Skin wounds are among the most common conditions treated by equine veterinarians, and these injuries have a negative financial impact on the equine industry. A 2005 report published by the USDA APHIS indicated that 19% to 24% of euthanasias of horses in the United States were the result of skin wounds or trauma. In a study in New Zealand, wounds at the distal aspect of the limbs accounted for 56 of 834 (7%) musculoskeletal injuries that led to retirement of Thoroughbred racehorses over 2.75 years. Wounds at the distal aspects of limbs have a longer preparatory phase of healing, a greater retraction of the wound margins, and a slower rate of wound contraction and epithelialization, compared with wounds on the trunk of horses.3,4 Wounds on the distal aspect of the limbs in horses are often contaminated and are prone to EGT formation.5 Healing of such wounds is delayed, in part, because the protrusion of EGT above the skin margins of the wound inhibits reepithelialization. A complex pathway and multiple factors are associated with the formation of EGT, which is thought to originate from a chronic state of low-grade inflammation.6 One of the key

**Effects of topical application of silver sulfadiazine cream, triple antimicrobial ointment, or hyperosmolar nanoemulsion on wound healing, bacterial load, and exuberant granulation tissue formation in bandaged full-thickness equine skin wounds**

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OBJECTIVE
To determine the effects of 3 topically applied treatments (1% silver sulfadiazine cream [SSC], triple antimicrobial ointment [TAO], and hyperosmolar nanoemulsion [HNE]) on microbial counts, exuberant granulation tissue (EGT) development, and reepithelialization of contaminated wounds at the distal aspect of the limbs of horses.

ANIMALS
8 healthy adult horses.

PROCEDURES
A 2.5 × 2.5-cm, full-thickness, cutaneous wound was created at the dorsal aspect of each metacarpus and metatarsus (1 wound/limb/horse), covered with nonadhesive dressing, and bandaged. Wounds were inoculated with bacteria and fungi the next day. Each wound on a given horse was randomly assigned to 1 of 4 treatment groups (SSC, TAO, HNE, or no topical treatment [control]). Bandage changes, culture of wound samples, treatments, photography for wound measurements, and biopsy were performed at predetermined time points. Time (days) until wound closure, number of EGT excisions, microbial counts, and scores for selected histologic characteristics were compared among groups.

RESULTS
Median time to wound closure for all groups was 42 days. Time to wound closure and histologic characteristics of wound healing did not differ among groups. Least squares mean microbial counts were significantly higher for HNE-treated wounds on days 9 and 21, compared with SSC-treated and TAO-treated wounds, but not controls. Proportions of SSC-treated (7/8) or HNE-treated (5/8) wounds needing EGT excision were significantly greater than that of TAO-treated (1/8) wounds. The proportion of SSC-treated wounds with EGT excision was greater than that of controls (3/8).

CONCLUSIONS AND CLINICAL RELEVANCE
None of the treatments resulted in more rapid wound closure, compared with that for untreated control wounds under the study conditions. When treatment is warranted, TAO may help to limit EGT formation. (Am J Vet Res 2017;78:638–646)
factors implicated in EGT formation is wound contamination.\textsuperscript{5,6} Excessive fibrosis and EGT can be detrimental to functional and aesthetic outcomes.\textsuperscript{5}

Many different treatments have been investigated to combat EGT, but none have proven universally successful.\textsuperscript{7–10} Although various treatments have been associated with improvements in the early stages of healing for wounds in the distal aspect of horses’ limbs, none have prevented EGT formation or reduced the time to wound closure, compared with that of untreated control wounds.\textsuperscript{7–10}

Hyperosmolar nanoemulsion is a novel compound that combines lipophilic (membrane disrupting) nanoemulsions with hyperosmotic saccharides.\textsuperscript{6} The hyperosmotic saccharides are similar to sugar or honey treatments, but when these are combined with lipophilic emulsions (eg, thymol), the 2 components are shown to act synergistically to inactivate isolates of \textit{Escherichia coli}, \textit{Enterococcus faecalis}, and \textit{Staphylococcus aureus}, including methicillin-resistant \textit{S aureus}.\textsuperscript{11} Together, the components disrupt the membrane and cause dehydration of microorganisms. Hyperosmolar nanoemulsion is also shown to decrease surface bacterial flora counts 24 hours after application and to shorten wound healing time, compared with that for untreated control wounds, in guinea pigs and swine.\textsuperscript{6} However, these species rarely produce EGT. The novel properties of HNE could be useful in the management of contaminated wounds in horses, but its effectiveness as a topical wound medication in this species has not been previously investigated.

Triple antimicrobial ointments (eg, neomycin-bacitracin-polymyxin B) have been used to decrease bacterial contamination and shorten wound healing times in people.\textsuperscript{12–15} The broad-spectrum antimicrobial combination is effective against most naturally occurring flora on human skin, and these products provide a moist environment, decrease transdermal water loss through occlusion of the wound, and create a nonstick surface for bandaging.\textsuperscript{12–14} However, the effects of TAOs on healing of equine wounds are not known, despite their common use in equine medicine.\textsuperscript{15}

Silver sulfadiazine preparations are also commonly used for topical wound treatment. Topical application of 1% SSC to incisional wounds in rats is associated with faster wound healing versus that in control wounds.\textsuperscript{16} Similarly, treatment with 1% silver sulfadiazine results in improved neovascularization and epithelialization, compared with results for 0.25% sodium hypochlorite and 10% povidone-iodine solutions in mice with ear injuries.\textsuperscript{17} However, in horses with experimentally induced skin injuries over the metacarpus and metatarsus, no significant differences have been identified in epithelialization rates or contraction rates between wounds treated with silver sulfadiazine–containing products and untreated control wounds.\textsuperscript{8} In that same study,\textsuperscript{8} wounds that were bandaged produced EGT and exudate, regardless of other treatments, whereas unbandaged wounds did not form EGT.

Bandages are commonly used on wounds at the distal aspects of limbs to reduce contamination and edema, minimize movement, and protect the wound from further trauma.\textsuperscript{6,18} It has been concluded that the presence of a bandage alone may lead to EGT formation owing to low ambient oxygen tension.\textsuperscript{7,8} Decreased oxygen tension stimulates angiogenesis and fibroplasia, which contribute to EGT formation.\textsuperscript{19,20}

The objective of the study reported here was to evaluate the effects of HNE, SSC, and TAO on healing of experimentally induced skin wounds on the distal aspects of the limbs of horses. The investigation included contamination of wounds with bacterial and fungal loads and tracking the microbial load of each wound over time to assess their associations with wound healing and the presence of EGT. Because microbial wound contamination has been implicated in the development of EGT, we hypothesized that, for wounds managed with nonadhesive dressings and bandages, topical administration of antimicrobials would result in lower wound microbial counts, a lower frequency of EGT requiring trimming, and more rapid reepithelialization, compared with results for control wounds that did not receive topical medications.

\section*{Materials and Methods}

\section*{Horses and study design}

Eight horses (5 geldings and 3 mares; median age, 8 years [range, 4 to 11 years]) were included in the study. Breeds included Appaloosa (n = 2), Quarter Horse (2), Standardbred (1), and Thoroughbred (1), with 1 warmblood-type horse and 1 other mixed-breed horse. The horses were part of a teaching herd or had been donated to the university. All horses underwent a physical examination and a brief lameness examination to rule out any systemic condition or lameness that could impede wound healing. Horses were included in the study if the heart and lungs were deemed normal by auscultation, the horse appeared systemically healthy, and no scars or defects were present on the distal regions of the limbs. The horses were determined to be in good body condition but did not have a CBC or serum biochemical analysis performed. All procedures were approved by the Purdue Animal Care and Use Committee.

Each horse had 1 wound created surgically on the dorsodistal aspect of each limb (for a total of 4 wounds/horse) on day 0. Metacarpal and metatarsal wounds were created approximately halfway between the metacarpophalangeal or metatarsophalangeal joint and the distal aspect of the carpus or tarsus, respectively. Wounds on each horse were assigned to the 4 treatment groups (SSC, TAO, HNE, and control [no topical medication]) by means of randomly drawing treatment group assignments from a hat. However, treatment group assignment was constrained, so that each limb (eg, the 8 left forelimbs) was assigned...
to each treatment group twice. Microbial inoculation, bandage change, treatment, and imaging schedules were summarized (Appendix). The horses’ general health was overseen by 1 author (CGH). Horse health was monitored with physical examinations, including measurements of body temperature, pulse, and respiratory rate while in stalls and by visual evaluation while in paddocks.

**Surgical procedures**

Horses were sedated with detomidine hydrochloride (0.01 mg/kg, IV) and butorphanol tartrate (0.01 mg/kg, IV). Hair was clipped at the surgical site on all 4 limbs. For each surgery, the area was aseptically prepared by washing with 4% chlorhexidine scrub diluted with sterile saline (0.9% NaCl) solution followed by rinsing with alcohol. Regional anesthesia (a dorsal ring block) was administered at the proximal aspect of the clipped area with 12 mL of 2% meipivacaine/lignocaine/lignocaine. A rubber template with a 2.5 × 2.5-cm square outline was fixed to the limb, and the skin was incised with a No. 10 scalpel blade. A full-thickness, 2.5 × 2.5-cm section of skin was removed, and the template was removed. The wounds were bandaged without inoculation or treatment for the first 24 hours. The bandage consisted of a nonadherent dressing placed over the wound and covered with 10.1-cm gauze squares. Sterile rolled gauze was used to wrap the limb and hold the nonadherent dressing and gauze in place. Elastic tape was used to cover the bandage and partially fix the bandage to the skin. Phenylbutazone (4.4 mg/kg, PO) was administered on the day of surgery; phenylbutazone treatment was continued at a lower dosage (2.2 mg/kg, PO, q 24 h) for 3 days after wound creation.

**Bandage changes and wound inoculation**

Horses were sedated as needed for bandage changes with xylazine hydrochloride (0.4 to 0.5 mg/kg, IV) or detomidine hydrochloride (0.01 mg/kg, IV). On day 1, the bandages were removed and wounds were contaminated by applying a filter paper inoculated with a combination of bacteria (*Staphylococcus aureus*, *Enterococcus faecalis* and *Escherichia coli*) and fungus (*Candida albicans*). A total of 5.0 × 10^6 CFUs were applied to each wound in a 1:1:1:1 ratio. The wounds were rebandaged as described for postsurgical application. Bandage changes continued according to the schedule until the wounds had decreased from the original size by approximately 25% (as measured from the digital photographs) and ≥ 50% of the wounds no longer had exposed granulation tissue.

**Culture and microbial evaluation**

On day 2, the bandages were removed, and samples were obtained from each wound for culture. The wound was first wiped with dry sterile gauze to remove visible exudate, and the tip of a sterile, cotton-tipped applicator was rolled across the wound and then used to directly inoculate a tryptic soy agar plate. The wounds were rebandaged, and plated culture samples were placed in a standard atmospheric microbiological incubator for 24 hours at 32° to 37°C. Afterward, the plates were scanned with a flatbed scanner and the colonies counted by means of image analysis software. Samples were subsequently obtained for cultures in the same manner immediately prior to the assigned study treatment (as applicable) on days 3, 9, and 21.

**Topical treatments**

The 4 wounds/horse each received a different topical treatment (TAO [neomycin sulfate, bacitracin, zinc, and polymyxin B sulfate], 1% SSC, or HNE at 0.063% [wt:wt] thymol concentration) or were used as a control (with no topical medication applied) according to the described random assignment procedure. Topical antimicrobial treatments, when applicable, began with the bandage change on day 2. The volume of each topical application was estimated as approximately 1 mL. Bandages were reapplied after the wound treatment or after inspection of untreated wounds. On days when culture, photography, or both were performed, the treatment was applied immediately prior to rebandaging. All treatments were discontinued on day 21; this predetermined time point was selected on the basis of information gained in a pilot study, in which wounds had decreased in size by one-fourth of the initial size and had a healthy granulation bed at this time.

**Excision of EGT**

If EGT protruded above the level of the skin or protruded over advancing epithelium at any time during the bandaging phase, the EGT was trimmed level with the skin by use of a No. 10 scalpel blade, and the new bandage was applied without treatment. The horses were sedated as needed for this procedure, which was usually the same sedative treatment that was required for a bandage change for the same horse. If a topical treatment was scheduled, it was not applied until the next scheduled bandage change because of bleeding at the site of EGT trimming. Photography took place as scheduled and was always performed prior to excision, as subsequent bleeding would have interfered with image evaluation. All EGT excisions were performed by 1 author (CGH), who was not blinded to the treatment group of wounds.

**Evaluation of wound size**

Digital photographs were obtained during bandage changes beginning on day 2, prior to treatment of the wounds. After bandaging was discontinued, photography was performed according to the predetermined procedure schedule. Photographs were obtained by use of 90-mm macro lens with a set focal distance of 0.45 m, and a flexible ruler was used for calibration. The ruler was also used as a label to identify the horse (with a number), wound location,
and day of imaging. Total wound area, wound perimeter, and granulation bed area were measured from the digital photographs by an image analysis software program. The initial wound area was determined by tracing the hair margin of the wound periphery. After granulation tissue formation, the granulation tissue area was determined by tracing the margin between granulation tissue and the epithelial edge. Each measurement was repeated 3 times by an author (MS) who was blinded to the treatment group assignment for the wound. A mean was determined for the 3 measurements and used in subsequent analysis. The percentage wound closure was calculated by determining the percentage difference between the initial wound area on day 0 and wound area on each day of imaging. Healing was assessed by the number of days until closure and the number of EGT excisions. Closure was defined as the wound having an eschar covering the nonepithelialized area with no visible granularity. Removal of eschars to evaluate healing would impede epithelialization and thus was not done. Additionally, the wounds no longer needed treatment once protected by eschars.

**Wound biopsy and histologic examination**

On day 135, a 6-mm biopsy sample, including the center of the wound, was obtained from each wound and placed in neutral-buffered 10% formalin solution for histologic examination. An additional 6-mm biopsy sample was obtained from adjacent, grossly normal skin of each horse and was treated in the same manner as the wound samples. The time point was selected on the basis of results reported by Berry and Sullivan; in that study, all experimentally induced equine skin wounds had healed by day 135. Horses were sedated for this procedure with detomidine (0.01 mg/kg, IV). To avoid disrupting the cells on the surface of the tissue, the wounds were not aseptically prepared prior to the procedure. A local block was administered with 4 mL of 2% mepivacaine, SC, at each site prior to sample collection with a 6-mm biopsy punch. Biopsy sites were allowed to heal by second intention. Formalin-fixed, paraffin-embedded sections (thickness, 4 μm) were stained with H&E and Masson trichrome stain. Stained sections were examined with a microscope, and digital images were obtained at 400X magnification with a scanning system. Digital images were analyzed with commercially available software.

All histologic evaluations were performed by a board-certified veterinary pathologist (MM) who was blinded to the treatment group information. Biopsy specimens were evaluated for epidermal healing, inflammation, granulation tissue, collagen, and vascular density. Epidermal healing was scored as follows: 0 = absent, 1 = present only at edge of section, and 2 = spanning the section. Inflammation was recorded as present or negligible to absent. Granulation tissue was scored as 1 = minimally organized, densely cellular fibrovascular proliferation; 2 = organized with a plane of neovascularization (still the predominant component) perpendicular to plane of fibroplasia; and 3 = well organized with increased fibrous collagen and diminished vascularity. Mitotic index in granulation tissue was recorded as the number of mitotic figures in fibroblasts or vascular wall cells/10 fields at 400X magnification. All biopsy specimens of grossly normal (nonwounded) skin were considered to be histologically normal and were not graded but were used as standards when grading wounded skin from the same horse.

The granulation tissue in healing wounds was further evaluated by color deconvolution of collagen staining (Masson trichrome stain) and vascular density morphometric evaluation (H&E stain). The normal skin specimens were used as the dermal standard for color deconvolution image analysis scoring, but were not included in the vascular density morphometric analysis because granulation tissue was not present in the normal skin specimens. The normal skin specimens were not used in statistical analysis.

For color deconvolution of collagen staining in Masson trichrome-stained digitally scanned images, the perimeter of each histologic section (both halves of the biopsy specimen or thus 2 histologic sections/wound) was traced with the pen tool, omitting epidermis and any serocellular crusting. An algorithm was then applied to calculate the area with positive staining results within the tracing as well as the percentage of weak, moderate, and strong staining. The algorithm was calibrated for concordance with visual observation to set the intensity thresholds for a positive result and separation into weak, moderate, and strong staining. The algorithm also calculated an overall score (1X [percentage of area with weak staining] + 2X [percentage of area with moderate staining] + 3X [percentage of area with strong staining]) for each section.

To measure vascular density in the wounds, 2 squares (1 mm in diameter) were drawn in the dermal granulation tissue in H&E-stained, digitally scanned images. One superficial square was placed near to, but not contiguous with, the ulcerated surface or just beneath intact epidermis. The second square was placed just deep to the superficial square. Once the squares were drawn, all clusters of neovascularization or individual immature vessels were traced with the pen tool at 7X to 8X magnification so that the 1-mm square filled the monitor screen. Vascular density was recorded for each square as cross-sectional vascular area divided by the area within the 1-mm square.

**Statistical analysis**

Considering all treatments on each horse, a priori power analysis indicated that 8 horses were required...
to demonstrate, with 80% power and 95% confidence, a significant difference in days to closure between treatments (with mean days to closure of 83.6 vs 101.6 from Berry and Sullins\(^8\) and an estimated SD of 15 for the difference of days to closure).\(^9\) Wound trimming, epidermal healing, cutaneous adnexa, granulation tissue organization, and presence of inflammation were analyzed with the Friedman test procedure.\(^9\) This rank-based nonparametric test was chosen because it allows comparisons of > 2 groups with ordinal data. Post hoc pairwise comparisons of data with significant Friedman test results were run with a Holm adjustment.\(^8\) Wound closure, CFU counts (logarithmically transformed), mitotic index, color deconvolution of collagen staining, and vascular density morphometry data were analyzed with 1-way repeated-measures ANOVA.\(^8\) The data were linear and normally distributed according to both Pearson and Studentized residual analysis. Individual horse was included as the covariate in all models to control for individual variation. For all analyses, values of \(P < 0.05\) were considered significant.

**Results**

All of the wounds expanded during the first week after wound creation (on the basis of wound measurements) and then began to contract and epithelialize. The mean ± SEM percentage increase in size for wounds of each treatment group from day 1 to day 7 was as follows: TAO, 9.65 ± 3.34%; control (no topical treatment), 10.45 ± 5.50%; HNE, 14.34 ± 4.69%; and SSC, 18.74 ± 4.91%. There was no significant (\(P = 0.502\)) difference among treatment groups for the percentage change in wound size between days 1 and 7. The mean ± SEM percentage change in size for wounds of each treatment group from day 1 to day 21 was as follows: TAO, -31.10 ± 7.88%; control, -25.15 ± 6.57%; HNE, -23.54 ± 7.83%; and SSC, -26.84 ± 5.43%). There was no significant (\(P = 0.694\)) difference among groups in the percentage change in wound size between days 1 and 21. The bandages were discontinued on day 45 because wound size was appropriately decreased, and 25 of 32 (78%) wounds no longer had exposed granulation tissue. The wounds that were not fully closed had all healthy epithelium and a coagulum in the center of the wound. The mean ± SEM number of days until wound closure for each group was as follows: HNE, 42.50 ± 1.54; SSC, 42.71 ± 1.44; TAO, 43.38 ± 1.41; and control, 43.43 ± 2.20. Median time to wound closure for all groups was 42 days (range, 36 to 50 days). All of the wounds developed a dry eschar after the use of bandages was discontinued. There was no significant (\(P = 0.730\)) difference in time to wound closure among the 4 treatment groups. Representative images depicting wound closure over time for each treatment group were obtained (Figure 1).

For HNE-treated wounds, the mean ± SEM microbial load increased (\(P = 0.005\)) between days 2 (3,136.38 ± 1,164.4 CFUs) and 21 (5,393.63 ± 1,451.7 CFUs). For TAO-treated and SSC-treated wounds, the mean ± SEM microbial load decreased (\(P < 0.001\) and \(P = 0.004\), respectively) between days 2 and 21. For TAO-treated wounds, there were 2,228.25 ± 1,157.3 CFUs on day 2, compared with 922.38 ± 616.0 CFUs on day 21. For SSC-treated wounds, there were 1,978.88 ± 683.4 CFUs on day 2 and 808.0 ± 358.8 CFUs on day 21. Results for control wounds did not differ significantly (\(P = 0.531\)) between days 2 (2,802.75 ± 1,157.3 CFUs) and 21 (2,431.13 ± 978.2 CFUs).

The differences among groups were nonsignificant on days 2 (\(P = 0.959\)) and 3 (\(P = 0.674\)) and were significant on days 9 (\(P < 0.001\)) and 21 (\(P = 0.001\)). Least squares mean CFUs were compared among groups for days 9 and 21 because of the differences detected. On day 9, HNE-treated wounds had a significantly higher least squares mean ± SEM microbial load (6,195.63 ± 1,343.9 CFUs) than did those treated with SSC (257.88 ± 125.0 CFUs; \(P < 0.001\)) or TAO (270.13 ± 183.0 CFUs; \(P < 0.001\)), but not control wounds (2,464.0 ± 1,241.9 CFUs; \(P = 0.15\)). Additionally, microbial loads were significantly (\(P = 0.017\) and \(P = 0.002\), respectively) lower for SSC-treated (257.88 ± 125.0 CFUs) and TAO-treated (270.13 ± 183.0 CFUs) wounds than for control wounds (2,464.0 ± 1,241.9 CFUs). On day 21, HNE-treated wounds had a significantly higher microbial load (5,393.63 ± 1,451.7 CFUs) than did SSC-treated (808.0 ± 358.8 CFUs; \(P = 0.015\)) and TAO-treated (922.38 ± 616.0 CFUs; \(P = 0.003\)) wounds, but not control wounds (2,431.13 ± 978.2 CFUs; \(P = 0.37\)).

Overall, 16 of 32 wounds required trimming of EGT, and none required > 1 trimming. Each time EGT was trimmed, the wounds were not treated; therefore, 1 treatment was missed for each EGT excision. There was a significant (\(P = 0.007\)) difference in the number of wounds that required EGT trimming among groups. Seven of 8 wounds treated with SSC, 5 of 8 wounds treated with HNE, 3 of 8 control wounds, and 1 of 8 wounds treated with TAO required trimming of EGT. Each EGT excision took place between days 11 and 19, with most (10/16) performed on day 15. A greater proportion of SSC-treated wounds were trimmed, compared with TAO-treated and control wounds (\(P < 0.001\) and \(P = 0.029\), respectively), and the proportion of HNE-treated wounds trimmed was greater than that of TAO-treated wounds (\(P = 0.004\)) but not control wounds (\(P = 0.182\)).

There was no difference among groups for the presence of wound inflammation (\(P = 0.223\)). Furthermore, evaluation of H&E-stained tissues revealed no difference among groups for epidermal healing (\(P = 0.207\)), cutaneous adnexa (\(P = 1.0\)), or granulation tissue organization (\(P = 0.568\)) scores or for mitotic index in granulation tissue (\(P = 0.828\)). There was no difference in color deconvolution scores for Masson trichrome-stained tissue sections among groups (percentage of section with weak staining [\(P = 0.868\)], moderate staining [\(P = 0.162\]), or strong staining [\(P = 0.868\]).
Figure 1—Representative photographs depicting healing of surgically created wounds at the distal aspect of the left forelimb in horses treated topically with HNE (A to D), TAO (E to H), 1% SSC (I to L), or no medication (control; M to P). On day 0 for each horse (n = 8), a wound was created over each metacarpus and metatarsus, covered with a nonadherent dressing, and bandaged. On day 1, all wounds were contaminated by application of a filter paper inoculated with a combination of *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, and *Candida albicans*. Each wound on a given horse was assigned to a treatment group by random selection from a hat; however, assignments were constrained so that each limb (eg, the 8 left forelimbs) was assigned to each treatment twice. Treatments were applied during bandage changes according to a predetermined schedule from day 2 until day 21. Bandage changes continued at predetermined intervals until day 45; photographs were obtained at each bandage change and at predetermined intervals afterward until evaluation of wound healing was completed (day 135). The images shown were obtained on days 1 (A, E, I, and M), 7 (B, F, J, and N), 19 (C, G, K, and O), and 45 (D, H, L, and P).
Discussion

In the present study, full-thickness excisional skin wounds were created on the distal aspects of the limbs of horses, inoculated with bacteria and fungi, and managed with 1% SSC, TAO, or HNE applied topically or no medical treatment (control). All wounds were covered with bandages, including a nonadherent dressing, for 45 days. There was no significant ($P = 0.730$) difference in mean time to wound closure for wounds treated with SSC, TAO, or HNE, compared with control wounds. This finding was consistent with those of previous studies$^{19,24}$ and suggested that application of topical medications to equine skin wounds under bandages is unnecessary in most instances.

Between days 2 and 21 (with the day of surgery considered day 0), the mean microbial load decreased for TAO-treated ($P < 0.001$) and SSC-treated ($P = 0.004$) wounds and increased for HNE-treated wounds ($P = 0.005$) in the present study, whereas this variable did not change significantly for control wounds during the same interval. These results suggested that the HNE product, which was developed to help decrease microbial load in wounds, may have lower antimicrobial activity, compared with the other treatments evaluated, for these types of wounds. The fact that there was no difference in wound closure times despite significant ($P < 0.05$ for all comparisons) differences in mean microbial load on days 9 and 21 (with higher CFU counts for HNE-treated than for SSC- or TAO-treated [but not control] wounds on both days and lower CFU counts for SSC- or TAO-treated wounds than for control wounds on day 9) was unexpected because wound contamination is known to delay wound healing.$^{15}$

In addition to a lower microbial load than control and HNE-treated wounds on day 9, TAO-treated wounds had the smallest proportion of EGT excisions (1/8 wounds). Although this product has been tested in human medicine, there is little research evaluating its use for skin wounds on the limbs of horses. Our results suggested that TAO decreased surface contamination of wounds while allowing the wounds to heal and that it may be a valid choice for application on skin wounds at the distal aspects of the limbs of horses when bandages are used and such treatment is warranted.

The number of wounds requiring excision of EGT in this study was notable. The procedure was performed for 7 of 8 SSC-treated wounds and 5 of 8 HNE-treated wounds, compared with 3 of 8 control wounds and only 1 of 8 treated with TAO. Although other significant differences between groups were identified, considering all treatments on the same horses, post hoc power analysis indicated that 61 horses would have been needed to detect, with 80% power and 95% confidence, a significant difference in EGT excisions between TAO-treated and control wounds.$^7$ Recommendations to treat EGT include excision or topical application of corticosteroids because EGT interferes with orderly progression of wound healing.$^8$ In the present study, significantly ($P \leq 0.004$) fewer TAO-treated wounds had EGT requiring excision, compared with wounds receiving other treatments (HNE and SSC) but not control wounds, when bandaged as described. We postulate that in addition to broad-spectrum antimicrobial activity, TAO helps to maintain a moist environment with reduced inflammation.$^{12}$ Even though HNE has been shown to have antimicrobial properties, it did not reduce microbial counts and slightly encouraged EGT formation, compared with TAO treatment.

The present study was designed to evaluate 3 topical medications that might be used when a bandage is necessary. Although the mean time to wound closure was not significantly shorter for wounds treated with any of the products tested than for control wounds, topical application of TAO reduced the number of wounds requiring excision of EGT. The formation of EGT did not appear to be associated with the microbial load on wounds, because SSC treatment decreased the number of CFUs in wounds in a manner similar to TAO, but more of the SSC-treated wounds required excision of EGT. The HNE-treated wounds were among those with the highest microbial counts and were intermediate in regard to the proportion that required excision of EGT.

No significant difference in histologic properties of wounds at the time of biopsy was identified among groups. This indicated that none of the tested agents altered the histologic properties of interest with the methods used; however, the methods provided an objective measure of histologic scoring for wound healing in horses.

The present study had some inherent limitations. The low number of horses may have limited the authors' ability to determine additional significant differences between wound treatment groups. Including all treatments on the same horses, post hoc analysis indicated that 438 animals would have been required to detect, with 80% power and 95% confidence, a significant difference in mean days to closure between the HNE-treated and control wounds in this study.$^9$ Individual variation among the horses was a potential confounding factor but was included as a covariate in the statistical model. Inclusion of more horses might have been beneficial for this reason, but the number 8 was determined a priori by statistical methods on the basis of mean and SD data from Berry and Sullins.$^8$ The wounds were surgically created under experimental conditions and thus may not have been representative of wounds treated in clinical practice, particularly in terms of size and the degree of tissue damage. Nonetheless, similar methods have been commonly used to assess treatment protocols for wounds at the distal aspects of the limbs.$^7,8,10,24–29$
In a clinical setting, EGT would be trimmed or treated with a topical application of corticosteroids. Therefore, trimming of the EGT was the best option and reflective of clinical practice when the EGT was impeding wound healing. The author performing this task was not blinded to treatments, and this was a potential source of bias. The timing of all treatments, discontinuation of treatments and bandages, and biopsies were the same for all wounds. This meant that decisions were not made according to the clinical appearance of each wound, and this could have impeded wound healing. The methods were chosen for consistency, to allow comparisons among treatments under similar conditions, and to eliminate the influence of additional subjective determinations. The use of NSAIDs is controversial owing to the potential inhibition of wound healing. Phenylbutazone was used for its analgesic and anti-inflammatory properties. Finally, the bacteria and fungi used for topical inoculation of wounds in this study were chosen because they are commonly found on wounds in a clinical situation. However, these may not have been representative of the various organisms that could contaminate equine wounds.

Our hypothesis that topical administration of antimicrobials for bandaged wounds would reduce wound bioburden, minimize EGT formation, and promote reepithelialization was not supported by the results of the study. In fact, all wounds had similar healing times despite significant differences in microbial load and EGT excisions among treatment groups. However, on the basis of the study results, we recommend treating wounds at the distal aspects of the limbs of horses that require a nonadherent dressing and bandage with TAO when warranted or with no topical medication until a granulation tissue bed is present; although 1% SSC and TAO treatment had similar antimicrobial effects against the bacteria and fungi used in this study, TAO may be a better choice to help limit EGT formation. Further investigation may be warranted for the use of TAO versus no topical treatment for wounds that do not require a bandage.

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Footnotes


b. Curad nonadherent pad, Medline Industries, Mundelein, Ill.

c. Accu-Sorb Gauze Sponge, Medline Industries, Mundelein, Ill.

d. Sof-Form Conforming Stretch Gauze Bandages, Medline Industries, Mundelein, Ill.

e. 3M Veterinary elastic adhesive tape, 3M Animal Care, Saint Paul, Minn.

f. ATCC 6538, American Type Culture Collection, Manassas, Va.

g. ATCC 29212, American Type Culture Collection, Manassas, Va.

h. ATCC 8739, American Type Culture Collection, Manassas, Va.

i. Candida albicans. Carolina Biological, Burlington, NC.


k. TAO, G&W Laboratories, South Plainfield, NJ.

l. SSC USP 1%, Ascend Laboratories, Montvale, NJ.

m. ImageScope, Aperio Technologies, Vista, Calif.

n. Spectrum Analysis algorithm package and ImageScope analysis software, version 9, Aperio Technologies Inc, Vista, Calif.

o. Power paired means, STATA SE, version 14.1, StataCorporation, College Station, Tex.

p. SAS, version 9.4, SAS Institute, Inc, Cary, NC.


r. Power paired proportions, STATA SE, version 14.1, Stata-Corporation, College Station, Tex.

References


15. Hendrickson DA. Management of superficial wounds. In:


Appendix

Schedule of procedures in a study to evaluate the effects of 3 topically applied treatments (1% SSC, TAO, and HNE) on bacterial counts, development of EGT, and reepithelialization of contaminated wounds at the distal aspect of the limbs of horses.

<table>
<thead>
<tr>
<th>Day</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Wound creation, bandage placement, and phenylbutazone administration</td>
</tr>
<tr>
<td>1</td>
<td>Photography of wound, inoculation of wound with bacteria and fungi, bandage change, and phenylbutazone administration</td>
</tr>
<tr>
<td>2</td>
<td>Photography of wound, sample collection for culture, wound treatment, bandage change, and phenylbutazone administration</td>
</tr>
<tr>
<td>3</td>
<td>Photography of wound, sample collection for culture, wound treatment, bandage change, and phenylbutazone administration</td>
</tr>
<tr>
<td>4–8</td>
<td>Photography of wound, wound treatment, and bandage change</td>
</tr>
<tr>
<td>9</td>
<td>Photography of wound, sample collection for culture, wound treatment, and bandage change</td>
</tr>
<tr>
<td>11, 13, 15, 17, 19</td>
<td>Photography of wound, wound treatment, and bandage change</td>
</tr>
<tr>
<td>24, 27, 30, 33, 36, 39, 42</td>
<td>Photography of wound, sample collection for culture, and bandage change</td>
</tr>
<tr>
<td>45</td>
<td>Photography of wound (bandaging discontinued)</td>
</tr>
<tr>
<td>50, 61, 71, 81, 91, 101, 112, 131</td>
<td>Photography of wound</td>
</tr>
<tr>
<td>135</td>
<td>Photography of wound and biopsy of wound and adjacent grossly normal skin</td>
</tr>
</tbody>
</table>

Each horse had 4 wounds (1/limb) created surgically at the dorsal aspect of the metacarpus or metatarsus. Wounds were inoculated with Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, and Candida albicans; samples for culture were collected by use of a sterile, cotton-tipped applicator and cultured on tryptic soy agar plates. Each wound on a given horse was assigned to receive 1 of the 3 topical treatments or no medication (control). Treatments were performed as described, except that if EGT protruded above the level of the skin or over advancing epithelium at any time during the bandaging phase, the EGT was trimmed level with the skin by use of a No. 10 scalpel blade, and the new bandage was applied without treatment. Phenylbutazone was administered at 4.4 mg/kg on day 0 and subsequently reduced to 2.2 mg/kg every 24 hours for the days indicated.