Evaluation of the number of colony forming units on the skin of dogs after clipping the hair with two sizes of clipper blades

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OBJECTIVE
To evaluate erythema and number of CFUs on the skin of dogs with hair clipped by use of 2 sizes of clipper blades.

ANIMALS
67 client-owned dogs receiving an epidural.

PROCEDURES
Hair was clipped with a No. 10 blade (approx hair length, 1.5 mm) on one half and a No. 40 blade (approx hair length, 0.25 mm) on the other half of each epidural site. Skin was surgically scrubbed with 2% chlorhexidine gluconate and 70% isopropyl alcohol. Samples were obtained immediately after clipping, after skin was scrubbed, and again 24 hours after clipping. Number of CFUs for both sides of the clipped areas, types of microorganisms, and growth on MacConkey agar were evaluated every 24 hours for 72 hours. Colonies were evaluated for bacterial morphology and Gram stain characteristics. Sites were evaluated 24 hours after clipping for evidence of erythema.

RESULTS
24 hours after hair was clipped, there was a significantly higher incidence of erythema and higher number of Micrococcaceae bacteria for the side clipped with the No. 40 blade than the side clipped with the No. 10 blade. Number of CFUs did not differ significantly between size of clipper blades.

CONCLUSIONS AND CLINICAL RELEVANCE
Clipping hair with a No. 40 blade resulted in a significant increase in the incidence of erythema and higher number of Micrococcaceae bacteria, compared with results for clipping with a No. 10 blade. These results supported use of a No. 10 clipper blade to prevent erythema and reduce variation in the skin microbiome. (*Am J Vet Res* 2019;80:862–867)

Distribution of bacteria within the layers of the integument of dogs has been described.[1] Depending on the bacterial species, microflora can thrive or be inhibited by the presence of hair.[2] Colonization of the skin after preparation with chlorhexidine has been evaluated in preterm infants.[3] In that study,[3] similar numbers of gram-negative bacteria were detected before and up to 48 hours after preparation with chlorhexidine, compared with the number of bacteria at those same times after bathing with water. In adults, daily scrubbing with chlorhexidine has been associated with a reduction of skin colonization by antimicrobial-resistant bacteria.[4] However, no studies have been conducted in humans or veterinary patients to evaluate changes in skin flora immediately after clipping hair and 24 hours after surgical preparation (ie, surgical scrub). Furthermore, no studies have been conducted to investigate whether the length of hair after clipping influences the bacterial flora before scrubbing the skin or after recolonization. Although multiple variables have a role in development of surgical site infections, changes in the bacterial population of healthy skin after clipping could increase this risk by promoting a favorable environment for proliferation of certain types of bacteria.

With few exceptions, hair of veterinary patients is clipped before surgery to prevent it from interfering with the surgical site. This is in accordance with current preoperative guidelines recommended by the CDC and the World Health Organization to reduce the risk of surgical site infections. It is generally accepted that clipping the hair leads to fewer infections than shaving with a razor because razor blades result in microtrauma to the skin before surgery.[5–7] In veterinary patients, inflammation caused by clipper-induced trauma has anecdotally been observed for several days after clipping of the hair, which indicates a potential for prolonged impairment of the skin's natural barriers. Inflammation leads to changes in lipid synthesis and aggregation on the skin, a higher pH, and disruption of differentiation and desquamation of the epidermis. In experimental settings, this

ABBREVIATIONS
BCS Body condition score

barrier returns to normal within a few hours after the cause is removed.[8]

No standard has been established for the size of clipper blades used to remove hair before surgery. However, one of the most commonly used is a No. 40 blade, which leaves hair with a length of approximately 0.25 mm, depending on the brand of clipper blade. Another commonly used size is a No. 10 blade, which leaves hair with a length of approximately 1.5 mm, again depending on the brand of clipper blade. Thus, hair length after clipping with a No. 10 blade is 6 times the length of hair after clipping with a No. 40 blade. Both lengths of hair would minimally interfere with the surgical site.

Distribution of bacteria differs depending on the location within the integument and coat, including the hair, skin surface, and hair follicles. In humans and dogs, bacteria from the family Micrococcaceae represent the largest portion of the flora within the hair follicles. This family includes *Staphylococcus* spp and *Micrococcus* spp, various soil and water bacterial species, and airborne bacterial species. The pathogenic potential of *Staphylococcus* spp has been recognized, and investigation into ways to reduce bacterial load of staphylococci during recolonization of the skin is warranted.[9]

The objective of the study reported here was to evaluate at various time points the number of CFUs and types of microorganisms after clipping of the hair by use of a No. 10 blade and a No. 40 blade. Our hypothesis was that clipping hair closer to the skin would lead to increased irritation of the skin and bacterial dislodgement from the hair follicles, thereby increasing the bacterial load on the skin immediately after clipping and during the recolonization of the skin, compared with results for clipping that resulted in longer hair.

Materials and Methods

Animals

Client-owned dogs scheduled to have an epidural as part of their anesthetic protocol were eligible for inclusion in the study. Size of the dogs in the study was limited by the ability to fit a 10 X 7-cm template over the dorsum, rather than by the patient's body weight. Dogs were excluded when the template did not fit between the ilial wings or when bending of the template would have been necessary to allow for a good fit.

Dogs that met specific criteria were enrolled in the study. Enrollment criteria included undergoing a procedure for which an epidural would routinely be used, no epidural during the 6 months preceding the study, no antimicrobial or corticosteroid treatment within the 2 months preceding the study, no known skin allergies, no evidence of pyoderma or other skin disease at the time of sample collection, and no recent trauma. Patients that did not meet these criteria were excluded from the study because changes in microflora have been associated with allergic conditions and the use of various drugs.[10,11] Owners provided informed consent for inclusion of their dogs in the study for collection of samples at the epidural site.

Experimental procedures

The epidural site was selected for this proof-of-concept study because of repeatability for hair-clipping clipper pattern and the fact the site was located distant from the intended surgical incision (ie, did not interfere with the surgery).

One individual collected and analyzed all data to reduce interobserver bias. Data were collected regarding each patient's BCS (scale, 1 to 9). Type of coat (single coat or double coat) and coat length (short or long) were recorded. A short coat was defined as hair that typically grew to < 2 cm in length at the epidural site, whereas a long coat was defined as hair that typically grew to > 2 cm in length at the epidural site.

Dogs were placed in sternal recumbency with the hind limbs positioned cranially to allow access to the L7-S1 epidural space. A 10 X 7-cm sterile template was placed over the epidural site. Half of the site was clipped by use of a sterile No. 10 blade[a] (approx hair length, 1.5 mm), and the other half was clipped by use of a sterile No. 40 blade[a] (approx hair length, 0.25 mm). Size of the clipper blade and side on which the clipper was used (left vs right) were randomly assigned with randomization software.[b]

A second sterile template with 2 sampling openings (each opening was 1 X 3 cm) was aligned over the first template such that 1 sample opening was centered on each side of the clipped area. Sterile swabs were moistened with 5 drops of saline (0.9% NaCl) solution by use of an 18-gauge needle attached to a syringe; a moistened swab was then rolled on the skin of each of the sampling areas (one area for hair clipped with the No. 10 blade and the other area for hair clipped with the No. 40 blade). Samples were plated on 5% sheep blood agar by rolling a swab over the entire surface of the agar plate. Templates were removed, and the site was then prepared for the epidural by use of 3 swabs containing 2% chlorhexidine gluconate and 70% isopropyl alcohol. Contact time was 5 minutes. Another sterile template with two 1 X 3-cm sampling openings was positioned, and swab samples were obtained after antiseptic preparation; these samples also were plated on 5% sheep blood agar. Each plate was labeled with the date and time of the sample collection, length of hair (ie, blade size), and patient identification. Plates were incubated at 37°C for 72 hours after sample collection.

Dogs were moved to the operating room. Dogs were scheduled to receive cefazolin (22 mg/kg, IV) before the beginning of the procedure, every 90 minutes during the procedure, and 4 hours after completion of the procedure. However, all dogs received ≤ 2 doses of cefazolin during the study because the procedures lasted < 90 minutes.

Twenty-four hours after the initial samples were collected, another sample was obtained from each

side of the epidural area by use of the same procedures and sterile template. Plates were incubated at 37°C for 72 hours.

Twenty-four hours after the epidural sites were clipped, 1 observer evaluated the areas for evidence of erythema, which, if present, was graded as mild (< 50% of the surface area), moderate (50% to 75% of the surface area), or severe (75% to 100% of the surface area). The 24-hour period between clipping of the hair and evaluation for erythema was intended to eliminate evidence of potential erythema caused by an immediate skin reaction to the chlorhexidine-alcohol preparation.

Data regarding the number of CFUs were collected every 24 hours for 72 hours for each plate. At the end of the incubation period, the species of bacteria were identified on the basis of colony morphology. A sample from each colony was placed on a microscope slide, and a catalase test was performed by placing 1 drop of 3% hydrogen peroxide on the sample. The test result was considered positive when gas bubbles were detected. Slides were prepared for each colony type by collecting a portion of the growth and placing it in a drop of sterile saline solution. Each slide was heat-fixed with a flame, and staining with Gram stain was performed by use of a standard procedure (immersion in crystal violet for 60 seconds, Gram iodine for 60 seconds, alcohol for 3 seconds, and safranin O for 60 seconds; slides were rinsed with tap water for 5 seconds between each step).

Slides were evaluated by use of immersion oil at 100X. Bacteria and other organisms were classified into the following categories: gram-positive rods, gram-negative rods, gram-positive cocci, and other. Bacteria in the gram-positive cocci category were categorized on the basis of results for the catalase test into Micrococcaceae bacteria (which included *Micrococcus* spp and *Staphylococcus* spp) and *Streptococcus* spp; further subclassification was not performed because speciation of bacteria was not the primary focus of the study. Thus, gram-positive species were subdivided into 3 categories: Micrococcaceae family, *Streptococcus* spp, and gram-positive rods. When gram-negative bacteria were identified, a sample was collected from the original colony and plated on MacConkey agar. Plates were incubated for 3 days to allow us to assess microbial growth. When growth was detected, it was categorized as a lactose fermenter when the growth was pink or a nonlactose fermenter when the growth was colorless.

Statistical analysis

Normality of the data distribution was determined by use of the D'Agostino-Pearson method. Number of CFUs was compared between the 2 blade sizes by use of a paired *t* test. Categorical data (incidence of erythema and number of cultures that yielded microbial growth) were compared by use of the McNemar test. The relationship between independent predictors (age, breed, sex, BCS, coat length, coat type, and erythema) and the dependent variable of number of CFUs at 72 hours was analyzed by use of multiple linear regression. The relationship between independent predictors (age, breed, sex, body weight, BCS, coat length, and coat type) and the dependent variable of erythema was analyzed by use of multiple linear regression. Number of types of bacteria at each time point was compared by use of the Friedman test with the Dunn test for post hoc analysis. Significance was set at $P \leq 0.05$.

Results

A total of 67 dogs were included in the study. The study population consisted of 31 males (6 sexually intact and 25 neutered) and 36 females (2 sexually intact and 34 neutered). Median age of the dogs was 7 years (range, 4.5 months to 12.5 years), median body weight was 31.5 kg (range, 4.9 to 68.8 kg), and median BCS was 5 (range, 4 to 8).

Dogs of 26 breeds as well as mixed-breed dogs were represented. Dogs with a long coat consisted of 17 Labrador Retrievers, 5 Golden Retrievers, 4 German Shepherd Dogs, 4 Siberian Huskies, 2 Border Collies, 2 Rottweilers, 1 Australian Cattle Dog, 1 Australian Shepherd, 1 Bichon Frise, 1 Great Pyrenees, 1 Newfoundland, 1 Papillon, 1 Poodle, 1 Schnauzer, and 1 Yorkshire Terrier. Dogs with a short coat consisted of 3 Chihuahuas, 2 Boxers, 2 Jack Russell Terriers, 1 Basset Hound, 1 Beagle, 1 Black and Tan Coonhound, 1 English Pointer, 1 Great Dane, 1 Shiba Inu, and 1 Whippet; the 10 mixed-breed dogs were terrier crosses with a short coat and were placed into the terrier group for statistical analysis.

Of the 67 dogs, 42 had a double coat and 25 had a single coat. Dogs with a double coat consisted of 17 Labrador Retrievers, 5 Golden Retrievers, 4 German Shepherd Dogs, 4 Siberian Huskies, 2 Border Collies, 2 Rottweilers, 1 Australian Cattle Dog, 1 Australian Shepherd, 1 Bichon Frise, 1 Great Pyrenees, 1 Newfoundland, 1 Schnauzer, 1 Shiba Inu, and 1 Yorkshire.

Of the 67 dogs, 34 were clipped with a No. 10 blade (approx hair length, 1.5 mm) on the left side of the epidural site. The other 33 dogs were clipped with a No. 10 blade on the right side of the epidural site.

Erythema was detected in 13 dogs at 24 hours after clipping of the hair at the epidural site. Erythema was not detected in any dogs immediately after surgical scrubbing of the area with the swabs, which indicated that there was no reaction to the chlorhexidine or alcohol or to the swabs. Therefore, erythema was deemed to be a direct cause of clipping the hair. There was a significant ($P = 0.003$) difference in the number of dogs that developed erythema on the side clipped with the No. 10 blade (2/67 [3.0%]), compared with the number that developed erythema on the side clipped with the No. 40 blade (13/67 [19.4%]). Both dogs that developed erythema on the side clipped with the No. 10 blade also developed erythema on the side clipped with the No. 40 blade **(Figure 1)**.

Of the 13 dogs that developed erythema, 10 had mild erythema, 1 had moderate erythema, and 2 had severe erythema. Both dogs that developed erythema on the side clipped with the No. 10 blade had mild erythema. Clipping with a No. 40 blade was associated with a significantly (P = 0.004) higher erythema grade. Eight of the dogs that developed erythema had a long double coat, and 5 had a short single coat.

Evaluation of the bacterial colonies revealed that gram-positive bacteria were the most common, with at least 1 bacterial species cultured from swab samples obtained before scrubbing of the epidural site and 24 hours later for 62 (92.5%) and 65 (97.0%) dogs, respectively. Of the 62 dogs that had bacterial growth for swab samples obtained before scrubbing of the epidural site, 58 (93.5%) had growth for samples obtained from the side clipped with a No. 10 blade and 55 (88.7%) had growth for samples obtained from the side clipped with a No. 40 blade. For swab samples obtained after scrubbing of the epidural site, at least 1 type of gram-positive bacteria was cultured from the side clipped with a No. 10 blade for 5 of 67 (7.5%) dogs, and at least 1 type of gram-positive bacteria was cultured from the side clipped with a No. 40 blade for 6 of 67 (9.0%) dogs. For swab samples obtained 24 hours after scrubbing of the epidural site, gram-positive bacteria were cultured from the side clipped with a No. 10 blade for 58 of 67 (86.6%) dogs and on the side clipped with a No. 40 blade for 63 of 67 (94.0%) dogs. The mean number of each type of gram-positive bacteria (Micrococcaceae family, *Streptococcus* spp, and gram-positive rods) cultured from swab samples obtained at any time point were calculated **(Table 1)**.

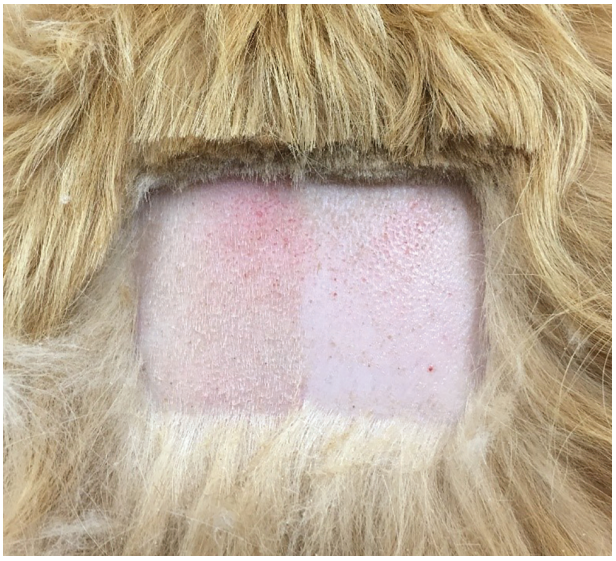

Figure 1—Photograph of the site of an epidural obtained 24 hours after the hair was clipped by use of a No. 10 blade (left side) and a No. 40 blade (right side) and surgically scrubbed with chlorhexidine and alcohol. Notice the mild erythema (< 50% of the surface area) on both the site clipped with the No. 10 blade and the site clipped with the No. 40 blade.

Fungi were the only organisms classified as other. Fungal organisms were the least common, with a single species cultured from swab samples obtained from 9 (13.4%) dogs and 2 species cultured from swab samples obtained from 2 (3.0%) dogs. All fungal organisms were of the mycelium form. No yeast organisms were observed.

Gram-negative rods were cultured from swab samples obtained from 13 (19.4%) dogs. Gram-negative cocci were not detected during the study. Fifteen samples from the gram-negative colonies were plated on MacConkey agar. Of these, 6 were lactose fermenters, and 1 was a nonlactose fermenter; the other 8 samples did not grow on the MacConkey agar.

There was no significant difference in the number of CFUs between blade sizes for swab samples obtained after clipping (P = 0.95) or immediately after scrubbing (P = 0.21). Number of CFUs for swab samples obtained 24 hours after scrubbing of the epidural site did not differ significantly (P = 0.45) between the sides clipped with a No. 10 blade and a No. 40 blade. Number of colonies classified as *Streptococcus* spp, gram-positive rods, and gram-negative rods did not differ significantly (P = 0.25, P = 0.34, and P = 0.80, respectively) between the side clipped with a No. 10 blade and the side clipped with a No. 40 blade at any time point during the study.

Table 1—Mean number of gram-positive bacteria (No. of CFUs) on the skin of dogs that had microbial growth for swab samples obtained at the site of an epidural, on the basis of time of the sample collection and size of the blade used to clip the hair.

Time	Blade size (No.)	Micrococcaceae family*	*Streptococcus* spp	Gram-positive rods
After clipping	10	2.41	1.00	2.11
	40	2.33	1.00	2.09
After scrubbing	10	1.00	—	1.00
	40	1.20	—	1.00
24 h after clipping	10	2.69	1.06	1.58
	40	2.96†	1.09	1.95

Swab samples were obtained after hair was clipped from the site of the epidural (after clipping), after the skin of the clipped area was surgically scrubbed with chlorhexidine and alcohol (after scrubbing), and 24 hours after hair was clipped (24 hours after clipping).

*Micrococcaceae family included *Micrococcus* spp and *Staphylococcus* spp. †Within a time point, value differs significantly (P < 0.05) from the value after clipping with the No. 10 blade.

— = No growth observed for this type of bacteria on any agar.

Number of CFUs at sites that developed erythema after clipping with a No. 10 blade or No. 40 blade could not be compared because of the low number of dogs (n = 2) that developed erythema on the side clipped with a No. 10 blade. Evaluation of the number of bacterial species obtained from the swab sample collected 24 hours after clipping revealed significantly (P = 0.006) more colonies classified as Micrococcaceae family on the side clipped with a No. 40 blade.

Breed, sex, BCS, body weight, coat length, coat type, and presence of erythema were not associated with a difference in CFU counts or number of bacterial species identified. Multivariate linear regression analysis revealed that an increase in age was associated with a significant decrease in the number of CFUs (R^2 = 0.05; P = 0.007) and a significantly (P = 0.04) lower incidence of Micrococcaceae bacteria. Age, breed, sex, BCS, body weight, coat length, coat type, and presence of erythema did not influence the number of CFUs for any of the other bacterial categories.

The most common types of bacteria were evaluated by use of the Friedman test and were the same for sides clipped with a No. 10 blade and a No. 40 blade. After hair was clipped (but not surgically scrubbed) and 24 hours after clipping, Micrococcaceae bacteria were present in the highest number, followed by the number of gram-positive rods, for both clipper blades. In addition, no difference in the number of *Streptococcus* spp and gram-negative bacteria was detected, and no bacterial species was more common for either clipper blade after scrubbing of the epidural site.

Discussion

For the study reported here, bias in sample collection and observations was eliminated because only 1 investigator collected samples, counted colonies, and evaluated colony morphology. Chlorhexidine was selected because of the lower number of skin reactions following use, compared with the number of skin reactions after the use of povidone-iodine scrub, and the minimal inhibition of chlorhexidine by organic matter (eg, hair debris and desquamated skin cells) that may be present at the epidural site after clipping.[12,13] By decreasing the risk of a skin reaction to the scrub solution, the erythema noted 24 hours after application of chlorhexidine at the epidural site could be attributed to the clipper blades, rather than to the chlorhexidine, although a delayed skin reaction to the solution could not be fully excluded. However, dogs that developed erythema did so more frequently on the side clipped with a No. 40 blade. If a delayed reaction to the scrub solution were to have developed, we would have expected to see it on both sides, regardless of the size of the blade used for clipping. The impact of longer hair as a protective effect against chlorhexidine-induced contact dermatitis has not been evaluated; therefore, it cannot be excluded as a possible explanation for the fact erythema was primarily observed on the side clipped with a No. 40 blade (shorter hair length).

The study had several limiting factors. One factor was identification of colony morphology for classification of the bacteria. For plates with high numbers of colonies of various morphologies or high numbers of colonies with similar morphologies, some of the bacteria types may have not been recorded. In addition, some bacterial species may have been overcrowded by larger colonies, which would make identification impossible. Variations or mutations of certain strains of bacteria may lead to differences in colony morphologies, but it remains important to differentiate bacterial colonies on agar cultures because of the possibility of differences in pathogenic potential for the various phenotypes.[14]

Another factor was potential contamination of the agar plates by environmental air despite the use of sterile technique and the limited amount of time each plate was exposed during quantification of the colonies. This could have led to an increase in the number of bacterial and fungal colonies found on the plates as well as colonies that were not from the skin of the dogs.

Surgical scrubbing with chlorhexidine was always conducted from left to right, which resulted in overlapping both clipped areas. Despite this, bacteria were not cultured from most sites after the sites were scrubbed. When bacteria were cultured, only 1 side (clipped with a No. 10 blade or a No. 40 blade) yielded bacteria. Therefore, it was unlikely that the manner in which scrubbing was performed affected the results. Some of the other types of organisms (eg, fungi or fastidious organisms) may not have been detected during evaluation of the agar plates because of the need for additional incubation time or use of special culture media.

Pressure exerted by clipper blades on the skin during clipping may have influenced the results if more pressure were applied to remove hair with the No. 10 blade or No. 40 blade. Measurement of the force used was not possible. However, because of the clinical setting, it was likely that even if more pressure had been applied to either site, the difference in bacterial recolonization of the skin would remain relevant because the same force would likely be used for that specific blade size to clip and remove hair from patients.

MacConkey culture medium is used to differentiate gram-negative bacteria on the basis of their capacity for lactose fermentation. Some of the gram-negative bacteria that were transferred to MacConkey agar plates did not have evidence of growth after culture for 72 hours. Lack of growth could be explained by the inability of some gram-negative bacteria to grow on MacConkey agar or poor growth of certain bacterial strains.[15]

Dogs with different coat length or coat type (single coat vs double coat) did not harbor more bacteria before the chlorhexidine-alcohol surgical scrub or during the recolonization period. Therefore, it was unlikely that these criteria would play a role in the development of surgical site infections. The BCS did not affect the number of CFUs or bacterial species. An increase in the number of surgical site infections has been noted

for obese patients,[16,17] but the underlying cause of the infections may not be related to bacterial numbers or species; rather, the infections may be related to other metabolic changes associated with obesity that change the ability of a wound to heal or affect the integrity of the skin barrier. Lower numbers of bacterial colonies and Micrococcaceae bacteria were detected in older dogs of the present study. This may have been attributable to changes in the microbiome that occur with age, similar to the situation described for humans.[18]

The higher incidence of erythema after hair was clipped with a No. 40 blade was not surprising because of the close contact between the blade and the patient's skin. Heat generated from the blade or the mechanical action of the blade on the skin surface could have led to microtrauma and resulted in an inflammatory response.

A larger number of Micrococcaceae bacteria were found on the skin 24 hours after scrubbing with chlorhexidine on the side clipped with the No. 40 blade. This could be explained by the blade generating heat closer to the skin during clipping, which would lead to changes in skin pH, the lipid layer, and desquamation. These alterations in the natural defensive barrier of the skin could have led to variations in the microbiome between sides clipped with the No. 10 and No. 40 blades as well as selection for certain species of bacteria during the recolonization period. However, the increase in the number of Micrococcaceae bacteria could have been secondary to dislodgement of bacteria from the hair follicles by clipping the hair to a length of approximately 0.25 mm with the No. 40 blade. This finding is consistent with results for studies of dogs[1] and humans[19] in which the largest fraction of Micrococcaceae bacteria were found within the hair follicles instead of on the skin surface. Dislodgement of these bacteria could explain the difference in bacterial populations at the skin surface during the recolonization phase. Use of the chlorhexidine product cannot totally be ruled out as causing the change in the population of bacteria, although it would have been expected that these changes would have been on both sides clipped with either blade. A combination of these factors could explain the difference in numbers obtained from the sides clipped with the No. 10 and No. 40 blades. Additional studies would be required to evaluate these variables.

Flora on the skin of dogs can be affected by multiple factors, and the size of the blade used to clip the hair should be considered because of its impact on the recolonization phase. Complete speciation of the bacteria would be helpful in determining their pathogenic potential. Although results of the present study supported the use of a No. 10 clipper blade to prevent erythema and reduce variation in the skin microbiome, further studies are required to determine whether clipping the hair at a length > 0.25 mm at a surgical site would affect the incidence of surgical site infections.

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The authors declare there were no conflicts of interest related to this study.

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Footnotes

a. Oster CryogenX, Oster, Boca Raton, Fla.
b. Microsoft Excel, version 2010, Microsoft Corp, Redmond, Wash.

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