Topical Antimicrobial and Bandaging Effects on Equine Distal Limb Wound Healing

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(ABSTRACT)

The objective of this study was to determine if topical antimicrobials silver sulfadiazine and povidone-iodine ointment increase rates of healing of equine distal limb wounds that heal by second intention. Second, to determine the effect of bandaging with these topical antimicrobials. Six healthy adult horses were used to create thirty-six, 2.5 cm^2 standardized full-thickness metacarpal/tarsal skin wounds. Each wound was exposed to a single treatment: 1.0 % silver sulfadiazine cream bandaged (SSD-B), 1.0 % silver sulfadiazine slow release matrix bandaged (SDX-B), 1.0% silver sulfadiazine slow release matrix not bandaged (SDX-NB), povidone-iodine ointment bandaged (PI-B), untreated control bandaged (C-B) and untreated control not bandaged (C-NB) until healing. Wound area, granulation tissue area and perimeter were measured with planimetry software from digital images obtained at each observation. Exuberant granulation tissue was excised when present. The days until healing, rate of healing parameter, rates of contraction and epithelialization were compared among groups using pair-wise analysis of least square means.

The healing parameters and mean days to healing did not statistically differ between groups. Analysis of percent wound contraction and rate of epithelialization between groups was similar. Mean number of days to healing ranged from 83 (PI-B and C-B) to101 (SSD-B). All bandaged wounds produced exuberant granulation tissue requiring excision compared to none of the unbandaged. The identified rates of epithelialization and wound contraction found insignificant differences between antimicrobial treated versus untreated wounds. Similarly, rates of epithelialization and wound contraction found insignificant differences between bandaged versus unbandaged wounds. Topical povidone-iodine and silver sulfadiazine did not increase rates of healing under bandage.

The 1.0% silver sulfadiazine slow release matrix not bandaged (SDX-NB) adhered well to dry wounds. Silver sulfadiazine slow-release matrix provides does not impede wound healing and provides good adherence to dry wounds not amenable to bandaging.

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List of Abbreviations

Co.	Company
IU	International Units
IV	intravenous
IM	intramuscular
kg	kilogram
mg	milligram
PO	*per os*

1.0 Introduction to Wound Healing and Horses

Wounds of the distal equine limb are of concern due to loss of performance and potential loss of commercial value (Cochrane 1997). Compared with wounds of the trunk, distal limb wounds have a longer preparatory phase characterized by greater wound retraction, slower rates of epithelialization and earlier cessation of wound contraction (Jacobs et al. 1984, Wilmink et al. 1999a). Anatomic location, specifically, body versus distal limb, contributes to physiologic differences in organization of the cellular inflammatory response have been found between the horse and pony to explain some of this phenomenon (Wilmink et al. 1999a, Wilmink et al. 1999b). Recent investigations focus on the roles of epidermal growth factor, fibroblastic growth factor-B1 and the matrix metalloproteinases regulation of fibroplasia (Cochrane 1997, Knottenbelt 1997). These studies suggest that the persistence of inflammation contributes to longer healing times in the horse compared to the pony. These findings support earlier research, which demonstrated that horses have inherent dermal and subcutaneous precursors predisposing them to exuberant granulation tissue formation (Chvapil et al. 1979). Exuberant granulation tissue delays healing by inhibiting wound contraction and subsequent epithelialization and eventually resulting in a larger scar (Jacobs et al. 1984, Lees et al. 1989, Fretz et al. 1983). Where infection contributes to the prolonged lag phase of wound healing, topical antibacterial therapy may improve the quality and rate of wound healing. Topical medications may alter the wound environment by antimicrobial activity, corticosteroid-induced reduction of fibroplasia or promotion of epithelialization (Stashak 1991b). Limited controlled studies have been performed on horses.

Because wound healing is a dynamic process, a primary goal should be management of local factors to provide an environment that promotes acceptable wound healing. Requirements

for this include microcirculation for oxygenation and nutrition, moisture, increased temperature, neutral to slightly acidic pH, and a bacterial population less than 10^5 organisms per gram of tissue (Wilmink et al. 1999a, Peacock Jr. 1984). A bandage with a topical antimicrobial is a commonly used means of providing this environment. Silver sulfadiazine cream (1.0%) has been reported to increase the rate of epithelialization in human burn patients, mice and pigs (Lansdown et al. 1997, Kjolseth et al. 1994, Geronemus et al. 1979) when compared to other topical medications including povidone-iodine.

Two antimicrobial preparations used commonly in equine wound management are 10% povidone-iodine ointment and 1% silver sulfadiazine cream. A 1% silver sulfadiazine slow-release matrix has recently developed that adheres to the wound bed when dry and can remain in place on unbandaged wounds. The topical application of either antimicrobial provides broad spectrum of antibacterial and antifungal activity *in vitro*. *In vivo* studies evaluating silver sulfadiazine, neomycin, bacitracin, polymyxin combinations or iodine containing ointments in murine and porcine models have shown varying degrees of improved wound healing primarily through increased epithelialization (Kjolseth et al. 1994, Geronemus et al. 1979). The assessment of these treatments in the equine assessment have not been reported. Controlled trials to examine the effect of commonly used topical antimicrobials in equine wounds would be beneficial (Stashak 1991b).

The objective of this study was to evaluate rates of contraction, epithelialization and days to healing of the equine distal limb wounds treated with either of two topical preparations of 1.0% silver sulfadiazine or 10% povidone iodine compared to untreated controls. The additional influence of bandaging of with these antimicrobials on granulation tissue formation was evaluated.

1.1 Clinical Incidence

Distal limb wounds are of common concern to the horse owner primarily from temporary performance restriction and potential loss of commercial value (Cochrane 1997). In a large, multicentered retrospective study of 122,642 cases presented to nineteen veterinary colleges in a four-year period, 2% of all cases required wound treatment and 61% of these wounds were treated with debridement only and healed by second intention (Lindsay 1990). This was because of significant tissue loss, degree of contamination or both. Distal limb wounds requiring second intention healing therefore presents a management concern because once the protective dermal barrier is lost, the remaining tissue may become susceptible to infection (Peacock Jr. 1984, Stashak 1991b). In another retrospective report of 452 cases, a 52% infection rate occurred in clean contaminated orthopedic wounds (Macdonald et al. 1994). This degree of morbidity makes management of distal limb wounds an important clinical concern.

1.2 Physiological Differences

Compared with wounds of the trunk, distal limb wounds have a longer preparatory phase of second intention healing characterized by greater wound retraction, slower rates of epithelialization and earlier cessation of wound contraction (Jacobs et al. 1984, Wilmink et al. 1999a). Regional anatomic and physiologic differences in organization of the cellular inflammatory response explain some of this phenomenon (Wilmink et al. 1999b, Wilmink et al. 1999a). Most recent investigations focus on the roles of epidermal growth factor, fibroblastic growth factor-B1 and the matrix metalloproteinases abilities to regulate fibroplasia (Knottenbelt 1997, Cochrane 1997). These studies suggest the persistence of inflammation contributes to the prolonged healing times between the pony and the horse. This supports earlier research which

determined horses have inherent connective tissue precursors, which activate in the dermis and subcutaneous tissues predisposing them to the formation of exuberant granulation tissue (Chvapil et al. 1979). The result of uninhibited fibroplasia is exuberant granulation tissue which delays the healing process by inhibiting epithelialization and contraction subsequently resulting in a larger granulation epithelium scar (Jacobs et al. 1984, Lees et al. 1989, Fretz et al. 1983).

The goal of wound healing by second intention is to repair the tissue defect through granulation tissue formation, which allows a matrix for epithelial cell migration. Tissue repair requires a good blood supply and an adequate inflammatory response, which attracts macrophages and fibroblasts that are necessary for clearance of debris and initiation of repair. These cells are supported by the vascular endothelium (Silver 1982).

During the initial response to injury, platelet accumulation provides chemotactic attraction through platelet derived growth factor. Macrophages and other leukocytes are activated and infiltrate the wound. This initial inflammatory phase of wound healing including debridement of necrotic tissue and foreign debris is crucial for initiation of the repair phase. Once this macrophage directed phagocytosis clears the tissue debris, perivascular pleuropotential mesenchymal cells differentiate into fibroblasts and myofibroblasts. These cells produce collagen, protein polysaccharide, and glycoprotein ground a process which reaches a peak around the fifth day after wounding (Chvapil et al. 1991, Stashak 1991a). If the inflammation does not subside, prolonged stimulation of these cells will promote continued, and in the case of the equine leg wound, excessive fibroplasia.

Recent research in horses focused on exogenous cytokine therapy. This used activated macrophage supernatant to influence the wound environment (Wilson et al. 1996) in an attempt to inhibit excessive fibroplasia. Though there was an inhibition of the equine fibroblasts in vitro,

in ponies little effect was observed when wounds were exposed to this growth factor milieu. To be effective second intention healing relies heavily upon the acute inflammatory phase following injury (Chvapil et al. 1991, Knottenbelt 1997). This was recently demonstrated in a comparison between the histologic aspects of second-intention wound healing in the horse versus the pony (Wilmink et al. 1999c). In that study, there was a difference between the horse and pony inflammatory response as measured by presence of leukocytes and the differentiated fibroblast, the myofibroblast. Furthermore there was reduced and prolonged inflammatory response in the horse compared to the pony. This finding correlated with the clinical presence of purulent exudate on the wound surface but the reason for the prolonged inflammation in the horse was unclear. In a second part of this work (Wilmink et al. 1999c), the researchers determined it was the degree of organization of myofibroblasts that determined the extent and rate of wound contraction pony. This result supports the notion that once effective inflammation has occurred, the repair and maturation processes can initiate rapid and efficient extracellular matrix reorganization, wound contraction and epithelialization (Stashak 1991b). Persistent stimulation of the wound that occurs with tissue infection, necrotic tissue, foreign body, movement, and iatrogenic irritants placed on the wound leads to chronic inflammation that encourages the development of proliferating granulation tissue and delayed wound healing (Blackford & Blackford 1995).

During the proliferative phase of wound healing rapid reduction in wound size is due to contraction of actin containing myofibroblasts. The rates of contraction vary according to wound location on the horse (Wilmink et al. 1999c). The clinical observation suggests the rate of healing of equine limb wounds is approximately half that of body wounds (Walton & Neal 1972, Jacobs et al. 1984). The physiologic explanation of this is not fully understood but is centered on

regional inherent differences in wound contraction. Contraction ceases when epithelialization is complete or when tension in the surrounding skin exceeds the exertion by the myofibroblasts (Swaim 1980). However, once the myofibroblasts encounter tensile forces which can not be overcome, epithelialization must be relied upon to cover the remaining granulation bed. Optimally, second intention wound healing contracts rapidly during the rapid healing phase, approximately days 14 through 35 in a horse, to minimize amount of epithelialization, which is often referred to as the scar epithelium (Jacobs et al. 1984). The differences in wound contraction appears to be determined by local environments such as oxygenation, moisture, temperature rather than by inherent contraction capacity of the cells (Wilmink et al. 1999b).

1.3 Wound Healing Kinetics

All cutaneous wounds heal by two independent processes, contraction and epithelialization. Wound contraction reduces the perimeter by centripetal movement of dermis and epidermis and occurs as long as the contractive forces of the wound myofibroblasts exceed the reactive forces of the surrounding skin on the wound edge (Lees et al. 1989, Jacobs et al. 1984). Epithelialization is the process that keratocytes proliferate and migrate to cover the surface of the cutaneous defect. The sum of these processes determines the rate of wound healing.

Second intention skin healing in horses and occurs in an ordered process and has been mathematically computed following first-order differential equation (Mcgrath & Simon 1983, Madison & Gronwall 1992, Schumacher et al. 1992). This process progresses at a rate proportional to the amount of area remaining to contract in a logarithmic linear rate. Schumacher deduced that equine lower limb wound contraction could be described by using a bi-

exponential mathematically derived rate constant while epithelialization, which is an advancing process, is described by a monoexponential equation. Madison and Gronwall used a similar exponential equation and concluded wound contraction is a complex process and may not be adequately described by simple mathematics equations.

Before these studies, rates of wound healing were derived from a linear regression equations that analyzed the square root of the area (Snowden 1981, Fretz et al. 1983, Bertone et al. 1985). The researchers determined a rate of wound healing but not the components of contraction and epithelialization, therefore, making comparison to later studies difficult. Using this earlier method, the overall rate of second intention healing in the pony was determined to be 0.56mm/day(is this area or linear) for open surgically created wounds on the dorsal fetlock of ponies (Fretz et al. 1983). This is similar to limb wounds of humans 0.58mm/day (Snowden 1981). These pony wounds were treated with excision of excessive granulation tissue only and rate of healing was a constant during the rapid phase of wound closure. Fretz et al. determined the rate of healing was dependent on the treatment applied to the wound with reference to bandaging alone. However, there was no effort made to minimize infection other than prevention of contamination by the varied treatment bandages.

1.4 Topical Medications

In attempts to reduce exuberant granulation tissue formation, application of topical medications either alter the wound environment by suppression of opportunistic bacterial infection, reduction of mitotic activity or promotion of epithelialization through fat-soluble vitamin supplementation. Many topical therapies have been applied to control the inflammatory

response leading to delayed healing but limited controlled studies with antimicrobials have been evaluated on horses.

Topical wound preparations have been studied on the horse to promote increased rates of wound healing, decrease the incidence of exuberant granulation tissue and produce an optimal cosmetic result (Bertone et al. 1985, Madison et al. 1991, Blackford et al. 1991, Bigbie et al. 1991, Barber 1989, Wilson et al. 1996, Woollen et al. 1987, Yvorchuk-St. Jean et al. 1995). Often, this employs the useof a topical antimicrobial for prevention of wound infection therefore avoiding systemic antibiotic toxicity and expense. This does require avoidance of local toxicity or contact dermatitis in the antimicrobial preparation. Among the three published studies using antimicrobials in horses, two of the three assessed the effect of a topical antimicrobial combination with the presence of a glucocorticosteroid (Barber 1989, Blackford et al. 1991, Woollen et al. 1987). Two additional, but not studied, antimicrobial preparations used commonly in equine wound management are 10% povidone-iodine ointment and 1% silver sulfadiazine cream or a recently developed slow-release silver sulfadiazine matrix. The topical application of either antimicrobial provides broad antibacterial and antifungal spectrum *in vitro*and is economical for routine use. The *in vivo* studies evaluating silver sulfadiazine, neomycin, bacitracin, polymyxin combinations or iodine containing ointments in murine and porcine models have shown varying degrees of improved wound healing primarily through increased epithelialization (Geronemus et al. 1979, Kjolseth et al. 1994) but *in vivo* assessment of their effect on healing has not been determined in the horse. Based on the widespread use, controlled trials to examine the effect of topical antimicrobials on equine wound healing are necessary (Stashak 1991b).

The clinical rationale for topical antimicrobial therapy is suppression of opportunistic bacterial tissue damage which decreases rates of wound healing thereby increasing morbidity and cost. Because wound healing is a dynamic process, a primary goal should be management of local factors to provide an environment that promotes acceptable wound healing. Requirements for this include a wound environment that provides microcirculation for oxygenation and nutrition, moisture, increased temperature, neutral to slightly acidic pH, and a bacterial population less than 10^5 organisms per gram of tissue (Peacock Jr. 1984, Knottenbelt 1997). A bandage with a topical antimicrobial is a common means of providing this environment. Beneficial effects from 1.0% silver sulfadiazine cream have been reported in human burn patients, mice and pigs (Lansdown et al. 1997, Kjolseth et al. 1994, Geronemus et al. 1979) by increasing the rate of epithelialization when compared to other topical medications, including povidone-iodine, and untreated controls.

The experimental hypothesis is to determine if equine distal limb wounds treated with either 1.0% silver sulfadiazine and bandaging demonstrated increased rates of second-intention healing pertaining to the equine distal limb

2.0 Experimental Design

2.1 Horses

The Institutional Animal Care and Use Committee approved the protocol used in this study. Six adult geldings (4 Thoroughbreds, 1 Warmblood, 1 Quarter horse) 5 to 11 years (mean 9.2) old and weighing 527 to 645 kg (mean, 593.5) were used. The horses were vaccinated routinely (Equiloid®, Ft. Dodge Laboratories, Ft. Dodge, IA.) and received ivermectin (Eqvalan®, Merck & Co. AgVet division, Rahway, NJ.) (0.2 mg/kg of body weight, PO). The horses were grouped in pairs and housed in covered stalls with an adjoining small dry-lot paddock. All horses were allowed free choice timothy/orchard grass hay and one kilogram of a concentrate (Priority 10®, Southern States Co-op, Cooperative Milling, Gettysburg, PA) per day.

2.2 Wound Creation

Each horse received procaine penicillin (Pfi-Pen G®, Pfizer Animal Health, New York, NY.) (22,000 IU/kg, IM) preoperatively then twice daily and phenylbutazone (phenylbutazone tablets, Vedco, St. Joseph, MO.) (4.4mg/kg, PO) once daily for 72 hours. Following a 12-hour fast, each horse was pre-medicated with xylazine hydrochloride (Sedazine®, Ft. Dodge Laboratories, Ft. Dodge, IA.) (0.5 mg/kg, IV) and guaifenesin (5% IV to effect). Anesthesia was induced with ketamine hydrochloride (Ketaset®, Ft. Dodge Laboratories, Ft. Dodge, IA.) (2.2 mg/kg IV) and maintained with the intravenous combination of xylazine, ketamine and guaifenesin (500mg, 2000mg, and 50 grams respectively) in 1 liter of sterile saline administered to effect.

The dorsomedial metacarpi and metatarsi were aseptically prepared with chlorhexidine gluconate and water. A 6.25-cm^2 full-thickness skin wound was created on each proximal and

distal metacarpus and on each proximal metatarsus avoiding the underlying long or common extensor tendons for a total of six wounds per horse (Figure 2). Following a 2.5-cm flexible square stainless steel template with a #10 scalpel blade created each standardized wound. The tissue within the template was excised with scissors to the level of the periosteum. Permanent tattoo ink was injected intradermally through predrilled holes in the template 10 mm from and parallel to the margin of the wound (Wilmink et al. 1999a).

2.3 Assignment of Treatment Groups

One of six treatments was administered to each of the wounds on each horse. The treatments (Table 1) included 1.0 % silver sulfadiazine cream (Silvadene®, Hoechst-Marion-Roussel, Kansas City, MO.) bandaged (SSD-B), 1.0 % silver sulfadiazine slow release matrix (Silvadex SR®, Buford Biomedical Inc, Frederick, MD.) bandaged (SDX-B), 1.0% silver sulfadiazine slow release matrix not bandaged (SDX-NB), 10% povidone-iodine ointment (Biozide® gel, Performance Products Inc, St. Louis, MO.) bandaged (PI-B), untreated control bandaged (C-B) and untreated control not bandaged (C-NB). Treatments were continued until complete epithelialization of the wound had occurred. The bandaged wounds were covered with a rayon-polyethylene non-adherent dressing (Release®, Johnson & Johnson, Arlington, TX.) and elastic adhesive tape (Elastikon®, Johnson & Johnson, Arlington, TX.)

Table 1. Summary of Treatment Groups

Treatment Groups	Explanation
All treatments were applied to each horse	**Treatment applications rotated between the six locations**
SDX-NB	**silver sulfadiazine slow-release matrix without bandage**
SSD-B	**silver sulfadiazine cream under bandage**
SDX-B	**silver sulfadiazine slow-release matrix under bandage**
PI-B	**povidone-iodine under bandage**
C-NB	**untreated control without bandage**
C-B	**untreated control without bandage**

The elastic tape was wrapped until pressure was firm. Bandages were changed and each wound was photographed on the first postoperative day, every other day for 17 days then every third day thereafter until the wound had healed. Wound treatments consisted of 500 mg total weight of each medication applied to the non-adherent pad. The SDX-NB group had equal amount of preparation applied with a clean tongue depressor and massaged into the wound bed or eschar. The SDX-NB group was further evaluated on ability of the medication to adhere to the wound bed.

2.4 Assessment of Wound Healing

Wounds were considered to have healed when visible epithelium covered the wound. Wounds were considered to have exuberant granulation tissue when the periphery of the granulation bed was above the surrounding level of the skin or advancing the epithelium. When exuberant granulation tissue was present, it was excised to the level of the surrounding skin or migrating epithelium with a # 10 scalpel blade (Howard et al. 1993).

Photographs were taken with a digital camera (Sony Mavica® MVC-FD83, Sony Corp, Tokyo, Japan.) through a macro lens at a 22-cm focal distance after wiping the wound clean of any exudate and clipping any hair interfering with identification of the wound margin and prior to any exuberant granulation tissue excision. A horizontal metric scale was attached just proximal to the wound to provide a calibration reference. A label was placed below the wound to identify wound location, date and horse. The total wound area, wound perimeter and granulation area was determined using planimetry software (NIH Image, version 1.62. Available at: http://rsb.info.nih.gov/nih-image. May 1999.)

Each digital image was displayed on a 40-cm color monitor (Apple Multiple Scan® 17 display, Apple Computer Inc, Cupertino, CA.). Each image was scaled to fit the window before the program was calibrated to a 1.0 cm distance from the metric scale in the image. The wound area and perimeter were determined by tracing the hair margin of the wound periphery. The granulation tissue area was determined by tracing the cursor around the margin between granulation tissue and epithelium when present. Each wound was measured three times, and the average of the three was recorded. The epithelial area was calculated by subtracting the granulation tissue area from the wound area. The area of the epithelium and total wound area were equal when wound healing was complete.

Wound contraction was expressed as a percentage of the wound area from the maximal wound size[3] following wound creation. The healing parameter describes the linear advance of the wound margin toward the wound center for comparison of healing rate between differently shaped wounds. It was calculated by dividing the final wound area by the average of the initial and final wound perimeter (Gilman 1990). The rate of epithelialization was derived from the slopes of the linear regression for plots of epithelial area versus time (Jacobs et al. 1984). For analysis of contraction during the rapid phase from 20 to 62 days (Geronemus et al. 1979), the natural log of the wound area was taken as the unit of analysis. Linear regression was then used to identify the rate constant for each treatment group.

2.5 Statistical Analysis.

The treatment groups were randomized in a Latin square design balanced for wound location on each horse. For each treatment group, Fisher's square least square means comparisons for analysis of variance was performed for days to healing, wound healing

parameter, rate of epithelialization and rate constant for contraction between groups. For all analyses, $P<0.05$ was considered significant. Statistical software (PROC GLM and PROC REG, SAS Institute Inc, Cary, NC.) was used for all analyses.

3.0 Experimental Results

3.1 Subjective Assessment

Horses remained comfortable and did not disturb their wounds. All wounds underwent linear expansion in the first weeks followed by rapid exponential contraction (Figure 1). Each retained the original square periphery to slightly stellate with a round, central granulation bed until complete epithelialization. The wound tattoos were only visible 29 of 36 sites. Therefore, were not useful in determining calculations. This did not affect the ability to accurately measure the wounds through out the protocol.

3.2 Days to Healing

Mean days to complete healing, healing rate parameter, number of times granulation tissue was excised and percent wound healing attributable to contraction, for all treatment groups are shown in Table 2. The mean days to complete healing for the treatment groups ranged from 83 days for C-B and PI-B to 101 days for SSD-B with a combined mean for all groups of 92 days. Significant differences were not detected in healing, healing parameter and percent wound contraction treatment groups and controls. All the wounds of horse 3 healed significantly faster (mean 57 days) than the remaining five horses (P=0.01). Horse 3 was the smallest of the group; 527 kg vs group mean of 593.5 kg.

3.3 Effect of Bandaging

All bandaged wounds eventually produced exuberant granulation tissue that required excision. The unbandaged wounds became covered with a dry eschar beginning 14-21 days after wounding and did not produce exuberant granulation tissue. The eschar

remained in place and reduced in size as contraction and epithelialization progressed until it fell off when epithelialization was visibly complete. Among the bandaged wounds, the mean number of times the exuberant granulation tissue was excised was 5 (range 1-9). These wounds would remain moist then progress to purulent exudate prior to becoming exuberant, regardless of treatment. Following exuberant granulation tissue excision, wound surface appearance returned to moist and clean at the next bandage change.

3.4 Epithelialization

Epithelium was first present in 15 of the 36 wounds by day 7 following wounding. There were no differences between groups for initiation of epithelialization. The rate of epithelialization ranged from 2.96+/-0.50 mm^2/day for PI-B to 4.21+/-0.50 mm^2/day for C-NB. These calculated differences were not determined to be of sufficient statistically significance (P=0.09)(power 0.39).

3.5 Contraction

Maximal wound size was attained during the second week after which the wounds in each group contracted to 75 percent of their maximal size when healed. Significant differences in wound area were not present in all measurements of wound areas between any treatment groups. The slope from the exponential regression model from the maximal wound size through the rapid phase of contraction ending at 62 days post wounding ranged from –0.023 for PI-B to –0.030 for C-NB.

4.0 Discussion

In this study, topical medication when compared to controls did not affect the rate of healing of the distal limb wounds. The increased rate of epithelialization (Kjolseth et al. 1994, Geronemus et al. 1979) or decreased rate of wound contraction (Leitch et al. 1993) with 1.0% silver sulfadiazine found in other species was not observed in these horses.

This discussion of these findings are related to experimental, small wounds with easily managed wounds. Larger wounds will have variables not present in these small controlled wounds. Further, larger granulating areas will have more tendency to become exuberant as the time required to complete contraction is prolonged.

Lastly, the results of this study could not identify a positive or detrimental effect of the treatments employed. Retrospective evaluation determined the sample size was not sufficient enough to account for the small differences present in healing rates between groups. Though trends exist for a difference to be present between PI-B and C-B compared to SSD-B, the small population size may have contributed in preventing us from disproving the null hypothesis.

4.1 Effect of Topical Antimicrobials

The rationale for application of topical antimicrobials is prophylaxis against and treatment of infection in compromised skin. These preparations allow high local antimicrobial efficacy while avoiding systemic toxicity and are most effective in the earlier stages of healing prior to a solid granulation bed. Our experimental protocol was designed to identified the effect of medication, particularly 1.0 % silver sulfadiazine, and not designed to maximize the

antimicrobial effects of the individual preparations. Which, the proposed application frequency for burn wounds is twelve hours (Kaye 2000).

Importantly, a negative effect from either 1.0% silver-sulfadiazine or 10% povidone-iodine medication was absent. We believe the use of either is advisable in the clinical situation where wounding is less controlled. However, prolonged application of a bandage or either antimicrobial tested under bandage was associated with exuberant granulation tissue formation. Therefore, if a bandage were not necessary for wound protection or mechanical stabilization based on location, the topical use of the silver sulfadiazine slow-release matrix could provide antimicrobial protection.

4.2 Effect of Exuberant Granulation Tissue

Recurrent exuberant granulation tissue production specific to the bandaged groups has been reported (Fretz et al. 1983, Howard et al. 1993, Theoret et al. 2001). The reason for the exuberant granulation tissue formation was not the design of this study and therefore was not determined. It is proposed that treatments that decrease the ambient oxygen tension stimulate angiogenesis through a variety of cytokines and growth factors promoting fibroplasia leading to exuberant granulation tissue (Howard et al. 1993, Knighton et al. 1981, Theoret et al. 2001). This may have occurred in our study when the three preparations are placed under bandage simulating a semi-occlusive type dressing. A second observation from this study is the accumulation of mild fibrinous exudate under these bandages reflective of persistent inflammation. This proteinaceous covering over developing granulation tissue remains intimately associated with contain profibrotic mediators encouraging unimpeded growth. This is a direct result of bandaging, as the unbandaged groups would desiccate into a dry eschar. To best assess

the effect of the antimicrobial treatments, this study design implemented unbandaged SDX-NB and C-NB groups.

4.3 Effect of Bandaging

The bandages were applied for uniform delivery of the treatments without adhering to the wound sites and providing protection from environmental contamination or secondary trauma. The bandages did confine the fibrinous exudate to the immediate area of the wound. That effect appears significantly related to the production of exuberant granulation tissue formation. All bandaged wounds produced exuberant granulation tissue.

The protocol in this study was patterned from previous reports to closely mimics typical clinical management of granulating wounds (Howard et al. 1993, Blackford & Blackford 1995) Subsequently, the every third day bandage change could have permitted enough exudate accumulation to affect the environment and facilitate exuberant granulation tissue formation (Fretz et al. 1983, Howard et al. 1993). Further studies designed with similar and varying bandage change protocols could facilitate understanding the complex intra-cellular communication differences in wound healing of similar wounds. In comparison with unbandaged wounds, once the granulation bed was established in an unbandaged wound, superficial contamination appears to play a minimal role in the course of healing. The absence of overt infection characterized by reddened granulation tissue and noticeable purulent exudate is supportive. The conclusion of the effect of bandaging is that the wound healing components of contraction and epithelialization would be impeded by the continual production of exuberent granulation tissue. Any medication requiring bandaging will need to consider theses effects before claiming efficacy.

4.4 Clinical Application and Further Research

Further studies should continue to focus on the efficacy of other antimicrobial preparations for equine distal limb wound healing. The use of silver containing dressings continue to promise antibacterial properties and a silver-chloride nylon wound dressings may provide antibacterial activity without the deleterious effects of bandaging previously outlined (Adams et al. 1999).

From the results of this study we determined the application of either silver sulfadiazine or povidone-iodine does not improve the healing compared to controls; that may not hold true in other uncontrolled wounds that are contaminated. In infected wounds, the silver sulfadiazine slow-release paste may offer convenient antimicrobial action without bandaging.

The lack of benefit from bandaging demonstrated herein cannot be universally applied to naturally-occurring equine wounds. Bandages are necessary to protect the underlying tissue until granulation tissue formation provides an opportunity to determine the best course of further treatment.

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Figure 1. Mean area of each treatment group versus time. The insignificant differences in area during the first two weeks after wounding, the preparatory phase, were followed by a uniform contraction phase.

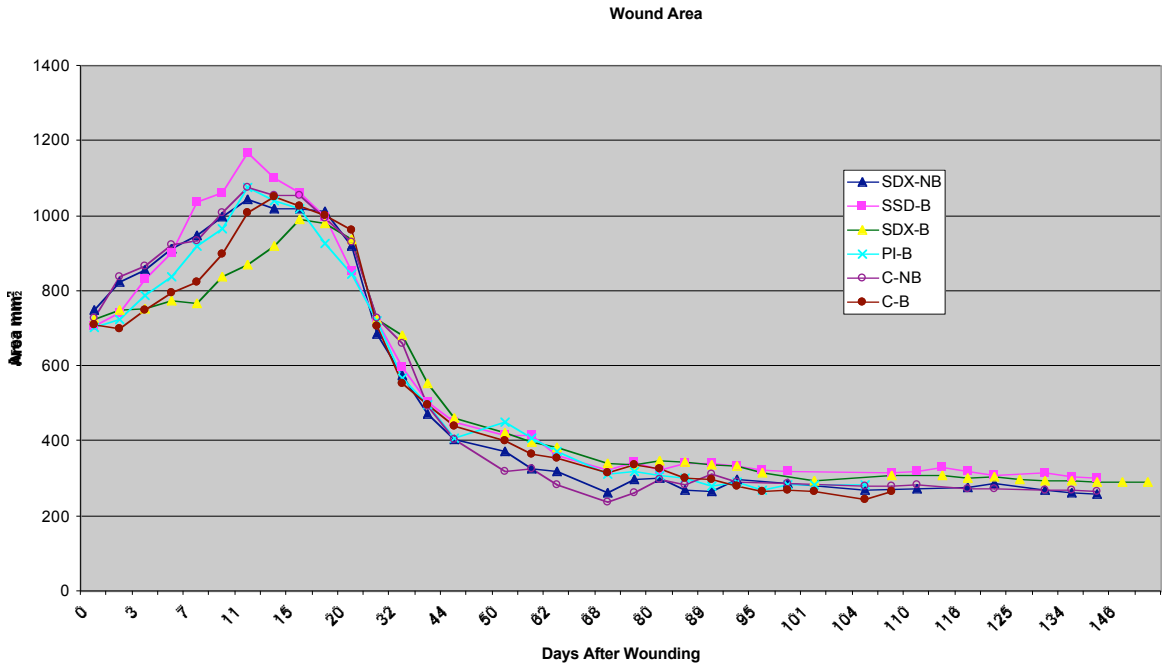

Figure 2. Wound tracings outlined the margin of granulation tissue from the epithelium to measure granulation tissue area. The total wound area was obtained from tracing the haired margin adjacent to the epithelium.

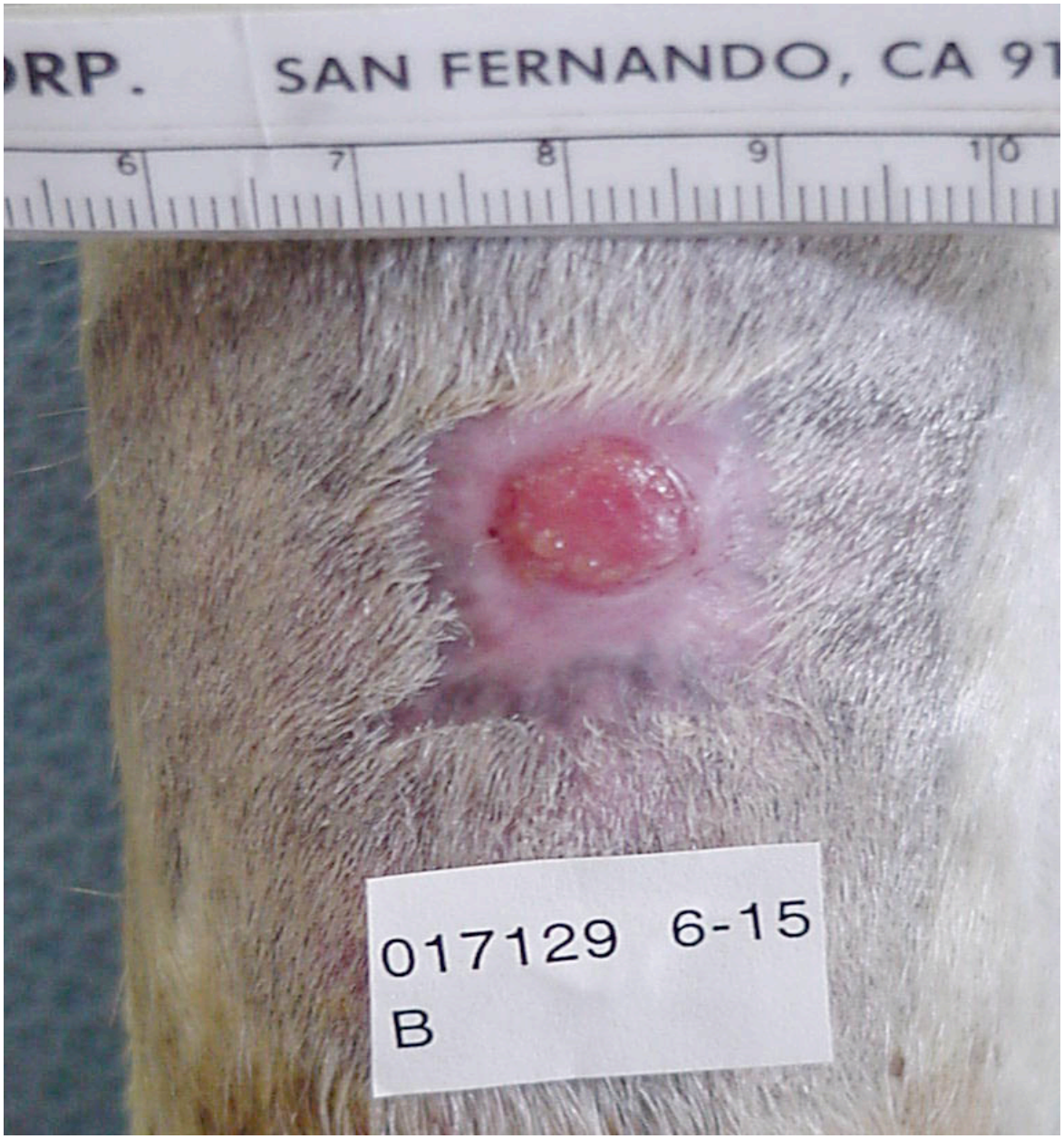

Table 2. Mean (± SD)values for wound healing in the six treatment groups

Wound healing variable	Treatment Groups					
	SDX-NB	**SSD-B**	**SDX-B**	**PI-B**	**C-NB**	**C-B**
# days until healing	95 ± 25	101 ± 32	96 ± 35	83 ± 18	95 ± 31	83 ± 23
Initial size (cm^2)	7.51 ± 0.96	7.39 ± 0.80	7.23 ± 0.96	7.04 ± 0.47	7.27 ± 0.61	7.11 ± 0.65
Maximum size attained (cm^2)	11.37 ± 1.21	11.80 ± 2.56	11.83 ± 2.56	10.42 ± 1.86	11.34 ± 1.40	10.88 ± 2.27
Final size (cm^2)	2.87 ± 0.65	2.86 ± 0.53	2.74 ± 0.44	2.80 ± 0.80	2.63 ± 0.74	2.66 ± 0.67
Parameter (mm)	5.5 ± 1.4	5.3 ± 1.4	5.2 ± 1.3	5.0 ± 1.6	5.7 ± 1.4	5.2 ± 1.4
Percent contraction	74.7 ± 6.1	74.0 ± 5.3	73.7 ± 3.9	75.3 ± 8.2	76.3 ± 6.9	75.2 ± 5.2
Number of granulation tissue excisions	0	5.5 ± 2.9	4.0 ± 1.9	4.8 ± 2.8	0	4.5 ± 2.1
Rate of epithelialization (mm^2/day)	4.21 ± 0.5	3.48 ± 0.5	3.21 ± 0.5	2.96 ± 0.5	3.06 ± 0.5	4.22 ± 0.50
Rate constant for contraction (log area)	-0.0271	-0.0266	-0.0235	-0.0228	-0.0305	-0.0274

6.0 Curriculum Vitae

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I. Education:

9/91-6/95 Auburn University. Doctor of Veterinary Medicine.

8/88-6/91 University of Kentucky. Animal Science major

II. Professional Experience:

7/01-present Clinical Instructor in Large Animal Surgery/Emergency Critical Care. Virginia-Maryland Regional College of Veterinary Medicine. Blacksburg, Virginia.

7/98-6/01 Resident in Equine Surgery. Marion duPont Scott Equine Medical Center, Virginia-Maryland Regional College of Veterinary Medicine. Leesburg, Virginia.

7/97-6/98 Clinical Fellowship in Large Animal Medicine and Surgery. Oregon State University College of Veterinary Medicine. Corvallis, Oregon.

10/96-6/97 Resident Veterinarian Castleton Farm. Lexington, Kentucky.
Associate of Dr. H. Steve Conboy. Responsible for over 230 Standardbred broodmares and their offspring, stallion collection and processing of semen and elective minor surgical procedures.

7/95-9/96 Resident Veterinarian Walmac International. Lexington, Kentucky.
Resident veterinarian for a large Thoroughbred breeding farm. Responsible for broodmare, stallions and offspring overall herd health program. Duties involved; foaling oversight, broodmare obstetrics, sales preparation, and maintenance of 20 two-year-olds in training.

III. Veterinary Related Responsibilities:

10/00	Paddock Official, Morven Park Steeplechase, Leesburg, VA.
4/00	Chief Veterinary Official, Fair Hill Advanced Horse Trials at Menfelt, Frederick, MD.
9/99	Chief Veterinary Official, Fair Hill Advanced Horse Trials at Menfelt, Frederick, MD.
4/99	Chief Veterinary Official, Fair Hill Advanced Horse Trials at Menfelt, Frederick, MD.
10/98	Course Official, Morven Park Steeplechase, Leesburg, VA.

IV. Research Funded:

2000 Topical Antimicrobial Effects on Equine Wound Healing
Principal Investigators, D.B. Berry II, K.E. Sullins
Funded July 2000 by Virginia Horse Industry Board $4,330
Completed May 2001.

1997 Age Dependence of Horses for Immunization for Viral Infectious Disease.
Principal Investigator, T. Chambers PhD., Co-investigators include D.B. Berry
Funded April 1997 by A.Q.H.A. Research $12,130. Completed April 1998.

V. Refereed Publications:

Berry II DB, Lyons ET, Drudge H, et al. Observations in Horses on the Effects of Ivermectin Treatment on Strongyle Egg Production and Larval Development. *Journal of the Helminthological. Society of Washington.* 1993;60(1):89-92.

VII. Proceedings:

Holland RE, Conboy HS, Berry II DB et al. Age dependence of foal vaccination for equine influenza. *Proceedings of the Eighth International Conference on Equine Infectious Diseases.* 1998; pg.190.

Conboy HS, Berry II DB, Chambers T. and Holland RB. Failure of Foal Seroconversion Following Equine Influenza Vaccination. *Proceedings of the 43rd Annual Convention of the A.A.E.P.* 1997; pg. 22-23.

VIII. Abstracts:

Berry II DB and Sullins, KE. Topical Antimicrobial and Bandaging Effects on Equine Distal Limb Wound Healing. *Proceeding of the 11th Annual A.C.V.S. Veterinary Symposium.* 2001 (accepted)

IX. Academic Honors:

1995	Lloyds of London Scholarship recipient. Auburn University
1994	Dean's List. Auburn University
1994	East Alabama V.M.A. Leadership Award recipient. Auburn University
1991	Oswald Research and Creativity Program award recipient. University of Kentucky
1988	Wayland Rhodes Scholarship recipient. University of Kentucky

X. Teaching Experience:

A. <u>Professional Curriculum OSUCVM</u>

VM 724 Large Animal Surgery
1998 2 lecture hours

XI. Graduate School Presentations:

2000 Mechanisms Contributing to Chronic Wound Healing. Fall seminar

Collateral Ligament Desmitis of the Equine Tarsus (7 cases). Case series

1999 Peri-operative Analgesia for Equine Orthopedic Surgery. Fall seminar

Equine Wound Contraction and Epithelialization. Spring seminar

Surgical Management of Intra-Uterine Cysts in a Mare. Case report

1998 Equine Wound Physiology. Fall seminar

Thoracic Vertebral Compression Fracture in a Foal. Case report

XII. Affiliations and Professional Associations:

American Veterinary Medical Association
American Association of Equine Practitioners
American College of Veterinary Surgeons
Wound Healing Society

XIII. Continuing Education:

Association for the Study of Internal Fixation. Equine Basic Internal Fixation Course. 1999
American Veterinary Medical Association Annual Convention. 1997
American Association of Equine Practitioners Annual Convention. 1996
Mid-American Veterinary Convention. 1995
Ultrasound Short-Course by Dr. Norman Rantanen. 1995

XIV. State Veterinary License: Kentucky, Maryland