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Review Article

A review of research into second intention equine wound healing using manuka honey: Current recommendations and future applications

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Summary

In addition to the generic properties of honey, manuka honey has a nonperoxide antimicrobial activity largely attributed to methylglyoxal. Commercially, manuka honey is graded against a standard antiseptic, phenol, to provide a measure of antimicrobial activity referred to as the unique manuka factor (UMF). The higher the UMF, the greater the antimicrobial activity. However, more recently, there is evidence that manuka honey can also modulate the initial inflammatory response through activation of toll-like receptor 4 on monocytes to enhance production of cytokines important in tissue repair and regeneration. Recent studies investigating the effects of manuka honey on second intention healing of lower limb wounds in horses have shown that wounds treated with UMF 20 manuka honey retracted less and healed faster than untreated wounds. Using this wound healing model, the primary effects of manuka honey appeared to be associated with the modulation of the initial inflammatory reaction rather than its antimicrobial effects. Based on the current knowledge, treatment with manuka honey should be instituted as soon as possible after injury. Where bacterial contamination is substantial, manuka honey with a UMF ≥15 should be used. While bandages will improve the contact between the honey and the wound and may be indicated in the early stages of wound healing, prolonged bandaging may lead to the production of excessive granulation tissue. If topical treatment without a bandage is to be used, more honey is not necessarily better. Using a thin film combined with regular application, contact times may be optimised. Application 2–3 times daily to open wounds may improve efficacy. Manuka honey should be applied for at least 21 days after wounding but there may be beneficial effects if it is applied until wound healing is almost complete.

Introduction

The practice of using honey as a topical treatment for open wounds dates back thousands of years (Majno 1975). The ancient Egyptians combined wild honey with animal fats and lint to promote second intention wound healing (Majno 1975). However, the application of honey to open wounds lost favour in many countries when modern antimicrobial agents were developed. More recently, the emergence of bacterial species resistant to antimicrobial drugs has initiated a renewed interest in the antimicrobial properties of different varietals of honey, particularly within the medical profession. However, while most of the recent research has been focused on the antimicrobial properties of honey, there is emerging evidence to suggest many honey varietals can modulate the process of wound healing directly (Molan 2006; Lee *et al.* 2011a,b). New bioactive components in honey are being discovered and it would appear that variability in the profile and concentrations of bioactive components in different honey varietals suggest all honeys do not behave equally (Allen *et al.* 1991; Cooper and Jenkins 2009; Kwakman *et al.* 2011; Carnwath *et al.* 2014; Cooper 2014).

The *Leptospermum* species of plants include approximately 83 species found primarily in Australia and New Zealand. Honey derived from the *Leptospermum* species of plants have been found to have superior antimicrobial activity compared with many other honey varietals (Allen *et al.* 1991; Snow and Manley-Harris 2004). In terms of the properties of honey from these plants, the manuka bush (*L. scoparium*) found in New Zealand has been the most extensively studied. Unlike other honey varietals, the commercial production of manuka honey is standardised for antimicrobial activity and is often used in human medicine for the treatment of open wounds. However, there have only been a few studies investigating the potential applications for manuka honey in veterinary medicine and these have focused on second intention healing of equine distal limb wounds (Bischofberger *et al.* 2011, 2013, 2015). The purpose of this review is to provide a summary of the current knowledge on the bioactivity of manuka honey, its potential role and application in second intention wound healing in horses and possible areas for future investigation.

Features of manuka honey

General properties of honey

A high sugar and low water content and a pH generally between 3.2 and 4.5, but may range from 3.2 to 6.1, are common to all honey varietals (Cooper 2014). Acidity is generally associated with gluconic acid and contributes to the antibacterial activity of honey (Molan 1992). Ripened honey consists of 80% sugars, mainly glucose and fructose and <18% water which is tightly bound to the sugars and not available to microorgansims (Kwakman and Zaat 2012). High osmolality creates an osmotic gradient initiating fluid shifts from adjacent tissues and the circulation into the wound (Molan 1999, 2011). An influx of fluid creates a moist environment and supplies nutrients. Together these properties promote autolytic wound debridement and enhance tissue healing, while inhibiting bacterial growth by causing osmotic

stress and shrinking the bacterial cell wall (Molan 1999, 2006). Furthermore, the activity of bacterial proteases are reduced in low pH environments (Gethin 2008). Proteases are responsible for destroying cytokines and growth factors and damage extracellular matrix contributing to nonviable tissue and poor tissue healing (Tarnuzzer and Schultz 1996).

Glucose oxidase is produced by bees and is found in many honey varietals in low concentrations (Cooper 2014). Glucose oxidase is responsible for converting glucose to gluconic acid and thereby releasing hydrogen peroxide. Generally, hydrogen peroxide is not detectable in undiluted honeys but dilution of honey activates glucose oxidase (White *et al.* 1963; Bang *et al.* 2003). Honey diluted to 30–50% optimises the generation of hydrogen peroxide so in the presence of wound exudate the antimicrobial activity of peroxide may contribute signficantly to the antibacterial activity in some honey varietals (Bang *et al.* 2003; Irish *et al.* 2011; Cooper 2014). However, the hydrogen peroxide activity of honey varies substantially and may be related to plant species, environmental conditions and entomological conditions, including the age of the bee, foraging patterns and activity and enzymes secreted (Irish *et al.* 2011). Processing and storage conditions, including heat, light and catalases produced by damaged cells, pollen and some bacteria can inactivate hydrogen peroxide which contributes to variable antibacterial activity of some honey varietals under different conditions (Chelikani *et al.* 2004; Irish *et al.* 2011; Chen *et al.* 2012; Kwakman and Zaat 2012; Brudzynski *et al.* 2013).

Nonperoxide antimicrobial activity of manuka honey

More recently, it has been shown that even in the presence of high concentrations of catalase, honey derived from some plant sources still show remarkable nonperoxide, antimicrobial activity (Molan 2006; Carnwath *et al.* 2014). Honey from the *Leptospermum* species of plants including manuka honey, has been one group of honey varietals found to have superior antimicrobial activity compared to honey dervied from many other plant sources (Mavric *et al.* 2008). Methylglyoxal (MGO) is produced from dihydroxyacetone found in high concentrations in the flower of the manuka bush (Kwakman and Zaat 2012). Methylglyoxal is responsible for most of the the antimicrobial activity of manuka honey (Mavric *et al.* 2008; Adams *et al.* 2009; Atrott *et al.* 2012; Kwakman and Zaat 2012). Recently, it was found that adding MGO to honey varietals known to produce high concentrations of hydrogen peroxide reduced the production of hydrogen peroxide by inhibiting glucose oxidase (Majtan *et al.* 2014). This may explain why some researchers have failed to detect hydrogen peroxide as a bioactive component contributing to the antibacterial activity of manuka honey (Molan 1992; Kwakman *et al.* 2011). More recently, a glycoside, leptosin, has been discovered in manuka honey (Kato *et al.* 2012). Leptosin and perhaps other undiscovered bioactive compounds may contribute to the antimicrobial activity (Kato *et al.* 2012).

While stored, the dihydoxyacetone in manuka honey slowly decreases over time while the concentrations of MGO increase at a similar pace. This nonperoxide activity of manuka honey is used to determine the unique manuka factor (UMF) which is commonly included on the label of commercially available manuka honey. The UMF is a rating that refers to the percentage concentration of a standard antispetic (phenol) with the same antimicrobial activity as the honey when tested in a radial diffusion assay with *Staphylococcus aureus* as the targeted microorganism (Mavric *et al.* 2008; Adams *et al.* 2009; Atrott *et al.* 2012). Batches of manuka honey are individually tested for antimicrobial activity. In general, honey with UMF 0–4 has no appreciable antimicrobial activity, UMF 5–9 has minimal antimicrobial activity and is not recommended for therapeutic use as an antimicrobial, UMF 10–15 is considered useful therapeutically and UMF 16–30 has superior activity with high antimicrobial efficacy (Molan 2001).

The action of MGO has been associated with a combination of enzymatic and nonenzymatic processes that are yet to be fully elucidated (Mavric *et al.* 2008; Adams *et al.* 2009). The antimicrobial effects of MGO are suggested to be related to an ability to interact with the nucleophilic centres of macromolecules such as DNA (Mavric *et al.* 2008; Adams *et al.* 2009). In Gram-positive organisms, MGO leads to the downregulation of autolysin, an enzyme involved in cleavage of bacterial cell wall components and cell division (Jenkins *et al.* 2011a,b). In Gram-negative bacteria, MGO appears to affect gene expression, particularly those genes involved in regulating a protein that contributes to structural stability of the cell wall, resulting in cell lysis (Henriques *et al.* 2011; Jenkins *et al.* 2011a,b; Roberts *et al.* 2011). Furthermore, examination of proteins in methicillin-resistant *Staphylococcal aureus* exposed suggest manuka honey acts to downregulate a protein that limits the ability of the microorganism to withstand exposure to conditions that induce stress (Jenkins *et al.* 2011a,b).

Effects of manuka honey on bacterial biofilms

Bacterial biofilms have been associated with delayed healing and chronic wound infection in horses and man and have been shown to interfere with the antimicrobial activity of many honey varietals (Serralta *et al.* 2001; Freeman *et al.* 2009; Merckoll *et al.* 2009). Manuka honey has been shown to prevent biofilm formation and disrupt established biofilms (Jervis-Bardy *et al.* 2011a,b; Maddocks *et al.* 2012). While the exact mechanism of action requires further investigation, it would appear that *in vitro*, MGO can downregulate genes coding for surface-binding proteins which are important in biofilm formation (Jervis-Bardy *et al.* 2011a,b; Maddocks *et al.* 2012). *In vitro* manuka honey has been shown to depress the activity of a global regulator in *Eschericia coli* that has widespread knock-on effects on the expression of genes controlling virulence, cell to cell communication and biofilm formation (Jenkins *et al.* 2014). Disrupting cellular communications may prevent biofilm maturation by affecting virulence genes when microorgansims are exposed to low concentrations of manuka honey and at high concentrations manuka honey may act to inhibit bacterial growth (Lee *et al.* 2011a,b; Wang *et al.* 2012; Kronda *et al.* 2013). Higher concentrations of manuka honey are required to penetrate and disrupt established biofilms (Alandejani *et al.* 2009; Merckoll *et al.* 2009; Cooper *et al.* 2011, 2014; Maddocks *et al.* 2012). It would appear MGO plays a critical role but is not exclusively responsible for the disruption of biofilms (Jervis-Bardy *et al.* 2011a,b; Kilty *et al.* 2011).

Effects of manuka honey on modulating second intention healing

While most of the interest in manuka honey has focused on its antimicrobial effects there is evidence that manuka honey

can modulate the initial inflammatory response by enhancing the production of cytokines that regulate fibroblast production and angiogenesis (Molan 2006; Tonks *et al.* 2007). A 5.8 kDa active component of manuka honey has been found to be responsible for activating toll-like receptor 4 on monocytes (Tonks *et al.* 2007). Activation of toll-like receptor 4 enhances the production of IL- 1β, IL-6 and TNF-α from monocytes which are important in tissue repair and regeneration (Tonks *et al.* 2001, 2003, 2007).

Studies investigating topical UMF 20 manuka honey on wound healing in the horse

Study 1: effects on early second intention wound healing in horses

Recently, a series of studies investigating the effects of topical UMF 20 manuka honey on second intention healing of lower limb wounds in the horse have been reported (Bischofberger *et al.* 2011, 2013, 2015). These studies were conducted using a contaminated, surgical wound healing model. An initial pilot study created a 2.5 × 2.5 cm full thickness wound on the metacarpus of both front legs of 8 Standardbred geldings aged between 5 and 10 years (Bischofberger *et al.* 2011). The horses had no evidence of previous injuries on the lower legs and were kept in small grass yards. Wounds were bandaged for 12 days and the bandages changed daily. One wound on each horse was treated with UMF 20 manuka honey while the other was left untreated. On Day 13, treatment was stopped and wounds were left open to heal. Wound area was measured weekly for 8 weeks and overall time to healing was recorded. Wounds treated with UMF 20 manuka honey for 12 days retracted less than untreated wounds and remained smaller than untreated wounds until Day 42. There was no difference in overall healing time between treated and untreated wounds. The authors reported that treated wounds appeared to develop a healthier bed of granulation tissue earlier than untreated wounds.

Study 2: the effects of a 66% manuka honey gel and duration of treatment on second intention would healing in horses

Manuka honey assumes a liquid form at room temperature so application under a bandage enhances the duration of contact between the wound and the honey when treating lower leg wounds in horses. However, bandages have been shown to promote dysregulated healing and the production of excess granulation tissue if applied to wounds for prolonged periods of time (Berry and Sullins 2003; Dart *et al.* 2009). Experimentally, it would appear that if bandages are removed before Day 12 after wound creation, early visible signs of dysregulated healing generally resolve and healing progresses normally (Dart *et al.* 2009). To address the liquid consistency of manuka honey, a gel mixture consisting of 66% UMF 20 manuka honey and 34% water-based, pH neutral gel was developed (Bischofberger *et al.* 2013). The consistency of this gel allowed application of the gel to leg wounds without the need for a bandage. A second study compared the efficacy of commercially available UMF 20 manuka honey to the 66% UMF 20 manuka honey gel when applied to wounds daily under a bandage for 12 days (short-term). This study also evaluated the effect of daily application of 66% UMF 20 manuka honey gel when applied to wounds throughout healing (long-term) compared to untreated control wounds (Bischofberger *et al.* 2013). Ten Standardbred geldings without evidence of previous injury on their distal limbs were used. Horses were kept in small grass yards. Five, full thickness wounds (2 × 2 cm) were created surgically on both metacarpi. Wounds were assigned to five different treatment groups; (i) UMF 20 manuka honey, (ii) 66% manuka honey gel applied for 12 days, (iii) gel alone applied for 12 days, (iv) manuka honey gel applied throughout healing and (v) untreated control. For the first 12 days bandages were changed daily and wounds treated as assigned. On Day 13 bandages were removed and wounds left open to heal. Treatment with honey gel was continued daily on one wound on each limb until the wounds were completely healed. Wound area was measured on Day 1 then weekly until Day 42. The time to complete healing for all wounds was recorded.

There were differences in the wound area between treatment groups on Days 7, 14, 21, 28 and 35. During the first 7 days after wound creation, wounds treated with manuka honey and those treated with 66% manuka honey gel retracted less than control wounds and wounds treated with the gel alone. The area of the wounds treated with manuka honey for 12 days, manuka honey gel for 12 days and manuka honey gel throughout healing were significantly smaller than control wounds and wounds treated with gel alone between Days 7 and 35. By Day 42 the difference in wound area between groups was no longer significant. The area of the wounds treated with gel alone and control wounds were not different at any time point during healing. Wounds treated with 66% manuka honey gel throughout healing healed faster than all other wounds. Wounds treated with manuka honey and 66% manuka honey gel for 12 days healed faster than control wounds and wounds treated with gel alone.

The results of these studies support a beneficial effect of manuka honey on second intention healing of distal limb wounds in horses. Using the surgical model, the principal therapeutic effect of manuka honey appeared to be in the early stages of wound healing and topical treatment should be initiated early to achieve the optimal results in clinical patients. The advantage of 66% manuka honey gel is that it can be applied topically without a bandage while maintaining good wound contact. This preparation lends itself to application throughout the entire healing process; however, currently it is not commercially available. Although, statistically, daily application did improve the overall healing time in this study, the overall improvement in healing time was not clinically relevant. Nonetheless, in naturally occurring wounds which are often more traumatic, substantial and contaminated, the effect of manuka honey on overall time to healing may be more apparent.

Study 3: manuka honey and wound healing in horses: growth factors, bacterial counts and histomorphology

The growth factors TGF-β1 and TGF-β3 are known to play a pivotal role in orchestrating the process of wound healing in horses and an imbalance in expression of the TGF isoforms have been implicated in the production of excessive granulation tissue in distal limb wound in horses (Shah *et al.* 1995; Theoret *et al.* 2001; Van den Boom *et al.* 2002; Grose and Werner 2003; Wilmink *et al.* 2003; De Martin and Theoret 2004; Theoret and Wilmink 2008). A third study was performed

using 10 Standardbred geldings without evidence of previous injuries on their distal limbs. The study undertook to investigate the effect of 66% manuka honey gel on the concentrations of transforming growth factor TGF-β1 and TGF-β3, bacterial counts and wound histomorphology during the first 10 days of healing of contaminated equine, distal limb wounds (Bischofberger *et al.* 2015). Wounds were created on both metacarpi of each horse and were assigned to 3 groups; (i) wounds contaminated with faeces for 24 h and treated topically with manuka honey gel daily, (ii) untreated, noncontaminated control wounds and (iii) untreated control wounds contaminated with faeces for 24 h. All wounds were bandaged for the first 24 h and horses were housed in grass yards. In 5 horses wounds were bandaged for the remainder of the study while the wounds in the remaining 5 horses were left to heal without a bandage. Wound biopsies were taken from different wounds on Days 1, 2, 7 and 10 to evaluate the effects of manuka honey gel, wound contamination and bandaging on TGF-β1 and TGF-β3 concentrations, aerobic and anaerobic bacterial counts and histomorphology of the wound bed.

The study found manuka honey gel had no effect on TGF-β1 and TGF-β3 concentrations within the wounds. Using this model of wound healing there was a transient increase in the aerobic and anaerobic bacterial counts for up to 48 h in contaminated wounds compared to noncontaminated wounds; however, there was no evidence of an effect of manuka honey gel on wound bacterial counts. Histologically, manuka honey gel decreased wound inflammation (Days 7, 10), increased angiogenesis (Days 2, 7, 10), increased fibrosis and collagen organisation (Day 7) and increased epithelial hyperplasia (Days 7, 10). Using this model of wound healing, daily treatment with manuka honey gel resulted in a more organised granulation tissue bed early in wound repair. The authors of this study proposed that the efficacy of myofibroblasts and fibroblasts may be enhanced in a more mature wound environment thereby reducing wound retraction.

There were potential limitations of the wound healing model used in this study. It is likely that the degree of wound contamination achieved is not representative of many naturally occurring wounds that are associated with significant tissue avulsion and may be heavily contaminated with microorganisms. It is therefore possible that the reported antibacterial effects of manuka honey were not detected. The beneficial effects of manuka honey on wound healing identified in this study appeared to be mediated through the effects of the honey on the early inflammatory response rather than its antibacterial properties. However, it is also worth noting biopsies for culture were taken immediately prior to application of the honey on each collection day. It is also possible that to achieve the optimal antibacterial effect of manuka honey on wound healing, daily application may not be sufficient to maintain low bacterial counts for 24 h and more frequent application is required, particularly in open wounds.

Manuka honey as a wound dressing in second intention healing of equine distal limb wounds

Based on the current knowledge, some recommendations can be made for using manuka honey as a wound dressing for distal limb wounds healing by second intention in horses (**Table 1**). Contaminated or severely traumatised wounds should be surgically debrided to remove necrotic tissue and debris to optimise healing (Theoret and Wilmink 2008; Caston 2012) (**Fig 1**). Treatment with manuka honey appears to be most effective if commenced soon after wounding, preferably within the first 24 h. Where bacterial contamination and tissue trauma is substantial, manuka honey with a UMF ≥15 should be used (Bischofberger *et al.* 2013). However, it should be noted that as the UMF of manuka honey increases so does the cost. While lower UMF manuka honey may not have the same antimicrobial properties it remains unclear at this time as to whether it has similar effects on the initial inflammatory response in wounds. So economically, if cost is a factor, where there is minimal contamination, using a lower UMF honey may reduce treatment costs.

If manuka honey is not being used as a 66% gel, it is best applied at normal room temperature for ease of application, with or without bandages. Bandages will improve the contact between the honey and wound and may be useful in the early stages of wound healing. However, ongoing bandaging may be associated with a higher incidence of EGT development (Berry and Sullins 2003; Dart *et al.* 2009). Where wounds are bandaged, apply 30 ml of honey to each 10 × 10 cm dressing area (Matthews and Binnington 2002; Dart *et al.* 2005). Initially, bandage changes will be required daily or every other day. These can be reduced as wound exudate decreases (Matthews and Binnington 2002; Nisbet *et al.* 2010). If a bandage is applied, the duration of application should be kept to a minimum, preferably under 12 days, to reduce the chances of EGT (Dart *et al.* 2009). Where honey is placed on open wounds, more is not necessarily better. By using a thin film combined with regular application, contact times may be optimised so application 2–3 times daily to open wounds may improve efficacy. Manuka honey should be applied for at least 21 days after wounding but there may be beneficial effects if it is applied until wound healing is almost complete (Bischofberger *et al.* 2013). Excessive granulation tissue should be excised as it arises and application of honey continued.

Need for future studies

Most studies in man have focused on the antibacterial properties of manuka honey. It has been established that the higher the UMF, the greater the efficacy against antibiotic resistant bacteria. However, the ability of manuka honey and other honey varietals have been found to stimulate cytokine production and enhance healing by modulating the initial inflammatory response (Tonks *et al.* 2001, 2003, 2007; Nisbet

TABLE 1: Optimal treatment plan using manuka honey topically to treat equine distal limb wounds left to heal by second intention

1. Surgically debride wounds to remove contaminated, necrotic, devitalised or infected tissues
2. Apply ≥UMF 15 manuka honey at room temperature within 24 h of injury or debridement
3. Apply 30 ml honey to each 10 × 10 cm dressing area
4. Change bandage each day and reply the honey for up to 12 days
5. Remove bandage at 12 days and leave wound open
6. Apply a thin film of manuka honey to the wound 2–3 times daily for at least 21 days

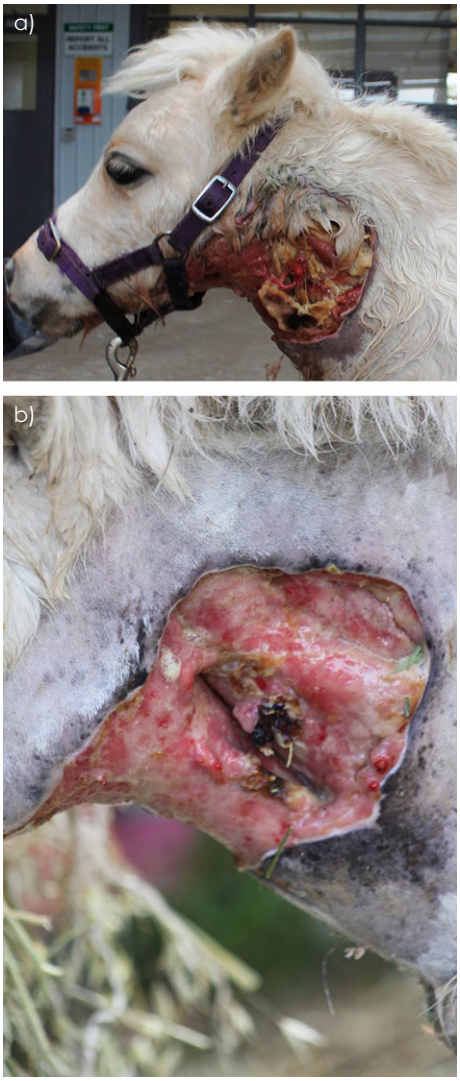

Fig. 1: (a and b) The neck of a 6-year-old miniature horse 10 days after receiving a perivascular injection of flunixin meglumine (Fig 1a). There was extensive sloughing of the soft tissues thrombosis of the jugular vein. The wound was debrided surgically to remove necrotic tissue and lavaged. The horse was placed on broad spectrum antimicrobial treatment and non steroidal anti-inflammatory therapy and the wound was cleaned and UMF 20 manuka applied to the wound twice daily. (b) Shows the same wound 7 days after debridement. The wound is clean and has a healthy bed of granulation tissue.

et al. 2010). It would appear that different honey varietals possess a range of bioactive components that can affect wound healing and the range and relative concentrations of the bioactive components in any varietal may influence the therapeutic value of that honey in different wounds. Currently, limited research using wound healing models suggest that the effects of manuka honey on the initial inflammatory response plays an important role in enhancing the early stages of second intention healing of distal limb wounds in horses.

In terms of manuka honey, more information is needed to refine the therapeutic approach to manipulating the process of second intention wound healing in horse. Some of the immediate information that would be of value include:

- As the UMF of manuka honey increases so does the antimicrobial activity and cost of treatment. Further study is needed to investigate whether UMF 20 honey is required to achieve the benefits of treatment in all equine lower limb wounds or only wounds with excessive contamination or antibiotic resistant organisms.
- In wounds that are not heavily contaminated or surgically debrided, could generic and less expensive honey be as effective as manuka honey in improving wound healing variables?
- Diluted UMF 20 manuka honey (66% manuka honey gel) has delivered the similar effects to undiluted honey in a distal limb wound model. At what dilution point does manuka honey lose its beneficial effects and does dilution shorten the shelf life of manuka honey? It is possible more cost effective treatment could be achieved by further diluting the honey.
- Could manuka honey be delivered in a variety of dressings specific for wound types and in a cost effective manner?

Conclusion

Manuka honey represents a step forward in the area of equine wound healing. As a dressing, it has the potential to address some of the challenges associated with second intention healing in horses, particularly wounds to the distal limb and holds significant promise as a therapeutic agent. The current recommendations outlined for treating equine wounds with manuka honey are based on the present body of knowledge and provides a protocol for veterinarians to follow. However, there is still substantial research to be done that should be aimed at understanding the mechanism of action of manuka honey and to develop recommendations that match the specific characteristics of the honey to the specific features of the wounds. Furthermore, as new information becomes available on the bioactivity of different honey varietals, it may be possible to match the features of different varietals of honey to treatment of different stages of the wound healing process. There is also a need to commercialise different honey dressings and products to meet the different features of wounds so the honey can be applied and maintain wound contact.

Authors' declaration of interests

No conflicts of interest have been declared.

Ethical animal research

Ethical review not applicable for this review article.

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Authorship

A.J. Dart, C.M. Dart and L.B. Jeffcott all currently work within the Research and Clinical Training Unit (REaCT) at the University of Sydney. A.S. Bischofberger was working within the REaCT unit as a an equine surgery resident. Drs Dart, Jeffcott and Bischofberger were the primary investigators into the

series of manuscripts published into the effects of manuka honey on second intention wound healing in the horse. All authors had input into the design, execution data analysis and interpretation and preparation of those manuscripts. The review manuscript was written by A.J. Dart and was circulated to all authors for comment and review. All authors had input to the final submission and all authors approved the final version.

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