# Veterinary use of medical grade Mānuka honey; a review of key healing features, spectrum of activity, indications and antimicrobial resistance.

Nerina Sommerville, BVSc., MPhil.

## INTRODUCTION

Wounds involving significant tissue trauma are common in equine practice, particularly wounds to the distal limb. Where possible, primary closure is ideal but is rarely successful due to poor circulation, joint movement and minimal soft tissue cover[1]. Compounding factors such as excessive contamination and tension[2] as well as the involvement of bone, tendons and joints lead to wounds largely being managed by secondary intention healing, which impacts the welfare of equine patients.

The antimicrobial and anti-inflammatory activity of mānuka honey is now widely known, providing a moist and more regenerative wound environment. Mānuka honey promotes autolytic debridement[3] with enhanced angiogenesis, accelerated re-epithelialisation and wound closure[4,5]. Antimicrobial resistance (AMR) which is being experienced worldwide is reaching a crisis point due to the lack of novel therapeutics. For this reason, mānuka honey is now becoming increasingly beneficial to wound healing[6], improving the financial, welfare and AMR implications facing the management of wounds in veterinary practice.

## KEY HEALING FEATURES

Wounds involving the distal limb in horses have been found to exhibit a weak and persistent inflammatory phase of healing, compared to wounds involving the body[7]. This prolonged low grade inflammation, in combination with the lack of soft tissues and a relatively poor vascular supply, can promote the formation of exuberant granulation tissue (EGT) which results in a complicated, and often delayed healing process[8].

Mānuka honey creates a favourable environment in the wound, promoting an accelerated and healthy bed of granulation tissue[9], thus decreasing wound-healing time[10]. When applied to the wound as part of a wound dressing, a physical barrier is created with the provision of a moist environment, preventing further contamination[11]. Due to its high osmolarity and subsequent osmotic action, wound oedema is reduced[6] as well as generating a flow of bacteria, necrotic tissue and debris out the wound[12]. This results in the stimulation of autolytic debridement which may in turn activate plasminogen, which breaks down wound fibrin, releasing attachments of devitalised tissue from the wound bed[3].

*“Mānuka honey creates a favourable environment in the wound, promoting an accelerated and healthy bed of granulation tissue”*

On the wound bed itself, honey has been shown to have several anti-inflammatory and immune-modulating properties. This is achieved firstly by an increase in the production of inflammatory cytokines in leukocytes which modulate fibroblast proliferation and angiogenesis[6,10,13], and secondly by actively drawing exudate and lymph out of the wound, physically reducing swelling, inflammation and symptomatic pain[14,15]. Furthermore, honey has been shown to neutralise reactive oxygen species during the inflammatory phase of healing, protecting the wound tissue from potential damage from inflammatory cells[16].

Finally, during the repair phase, honey has been shown to further stimulate angiogenesis, preserving

## KEY FEATURES OF MĀNUKA HONEY IN WOUND HEALING

- *Broad antimicrobial action*
- *Anti-inflammatory effect*
- *Antioxidant*
- *Antibiofilm*
- *Autolytic debriding action*
- *Deodorising properties*
- *Accelerates wound healing*
- *Encourages formation of healthy granulation tissue*
- *Provides optimal moist wound environment*
- *Beneficial properties for cellular activity*

the healthy granulation tissue bed, and promoting re-epithelialisation[17]. Honey provides a rich nutrient source for proliferating cells that are rebuilding the lost tissue and promotes the migration of new skin cells[18].

# UMF RATING

Mānuka honey possesses several substances that have a direct antioxidant and antimicrobial action which includes bee product propolis, bee defensin and phytochemicals such as methylglyoxal (MGO), the dominant antibacterial component of mānuka honey[19-21]. A higher concentration of MGO and its associated antibacterial properties, increases the antibacterial potency of the honey.

In the hive, high levels of dihydroxyacetone (DHA) are detected[22,23], which is harvested from the mānuka tree *(Leptospermum scoparium)* and converted non enzymatically to MGO[24]. The presence of MGO in mānuka honey is therefore determined by the concentration of DHA. A higher DHA content increases the shelf life of mānuka honey due to this ongoing conversion of DHA to MGO. Additionally, a compound unique to mānuka honey, leptosperin, has been recently discovered; it contributes to mānuka honey bioactivity and its anti-inflammatory properties, and is now incorporated into the UMF™ rating[25,26]. As leptosperin is a compound found solely in mānuka flower nectar and can only be created by nature, its presence is measured for the authenticity of mānuka honey. Lastly, a test for hydroxymethylfurfural (HMF) is included, which assures the honey has not been overheated or stored too long.

| UMF Rating | MGO (mg/kg) *Potency* | Leptosperin (mg/kg) *Authenticity* | DHA (mg/kg) *Shelf Life* | HMF (mg/kg) *Freshness* |
|---|---|---|---|---|
| 5+ | 93 | >100 | 150 | <40 |
| 10+ | 261 | >150 | 250 | <40 |
| 15+ | 512 | >200 | 400 | <40 |
| 20+ | 826 | >200 | 500 | <40 |
| 25+ | 1197 | >200 | 500 | <40 |

*Table 1. UMF™ rating based on MGO, Leptosperin, DHA and HMF concentration (UMF™ Honey Association[26])*

# ANTIMICROBIAL ACTION

Broadly speaking, the antibacterial activity of honey is linked to its low pH which impedes pathogens to thrive, its hygroscopicity which leads to dehydration of the microorganism and the constant release of low amounts of hydrogen peroxide followed by catabolism of glucose which is a potent antimicrobial[27]. A 100-fold difference in antimicrobial activity between honey types is reported and depends on geographical, seasonal and botanical source as well as harvesting, processing and storage conditions[28]. Furthermore, this activity is dependent on various factors working singularly or synergistically, such as hydrogen peroxide and phenolic compound activity, wound pH, pH of honey and the osmotic pressure exerted by the honey[29].

*"A 100-fold difference in antimicrobial activity between honey types is reported"*

This provides evidence that the choice of honey for wound dressings is of utmost importance. A recent study examining the antibacterial activity of a variety of honeys against common equine wound isolates reaffirmed the general recommendation that only medical grade mānuka honey above UMF 12 has therapeutic benefits[13]. Where bacterial contamination

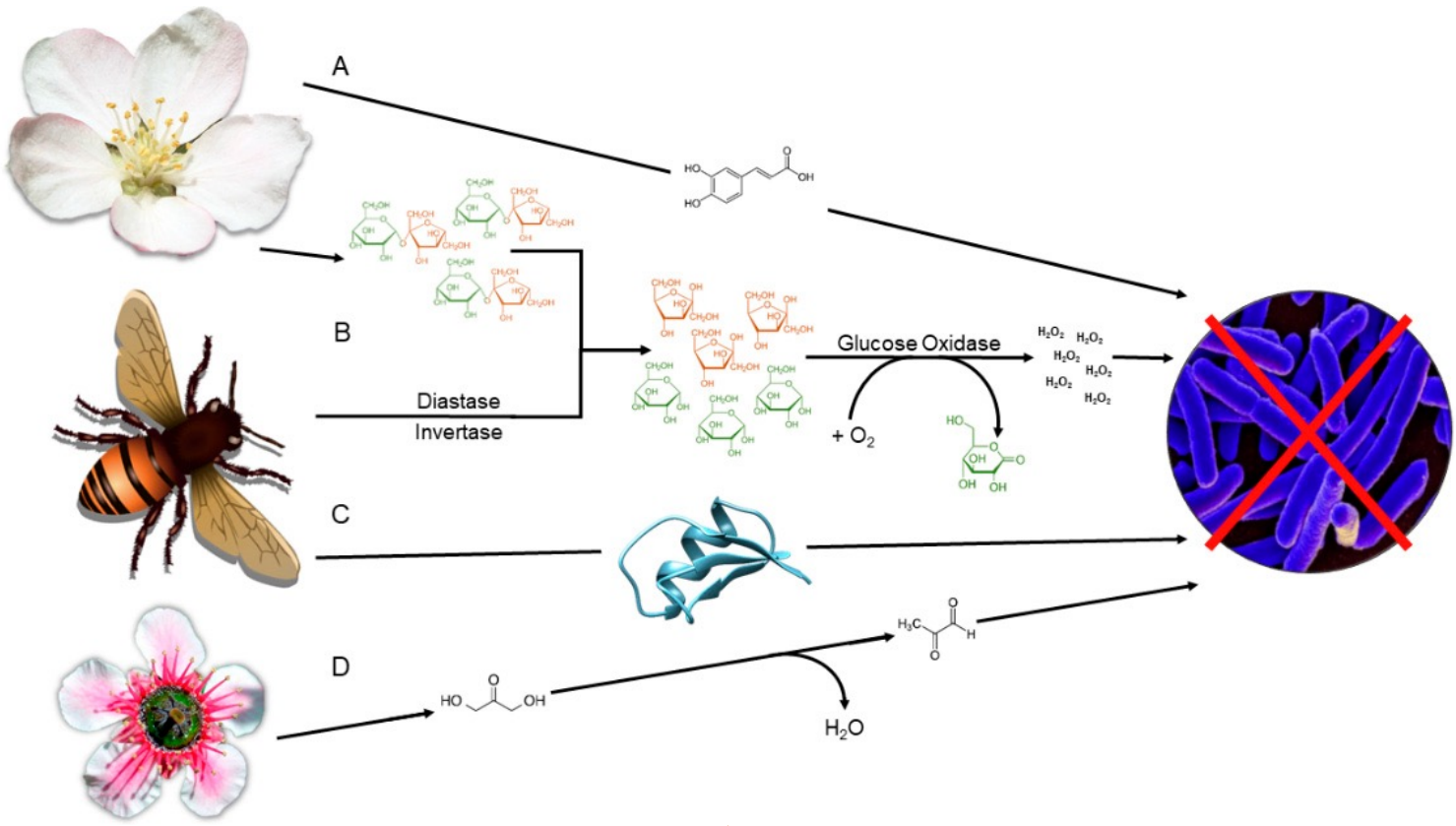


*Figure 1. Acquisition of antimicrobial compounds within honey. (A) Polyphenolic compounds derived from the plant are transferred by the bee. (B) Sucrose from the flower is ingested by the bee and broken down into glucose and fructose upon addition of diastase and invertase by the bee. The glucose is oxidised by glucose oxidase upon the addition of oxygen, producing D-glucono-δ-lactone and hydrogen peroxide. The hydrogen peroxide has antimicrobial activity. (C) Bee defensin-1 is added to honey by the bee (Swissmodel 6mry.5.A). (D) Dihydroxyacetone is harvested from Leptospermum sp. and converted non-enzymatically to methylglyoxal through dehydration reaction. Source: Nolan et al, 2019[24]*

and tissue trauma is substantial, mānuka honey with a UMF of >15 should be used, particularly in wounds of the equine limb[30]. Furthermore in 2017, Tsang et al. compared the effects of topical application of UMF 20 and UMF 5 mānuka honey with a generic multi-floral honey. The results showed that when using the UMF 20 the total healing time was reduced when compared to UMF 5 and general honey (GH)[31]. Healing times of wounds treated with GH and UMF 5 were not significantly different.

In addition to UMF choice, as honey contains particulate contaminants, such as pollens, and microorganisms from the honeybee gastrointestinal tract, most notably *Clostridium spp.*, a medical-grade, filtered, commercially sterile product is recommended for wound treatment[32]. Multiple brands of honey purchased from a local supermarket and UMF 5 mānuka honey were among contaminated samples, which reiterates the further importance of product sterility before administration to wounds[13].

## SPECTRUM OF ACTIVITY

To date there are many publications reporting the therapeutic properties of mānuka honey, confirming its activity against a wide range of bacteria[33,34]. There is a reported inhibitory effect on around 60 bacterial species, including aerobes and anaerobes, gram positives and gram negatives[35]. Recent studies[13,36-38] highlight the benefit of honey to wound healing due to the high bactericidal activity at low concentrations against common wound isolates. Almasaudi[37] compared the effects of 5 types of honey including mānuka honey UMF 20+ and UMF 16+ against 2 strains of *Staphylococcus aureus*. The inhibition of bacterial growth by all types of honey was evident at 20% and

| Bacterial Species | Type of Honey | MIC (%v/v) | Reference |
|---|---|---|---|
| Enterococcus faecalis | Medical manuka 1 | 8 | |
| | Medical manuka 2 | 12 | |
| | Manuka 20+ | 6 | Carnwath et al., 2014[13] |
| | Manuka 10+ | 10 | |
| Escherichia coli | Medical manuka 1 | 6 | Carnwath et al., 2014[13] |
| | Medical manuka 2 | 10 | |
| | Manuka 20+ | 4 | |
| | Manuka 10+ | 8 | |
| | Manuka 25+ | 12.5 | Sherlock et al., 2010[45] |
| | Woundcare 18+ (CUK/NZ) | 12 | Cooper et al., 2010[47] |
| Kliebsiella pneumonia | Manuka | 10 | Allen and Molan, 1997[48] |
| | Medihoney® (AUST) | 6-8 | George and Cutting., 2007[49] |
| | Medihoney® (AUST) | 13 | Blair et al., 2009[50] |
| Proteus mirabilis | Manuka | 10.8 | Willix et al., 1992[32] |
| Pseudomonas aeruginosa | Manuka | 4-9 | Cooper et al., 2002a[51] |
| | Manuka | 15.7 | Willix et al., 1992[32] |
| | Medihoney® (AUST) | 12-14 | George and Cutting., 2007[49] |
| | Woundcare 18+ (CUK/NZ) | 7.3 | Jenkins et al., 2015b[52] |
| | Medical manuka 1 | 8 | Carnwath et al., 2014[13] |
| | Medical manuka 2 | 10 | |
| | Manuka 20+ | 8 | |
| | Manuka 10+ | 10 | |
| | Woundcare 18+ (CUK/NZ) | 6 | Jenkins and Cooper., 2012[53] |
| | Medical Manuka | 12 | Roberts et al., 2012[54] |
| | Medihoney® (CNZ) | 10-30 | Maddocks et al., 2013[55] |
| | Manuka | 9.5 | Henriques et al., 2011[56] |
| | Manuka 25+ (CNZ) | 12.5 | Sherlock et al., 2010[45] |
| | Woundcare 18+ (CUK/NZ) | 15.7 | Cooper et al., 2010[47] |
| Staphylococcus aureus | Manuka | 5 | Allen and Molan, 1997[48] |
| | Manuka | 2.7 | Willix et al., 1992[32] |
| | Medihoney® (AUST) | 4 | George and Cutting., 2007[49] |
| | Medical manuka 1 | 6 | Carnwath et al., 2014[13] |
| | Medical manuka 2 | 10 | |
| | Manuka 20+ | 2 | |
| | Manuka 10+ | 10 | |
| | Medihoney® (CNZ) | 8 | Maddocks et al., 2013[55] |
| | Manuka | 1.2-3.4 | Henriques et al., 2011[56] |
| | Woundcare 18+ (CUK/NZ) | 3 | Cooper et al., 2010[47] |
| MRSA | Manuka | 2.7-3 | Cooper et al., 2002b[57] |
| | Medical manuka 1 | 4 | Carnwath et al., 2014[13] |
| | Medical manuka 2 | 10 | |
| | Manuka 20+ | 3 | |
| | Manuka 10+ | 10 | |
| | Manuka 25+ (CNZ) | 12.5 | Sherlock et al., 2010[45] |

*Table 2. Spectrum of activity (MIC) of Leptospermum (mānuka) honeys registered as human wound care products of common wound isolates in animals. Adapted from Carter, 2016[80]*

10% concentrations (v/v). Researchers found that mānuka honey had a higher antibacterial activity against both strains, with a stronger effect from the higher UMF. This provides significant evidence for a correlation between MGO concentration and antibacterial activity. Further studies have shown a bactericidal or bacteriostatic effect against wound pathogens such as multidrug-resistant *S. aureus*, methicillin resistant *Staphylococcus pseudintermedius, Pseudomonas, Pasteurella multocida, Enterococcus, Proteus, and Escherichia coli*[21,39] *as well as antifungal properties*[40].

*"There is a reported inhibitory effect on around 60 bacterial species, including aerobes and anaerobes, gram positives and gram negatives"*

Mānuka honey is effective against common equine wound isolates (including ones that form biofilms) namely *S. aureus* (MSSA), methicillin-resistant *S. aureus* (MRSA), *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*[41], with activity also extending to *Streptococcus pyogenes, Staphylococcus epidermis, E. coli, Enterobacter aerogenes* and *Salmonella typhimurium*[29,41-46].

Honey has been shown to affect bacteria through multiple mechanisms, such as changing their cell structure, decreasing membrane potential, disrupting the cell-cycle and metabolism, decreasing cellular growth, affecting efflux pump mechanisms, disrupting quorum sensing, and disrupting biofilms[58]. Interestingly, the sugars such as those found in mānuka honey, change the way in which bacterial organisms metabolise, resulting in less odorous wounds[3].

The mode of action also appears to differ depending on the microorganism involved. Exposure of gram-positive organisms to MGO leads to the downregulation of autolysin, an enzyme involved in cell division, and the cleavage of bacterial cell wall components[59,60]. Exposure of gram-negative bacteria to MGO appears to lead to altered gene expression of proteins involved in the structural integrity of the cell wall and cell lysis[8,60]. A decrease in virulence factors of bacteria has also been observed following treatment with mānuka honey, including downregulation of flagella-associated proteins[54], inhibition of siderophore

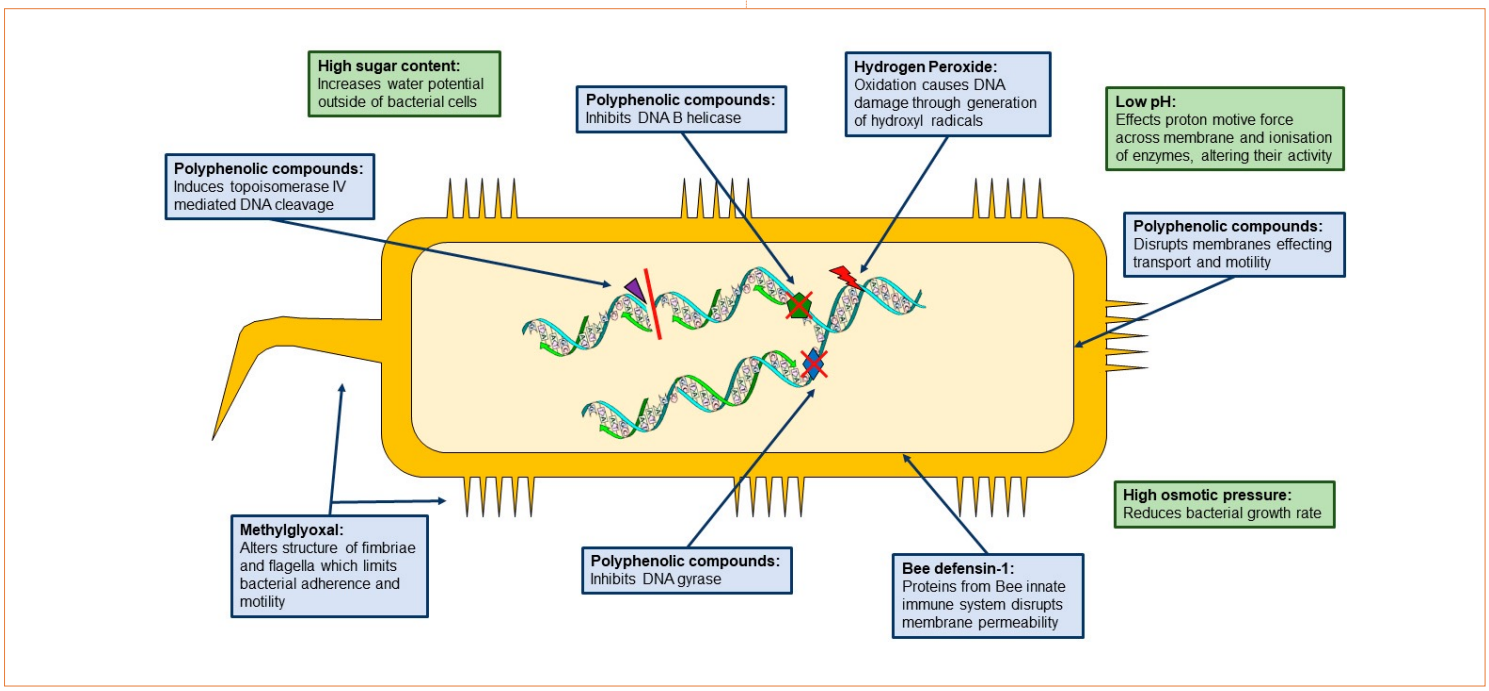


*Figure 2. The main constituents attributed to honey's antimicrobial activity and their mechanism of action. Direct inhibitory factors affect cellular mechanisms (blue), indirect inhibitory factors have a wider ranging effect on the bacterial cell (green). Source: Nolan et al, 2019*[24]

formation[61], and reversal of antibiotic resistance[5,62]. The collaborative and multifactorial benefits of using mānuka honey, in turn reduces the reliance on oral antibiotics while still aiding in wound treatment[27,63].

## EFFECT ON BACTERIAL BIOFILMS

Bacterial biofilms are known to impair healing of wounds in both humans and horses due to the ability of biofilms to interfere with the activity of many antibiotics[64-66]. In wounds in the distal equine limb, biofilms may then result in chronic wound healing which can lead to the development of excessive granulation tissue (EGT)[67]. Bee defensin and MGO both impair biofilm formation[68-70] suggesting mānuka honey not only has potent antimicrobial activity but also inhibits the formation of biofilms and causes the detachment of established biofilms[55].

Mānuka honey at a concentration of 40% has been found to give significantly reduced biofilm mass with in vitro testing of clinical isolates of *P. aeruginosa* that had developed into a biofilm[71]. Similar findings were seen in studies with *S. pyogenes*[46] and MRSA[72], where it was found biofilms were sensitive to mānuka honey treatment. In another study conducted with biofilms developed from 11 isolates each of MSSA, MRSA, and *P. aeruginosa*, it was found that 50% mānuka honey killed 9 out of 11, 7 out of 11 and 10 out of 11 of the isolates, respectively[73]. Greater sensitivity was found in another study on biofilms formed in vitro where the MIC for mānuka honey was 6% honey for MRSA and methicillin-resistant *S. epidermidis*, and 12% honey for *P. aeruginosa* and *K. pneumoniae*[66].

MGO in mānuka honey appears to play a critical role in disruption of biofilms but it is not exclusively responsible for this property[74]. Although the exact mechanism of action is currently unknown, exposure of bacteria to mānuka honey in vitro has demonstrated downregulation of gene coding for surface-binding proteins important in biofilm formation, virulence, and cell to cell communication[55,75]. Low concentrations of mānuka honey may prevent biofilm maturation by disrupting cellular communications whilst higher concentrations of mānuka honey are able to penetrate and detach established biofilms[55,73].

*"Higher concentrations of mānuka honey are able to penetrate and detach established biofilms."*

## ANTIMICROBIAL RESISTANCE

As we face the challenge of increasing AMR in veterinary medicine, there is a pressing need for a prudent and more directed use of antimicrobial drugs. Currently, some degree of antimicrobial resistance has been documented within all *Staphylococcus* species that infect humans and domestic animals[76,77]. Topical therapy should be used as the sole on-animal antibacterial treatment for surface and superficial infections whenever a pet and owner can be expected to be compliant[78].

It has been observed that no organism has gained resistance to honey[55] which is likely due to the multiple antibacterial properties which act synergistically, enhancing its potency[19,24]. Mānuka honey exhibits antimicrobial activity against a broad spectrum of organisms including those with multidrug resistance[43], and it is now widely recognised for its ability to eliminate problematic methicillin-resistant *S. aureus* and multi-drug resistant *P. aeruginosa* for which few to no effective antibiotics currently exist[79,80]. Furthermore, the multi drug resistance status of each bacterial strain has no impact on the susceptibility of the organism to honey[81].

Interestingly, recent in vitro studies provide evidence of the potential of mānuka honey to increase the efficiency of FDA- approved antibiotics by restoring resistant bacterial strains susceptibility to antimicrobial action[42,82-85]. These findings provide a strong basis for the use of honey in the clinical setting especially advantageous during chronic infections.

*"Studies provide evidence of the potential of mānuka honey to increase the efficiency of FDA - approved antibiotics by restoring resistant bacterial strains susceptibility to antimicrobial action."*

## INDICATION FOR USE

Ideally, topical wound treatment products must have a wide range of antibacterial properties while at the same time protect the wound environment from desiccation and other environmental factors and therefore enhance the healing process[86]. When used as a topical dressing, honey decreases inflammatory edema, accelerates sloughing of devitalised tissue, and nourishes the wound: these factors contribute to enhanced granulation and epithelialisation[87]. Several studies have described the effectiveness of mānuka honey clearing wound infection without slowing the healing process and it may actively promote healing. These studies also outline the different uses of honey within a clinical setting and that its implementation in promoting healing is not only due to its antimicrobial action, but also to reduce healing times and patient discomfort.

The general convention in veterinary practice is to use honey in the inflammatory and debridement stages of wound healing only[67].

In reviewing the studies by Bischofberger, Guisto and Tsang, which all utilised control groups, mānuka honey had an efficient anti-inflammatory activity during the treatment, but more specifically during the first three weeks (21 days), acting more successfully during the initial phases of inflammation, assessed by the observation of the development of a healthier bed of granulation tissue, increased angiogenesis, fibrosis, collagen organisation, in the first 10 days, and by moisturising the wound throughout the treatment enhancing the debridement activity[31,88].

### COMMON WOUND APPLICATIONS

**PURE MEDICAL GRADE MĀNUKA HONEY IS INDICATED FOR DIRECT APPLICATION (WITH OR WITHOUT A DRESSING)**

- *Abscesses*
- *Bites*
- *Boils*
- *Burns*
- *Field wounds*
- *Skin grafts*
- *Surgical wounds*

In the study by Bischofberger, the results conclude that the most beneficial time to apply mānuka honey is in the first 3 weeks after wounding[30]. However, a study was undertaken to try to understand the outcomes between application of mānuka honey for 21 days, and application until the wound healing is complete. It showed that a wound heals faster if the treatment continues until the healing is complete[6].

Many studies have provided evidence of the beneficial effects of honey on excisional and burn wounds in rabbits[89], rats[90-93], dogs[86,94-95], sheep[96] and goats[97]. Benefits include a reduction of bacterial counts, enhanced wound closure, accelerated rate of re-epithelialisation and reducing the expression of pro-inflammatory cytokines.

Studies specifically mentioning use of medical grade honey or mānuka honey in rats[88], dogs[36], sheep[15], rhinos[27] and horses[31,98-101] displayed faster healing times with more organised granulation tissue beds, resolving infection with evidence of reducing dependance on oral antibiotics.

Indications other than wound healing that have featured in veterinary literature to date include periodontitis[102], otitis externa in dogs[40], mastitis in cats[103] and cattle[48], foot and mouth disease in cattle[104] and endometritis in cattle[105,106] and horses[107]see the potential of various types of honey across many clinical settings.

| Species | Clinical Significance | Reference |
|---|---|---|
| Dog | Otitis Externa<br>Excisional and burn wounds<br>Wound infection<br>Wound healing while immunocompromised | Maruhashi et al., 2016<br>Jalali et al., 2007, Esmaeelian et al., 2012<br>Tramuta et al., 2017<br>Alshehabat et al., 2020 |
| Cat | Feline gangrenous mastitis | Wilson, 2013 |
| Rabbit/Rat | Excisional and burn wounds | Kundu et al., 2005, Lusby et al., 2006<br>Sukur et al., 2011, Zohdi et al., 2012<br>Aljady et al., 2000, Giusto et al., 2017 |
| Horse | Second intention healing<br>Excisional wounds<br>Chronic wounds<br>Endometritis<br>Intra-lesional application prior to wound closure<br>Post-operative colic surgery- incisional infection | Bischofberger et al., 2011, 2013, 2015, 2016<br>Khiati et al, 2014,<br>Tsang et al., 2017<br>Ali et al., 2011<br>Abidine and Bouabdellah, 2018<br>Mandel et al., 2020<br>Gustafsson et al., 2020 |
| Sheep/Goat | Full thickness incisional wounds | Al Khazraji et al., 2012, Iacopetti et al., 2020, Ajibola, 1995 |
| Cattle | Foot and mouth disease<br>Mastitis<br>Endometritis | Gakuya et al., 2011<br>Allen and Molan, 1997<br>Maarouf et al., 2018, Abdul-Hafeez et al., 2019 |

*Table 3. Summary of the clinical significance of the use of honey in domestic animal species.*

# CONCLUSION

The use of mānuka honey for wound healing has gained special focus over the years, with the development of further clinical applications, taking advantage of its unique chemical characteristics. In contrast to modern antibiotics which target specific cell mechanisms and structures, honey holds multifaceted intrinsic activities including broad spectrum antimicrobial actions attributed to more than 200 different constituents[108]. Unlike some of the biocides, high amounts of mānuka honey are not cytotoxic[47], and to date no organism has gained resistance to honey.

There is also a promising potential that the use of honey, particularly mānuka honey, may indeed reduce the reliance on oral antibiotics, contributing to the collaborative effort of tackling antimicrobial resistance worldwide.

# DECLARATION

This article was written with the support of Manuka Vet NZ Limited.

# REFERENCES


1. Derham, A. and O'Leary, J.M. (2017). Equine limb wound management. Veterinary Ireland Journal, 7(2), 65-67.

2. Alford, C.G., Caldwell, F.J. and Hanson, R.R. (2012). Equine Distal Limb Wounds: New and Emerging Treatments. Compendium [online], 34(7).

3. Mitchell, T. (2018). Use of manuka honey for autolytic debridement in necrotic and sloughy wounds. Journal of Clinical Nursing, 32(4).

4. Tonks, A.J., Cooper, R.A., Jones, K.P., Blair, S., Parton, J., and Tonks, A. (2003). Honey stimulates inflammatory cytokine production from monocytes. Cytokine, 21, 242-247.

5. Tsang, A. S., Dart, A. J., Dart, C. M., and Jeffcott, L. (2018). Mechanisms of action of manuka honey in an equine model of second intention wound healing: current thoughts and future directions. Medical Research Archives, 6(7).

6. Dart, A.J., Bischofberger, A.S., Dart, C.M. and Jeffcott, L.B. (2015). A review of research into second intention wound healing using manuka honey: Current recommendations and future applications. Equine Veterinary Education, 27(12), 658-664.

7. Wilmink, J.M., Stolk, P.W.T.H., Van Weeren, P.R., and Barneveld, A. (1999). Differences in second intention wound healing between horses and ponies: macroscopic aspects. Equine Vet J, 31(1), 53-60.

8. Dart, A.J., Perkins, N.R., Dart, C.M., Jeffcott, L.B., and Canfield, P. (2009). Effect of bandaging on second intention healing of wounds of the distal limb in horses. Aust Vet J, 87(6), 215-218.

9. Thompson E. (2017). Debridement Techniques and Non–Negative Pressure Wound Therapy Wound Management. Vet Clin North Am Small Anim Pract., 47(6), 1181-1202.

10. Scagnelli, A.M. (2016). Therapeutic review: Manuka honey. Journal of Exotic Pet Medicine [online], (25).

11. Molan, P.C. (2002). Re-introducing honey in the management of wounds and ulcers-theory and practice. Ostomy/Wound Management, 4811, 28-40.

12. Minden-Birkenmaier, B.A. and Bowlin, G.L. (2018). Honey-based templates in wound healing and tissue engineering. Bioengineering (Basel), 5(2):46.

13. Carnwath, R., Graham, E. M., Reynolds, K., and Pollock, P. J. (2014). The antimicrobial activity of honey against common equine wound bacterial isolates. Veterinary Journal, 199(1), 110–114.

14. Hollis, G. (2010). Manuka honey: an effective wound healing environment. The Veterinary Nurse, 1(1), pp.42-49.

15. Iacopetti, I., Perazzi, A., Martinello, T., Gemignani, F., and Patruno, M. (2020). Hyaluronic acid, manuka honey and acemannan gel: Wound-specific applications for skin lesions. Res Vet Sci, 129, 82-89.

16. Dart, A.J., Dowling, B.A. and Smith, C.L. (2005). Topical Treatments in Equine Wound Management. The Veterinary Clinics of North America, Equine Practice, 21(1), 77-89.

17. Matjan, J. (2014). Honey: an immunomodulator in wound healing. Wound Repair Regen, 22(2), 187-92.

18. Smaropoulos, E. and Cremers, N.A.J. (2020). Treating severe wounds in pediatrics with medical grade honey: A case series. Clin Case Rep, 8(3), 469-476.

19. Mavric, E., Wittmann, S., Barth, G., and Henle, T. (2008). Identification and quantification of methylglyoxal as the dominant antibacterial constituent of Manuka (Leptospermum scoparium) honeys from New Zealand. Molecular Nutrition & Food Research, 52(4), 483–489.

20. Roberts, A.E.L., Maddocks, S.E., and Cooper, R.A. (2015). Manuka honey reduces the motility of Pseudomonas aeruginosa by suppression of flagella-associated genes. J Antimicrob Chemother, 70(3), 716-25.

21. Johnston, M., Mcbride, M., Dahiya, D., Owusu-Apenten, R., and Nigam, P.S. (2018). Antibacterial activity of Manuka honey and its components: An overview. AIMS Microbiol, 4(4), 655-664.

22. Adams, C. J., Manley-Harris, M., and Molan, P. C. (2009). The origin of methylglyoxal in New Zealand manuka (Leptospermum scoparium) honey. Carbohydrate research, 344(8), 1050–1053.

23. Majtan, J., Klaudiny, J., Bohova, J., Kohutova, L., Dzurova, M., Sediva, M., Bartosova, M., and Majtan, V. (2012). Methylglyoxal-induced modifications of significant honeybee proteinous components in manuka honey: Possible therapeutic implications. Fitoterapia, 83(4), 671–677.

24. Nolan, V. C., Harrison, J., and Cox, J. (2019). Dissecting the Antimicrobial Composition of Honey. Antibiotics (Basel, Switzerland), 8(4), 251.

25. Greiner, N. (2022). The use of medical-grade honey in veterinary medicine. Innovative Veterinary Care J, Available from: https://ivcjournal.com/medical-grade-honey-veterinary-medicine/

26. UMF website Grading System Explained. Unique Manuka Factor: Honey Association. 2021.

27. Marais, H. J., Glyphis, Z. G., and Cremers, N. (2021). Medical grade honey: Hope for wounded white rhinos. Veterinary and animal science, 13, 100196.

28. Molan, P.C. and Cooper, R.A. Honey and Sugar as a Dressing for Wounds and Ulcers. Tropical Doctor. 2000;30(4):249-250.

29. Mandal, M. D., and Mandal, S. (2011). Honey: its medicinal property and antibacterial activity. Asian Pacific Journal of Tropical Biomedicine, 1(2), 154–160.

30. Bischofberger, Andrea S., Dart, C. M., Perkins, N. R., Kelly, A., Jeffcott, L., and Dart, A. J. (2013). The Effect of Short- and Long-Term Treatment with Manuka Honey on Second Intention Healing of Contaminated and Noncontaminated Wounds on the Distal Aspect of the Forelimbs in Horses. Veterinary Surgery, 154-160.

31. Tsang, A. S., Dart, A. J., Sole-Guitart, A., Dart, C. M., Perkins, N. R., and Jeffcott, L. B. (2017). Comparison of the effects of topical application of UMF20 and UMF5 manuka honey with a generic multifloral honey on wound healing variables in an uncontaminated surgical equine distal limb wound model. Australian Veterinary Journal, 95(9), 333–337.

32. Molan, P.C. and Allen, K.L. (1996). The effect of Gamma-irradiation on the antibacterial activity of honey. J Pharm Pharmacol, 48(11), 1206-1209.

33. Willix, D.J., Molan, P.C., and Harfoot, C.G. (1992). A comparison of the sensitivity of wound-infecting species of bacteria to the antibacterial activity of manuka honey and other honey. J Appl Bacterial, 73, 388-394.

34. Taormina, P. J., Niemira, B. A., and Beuchat, L. R. (2001). Inhibitory activity of honey against foodborne pathogens as influenced by the presence of hydrogen peroxide and level of antioxidant power. International journal of food microbiology, 69(3), 217–225.

35. Molan, P.C. (1992). The antibacterial activity of honey: The nature of the antibacterial activity. Bee World, 73(1), 5-28.

36. Tramuta, C., Nebbia, P., Robino, P., Giusto, G., Gandini, M., Chiadò-Cutin, S., & Grego, E. (2017). Antibacterial activities of Manuka and Honeydew honey-based membranes against bacteria that cause wound infections in animals. Schweiz Arch Tierheilkd, 159(2):117-121.

37. Almasaudi, S. B., Al-Nahari, A., Abd El-Ghany, E., Barbour, E., Al Muhayawi, S. M., Al-Jaouni, S., Azhar, E., Qari, M., Qari, Y. A., and Harakeh, S. (2017). Antimicrobial effect of different types of honey on Staphylococcus aureus. Saudi journal of biological sciences, 24(6), 1255–1261.

38. Grego, E., Robino, P., Tramuta, C., Giusto, G., Boi, M., Colombo, R., Serra, G., Chiadò-Cutin, S., Gandini, M., and Nebbia, P. (2016). Evaluation of antimicrobial activity of Italian honey for wound healing application in veterinary medicine. Untersuchung zur antimikrobiellen Aktivität von italienischem Honig für die Wundheilung in der Veterinärmedizin.

39. de Groot, T., Janssen, T., Faro, D., Cremers, N.A.J., Chowdhary, A., and Meis. J.F. (2021). Antifungal Activity of a Medical-Grade Honey Formulation against Candida auris. Journal of Fungi, 7(50), 50.

40. Maruhashi, E., Braz, B.S., Nunes, T., Pomba, C., Belas, A., Duarte-Correia, J.H., and Lourenço, A.M. (2016). Efficacy of medical grade honey in the management of canine otitis externa – a pilot study. Vet Dermatol, 27(2), 93-e27.

41. Abd El-Malek, F. F., Yousef, A. S., and El-Assar, S. A. (2017). Hydrogel film loaded with new formula from manuka honey for treatment of chronic wound infections. Journal of global antimicrobial resistance, 11, 171–176.

42. Deng, J., Liu, R., Lu, Q., Hao, P., Xu, A., Zhang, J., and Tan, J. (2018). Biochemical properties, antibacterial and cellular antioxidant activities of buckwheat honey in comparison to manuka honey. Food chemistry, 252, 243–249.

43. Girma, A., Seo, W., and She, R. C. (2019). Antibacterial activity of varying UMF-graded Manuka honeys. PloS one, 14(10), e0224495.

44. Lu, J., Cokcetin, N. N., Burke, C. M., Turnbull, L., Liu, M., Carter, D. A., Whitchurch, C. B., and Harry, E. J. (2019). Honey can inhibit and eliminate biofilms produced by Pseudomonas aeruginosa. Scientific reports, 9(1), 18160.

45. Sherlock, O., Dolan, A., Athman, R., Power, A., Gethin, G., Cowman, S., and Humphreys, H. (2010). Comparison of the antimicrobial activity of Ulmo honey from Chile and Manuka honey against methicillin-resistant Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa. BMC complementary and alternative medicine, 10, 47.

46. Maddocks, S. E., Lopez, M. S., Rowlands, R. S., and Cooper, R. A. (2012). Manuka honey inhibits the development of Streptococcus pyogenes biofilms and causes reduced expression of two fibronectin binding proteins. Microbiology (Reading, England), 158(Pt 3), 781–790.

47. Cooper R., Jenkins L., Henriques A., Duggan R., and Burton N. (2010). Absence of bacterial resistance to medical-grade manuka honey. Eur. J. Clin. Microbiol. Infect. Dis. 29 1237–1241.

48. Allen K. L., and Molan P. C. (1997). The sensitivity of mastitis-causing bacteria to the antibacterial activity of honey. N. Z. J. Agric. Res. 40 537–540.

49. George N. M., and Cutting K. F. (2007). Antibacterial honey (Medihoney): in-vitro activity against clinical isolates of MRSA, VRE, and other multiresistant gram-negative organisms including Pseudomonas aeruginosa. Wounds 19:231.

50. Blair S., Cokcetin N., Harry E., and Carter D. (2009). The unusual antibacterial activity of medical-grade Leptospermum honey: antibacterial spectrum, resistance and transcriptome analysis. Eur. J. Clin. Microbiol. Infect. Dis. 28 1199–1208.

51. Cooper R. A., Halas E., and Molan P. C. (2002a). The efficacy of honey in inhibiting strains of Pseudomonas aeruginosa from infected burns. J. Burn Care Rehabil. 23 366–370.

52. Jenkins R., Wootton M., Howe R., and Cooper R. (2015b). A demonstration of the susceptibility of clinical isolates obtained from cystic fibrosis patients to manuka honey. Arch. Microbiol. 197 597–601.

53. Jenkins R., Cooper R. (2012). Improving antibiotic activity against wound pathogens with manuka honey in vitro. PLoS ONE 7:e45600.

54. Roberts A. E., Maddocks S. E., and Cooper R. A. (2012). Manuka honey is bactericidal against Pseudomonas aeruginosa and results in differential expression of oprF and algD. Microbiology 158 3005–3013.

55. Maddocks S. E., Jenkins R. E., Rowlands R. S., Purdy K. J., and Cooper R. A. (2013). Manuka honey inhibits adhesion and invasion of medically important wound bacteria in vitro. Fut. Microbiol. 8 1523–1536.

56. Henriques A. F., Jenkins R. E., Burton N. F., and Cooper R. A. (2011). The effect of manuka honey on the structure of Pseudomonas aeruginosa. Eur. J. Clin. Microbiol. Infect. Dis. 30 167–171.

57. Cooper R. A., Molan P. C., and Harding K. G. (2002b). The sensitivity to honey of Gram-positive cocci of clinical significance isolated from wounds. J. Appl. Microbiol. 93 857–863.

58. Combarros-Fuertes, P., Fresno, J.M., Estevinho, M.M., Sousa-Pimenta, M., Tornadijo, M.E., and Estevinho, L.M. (2020). Honey: Another Alternative in the Fight against Antibiotic-Resistant Bacteria? Antibiotics, 9(11), 774.

59. Jenkins, R., Burton, N., and Cooper, R. (2011). Effect of manuka honey on universal stress protein A in methicillin Staphylococcus aureus. Int J Antimicrob Agents, 37, 373-376.

60. Jenkins, R., Burton, N., and Jenkins, R. (2011). Manuka honey inhibits cell division in methicillin resistant Staphylococcus aureus. J Antimicrob Chemother, 66, 2536-2542.

61. Kronda J. M., Cooper R. A., and Maddocks S. E. (2013). Manuka honey inhibits siderophore production in Pseudomonas aeruginosa. J. Appl. Microbiol. 115 86–90.

62. Jenkins R., and Cooper R. (2012). Synergy between oxacillin and manuka honey sensitizes methicillin-resistant Staphylococcus aureus to oxacillin. J Antimicrob Chemother, 67(6), 1405-7.

63. Molan, P.C. (2006). The evidence supporting the use of honey as a wound dressing. Int J Low Extrem Wounds, 5, 40-54.

64. Serralta, V.W., Harrison-Balestra, C., Cazzaniga, A.L., Davis, S.C., and Mertz, P.M. (2001). Lifestyles of bacteria in wounds: presence of biofilms? Wounds, 13, 29-34.

65. Freeman, K., Woods, E., Welsby, S., Percival. S.L., and Cochrane, C.A. (2009). Biofilm evidence and the microbial diversity of horse wounds. Can J Microbiol, 55,197-202.

66. Merckoll, P., Jonasson, T.O., Vad, M.E., Jeansson, S.L., and Melby, K.K. (2009). Bacteria, biofilm and honey: a study of the effects of honey on “planktonic”and biofilm-embedded chronic wound bacteria. Scand J Infect Dis, 41,41-347.

67. Kennedy, C. R. (2018). The role of Manuka honey in second intention healing of wounds of the equine distal limb. Veterinary Ireland, 8(11), 669–674.

68. Ganz T. (2003). Defensins: antimicrobial peptides of innate immunity. Nature reviews. Immunology, 3(9), 710–720.

69. Rabie, E., Serem, J. C., Oberholzer, H. M., Gaspar, A. R., and Bester, M. J. (2016). How methylglyoxal kills bacteria: An ultrastructural study. Ultrastructural pathology, 40(2), 107–111.

70. Sojka, M., Valachova, I., Bucekova, M., and Majtan, J. (2016). Antibiofilm efficacy of honey and bee-derived defensin-1 on multispecies wound biofilm. Journal of medical microbiology, 65(4), 337–344.

71. Okhiria, O.A., Henriques, A., Burton, N.F., Peters, A., and Cooper, R.A. (2009). Honey modulates biofilms of Pseudomonas aeruginosa in a time and dose dependent manner. J ApiProduct ApiMed Sci, 1(1), 6-10.

72. Kot, B., Sytykiewicz, H., Sprawka, I., and Witeska, M. (2020). Effect of manuka honey on biofilm-associated genes expression during methicillin-resistant Staphylococcus aureus biofilm formation. Scientific reports, 10(1), 13552.

73. Alandejani, T., Marsan, J., Ferris, W., Slinger, R., and Chan, F. (2009). Effectiveness of honey on Staphylococcus aureus and Pseudomonas aeruginosa biofilms. Otolaryngology - Head and Neck Surgery 141(1):114-8.

74. Kilty S. J., Duval M., Chan F. T., Ferris W., and Slinger R. (2011). Methylglyoxal: (active agent of manuka honey) in vitro activity against bacterial biofilms. Int. Forum Allergy Rhinol. 1 348–350.

75. Jenkins, R., Burton, N., and Cooper, R. (2014). Proteomic and genomic analysis of methicillin-resistant Staphylococcus aureus (MRSA) exposed to manuka honey in vitro demonstrated down-regulation of virulence markers. The Journal of antimicrobial chemotherapy, 69(3), 603–615.

76. Werckenthin, C., Cardoso, M., Martel JL et al. Antimicrobial resistance in staphylococci from animals with particular reference to bovine S. aureus, porcine S. hyicus, and canine S. intermedius. Vet Res 2001; 32: 341– 362.

77. Chambers, H.F. (1997). Methicillin resistance in staphylococci: molecular and biochemical basis and clinical implications. Clin Microbiol Rev, 10(4).

78. Morris, D. O., Loeffler, A., Davis, M. F., Guardabassi, L., and Weese, J. S. (2017). Recommendations for approaches to meticillin-resistant staphylococcal infections of small animals: diagnosis, therapeutic considerations and preventative measures.: Clinical Consensus Guidelines of the World Association for Veterinary Dermatology. Veterinary dermatology, 28(3), 304–e69.

79. Alvarez-Suarez, J. M., Gasparrini, M., Forbes-Hernández, T. Y., Mazzoni, L., and Giampieri, F. (2014). The composition and biological activity of honey: A focus on Manuka honey. Foods (Basel, Switzerland), 3(3), 420-432.

80. Carter, D. A., Blair, S. E., Cokcetin, N. N., Bouzo, D., Brooks, P., Schothauer, R., and Harry, E. J. (2016). Therapeutic Manuka Honey: No Longer So Alternative. Frontiers in microbiology, 7, 569.

81. Nolan, V. C., Harrison, J., Wright, J., & Cox, J. (2020). Clinical Significance of Manuka and Medical-Grade Honey for Antibiotic-Resistant Infections: A Systematic Review. Antibiotics (Basel, Switzerland), 9(11), 766.

82. Hayes, G., Wright, N., Gardner, S. L., Telzrow, C. L., Wommack, A. J., and Vigueira, P. A. (2018). Manuka honey and methylglyoxal increase the sensitivity of Staphylococcus aureus to linezolid. Letters in applied microbiology, 66(6), 491–495.

83. Campeau, M. E., & Patel, R. (2014). Antibiofilm Activity of Manuka Honey in Combination with Antibiotics. International journal of bacteriology, 2014, 795281.

84. Liu, M. Y., Cokcetin, N. N., Lu, J., Turnbull, L., Carter, D. A., Whitchurch, C. B., and Harry, E. J. (2018). Rifampicin-Manuka Honey Combinations Are Superior to Other Antibiotic-Manuka Honey Combinations in Eradicating Staphylococcus aureus Biofilms. Frontiers in microbiology, 8, 2653.

85. Kumar, N. D., Kalluru, R. S., Ahmed, S., Abhilashini, A., Jayaprakash, T., Garlapati, R., Sowmya, B., and Reddy, K. N. (2014). Comparison of the Antibacterial Efficacy of Manuka Honey Against E.faecalis and E.coli - An In vitro Study. Journal of clinical and diagnostic research : JCDR, 8(8), ZC29–ZC31.

86. Alshehabat, M., Hananeh, W., Ismail, Z. B., Rmilah, S. A., and Abeeleh, M. A. (2020). Wound healing in immunocompromised dogs: A comparison between the healing effects of moist exposed burn ointment and honey. Veterinary world, 13(12), 2793–2797.

87. Matthews, K.A., and Binnington, A.G. (2002). Wound Management Using Honey. Compendium Cont Edu for Pract Vet, 24(1), 53-60.

88. Giusto, G., Vercelli, C., Comino, F., Caramello, V., Tursi, M., and Gandini, M. (2017). A new, easy-to-make pectin-honey hydrogel enhances wound healing in rats. BMC Complementary and Alternative Medicine, 17(1), 1–7.

89. Kundu, S., Biswas, T.K., Das, P., Kumar, S., and De, D.K. (2005). Turmeric (Curcuma longa) Rhizome Paste and Honey Show Similar Wound Healing Potential: A Preclinical Study in Rabbits. The International Journal of Lower Extremity Wounds, 4(4), 205-213.

90. Lusby, P. E., Coombes, A. L., and Wilkinson, J. M. (2006). A comparison of wound healing following treatment with Lavandula x allardii honey or essential oil. Phytotherapy research : PTR, 20(9), 755–757.

91. Sukur, S. M., Halim, A. S., and Singh, K. K. (2011). Evaluations of bacterial contaminated full thickness burn wound healing in Sprague Dawley rats Treated with Tualang honey. Indian journal of plastic surgery : official publication of the Association of Plastic Surgeons of India, 44(1), 112–117.

92. Mohd Zohdi, R., Abu Bakar Zakaria, Z., Yusof, N., Mohamed Mustapha, N., and Abdullah, M. N. (2012). Gelam (Melaleuca spp.) Honey-Based Hydrogel as Burn Wound Dressing. Evidence-based complementary and alternative medicine : eCAM, 2012, 843025.

93. Aljady, A.M., Kamaruddin, M.Y., Jamal, A.M., and Mohd. Yassim, M.Y. (2000). Biochemical study on the efficacy of Malaysian honey on inflicted wounds: an animal model. Med J Islamic World Acad Sci, 13(3), 125-132.

94. Jalali, F. S. S., Tajik, H., Saifzaideh, S. and Fartash, B. (2007a). Topical Application of Natural Urmia Honey on Experimental Burn Wounds in the Dog: Clinical and Microbiological Studies. Asian Journal of Animal and Veterinary Advances, 2(3): 133–139.

95. Esmaeelian, B., Najafi, O., Aldavood, S.J., Jalali, F.S.S., Farshid, A.A., and Rahmani, S. (2012). Clinical and histopathological evaluations of local honey application in the healing of experimental wounds in dog. J Vet Res, 67, 265–271.

96. Al-Khazraji, K.I., Al-Byatee, A.J., and Falih, A.B. (2012). Therapeutic use of honey for skin wounds healing in Iraqi awassi sheep. Kufa J Vet Med Sci, 3, 17–22.

97. Ajibola, A. A. (1995). Honey in the post-surgical wound management in goats. (DVM dissertation) Nigeria.

98. Mandel, H. H., Sutton, G. A., Abu, E., and Kelmer, G. (2020). Intralesional application of medical grade honey improves healing of surgically treated lacerations in horses. Equine veterinary journal, 52(1), 41–45.

99. Bischofberger, A.S., Dart, C.M., Perkins, N.R., and Dart, A.J. (2011). A preliminary study on the effect of manuka honey on second-intention healing of contaminated wounds on the distal aspect of the forelimbs of horses. Vet Surg, 40, 898–902.

100. Bischofberger, A. S., Tsang, A. S., Horadagoda, N., Dart, C. M., Perkins, N. R., Jeffcott, L.B., Jackson, C.J., Dart, A. J. (2015). Effect of activated protein C in second intention healing of equine distal limb wounds: A preliminary study. Australian Veterinary Journal, 93(10), 361–366.

101. Bischofberger, A. S., Dart, C. M., Horadagoda, N., Perkins, N. R., Jeffcott, L. B., Little, C.B., and Dart, A. J. (2016a). Effect of Manuka honey gel on the transforming growth factor β1 and β3 concentrations, bacterial counts and histomorphology of contaminated full thickness skin wounds in equine distal limbs. Australian Veterinary Journal, 94(1–2), 27–34.

102. Eick, S., Schäfer, G., Kwieciński, J., Atrott, J., Henle, T., and Pfister, W. (2014). Honey - a potential agent against Porphyromonas gingivalis: an in vitro study. BMC oral health, 14, 24.

103. Wilson, C.R. (2013). Feline gangrenous mastitis. Can Vet J, 54, 292–294.

104. Gakuya, D.W., Mulei, C.M., and Wekesa, S.B. (2011). Use of ethnoveterinary remedies in the management of foot and mouth disease lesions in a dairy herd. Afr J Tradit Compl Altern Med, 8, 165–169.

105. Maarouf, A.A., Farag, E.A.H., and Fathy, L.A.M. (2018). Studies on endometritis in cows and honey as future alternative therapy. Animal Health Res. Institute, ARC. Available from: https://www.researchgate.net/publication/328319836_STUDIES_ON_ENDOMETERITIS_IN_COWS_AND_HONEY_AS_FUTURE_ALTERNATIVE_THERAPY_BY_Maarouf_AA_EnasAHFarag_and_LamyaAMFathy_Benha_Provincial_Lab_Animal_Health_Res_Institute_ARC

106. Addul-Hafeez, M.M., Abdul-Kadder, H.A., Sayed, A.M., and Shehata, S.H. (2019). Intrauterine honey infusion in Holstein Frisian cows with purulent endometritis. Int J Complement Alt Med, 12(1), 53-57.

107. Abidine, K.Z., and Bouabdellah, B. (2018). Diagnosis and treatment of endometritis with intra-uterine infusion of a solution of honey 70% in mares. J Vet Sci Technol, 9(1).

108. Hermanns, R., Mateescu, C., Thrasyvoulou, A., Tananaki, C., Wagener, F.A.D.T.G., and Cremers, N.A.J. (2020) Defining the standards for medical grade honey, Journal of Apicultural Research, 59:2, 125-135.

109. Khiati, B., Bacha, S., Aissat, S., and Ahmed, M. (2014). The use of Algerian honey on cutaneous wound healing: A case report and review of the literature. Asian Pac J Trop Dis, 4 (supply2), S867-S869.

110. Ali, M.M. and Radad, K. (2011). Cod liver oil/honey mixture: An effective treatment of equine complicated lower leg wounds. Vet. World, 4(7), 304-310.

111. Gustafsson, K., Tatz, A. J., Slavin, R. A., Sutton, G. A., Dahan, R., Ahmad, W. A., and Kelmer, G. (2021). Intraincisional medical grade honey decreases the prevalence of incisional infection in horses undergoing colic surgery: A prospective randomised controlled study. Equine veterinary journal, 53(6), 1112–1118.