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The Use of Waterless Alcohol-based Antiseptic for Surgical Skin Preparation in Rhesus Macaques (*Macaca mulatta*)

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Ensuring asepsis of the surgical site before surgery is an essential component of safe surgical practices to reduce the incidence of surgical site infections in veterinary medicine. The current accepted method of skin preparation is a multistep process that alternates either a povidone-iodine or chlorhexidine soap scrub with a 70% alcohol rinse. After cleansing, the site is left to dry before draping. The goal of this study was to assess the effectiveness of a waterless alcohol-based (WAB) antiseptic as part of a 2-step procedure after the soap scrub. WAB antiseptics are commonly used as a presurgical hand scrub for the surgeon as they evaporate quickly and provide effective antisepsis. Previous studies have examined the WAB antiseptics in small animal surgeries. We tested this approach in large animal surgery. Twenty-four rhesus macaques were divided into 4 groups that received one of the following treatments: saline and alcohol, iodine-alcohol-iodine, soap scrub/WAB, and chlorhexidine-alcohol-chlorhexidine. The surgical site was swabbed before and after treatment and plated to assess sterility. Overall, no colonies were recovered from skin treated with WAB antiseptic, establishing it as an effective alternative to the current standard protocol. This method will simplify the current 3-step procedure and reduce animal handling, the use of materials, and the time necessary for surgical preparation.

Abbreviations and Acronyms: CHG, chlorhexidine; PVI, povidone-iodine; SSI, surgical site infection; WAB, waterless alcohol-based

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Introduction

Performing proper skin preparation before surgery is essential to optimizing patient health outcomes. The primary goal of skin preparation in both human and veterinary medicine is to achieve adequate antisepsis; this is defined as creating a skin surface free of gross contamination and microbial flora to reduce the risk of surgical site infection (SSI).[10] SSIs can have detrimental health effects and are easily prevented by the use of appropriate techniques.

SSIs account for 15% of nosocomial infections in human patients and are the most common nosocomial infections in surgery patients.[13] Studies in small animal medicine have suggested SSI incidence rates of 2% in clean surgical procedures with the frequency of infection ranging from 0.8% -18.1%.[4,9] In equine surgery, SSI frequency can occur in as many as 50% of surgical procedures.[4] The frequency of these infections demonstrates the need for adequate focus on preparing the surgical area and removing contaminants. This simple step is essential before performing surgery in all areas of medicine.

The current gold-standard of skin preparation for veterinary patients is a multistep process using liquid chemical agents and rinses to prepare the incision site. First, the surgical area should be cleaned of debris and a rectangular area shaved around the incision site. Scrubbing should commence in the center of the site, moving outwards toward the periphery. The scrub solution, usually a povidone-iodine (PVI) scrub, is alternated with a 70% alcohol rinse. The gauze used to apply the solution should be handled with sterile forceps. Either chlorhexidine (CHG) or PVI can be used as the scrub solution as they both have characteristics of a preoperative antiseptic, including killing gram-positive and gram-negative bacteria, fungi, and having some activity against viruses.[5] In addition, preoperative antiseptic agents should be nontoxic, hypoallergenic, non-absorbable, and provide residual activity. The combination of alcohol with PVI or CHG has better antimicrobial activity than PVI, CHG, or alcohol alone.[6] Soap and alcohol are used alternately; each is applied 3 times for a total of 5 min of contact time. The final step is to spray on or paint a 10% PVI solution. After the site has received a uniform application, it should be left to dry for 2 to 3 min before draping.[6] One study suggested that CHG may be superior to PVI for perioperative antisepsis, with a 41% reduction in total SSI in patients who received a single chlorhexidine- alcohol scrub.[3] Overall, the rate of SSI was significantly lower in the chlorhexidine-alcohol group compared with the povidone-alcohol group, 19.5% and 16.1% respectively. In both large and small animal aseptic surgery, draping is highly recommended to minimize contact with parts of the animal that have not been scrubbed.[3]

Although this standard method of prepping the skin for surgery has been in practice for decades, refinements to the procedure may be possible. Previous studies have focused on presurgical scrub practice in small animals, including rodents.[5,8,10] These studies found that use of a waterless alcohol-based (WAB) antiseptic was effective and comparable to the traditional method of surgical site cleansing for mice and rats.[5,8,10] To provide a generalized view of the effectiveness of this refined procedure, large animals should also be studied.

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Notable differences to consider between large and small animals are the size of the area prepared for surgery and their susceptibility to microbial flora from their habitat. Focusing specifically on nonhuman primates in a research environment, their size, and the clean, controlled environment they inhabit could permit modifications to the current skin-preparation procedure. Repetitive application of chemical disinfectant agents may be unnecessary and alternative methods that produce the same overall outcome should be investigated.

WAB antiseptics are used as a presurgical hand scrub for surgeons and provide fast and effective antisepsis.[8,15] WAB products include alcohol-based hand-sanitizer gels that evaporate spontaneously without rinsing.[5,8] Using a WAB hand scrub in the skin prep procedure to replace the current second and third steps could reduce irritation, provide a more efficacious antimicrobial effect, reduce time, and cut down on tap water usage.[7] Simplifying the current 3-step procedure to a 2-step procedure would reduce animal handling, the use of materials, and the time spent scrubbing before surgery. The present study will assess the performance of a WAB antiseptic as the cleansing agent after the initial soap scrub in presurgical skin preparation. We hypothesize that the sole use of a WAB agent to cleanse the surgical site after the soap scrub will be as effective in skin asepsis as is the standard 3-step scrub protocol.

Performing surgery appropriately is an essential step in maintaining animal welfare, such that refinement and improvement of current protocols should be considered. If a modification to the current skin preparation method can improve the overall surgery experience for large, laboratory animals, future applications may be possible in other areas of veterinary medicine.

Materials and Methods

Animal housing and husbandry. All the procedures performed herein were approved by the University Animal Care Committee and followed the CCAC guidelines for nonhuman primates. Twenty-four female rhesus macaques (*Macaca Mulatta*) were used (4 to 6 y old and 5.2 kg to 7.9 kg). Macaques were housed in groups of 6 with a 12:12-h light:dark cycle in indoor pens with an overall dimension of 485 cm × 155 cm × 225 cm. The pens included 2 integrated customized squeeze-back cages (Tecniplast, Buguggiate, Italy). The floor of the pens contained laboratory grade, 100% virgin wood fiber Kiln-Dried Pine Shavings (Northeastern Products, Warrensburg, NY). Pens were spot cleaned daily to remove feces and wet areas of shaving, with the shavings topped-up as necessary to create an approximately 4-cm bedding depth. Every 2 wk, the integrated squeeze-back cages were removed and washed in a cage wash (Tecniplas. Buguggiate, Italy) that achieved 82 °C (180°F). The pens were completely emptied, and all surfaces foamed with Accelerated Hydrogen Peroxide 4.25% (Lighthouse Life Sciences, Woburn, MA) diluted to 1:40 using WorldChem Hydro Sanitation System (Hydro System, Cincinnati, OH) using hot domestic city water. After 5 min contact time, room walls were scrubbed and then hosed down with hot domestic city water. A variety of manipulanda were provided on a weekly rotating basis, including but not limited to Kong Toys, mirrors, stainless steel balls, dry logs, hose, and tire swings. All macaques had ad libitum water access and were fed LabDiet 5050 (St. Louis, MO). In addition to lab chow, all macaques received 1 piece of fresh fruit/vegetable per day including, but not limited to, apples, oranges, watermelons, and celery. In addition, 1 cup of foraging mixture was added to the bedding for every 6 animals on a daily basis. The foraging mixture was made inhouse from Jumbo Primate Foraging with additional food-grade sunflower seeds and shelled peanuts. The animals were randomly assigned to their groups using excel randomization. The 4 treatment groups were as follows:

A. Control (saline and alcohol – 70% ethanol)
B. Standard scrub procedure 1: Iodine – alcohol
C. WAB: WAB antiseptic
D. Standard scrub procedure 2: Chlorhexidine 4% scrub – alcohol

The products that were used in each treatment group are as follows: group A, NaCl 0.9% irrigation (Baxter Corporation, Mississauga, ON, Canada) and 70% isopropyl alcohol (Loris; Lernapharm, Montreal, QC, Canada); group B, povidone Iodine 10% solution (Teva Canada, Montreal, QC, Canada) and 70% isopropyl alcohol (Loris; Lernapharm, Montreal, QC, Canada); group C, Baxedin 2% to 70% Untinted (Omega Laboratories Ltd, Montreal, QC, Canada), WAB containing Chlorhexidine Gluconate 2% w/v and isopropyl alcohol 70% v/v;, and group D, Germi-Stat Gel 4% (Ceva Animal Health, Montreal, QC, Canada) and isopropyl alcohol 70% (Loris; Lernapharm, Montreal, QC, Canada).

All macaques were clinically healthy at the time of the study. All animals in the facility undergo physical exam at least twice a year, with biannual TB testing using Tuberculin Mammalian, Human isolates (Zoetis, Kalamazoo, MI). In addition to being tested yearly for enteric culture (*Shigella/Salmonella/Campylobacter*), the Simian Serological Profile (Charles River Laboratories) and fecal analysis are conducted inhouse. All macaques had negative skin tests for tuberculosis, with no growth on enteric culture and serologically negative for *Simian Immunodeficiency Virus*, *Simian Retrovirus Virus*, *Simian T-cell Lymphotropic Virus STLV 1 and 2*, *Herpes B Virus*, *Measles Virus MV*, *Simian Foamy Virus, Simian Cytomegalovirus*, *Macaque Rhadinovirus*, *Varicella Zoster Virus*, *and Simian Virus 40.* All animals were positive for SV40 and Measles Virus. The high measles virus rates were attributed to previous measles vaccination.

Macaques were anesthetized with 4 mg/kg ketamine (Vetiquoinol, Lavaltrie, QC, Canada) and dexmedetomidine (Zoetis, Kirkland, QC, Canada), 4.5 mcg/kg. Once anesthetized, they were removed from their cages. No inclusion/exclusion criteria were set for this group of macaques. The Kingston Health Sciences Centre Clinical Microbiology laboratory personnel performed the microbiology and were blind to the study groups.

Surgical preparation, sample collection, and bacterial cultures. A 2 × 2 in. (5.1 × 5.1 cm) area was shaved on the ventral abdomen with a 40-clipper blade, with the xiphoid process as the midline. First, a prescrub sample was taken, swabbing in the pattern pictured in Figure 1. Next, the skin was treated with one of the 4 treatments. For the standard 3-step procedure, we alternated between the 2 solutions, with 3 passages each. The scrub action began in the midline of the shaved area and then moved outwards in a concentric pattern. After 5 min of allowing the solution to remain on the skin, another swab was taken in the pattern shown in Figure 1. All samples were submitted to the Kingston Health Sciences Centre Microbiology Lab.

The swabs used were the BD CultureSwab System, in vitro Diagnostic (MD), and they were not moistened prior to use. Swabs were plated on Blood Agar and Columbia Nalidixic Acid Agar plates and incubated in both CO_2 atmosphere and a MacConkey's Agar plate in an O_2 atmosphere. All plates were incubated at 35 °C. Plates were examined daily. If no growth was seen at 24 h, the incubation continued for another 24 h. If organisms were detected, they were gram stained followed by catalases performed for gram-positive specimens

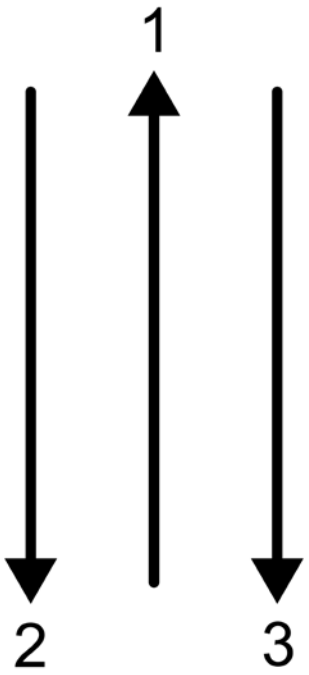

Figure 1. Swab pattern of the samples collected for pre- and postscrub treatments.

and oxidases performed for gram-negative specimens. A Matrix-Assisted Laser Desorption/Ionization (MALDI/ID) was performed with greater than or equal to 95% being acceptable for identification. All specimens were identified based on guidelines of the Kingston Health Sciences Centre Clinical Laboratory Services. The primary outcome that was measured was bacterial growth.

Statistical analysis. A 2-way ANOVA analysis with repeated measures was used to identify differences in the effectiveness of the 3 treatments and the control method. The data were entered as a repeated measure because the same group of macaques experienced a prescrub swab followed by a postscrub swab. Tukey's multiple comparison test was used. The Null hypothesis was rejected if P was less than 0.05, which was considered to indicate statistically significant effects and thus strong evidence against the null hypothesis. GraphPad Prism (V. 9.2.0; GraphPad Software, San Diego, California) was used for the analysis.

Results

Number of bacterial colonies detected before and after scrub. We observed a significant difference between the number of bacterial colonies generated before and after the scrub ($P < 0.05$). As seen in Table 1, the number of colonies detected was lower after all of the 4 treatments, with treatment C having the fewest colonies.

Between treatment groups. Statistical analysis of the data across treatment groups failed to reject the null hypothesis. Tukey's multiple comparisons test was used to compare the 4 groups and confirmed no significant difference between the treatment types ($P > 0.05$). This supports the effectiveness of using WAB antiseptic in a 2-step skin preparation procedure.

Discussion

Refining the method of presurgical skin preparation has several advantages, including reducing skin irritation, enhancing microbial asepsis, and conserving resources. The proposed modification to the current 3 step procedure has been studied in small animals, including rodents, but its use to large animals requires further study. Our goal was to validate the effectiveness of a modified skin-prep procedure for surgical preparation of rhesus macaques. The primary goal of pre-surgery skin preparation is to achieve complete asepsis of the incision site to eliminate the risk of postoperative SSI. SSIs lead to increased morbidity, mortality, and hospitalization, and thus are serious and costly.[11] Refinements should be considered for all commonly practiced techniques in all areas of veterinary medicine. Refinements can promote animal welfare and good outcomes after surgery.

In our experiment, we performed a skin swab before scrubbing the area to identify the bacteria that were present. A complete list of the bacteria that were identified is provided in Figure 3. Only 1 of 24 macaques had no growth before the scrub (Table 1). The number of positive cultures collected for each genus is shown for swabs taken both before and after the scrub treatment (Table 2) and postscrub treatment (Table 3). The macaques were divided into 4 groups of 6 and each group had a different skin treatment. The control treatment was a saline solution followed by alcohol. After this treatment (A), 2 animals had bacterial growth after the scrub. This was the largest number of positive cultures among the 4 treatment groups.

Treatments B and D were versions of our current scrub practice: (1) Chlorhexidine soap scrub (4%) followed by an alcohol rinse and finished with an application of chlorhexidine solution and (2) iodine soap (7.5%) followed by an alcohol rinse and finished with an application of iodine solution, respectively. Both treatments reduced the positive cultures to only 1 positive

Table 1. The number of bacterial colonies detected before and after surgical scrub for each treatment group

	Treatment A		Treatment B		Treatment C		Treatment D	
Culture:	Before	After	Before	After	Before	After	Before	After
Staphylococcus	3	0	7	1	4	0	9	0
Coagulase-Negative *Staphylococcus*	0	2	0	0	1	0	0	0
Aerococcus	2	0	2	0	3	0	4	1
Corynebacterium	3	0	0	0	3	0	2	0
Acinetobacter	0	0	3	0	1	0	3	0
Streptococcus	4	0	5	0	0	0	1	0
Lactobacillus	1	0	2	0	2	0	1	0
No growth	0	4	0	5	1	6	0	5
Total	13	2	19	1	14	0	17	1

*The data reported includes all animals that had at least 1 colony of the species listed.

Table 2. Numbers of colonies detected before treatment among the 24 macaques.

Genus	No. of Positive Swabs (of 24)	%	Organisms detected
Staphylococcus	18	75	*S. epidermidis*, *S. cohnii*, *S. capitis*, *S. warreri*, *S. petterikoferi*, *S. xylosus*, *S. simulans*, *S. aureus*, *S. saprophyticus*, *S. equorum*
Coagulase-Negative *Staphylococcus*	1	4	Not speciated
Aerococcus	11	45	*A. viridans*
Corynebacterium	8	33	Not speciated
Acinetobacter	7	29	*A. lwoffii*, *A. johnsonii*
Streptococcus	10	41	Not speciated
Lactobacillus	6	25	Not speciated
None detected	1	4.	None detected

*The data show numbers of colonies for all macaques that were positive for at least 1 species in the category of organisms listed.

Table 3. Number of colonies detected after treatment among the 24 macaques.

Genus	No. of Positive swabs (of 24)	%	Organisms detected
Staphylococcus	1	4	*S. epidermidis*
Coagulase-Negative *Staphylococcus*	2	8	Not speciated
Aerococcus	1	4	*A. viridans*
None detected	20	83	None detected

*The data show numbers of colonies that were positive for at least 1 species in the category of organisms listed.

animal after the scrub. Overall, the WAB scrub was the most effective, eliminating all bacteria from the surgical site. This treatment consisted of a chlorhexidine soap application (4%) followed by the application of the WAB antiseptic.

Our data showed a significant reduction in numbers of bacterial colonies between before and after treatments ($P < 0.05$), demonstrating the effectiveness of these protocols. This was expected as we know our current protocol is effective in cleaning the surgery site and has been used for many decades. The control treatment of saline and alcohol also provided effective asepsis, reducing the number of colonies from 13 total colonies to 2 colonies of Coagulase-Negative *Staphylococcus*. The results are likely due to the effectiveness of alcohol as a disinfectant; 70% to 80% ethanol applied for a minimum of 5 min efficiently sterilizes a surface.[1,14] Ethanol concentrations ranging from 30% to 90% are bactericidal.[1] Organisms incubated for 16 h after inoculation and exposed to 70% ethanol for 1 to 24 h did not grow.[1] The study also reported that ethanol does not compromise the integrity of materials such as the rubber and shellac when it is used for cleaning.[1]

We did not find a statistically significant difference ($P > 0.05$) between the treatments, and so were not able to reject the null hypothesis: there is no difference in effectiveness between the standard treatments and the proposed 2-step procedure. This is not surprising because WAB antiseptics are currently used as a hand scrub for surgeons due to the strong antimicrobial effects of alcohol.

The species cultured from the swabs are listed in Figure 2. These species were compared with what has previously been deemed a baseline skin flora for nonhuman primates from 2 sources.[2,12] One study identified a core primate axillary microbiome that encompasses taxa common to 95% of individuals.[2] The most abundant taxa cultured based on read number were *Corynebacterium*, *Prevotella*, *Anaerococcus*, and *Staphylococcus*. Nonhuman primates that are more evolutionary distant from humans have a greater diversity of Operational Taxonomic Units outside of these 4, which is attributed to hygiene practices and the evolutionary differences of the hosts.[2] In addition, an analysis of 144 colonies from the abdominal skin of cynomolgus macaques (*Macaca fascicularis*) revealed the following 5 genera:

Species Cultured				
Genus	***Staphylococcus***	***Acinetobacter***	***Streptococcus***	***Lactobacillus***
Species	*Epidermidis*	*Lwoffii*	*Alactolyticus*	*Not speciated*
	Cohnii	*Johnsonii*	Equirius	
	Capitis		Not speciated	
	Warreri			
	Petterikoferi			
	Xylosus			
	Simularis			
	Aureus			
	Saprophyticus			
	Equorum			
	Coagulase-negative (not speciated)			

Figure 2. List of all species cultured during the experiment

Staphylococcus, *Kocuria*, *Micrococcus*, *Corynebacterium*, and *Rothia*, which are common bacteria on animal skin.[12]

All of the species identified in the samples swabbed from the ventral abdomen of the rhesus macaques *(Macaca Mulatta*) were considered normal for nonhuman primates except for lactobacillus. *Lactobacillus* was not listed in the typical normal skin flora for axilla or the ventral abdomen of macaques.[2,12] Although species from the lactobacillus genus were not identified in previous studies[2,12] this genus is present in the vaginal microbiome of nonhuman primates.[10] Another study examined the effect of *L. crispatus* colonization on the vaginal microbiome using vaginal swab samples collected for the duration of 3 menstrual periods.[11] DNA extraction and analysis using qPCR detected stable and low levels of *L. crispatus* that were not influenced by the animal's menstrual cycles. *Lactobacillus* in total made up 0.1% of the rhesus macaque vaginal microbiome. When the rhesus macaques were colonized with *Lactobacillus*, the composition of the microbiome was not drastically affected nor was the immunologic milieu.[11] These findings suggest that *Lactobacillus* could be considered normal in the composition of the vaginal bacterial flora of rhesus macaques.

As our study showed, many of the samples that were treated with the WAB antiseptic had no bacterial cultures, demonstrating the effectiveness of WAB as compared with our standard protocol. Recent studies in small research animals have had similar findings.[5,10] With the addition of our data, we can confidently use a WAB antiseptic in the presurgical scrub of both small and large animals.

A limitation of this research is its application to other areas of large animal veterinary medicine. Our research was conducted on rhesus macaques housed in a controlled, clean environment; however, different conditions might apply to farm animals and species with more contaminated environments. Large animals, including cows, horses and pigs, may require a more intensive scrub to effectively remove the microbial agents that are present in their habitat. In addition, our samples were only collected from the ventral abdomen of the rhesus macaques. In future studies, other common surgical sites should be evaluated. The efficacy of WAB antiseptic agents could vary depending on the incision site due to differing levels of contaminants in the environment. Other areas to collect skin flora samples from include dorsal surfaces, the perianal area, the thorax, and any other common areas where incisions would be made. Our research provides a stepping stone to future studies that consider the role of WAB antiseptic agents in incision infections, wound healing, and skin irritation. Future studies could also evaluate the effect of WAB antiseptic agents on thermoregulation in nonhuman primates undergoing surgery. We did not evaluate this in the present study as we believe the thermal effects on large animals would be minor as compared with rodents. Our research focused on antisepsis of the surgery site. Another consideration is the spontaneous evaporation of alcohol. In our procedure, the antiseptic remained on the surgery site for 5 min before the sample was collected; however, our study did not evaluate the residual or long-term presence of microbial flora that may remain after the alcohol has evaporated. We suggest that future studies consider the residual effects of chlorhexidine and iodine beyond the 5-min time-period that was assessed in our study.

Most research on presurgical asepsis protocols in veterinary medicine has been limited to small animals. We tested the effectiveness of a 2-step scrub procedure on rhesus macaques. The data showed that using a WAB antiseptic as the second and final step in skin preparation effectively eliminates bacteria from the abdomen of rhesus macaques and justifies using this procedure in large animal surgery.

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