Equine Veterinary Journal

Equine Veterinary Journal ISSN 0425-1644
DOI: 10.1111/evj.13111

Intralesional application of medical grade honey improves healing of surgically treated lacerations in horses

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Summary

Background: Infection and dehiscence of simple lacerations is common in horses, and consistently effective methods of prevention are yet to be found. Honey has been shown to promote wound healing when applied topically; however, intralesional application prior to wound closure has not been reported.
Objectives: To examine whether intralesional application of medical grade honey (MGH) would reduce the incidence of infection and dehiscence following wound closure.
Study design: Prospective, open-label randomised block design clinical study.
Methods: Lacerations, treated by field practitioners, were divided into treatment and control groups using block randomisation. Horses in the treatment group received a single intralesional treatment with l-mesitran gel (MGH). Data were collected at the time of wound closure and at suture removal.
Results: Data from 127 horses were included, 69 MGH-treated and 58 control cases. No adverse effects of the MGH were recorded. MGH-treated horses were more likely to completely heal (P = 0.006, odds ratio [OR] 3.40 95% confidence interval [CI] 1.41–8.20), to have no signs of infection (P = 0.007, OR 3.64, CI 1.42–9.26) and for the veterinarians to report some degree of satisfaction (P = 0.04, OR 2.72, CI 1.05–7.09) compared to control cases. Numbers needed to treat for complete healing was 5.1 (CI 2.8–40).
Main limitations: Clinical studies have inherent flaws compared to wound healing models, because of variability between wounds. There were more horses with limb injuries in the control group, although not statistically significant, this may have biased the results. Clinical satisfaction and signs of infection were subjective evaluations and evaluators were not blinded to the treatment group.
Conclusions: Intralesional application of MGH to lacerations prior to wound closure may be beneficial in preventing infection and dehiscence. Larger, blinded studies focusing on wounds at a specific location with more objective assessment should be pursued.

Keywords: horse; laceration; primary repair; honey; intralesional

Introduction

Dehiscence is a common complication following wound repair in horses with reported dehiscence rates as high as 74% on the lower limb [1]. The main reasons for dehiscence are tension and infection [1]. In horses, wound location influences healing, with distal limb wounds having more complicated healing than wounds at other sites [2].

Studies in human medicine have focused on using local, intralesional antimicrobial treatment, prophylactically to reduce the incidence of surgical site infection [3–5]. In horses, regional limb perfusion (RLP) is a well-established method for delivering high antimicrobial concentrations to the distal limb for preventing and treating orthopaedic and soft tissue infections [6]. In a recent study [7], RLP with amikacin failed to decrease the bioburden in wounds. In the latter study, however, the authors did not measure antimicrobial pharmacokinetics limiting their ability to truly assess RLP efficacy. Applying an antimicrobial into the wound prior to wound closure is simple compared to RLP and suitable to any region of the body. Very little information is available in the veterinary literature regarding the efficacy of local, intralesional antimicrobials applied at the time of wound repair.

Studies have demonstrated that honey has broad antibacterial activity against common equine pathogens including multiple drug-resistant (MDR) strains such as methicillin-resistant *Staphylococcus aureus* (MRSA) [8]. Honey has proven efficacy in both preventing and eradicating biofilm [9,10]. In addition, honey has other wound healing properties, such as an anti-inflammatory effect [11] and providing the wound with the required nutrients for healing [11]. In a contaminated equine wound model, Bischofberger *et al.* [12] demonstrated better angiogenesis in wounds treated with honey compared with control wounds, and honey significantly reduced healing time [13].

Raw honey, however, may contain bacterial spores, mainly those of *Bacillus* spp. and *Clostridium* spp. *Clostridium botulinum* is known to be present in honey and may cause wound botulism or gangrene; therefore, medical grade honey (MGH) is sterilised using gamma irradiation to eradicate any bacteria or bacterial spores present in raw honey [14].

We elected to investigate whether application of MGH within the wound prior to closure, would reduce the incidence of wound dehiscence. Our hypothesis was that incorporating MGH into primary closure of lacerations in horses will have a noticeable, positive effect on wound healing.

Materials and methods

Study design

This was a prospective, open-label block randomised controlled clinical study. Horses were divided into control or treatment groups by using block randomisation. Each participating field service practitioner received six envelopes, consecutively marked, so that each envelope provided the random assignment to either control or treatment group. Therefore, there were three treatment and three control horses for each block of six cases. Each envelope contained two questionnaires (Supplementary Item 1): one to be filled out at the time of primary wound repair and the other at the time of suture removal. Practitioners were instructed to open the envelope consecutively by number order, after they had arrived at the location of the horse. Horses were enrolled only if the practitioners decided that they were going to repair the wound using primary closure and providing that the owner consented to study participation. Neonatal foals (under 1 month of age) and horses with any major systemic illness, penetrating wounds requiring hospitalisation and eyelid lacerations were excluded. Practitioners were asked to repair the wounds similarly in horses from both control and treatment groups (Supplementary Item 2), with the addition of applying sterile MGH (L-Mesitran gel)[a] prior to skin closure in the treatment group. There were two techniques for gel application: 1) directly to the subcutaneous tissue along the length of the laceration prior to any wound closure (Fig 1a) or 2) by leaving the proximal and distal aspect of the wound open, and applying the gel through the wound opening, using a sterile 5 mL syringe to completely fill the subcutaneous space until gel was seen exiting at the other end of the sutured wound. Gel that exited the wound was removed using a sterile gauze pad followed by completing the wound closure (Fig 1b).

Data collection

Data were collected between August 2015 and February 2017, from the participating practitioners by questionnaires, supplemented by phone conversations, and included information about the horse, wound, treatment and results of the repair (Supplementary Item 1). Evaluators were not blinded to the treatment group. The result of the repair was evaluated in three categories each with three optional answers: 1) Wound healing (complete, partial and failure), 2) Signs of infection (obvious, suspected and no signs) and 3) Level of satisfaction of veterinarian from the results, considering the details of each case (full, partial and none at all). At least two pictures were submitted for each case, taken from each veterinary visit (at laceration repair and suture removal).

Data analysis

Continuous variables (age and length of wound), were evaluated by Shapiro–Wilk test of normality. Since they were not normally distributed, they were converted into categories. Age was divided into categories based on clinical age groups (birth to yearlings, yearlings to adults <5 years old), young adults (5–15 years old) and geriatrics (15 years old and older). Length of wound categories was based on quartiles: minimum to lower quartile (<4 cm), lower quartile to median (4–5.5 cm), median to upper quartile (5.6–10 cm) and upper quartile to maximum (>10 cm). Characteristics of the cases were described as frequencies and proportions. Univariable assessment of associations between parameters and three outcomes (each with three levels of severity) were performed by Chi-square (or Fisher's Exact Test if any expected cell counts were less than 5) as well as for linear trend (Mantel Haenszel Chi-square test for trend) where appropriate. Outcomes were reclassified dichotomously in order to increase the power, for logistic regression, and to calculate the Numbers Needed to Treat (NNT), thus: 1) Complete healing vs. partial healing/failure to heal, 2) No signs of infection vs. suspected or obvious infection, 3) Some degree of satisfaction vs. dissatisfaction of the attending veterinarian. Bivariable logistic regression was used to identify significant interactions. For each of the three outcomes, each of the characteristics of the horses was evaluated together with the treatment group and the interaction term between them. If the interaction term remained significant, that variable, as well as the interaction term was included in the multivariable model. Potential confounding variables were identified by significant association with each outcome as well as by differential distribution of the variable between the two treatment groups. Potential confounding variables with $P<0.1$ were considered for inclusion in the multivariable models. Three multivariable models were performed by backward stepwise (Wald) elimination approach, one for each outcome. They included variables significant on univariable analysis, variables with significant interactions identified by bivariable analysis, as well as potential confounding variables. The multivariable models were evaluated by improvement in percentage correct in classification table comparing block 0 to the final block, Omnibus Test of <0.05, Hosmer and Lemeshow test of >0.05 and by the R squares. All analyses described above were performed using IBM SPSS 24. The NNTs were calculated as the inverse of the rate differences of the outcome for each of the three dichotomous outcome groups in the overall data (Table 2). These values were not adjusted for tissue damage, other parameters or interactions. The 95% confidence intervals of the raw data were calculated by Wilson's Score Method (WinPEPI version 11.15, July 2011, Compare2 A). Other than for inclusion in the multivariable models, significance was set at $P<0.05$.

Fig 1: Methods for intrawound application of medical grade honey in closure of equine lacerations: directly onto the subcutaneous tissue prior to skin closure a) and after partial wound closure b).

Results

Study population

A total of 135 cases, treated by 11 equine practitioners were included. Information at the time of suture removal was available for 127 cases. Eight cases were lost to follow-up (Supplementary Item 3). Of 127 cases, 69 were in the MGH-treated group and 58 were in the control group. No significant differences were found when epidemiological and clinical findings were compared between MGH-treated and control groups (Supplementary Table 1). The ages ranged from 4 months to 23 years (median 5 years, interquartile range 2.5, 10 years). More than half the study population were mares (74, 58.3%), 28 were geldings (22%) and 25 were stallions (19.8%). The most common breeds were Arabian (33, 26%) and 26 were local Arabian crosses (20.5%), followed by Quarter Horse (28, 21.3%). Other breeds included Tennessee Walking Horse (8), German/Belgian Warmbloods (7), Ponies and Pony crosses (4), Single-Footing horse (4), Appaloosa (3), Missouri Fox Trotter (2), Appendix (2), Thoroughbred (2), Rocky Mountain (1), Azteca (1), Paint (1), Friesian (1) and Miniature Horse (1). Three horses were of unknown breed. Time from injury to treatment did not exceed 6 h for 100 (78.7%) of the wounds. Wound length ranged from 2 to 37 cm (median 5.5 cm). The distribution of the study population according to wound location is illustrated in Supplementary Item 4. There was no significant difference between the MGH-treated and control groups in regards to wound characteristics (Table 1). Prior to MGH application, all wounds were cleansed thoroughly using either diluted chlorhexidine (114, 89.8%) or diluted povidone iodine (4, 3.1%) followed by balanced sterile electrolyte solution (113, 89%) and debrided as needed. Systemic antibiotics were administered to 118 horses (93%), most commonly penicillin alone (74, 58.3%) or in combination (15, 11.8%) with either streptomycin (7, 5.5%), enrofloxacin (4), marbofloxacin (2), sulfamethoprim (1) or gentamicin (1). Other antibiotic used includes: sulfamethoprim (14, 11%), cefquinome (9, 7.1%) and enrofloxacin (6, 4.7%). Most horses (115, 90.6%) received a nonsteroidal anti-inflammatory drug (NSAID) when the wounds were sutured (either flunixin meglumine or phenylbutazone). Owners were asked to continue with oral administration of phenylbutazone in 88 horses (69.3%). When possible, limb wounds were bandaged (36/73, 49.3%). In 37 wounds (30.3%), a distal opening was left for drainage and in 10 wounds (8.2%) a rubber tube was placed for drainage. There was no significant difference between the MGH-treated and control groups with regards to antimicrobial drug use, NSAID treatment, bandaging and drainage.

No adverse effects of the MGH were recorded in any of the horses participating in the study. Overall complete healing in this study was 53/127 (41.7%) with a 95% confidence interval (31.1–53.1%). Results for MGH-treated and control cases for each of the outcome variables are shown in Table 1. Results of univariable analysis on the association between epidemiological and clinical variables on all three outcomes (healing, infection and satisfaction) are provided in Supplementary Items 5–8 respectively. Variables included in the initial iteration of the multivariable models were age in categories, breed and tissue damage as well as treatment group and interactions between treatment group and age or breed. The final multivariable models for the three outcome variables included treatment group and tissue damage (Tables 2, 3, 4). Treatment with MGH significantly improved healing, prevented infection and resulted in a more satisfactory outcome compared to controls whereas tissue injury significantly decreased healing and increased infection.

Discussion

In the current study, intralesional application of MGH significantly improved healing with more wounds reaching complete healing, less signs of infection, and higher practitioner satisfaction in the MGH-treated compared to control horses. Despite the wide variation in clinical cases, the treatment effect remained significant in the multivariable model. The overall rate of wound healing in control horses in our study was 31% (CI 21–44%) which is similar to that reported by Wilmink *et al.* [1] with 21% of horses and 37% of ponies achieving complete wound healing.

In the current study, none of the wounds that showed complete healing had any signs of infection. This leads us to postulate that infection played a major role in dehiscence of sutured lacerations in the study population. Signs of infection during healing were more prevalent in the control group compared to the horses treated with intralesional MGH.

It is crucial to prevent infection, thereby removing a major source of failure for laceration healing [15]. Local administration of antibiotic substances has several advantages over systemic administration; such as decrease in cost and reaching high concentrations in the target location while avoiding the risk of side effects that might occur with systemic antimicrobial administration [16]. Large-scale systematic reviews support the positive effect of intrasurgical application of an antimicrobial for preventing superficial surgical site infection (SSI) [3–5]. In equine surgery, Mair *et al.* demonstrated beneficial results for the administration of an antimicrobial drug into the laparotomy wound prior to closure after colic surgery [17].

TABLE 1: The overall data are presented for the three outcomes of the MGH-treated and the control groups and the numbers needed to treat (NNT) to achieve complete healing, no infection or some degree of satisfaction due to treatment. [Data are shown as number of cases with the outcome/number of cases in the group, percentage of cases with the outcome and 95% confidence intervals of the percentage]

Complete healing		No signs of infection		Degree of dissatisfaction	
MGH-treated	Control	MGH-treated	Control	MGH-treated	Control
35/69 (50.7%)[a] 39.2–63.2	18/58 (31%)[b] 20.6–43.8	57/69 (82.6%)[a] 72.0–89.8	37/57 (64.9%)[b] 51.9–76.0	10/68 (14.7%)[a] 8.2–25.0	19/58 (32.8%)[b] 22.1–45.6
NNT: 5.1 (2.8–40)		NNT: 5.6 (3.1–41.7)		NNT Treat: 5.5 (3.1–31.3)	

Results marked ‘a’ differ from results marked ‘b’ at P<0.05 in univariable analysis.

TABLE 2: Multivariable model for complete healing

Variable	Category	Numbers in group	Odds ratio	95% confidence interval	P-value
Treatment group	Control	57	Ref.	N/A	
	Treatment	66	3.40	1.41–8.20	0.006
Tissue damage	Mild	63	Ref.	Overall P<0.001[1]	
	Moderate	37	0.20	0.08–0.54	0.001
	Severe	23	0.10	0.03–0.36	0.006

[1]Overall P-value for parameter with more than two categories.

TABLE 3: Multivariable model for healing without infection

Variable	Category	Numbers in group	Odds ratio	95% confidence interval	P-value
Treatment group	Control	57	Ref.	N/A	
	Treatment	66	3.64	1.42–9.26	0.007
Tissue damage	Mild	63	Ref.	Overall P = 0.011[1]	
	Moderate	37	0.43	0.16–1.17	0.099
	Severe	23	0.17	0.05–0.55	0.003

[1]Overall P-value for parameter with more than two categories.

TABLE 4: Multivariable model for the outcome of some degree of satisfaction.

Variable	Category	Numbers in group	Odds ratio	95% confidence interval	P-value
Treatment group	Control	57	Ref.	N/A	
	Treatment	66	2.72	1.05–7.09	0.040
Tissue damage	Mild	63	Ref.	Overall P = 0.125[1]	
	Moderate	37	0.51	0.18–1.41	0.195
	Severe	23	0.29	0.08–1.00	0.051

[1]Overall P-value for parameter with more than two categories.

Despite potential benefit of local antimicrobial drug administration, MDR bacteria are a major, rapidly growing problem, in human and veterinary medicine. Thus, as there is an ethical concern regarding wide spread prophylactic use of antimicrobials, and its contribution to the development of bacterial resistance, use of an alternative treatment, such as honey, is preferred [18]. In contrast to antibiotics, sublethal concentrations of honey, did not produce permanent resistance when tested with cultures of *S. aureus* and *Pseudomonas aeruginosa* [19]. The reason might be that while antibiotics usually target a particular process, causing a specific evolutionary pressure for the emergence of resistance genes, honey contains various compounds acting synergistically by different routes [10].

Honey has three antibacterial mechanisms, it is an osmotically active substance (approximately 80% sugar), with low pH (generally 3.2–4.5) and contains the enzyme glucose oxidase. Glucose oxidase transforms sugars into a well-known antiseptic, hydrogen peroxide [10,14]. As an antiseptic, hydrogen peroxide can also cause damage to tissue, such as cartilage [20]. It is worth investigating whether these negative traits also play a role when honey is placed in a wound, in conjunction with all of the positive effects of honey on wound healing.

In recent years, additional specific antibacterial substances were identified in honeys of various sources. Two of the molecules discovered recently are methylglyoxal (MGO) from Manuka honey and Bee Defencin-1 from Revamil honey. The first is of plant origin and the latter of bee origin [14]. Honey is also known to contain polyphenolic compounds and flavonoids that have an antibacterial effect [10].

Previous data indicate that honey has an antimicrobial action against common pathogens isolated from equine wounds, and that it has beneficial action in wound healing in horses [8,13,21]. Carnwarth *et al.* compared the efficacy of 11 different honey products on 10 strains of bacteria recovered from wounds of horses, including MRSA and *P. aeruginosa* and found that eight products were effective against all bacterial isolates [8]. A recent study in horses, showed that bacterial bee symbionts that reside in honey (LAB- Lactic Acid Bacteria) produce specific formulations that proved to have effective antibacterial properties both in vitro and in vivo [22].

In models of contaminated and noncontaminated equine wounds allowed to heal by second intention, Manuka honey/Manuka honey gel-treated wounds healed faster than control wounds [13,23]. Clinical studies, investigating naturally occurring lacerations have many inherent weaknesses. The main disadvantage is that each wound is unique and there are many variables affecting each wound necessitating a much larger study population compared to wound model studies to address the variability between animals and wounds. On the other hand, wounds created in the same location, by a surgical blade are not an accurate representation of the clinical scenario. In our study, differences in the characteristics of the horses or the wounds, although not significantly different between groups, may have skewed the results (e.g. slight differences between groups in duration of injury, wound location and age), The most significant of these differences were addressed with the multivariable model, however, it is not possible to put all of the potential confounding variables in the model due to the small sample size [24].

When designing the study, we were aware of the impact of wound variability and asked the participating practitioners about their satisfaction from the results of the repair. This allowed each clinician to take into consideration many factors that might influence the results of the repair. Clinician satisfaction in our study supported the beneficial effect of MGH treatment, yet the results should be interpreted with caution. Clinician satisfaction is a subjective assessment and combined with a nonblinded design, it may have biased the results.

In addition to the above limitations of the study, considering the increasing emphasis in the veterinary world regarding antimicrobial stewardship, it was an unfortunate consequence of the design of this study, that some of the antimicrobials used, are not recommended for use as a first line of defence. However, due to the nature of this study, as a field study involving experienced practitioners, we were limited to accepting their choice of antimicrobials, to obtain clinician cooperation.

To conclude, intralesional application of MGH in repaired lacerations of horses is a simple, easy and innovative method that markedly improved the descriptive observations; in the healing of lacerations in horses in the current nonblinded study. However, this conclusion must be tempered by the lack of blinding in this study and subjective outcome variables which are likely to have introduced an unquantifiable level of bias. Hence, we suggest further research. Both a multicentre study and a wound model study should be undertaken to further evaluate this novel application of MGH both in traumatic and in elective surgical wounds. Such studies should be careful to avoid the bias that was inherent in this preliminary study.

Authors' declaration of interests

No competing interests have been declared.

Ethical animal research

The study received approval from the University Hospital Internal ethical review committee. Approval number: KSVM-VTH/02_2017.

Owner informed consent

Owners gave consent for their animals' inclusion in the study.

Source of funding

The L-Mesitran soft syringes were generously provided for the study by Triticum, Maastricht, the Netherlands.

Acknowledgements

The authors would like to express their gratitude to all the equine practitioners that participated in the study and communicated their results. The product containing medical grade honey, l-mesitran®, was donated by the manufacturer Triticum, Maastricht, Netherlands. Presented in part at the European College of Veterinary Surgeons, Annual Scientific Meeting, Edinburgh, Scotland, 2017 and at the American College of Veterinary Surgeons, Annual Scientific Meeting, Indianapolis, IN, United States, 2017.

Authorship

H. Mendal and G. Kelmer contributed to all stages of manuscript preparation. G. Sutton contributed to study design, data analysis and interpretation. E. Abu contributed to study execution, data analysis and interpretation and final approval of the manuscript.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Supplementary Item 1: Questionnaire.
Supplementary Item 2: Clinician instructions.
Supplementary Item 3: Recruitment and follow-up.
Supplementary Item 4: Repaired lacerations by anatomical regions.
Supplementary Item 5: Population characteristics: Univariable analysis.
Supplementary Item 6: Associations with signs of infection: Univariable analysis.
Supplementary Item 7: Associations with recovery: Univariable analysis.
Supplementary Item 8: Associations with veterinary satisfaction: Univariable analysis.