day 7 in both groups ( $P < .05$ ).

## DISCUSSION

Bacterial cystitis, which is characterized by hematuria, pollakiuria, periuria, and strangury in cats, is usually seen in middle-aged and elderly female cats.<sup>23</sup> Although there was no statistically significant difference in terms of age and gender in this study, a significant part of the cats included in the study consisted of neutered middle-aged male cats.

The prevalence of positive urine cultures in cats with urethral obstruction rises to 33%.<sup>24</sup> Ureteral plugs were detected in 36% (5/14) of the cats in this study. Hugonnard et al reported that bacterial growth increased 24-48 hours after catheter placement in cases of urethral obstruction.<sup>25</sup> There was no increase in bacterial growth observed after the urinary system catheter was placed in the cats with urethral obstruction in this study.

Most cats with bacterial cystitis have a comorbidity that predisposes them to bacterial infection. The most common comorbidities include chronic kidney disease and various endocrinopathies.<sup>10,11</sup> In this study, no comorbidity predisposing to bacterial infection was detected in any of the cats in both groups.

*E. coli* was the most common bacteria that was isolated from urine samples from cats with bacterial cystitis. Other microorganisms reported to be frequently isolated are *Streptococcus* species and *Staphylococcus* species.<sup>6,9,26</sup> In this study, *E. coli* growth was determined in 86% of all cats according to bacterial culture results from urine samples taken by cystocentesis, and *E. coli* was grown alone in only 4 of these cats. *Staphylococcus spp.*, *Streptococcus spp.*, and *Proteus spp.* were found to be grown in urine cultures in this study as well.

Only the urinary system is usually affected, and there are no significant changes in hematological findings in UTIs of cats.<sup>27</sup> An increase in total leukocyte counts was determined in a significant part of the cats in this study, but it could not be revealed that the reason for this increase was bacterial cystitis. Total leukocyte counts and other hematological abnormalities returned to normal values on the third day of treatment in both groups.

Pomba et al<sup>28</sup> reported that oral administration of nitrofurantoin was effective in UTIs with multidrug resistance in cats. Consistent with this finding, in this study, it was determined that clinical improvement and bacterial growth were decreased in all the cats in group I who were administered enrofloxacin together with nitrofurantoin.

There is no evidence of the effect of direct administration of antimicrobials and antiseptics on the urinary bladder in cats.<sup>29,30</sup> There is also no evidence for the efficacy of hypochlorous acid in cases of bacterial cystitis in cats. In this study, clinical improvement was achieved in all of the cats in group II, in which hypochlorous acid was administered. In addition, an effective decrease in the number of bacterial species grown in bacterial cultures in group II cats, in which hypochlorous acid was administered intravesical, was achieved on the third day compared to group I. In cases where mixed growth was determined, bacterial growth could not be prevented in the cultures performed on the seventh day in the cats in group I, whereas in 1 of the cats in group II, which was administered hypochlorous acid, evidence of the effectiveness of hypochlorous acid was revealed by completely inhibiting bacterial growth on the seventh day.

Weyler et al<sup>22</sup>, in their study, applied urinary bladder irrigation with hypochlorous acid in human patients with recurrent UTIs and revealed that bacteriuria was eliminated as a result of this application and that hypochlorous acid and urinary bladder irrigation could replace antibiotic treatment in recurrent UTIs. In this study, hypochlorous acid was administered intravesical together with parenteral administration of enrofloxacin to the cats in group II, and bacteriuria was largely eliminated after the treatment. Clinical improvement and bacterial growth in urine cultures were faster and more pronounced in cats in group II than in cats in group I. Weyler et al suggest that irrigation of the urinary bladder with hypochlorous acid may replace antibiotic therapy in cats with bacterial cystitis.

Severing et al<sup>21</sup> revealed in their study that hypochlorous acid has antimicrobial activity and showed that the time of emergence of this activity is related to the concentration of hypochlorous acid. In this study, 20% hypochlorous acid diluted with 0.9% NaCl solution was used, and it is thought that the decrease in bacterial growth during the treatment process may be related to the antimicrobial properties of hypochlorous acid. Since hypochlorous acid was used only in a single concentration, it has not been demonstrated to what extent more concentrated and more diluted hypochlorous acid solutions will work.

Diagnosis of bacterial cystitis cases in cats can be challenging because cystitis cases are multifactorial. The fact that other etiologies causing cystitis in cats predispose to bacterial growth can complicate the cases of idiopathic cystitis with bacterial cystitis. This study revealed that bacterial cystitis cases in cats generally progressed with multiple bacterial growth. It has been shown that direct application of hypochlorous acid, which has been shown to have antimicrobial activity, into the urinary bladder accelerates clinical recovery and reduces bacteriuria more than the widely used nitrofurantoin. It has been shown that hypochlorous acid inhibits bacterial growth faster than nitrofurantoin and vitamin C; it reduces and inhibits bacterial growth in cases with multiple bacterial growth. Another potential benefit of using hypochlorous acid is that it is an effective treatment method that can be used in the treatment of multi-drug-resistant UTIs without causing new drug resistance. Since the effectiveness of hypochlorous acid varies at different concentrations, the study can be repeated by increasing the sample group and using solutions at different concentrations, and the effectiveness of antibiotics can be compared. According to the results of this study, it would be beneficial to add intravesical administration of hypochlorous acid to the treatment protocols for cystitis cases in cats. The clinical efficacy of hypochlorous acid on bacterial cystitis in cats was demonstrated for the first time in this study.

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