The Veterinary Journal 199 (2014) 110–114

Contents lists available at ScienceDirect

The Veterinary Journal

journal homepage: www.elsevier.com/locate/tvjl

The antimicrobial activity of honey against common equine wound bacterial isolates

R. Carnwath [a], E.M. Graham [b], K. Reynolds [b], P.J. Pollock [a,*]

[a] Weipers Centre for Equine Welfare, Division of Large Animal Sciences and Public Health, School of Veterinary Medicine, University of Glasgow, 464 Bearsden Road, G61 1QH Glasgow, Scotland, United Kingdom
[b] Division of Veterinary Biosciences, School of Veterinary Medicine, University of Glasgow, 464 Bearsden Road, G61 1QH Glasgow, Scotland, United Kingdom

ARTICLE INFO

Article history:
Accepted 4 July 2013

Keywords:
Honey
Bees
Equine
Bacteria
Wound
Healing
Resistance

ABSTRACT

Delayed healing associated with distal limb wounds is a particular problem in equine clinical practice. Recent studies in human beings and other species have demonstrated the beneficial wound healing properties of honey, and medical grade honey dressings are available commercially in equine practice. Equine clinicians are reported to source other non-medical grade honeys for the same purpose. This study aimed to assess the antimicrobial activity of a number of honey types against common equine wound bacterial pathogens. Twenty-nine honey products were sourced, including gamma-irradiated and non-irradiated commercial medical grade honeys, supermarket honeys, and honeys from local beekeepers. To exclude contaminated honeys from the project, all honeys were cultured aerobically for evidence of bacterial contamination. Aerobic bacteria or fungi were recovered from 18 products. The antimicrobial activity of the remaining 11 products was assessed against 10 wound bacteria, recovered from the wounds of horses, including methicillin resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Eight products were effective against all 10 bacterial isolates at concentrations varying from <2% to 16% (v/v). Overall, the Scottish Heather Honey was the best performing product, and inhibited the growth of all 10 bacterial isolates at concentrations ranging from <2% to 6% (v/v).

Although Manuka has been the most studied honey to date, other sources may have valuable antimicrobial properties. Since some honeys were found to be contaminated with aerobic bacteria or fungi, non-sterile honeys may not be suitable for wound treatment. Further assessment of gamma-irradiated honeys from the best performing honeys would be useful.

Introduction

Substances produced by Honey bees (*Apis mellifera*), including propolis, honey, wax and venom have been used for their medicinal properties throughout history. However, it is the potential wound-healing benefit of honey that has been the primary focus of interest in recent times (Allen et al., 1991). In human beings, research into the therapeutic effect of honey has largely focused on its antimicrobial properties, which are attributed to many factors including acidity, hydrogen peroxide content, osmolarity and phytochemical components (Moore et al., 2001). In addition to inhibiting microbial growth, some of these factors may also have a role to play in controlling inflammation and promoting the healing process through the modulation of cytokines, fibroblast proliferation and angiogenesis (Tonks et al., 2003).

Many varieties of honey are available, differing in constitution and quality between types, and even between batches (French et al., 2005). Some of this variation is due to the type of plant from which the nectar and pollen is collected, the country of origin, and the method of production. The most commonly used medicinal honey is produced by bees foraging Manuka plants (*Leptospermum scoparium*), native to Australia and New Zealand. Manuka honey is believed to have superior antimicrobial properties due to factors other than hydrogen peroxide content. These factors may be due to an as yet poorly understood set of phytochemical properties, and/or to the presence of methylglyoxal, which is derived from dihydroacetone in the nectar of the Manuka flower (Mavric et al., 2008). This non-peroxide property of Manuka honey has been classified as the Unique Manuka Factor (UMF), which is determined by comparison to a standard phenol concentration (Snow and Manley-Harris, 2004).

Equine wounds, particularly those involving the distal portion of the limbs, often undergo prolonged complex healing and may enter a non-healing state with obvious financial and welfare implications. There are many factors which lead to delayed wound healing in horses, and among the most common of these is infection (Hendrickson, 2012). Many chronic equine wounds heal by

* Corresponding author. Tel.: +44 141 330 5999.
E-mail address: patrick.pollock@glasgow.ac.uk (P.J. Pollock).

1090-0233/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.tvjl.2013.07.003

second intention and it has been suggested that honey should allow for a better quality of wound repair as it stimulates the initial inflammatory response in leukocytes, increasing the production of cytokines that modulate fibroblast proliferation and angiogenesis (Tonks et al., 2003; Bischofberger et al., 2011).

The protocol for using honey to manage wounds in veterinary practice is highly variable. Some clinicians purchase inexpensive honeys intended for human consumption, and others opt to use standardised, medical grade, gamma-irradiated Manuka honey which in some cases has been incorporated directly into a wound care product or dressing. Since most research studies have been based on medical grade Manuka honey, the properties of other types of honey and bee products are poorly understood.

The aim of this study was to determine the effect of a number of different types, sources and preparations of uncontaminated honey on the growth of common equine wound bacterial pathogens.

Materials and methods

Approval for the project was granted from the Ethics and Welfare Committee at the School of Veterinary Medicine, University of Glasgow.

Honey samples

In total, 28 individual honeys and one commercial sugar solution (Honey Bee Feed) were obtained from a variety of sources ranging from commercial sources of medical grade honey, supermarkets or local beekeepers, and products were refrigerated prior to use (Table 1). Gamma-irradiated and non-irradiated preparations of the same medical grade honey were available and tested separately (Medical Brand 1). All 29 products were cultured aerobically on 5% sheep blood agar and MacConkey agar (E & O Laboratories) overnight at 37 °C; any contaminated honeys were excluded from the second part of the study.

Table 1
Source and culture results of products tested.

	Products tested	Source	Aerobic contamination	
1	Medical brand 1 Manuka honey sterilised (gamma-irradiated)	Manufacturer	No	
2	Medical brand 1 Manuka honey non-sterile (non-irradiated)	Manufacturer	No	
3	Medical brand 2 Manuka honey	Manufacturer	No	
4	Manuka honey 20+	Shop bought	No	
5	Manuka 10+	Shop bought	No	
6	Manuka 5+	Shop bought	Yes	*Bacillus* spp.
7	Heather honey (local)	Bee keeper	No	
8	Heather honey (local)	Shop bought	Yes	*Bacillus* spp.
9	Heather honey	Shop bought	Yes	*Bacillus* spp.
10	Blossom honey	Shop bought	No	
11	Clover honey	Shop bought	Yes	*Bacillus* spp.
12	Orange Blossom honey	Shop bought	Yes	*Bacillus* spp.
13	Lime honey	Shop bought	Yes	*Bacillus* spp.
14	Vipers Bugloss honey	Shop bought	No	
15	Inverness floral (from hive frame)	Bee keeper	No	
16	Inverness floral (from jar)	Bee keeper	Yes	*Bacillus* spp.
17	Glasgow floral (derived from jar)	Bee keeper	No	
18	Supermarket honey 1	Shop bought	Yes	*Bacillus* spp.
19	Supermarket honey 2	Shop bought	Yes	*Bacillus* spp.
20	Supermarket honey 3	Shop bought	Yes	*Bacillus* spp.
21	Supermarket honey 4	Shop bought	Yes	*Enterobacteriaceae* spp.
22	North African Thyme honey	Shop bought	Yes	*Bacillus* spp.
23	North African Coriander honey	Shop bought	Yes	*Bacillus* spp.
24	North African Eucalyptus honey	Shop bought	Yes	*Bacillus* spp.
25	North African Lavender honey	Shop bought	Yes	*Bacillus* spp.
26	North African Ziziphus honey	Shop bought	Yes	*Proteus* spp.
27	North African Euphobia honey	Shop bought	Yes	Fungus
28	Middle Eastern honey	Shop bought	Yes	*Bacillus* spp.
29	Sugar solution (commercial bee winter feed)	Shop bought	No	

Microbe collection and characterisation

The 10 bacterial isolates selected for testing came from a variety of sources, including the wounds of horses presented to the Weipers Centre Equine Hospital at the University of Glasgow; healthy skin samples from a group of horses at livery; equine wounds presented to external veterinary practitioners, and from the University Veterinary Hospital, University College Dublin (Table 2). All samples submitted to the University of Glasgow were cultured aerobically on 5% sheep blood agar and MacConkey agar for 48 h. Isolates were identified by their morphology and analytical profile index (API; BioMérieux,) testing. An antibiogram for each isolate was generated using the disc diffusion method on Diagnostic Sensitivity Test agar (DST; E & O Laboratories); antimicrobial susceptibility discs were sourced from Oxoid. Isolates were stored at −80 °C using a commercial microbead preservation system (Pro-lab Diagnostics).

Preparation of honey-agar solution

The method we used was slightly modified from that described by Cooper et al. (2002). The density of all honeys was assumed to be 1.37 g/mL (Cooper et al., 2002), and only uncontaminated honeys were selected. Double-strength nutrient agar solution was prepared, sterilised and held at 50 °C in a water bath. A 32% (v/v) solution of each honey was prepared in sterile distilled water (dH_20) using aseptic techniques. If required, honeys were dissolved using a sterile magnetised stirrer at 37 °C. Serial dilutions of each honey were prepared in sterile dH_2O at 4% increments (28–4% v/v), and mixed with an equal volume of double-strength nutrient agar. A final volume of 20 mL was poured into each of 3 × 90 mm labelled Petri dishes, which were left to dry. The final concentrations of honey used in the study were 16%, 14%, 12%, 10%, 8%, 6%, 4% and 2% (v/v). The final concentrations of sugar solution used were 45%, 40%, 35%, 30%, 25%, 20%, 15%, 10% and 5% (v/v).

Preparation and inoculation of bacterial isolates

Ten bacterial isolates were used in this study; the origin and antibiogram of each isolate is shown in Table 2. To prepare samples for inoculation, each isolate was recovered from a microbead, streaked onto nutrient agar plates and incubated aerobically at 37 °C for 18–24 h. For each isolate, between three and five colonies were selected and transferred into a sterile glass-capped tube containing dH_20 and vortexed. The turbidity was adjusted to a 0.5 McFarland standard (BioMérieux; 1.5×10^8 cfu/mL), and confirmed using a colorimeter (Viek). A suspension of each isolate was prepared (1.5×10^7 cfu/mL) and used within 30 min. The honey-agar plates were inoculated in duplicate with 10 isolates in 1 μL volumes each containing 1.5×10^4 cfu organisms (Denley Multipoint Inoculator A400), and incubated aerobically overnight at 37 °C. An uninoculated plate at each honey concentration was used as a negative control to detect contamination. Three single-strength nutrient plates were inoculated with all 10 isolates as a positive growth control.

Interpretation of results

The plates were examined after 16–24 h culture, and the presence or absence of visible colony formation was recorded for each isolate at each honey concentration. The honey minimum inhibitory concentration (MIC) value was recorded as the lowest concentration of honey at which bacterial growth was absent. MIC values were obtained for each honey against each bacterial isolate. If an isolate was inhibited at the lowest concentration (2%), the MIC was recorded as <2%. Growth at the highest concentration tested i.e. 16%, meant that the MIC was recorded as >16%. The MIC values reported are the mean of four replicates except Heather and Inverness Floral honeys (two replicates).

Results

Bacterial contamination

Of the 29 products tested, 18 were contaminated with pure cultures of aerobic bacteria or fungi (Table 1). *Bacillus* spp. were recovered from 15 products, *Proteus* spp. was recovered from a single supermarket honey, an unidentified *Enterobacteriaceae* organism was recovered from a commercial North African honey, and an unidentified fungus was recovered from a second commercial North African honey.