

Evaluation of Urinary Catheters Coated with Sustained-Release Varnish of Chlorhexidine in Mitigating Biofilm Formation on Urinary Catheters in Dogs

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Background: Biofilm formation occurs commonly on urinary catheters.

Objectives: To assess the efficacy of urinary catheters coated with sustained-release varnish of chlorhexidine in decreasing catheter-associated biofilm formation in dogs.

Animals: Thirty client-owned dogs.

Methods: Prospective study. Thirteen dogs were catheterized with urinary catheters coated with sustained-release varnish of chlorhexidine (study group), and 13 dogs were catheterized with an untreated urinary catheter (control group). Presence and intensity of biofilm formation on the urinary catheters were assessed and compared between the groups by evaluating colony-forming units (CFU) of biofilm bacteria, and semiquantitatively, using confocal laser scanning microscopy and electron microscopy.

Results: None of the dogs experienced adverse effects associated with the presence of the urinary catheters. Median CFU count of biofilm bacteria at all portions of the urinary catheter was significantly ($P < .001$) lower in the study compared with the control group. The degree of biofilm formation on the urinary catheters, as evaluated by confocal laser scanning microscopy and electron microscopy, was significantly lower in the study compared with the control group. Electron microscopy examination identified crystals on some of the urinary catheters. The proportion of catheters on which crystals were observed was significantly lower on the distal part of the urinary catheter in the study group compared with the control group (16.7% versus 66.7%, respectively; $P = .04$).

Conclusions and Clinical Importance: Chlorhexidine sustained-release varnish-coated urinary catheters effectively decrease urinary catheter-associated biofilm formation in dogs.

Key words: Coating material; Urinary tract infection; Urine.

Urinary tract infection (UTI) is a common disorder in dogs, and is expected to occur in 14% of all dogs during their lifetime.¹ Because of a relatively short and wide urethra, UTIs occur more commonly in females compared with males.² Most UTIs result from ascending infection of bacteria originating from the perineal area and the gastrointestinal tract.³ Several natural defense mechanisms clear bacteria and prevent UTI.⁴ These include anatomic barriers such as urethral length, high pressure zones, normal urethral peristaltics, and unidirectional valves, as well as mucosal properties such as the presence of glycosaminoglycan and local antibodies. Urine properties (eg, osmolarity) and its complete elimination also are important factors in preventing and eliminating UTI.

Urinary catheter placement eliminates many of the aforementioned defense mechanisms, thereby allowing introduction of bacteria into the urinary tract during the procedure, especially if a strict aseptic technique is

Abbreviations:

CFU	colony-forming unit
CLSM	confocal laser scanning microscopy
PBS	phosphate-buffered saline
SRV	sustained-release varnish
UTI	urinary tract infection

not applied. Almost 50% of catheterized dogs develop UTI.^{2,5} The incidence of catheter-associated UTI increases with age and the duration of time for which the urinary catheter is kept in place, with each additional day increasing the incidence of UTI by 27%.² Despite these potential complications, both in human and animal patients, placement of urinary catheters is indicated frequently.

Urinary tract infection also is a major problem in human medicine, and is the most common nosocomial infection, accounting for 40% of all nosocomial infections.^{6–8} Previous use of antibiotics, female sex, duration of hospitalization, and reason for hospitalization all were found to be risk factors for hospital-acquired UTI in human patients.⁹ The cost of urinary catheter-associated UTI in human patients is estimated at \$589 per case, further demonstrating the importance of this problem and the urgent need for a solution.¹⁰

Urinary tract infections may have severe clinical consequences both in human and animal patients, because the infection may not be limited to the urinary bladder, and may ascend to the kidneys and predispose to pyelonephritis and urosepsis. This risk is further increased because many patients requiring catheterization also have other predisposing factors

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(eg, recumbency), and therefore are at a greater risk for severe clinical consequences of catheter-associated UTI.²

To date, methods to prevent urinary catheter-associated UTI in dogs have been unsuccessful. The use of systemic antibiotics does not prevent catheter-associated UTI, and may even predispose to the emergence of resistant bacteria.¹¹ One speculated reason for the failure of systemic antibiotic administration in preventing urinary catheter-associated UTI is biofilm formation.¹² Bacteria within the biofilm are more protected from antibiotics.¹³ The prevention of catheter-associated UTI also is hindered by the number and types of organisms present on the urinary catheter.¹⁴

Because of the low efficacy of antibiotics in preventing catheter-associated UTI,¹¹ antimicrobial-impregnated urinary catheters have been considered as a preventative therapy for catheter-associated UTI and biofilm formation. Antiseptic and antimicrobial agents such as silver and nitrofurazon previously have been assessed as urinary catheter-coating materials, but were associated with variable success.^{15–17} Chlorhexidine is widely used as an antiseptic agent in veterinary medicine. It has low toxicity to mammals and a broad antimicrobial spectrum.¹⁸ Thus chlorhexidine potentially is an effective coating material to prevent catheter-associated UTI. The development of sustained-release varnish (SRV) of chlorhexidine for use on urinary catheters has been reported recently.¹⁹

We hypothesized that SRV of chlorhexidine will decrease biofilm formation on urinary catheters. Therefore, the aim of this study was to assess the efficacy of urinary catheters coated with a SRV of chlorhexidine in decreasing biofilm formation in dogs.

Materials and Methods

Animals and Study Design

The study was approved by the Institutional Animal Care and Use Committee of the Hebrew University Veterinary Teaching Hospital (HUVTH). It was a prospective study including 30 dogs that were hospitalized at the HUVTH for various medical conditions, and that had urinary catheters placed for at least 24 hours, based on decisions made by their attending clinicians.

The 1st group was used to assess adverse effects associated with the presence of coated urinary catheters and included dogs that were hospitalized in the Intensive and Critical Care Unit of the HUVTH. A urinary catheter coated with chlorhexidine SRV was introduced as part of their medical management, after their owners had signed a consent form. Four dogs that did not survive and underwent complete postmortem examinations at the request of their owners were included and used to assess potential adverse effects associated with the presence of chlorhexidine-coated urinary catheters. After completion of this part of the study, the 2nd part evaluating the efficacy of the coated catheters was initiated.

The 2nd group of dogs (26 dogs) had urinary catheters placed as part of their management, and were used to assess the efficacy of the chlorhexidine-coated urinary catheters in decreasing biofilm formation. Only dogs presented during the regular working hours of the HUVTH were included to assure strict

aseptic technique when placing the urinary catheters. Dogs were included regardless of their underlying disease. Once the decision to place a urinary catheter was made, either an untreated catheter (13 dogs) or a urinary catheter coated with SRV of chlorhexidine (13 dogs) was placed after the owners had signed a consent form. Randomization was performed by placing all urinary catheters (coated and uncoated) covered in a single box and selecting an appropriate size catheter. Dogs with pyuria, bacteruria, or both before catheter placement, based on urinalysis, were excluded from the study. Dogs with positive urine culture before catheterization, regardless of when the result was obtained, were excluded from the study. The attending clinicians and staff collecting the data during the study were blinded to the type of catheter placed.

Preparation and Placement of Urinary Catheters

The catheters were coated with SRV prepared as previously described.¹⁹ Briefly, ethyl cellulose (5 g), polyethylene glycol (4 g), and chlorhexidine to a final concentration of 1% were dissolved in 100 mL of ethanol. The varnish was prepared by dissolving the active ingredient and the polymers until a homogeneous preparation was achieved.²⁰ The SRV was applied to the catheters by a brush as an outer coat, resulting in a uniform coat on the catheters as previously demonstrated.¹⁹ After application, the solvent (ethanol) was allowed to evaporate and the coat remained as a uniform cover of the catheter. After the coating procedure was completed, the catheters were wrapped and sterilized using ethylene oxide gas.

Urinary catheters were placed in all dogs by trained personnel, using strict aseptic technique. Briefly, hair was clipped from the vulva or prepuce and the area was prepared with chlorhexidine. Aseptic technique was maintained throughout the procedure by using a sterile barrier and gloves. Females and males had their vestibule and prepuce, respectively, flushed with 5 mL of 0.05% chlorhexidine. A silicone or latex Foley catheter of appropriate size then was introduced and the balloon inflated. A sterile closed collection system was connected to the catheter. A urine sample for urinalysis was collected using the infusion port at the time of catheterization and immediately before catheter removal. The urinary catheter was left in place as indicated by the attending clinician.

Urinalysis

Urinalysis was performed at the hospital laboratory. Analysis included dipstick analysis and sediment evaluation after centrifugation of 5 mL of urine. Urine samples for aerobic culture were collected by cystocentesis before catheterization. Urine was cultured within 30 minutes of collection on blood agar and MacConkey's agar plates, which were incubated at 37°C for 48 hours.

Biofilm Assessment

When indicated, urinary catheters were removed sterily as follows: the area of the prepuce or the vulva was cleaned. One technician deflated the balloon and pulled out the catheter several inches. Once the part of the catheter that was inside the body became visible, a second technician, wearing sterile gloves, held the catheter and removed it entirely, ensuring that the catheter did not become contaminated. The catheter then was cut to remove the nonsterile area (ie, the portion that was outside of the body while the catheter was in place), and the remaining catheter was placed on a sterile barrier. The catheter then was divided into 3 segments, which were obtained from the proximal (near the urinary bladder), middle,

and distal end of the urinary catheter. The segments were placed in media as described below. From each of the segments, 3 adjacent samples, each 15 mm long, were cut for colony-forming unit (CFU) count, semiquantitative evaluation under confocal laser scanning microscopy (CLSM), and evaluation by electron microscopy as indicated below.

Colony-Forming Unit Assessment

The 3 samples, representing the proximal, middle, and distal portions of each catheter, were rinsed twice using 500 μ L of refrigerated (4°C) phosphate-buffered saline (PBS) solution to remove any debris and bacteria not associated with the biofilm. Each sample then was placed in a miniplast tube and 500 μ L of PBS solution were added. The tube then was placed in an ultrasonic water bath^a for 10 minutes to remove biofilm-associated bacteria from the catheter. Forty microliters of the suspension was plated on brain heart infusion agar plates. Plating was performed in 10^{-2} , 10^{-4} , 10^{-6} dilutions. Plates then were incubated at 37°C for 24 h and a CFU count performed using a magnifying viewer.

Confocal Laser Scanning Microscopy

The above 3 samples representing the proximal, middle, and distal portions of each catheter were rinsed with PBS solution as described above to remove any debris and bacteria that were not associated with the biofilm. Each 1 of the 3 samples was placed in 4% formaldehyde solution for 10 min and then rinsed with distilled water.¹⁹ Each sample was immersed in 0.1% propidium iodide^b solution for 30 min in a dark room and washed. All samples were wrapped in aluminum paper and refrigerated at 4°C pending evaluation.^c

The degree of fluorescence was evaluated semiquantitatively using 0.5/ $\times 10$, $\times 40$, $\times 63$ lenses after laser excitement (wave length, 532 nm). A coated and an uncoated urinary catheter were compared for autofluorescence. Each sample was evaluated by 2 investigators who were blinded to the type of catheter evaluated. Each catheter was evaluated in 3 distinct random areas, and the degree of fluorescence was categorized as none, mild, moderate, or severe (Fig 1).

Scanning Electron Microscopy

Sample preparation for electron microscopy was performed as described for the CLSM. Each sample was placed in 2.5% glutaraldehyde solution for 2 hours and then rinsed with PBS solution. All samples were wrapped in aluminum paper and refrigerated at 4°C pending evaluation. Before evaluation, samples were rinsed in ethanol solution and left to dry. Samples then were placed in a sputter coater^d to be coated with gold-palladium and then scanned under electron microscope at 1500, 3000, and 6000 magnification.^e Each sample was evaluated in 3 distinct random areas and the degree of bacteria present on the urinary catheter was evaluated semiquantitatively as none, mild, moderate, or severe (Fig 2), as previously described.¹² As for the CLSM, each catheter was evaluated by 2 investigators who were blinded to the type of catheter evaluated.

Statistical Analysis

Normality of distribution of continuous parameters was assessed using the Shapiro-Wilk test. Continuous parameters were compared between the study and the control group using the Student's *t*-test or the Mann-Whitney *U*-test based on data distribution. The categories representing the magnitude of bacterial colonization on the urinary catheter as evaluated by CLSM and electron microscopy were compared between the groups

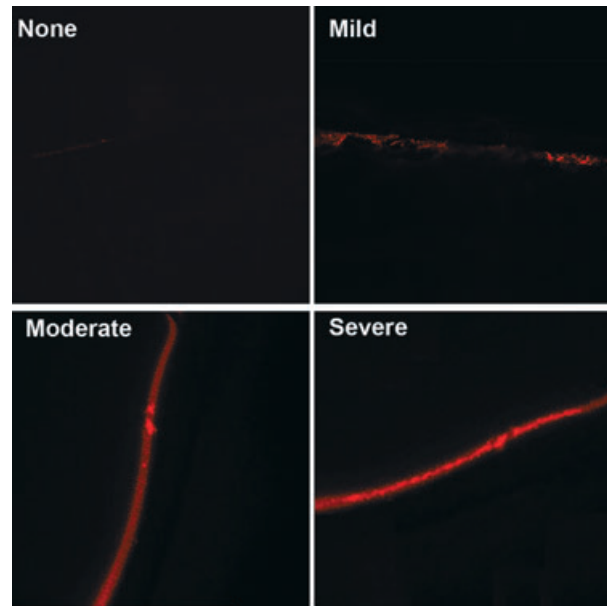


Fig 1. Fluorescence intensity was evaluated on each one of the coated and the control urinary catheters. Magnitude of fluorescence on the urinary catheters was defined as none/mild and moderate/severe. Magnification: 10 $\times$.

using the Chi-Square test. $P < .05$ was considered statistically significant. All calculations were performed using statistical software.^f

Results

Seven dogs (group 1) were presented to the Emergency Service and had a coated urinary catheter placed as part of their management. Of those, 4 dogs did not survive, and were included in the study. The latter 4 dogs included 3 mixed breed dogs and 1 Weimaraner. Two were males (1 castrated) and 2 were females (1 spayed). Median age was 11 years (range, 8–14 years). Underlying diseases included pancreatitis, acute or chronic kidney disease, congestive heart failure, and seizures. The median time that the coated urinary catheters were left in place in these 4 dogs was 48 hours (range, 36–144 hours). The gross and microscopic postmortem examination of the lower urinary system (ie, urinary bladder and urethra) in all 4 dogs was unremarkable.

Twenty-six dogs (group 2) fulfilled the inclusion criteria and were enrolled into the study, of which 16 (62%) were males (5 castrated) and 10 (38%) were females (7 spayed). The sex proportion was not statistically different between the study and the control groups (9/13 versus 7/13 males, respectively; $P = .42$). The median age of the study group was 6.5 years (mean, 7.7 years; range, 1.5–13 years) compared with 7 years (mean, 7.3 years; range, 3–13 years) of the control group, with no statistically significant differences between the groups ($P = .65$). Ten dogs (38%) were mixed breed dogs and the remainder included Labrador Retriever (6 dogs, 23%), Dachshund (3 dogs,

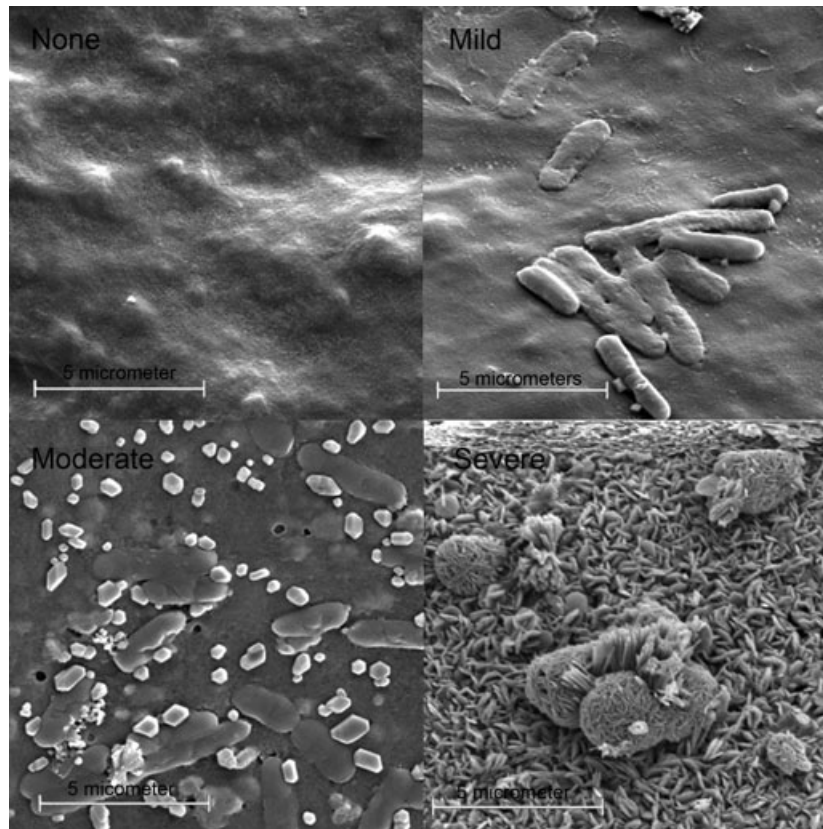


Fig 2. The degree of bacteria was evaluated on each one of the coated and the control catheters. Catheters were defined as none/mild and moderate/severe. Magnification: $\times 6000$

12%), French Bulldog and Doberman Pinscher (2 dogs each, 7.5%), Pekinese, Golden Retriever, and Great Dane (1 dog each, 4%). Medical conditions for which dogs were presented to the HUVTH included paraplegia (8 dogs, 30.8%), paraparesis (8 dogs, 30.8%), seizures (3 dogs, 11.6%), gastroenteritis (2 dogs, 7.7%), brain disorder (2 dogs, 7.7%), heat stroke and snake bite (1 dog each, 3.8%). One dog (3.8%) did not have a final diagnosis. The proportion of dogs with neurologic disorders was not statistically different between the study and the control groups (7/13 versus 9/13, $P = .42$). All dogs were treated systemically with antibiotics during their hospitalization because of reasons unrelated to the urinary condition. In the control group, 8 dogs received first-generation cephalosporins, 4 dogs amoxicillin clavulanic acid, 1 dog amoxicillin, and 1 dog clindamycin (1 dog received 2 antibiotics). In the study group dogs, 8 dogs received first-generation cephalosporins, 4 dogs amoxicillin clavulanic acid, and 1 dog chloramphenicol.

The urinary catheters were left in place in the study group for a median of 72 hours (range, 24–168 hours) and in the control dogs for 36 hours (range, 24–144 hours) with no statistically significant difference between the groups ($P = .19$). No adverse effects that could have been attributed to the presence of the urinary catheter were detected in any of the dogs.

Median values of dipstick analysis and sediment evaluation at the time the urinary catheters were placed, and just before their removal, were not statistically different between groups. None of the dogs in this study developed macroscopic hematuria while the urinary catheters were left in place. Only 1 dog in the control group and none of the dogs in the study group had evidence of pyuria. All urine cultures at the time of urinary catheter placement were negative.

Median CFU count of biofilm bacteria in the proximal portion of the urinary catheters was significantly lower in the study compared with the control group (median, 6.25×10 CFU/15-mm catheter [range, $0-3.75 \times 10^3$ CFU/15-mm catheter] versus median, 5×10^5 CFU/15-mm catheter [range, $0.375-3.75 \times 10^7$ CFU/15-mm catheter]; $P < .001$; Fig 3). Median CFU of biofilm bacteria at the middle portion of the urinary catheters also was significantly lower in the study compared with the control group (median, 3.75×10 CFU/15-mm catheter [range, $0-3.75 \times 10^3$ CFU/15-mm catheter] versus median, 5×10^5 CFU/15-mm catheter [range, $1.25-5 \times 10^7$ CFU/15-mm catheter]; $P < .001$). Median CFU of biofilm bacteria at the distal portion of the urinary catheters also was significantly lower in the study compared with the control group (median, 2.5×10 CFU/15-mm catheter [range, $0-2.5 \times 10^3$ CFU/15-mm catheter] versus median,

2.5×10^3 CFU/15-mm catheter [range, $0-4.35 \times 10^7$ CFU/15-mm catheter]; $P < .001$; Fig 3).

Isolation of bacteria from the biofilm was performed only in 9 catheters. Bacteria isolated from the biofilm included *Escherichia coli* (3 catheters in the study group and 1 catheter in the control group), *Pseudomonas aeruginosa* (1 catheter from each group), *Proteus mirabilis* (1 catheter from the study group), Gram positive *Bacillus* and mixed bacterial population (1 catheter each from the study group).

The CFU count in the study group was higher on the proximal portion of the urinary catheter compared with the middle and distal portions of the catheter. The CFU count was higher on the proximal portion compared with the middle portion in 35% of the cases. The CFU count was higher on the proximal portion compared with the distal portion in 54% of the cases. In only 11% of the cases, the CFU count was higher on the middle and distal portions compared with the proximal portion. In the control group, the CFU count was higher on the proximal portion compared with the middle portion in 23% of the cases. The CFU count was higher on the proximal portion compared with the distal portion in 62% of the cases. In only 8% of the cases, the CFU counts were higher on the middle and distal portions compared with the proximal portion.

The proportion of catheters that were classified as none or mild intensity based on results of CLSM was significantly higher in the study compared with the control group in all evaluated portions of the urinary catheter (Table 1). The proportion of catheters that were classified as none or mild based on results of scanning electron microscopy also was significantly higher in the study compared with the control group in the evaluated portions of the urinary catheter (Table 2).

The electron microscopy examination disclosed the presence of crystals on some of the urinary catheters. In the proximal part of the catheter, the proportion of catheters on which crystals were detected was lower in the study compared with the control group, but not significantly (7.7% versus 46.2%, respectively, $P = .07$). In the middle part of the catheter, the proportion of crystals also was not statistically different between the study and the control group (7.7% versus 15.4%, respectively, $P = .13$). In the distal part of the catheter, the proportion of crystals was significantly lower in the study compared with the control group (16.7% versus 66.7%, respectively, $P = .04$).

Discussion

Urinary catheter-associated UTI is a major clinical complication of urinary catheter placement, resulting in considerable morbidity and mortality both in humans and in dogs.^{5,21-23} One of the speculated reasons for the high incidence of catheter-associated UTI is the lack of antibiotic effectiveness in preventing biofilm formation, which may occur as early as few hours after urinary catheter placement.^{12,24} Biofilm is the

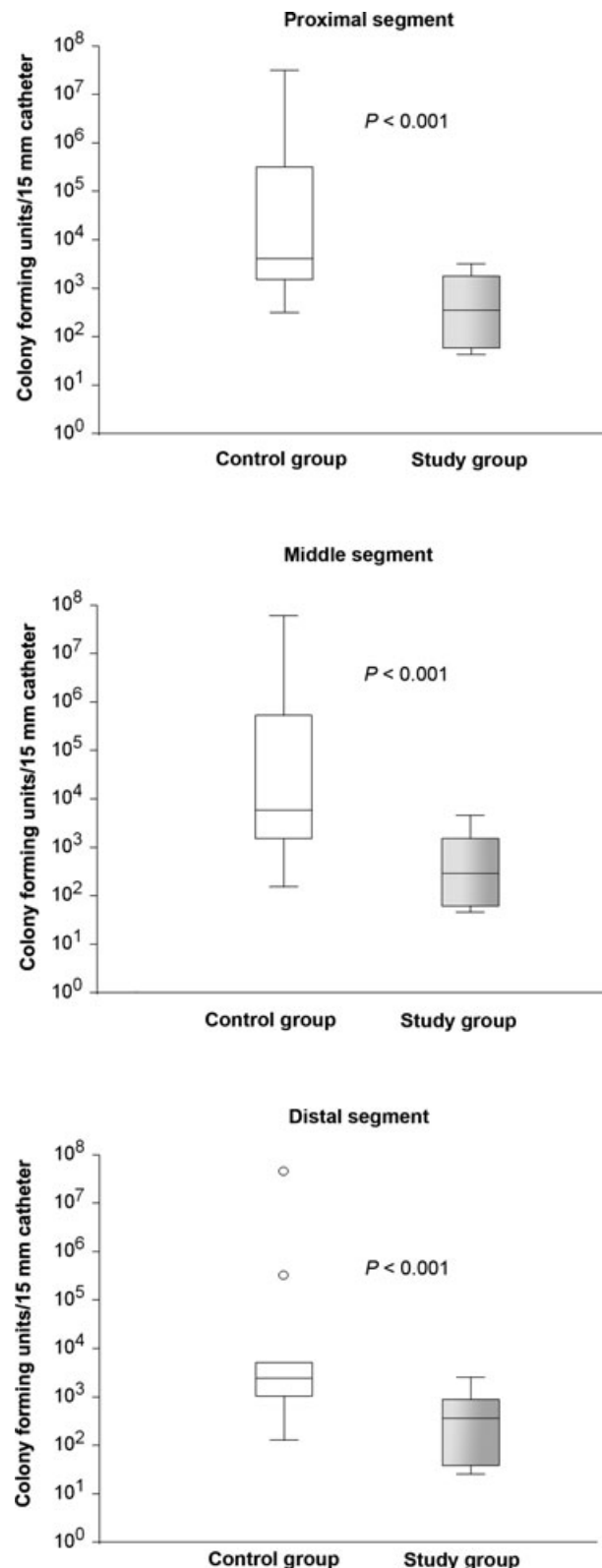


Fig 3. Colony-forming unit count of biofilm bacteria at the proximal, middle, and distal segment of the urinary catheter. Data are presented as boxes and whiskers. Each box includes the interquartile range, whereas the line within a box represents the median, and the whiskers represent the range, extending to a maximum of 1.5 times the interquartile range. Outliers are represented as open circles.

Table 1. Degree of bacteria present on the different portions of the urinary catheters as evaluated by confocal laser scanning microscopy

	Fluorescence	Study Group (n%)	Control Group (n%)	<i>P</i> Value
Proximal portion	None/mild	10 (76.9)	2 (15.4)	.0016
	Mod/sev	3 (23.1)	11 (84.6)	
Middle portion	None/mild	11 (84.6)	5 (38.5)	.015
	Mod/sev	2 (15.4)	8 (61.5)	
Distal portion	None/mild	11 (84.6)	6 (46.2)	.039
	Mod/sev	2 (15.4)	7 (53.8)	

Mod, moderate; sev, severe.

Table 2. Degree of bacteria present on the different portions of the urinary catheter as evaluated by electron microscopy

	Fluorescence	Study Group (n%)	Control Group (n%)	<i>P</i> Value
Proximal portion	None/mild	13 (100)	9 (69.2)	.030
	Mod/sev	0 (0.0)	4 (30.8)	
Middle portion	None/mild	13 (100)	8 (61.5)	.013
	Mod/sev	0 (0.0)	5 (38.5)	
Distal portion	None/mild	13 (100)	9 (69.2)	.030
	Mod/sev	0 (0.0)	4 (30.8)	

Mod, moderate; sev, severe.

predominant form of life for the majority of microorganisms in hydrated biologic systems.¹³ Biofilm formation includes adherence of the microorganisms either to a surface or to one another, subsequent alteration of gene expression, and formation of an extracellular matrix.^{25,26} All of the above render the bacteria in the biofilm less susceptible to antibiotics; thus antibiotic use is not effective.¹² Biofilm also may be the source of repeated and continuous bacterial shedding into the urinary system.¹⁴

Previous studies have evaluated various interventions to decrease the incidence of biofilm formation on urinary catheters, but many of these studies used *in vitro* models or laboratory animals.^{12,16,24,27} To date, methods to prevent catheter-associated UTI have only had limited success; thus, alternative novel means to prevent UTI continue to be explored.¹¹ In 1 *in vivo* study, biofilm formation was not prevented using various antibiotics placed into the catheter lumen before placement, and biofilm was documented as early as 24 hours after catheter placement.¹² The use of catheters impregnated with various antibiotics can be very expensive, and even may promote the emergence of bacterial resistance. Nitrofurazone-containing urinary catheters were proven effective *in vitro* against multi-drug-resistant strains of bacteria, and a clinical trial found a lower rate of bacteriuria in human patients who had nitrofurazone-impregnated urinary catheters placed compared with controls.^{17,28} A meta-analysis of

8 trials of silver-coated urinary catheters in human patients found that the odds ratio for developing bacteriuria was 0.59 (95% confidence interval, 0.42–0.84) when a coated urinary catheter was placed.²⁹

This study is the first to evaluate the use of an SRV of chlorhexidine as a coating material for urinary catheters in dogs. We have demonstrated, using different modalities, that biofilm formation can be decreased substantially when using an SRV of chlorhexidine-coated urinary catheters. It is yet to be determined if the SRV by itself has any effect on biofilm formation on urinary catheters.

The local application of a sustained-release delivery system has many advantages. An important benefit of the SRV is that the active agent is targeted to the desired site, while minimizing its systemic effect, and thereby decreasing potential adverse effects. The drug in an SRV is being released over a long period of time in a controlled manner,³⁰ which bears many clinical and pharmacologic advantages including low frequency of drug application, improved compliance, and reduction of care by the professional staff during hospitalization.

None of the dogs in this study developed macroscopic hematuria during the time that the urinary catheters were in place, thus most likely chlorhexidine in the SRV did not irritate the mucosa of the urinary system. Nonetheless, because hematuria is not a sensitive marker of urethral lesions, irritation cannot be completely excluded. Although lack of macroscopic post-mortem lesions in the 4 dogs evaluated and lack of clinical signs in the study dogs suggest that presence of chlorhexidine-coated urinary catheters is not associated with adverse effects, a well-designed safety and toxicity study is necessary before safety can be confirmed.

The CFU counts of biofilm bacteria in this study were significantly lower in the study group compared with the control group for all portions of the urinary catheter. Because the urinary catheters were left in place in this study as dictated by the medical condition of the dogs, there was a difference in the length of catheterization between the groups, but this difference was not statistically significant. There is an association between the time urinary catheters are left in place and the degree of biofilm formation³¹; but all dogs in the control group, including dogs in which urinary catheters were left in place for a relatively short period of time, had very high CFU counts, whereas all dogs in the study group, including those in which the urinary catheter was left in place for a relatively long period of time, had relatively low CFU counts. This observation along with the lack of statistical difference in the time of catheterization between the groups further supports the conclusion that the coating likely affected biofilm formation.

The reduction of biofilm formation on the coated urinary catheters was further supported by the CLSM results. Of the sections of catheter evaluated, 77–85% of the coated catheters were in the none or mild-intensity category. In contrast, 54–85% of the sections of untreated catheters were in the moderate or severe cat-

egory. This observation also is consistent with the results of the scanning electron microscopy, in which 100% of the coated catheter sections were classified in none or mild category for the degree of bacteria present of the urinary catheters and no sections were classified as moderate or severe (compared with 31–39% for the untreated catheters). These observations, along with the CFU count, indicate that biofilm formation can be decreased using SRV of chlorhexidine applied on urinary catheters.

In this study, the longest period of time a coated catheter was left in place was 144 hours with a CFU count of 15–40 CFU/15-mm catheter in the different segments of the catheter. Based on this single observation, it seems that the coating may be effective for at least 1 week, but because in most of the dogs the duration of catheterization was shorter, we cannot speculate on the maximal period of time a chlorhexidine-coated catheter can be left in place and still be effective.

As ours was a clinical study, dogs were treated according to their medical condition and thus a standard protocol was not used. Antibiotics were administered systemically to dogs as dictated by their medical conditions (and unrelated to the presence of the urinary catheter). In human patients, catheter-associated UTIs are not prevented by systemic administration of antibiotics.³² This observation further emphasizes the need to seek methods to prevent catheter-associated UTIs other than systemic administration of antibiotics, which in addition to not being effective, also may promote resistance.

Based on the CFU counts, the proximal portion of the catheter was more populated with bacteria compared with the other parts. This observation may be related to the fact that the first step in biofilm formation is the formation of a conditioning film,²⁵ which may be composed of crystals. As early as 1 hour after exposure of a urinary catheter to urine, its surface is covered by a layer of material comprised of microcrystals.¹⁶ Bacterial colonization then may occur as early as 4 hours on the crystalline layer.¹⁶ This phenomenon may render a coated catheter less effective because the bacteria can adhere to the crystalline material rather than to the urinary catheter, which is coated with antimicrobial material.

This study has several limitations. First, our study has shown that biofilm can be decreased for a period of up to 1 week, but long-term studies need to evaluate the maximal period of time that SRV of chlorhexidine can impact biofilm formation on urinary catheters. Second, urinalyses were not performed in all dogs on a routine basis while the urinary catheters were in place. Despite the fact that no adverse effects were noted, the latter cannot be completely ruled out based on the urinalysis results. Third, all the dogs in this study received antibiotics systemically as part of their medical management, thus it is possible that antibiotics have affected biofilm formation. Fourth, despite the fact the none of the dogs in this study developed adverse effects associated with the presence of a coated urinary catheter,

only 4 dogs (group 1) underwent complete postmortem examination to assure the absence of macroscopic and histopathologic changes in the sections of urethra and urinary bladder examined. Fifth, evaluation of the CLSM and the electron microscopy results was subjective. Finally, our study did not evaluate the presence and occurrence of UTI days and weeks after catheter removal, thus long-term effectiveness cannot be evaluated.

In conclusion, this study suggests that the use of urinary catheters coated with SRV of 1% chlorhexidine may decrease the formation of biofilm on urinary catheters in dogs.

Footnotes

^a Transsonic T-460, ELMA, 37°C, 37kHz

^b Sigma-Aldrich, St. Louis, MO

^c Leica DMI 4000 Confocal Microscope

^d CS7640, Polaron, UK

^e Sirion, Field Emission Instruments, Netherlands

^f SPSS 17.0 for Windows, Chicago, IL

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Conflict of Interest: Authors disclose no conflict of interest.

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