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Efficacy and Dermal Tolerance of a Novel Alcohol-Based Skin Antiseptic in Horses

Victoria J. Tannahill BVMS

Tristan Cogan BSc PhD

Kate Allen BVSc PhD DipACVSMR

Elizabeth Acutt BVSc

Evita Busschers DVM MVR DipACVS DipECVS DipACVSMR

University of Bristol, School of Veterinary Sciences, Bristol, United Kingdom.

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Corresponding Author: Victoria J. Tannahill, University of Bristol, School of Veterinary Sciences, Bristol, BS40 5DU, United Kingdom. victoria.tannahill@bristol.ac.uk

ABSTRACT

Objective: To determine the efficacy and dermal tolerance of a novel alcohol-based skin antiseptic (ABSA) in horses.

Study Design: Experimental study.

Animal Population: Systemically healthy horses (n=25) with no history or clinical signs of skin disease.

Methods: Four clipped sites on the abdomen were randomly assigned to a skin preparation protocol: saline (*NC*), chlorhexidine gluconate followed by isopropyl alcohol (*PC*), saline followed by the ABSA (*ABSA A*), or a commercially available horse shampoo followed by the ABSA (*ABSA B*). Microbiology swabs were taken from each site and cultured on MacConkey and mannitol salt agar plates. Colony forming units were counted 18-24 hours later. All sites were scored for signs of skin reaction before, immediately after, 1 hour after, and 24 hours after skin preparation.

Results: The *PC*, *ABSA A* and *ABSA B* methods were effective at reducing skin microbial burden when compared to the *NC* method ($P < 0.001$) and there was no difference between antiseptic products. Both the *ABSA A* ($P < 0.001$) and *ABSA B* ($P < 0.001$) methods took less time than the *PC* method but there was no difference between the ABSA methods ($P = 0.108$). Skin reactions were most abundant at 24 hours after skin preparation (30.5%), but there was no significant association with antiseptic used and no horses required veterinary treatment.

Conclusions: The alcohol-based skin antiseptic used in this study is an efficacious, fast-acting and well tolerated antiseptic for achieving skin antisepsis in horses. Further validation is required to determine its safety and effectiveness in clinical cases.

INTRODUCTION

Aseptic preparation of the patient's skin is an essential step in the prevention of surgical site infections (SSI).[1-3] Many studies have attempted to establish the optimum pre-operative skin preparation protocol for the patient, both human[2] and veterinary,[1,3-10] as well as for the surgeon.[11-13] The variables most commonly investigated are total time required and product used.

In an ideal situation, the total time required to achieve a sterile surgical field is as short as possible. This is especially important in equine patients undergoing general anaesthesia as perioperative mortality increases with anaesthetic duration.[14] Protocols as short as 4 minutes for the patient,[1] and 2 minutes for the surgeon,[13] have been proven to be effective using 4% chlorhexidine gluconate (CHXG). Similarly, Zubrod *et al*[10] showed that three 30 second scrubs with povidine-iodine (PI) was as effective as a 10 minute scrub or the application of a commercial one-step PI solution in horses.

Recently, the use of alcohol-based skin antiseptics (ABSAs) has gained interest[11-12,15]; studies have shown that they are as effective as and faster than traditional antiseptic products in preparing the veterinary surgeon.[12] However, no studies have looked at the efficacy of such products on the veterinary patient where they may also be effective and require a shorter preparation time.

Another consideration for the use of antiseptics on the patient and surgeon is dermal tolerance. Aseptic skin preparation is not always innocuous, with some patients[1-3] and surgeons[13] suffering from skin reactions to certain products. Osuna *et al*[7-8] established that CHXG is better tolerated than PI in the pre-operative preparation of dogs but no objective data exists regarding the tolerance of either traditional antiseptics or ABSAs in horses.

The alcohol-based products previously investigated[11-12] are designed for pre-operative preparation of the surgeon and are not intended for use on the patient.[16-17] The ABSA used in this study (Cutasept F; BODE Chemie GmbH, Hamburg, Germany) is specifically designed for pre-operative skin preparation in humans patients and is reported to be both efficacious and well tolerated.[18] It is a non-coloured skin antiseptic containing propan-2-ol (72%) and benzalkonium chloride (<1%) with a recommended contact time of 2 minutes for skin that is rich in sebaceous glands,[19] as is the case with equine skin.

The aim of this study was to determine the efficacy of an ABSA in reducing the skin microbial burden in horses compared to another commonly used skin antiseptic (CHXG) and a control (saline), and to report on the dermal tolerance of these methods. We hypothesised that the ABSA would be as effective as CHXG, faster than a standard CHXG skin preparation method, and well tolerated by equine patients.

MATERIALS AND METHODS

The study was approved by the University of Bristol's Animal Welfare and Ethical Review Body. Written informed consent was obtained from the owner of each horse prior to participating in the study.

Skin Preparation

Systemically healthy horses with no history or clinical signs of skin disease were included in the study. The mid-to-ventral abdomen was brushed five times with a clean, soft brush in the direction of hair growth to remove any gross debris. Four sites measuring 5 x 5 centimetres were then clipped using a number 40 clipper blade, approximately 5 centimetres apart from cranial to caudal, on the mid-to-ventral abdomen (Figure 1) and numbered consecutively from 1 (closest to elbow) to 4 (closest to flank). Each site was randomly assigned (www.randomizer.org) to one of 4 skin preparation methods and all skin preparations were conducted by a single operator wearing sterile gloves. The time required to obtain grossly clean swabs during the *NC*, *ABSA A* and *ABSA B* methods was noted.

- *Negative control* [*NC*] – the site was scrubbed in a circular motion, focused around the central point with gauze swabs soaked in sterile saline until the used swabs appeared grossly clean after use. The site was then dried with 2 dry sterile gauze swabs.

- *Positive control* [*PC*] – the site was scrubbed in a circular motion, focused around the central point with gauze swabs soaked in 4% chlorhexidine gluconate (Vetasept Chlorhexidine Surgical Scrub; Animalcare Ltd, York, United Kingdom). Each swab was used for 30 seconds and 10 swabs were used, giving a total scrub time of 5 minutes. Excess lather was then removed from the site using 2 sterile gauze swabs soaked in 70% isopropyl alcohol and the site was allowed to dry by evaporation.
- *ABSA A* – the site was scrubbed in a circular motion, focused around the central point with gauze swabs soaked in sterile saline until the used swabs appeared grossly clean after use. The site was then dried with 2 dry sterile gauze swabs. Sterile gauze swabs soaked in the ABSA (Cutasept F) were applied to the site, ensuring continual wetting for 2 minutes, and the site was allowed to dry by evaporation.
- *ABSA B* – the site was scrubbed in a circular motion, focused around the central point with gauze swabs soaked in a commercially available horse shampoo (Gallop Conditioning Shampoo; Carr & Day & Martin Ltd, Lytham, United Kingdom) until the used swabs appeared grossly clean after use. Excess lather was then removed from the site using 2 swabs soaked in tap water and the site was dried with 2 dry gauze swabs. Gauze swabs soaked in the ABSA (Cutasept F) were applied to the site, ensuring continual wetting for 2 minutes, and the site was allowed to dry by evaporation.

Microbiological Sampling[5-6,10]

Immediately following the completion of skin preparation at each site, a sterile microbiological swab was placed into 1ml of liquid Amies preservation medium (ESwab; Copan Diagnostics Inc, Murrieta, California) within a sterile tube. Excess moisture was removed from the swab by pressing it against the side of the tube during withdrawal. The moistened swab was then rolled 360° on the middle of the prepared site before being returned and sealed within the tube containing the preservation medium. The process was repeated for each site and the swabs were stored at 4°C for up to 24 hours prior to laboratory testing. All sampling was conducted by a single operator.

Microbiological Testing

Each tube containing a microbiological swab was agitated for 30 seconds to distribute microbes throughout the preservation medium. A MacConkey agar plate (selective for Gram negative species) and a mannitol salt agar plate (selective for Gram positive species) were each spread evenly with 100µl of preservation medium and incubated aerobically at 37°C for 18-24 hours before the plates were inspected. Colony-forming units (CFU) were counted manually by a single operator who was blinded to the skin preparation method used.

Dermal Tolerance Scoring

All sites were scored for clinical signs of a skin reaction (Table 1) by a single operator who was blinded to the skin preparation method used at each site:

- *Time A* – immediately after clipping but before skin preparation.
- *Time B* – immediately after skin preparation and microbiological sample collection.

- *Time C* – 1 hour after skin preparation.
- *Time D* – 24 hours after skin preparation.

If any horse was deemed to require veterinary treatment for a skin reaction occurring as a result of skin preparation, then this was undertaken at the discretion of the qualified veterinary surgeons undertaking the skin preparation and dermal tolerance scoring.

Data Analysis

Data were analysed using SPSS Statistics for Windows version 23.0 (IBM Corp, Armonk, New York). Categorical data were analysed using cross-tabulation methods (Pearson Chi-Square and Fisher's Exact). Numerical data were tested for normality (Shapiro-Wilk) and were not found to follow a Gaussian distribution; therefore these data were analysed using non-parametric tests (Mann-Whitney U and Kruskal-Wallis). Significance was set at $P < 0.05$. For multiple, simultaneous analyses between groups, a Bonferroni correction was applied.

RESULTS

Twenty-five horses were recruited to the study and each horse was tested on both the left and right side, providing a total of 50 data sets. Thirteen geldings and 12 mares were included, ranging from 1 to 25 years of age (mean 9 years). There were 11 native breed ponies, 9 cobs, and 5 Thoroughbreds. Nine horses had a light coat colour, 8 were dark and 8 were multi-coloured.

Microbiological Testing

Site preparation method was correlated to the number of bacteria detected on both MacConkey (P <0.001) and mannitol salt (P <0.001) agar plates. The microbial burden was significantly reduced by all of the skin preparation methods using an antiseptic product when compared to the *NC* method, and there was no difference between antiseptics (Figures 2 and 3).

The *PC* method (300 seconds) took longer than both the *ABSA A* (178.1 seconds; range 140-220; P <0.001) and *ABSA B* (185.7 seconds; range 140-230; P <0.001) methods, but there was no difference between the *ABSA A* and *ABSA B* methods (P = 0.108).

There was no significance of breed, coat colour, side of horse, or site number on the number of bacteria on either MacConkey or mannitol salt agar plates.

Dermal Tolerance Scoring

There was no correlation between site preparation method and dermal score at any time when considered overall, or when analysed between groups.

97.5%, 83.5%, 82% and 69.5% of sites recorded a dermal tolerance score of '0' at *Time A, B, C* and *D* respectively. The highest dermal tolerance score recorded was '2' (Table 1) at *Time D* only. The mean dermal tolerance score at each time was: 0.03 at *Time A*; 0.16 at *Time B*; 0.18 at *Time C*; and 0.37 at *Time D*.

Breed had a significant effect on dermal score at *Time A* ($P = 0.038$), although only one horse (a native breed pony) recorded all of the skin reactions at this time. At *Time D*, breed was also significant ($P = 0.048$) with Thoroughbreds demonstrating considerably more skin reactions (50.0%) than cobs (27.8%) or native breed ponies (23.9%). At *Time B* and *Time C* there was no difference between breed and dermal score.

Coat colour was not a significant variable in relation to dermal score at any time.

At *Time C*, 75% of skin reactions occurred on the right side of the horse ($P = 0.001$) but at all other times, there was no difference between the side of the horse and dermal score.

Site number had a significant effect on dermal score at *Times B* ($P = 0.028$), *C* ($P = 0.001$) and *D* ($P < 0.001$) but not at *Time A*. Most skin reactions occurred at site number 1 at *Time B* (45.5%), *Time C* (50.0%) and at *Time D* (44.3%).

DISCUSSION

The results of this study show that the alcohol-based skin antiseptic (ABSA) was as efficacious in reducing the microbial burden of equine skin as CHXG, requires significantly less time for patient skin preparation than a standard CHXG skin preparation method, and is well tolerated by horses.

The ABSA tested in this study contains propan-2-ol which acts by coagulating proteins, causing them to denature. Its effect is mainly on the cell wall, cytoplasmic membrane and plasma proteins, causing a loss of cellular functions, increased permeability of the cell wall, and subsequent lysis of the organism.[20] When combined with benzalkonium chloride, which disrupts intermolecular interactions leading to cell lysis as well,[21] the overall action is bactericidal, yeasticidal, tuberculocidal and virucidal.[19]

The ABSA was faster than CHXG with an average preparation time of approximately 3 minutes (60 second scrub + 2 minute contact time) when compared to a standard 5 minute CHXG skin preparation method. This reduction in skin preparation time is beneficial as it may contribute to a shorter overall anaesthetic time, which is pertinent in equine patients as perioperative mortality increases with anaesthetic duration.[14] Another benefit is the reduced cost in terms of both staffing and materials to perform the skin preparation. We did not measure the exact volume of the ABSA used for each skin preparation in this study, which would have allowed a direct comparison of cost per unit area for each of the skin preparation methods used. However, the purchase price of the

ABSA was less per millilitre when compared to the CHXG product used, and the overall volume used for the study was comparable for both products. Therefore, the cost of the ABSA seems comparable to that of CHXG which is commonly used in veterinary practice. It is possible that shorter scrub times using CHXG are achievable in equine patients, as in other species,[1,13] but there are no studies documenting this finding to date.

Both the ABSA and CHXG were well tolerated on equine skin which correlates well with studies using these products in other species. Dermal tolerance is commonly reported in human-based antiseptic studies,[2,18,22] and occasionally reported subjectively in veterinary studies,[1,3,7-8,10] but there are no objective reports for any products in equine patients. If the skin surrounding a surgery site becomes irritated, for instance from shaving to remove hair instead of clipping, bacteria may colonise the skin which increases the risk of an SSI.[23] It is therefore pertinent to use an antiseptic that will minimise the risk of skin irritation surrounding the surgery site. The highest dermal score at any time point was a grade 2 out of 4 (Table 1) but no horses required any veterinary intervention to resolve their skin irritation.

The effect of breed on dermal score at *Time D* only and that the side of horse was related to dermal score at *Time C* only is unclear in this sample set, especially given that these variables were not deemed significant at any other time point. A post-hoc analysis of power was not conducted as extrapolating from our data to predict what would happen if larger numbers were included would simply maintain these significant differences. The

only way to determine whether our findings were true anomalies would be to repeat the study to establish whether fresh data showed lower variance.

The skin preparation method used at each site was randomised but there was still an effect of site number on dermal score, with more skin reactions recorded at site number 1. This site was always the first to be clipped when starting skin preparation; dirt and/or hair were removed from the clippers with a clean stiff brush between horses and between each side of the same horse but no clipper lubricant was applied. A new clipper blade was applied if the current blade was deemed to be blunt and not cutting the hair adequately. We do not feel that the use of clean, sharp clippers, especially those without clipper lubricant applied, would be a contributing factor to the number of skin reactions seen at this site however other unidentified clipper-related factors may be responsible. Alternatively, this site was closest to the elbow and therefore it is hypothesised that the skin in this area is more sensitive than that closer to the flank; further studies are required to establish the dermal tolerance of both the ABSA and CHXG on other body areas in equine patients.

In devising our study protocol, we chose to compare a standard 5 minute CHXG scrub, which is the method used in our equine hospital, to the selected ABSA (Cutasept F), a product designed to be used in the pre-operative preparation of human patients. Although the sustained action of CHXG may be reduced by rinsing with 70% isopropyl alcohol,[8-9] its use has become common in many hospitals and other studies[1-3,5,7-9]; as it was not

within the aims of our study to report on the residual action of the antiseptics we therefore adopted this method of rinsing CHXG to maintain clinical applicability.

The protocol for microbiological testing is similar to that used in other veterinary studies comparing the efficacy of antiseptic methods.[5-6,10] However, unlike other studies,[1,3-10] we chose not to conduct pre-antiseptic microbiological sampling, allowing determination of the percentage of CFU reduction as our aim was to compare the activity of four skin antiseptic methods, not to establish the final efficacy of the methods in preventing SSIs. The physical process of conducting pre-antiseptic microbiological sampling will inherently reduce the microbial burden of the skin, thereby giving a false result in terms of the overall number of CFUs and of the percentage of CFU reduction. Also, to the best of our knowledge, the number of CFUs required to initiate a SSI remains unknown and so calculation of the percentage of CFU reduction is unnecessary in this experimental study if we do not know what level of post-antiseptic CFUs is deemed acceptable. However, if the ABSA methods proved to be as effective at reducing skin microbial burden as CHXG at a single time point in this study, we could extrapolate that the ABSA would be safe enough to use in a clinical trial to establish its efficacy in reducing SSIs.

We chose to examine the microbiological samples for common bacteria – Gram positive and Gram negative – to establish the basic effect of the skin preparation methods. In choosing to culture microbial growth on MacConkey (selective for *Enterobacteriaceae* species) and mannitol salt (selective for *Staphylococcus* species) agar plates, we accept that we would have not cultured the full complement of bacteria that may be involved in

SSIs. However, as previously stated, it was not our aim to establish the efficacy of our skin preparation methods in preventing SSIs. A larger study would be necessary if examining for specific bacteria as these would not necessarily be as commonly present. Furthermore, an initial microbiological sample would have to be taken from each site to do this, which has problems as explained above.

The limitations of this study include the small sample of horses used, and that it was conducted during summer months when all the horses were relatively clean and dry. It is known that the number of CFUs cultured from unclipped contaminated skin is higher than from unclipped clean skin[10] and that clipping, as we did, can result in a higher number of pre-scrub CFUs compared to non-clipped skin.[6] However there is no difference in the number of post-scrub CFUs when comparing clipped versus non-clipped skin[6] and increased skin contamination is unlikely to affect the overall efficacy of the skin antiseptic.[10] Our method of skin preparation required all swabs to be grossly clean after use and so the only potential effect of increased skin contamination is that the average scrub time in advance of applying an ABSA may become longer when used on horses during the winter months. Finally, we cannot rule out that the number of CFUs was reduced by continued antiseptic activity within the preservation medium or on the agar plates as we did not use antiseptic-neutralising medium or agar. However, it was assumed that this would be minimised by the dilution effect of the preservation medium and therefore would not negatively impact our results.

We concluded that the alcohol-based skin antiseptic tested in this study is an efficacious, fast and well tolerated antiseptic for achieving skin antisepsis in horses. It now requires further validation to ensure it is safe and effective in clinical equine cases.

DISCLOSURE STATEMENT

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

REFERENCES

1. Bourel C, Buczinski S, Desrochers A, *et al*. Comparison of two surgical site protocols for cattle in a field setting. *Vet Surg*. 2013;42:223-228.
2. Darouiche RO, Wall MJ, Itani KMF, *et al*. Chlorhexidine-alcohol versus povidine-iodine for surgical-site antisepsis. *N Engl J Med*. 2010;362:18-26.
3. Desrochers A, St-Jean G, AndersonDE, *et al*. Comparative evaluation of two surgical scrub preparations in cattle. *Vet Surg*. 1996;25:336-341.
4. Davids BI, Davidson MJ, TenBroeck SH, *et al*. Efficacy of mechanical versus non-mechanical sterile preoperative skin preparation with chlorhexidine gluconate 4% solution. *Vet Surg*. 2015;44:648-652.
5. Geraghty TE, Love S, Taylor DJ, *et al*. Assessing techniques for disinfecting sites for inserting intravenous catheters into the jugular veins of horses. *Vet Rec*. 2009;164:51-55.
6. Hague BA, Honnas CM, Simpson RB, *et al*. Evaluation of skin bacterial flora before and after aseptic preparation of clipped and non-clipped arthrocentesis sites in horses. *Vet Surg*. 1997;26:121-125.
7. Osuna DJ, DeYoung DJ, Walker RL. Comparison of three skin preparation techniques in the dog. Part 1: experimental trial. *Vet Surg*. 1990;19:14-19.
8. Osuna DJ, DeYoung DJ, Walker RL. Comparison of three skin preparation techniques in the dog. Part 2: clinical trial in 100 dogs. *Vet Surg*. 1990;19:20-23.
9. Wilson DG, Hartmann F, Carter VR, *et al*. Comparison of three preoperative skin preparation techniques in ponies. *Equine Vet Educ*. 2011;23:462-465.

10. Zubrod CJ, Farnsworth KD, Oaks JL. Evaluation of arthrocentesis site bacterial flora before and after 4 methods of preparation in horses with and without evidence of skin contamination. *Vet Surg*. 2004;33:525-530.
11. 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.
12. 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;190:372-277.
13. Wheelock SM, Lookinland S. Effect of surgical hand scrub time on subsequent bacterial growth. *AORN J*. 1997;65:1087-1098.
14. Johnston GM, Taylor PM, Holmes MA, *et al*. Confidential enquiry of perioperative equine fatalities (CEPEF-1): preliminary results. *Equine Vet J*. 1995;27:193-200.
15. Verwilghen D, Grulke S, Kampf G. Presurgical hand antisepsis: concepts and current habits in veterinary surgeons. *Vet Surg*. 2011;40:515-521.
16. 3M™. Avagard™ Drug Facts. USA. (http://multimedia.3m.com/mws/media/818676O/avagard-drug-facts.pdf) 2012.
17. Chemie B. Sterillium® Product Information. Germany. (http://www.productcatalogue.bode-chemie.com/products/hands/product-information/sterillium_int.pdf) 2012.
18. Kampf G, Wigger-Alberti W, Schoder V, *et al*. Dermal tolerance of two ethanol-based skin antiseptics and their auxiliary agents. *Hyg Med*. 2010;35:112-115.

19. Chemie B. Cutasept® F / Cutasept® G Product Information. Germany. (http://www.productcatalogue.bode-chemie.com/products/skin/product-information/cutasept_fg_int.pdf) 2014.
20. Oro Clean Chemie AG. 2-Propanol. Switzerland. (https://www.oroclean.com/gb-en/Infection_Control_Resources/Active_Substances/2-Propanol) 2017.
21. Oro Clean Chemie AG. Benzalkalonium chloride. Switzerland. (https://www.oroclean.com/gb-en/Infection_Control_Resources/Active_Substances/Benzalkalonium_chloride) 2017.
22. Kampf G, Muscatiello M, Häntschel D, *et al*. Dermal tolerance and effect on skin hydration of a new ethanol-based hand gel. *J Hosp Infect*. 2002;52:297-301.
23. Tanner J, Norrie P, Melen K. Preoperative hair removal to reduce surgical site infection. *Cochrane Database of Systematic Reviews*. 2011;11:CD004122.

FIGURE LEGENDS

Figure 1 Four clipped sites on the left side of a horse prior to skin preparation.

Figure 2 Number of colony forming units (CFU) per swab against each skin preparation method – MacConkey agar.

Figure 3 Number of colony forming units (CFU) per swab against skin preparation method – mannitol salt agar.

TABLES

Table 1 Dermal Tolerance Scoring.

Score	Description
0	No clinical evidence of dermatitis
1	Mild diffuse erythema and/or mild skin oedema
2	Moderate diffuse erythema and/or skin oedema with/without small vesicle formation
3	Severe diffuse erythema and/or skin oedema with/without large vesicle formation
4	As for 3 but with signs extending beyond the treated area