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Effectiveness of conventional and hydrosurgical debridement methods in reducing *Staphylococcus aureus* inoculation of equine muscle *in vitro*

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Summary

Reasons for performing study: The success of primary healing of equine traumatic wounds is dependent on thorough debridement. A specific hydrosurgical debridement device (Versajet™)[a] is gentle to viable tissues, yet effectively removes macroscopic contaminants and debris. We wished to investigate whether it is effective in reducing bacterial burden and whether it differs from traditional methods. No previous reports compare hydrosurgical debridement and conventional wound debridement with regard to bacterial reduction from *in vitro* inoculated soft tissue.
Objectives: To assess the effectiveness of hydrosurgical debridement in reducing the *Staphylococcus aureus* load from *in vitro* inoculated equine muscle compared with conventional wound debridement methods.
Study design: *In vitro* experimental study.
Methods: The surface of equine masseter muscle was inoculated with a *S. aureus* broth and subsequently debrided using one of the following 4 methods: saline irrigation; sharp debridement; saline irrigation and sharp debridement; or hydrosurgical debridement. Tissue samples for quantitative cultures were collected before and after debridement, and the colony-forming units per gram of tissue were calculated and log transformed. The reductions in bacterial counts were analysed statistically using Wilcoxon signed-rank tests and Friedman two-way ANOVA.
Results: Hydrosurgical debridement was more effective than conventional debridement methods in reducing the *S. aureus* load ($P<0.05$). Hydrosurgical debridement reduced the bacterial load by 99.7%, in comparison to saline irrigation and sharp debridement (87.4%), sharp debridement (82.2%) and saline irrigation (46.0%).
Conclusions: Hydrosurgical debridement reduces the *S. aureus* load from *in vitro* contaminated equine muscle significantly more than conventional debridement methods.

Keywords: horse; wound; debridement; bacterial reduction; hydrosurgery; Versajet

Introduction

Horses often sustain traumatic wounds, commonly associated with tissue loss, necrosis and heavy contamination [1]. Whenever possible, primary closure is preferred over healing by second intention. Primary closure reduces the risk of further wound contamination and infection and, if successful, healing is faster, with a better cosmetic and functional outcome [2–4], whereas second-intention healing, of distal limb wounds in particular, is at high risk of complications such as chronic inflammation, the development of exuberant granulation tissue, poor wound contraction and slow epithelialisation [4].

When primary closure is attempted, there is, however, a high risk of dehiscence. In a retrospective study, complete primary healing after closure of traumatic wounds was successful in only 26% of horses and 41% of ponies [5]. Localisation of the wound, structures involved, movement and tension on wound margins could all play a role, but infection is considered to be a major cause of dehiscence and impaired healing overall [5–7]. To control infection, it is critical that debridement reduces the bacterial load and removes factors facilitating colonisation, such as devitalised tissue and foreign material [2,8,9]. For successful healing, it is also essential to preserve a maximum of viable tissue [3,9].

Traditional wound debridement involves sterile saline irrigation and sharp resection [6,7,10]. Low-pressure irrigation does not dislodge contaminants effectively [10,11], whereas high-pressure irrigation might damage tissues [12–14]. With sharp debridement, there is always a risk of removing viable tissue or leaving necrotic tissue in the wound, especially when the margin between the two is ill defined [2,7,15]. A potentially more effective hydrosurgical device (Versajet™)[a] has became available during the last decade and is widely used in the human field to manage burns [16–18] and a variety of other wounds [19–21]. It consists of a foot-pedal-activated power console, a handpiece connected to a sterile saline supply and a waste tube that can be connected to a receptacle (Fig 1). A high-pressure stream of sterile saline jets across the operating window of the handpiece and, while cutting, creates a localised suction (Venturi) effect (Fig 2). The closer to 90 degrees that the aperture is held to the tissue, the more it excises, while the primary effect from an oblique angle is irrigation and aspiration. The system has 10 power settings, where the lowest gently cleans and debrides, while higher settings allow tougher tissue to be excised. There are handpieces available with various tip angles and sizes of operating window.

Results of previous studies looking at bacterial reduction achieved by hydrosurgical debridement have been equivocal [20,22–24], and there has been no comparison made with traditional methods regarding direct bacterial reduction on inoculated soft tissue. The aim of the present study was to quantify the effect of hydrosurgical debridement on *Staphylococcus aureus* counts in an *in vitro* equine muscle model, in comparison with conventional debridement methods.

Materials and methods

Specimen preparation

Both masseter muscles from 6 recently slaughtered horses were placed in sterile trays for transport to the laboratory. The outer fascia and a thin underlying muscle layer were removed in a sterile manner. Each muscle was halved, i.e. 4 sections were obtained from each horse, one for each debridement method.

Surface inoculation

Using a *S. aureus* strain isolated from an equine wound infection, a broth was prepared from 1 μl of colony material mixed in 10 ml of sterile beef

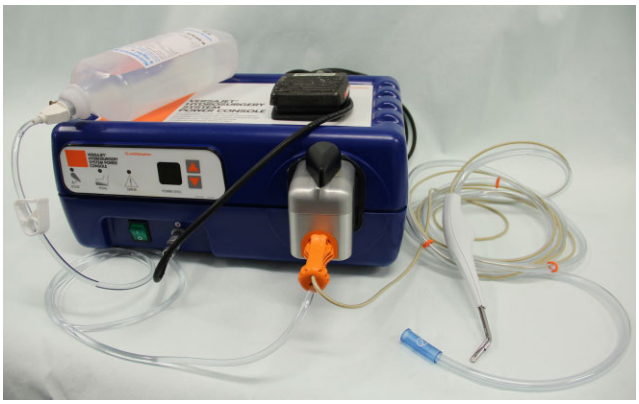

Fig 1: The hydrosurgical system (Versajet™)[a] consists of a foot-pedal-activated power console, a handpiece connected to sterile saline and a waste tube.

broth, which after 6 h of incubation at 37°C (Termaks)[b] resulted in a bacterial concentration of 3.6×10^7 colony-forming units (CFU)/ml. Each muscle section was inoculated with 0.25 ml of the broth, corresponding to a total of 9×10^6 CFU. In order to disperse the broth in a uniform manner, a 35 cm^2 metal template net divided into 25 squares was used, and 10 µl of broth was pipetted onto each square. The muscle was labelled so that the position of the template was consistent throughout the procedure. The inoculated sections were placed in sterile trays and incubated at 37°C (Termaks)[b] for 6 h.

Debridement techniques

Debridement was performed on the 4 specimens from each horse using one of 4 methods. The debridement time was set to 3 min for each method in order to compare the efficacy with which the different methods reduced the bacterial load. For the combined method (saline irrigation [IR]+sharp debridement [SD]), the resulting total time was 6 min.

Saline irrigation (IR): The specimen was placed at a 15 degree angle and the surface irrigated with 0.9% sterile saline (B Braun)[c]. A 1 l bag of saline was placed in a pressure cuff (Infusable®)[d], manually maintained at 300 mmHg, and the irrigation was performed using an infusion set (V 86)[e], with an internal diameter of 3 mm. The tip was held 1–2 cm above the muscle at an approximate angle of 45 degrees to the tissue. Irrigation was repeated from top to bottom, back and forth between sides.

Sharp debridement (SD): The muscle surface was scraped with a no. 10 scalpel blade (B Braun)[f] held at an approximate angle of 30 degrees to the tissue. The blade was used repeatedly from top to bottom and from one side to the other and was wiped on a sterile cotton swab between every scraping.

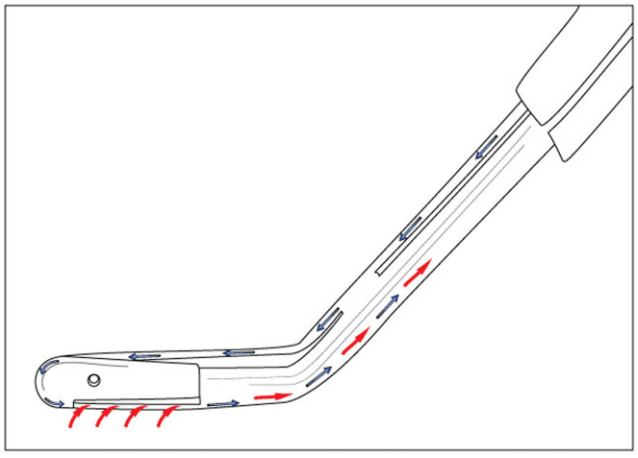

Fig 2: The tip of a hydrosurgical handpiece (Versajet™)[a]. A high-pressure stream of sterile saline jets across the operating window of the handpiece, creating a localised suction effect. (Illustrated by Susanne Lindeborg.)

TABLE 1: *Staphylococcus aureus* counts[1] from equine mandibular muscle before and after 4 different debridement methods[2]

	Before debridement			After debridement		
Method	Q1[3]	Median	Q3[3]	Q1	Median	Q3
IR	9.93	10.03	10.07	9.58	9.76	9.83
SD	9.89	10.08	10.20	8.72	9.21	9.39
IR+SD	9.88	10.00	10.07	8.73	8.92	9.17
HD	9.85	10.13	10.34	6.92	7.54	8.09

[1]Expressed as $\log_{10}$ colony-forming units per gram of tissue.
[2]IR = irrigation, SD = sharp debridement and HD = hydrosurgical debridement.
[3]Q1 represents the 25th percentile and Q3 represents the 75th percentile.

Hydrosurgical debridement (HD): Power setting 3 and a handpiece with a 14 mm operating window in a 45 degree angled tip was used. The aperture was kept at an approximate angle of 60 degrees to the tissue surface and moved in parallel strokes, repeatedly from top to bottom, back and forth between sides.

Quantitative cultures

Tissue samples for quantitative cultures were collected before and after debridement. Five biopsies were taken diagonally along the template squares using a 4 mm biopsy punch (Kruuse)[g], obtaining ~0.4 g of muscular tissue in total. Areas containing fascia were avoided in order to achieve consistent amounts of muscular tissue. The tissue was placed into a tube containing 3.6 ml of sterile saline and mixed for 1 min on a Vortex blender (Merck®)[h] before a 1:10 dilution series was performed. The spread plate technique was used, i.e. 100 µl from each dilution was spread onto a blood agar that was then incubated aerobically at 37°C (Termaks)[b] for 24 h. The resulting numbers of CFUs were counted manually from the plates growing 30–300 colonies, and the number of CFUs per gram of tissue was calculated.

Data analysis

A Wilcoxon signed-rank test was used to evaluate whether the individual methods reduced the bacterial load. A Friedman two-way ANOVA was then applied to analyse the potential difference in reducing bacterial counts between the 4 methods. Finally, the magnitude of bacterial reduction was compared between hydrosurgical debridement and the 3 conventional methods, respectively, using Wilcoxon signed-rank tests. The distribution of bacterial counts is known to be skewed, and therefore the counts were transformed to the logarithmic scale with base 10 prior to statistical analysis. A critical probability of 0.05 was used to determine significant effects.

Results

Counts of *S. aureus* (expressed as $\log_{10}$ CFU per gram of tissue) pre- and post debridement are shown in Table 1. All debridement methods significantly reduced the *S. aureus* load ($P<0.05$), but the Friedman two-way ANOVA demonstrated significant differences in bacterial reduction between the different methods ($P = 0.04$). The median $\log_{10}$ reduction achieved by HD was 2.62 CFU/g (Fig 3), corresponding to 99.7%. This reduction was greater than the other methods, respectively ($P = 0.03$). The $\log_{10}$ reduction achieved by IR+SD was 0.90 CFU/g, SD, 0.77 CFU/g and IR, 0.27 CFU/g, corresponding to 87.4, 82.2 and 46.0%, respectively.

Discussion

This study compared the *in vitro* effectiveness of different debridement methods in reducing *S. aureus* load on equine muscle specimens. From a

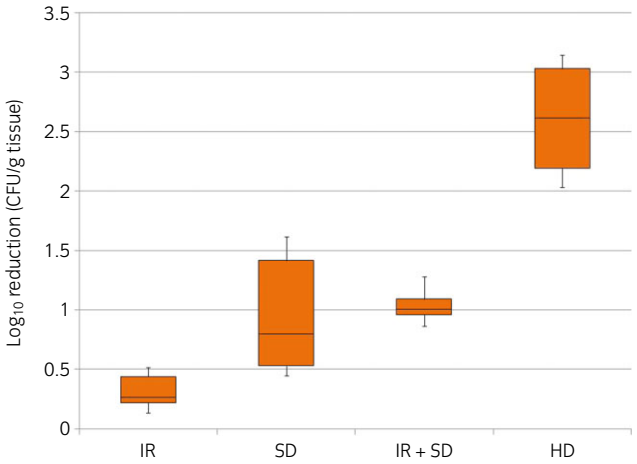

Fig 3: Median (represented by the line through the box), interquartile range (represented by the box itself), minimum and maximum (represented by the whiskers) log_{10} reduction of *Staphylococcus aureus* (expressed as colony-forming units [CFU] per gram of tissue) from equine mandibular muscle by saline irrigation (IR), sharp debridement (SD), IR+SD and hydrosurgical debridement (HD).

clinical point of view, this is extremely relevant because the lower the bacterial burden left in a wound after debridement, the lower the risk for development of wound infection [6,7,25]. Hydrosurgical debridement significantly ($P<0.05$) reduced the predebridement load by 99.7%. In previous studies, the reduction of bacterial load has varied. The reduction was not significant when using HD *in vitro* on contaminated porcine joints [22]. In that study, however, only 4 specimens were inoculated, each with different bacterial species. A randomised human clinical study found that the reduction by HD of 12 acute traumatic and surgical wounds was 90.8% [20]. The fact that clinical wounds were debrided instead of inoculated muscle specimens may be a reason for the discrepancy compared with our results. Damage to vital structures had to be avoided and tissue removal was limited because viability could be evaluated, both of which increased the chance of bacteria being left behind. Although their results represent a clinical situation, the low initial bacterial load in the wounds (2–100 CFU/g tissue) is not likely to be representative of heavily contaminated equine traumatic wounds. Recently, Hughes *et al*. [23] showed significant reductions of *S. aureus* from incubated stainless-steel fracture plates irrigated with one litre saline using a bulb syringe, pressurised pulsed lavage or the hydrosurgical system. The results were similar to those of the present study. All methods decreased the bacterial load significantly, but hydrosurgery was the most effective. In another study, Nusbaum *et al*. [24] demonstrated a persistent effect of HD; methicilllin-resistant *S. aureus* counts in inoculated deep dermal porcine wounds were significantly reduced over a 3 week period after HD. The sustained reduction is probably representative of other bacteria as well and is likely to be explained by the efficient debridement, which at the same time preserves viable tissue [26,27]. In contrast, bacterial load rebounded after 48 h, when using high-pressure pulsed lavage to debride inoculated wounds in goats [13]. The efficient reduction of bacteria in a wound by HD should lead not only to more successful wound healing, but also to a decreased requirement for antibiotics.

The experimental set-up in this study may not completely mimic the clinical situation in equine practice, where traumatic equine wounds are usually highly contaminated with various bacterial species [28] as well as dirt, contusion of tissues occurs, and the depth of debridement is limited at certain locations in comparison with muscle specimens. However, we aimed to mimic the clinical situation as closely as possible by choosing *S. aureus* for inoculation, because it is representative of equine wound infection [29]. Heavy contamination was simulated by inoculating each muscle surface with ~9×10^6 CFU [6,25]. Additionally, the specimens were incubated at 37°C for 6 h to allow time for bacterial adhesion and invasion of the tissue [7,30,31], mimicking a starting infection. Moreover, the duration of wounds presented to referral hospitals is seldom shorter.

Quantitative cultures were performed from tissue biopsies, which is the gold standard method for determining wound bioburden, clinical infection [32,33] and measuring the efficacy of bacterial reduction [10]. Biopsies were also considered to be more representative than surface swabs because the debridement methods were expected to reach different depths in our specimens. Five biopsies were taken, spread out over the specimen surface in order to decrease the risk of sampling error [10,34–36].

The effectiveness of debridement depends on the method used, the amount of tissue removed and the duration of debridement; therefore, protocols were designed to facilitate repeatability and would not necessarily be optimal in a clinical wound. The irrigation pressure was chosen to be low, in order to avoid fluid dispersion into the tissue. Although higher pressure irrigation has been shown to reduce wound bioburden more effectively [11,37,38], several reports have demonstrated its potentially deleterious effects on musculoskeletal tissues [12,14,39–42]. The volume used for irrigation was constant, due to the preset debridement time and irrigation pressure. Using a larger volume may have been more effective [9,10,13].

Scraping the tissue with a scalpel blade has been suggested to be ineffective and also results in tissue trauma [3] in comparison with surgical excision. However, in this study scraping was used instead of surgical excision because standardisation of the depth of excision and the amount of tissue removed, including contamination, would have been more difficult. Moreover, scraping is often used in clinical equine practice.

Some authors believe that moving the hydrosurgical handle in circles is better than back and forth, because the cut deepens during the short time required to change direction [17]. This effect cannot have influenced the results in the present study, because the turning points were outside the sampled area. Power setting 3 of 10 was used for HD, because we often find this setting clinically appropriate when debriding traumatic wounds, and the amount of tissue removed is limited. The results may have been different for other settings.

For the above-mentioned reasons, the debridement time was preset to limit the variation caused by the subjective decision of when to stop debridement and thus the amount of tissue removed. Time was also considered highly relevant, because in the clinical situation the speed of debridement is important for patients in order to reduce the duration of surgery and anaesthesia and concurrent complications [43,44]. In a clinical wound, debridement stops when healthy tissue is reached, indicated by pin-point bleeding [16,19], which cannot be replicated in a muscle specimen. The better reduction in bacterial load achieved by HD is probably due to the more efficient method of debridement and possibly due to a larger amount of tissue being removed, whereas IR+SD and SD would certainly have been more effective if performed for longer.

In the authors' experience, the hydrosurgical debridement device (Versajet™)[a] improves wound healing in equine cases. The system is well tolerated and easy to use in horses, both under general anaesthesia and in standing procedures. Hydrosurgical debridement is thorough, enabling the primary closure of wounds that would otherwise be left to heal by second intention. This results in significant benefit to both the horse and its owner, because primary wound healing is quicker and results in a superior cosmetic appearance and functional outcome [2–4]. Without compromising healing, HD is faster than traditional debridement [19], which is of great importance particularly for the equine case under general anaesthesia [43,44]. Another advantage of HD is the preservation of viable tissues [3,9]; adjustable settings and the variable orientation of the handpiece make the instrument precise and give the surgeon good control over excisional depth [26,27]. It maintains a cool temperature [8], preventing thermal damage, and the saline stream is oriented parallel to the tissue, preventing deeper damage and penetration of contaminants into the tissue.

Hydrosurgical debridement also may stimulate healing in chronic and infected wounds. Polymicrobial biofilms are prevalent in chronic wounds in humans [45,46], and evidence of their presence in equine chronic wounds also has been documented [28,47]. Biofilms are considered to play a role in delayed wound healing [45], and thorough debridement in combination with appropriate antimicrobial treatment is needed to restore an optimal wound environment for healing [48]. The adherent nature of biofilms makes them a debridement challenge, and it will be important to elucidate

whether our positive clinical experience from HD is due to effective removal of biofilms.

The device should, however, be used with caution, especially in chronic and infected wounds, with the increasing prevalence of multiresistant bacteria [49]. A disadvantage with HD is a potential risk for bacterial air contamination, illustrated in 2 recent reports by active and passive air sampling of the perioperative environment during and following debridement of inoculated porcine specimens [22,49].

In conclusion, this study demonstrates that HD reduces *S. aureus* inoculation from equine muscle specimens and is significantly better than conventional debridement methods. It is likely that HD is also more effective in reducing the bacterial load from equine traumatic wounds in clinical practice, and therefore supports a better overall healing process. The findings of this study are consistent with the clinical experience of using HD.

Authors' declaration of interests

No competing interests have been declared.

Ethical animal research

Ethical review not required by this journal: the study was performed on abattoir material.

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Authorship

E.M. Skärlina contributed to study design, study execution, data analysis and interpretation and preparation of the manuscript. J.M. Wilmink contributed to preparation of the manuscript. N. Fall contributed to data analysis and interpretation and preparation of the manuscript. D.A. Gorvy contributed to study design and preparation of the manuscript. All authors gave their final approval of the manuscript.

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[c]B Braun, Melsungen AG, 34209 Melsungen, Germany.
[d]Vital Signs Inc., GE Healthcare, Totowa, New Jersey, USA.
[e]CODAN Medizinische Geräte GmbH & Co. KG, 23738 Lensahn, Germany.
[f]B Braun, Aesculap AG, 78532 Tuttlingen, Germany.
[g]Kruuse Svenska AB, 75651 Uppsala, Sweden.
[h]Merck Eurolab AB, 69180 Karlskoga, Sweden.

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