Comparative efficacy of three antiseptics as surgical skin preparations in dogs

Charles Boucher BVSc (Hons), MMedVet (Small Animal Surgery)1 | Maryke M. Henton BVSc, MMEdVet (Bacteriology)2 | Piet J. Becker BSc, MSc, PhD3 | Robert M. Kirberger BVSc, DVSc, MMEdVet (Radiology), DECVDI1 | Marthinus J. Hartman BVSc (Hons), MSc, MMEdVet (Small Animal Surgery), PhD1

1 Department of Companion Animal
Clinical Studies, Faculty of Veterinary
Science, University of Pretoria, Pretoria,
South Africa
2 Vetdiagnostix Gauteng, Midrand, South
Africa
3 Research Office, Faculty of Health
Sciences, University of Pretoria, Pretoria,
South Africa

Correspondence
Charles Boucher, Department of
Companion Animal Clinical Studies,
Onderstepoort Veterinary Academic
Hospital, Private Bag X04,
Onderstepoort 0110, South Africa.
Email: charlie.boucher@up.ac.za

Funding information
South African Veterinary Foundation;
National Research Fund of Prof Robert
Kirberger; Companion Animal Clinical
Studies Research Fund of the University
of Pretoria

Abstract

Objective: To compare the antimicrobial efficacy of a 2% chlorhexidine gluconate and 70% ethanol solution (CG+A) with that of F10 Skin Prep Solution (F10) and electrochemically activated water (EAW) when used as a surgical preparation in canine patients.

Study design: Prospective randomized clinical study.

Sample population: One hundred sixteen dogs presented for ovariohysterectomy.

Methods: Dogs were randomly divided into 1 of the 3 antiseptic groups (CG+A, F10, EAW). Skin samples with replicating organism detection and counting plates were taken at 4 different perioperative sites and time intervals (postskin preparation, postskin antisepsis, 2 hours after the second sample, and at the end of surgery) during ovariohysterectomies performed by students. The colony forming unit (CFU) counts from each sample were quantified according to the level of bacterial contamination. Zero CFU was defined as no contamination, 1-12 CFU was defined as low contamination, and greater than 12 CFU was defined as high contamination. The 3 antiseptics were compared with respect to the level of contamination.

Results: There was no difference in the level of colonization between the antiseptics at the first sampling time ($P = .454$). However, the level of contamination for CG+A was lower compared with F10 and EAW at the second, third, and fourth sampling times ($P = .001, P = .01, P = .02$, respectively).

Conclusion: CG+A was more effective at achieving a zero CFU count and low levels of contamination compared with F10 and EAW for surgical preparation in dogs undergoing ovariohysterectomy.

Clinical significance: This study does not provide evidence to support the use of F10 and EAW instead of CG+A for the surgical skin preparation of dogs undergoing ovariohysterectomy.

1 | INTRODUCTION

Surgical site infections remain a serious complication in both human and veterinary surgery.1 Despite implementing modern principles of preoperative preparation of the patient and the surgical team, the use of perioperative antibiotics, and the refinement of surgical techniques, surgical site infections remain a substantial cause of morbidity in veterinary
isms on the patient are caused by a continuing source of endogenous microorganisms on the patient’s skin. Preoperative skin preparation with a topical antiseptic on the surgical site is recognized as the most important measure in minimizing potential infection by reducing endogenous skin microflora.

Skin preparation of animals may be more challenging compared with human patients. The thick hair coat, infrequency of bathing, and more contaminated environment of animals may challenge the efficacy of surgical preparation techniques that work well in man. Preparation of the surgical site generally involves a series of steps that includes the clipping of hair, removal of dirt and oils, and removal or reduction of microbes. Included in the characteristics of an ideal skin antiseptic are a broad spectrum of antimicrobial activity, especially against vegetative and spore-producing bacteria, rapid killing of pathogenic microbes, and a residual lethal effect against microorganisms. Cleansing capacity, lack of skin irritation or toxicity, an ability to retain antimicrobial efficacy in the presence of organic material, and no teratogenic or carcinogenic effects or systemic side effects in the patient or user are other ideal attributes. Chlorhexidine gluconate has traditionally been considered the skin antiseptic of choice because it possesses many of these ideal characteristics. However, currently, no skin antiseptic possessing all of these characteristics exists.

The residual effect of chlorhexidine has been well described, but this claim has recently been challenged. Bacterial resistance to chlorhexidine has also been described for a number of bacteria and bacterial strains. Resistance of bacteria to most of the commonly used biocides has serious economic, health, and environmental implications in various applications and has led to the pursuit of newer and more effective antiseptics. Two antiseptics that could provide relief to this area of concern are F10 Skin Prep Solution (F10; Health and Hygiene, Johannesburg, South Africa) and electrochemically activated water (EAW; IQ Green Solutions, Cape Town, South Africa).

F10 is an antiseptic marketed for the veterinary profession and is commonly used in Australia, the United Kingdom, South Africa, and New Zealand as a surgical skin preparation. It contains 0.05% quaternary ammonium compounds (alkyl benzyl dimethyl ammonium chloride and didecyl dimethyl ammonium chloride), 0.05% biguanide compound (polyhexamethylene biguanide [PHMB] hydrochloride), and 20% alcohol (ethanol). PHMB has been reported as one of the most promising modern day substances from a clinical perspective with regard to its efficacy, safety, and clinical applications.

EAW is produced from tap water and saline solution by a special unit that contains a cylindrical electrolytic cell containing an anode and a cathode separated by a permeable membrane. The EAW production process has been described in detail by various researchers. EAW has a strong antimicrobial activity against a wide spectrum of microorganisms, and it can potentially be an environmentally friendly substitute for chemical agents that can be produced in large quantities on site.

The routine use of biocides is regulated far less than antibiotic use, leading to major concern over the development of biocide resistance and the possible role that these agents play in driving the emergence of multidrug-resistant bacteria. A comprehensive understanding of the clinical efficacy and evidence of bacterial resistance to specific antiseptics will inform the optimal use of these agents to preserve their activity in the future and prevent their indiscriminate use.

The objective of our study was to compare the antimicrobial efficacy of a 2% chlorhexidine gluconate and 70% ethanol solution (CG+A; Dismed Pharma, Midrand, South Africa) with that of F10 and EAW. We hypothesized that there would be no difference in antimicrobial efficacy between the 3 products.

## 2 | MATERIALS AND METHODS

### 2.1 | Model system

A live animal model of intact female dogs that had been admitted to our Veterinary Academic Hospital for student ovariohysterectomy was included in the study. Patients were clinically healthy, >4.5 kg in weight; free of any clinical skin condition; and fully vaccinated, with normal haematology and basic serum chemistry. This study was approved by the Animal Ethics Committee of the University of Pretoria (project number V064-15). The dogs were assigned to 1 of 3 skin antiseptic groups (CG+A, F10, EAW) by using simple random sampling by drawing numbers out of a hat. The dogs’ age and weight were recorded.

### 2.2 | Study procedure

The dogs were anesthetized according to the protocol set out by the teaching hospital. No antibiotics were administered in the perioperative period. The dogs were positioned in dorsal recumbency, and the surgical site was clipped with electric clippers and a sterilized blade. Final-year veterinary students wearing scrub suits, surgical caps, latex gloves, and masks performed the skin asepsis. The ventral abdomen was washed with abdominal sponges soaked in a neutral detergent solution (supplied by Health and Hygiene) with no proven antibacterial properties and sterile water to remove surface dirt. The wash started at the proposed incision site.
and then moved outward in an elliptical fashion. The area was then rinsed with a sponge soaked in sterile water (Sabax Pour Water, Adcock Ingram Critical Care, Midrand, South Africa) before the second wash was started. This procedure was repeated a minimum of 3 times until the skin was clear of visible hair, dirt, and debris. The surgical site was dried with a sterile paper towel, and the time for the wash was recorded. The first skin culture sample (Figure 1A) was taken at this time by using replicating organism detection and counting plates\(^4,6,34\) from the caudal midline just cranial to the pubic bone. The patient was then transferred in dorsal recumbency to the operating room where 1 of the 3 antiseptic solutions was sprayed onto the entire clipped area until the skin was saturated. After 3 minutes, sterile dry gauze swabs (Akacia Medical, Cape Town, South Africa) handled with sterile Cheatle forceps were used to soak up the pooled antiseptic. The second skin culture sample (Figure 1B) was taken at this time from the centre of the surgical field, over the umbilicus. After draping of the patient, final-year veterinary students performed a routine, standardized, ventral median ovariohysterectomy. The third skin culture sample (Figure 1C) was taken 2 hours after the second sample from the cranial aspect of the surgical field. The duration of the ovariohysterectomy was recorded. The fourth sample (Figure 1D) was taken at the end of surgery, before removing the surgical drapes, from the right paramedian aspect of the surgical field adjacent to the centre of the incision. Care was taken to ensure that there was no overlapping of the skin samples to avoid mechanical stripping of bacteria and the inhibition of the antiseptics by the polysorbate and lecithin residues, which may have artificially influenced subsequent colony forming unit (CFU) counts. The senior investigator

FIGURE 1  A-D, Anatomic locations of first, second, third, and fourth skin culture samples, respectively
and the head theatre nurse took all the samples wearing theatre attire. Sterile gloves were used to take the third sample to avoid contamination of the surgical field.

The agar plate preparation, quality controls, incubation, and CFU counting were performed by the institution’s microbiologists by using standardized, prescribed, validated methods. The result of each culture was classified according to the level of contamination. Negative cultures were classified as “no contamination” (0 CFU). Positive cultures were classified as “low contamination” (1-12 CFU) or “high contamination” (>12 CFU), as described by Andrade. Cutaneous reactions were recorded after the initial wash with the neutral detergent, after the application of the antiseptic, and at the end of surgery. Dogs were classified as having skin reactions if they showed evidence of urticaria, erythema, or rash. The surgical wounds were examined within 24 hours of the surgery and again at suture removal (10-14 days); if the sutures were removed elsewhere, follow-up information was obtained by a telephone interview with the owner. Criteria that were used to identify surgical site infections were a purulent exudate and/or signs of inflammation, and at the end of surgery. Dogs were classified as having skin reactions if they showed evidence of urticaria, erythema, or rash. The surgical wounds were examined within 24 hours of the surgery and again at suture removal (10-14 days); if the sutures were removed elsewhere, follow-up information was obtained by a telephone interview with the owner. Criteria that were used to identify surgical site infections were a purulent exudate and/or signs of inflammation. The antiseptics were stored in the theatre complex in a secure dark cupboard at room temperature. The EAW was discarded 60 days after opening the bottle and replaced with a fresh solution. The solution of EAW contained free available chlorine of more than 180 mg/L, a pH of between 6.5 and 8.5, and an oxidative reduction potential of more than 700 mV.

2.3 Statistical analysis

The sample size calculation was performed at the 2-hour reference point. Success was defined as a zero CFU count. Under the assumption of expected success rates that range from 60%-90% for the 3 antiseptics, a sample size of at least 34 dogs per group would have power in excess of 90% to detect a trend in success rates over the treatments. One-way ANOVA was initially used to compare means of CFU between antiseptics at different time intervals. Levene’s test, however, demonstrated unequal variances between the antiseptics. Data analyses then resorted to the use of nonparametric methods for the analysis. The Kolomogorov-Smirnov test was used to test for normality. Age, body weight, duration of wash, surgical time, and CFU counts at the different sampling times were reported as median and interquartile range (IQR). The Kruskal-Wallis test was used to compare medians of body weight, total surgery time, duration of wash, and CFU counts across the antiseptics at each of the sampling times. Wilcoxon’s matched pairs signed-rank test was employed to compare 2 stages within a treatment. The Mann-Whitney U test, with pair-wise comparisons between the antiseptics, was used to compare CFU counts at the 4 sampling times. The levels of contamination of the 3 antiseptics were compared with Fisher’s exact test. The odds ratio for a clean or contaminated outcome of each antiseptic, with CG+A as the reference, was calculated for each antiseptic. Fisher’s exact test was used to compare cutaneous reactions and postoperative infections among the antiseptics. The fourth sample was adjusted for surgery time by using logistic regression. A logistic regression model was also used to determine the correlation between postoperative wound infections and CFU at the end of surgery and postoperative wound infection and surgery time. A simple linear regression model was used to determine the correlation between total surgery time and CFU. The statistical analyses were performed in SPSS v.17 (SPSS, Chicago, Illinois) and StataCorp 2015 (Statistical Software Release 14; StataCorp, College Station, Texas) statistical software. Significance was set at $P < .05$.

3 RESULTS

One hundred sixteen dogs were enrolled for the study. Thirty-nine dogs were included in the CG+A and F10 groups, and 38 dogs were included in the EAW group. The median age for all dogs was 11 months (IQR 18), and median weight was 7.8 kg (IQR 8.6). The median duration of the neutral detergent wash was 8 minutes (IQR 4), and the total surgical time was 146 minutes (IQR 55). There was no difference in body weight ($P = .983$), total surgery time ($P = .875$), duration of wash ($P = .222$), or age ($P = .942$) among the 3 antiseptic groups. The total CFU count for all 116 dogs and the median CFU counts at the 4 sampling times are illustrated in Figure 2. There was no difference in the CFU counts at the first sampling time ($P = .779$) across the 3 antiseptic groups. However, there were differences at
the second (P = .001), third (P = .002), and fourth (P = .005) sampling times. There was a significant reduction in CFU counts between the first and the second sample for all antiseptics (P = .001). When comparing the 3 antiseptics pair wise, there were fewer CFU in the CG+ A group compared with F10 (P = .003, P = .001, P = .005) and EAW (P = .001, P = .008, P = .004) groups at the second, third, and fourth sampling times, respectively (Table 1). However, there were no differences in the CFU counts between F10 and EAW at these times (P = .667, P = .527, P = .785). There was no difference in the level of colonization among the antiseptic groups at the first sampling time (P = .454). However, the level of contamination for CG+ A was lower compared with F10 and EAW at the second, third, and fourth sampling times (P = .001, P = .01, P = .02; Table 2, Figure 3). Classification according to a clean (CFU = 0) or contaminated (CFU ≥ 1) outcome revealed that EAW had a 7-fold increased risk for bacterial contamination (P = .001), and F10 had a 10-fold increased risk for bacterial contamination (P = .001) relative to CG+ A at the second sampling time. Similar results were obtained at the third and fourth sampling times when EAW had 3- and 4-fold increased risks for bacterial contamination, respectively (P = .015), and F10 had 5- and 4-fold increased risks, respectively (P = .001).

There was no difference in the distribution of skin reactions after the initial wash with the neutral detergent (P = .441) after the application of the antiseptic (P = .441) or at the end of surgery (P = .118) across the antiseptic groups. Forty-two of the 116 dogs developed skin reactions after the initial wash with the neutral detergent. Forty-three percent of dogs that reacted initially experienced an improvement in the severity of the skin reaction by the end of surgery. No dogs that reacted normally to the neutral detergent wash showed any evidence of skin irritation either immediately after the application of the antiseptic or postoperatively. Forty-three percent of dogs that reacted initially experienced an improvement in the severity of the skin reaction by the end of surgery. No dogs that reacted normally to the neutral detergent wash showed any evidence of skin irritation either immediately after the application of the antiseptic or after surgery.

Six (5%) dogs, 3 from the CG+ A group and 3 from the F10 group, developed postoperative wound infections. However, no difference in the postoperative infections across the antiseptics (P = .161) was observed. One dog from the F10 group returned to the hospital after 5 days with a purulent discharge from the surgical site. This dog was treated with amoxicillin/clavulanic acid at 20 mg/kg orally twice daily, and the infection had cleared by the time of suture removal.

**Table 1** Median CFU counts at the second, third, and fourth sampling times for the 3 antiseptics (pair-wise comparisons)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Antiseptic</th>
<th>CG+ A</th>
<th>F10</th>
<th>EAW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 2</td>
<td>Median/IQR</td>
<td>0 (0.0-0.0)</td>
<td>0 (0.0-1.0)</td>
<td>0 (0.0-1.0)</td>
</tr>
<tr>
<td>P value</td>
<td>.003</td>
<td>.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample 3</td>
<td>Median/IQR</td>
<td>0 (0.0-0.0)</td>
<td>1 (0.0-3.5)</td>
<td>0.5 (0.0-2.0)</td>
</tr>
<tr>
<td>P value</td>
<td>.011</td>
<td>.008</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample 4</td>
<td>Median/IQR</td>
<td>0 (0.0-1.0)</td>
<td>1 (0.0-3.0)</td>
<td>1 (0.0-4.3)</td>
</tr>
<tr>
<td>P value</td>
<td>.005</td>
<td>.004</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CFU, colony forming unit; CG+ A, chlorhexidine gluconate and ethanol; EAW, electrochemically activated water; F10, F10 Skin Prep Solution; IQR, interquartile range.

**Table 2** CFU counts and levels of bacterial contamination for the 3 antiseptics

<table>
<thead>
<tr>
<th>Sample</th>
<th>CFU count</th>
<th>Level of contamination</th>
<th>CG+ A (n = 39)</th>
<th>F10 (n = 39)</th>
<th>EAW (n = 38)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>0</td>
<td>No</td>
<td>3</td>
<td>6</td>
<td>2</td>
<td>.454</td>
</tr>
<tr>
<td></td>
<td>1-12</td>
<td>Low</td>
<td>23</td>
<td>21</td>
<td>27</td>
<td>.01</td>
</tr>
<tr>
<td></td>
<td>&gt;12</td>
<td>High</td>
<td>13</td>
<td>12</td>
<td>9</td>
<td>.02</td>
</tr>
<tr>
<td>Sample 2</td>
<td>0</td>
<td>No</td>
<td>36</td>
<td>21</td>
<td>24</td>
<td>.001</td>
</tr>
<tr>
<td></td>
<td>1-12</td>
<td>Low</td>
<td>2</td>
<td>18</td>
<td>11</td>
<td>.01</td>
</tr>
<tr>
<td></td>
<td>&gt;12</td>
<td>High</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>.02</td>
</tr>
<tr>
<td>Sample 3</td>
<td>0</td>
<td>No</td>
<td>30</td>
<td>15</td>
<td>19</td>
<td>.01</td>
</tr>
<tr>
<td></td>
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<td>16</td>
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<tr>
<td></td>
<td>&gt;12</td>
<td>High</td>
<td>0</td>
<td>5</td>
<td>3</td>
<td>.02</td>
</tr>
<tr>
<td>Sample 4</td>
<td>0</td>
<td>No</td>
<td>25</td>
<td>13</td>
<td>11</td>
<td>.02</td>
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<td>&gt;12</td>
<td>High</td>
<td>2</td>
<td>6</td>
<td>7</td>
<td>.02</td>
</tr>
</tbody>
</table>

CFU, colony forming unit; CG+ A, chlorhexidine gluconate and ethanol; EAW, electrochemically activated water; F10, F10 Skin Prep Solution.
Another dog from the CG+A group returned after 7 days with a purulent discharge at the cranial aspect of the wound that was treated with a topical antibacterial ointment and recovered uneventfully. The other 4 dogs showed signs of mild superficial skin infection at the time of suture removal. There was no correlation between postoperative wound infections and CFU at the end of surgery ($P = .774$) or postoperative wound infections and surgery time ($P = .802$; $r^2 = .002$). There was also no correlation between surgery time and postoperative CFU counts at the end of surgery ($P = .280$; $r^2 = .010$). No associations between skin reactions and postoperative infections were observed ($P = .963$).

4 | DISCUSSION

CG+A was more effective at achieving a zero CFU count and low levels of contamination compared with F10 and EAW for surgical skin preparation in dogs undergoing elective ovariohysterectomy. Two of the desirable characteristics of an ideal skin antiseptic are rapid killing capabilities and a persistent or residual lethal effect against bacteria. The combination of chlorhexidine gluconate and alcohol was more effective than F10 or EAW at maintaining zero CFU or low levels of contamination at the second, third, and fourth sampling times. The risk for bacterial contamination was between 3- and 10-fold higher for F10 and EAW, depending on the sampling time, compared with CG+A. This could be attributed to superior immediate and residual bactericidal properties of CG+A and its efficacy in the presence of organic material. Alcohol at the correct concentrations has long been known for its rapid bactericidal effect with short contact times. F10 contains alcohol (ethanol) but only at a 20% concentration. This concentration may be inferior for reducing immediate bacterial levels compared with the 70% ethanol used in the CG+A preparation, as indicated by the significantly higher number of negative cultures at the second sampling time for this antiseptic. Issues such as spectrum of activity, concentration levels, time of action, and formulations have been routinely debated in the literature, and the use of alcohol has varied widely. Larson reported that alcohols, when used in appropriate concentrations of between 60% and 90%, provide the most rapid and greatest reduction in microbial counts on the skin compared with other antiseptics. More research regarding the ideal concentration of alcohol in combination with another antiseptic is required.

The high degree of bacterial contamination at samples 3 and 4 of the EAW group may be attributed to the fact that contact with organic material, such as blood, has been shown to influence negatively its antimicrobial activity, and this raises questions regarding its use as a surgical antiseptic. The
high CFU counts at the same sampling times of the F10 antiseptic group may be attributed to poor residual activity of the active ingredients of this formulation. Quaternary ammonium compounds (QAC) have also been proved to be inactivated by organic material and soap residues and are generally not regarded as reliable surgical antiseptics.\textsuperscript{38,44} The value that the QAC add to the F10 formulation may be a topic of additional research. The residual effect of PHMB has also not been proved in a clinical setting. In reviewing PHMB, Fjeld and Lingaas\textsuperscript{28} suggested that better designed, larger scale clinical studies of efficacy and safety were required to give recommendations on the use of PHMB as an antiseptic.

Currently, this is the only antiseptic study to have reported skin reactions after the initial scrub with a neutral detergent and may thereby emphasize the role that washing alone or chemical detergents play in the development of skin reactions. Our study also had a higher prevalence of skin reactions (35%) compared with other similar studies.\textsuperscript{4,6,34,45,46} Because no dogs showed any differences in the grading of the cutaneous reactions between the wash and the application of the antiseptic and because of the insignificant distribution of the skin reactions across the antiseptics, it is our opinion that the skin reactions were caused by the mechanical wash and not by the application of the antiseptics. There was no correlation between skin reactions and high CFU counts in our study, which was similar to the findings reported by Osuna et al\textsuperscript{37} in the first part of their experimental trial.

Contact dermatitis has been correlated with a rise in post-surgical wound infections in man,\textsuperscript{47} and similar findings have been suggested in the veterinary literature.\textsuperscript{6} One explanation is that surface bacteria multiply more rapidly on irritated, damaged, or diseased skin.\textsuperscript{4,49} Five percent of our dogs developed postoperative wound infections. This infection rate is comparable to those reported in veterinary studies, which range from 3.6\% - 5.8\% for patients undergoing clean surgical procedures.\textsuperscript{3,4,6,7,50-52} The criteria used to evaluate wound infection in this trial were similar to those of other skin preparation studies and included those dogs that had signs of inflammation.\textsuperscript{5,6,53} A number of factors such as the degree of bacterial contamination, the virulence of the microbe, duration of surgery, local wound environment, contact dermatitis, and host defence mechanisms have been identified as risk factors for surgical site infections.\textsuperscript{3,7} We find it counterintuitive that, in our study, there was no correlation between the degree of bacterial contamination at the last sampling time and the dogs that developed postoperative infections. This was similar to reports of other antiseptic studies that found differing degrees of microbial reduction but did not report any correlation with infection rates.\textsuperscript{5,8,34,54,55} No dogs developed postoperative infections in the Lambrechts et al\textsuperscript{4} study, and these were not recorded in the Osuna et al\textsuperscript{34} study. Stubbs et al\textsuperscript{4} also reported that no dogs with unusually high CFU counts developed wound infections. The degree of microbial reduction that is required after the application of an antiseptic has been debated.\textsuperscript{3,56,57} The presence of bacteria in a surgical wound has, however, been quoted as the most important factor in the development of postoperative wound infections,\textsuperscript{58} and the goal of aseptic skin preparation is to reduce wound colonization to a level that the patient’s defences can control.\textsuperscript{7} There was no correlation between the total surgical time and the postoperative CFU counts on the skin. Similar findings were reported in the clinical antiseptic trials by Osuna et al\textsuperscript{34} and Lambrechts\textsuperscript{4} and appear to contradict some of the older literature in which the duration of surgery is reported to affect directly the number of bacteria that gain access to the surgical site.\textsuperscript{59,60} It is, however, important to realize that these previous studies were performed in very different clinical settings with different outcomes and on wounds with varying degrees of contamination and, therefore, cannot be directly compared. The results of the past and present studies provide substantial evidence that the development of postoperative wound infection is a multifactorial condition that cannot be attributed only to the number of CFU on the skin surface at the time of surgery.

Our surgical skin preparation technique was similar to those comparative antiseptic studies performed by Lambrechts et al\textsuperscript{4} and others\textsuperscript{44} in that the initial wash was performed with a neutral detergent solution with no specific antimicrobial properties. The goal was to separate the mechanical effect of skin preparation on reducing bacterial levels from the specific antibacterial effects of the antiseptic solution itself. No prewash sample was taken in our study because of the strong possibility that bacterial overgrowth would render accurate CFU counting impossible.\textsuperscript{5} Our study was unique in that a 2-hour, intraoperative skin culture sample was taken from the surgical field of a standardized surgical procedure. The addition of this sample provided valuable information regarding bacterial counts at a fixed time period as opposed to using postoperative samples only, for which the time periods differ significantly. The recommended contact time for a chlorhexidine single-agent solution is a minimum of 2 minutes prior to making the skin incision.\textsuperscript{39,61} However, no clinical studies on contact times are available for F10 or EAW, so this remains a subject for future research. An antiseptic contact time of 3 minutes, which was comparable to other clinical antiseptic studies,\textsuperscript{4,6,34,45} was regarded as sufficient in our study. Our study differs from most other antiseptic trials in that only 1 antiseptic application was performed.\textsuperscript{4,6,34,37,40} In practice, the skin of a patient is typically scrubbed according to one of a vast array of protocols recommended in human and veterinary literature.\textsuperscript{3} These protocols most commonly include an antiseptic-based scrub, most of which contain a surgical detergent with antibacterial properties,\textsuperscript{4} followed by alcohol as the initial...
preparation in the induction area, and then completed by a sterile scrub procedure in the operating room. By eliminating the antimicrobial agent in the initial wash, our study was completely reliant on the mechanical effect of reducing bacterial counts and the single antiseptic application in the operating room. The postantiseptic reduction in CFU of all the products to a median of zero provided sufficient evidence that 1 application of only antiseptic significantly reduced CFU counts. This was followed by a gradual increase in the total CFU counts at the subsequent sampling times. Our results also provide evidence that a single application of a preoperative antiseptic solution may achieve sufficiently low levels of bacteria; this requires additional investigation. Larger quantities of antiseptic are likely to be used with a single application, which may have a negative effect on the dog’s temperature.

The development of skin reactions were documented by the senior author, who was not blinded to the antiseptic that was used. Although criteria similar to those of other studies were used to assess this factor, the interpretation and grading of skin reactions remains subjective. More objective ways of accessing this factor may be indicated in future studies. A number of cases were lost to follow-up, with many clients returning to their regular veterinarians for suture removal. We had to rely on more subjective telephone interviews with these owners to assess and record postoperative wound infections or wound complications. Cytology and culture of the purulent discharge that developed from the surgical site in 2 of the dogs were not performed.

The results of this study provide sufficient evidence for us to conclude that, despite a number of disadvantages and ongoing debates associated with the use of CG + A as a surgical antiseptic, it can still be regarded as an appropriate surgical skin preparation in dogs and is more effective at achieving no or low levels of bacterial colonization compared with F10 or EAW. This study does not provide evidence to support the use of F10 and EAW instead of CG + A for the surgical skin preparation of dogs undergoing ovariohysterectomy.

ACKNOWLEDGMENT

The authors thank the South African Veterinary Foundation, the National Research Fund of Prof Robert Kirberger, and the Companion Animal Clinical Studies Research Fund of the University of Pretoria for their financial contributions towards this project.

CONFLICT OF INTEREST

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

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**How to cite this article:** Boucher C, Henton MM, Becker PJ, Kirberger RM, Hartman MJ. Comparative efficacy of three antiseptics as surgical skin preparations in dogs. *Veterinary Surgery.* 2018;00:1-10. [https://doi.org/10.1111/vsu.12913](https://doi.org/10.1111/vsu.12913)