Effectiveness of ozonized saline solution in the treatment of *Proteus* spp. bacterial cystitis

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Abstract

Bacterial cystitis is a common clinical problem among cats and dogs and is one of the main reasons for the administration of antimicrobials. This can cause serious damage to public and animal health, as this practice facilitates the selection of bacteria that are multidrug-resistant to antibiotics. In this context, it is urgent to understand and validate therapeutic modalities that complement antimicrobial treatment in cystitis cases. Ozone therapy has been proposed by scientists owing to the various mechanisms of action in a range of pathologies, both in human and animal medicine. This paper describes the bactericidal action of two different protocols of bladder irrigation with ozonized saline solution (59 µg/mL) in a paraplegic canine with recurrent bacterial cystitis caused by *Proteus* spp. In the first protocol, the bladder instillations were applied once a day for three consecutive days while in the second, successive lavages were performed throughout the day until a significant reduction in the presence of bacteria in the urine sediment. In this study, we were able to demonstrate that repeated bladder instillation within 24 hours was the most effective treatment for *Proteus* compared to a single instillation on successive days.

Key words: bactericidal effect; lower urinary tract infection; medicinal ozone; ozone therapy

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Introduction

Bacterial urinary tract infection (UTI) is a common clinical problem among cats and dogs and is the main reason for antimicrobial administration.[1,2] Approximately 14% of all dogs present with at least one episode of UTI during their lifetime.[3] In dogs, UTI occurs more often in females, elderly animals, and those with certain concomitant illnesses.[4-6] Spinal cord injury is a coexisting condition that increases UTI risk in both human beings and animals; this is because of an impairment of storage and voiding function in the lower urinary tract.[7]

With the emergence of antimicrobial resistance, treatment options for UTI and other infections are becoming increasingly limited. Unconventional treatments, such as oral immunostimulants, mannose, acupuncture, vaginal and oral estrogen, and, more recently, ozone therapy, have been proposed and studied.[8-15]

There have been medical reports of the resolution of refractory bacterial cystitis when treated with medicinal ozone, rather than antimicrobials.[13,15] An experimental study demonstrated the antimicrobial effect of bladder irrigation with ozonized saline solution (O_3SS) as a treatment for *Escherichia coli*-induced cystitis.[16] Given these promising results of ozone therapy in the treatment of bacterial cystitis and the lack of such studies in dogs, this study reported the effectiveness of two different protocols of bladder irrigation with O_3SS in a paraplegic canine with recurrent bacterial cystitis caused by *Proteus* spp.

Case Report

A 14-year-old female Dachshund, paraplegic, weighing 10.2 kg, was referred to the ozone therapy sector of the Veterinary Hospital of the Federal University of Santa Maria, Santa Maria, Brazil, because of recurrent bacterial cystitis caused by *Proteus* spp. Two months after treatment with enrofloxacin, amoxicillin, and potassium clavulanate, the patient developed adverse effects such as pharmacodermia and corneal ulcers.

Alternative treatments were discussed in our team and ozone therapy was selected, which consisted of bladder instillations of O_3SS once a day for 3 consecutive days. To obtain O_3SS, 1 L of 0.9% NaCl solution was ozonized for 10 minutes at a concentration of 59 µg/mL in an O_3 generator apparatus (model OeL1.5RM, Ozone and Life, São José dos Campos, Brazil), with an O_2 flux of 0.25 L/min. After antisepsis of the external genitalia with 1% chlorhexidine gluconate, a bladder catheterization was performed using a sterile 8-Fr urethral catheter (Mark Med, Bragança, São Paulo, Brazil) lubricated with lidocaine hydrochloride jelly (20 mg/g). First, the urinary bladder was drained through the urethral catheter aided by syringes and 10 mL of urine was separated for urinalysis, culture, and antibiogram. Next, within a 20-minute interval, the bladder was washed with 1 L of O_3SS at a concentration of 59 µg/mL.

Table 1 shows the results of the urine analysis (urinalysis, culture, and antibiogram) before the treatment (D0), and after the first (D1), second (D2), and third (D3) treatments. As the urinary bacterial load did not reduce after D3, we started a

Table 1: Urinalysis, urine culture, and antibiogram profile of the treatment protocol performed once a day for 3 consecutive days in a female Dachshund canine with bacterial cystitis before the treatment (D0), after the first treatment (D1), after the second treatment (D2), and finally after the third treatment (D3)

	D0	D1	D2	D3
Urinalysis				
Color	Yellow	Yellow	Yellow	Yellow
Odor	Sui generis	Malodorous	Malodorous	Sui generis
Turbidity	Cloudy	Cloudy	Cloudy	Cloudy
Specific gravity (kg/m^3)	1036	1032	1036	1030
pH[a]	8.5	8	8	8
Protein[a]	1+	2+	2+	1+
Transitional epithelial cells (/400× magnification field)[b]	1	2	1	0
Red blood cell (/400× magnification field)[b]	6.8	3.6	1.2	2.4
White blood cell (/400× magnification field)[b]	>50	24.2	30.8	10.2
Bacteria[c]	Many[d]	Many[d]	Many[d]	Many[d]
Struvite[c]	Many	Few	Few	Moderate
Uroculture (colony-forming unit/mL)				
Proteus spp.	1.2×10^7	6.5×10^7	5.9×10^7	2.5×10^7
Antibiogram				
Gentamicin	Susceptible	Susceptible	Susceptible	Susceptible
Marbofloxacin	Susceptible	Susceptible	Susceptible	Susceptible
Neomycin	Susceptible	Susceptible	Susceptible	Susceptible
Nitrofurantoin	Resistant	Resistant	Resistant	Resistant
Amoxicillin + clavulanic acid	Susceptible	Susceptible	Susceptible	Susceptible
Enrofloxacin	Intermediate	Intermediate	Intermediate	Intermediate
Cephalotin	Susceptible	Susceptible	Susceptible	Susceptible
Trimethoprim + sulfadiazine	Susceptible	Susceptible	Susceptible	Susceptible

Note: Superscript a was measured with a urine reagent strip. Superscript b indicates the data were the average between 10 slide fields. Superscript c was determined by microscopic examination. Superscript d indicates the presence of cocci and bacilli-type bacteria.

conventional treatment with amoxicillin and potassium clavulanate (12.5 mg/kg, twice a day, 10 days in total), which effectively resolved cystitis.

Because the animal had paraplegia, the tutor was instructed to perform daily bladder compressions to induce urine drainage. However, 6 months after the first treatment, the tutor noted color and odor alterations in the animal's urine. The patient underwent another physical examination, but no clinical alterations were observed. However, on ultrasound examination, the bladder presented medium repletion filled with suspended anechoic and echogenic material (sediment) and small hyperechogenic, punctiform, and linear structures suggestive of crystals or small clots. The bladder wall had thickened, with an irregular mucosal layer and evident stratification of the layers (diameter, approximately 0.82 cm; **Figure 1A**).

A bladder catheterization was performed following the aforementioned antisepsis procedure for urine collection and initiation of therapy. Due to the previous therapeutic failure (3 consecutive days of bladder irrigation), we adopted a new protocol in which successive lavages were performed throughout the day until a significant reduction in the presence of bacteria in the urine sediment. This was achieved after the third bladder irrigation, performed every two hours. For each irrigation, 1 L of O_3SS for 10 minutes at a concentration of 59 µg/mL in an O_3 generator device, with an O_2 flux of 0.25 L/min.

Table 2 shows the results of the urinalysis, which was performed on the same day before treatment (T0), and after the first (T1), second T2, and third (T3) lavages.

To report this case, we had the consent of the guardian of the animal and the veterinarians who participated in the case.

Discussion

Herein, we document the care provided to a paraplegic canine patient with recurrent cystitis caused by a multidrug-resistant strain of *Proteus* spp. None of the drug approaches - different combinations of antibiotics for different periods - could resolve the infection. Therefore, the patient was referred to our hospital for ozone therapy.

The ineffectiveness of conventional treatments due to microbial resistance to antibiotics has been extensively reported, and both veterinarians and guardians have been searching for alternatives to cure or improve the living conditions of companion animals. The literature has demonstrated the antimicrobial effects of ozone in dogs in preventing dental plaque formation and its prophylactic use to reduce bacterial colonization in the conjunctival sac and periocular skin.[17,18]

In our first therapeutic approach, we performed the conventional protocol of bladder irrigation, based on the results obtained in an experimental study.[16] On the 1st day, a urine sample was sent for culture and an antibiogram. Despite successive days of bladder irrigation, the infection did not respond to treatment - urinalysis showed the persistence of bacteria. At the end of the lavages, the urine culture results of the D0 sample revealed bacterial susceptibility to amoxicillin-

Table 2: Urinalysis, urine culture, and antibiogram profile of the treatment protocol performed on the same day in a female Dachshund canine with bacterial cystitis before treatment (T0), after the first bladder irrigation (T1), after the second bladder irrigation (T2), and finally after the third bladder irrigation (T3)

	T0	T1	T2	T3
Urinalysis				
Color	Brown	#	#	Red
Odor	Malodorous	#	#	Sui generis
Turbidity	Cloudy	#	#	Cloudy
Specific gravity (kg/m^3)	1040	#	#	1028
pH[a]	8.5	#	#	8
Protein[a]	3+*	#	#	3+*
Transitional epithelial cell (/400× magnification field)[b]	6*	#	#	1
Squamous epithelial cell(/400× magnification field)[b]	2	#	#	1
Red blood cell (/400× magnification field)[b]	>100*	#	#	>500*
White blood cell (/400× magnification field)[b]	>200*	#	#	18*
Bacteria[c]	Many[d]	#	#	Few*
Struvite[c]	Moderate	#	#	0*
Uroculture (colony-forming unit/mL)				
Proteus spp.	7.9×10^7*	9.0×10^6*	6.9×10^2*	5.6×10^2*
Antibiogram				
Gentamicin	Susceptible	Susceptible	Susceptible	Susceptible
Marbofloxacin	Susceptible	Susceptible	Susceptible	Susceptible
Neomycin	Intermediate*	Intermediate*	Intermediate*	Susceptible
Nitrofurantoin	Resistant	Resistant	Resistant	Resistant
Amoxicillin + clavulanic acid	Susceptible	Resistant*	Intermediate*	Intermediate*
Enrofloxacin	Susceptible*	Susceptible*	Susceptible*	Susceptible*
Cephalotin	Intermediate*	Intermediate*	Intermediate*	Susceptible
Trimethoprim + sulfadiazine	Resistant*	Intermediate*	Intermediate*	Intermediate*

Note: Superscript a was measured with a urine reagent strip. Superscript b indicates the data were the average between 10 slide fields. Superscript c was determined by microscopic examination. Superscript d indicates the presence of cocci and bacilli-type bacteria. Superscript * indicates the difference in the observation time points between Tables 1 and 2.

clavulanate; therefore, these drugs were prescribed. At this time, the infecting strain was identified as *Proteus* spp., which is characterized by invasive growth and rapid multiplication: the bacterial population doubles every 3.5 hours at 37°C.[19] Therefore, bacteria that were not eliminated through bladder irrigation could recolonize the bladder mucosa within 24 hours. The colony-forming unit (CFU) count increased from one day to the next as it did between D0 and D1 (**Table 1**). We hypothesized that the inability of ozone therapy to kill the residual bacteria might cause bacterial recolonization. In agreement with our hypothesis, another study observed the same effect following treatment with ozonized distilled water of root canals experimentally infected with *Enterococcus faecalis* and *Candida albicans*.[20] After 10 days of antibiotic treatment, a complete remission of the infection was observed.

In the second therapeutic moment, 6 months later, in a new recurrence in the same patient, due to the previous failure, we performed successive irrigations on the same day. At T3 (after 3 washes), the bacterial count dropped drastically, following which we stopped the washes. This result was confirmed by the urine culture, which showed a decrease in the bacterial population (from almost 80 million CFU/mL to 560 CFU/mL). According to the literature, to consider that bacteriuria is consistent with cystitis, a bacterial count of > 10,000 CFU/mL is needed for samples obtained by catheterization; any value < 1000 CFU/mL should be considered as contamination.[21] Therefore, we achieved the resolution of the clinical condition with the isolated use of bladder irrigation with O_3SS.

During the procedure, urinalysis showed an increase in the red blood cell count. We considered this event to be related to the removal of the biofilm that covered the bladder mucosa, visibly altered by a chronic inflammatory process (**Figure 1**), which facilitated the diapedesis of these cells into the bladder lumen. Persistent bleeding, probably due to the antioxidant and anti-inflammatory actions of ozone on biological membranes,[22,23] was not reported. After washing, the bladder environment changed considerably. This change was visible on the ultrasound image, characterized by a marked reduction in the suspended echogenic content (sediment) and small hyperechogenic, punctiform, and linear structures suggestive of crystals or small clots (**Figure 1B**). Furthermore, the moderately distended urinary vesicle was approximately 0.14 cm thick.[24] In this case, the thickness was significantly increased (0.82 cm), probably in response to the chronic inflammatory process.

O_3SS selected a population with intermediate susceptibility to amoxicillin-clavulanate. Conversely, it seems to have changed the pattern of susceptibility to cephalothin and neomycin, which could expand the therapeutic options. We did not find any publications that strongly linked this action to ozone therapy. Therefore, in a controlled experiment with a sufficient sample size, which is a limitation in this report, we

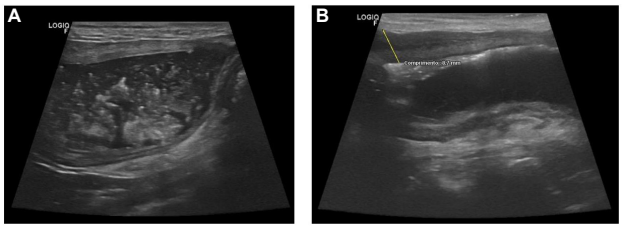

Figure 1: Ultrasound of the bladder of a female Dachshund canine with bacterial cystitis at 6 months after the first treatment.
Note: (A) The evident and accentuated stratification of the urinary bladder wall layers, in addition to the presence of anechoic and echogenic content in suspension (sediment) and small hyperechogenic, punctiform, and linear structures suggestive of crystals or small clots before the beginning of treatment. (B) The marked reduction in suspended echogenic content (sediment) and small hyperechogenic, punctate, and linear structures suggestive of crystals or small clots.

may be able to statistically perceive this correlation.

This report demonstrates that the effective bactericidal action of O_3SS in bacterial cystitis depends on the infecting agent. Therefore, it is important to know the characteristics of the pathogenic bacterial growth to determine the most effective protocol. Repeated bladder instillation within 24 hours was the most effective treatment for *Proteus* compared to a single instillation on successive days.

Author contributions
Study conception and design, and data collection and interpreting: AME and AB. Urine analysis and cooperation among the authors: NVB. Carrying out and interpreting the urine culture and antibiogram: CM and DC. Manuscript revision: JFC and CMA. All authors approved the final version of the manuscript.

Conflicts of interest
The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest.

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