Comparative antimicrobial efficacy of 4 surgical hand-preparation procedures prior to application of an alcohol-based hand rub in veterinary students

Hermien Viljoen BVSc, MSc[1,*], Johan P. Schoeman BVSc, MMedVet (Med), DSAM, Dipl. DECVIM, PhD[1], Geoffrey T. Fosgate DVM, PhD, Diplomate ACVPM [2], Charles Boucher BVSc (Hons), MMedVet (Small Animal Surgery[1]

[1] Department of Companion Animal, Clinical Studies, Faculty of Veterinary, Science, University of Pretoria, Pretoria, South Africa
[2] Epidemiology Section, Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Pretoria, South Africa

*Correspondence
Hermien Viljoen, Department of Companion Animal Clinical Studies, Onderstepoort Veterinary Academic Hospital, Private bag X04, Onderstepoort 0110 South Africa. Email: hermien.viljoen@up.ac.za

Funding information: Companion Animal Clinical Studies Research Fund of the University of Pretoria; South African Veterinary Foundation

ABSTRACT

Objective: To determine the influence of skin preparations before application of an alcohol-based hand rub (ABHR) on bacterial counts before and after elective surgery.

Study design: Clinical prospective study.

Sample population: Veterinary students (n = 103) performing ovariohysterectomies on 140 dogs.

Methods: Participants were randomly assigned to 1 initial surgical preparation on the day of surgery: A – hand preparation with medicated solution (4% w/v chlorhexidine bigluconate followed by an ABHR; B – application of a medication solution (benzalkonium chloride 0.1%-1% and polymeric biguanide hydrochloride 0.01%-0.1%) followed by an ABHR; C – nonmedicated pH-neutral soap hand wash followed by ABHR, and D – direct application of an ABHR. Samples were taken by pressing the distal finger tips to an agar plate before the hand preparation, after the hand preparation (n = 3), after ABHR application, and 120 minutes later. Colony-forming units (CFUs) for samples were determined. Total log CFU and CFU log10 reduction were calculated and used for comparison with $P < .05$.

Results: Two hours after surgery commenced, the participants of groups that performed a hand preparation had lower total CFUs than those that did not perform a hand preparation (P = .001). In particular, the number of CFUs was lower when ABHR was performed after application of pHN compared to direct ABHR (P = .001).

Conclusion: In this population, performing a hand preparation with pHN prior to applying an ABHR had better antimicrobial effect for the duration of surgery than not performing a hand preparation.

Clinical significance: Surgeons should wash their hands prior to ABHR before starting their first surgery of the day, even when hands appear clean.

1 INTRODUCTION

The skin microbiota on the hands of the surgeon and surgical staff are broadly classified as either transient or resident flora.[1] Transient flora are susceptible to removal by routine handwashing because they only colonize the superficial layer of intact skin.[1] Resident flora, found in the deeper layers of skin and hair follicles, are more resistant to removal and are not as likely to be associated with nosocomial infections.[1] In contrast, transient flora are acquired by contact with other people, animals, and/or contaminated surfaces and are the most common cause of surgical site infection (SSI).[2, 3]

Surgical site infection increases the cost of care in both human and veterinary hospitals significantly.[4, 5] It results in poor wound healing, increased use of antibiotics, extended hospital stays, and increased rates of morbidity and mortality.[6-8] When the bacterial count in a surgical wound is $\geq 10^5$ colony forming units (CFUs) per gram of tissue, the risk of SSIs increases significantly.[2] The incidence of SSI as a complication of small animal surgery has been reported to be as high as 18.1% depending on the type of surgery performed.[5] Surgical site infections cannot be completely eliminated but preventative strategies can be implemented to reduce their incidence and impact.[9] Pathogens responsible for SSI can originate from the patients' own resident flora but they can also originate from exogenous sources, such as the hospital environment and members of the surgical team.[2, 10] It is for this reason that surgical hand preparation prior to performing surgical procedures is an important strategy in minimizing the transition of potential pathogenic bacteria from the surgeon's hands to the surgical wound.

Surgical hand preparation is performed to reduce the risk of SSI by removing transient skin flora and minimizing resident skin flora before surgical glove donning.[8, 11] The ideal agent used for presurgical hand preparation, should adequately reduce micro-organisms on intact skin, have a broad-spectrum activity, and a residual effect.[11] While the use of sterile surgical gloves by surgical staff aids in preventing bacterial transfer from the surgical staff to the patient, studies show that between 4% to 80% of sterile gloves become perforated during surgical procedures, highlighting the importance of appropriate surgical hand preparation.[12-14] There are currently 3 main types of antiseptic solutions available for surgical hand preparation: aqueous medicated scrub solutions, alcohol-based hand rubs (ABHRs), and ABHRs containing additional active ingredients.[13]

Alcohol-based hand rubs have been shown to be as effective as, or even more effective than, the currently available surgical hand scrubs.[11, 14-20] Alcohol-based hand rubs were reportedly better tolerated (fewer reported incidences of dermatitis) than conventional surgical scrubs with medicated soap, required reduced application time, and led to better compliance and tolerance among surgical staff in human hospitals.[8, 15, 20] Alcohols also exhibit rapid broad-spectrum activity against vegetative bacteria, viruses, and fungi; yet alcohol does not have residual activity.[21] When it desiccates, regrowth of skin flora can occur in the humid environment under the surgical glove.[11, 22] To address this issue, long-acting compounds such as chlorhexidine gluconate (CHX), have been added to the alcohol-based hand rub formula to suppress bacterial growth under the gloves.[6, 15, 22] Alcohol-based hand rubs are widely used in

human hospitals across the globe, and their popularity is rising among human surgeons because of the aforementioned associated benefits.[13, 17] The use of ABHRs in the veterinary field has also grown as indicated by the number of recent publications.[3, 6, 18]

Most of the research on presurgical hand asepsis have been performed in human medicine and has generated relatively little interest in the veterinary field.[8] Accordingly, there are comparatively fewer studies assessing presurgical hand asepsis in the veterinary field and recommendations for veterinarians are largely extrapolated from the research performed in human medicine.[3, 7, 16, 18] One of the recommendations for using ABHRs for surgical hand preparation is that an approved hand rub should be applied to dry hands free of debris and gross organic material.[9] There are concerns, however, that this method of surgical hand preparation may not be sufficient to reduce bacterial contamination levels to acceptable low levels for the small animal surgeon.[3, 8] Consequently, there is a need for research on surgical hand asepsis that is specifically aimed at the veterinary surgeon to reduce transmission of pathogenic bacteria from the hands of the veterinary surgeon to the patient.

The efficacy of 4 different hand preparation protocols was compared. These were: A – hand preparation with medicated solution (4% w/v CHX followed by an ABHR); B – application of a medication solution with benzalkonium chloride 0.1%-1% and polymeric biguanide hydrochloride 0.01%-0.1% (BAC) followed by an ABHR; C – nonmedicated pH neutral soap (pHN) hand wash followed by ABHR, and D – direct application of an ABHR. The objectives of this project were to compare the number of and the reduction in CFUs after ABHR application using 3 hand preparation protocols and a no hand preparation protocol on visibly clean hands. The 4 protocols were used by veterinary students performing ovariohysterectomies in a teaching hospital. We hypothesized that application of an ABHR on visibly clean hands would not differ significantly in reduction of CFUs from using hand preparation protocols or no hand preparation prior to ABHR application.

2 MATERIALS AND METHODS

2.1 Experimental design

This study was conducted from April 1 to November 30, 2019, as a randomized field study. The study protocol was approved by the research ethics committee (reference no. 049-18), the animal ethics committee (reference no. V072-18), and the health ethics committee (reference no. 478/2018) of the University of Pretoria. Participants eligible for enrolment were final-year veterinary students performing elective ovariohysterectomies on client-owned dogs. All participants were required to sign informed consent forms and were permitted to withdraw from the study at any point. Owners of the dogs involved in the study were also fully informed about the study and were required to sign informed consent forms. Participants were required to have nails short (<1 mm), clean, and to be free of artificial nails or nail products in conformity with international guidelines concerning surgical hand hygiene.[13, 23] Participants with dermatitis, cuts, scrapes or grossly contaminated hands, fingernails or arms were excluded from the study. Participants were required to wear appropriate surgical attire including scrubs, gowns, shoes, and head covers while performing ovariohysterectomies. Participants were assigned to 1 of 4 groups (group A, B, C or D) using a simple random allocation method by drawing a number out of a bag. The different groups represented different hand-preparation protocols prior to applying an ABHR for all groups. Standardized instructions were used to prevent variations in technique. The day before participants were to perform the ovariohysterectomy, the primary investigator educated the participants on the

surgical hand-preparation protocols that they would be expected to perform and on the methodology of sample collection for this trial. Participants did not perform any other surgical hand preparation or surgical scrubbing on the day prior to their partaking in this trial.

2.1.1 Hand-preparation protocols

The following products were used in the hand-preparation protocols assigned to students: a hand scrub containing 4% w/v CHX (Bioscrub, Medinox, Johannesburg, South Africa), a hand scrub containing benzalkonium chloride 0.1%-1% and polymeric biguanide hydrochloride 0.01%-0.1% (BAC) (F10 Hand Scrub, Health and Hygiene (Pty) Ltd, Florida Hills, South Africa), a pH-neutral, nonmedicated soap (pHN) (a no name brand, produced by Kyron Laboratories (Pty) Ltd, Benrose, South Africa), and an ABHR antiseptic solution containing 0.5% CHX, 70% propyl alcohol and emollients (D-Germ, B Braun Medical (Pty) Ltd, Randburg, South Africa).

The protocol for surgical hand preparation and the timing of samples (samples 1-4) for group A to D are outlined and summarized in Table 1. Groups performed presurgical hand preparation as follows: A – hand preparation with medicated solution (4% w/v CHX) followed by an ABHR; B – hand preparation with a medication solution (BAC) followed by an ABHR; C – hand preparation with pHN followed by ABHR and D – direct application of an ABHR. To establish a baseline, all participants rinsed their hands and forearms up to their elbows under lukewarm running water for 30 seconds. Then they dried their hands and arms completely with nonsterile paper towel. Participants of groups A, B, and C performed a hand preparation by wetting and washing their hands and arms up to their elbows with CHX, BAC, or pHN, respectively, for 30 s. They subsequently rinsed them under water for a further 30 seconds before drying their hands and arms with nonsterile paper towel so that the total hand preparation procedure lasted 1 min. Once the hand preparation was completed, an ABHR was applied according to the manufacturer's recommendation for surgical hand preparation.[24] Participants assigned to group D did not perform a hand preparation and applied and ABHR following the rinsing step. It was accepted that all participants washed their hands at least once during the course of the day (eg, after going to the toilet), prior to entering the theater. The surgery performed by the participants were the first surgery for the day for the individual. The ABHR continued until the alcohol had completely evaporated, after which sterile surgical gown and closed glove donning was performed according to standardized protocols.

2.2 Experimental procedures and data collection

2.2.1 Sampling method

To collect samples (Table 1), participants gently pressed all the distal aspects of the phalanges of 1 or both hands on agar contact plates (Oxoid Ltd., Basingstoke, Hampshire, England) for 5 s. The person assisting with collection of samples decontaminated his hands by performing an ABHR procedure and wore examination gloves to prevent inadvertent contamination during the collection procedure. Packaging of agar contact plates remained sealed just prior to collection of samples and were resealed immediate after sample collection to prevent postsampling contamination. Agar contact plates consisted of tryptone soya agar with neutralizing agents' lecithin (5%) + Tween 80 (15%). Sample 1 was taken from the left and right hands after the rinse step. Sample 2 was taken after participants performed the hand preparation. An online random choice generator (www.textfixer.com/tools/random-

choice.php) was used to determine if the second sample was taken from the left or right hand. Sample 2 was not collected from Group D but a left or right hand was identified for the participant. Sample 3 was taken directly after a surgical ABHR procedure from the hand that was not selected in the previous round. To standardize the timing of the final sample (sample 4), 120 min was chosen as the time the final sample would be taken as this is typically the time a veterinary student takes to complete an ovariohysterectomy at the training facility. A timer was set for 120 min as soon as surgical gowning and gloving were completed. The students proceeded with performing an ovariohysterectomy on client-owned dogs under the supervision of a qualified veterinarian. At the end of 120 ± 5 min, students were asked to remove the surgical gloves, and the final sample (sample 4) was taken from the hand that was not sampled in the previous round, all while preserving sterility of the hands.

TABLE 1. Summary of the procedures followed for surgical hand preparation by 4 different test groups (Groups A-D) and the different sampling times (Sample 1-4)

Step 1:	**Participants rinse hands and forearms under running tap water for 30 seconds**			
	Sample 1: Sampling of fingertips of both left (L) and right (R) hands			
Step 2:	Group A: Hand preparation with CHX	Group B: Hand preparation with BAC	Group C: Hand preparation with pHN	Group D: No hand preparation performed
	Sample 2: Sample L or R hand depending on a random choice generator			Sample 2 omitted
Step 3:	Application of ABHR			
	Sample 3: Sample of the L or R hand that was NOT sampled directly after the prewash (Sample 2)			
Step 4:	Perform ovariohysterectomy (120 ± 5 minutes)			
	Sample 4: Resample of the L or R hand that was sampled directly after the hand preparation (Sample 2)			

Abbreviations: ABHR, alcohol-based hand rub; BAC, hand scrub containing benzalkonium chloride and polymeric biguanide hydrochloride; CHX, hand scrub containing chlorhexidine bigluconate; pHN, nonmedicated pH neutral soap.

Surgical gloves were collected and evaluated for the number of punctures per finger by filling it with colored water and sealing the cuff. The dominant hand of each participant was recorded. Students who performed an ovariohysterectomy in less than 115 min were excluded from the study. If students required intraoperative assistance from the supervising veterinarian, all samples obtained from the individual were discarded and excluded from the study. Sampling of alternating hands was performed to avoid potential interaction between neutralizer residue from agar and antiseptic residues from the previous round of sampling.

2.2.2 Incubation and counting of colony forming units

Agar contact plates were placed in an incubator for 48 h at 36-38°C under aerobic conditions within 4 h of collection. Samples were labeled in a coded manner so that laboratory staff that counted CFUs were blinded to the hand preparation protocol used. Colony-forming units were counted with the naked eye by a laboratory technician.

2.3 Statistical analysis

Hand CFU data were assessed for normality by plotting histograms, evaluating descriptive statistics, and performing the Anderson-Darling test in available statistical software (MINITAB Statistical Software, Release 13.32, Minitab Inc, State College, Pennsylvania). Hand CFU data were log10 transformed to improve the distributional form and descriptive statistics were presented as medians and interquartile ranges (IQR) for all quantitative data. The log10 reduction in the hand CFU caused by the surgical scrubbing procedures was

calculated as the simple subtraction of postscrub log10 CFU values from the values obtained for the same hand at baseline. Linear mixed models were used to compare hand log10 CFU (and log10 reductions) among the 4 surgical scrub treatment groups. "Student" was included as a random effect in these models, as some students were enrolled into the study more than once. The treatment group was included as a fixed effect and post hoc pairwise comparisons of means were adjusted using the Bonferroni correction. Independent models were fitted for each study time point. Gloves that developed holes during surgery were a potential source of contamination. The variables "whether or not the sample was collected from the dominant hand" and "whether holes developed in the gloves during surgery" were therefore forced into the postsurgery statistical model to adjust for potential confounding (not subject to removal based on tests of significance). Unless stated otherwise, all statistical analyses were performed using commercial software (IBM SPSS Statistics Version 25, International Business Machines Corp., Armonk, New York) and significance was set as $P < .05$.

3 RESULTS

A total of 103 final year veterinary students participated in the study. Each group was allocated 35 participants and of the total participants, 35 students participated twice and 1 student participated 3 times. None of the participants experienced a skin reaction to any product. One participant was excluded after reporting a skin reaction (mild rash) after applying the ABHR product to their hands and arms during a previous, separate occasion. All client-owned pets involved in the study recovered fully after the ovariohysterectomy and no SSIs were reported by owners or noted during follow-up examinations to remove skin sutures 14 days after surgery.

There was no difference in the number of CFUs between the 4 groups following the rinsing step "sample 1" (Table 2) ($P = .552$). There was no difference in the number of CFUs due to a hand preparation "sample 2" found between group A "CHX" and group B "BAC" or between group B and group C "pHN" (Table 2). There was, however, a difference found in the number of CFUs between group A and C due to prewash alone (Table 2) ($P = .32$). The number of CFUs just after performing an ABHR "sample 3" was higher in group D "ABHR alone" than group A (Table 2) ($P = .032$). There was no difference in total CFU just after performing an ABHR between the groups A-C (Table 2). The total CFU of sample 4 was higher in group D than in the groups that performed a hand preparation prior to an ABHR. (Table 2). No difference was found in the total log10 CFU reduction from the baseline to 120 min after surgery commenced between the 4 groups (groups A-D) (Table 2), but the benefit of performing the hand preparation (Group A-C) over the group that did not perform a prewash (group D) remained significant even after adjusting for potential confounding within a multivariable model (Table 3) ($P = .003$, $<.001$, 0.13 for Group A-C respectively).

TABLE 2. Comparison of colony-forming units (CFUs) on the fingertips of final year veterinary students randomly assigned to 1 of 4 surgical scrub treatment groups (140 total replicates) prior to performing an elective ovariohysterectomy (35 treatments within each groups)

Measurement	Group A CHX	Group B BAC	Group C pHN	Group D No hand preparation	*P*
	Median* (IQR)	**Median* (IQR)**	**Median* (IQR)**	**Median* (IQR)**	
Sample 1 CFU[a]	20^{x} (6, 44)	30^{x} (6, 46)	16^{x} (5, 43)	33^{x} (8, 65)	.552[†]
Log10 CFU reduction due to hand preparation only	0.59^{x} (0.00, 1.10)	$0.08^{x,y}$ (−0.31, 0.99)	-0.39^{y} (−1.61, 0.60)	NA	.012[†]
Sample 3 CFU[b]	0^{y} (0, 1)	$0^{x,y}$ (0, 3)	$0^{x,y}$ (0, 3)	2^{x} (0, 6)	.010[†]
Sample 4 CFU[c]	1^{y} (0, 5)	0^{y} (0, 2)	0^{y} (0, 10)	4^{x} (0, 28)	.001[†]
Log10 CFU reduction total	2.71^{x} (1.79, 3.26)	2.17^{x} (0.66, 3.57)	1.95^{x} (0.69, 2.94)	2.30^{x} (0.69, 2.92)	.362[‡]

Abbreviations: BAC, hand scrub containing benzalkonium chloride and polymeric biguanide hydrochloride; CHX, hand scrub containing chlorhexidine bigluconate; CFUs, colony forming units; IQR, interquartile range (25th to 75th percentile); NA, not applicable; pHN, nonmedicated pH neutral soap.

Note: Data log10 transformed prior to statistical analysis.

[†] Based on linear mixed models including student as a random effect and treatment group as a fixed effect.

[‡] Based on a linear mixed model including student as a random effect and treatment group as a fixed effect. Whether or not the sample was from the dominant hand, whether holes were present (thumb, index, middle, ring, pinky), and baseline CFUs were included in models to adjust for potential confounding.

* Medians, in the same line, without superscripts (x, y) in common are different ($P < .05$) after Bonferroni correction for multiple post hoc comparisons.

[a] Baseline after rinsing hands.

[b] Prior to surgical gowning and gloving (presurgery).

[c] Post glove removal (postsurgery).

TABLE 3. Multivariable analysis of the log10 colony forming units (CFUs) on the hands of final year veterinary students randomly assigned to 1 of 4 surgical scrub treatment groups (140 total replicates) after performing an elective ovariohysterectomy

Variable	Level	Estimate (95% CI)	*P*
Surgical scrub	CHX	−0.42 (−0.68, −0.15)	.003
	BAC	−0.57 (−0.83, −0.30)	<.001
	pHN	−0.34 (−0.61, −0.07)	.013
	No hand preparation	Referent	
Initial CFUs	Continuous	0.07 (0.00, 0.14)	.051
Glove	Dominant hand	−0.16 (−0.38, 0.06)	.146
Hole present in glove	Thumb	−0.25 (−0.49, −0.02)	.036
	Index	0.04 (−0.23, 0.30)	.792
	Middle	0.05 (−0.46, 0.57)	.840
	Ring	0.30 (−0.50, 1.09)	.464
	Pinky	Referent	

Abbreviations: BAC, hand scrub containing benzalkonium chloride and polymeric biguanide hydrochloride; CHX, hand scrub containing chlorhexidine bigluconate; CI, confidence interval; pHN, nonmedicated pH-neutral soap.

4 DISCUSSION

The number of CFUs on the hands of participants that performed a hand preparation prior to applying ABHR was lower compared to that of participants that performed no hand preparation. Our hypothesis that application of an ABHR on visibly clean hands without performing a hand preparation would not differ in total CFU reduction from using hand preparation protocols prior to ABHR was, therefore, rejected. The purpose of performing a hand preparation is to reduce the number of transient microbes that colonize the superficial layers of the skin.[1] The mechanical action of rinsing hands with water alone reduces the bacterial load to some extent and this effect is further amplified when hands are washed with scrub or soap and rinsed.[25] Scrubs with active antiseptic substances are more effective in reducing bacterial load on hands compared to solutions without but it is unclear whether there is a demonstrable, overall reduction in the bacterial load from washing with a medicated soap compared with a nonmedicated soap before performing an ABHR for surgical hand preparation.

Recommendations for alcohol-based hand rubbing protocols are made by several international organizations, including the World Health Organization (WHO) and Association of periOperative Registered Nurses (AORN).[7, 13, 23] Whether or not it is advisable to wash hands before applying an alcohol-based hand rub is unclear and remains a controversial topic.[6, 13, 26, 27] For surgical hand preparation, the WHO does not recommend a hand wash every time an ABHR is performed, but instead they recommend that the hands of the surgeon or members of the surgical team should be cleaned only once upon entering the theater by washing hands with a gentle, pH neutral, nonmedicated soap.[13] Alcohol-based hand rubs should then be applied before and between procedures, but hands should only be rewashed if they are visibly soiled or if there was contact with biologically hazardous material.[13, 28] There is even some evidence that hand preparation prior to applying ABHRs could negatively alter the effectiveness, especially if hands are not dried sufficiently before applying the ABHRs.[16, 28] The work environment of veterinary surgeons differs from that of human surgeons, with more opportunities to contaminate hands throughout a work shift. Veterinary surgeons also often examine patients in between surgeries and may therefore have different flora and/or level of contamination on their hands during the course of the day.[3] Recommendations for surgical hand preparation should be determined separately and specifically for veterinary surgeons.

In this study, the number of CFUs was lower after a 1 minute hand preparation with CHX compared to a hand preparation with pH neutral soap, but no difference compared to a hand preparation with BAC was found. Directly after an ABHR was performed, the participants of the group that washed hands with CHX had fewer total CFUs than those of the group that performed no hand preparation, but not in comparison with the other groups. Chlorhexidine gluconate is one of the most common antiseptics used for hand disinfection in both the medical and veterinary fields.[8, 22, 29] Concerns have been raised about the long-term use of CHX as a topical antiseptic on the skins of healthcare workers, with frequent reports of dry skin, eczema, or dermatitis attributed to CHX use.[29] Other adverse reactions that have been reported include asthma, contact dermatitis, and immediate and/or delayed hypersensitization.[29]

Despite its popularity as an antiseptic agent, the true efficacy of CHX has been questioned.[30-32] The effect of residual activity of CHX in particular has been questioned due to inadequate neutralizers used in studies evaluating its antiseptic efficacy.[30-32] If the neutralizers in the

sampling fluids or agar are not effective, residues of the active antiseptics will continue to reduce the number of the microorganisms after sampling causing false-positive antiseptic efficacy.[31, 32] The employed neutralizing agents used in this study (5% lecithin +15% Tween 80) are commonly used for neutralizing a wide variety of antiseptics in the medical and industrial fields. Lecithin neutralizes quaternary ammonia compounds such as those used in the BAC hand scrub and the combination of Tween 80 and l-α-lecithin is effective in neutralizing CHX.[33, 34] Regardless, Kampf argues that neutralizing agent validation tests should performed specifically for compounds used in tests for antiseptic efficacy.[35] A major shortcoming of this study is that the neutralizing agents were not validated specifically for the active ingredients used in this experiment. This could be why the group that washed hands with CHX outperformed the other groups, and therefore these results must be interpreted with extreme caution. In order to prevent false-negative antiseptic efficacy, this study was designed so that the sampling of alternating hands were performed, avoiding interaction between neutralizer residues and antiseptic residues from the previous round of sampling.

The most important result from this study is that 120 min after surgery commenced, the groups that performed hand preparations prior to ABHR had fewer CFUs than the group that performed no hand preparation. More significant, the group that performed a hand preparation with pHN also outperformed the group where no hand preparation was performed. This is an important finding considering that the lack of neutralization agent validation casts doubt on the reliability of CFU results in groups that used medicated scrubs. There were no significant differences in the total log10 CFU reductions among the 4 hand preparation groups from baseline to final sampling (P = .362). This could be as a result of the baseline of Group D being the highest of all the groups, even though the difference were not significant (P = .552).

Although the technicians responsible for counting CFUs on agar plates were blinded to the treatment of each group, it was not possible to blind participants to the different treatment groups that they belonged to. It is possible that this could have introduced potential bias (either recognized or unrecognized) in the behavior of students. Another limitation of the study is that only CFUs on the fingertips were sampled and counted, compared with collecting samples using the glove juice technique that would have probably yielded far more accurate results.[8] It is assumed that all participants of all groups (including group D) washed their hands prior the entrance to the theater complex following other activities, eg using lavatories, examining other patients. As the ovariohysterectomy performed by the participants was the very first surgery performed on the day, as opposed to doing a second or third surgery where hands would be washed several times, the question arises if the results would be different if participants partook in several consecutive surgeries prior that would have involved them washing their hands every time before applying ABHR or only applying ABHR in between surgeries.

The gold standard for determining the efficacy of hand asepsis protocols is by performing trials that ultimately measure the effects of hand preparation protocols on the incidence of SSIs.[8,32] Even though no surgical site infections were reported by owners in the population of dogs in this study, the study was not designed to identify different grades of SSI objectively. Furthermore, bacteria cultured in this study were not identified and therefore there is no information on the pathogenicity of the bacteria that was collected in the samples. This information could prove to be useful especially when correlating it with incidence of SSI.

In conclusion, the results of this study contribute to the crucial ongoing research on best practices for surgical hand preparation specifically for the veterinary surgeon. The results support the notion that performing a hand wash or scrub prior to an ABHR for surgical hand preparation is more effective at reducing bacterial load than omitting to perform a hand preparation prior to ABHR, even when hands appeared to be clean and free from organic material. Specifically, washing hands with a nonmedicated soap (pHN) was better than not washing hands at all prior to performing an ABHR. Unfortunately, due to the lack of neutralizing agent validation, no definite conclusions can be drawn regarding the efficacy of using medicated products prior to ABHR. Considering the potential for adverse skin reactions with the use of medicated scrubs, in particular scrubs containing CHX, the results support a 1 minute hand preparation with a pH-neutral soap prior to the application of an ABHR for the veterinary surgeon. A larger study in the same format is warranted, with more sensitive sample collection methods such as the glove juice technique, validation tests of neutralizing agents, and reporting on the incidence of SSIs.

ACKNOWLEDGMENTS

Author Contributions: Boucher C, BVSc, MMedVet: contributed significantly to the conception and design of the study and interpretation of the data. Fosgate GT, DVM, PhD, Diplomate ACVPM: contributed to the analysis and interpretation of the data for the work. All coauthors, including Schoeman JP, BVSc, MMedVet (Med), DSAM, Dipl. DECVIM, PhD: were involved in the revision of this work and the approval of the final version submitted for publication.

CONFLICT OF INTEREST

The authors declare no conflicts of interest related to this report.

REFERENCES

1. Pearson ML. Guideline for hand hygiene in healthcare settings. *J Am Coll Surg*. 2004; 198: 121- 127.
2. Owens CD, Stoessel K. Surgical site infections: epidemiology, microbiology and prevention. *J Hosp Infect*. 2008; 70: 3- 10.
3. Da Silveira EA, Bubeck KA, Batista ER, et al. Comparison of an alcohol-based hand rub and water-based chlorhexidine gluconate scrub technique for hand antisepsis prior to elective surgery in horses. *Can Vet J*. 2016; 57: 164- 168.
4. Kirkland KB, Briggs JP, Trivette SL, Wilkinson WE, Sexton DJ. The impact of surgical-site infections in the 1990s: attributable mortality, excess length of hospitalization, and extra costs. *Infect Control Hosp Epidemiol*. 1999; 20: 725- 730.
5. Nicoll C, Singh A, Weese JS. Economic impact of tibial plateau leveling osteotomy surgical site infection in dogs. *Vet Surg*. 2014; 43: 899- 902.
6. Chou PY, Doyle AJ, Arai S, Burke PJ, Bailey TR. Antibacterial efficacy of several surgical hand preparation products used by veterinary students. *Vet Surg*. 2016; 45: 515- 522.
7. Liu LQ, Mehigan S. The effects of surgical hand scrubbing protocols on skin integrity and surgical site infection rates: a systematic review. *AORN J*. 2016; 103: 468- 482.
8. Verwilghen D, Grulke S, Kampf G. Presurgical hand antisepsis: concepts and current habits of veterinary surgeons. *Vet Surg*. 2011; 40: 515- 521.

9. Nelson LL. Surgical site infections in small animal surgery. *Vet Clin Small Anim*. 2011; 41: 1041- 1056.
10. Andrade N, Schmiedt CW, Cornell K, et al. Survey of intraoperative bacterial contamination in dogs undergoing elective orthopaedic surgery. *Vet Surg*. 2016; 45: 214- 222.
11. Hsieh HF, Chiu HH, Lee FP. Surgical hand scrubs in relation to microbial counts: systematic literature review. *J Adv Nurs*. 2006; 55: 68- 78.
12. Meakin LB, Gilman OP, Parsons KJ, Burton NJ, Langley-Hobbs SJ. Colored indicator undergloves increase the detection of glove perforations by surgeons during small animal orthopedic surgery: a randomized controlled trial. *Vet Surg*. 2016; 45: 709- 714.
13. World Health Organization (WHO). WHO Guidelines on Hand Hygiene in Health Care: First Global Patient Safety Challenge Clean Care is Safer Care. Vol 30; 2009: 270.
14. Forer Y, Block C, Frenkel S. Preoperative hand decontamination in ophthalmic surgery: a comparison of the removal of bacteria from surgeons' hands by routine antimicrobial scrub versus an alcoholic hand rub. *Curr Eye Res*. 2017; 42: 1333- 1337.
15. Widmer AF. Surgical hand hygiene: scrub or rub? *J Hosp Infect*. 2013; 83: S35- S39.
16. Verwilghen DR, Mainil J, Mastrocicco E, et al. Surgical hand antisepsis in veterinary practice: evaluation of soap scrubs and alcohol based rub techniques. *Vet J*. 2011; 190: 372- 377.
17. Hamed Mahmoud M, Morad Asaad A, Ansar QM. Hand rubbing and scrubbing in relation to microbial count among surgical team members in a Saudi hospital. *Life Sci J*. 2013; 10(7): 198- 205.
18. Edwards R, Riley C, Howe L, Burrows E, Riley K, Frellstedt L. Comparison of an alcohol-based hand sanitation product with a traditional chlorhexidine hand scrub technique for hand hygiene preparation in an equine hospital. *N Z Vet J*. 2017; 65: 242- 247.
19. Parienti JJ, Thibon P, Heller R, et al. Hand-rubbing with an aqueous alcoholic solution vs traditional surgical hand-scrubbing and 30-day surgical site infection rates: a randomized equivalence study. *Jama*. 2002; 288: 722- 727.
20. Asadpoor-Dezaki Z, Barabady A, Abtahi D. Comparison of two surgical hand antiseptic techniques: hand rubbing and hand washing with alcohol-based agent and 7.5% povidone iodine. *J Crit Care Nurs*. 2016; 9(4):e8255.
21. McDonnel G. Antisepsis, Disinfection, and Sterilization: Types, Action and Resistance. ASM Press; 2007.
22. Olson LKM, Morse DJ, Duley C, Savell BK. Prospective, randomized in vivo comparison of a dual-active waterless antiseptic versus two alcohol-only waterless antiseptics for surgical hand antisepsis. *Am J Infect Control*. 2012; 40: 155- 159.
23. Braswell ML, Spruce L. Implementing AORN recommended practices for surgical attire. *AORN J*. 2012; 95: 122- 140.
24. B. Braun Medical AG www.bbraun.com. https://www.bbraun.co.za/content/dam/b-braun/za/website/za-photoes/surgical-hand-preparationtouchlessposter10230716.pdf. Published 2016. Accessed June 25, 2021.
25. Burton M, Cobb E, Donachie P, Judah G, Curtis V, Schmidt WP. The effect of handwashing with water or soap on bacterial contamination of hands. *Int J Environ Res Public Health*. 2011; 8(1): 97- 104.
26. Kampf G. What is left to justify the use of chlorhexidine in hand hygiene? *J Hosp Infect*. 2008; 70: 27- 34.

27. Rotter M, Sattar S, Dharan S, Allegranzi B, Mathai E, Pittet D. Methods to evaluate the microbicidal activities of hand-rub and hand-wash agents. *J Hosp Infect*. 2009; 73: 191- 199.
28. Kampf G, Reichel M, Hollingsworth A, Bashir M. Efficacy of surgical hand scrub products based on chlorhexidine is largely overestimated without neutralising agents in the sampling fluid. *Am J Infect*. 2013; 41: e1- e5.
29. Hübner NO, Goerdt AM, Stanislawski N, et al. Bacterial migration through punctured surgical gloves under real surgical conditions. *BMC Infect Dis*. 2010; 10: 192.
30. Larson EL, Aiello AE, Heilman JM, et al. Comparison of different regimens for surgical hand preparation. *AORN J*. 2001; 73: 412- 432.
31. Hübner NO, Kampf G, Kamp P, Kohlmann T, Kramer A. Does a preceding hand wash and drying time after surgical hand disinfection influence the efficacy of a propanol-based hand rub? *BMC Microbiol*. 2006; 6: 57.
32. Tanner J, Dumville JC, Norman G, Fortnam M. Surgical hand antisepsis to reduce surgical site infection. *Cochrane Database Syst Rev*. 2016; 2016(1):CD004288.
33. Engley FB Jr, Dey BP. A universal neutralizing medium for antimicrobial chemicals. *Chem Special Manuf Assoc Proc*. 1970; 100- 106.
34. Zamany A, Spangberg LSW. An effective method of inactivating chlorhexidine. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2002; 93(5): 617- 620.
35. Kampf G. Aqueous chlorhexidine for surgical hand disinfection? *J Hosp Infect*. 2018; 98(4): 378- 379.