

Efficacy of medical grade honey in the management of canine otitis externa – a pilot study

Emi Maruhashi, Berta São Braz, Telmo Nunes, Constança Pomba, Adriana Belas, José Henrique Duarte-Correia and Ana Mafalda Lourenço

Centre for Interdisciplinary Research in Animal Health (CIISA), Faculty of Veterinary Medicine, University of Lisbon, Avenida da Universidade Técnica, 1300-477 Lisboa, Portugal

Correspondence: Berta São Braz, Centre for Interdisciplinary Research in Animal Health (CIISA), Faculty of Veterinary Medicine, University of Lisbon, Avenida da Universidade Técnica, 1300-477 Lisboa, Portugal. E-mail: bsaobraz@fmv.ulisboa.pt

Background – The high prevalence of antimicrobial resistance within otic pathogens has created a need for alternative therapies of otitis externa (OE). Evidence suggests that medical grade honey (MGH) may be effective against drug-resistant pathogens.

Hypothesis/Objectives – The efficacy of a commercial MGH compound was assessed in an open clinical trial. We hypothesized that it would be an effective alternative to conventional treatments.

Animals – Client-owned dogs ($n = 15$) with a confirmed diagnosis of infectious OE were enrolled in this pilot study.

Methods – Dogs were prescribed MGH (1 mL daily per ear) until cure was achieved or for a maximum of 21 d. Evaluation was based on weekly clinical scores, cytological progression and owner assessments of pruritus. Swab samples were submitted for culture and susceptibility testing. MGH was tested for biocidal activity against the bacterial isolates.

Results – Medical grade honey promoted rapid clinical progress, with 70% of dogs achieving clinical cure between days 7 and 14 and over 90% having resolved by Day 21. There was a decrease in clinical scores throughout the duration of the trial ($P < 0.001$) and owner-assessed pruritus also decreased significantly ($P < 0.05$). *In vitro* assays of the biocidal activity of MGH showed activity against all bacterial isolates, including methicillin-resistant strains of *Staphylococcus pseudintermedius* (MRSP) and other species of drug-resistant bacteria.

Conclusion and clinical importance – Medical grade honey was successful in both clinical and laboratory settings, thus demonstrating its potential of becoming an alternative treatment for canine OE.

Introduction

The use of honey pre-dates mankind's knowledge of sugar. Its nonfood use throughout history has varied greatly among civilizations, ranging from ceremonial purposes to medicinal use as a drug and ointment.^{1,2} Western medicine has only recently turned its attention to this ancient medicinal use of honey. Protocols for the use of honey in wound management, for example, are highly variable and dependent on clinician preferences. Some practitioners purchase inexpensive honey intended for human consumption, whilst others opt for standardized irradiated medical grade honey (MGH). Although natural honey which originates from the comb has antibacterial properties, its potency is highly variable.^{3,4} Furthermore, utilization of honey that has not been subjected to gamma

irradiation poses the threat of introducing clostridial spores. Medical grade honey is also screened for traces of pesticides, herbicides, heavy metals and antibiotics used to treat diseases in bees.^{5–7} Additionally, and of relevance to the present study, concerns have been raised regarding the ototoxic potential of MGH.^{8,9}

Canine infectious otitis externa (OE) is a disease that often requires repeated and prolonged antimicrobial treatment regimens.¹⁰ The tendency towards chronicity and repeated therapy may thereby select for antimicrobial resistance.¹¹ The rapid increase in antimicrobial resistance creates the need for discovery and implementation of safe and effective alternative therapies. The predominant agents in canine OE include *Staphylococcus pseudintermedius*, which can be present in low numbers even in the normal ear, and the Gram-negative agents *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Klebsiella* spp. and *Escherichia coli*.¹² With regard to fungal agents, *Malassezia pachydermatis* is the most common pathogen but is regularly present in low numbers under normal conditions within the healthy external ear canal.¹³ Therefore, a positive culture does not necessarily indicate over-colonization or infection by *Malassezia*, and this assessment

Accepted 2 September 2015

Sources of funding: The product containing medical grade honey, Mesitran[®], was supplied as a free gift from the manufacturer Triticum, Maastricht, Netherlands.

Conflict of interest: No conflicts of interest have been declared.

is best made by cytological evaluation. Additionally, *Candida* spp. may occasionally be encountered.

The aim of this study was to assess the therapeutic efficacy of an MGH gel (L-Mesitran® Soft, Triticum; Maas-tricht, Netherlands) in the management of canine OE.

Materials and methods

Written informed consent was obtained from the owner of each dog prior to inclusion in the study, which was approved by the Committee for Ethics and Animal Welfare of the Faculty of Veterinary Medicine of the University of Lisbon (CEBEA).

A preliminary phase was conducted prior to the clinical trial in order to assess patient tolerance of MGH in the ear canal. Upon otoscopic evaluation and confirmation of intact tympanic membranes, MGH was applied to the ear canals of 10 healthy dogs owned by hospital staff and friends. The Short Form of the Glasgow Composite Measure Pain Scale was used to assess whether there was any discomfort associated with the application of MGH in the ear canal.¹⁴ Based on each of the six parameters assessed by this scale all of the scores obtained for each dog totalled 0, thus indicating 100% absence of discomfort. In this sense the administration of MGH proved to be tolerated by dogs at least as well as any other commonly prescribed otic therapy.

Study design

This was a prospective, open-label, nonblinded clinical trial, performed in accordance with the ethical guidelines established by our Faculty. In order to guarantee maximum safety and avoid potential risk of ototoxicity, only subjects with a diagnosis of OE which were confirmed to have visibly intact tympanic membranes were included in the study.

Subjects

Client-owned dogs of various breeds, which were 4 months of age or older and in general good health based on the initial physical examination at baseline (Day 0), were recruited. Animals were deemed eligible if they presented with OE of confirmed bacterial, fungal or mixed aetiology. Dogs with first-time or recurrent OE were eligible for inclusion and were enrolled without regard to the primary cause of otitis (such as endocrinopathy or allergic disease), as only management of the secondary infection was to be addressed. The diagnosis of OE was based on clinical aspects and cytological evaluation (presence of inflammatory cells and intracellular or abnormally large numbers of bacteria/yeast). In cases of bilateral OE, each ear was evaluated as one experimental unit. Dogs were excluded if they had been exposed to any of the following therapies: topical or systemic antifungal or antibacterial agents, glucocorticoids or ciclosporin within the prior 7 d; long-acting glucocorticoid injections within the prior three months; or an antiseptic topical product applied on the day of examination. Dogs with pyoderma, advanced stages of proliferative or occlusive otitis, parasitic otitis or presence of a foreign body, negative otic cytology, or evidence of a ruptured tympanic membrane (as confirmed by video otoscope exam) were excluded. Pregnant or lactating bitches were also excluded.

Treatment and monitoring

The Mesitran® product contains 40% MGH, medical grade hypoallergenic lanolin, propylene glycol, PEG 4000, and vitamins C and E (<http://www.l-mesitran.com/en/l-mesitran-soft>; accessed 05/01/2016); the product was supplied in pre-measured 1-mL syringes. The investigators administered the initial dose in the clinical setting to demonstrate the correct technique. Immediately after application, the ear was massaged to ensure uniform distribution throughout the external ear canal. Thereafter, owners applied 1 mL to each affected ear canal, once daily until clinical cure was achieved or until the end of the study at Day 21. Owners were instructed to refrain from use of any type of ear cleansing agent during the trial period. Following the baseline (enrolment) visit, dogs were scheduled for weekly

examinations, during which clinical scores, cytological examination of ear canal exudate and owner assessments were collected.

Clinical examination

The 0–3 Otitis Index Score (OTIS3) was utilized to monitor the clinical response to treatment and subjects were divided into cases of erythro-ceruminous or suppurative otitis based on the clinical signs and type of exudate present at enrolment.¹⁵ The score assesses erythema, oedema/swelling, erosion/ulceration, and exudate of both the horizontal and vertical ear canals, on a scale of 0–3. A total clinical score was calculated through the summation of the individual scores of each clinical sign at each weekly visit, which allowed for a maximum total score of 12, as depicted in Table 1.¹⁵ A prior study has shown that the most reliable cut-off score which differentiates clinically affected ears from healthy ears is ≥ 4 .¹⁵ Numerical scores were assigned at baseline to allow for weekly comparisons. During each visit the ears were examined and evaluated by the same investigator. In keeping with the previous study, a score of ≤ 3 was considered to be consistent with clinical success.¹⁵ Participants were instructed to report all adverse effects to the investigators.

Cytology assessment

A swab sample of the external ear canal was obtained at each visit for cytological examination. The content was smeared as a thin layer on a glass slide and the Diff-Quik® (Main S.L.; Barcelona, Spain) procedure was used. Mean counts of *M. pachydermatis* of ≤ 2 yeast cells per high-power dry field (400 \times) or bacteria with mean counts of ≤ 5 bacterial cells per field were considered to be normal. Mean counts of ≥ 5 yeast cells per high-power dry field or ≥ 25 bacterial cells per field were considered to be abnormal.¹⁶ Regardless of the specificity of these intervals in predicting pathological effect, the assessment of a pathogenic role for these organisms required consideration of other clinical criteria.¹⁶ Therefore, mean values lying between these stated intervals were considered to fall in the “grey-zone”.

Owner assessments

The subjective evaluation of otic pruritus by dog owners was considered to be a secondary outcome measure. Each owner indicated the intensity of their dog's pruritus on a 10-cm horizontal Visual Analog Scale (VAS). The scale at the left end (0) corresponded to “not itchy” and the right end (10) to “very itchy”.¹⁵ This assessment allowed the investigators to develop a sense for the effect of the treatment in the animals' home environments.

At the end-point of the trial, owners were asked to complete a five question survey. Aspects such as overall satisfaction with the treatment, ease of application and comparison to previously used treatments for the same problem were evaluated. Responses were graded on a five-point scale ranging from “very satisfactory” to “very unsatisfactory”. Additionally, owners were asked if they would perform the treatment again and whether they would recommend it to other pet owners. Although subjective, this enquiry was critical in revealing aspects in need of improvement for future trials and served as an indirect indication of owner compliance. Initiative was also taken to contact owners after the trial's conclusion, in order to assess the duration of resolution of clinical signs.

Antimicrobial culture and susceptibility testing

In cases of bacterial and/or mixed otitis, a sample was collected for bacteriological culture from each affected ear canal with a sterile swab. Isolation and characterization of bacterial species as well as determination of the minimum inhibitory concentrations of antimicro-

Table 1. Clinical signs and respective scores¹⁵

| | | | | |
|--------------------|---|---|---|---|
| Erythema | 0 | 1 | 2 | 3 |
| Oedema/swelling | 0 | 1 | 2 | 3 |
| Erosion/ulceration | 0 | 1 | 2 | 3 |
| Exudate | 0 | 1 | 2 | 3 |

0 = absent; 1 = slight; 2 = moderate; 3 = severe.

bial agents were conducted through the microdilution method in accordance with guidelines issued by the Clinical Laboratory Standards Institute.¹⁷

Biocidal activity testing

Bacterial isolates were tested against the MGH, in order to evaluate whether *in vitro* biocidal activity existed. The quantitative assay was conducted in accordance with the European standard NF EN 1040.¹⁸ All *in vitro* bacteriological testing was conducted by an in-house laboratory.

Withdrawal and clinical failure

Participating owners were entitled to withdraw their dogs from the trial at any time and for any reason. Such incidences were recorded as a study endpoint. Possible endpoints therefore included achievement of cure prior to Day 21, status at the Day 21 visit or date of withdrawal. Clinical failure was defined as persistence of OE beyond the pre-established time frame of 21 d, as defined by an OTIS3 score of >3. Cytological resolution was not a factor used to determine whether there was clinical cure or failure, because numbers may not always correlate with absence or presence of OE.

Statistical Analysis

Repeated Measures ANOVA, in addition to a *post hoc* test with Holm's correction, was used to evaluate the clinical scores across visits. Owner VAS scores were also analysed with the repeated measures ANOVA test. The statistical analysis was performed with R software (v3.1.2; ©2014 The R Foundation for Statistical Computing).

Results

Subjects

The 15 dogs enrolled in the study fulfilled the pre-established inclusion and exclusion criteria. Four of the 15 enrolled dogs presented with unilateral otitis, totalling 26 experimental units for evaluation. The enrolled dogs came from various home environments and presented differing histories in regard to clinical disease. Eight dogs had experienced recurrent episodes of OE prior to enrolment, whereas seven had first-time infections. Characteristics of the dogs and types of microbes associated with OE in each dog are presented in Table 2. Each subject's weekly scores were compared at the end of the trial. Some dogs

required less than 21 d of treatment, with three dogs requiring only 7 d to reach both clinical and cytological cure. The clinical scores and cytological results are detailed in Table 3.

Of the 15 dogs (26 ears) evaluated, one needed alternative treatment after completing the 21-d trial due to persistence of cocci and rods on cytology, as well as a corresponding clinical score of 4. In addition, one other dog was withdrawn by its owner due to difficulty in product administration. At the point of withdrawal on Day 14, this dog presented a score of 4 and abnormal cytology. The 13 remaining dogs showed resolution at various times throughout the stipulated trial period. Overall, 70% of the 15 original dogs achieved clinical cure between days 7 and 14, and over 90% had resolved by Day 21. Repeated measures ANOVA showed that there was a clear decrease in clinical scores throughout the trial duration ($P < 0.001$; Figure 1).

Cytology assessment

Of the 26 affected ears, a total of eight (30.7%) did not have low enough numbers of organisms, based on cytology, to meet the definition of 'normal'¹⁶ by the end of the trial, despite having achieved clinical cure using the definition of the clinical scoring method. Most ears/dogs required longer periods of time to achieve cytological cure in comparison with the time required to achieve clinical cure (Table 3).

Antimicrobial culture and biocidal activity testing

Isolation, speciation and antimicrobial susceptibility testing revealed a diverse group of bacterial agents, all of which have been reported as secondary causes of OE (summarised in Table 4).^{19,20} The MGH demonstrated clear biocidal activity within the first 5 min (± 10 s) of contact against all bacterial isolates *in vitro*.

Owner assessment

Owner-assessed VAS scores decreased over time ($P < 0.05$; see Figure S1); VAS scores were not obtained

Table 2. Details of the otitis condition for each canine subject

| Subject | Ear type | Aetiology | Agent | Inflammatory cells (upon presentation) | Classification | Location | History |
|---------|----------|-----------|---|--|-------------------|------------|---------------|
| 1 | E | B | <i>Staphylococcus pseudintermedius</i> | No | Erythroceruminous | Bilateral | Recurrent |
| 2 | P | F | <i>Malassezia pachydermatis</i> | No | Erythroceruminous | Bilateral | Recurrent |
| 3 | E | M | <i>S. pseudintermedius</i> | No | Erythroceruminous | Bilateral | First episode |
| 4 | P | B | <i>Enterococcus faecalis</i> | Yes | Suppurative | Bilateral | Recurrent |
| 5 | P | M | MRSP | No | Erythroceruminous | Unilateral | First episode |
| 6 | P | M | <i>S. pseudintermedius</i> , <i>Klebsiella pneumoniae</i> , <i>Enterococcus cloacae</i> | Yes | Erythroceruminous | Bilateral | Recurrent |
| 7 | P | F | <i>M. pachydermatis</i> | No | Erythroceruminous | Unilateral | First episode |
| 8 | P | F | <i>M. pachydermatis</i> | No | Erythroceruminous | Bilateral | First episode |
| 9 | P | B | MRSP | No | Erythroceruminous | Bilateral | Recurrent |
| 10 | P | F | <i>M. pachydermatis</i> | No | Erythroceruminous | Bilateral | Recurrent |
| 11 | P | F | <i>M. pachydermatis</i> | No | Erythroceruminous | Bilateral | Recurrent |
| 12 | P | F | <i>M. pachydermatis</i> | No | Erythroceruminous | Unilateral | First episode |
| 13 | P | F | <i>M. pachydermatis</i> | No | Erythroceruminous | Bilateral | Recurrent |
| 14 | E | F | <i>M. pachydermatis</i> | No | Erythroceruminous | Unilateral | First episode |
| 15 | E | B | <i>Pseudomonas aeruginosa</i> | No | Erythroceruminous | Bilateral | First episode |

E, Erect; P, Pendulous; R, Right; L, Left; F, Fungal; B, Bacterial; M, Mixed; MRSP, methicillin-resistant *Staphylococcus pseudintermedius*.

Table 3. Summary of the weekly clinical and cytology scores for 26 canine ears initially presenting with otitis externa

| Animal | Type | Day 0 | Cytology | Day 7 | Cytology | Day 14 | Cytology | Day 21 | Cytology |
|--------|------|-------|----------|-------|----------|--------|----------|--------|----------|
| 1 | B | 6 | Abnormal | 5 | Abnormal | 2 | Grey | 1 | Normal |
| | B | 6 | Abnormal | 4 | Grey | 2 | Grey | 1 | Normal |
| 2 | F | 5 | Abnormal | 4 | Abnormal | 2 | Abnormal | 1 | Normal |
| | F | 7 | Abnormal | 4 | Grey | 2 | Grey | 1 | Normal |
| 3 | M | 4 | Abnormal | 0 | Grey | 0 | Normal | – | – |
| | M | 4 | Abnormal | 0 | Grey | 0 | Normal | – | – |
| 4 | B | 5 | Abnormal | 3 | Grey | 1 | Normal | – | – |
| | B | 10 | Abnormal | 5 | Grey | 1 | Normal | – | – |
| 5 | M | 6 | Abnormal | 1 | Normal | – | – | – | – |
| 6 | M | 4 | Abnormal | 2 | Abnormal | 1 | Normal | – | – |
| | M | 6 | Abnormal | 3 | Abnormal | 1 | Normal | – | – |
| 7 | F | 4 | Abnormal | 1 | Abnormal | 1 | Grey | 0 | Normal |
| 8 | F | 4 | Abnormal | 1 | Abnormal | 1 | Abnormal | 1 | Abnormal |
| | F | 6 | Abnormal | 1 | Abnormal | 1 | Abnormal | 1 | Abnormal |
| 9 | B | 7 | Abnormal | 4 | Abnormal | 4 | Abnormal | 4 | Clinical |
| | B | 4 | Abnormal | 3 | Abnormal | 2 | Abnormal | 2 | Failure |
| 10 | F | 5 | Abnormal | 3 | Abnormal | 2 | Abnormal | 1 | Abnormal |
| | F | 4 | Abnormal | 3 | Abnormal | 1 | Abnormal | 1 | Abnormal |
| 11 | F | 8 | Abnormal | 3 | Abnormal | 2 | Abnormal | 2 | Abnormal |
| | F | 7 | Abnormal | 3 | Abnormal | 2 | Abnormal | 1 | Abnormal |
| 12 | F | 4 | Abnormal | 0 | Normal | – | – | – | – |
| 13 | F | 6 | Abnormal | 2 | Abnormal | 1 | Abnormal | 1 | Abnormal |
| | F | 4 | Abnormal | 2 | Abnormal | 1 | Abnormal | 1 | Abnormal |
| 14 | F | 8 | Abnormal | 5 | Abnormal | 4 | Drop-out | – | – |
| | M | 4 | Abnormal | 0 | Normal | – | – | – | – |
| 15 | M | 4 | Abnormal | 0 | Normal | – | – | – | – |

F, Fungal; B, Bacterial; M, Mixed; –, animals did not require further evaluation; Abnormal: ≥ 5 yeast cells, ≥ 25 bacteria per high-power dry field;¹⁶ Normal: ≤ 2 yeast cells, ≤ 5 bacteria per high-power dry field;¹⁶ Grey, Grey zone (3–4 yeast cells, 6–24 bacteria per high-power dry field). Clinical failure/withdrawal highlighted in red; clinical and cytological cure highlighted in grey.

from some owners whose dogs lived mostly outdoors or in cases in which different owners attended the weekly visits. Of the 11 dogs from which this information was obtained, four had demonstrated 50% reduction in pruritus by the end of treatment and seven demonstrated at least 90% reduction. Furthermore, 75% of the initial 15 owners indicated that they were “very satisfied” with the treatment, whereas the remaining 25% considered it “satisfactory”. All owners reported that they would repeat the treatment as well as recommend it to others.

Follow-up

With the exclusion of two cases (one clinical failure and one withdrawal), contact was maintained for at least

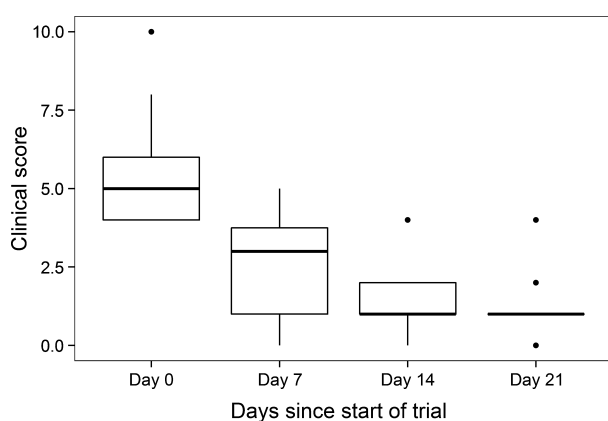


Figure 1. Clinical score evolution over time. Bars represent median score; boxes include 25th to 75th percentile; the whiskers represent 1.50 * (or x) the interquartile range (IQR); circles signify outliers.

1 month after the study completion with all pet owners, at which point none of the dogs had relapsed. Shortly after this initial month, one dog developed signs of otitis according to the owner, as did two other dogs by the 2-month point of contact. Two additional dogs had been lost to follow-up by this time. The remaining eight dogs remained normal for 3–5 months, beyond which contact with owners was discontinued.

Discussion

The use of MGH demonstrated overall effectiveness in the management of OE in the population studied. The rapid onset of clinical and cytological improvement, positive owner evaluations, *in vitro* biocidal results and durability of clinical resolution during follow-up is encouraging. It is also noteworthy that success was attained against multi-drug resistant bacteria in some cases. As with many products applied within the ear, there remains some concern regarding the ototoxic potential of MGH. Prior studies in which manuka honey was applied trans-tympanically in chinchillas concluded that a 4% concentration was nontoxic to cochlear cells, whereas a 50% concentration was severely toxic.^{8,9} Evaluation of the transtympanic effect of MGH will require additional studies and was outside the scope of this clinical trial. Therefore, subjects were enrolled only after careful video otoscopic examination to confirm the presence of intact tympanic membranes.

As shown in this study population, the cytological resolution of OE may lag behind clinical resolution as defined by the OTIS3 scoring system. By Day 21, four of 15 dogs,

Table 4. Antimicrobial culture and susceptibility

| Antibacterial agents | Bacterial Isolates | | | | | | | | |
|-------------------------------|--------------------|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| Fusidic Acid | S | S | S | S | – | – | S | S | S |
| Amoxicillin/Clavulanic acid | S | R | R | S | R | R | S | R | R |
| Ampicillin | R | R | R | R | R | R | R | R | R |
| Cefalotin | – | – | – | – | S | R | S | – | – |
| Cefotaxime | – | – | – | – | S | R | I | – | – |
| Clindamycin | S | R | R | S | – | – | – | S | S |
| Enrofloxacin | S | R | S | S | S | I | S | S | S |
| Erythromycin | S | R | I | S | – | – | – | I | I |
| Oxacillin | S | – | S | S | – | – | – | R | R |
| Gentamicin | S | R | S | S | S | S | S | S | S |
| Penicillin | R | R | R | R | – | – | – | R | R |
| Sulfamethoxazole/Trimethoprim | S | S | S | S | S | S | S | S | S |
| Tetracycline | S | R | S | S | S | I | S | R | R |
| Chloramphenicol | – | – | – | – | – | – | – | S | S |
| Florfenicol | – | – | – | – | – | – | – | S | S |
| Amikacin | – | – | – | – | – | – | – | S | S |

1, *Staphylococcus pseudintermedius*; 2, *Enterococcus faecalis*; 3, *S. pseudintermedius*; 4, *S. pseudintermedius*; 5, *Klebsiella pneumoniae*; 6, *Enterobacter cloacae*; 7, *Pseudomonas aeruginosa*; 8, MRSP, meticillin-resistant *S. pseudintermedius*; 9, MRSP; S, Susceptible; I, Intermediate; R, Resistant.

or eight of 26 ears, had not achieved cytological counts within the normal range despite having achieved clinical scores of ≤ 3 . The owners of these dogs also reported lack of clinical signs associated with OE in the home environment. Due to continuously improving cytology counts, it may be hypothesized that cytological resolution would have occurred with a trial period greater than 21 d. Only one dog without cytological resolution required additional therapy for persistent clinical signs following the 21-d trial period. In a prior study, cytology counts also decreased with successful therapy but neither adequately differentiated healthy from affected ears, nor correlated with clinical success.¹⁵ As a consequence of the multifactorial pathogenesis of canine OE, it may not always be possible to directly correlate cytology counts and clinical indicators of pathogenic effect.¹⁶

The effect of MGH against multi-drug resistant bacteria will require additional study. In this case population, MRSP was isolated from two dogs and a multi-drug resistant *E. faecalis* was isolated from another. Of these, one MRSP case experienced clinical failure despite a trend for improvement during the trial. This was an elderly dog with a life-long history of recurrent otitis, which had been subjected to numerous treatments. Despite failure according to the study parameters, the owner of this dog requested additional treatment with MGH two months after conclusion of the trial, reporting that it had been the most satisfactory therapy used to date. In the other case with an MRSP isolate (a first-time episode of unilateral OE), resolution was obtained by Day 7.

Although pet owners were generally quite satisfied with their pets' tolerance of MGH application, an inconvenient aspect of this product is the accumulation of sticky residue within the canal and on the pinna. The product was not formulated with the intention of otic application. A standard dose of 1 mL was used for all cases regardless of body size, but tailoring volume for smaller dogs may be helpful to reduce accumulation of residue. Some owners found it useful to cleanse the pinnae with cotton

moistened with water so as to remove the residue, whereas others resorted to bathing at the end of treatment. Due to the consistency and colour of MGH, which is highly similar to that of ear cerumen, it was sometimes difficult for the investigators to distinguish between the two during treatment. However, the distinctly sweet odour of the product does help to differentiate it from malodorous ceruminous exudate.

Weaknesses of this trial include the small sample size and lack of use of a control treatment with randomization. Although blinding would have been ideal for this study, intrinsic characteristics of this particular product would make it difficult, if not impossible to do so. In addition, the study population was heterogeneous with regards to primary disease processes and chronicity of disease. However, to the best of the authors' knowledge this is the first report of the use of MGH for treatment of canine OE. The initial positive experience within the setting of a pilot study suggests that MGH deserves more rigorous evaluation in a large-scale randomized, controlled trial.

Acknowledgements

The authors would like to thank staff of the Teaching Hospital of the Faculty of Veterinary Medicine, University of Lisbon, and in particular Lúcia Sales, Joana Cardoso and Gonçalo Vicente, for their involvement in the study.

References

1. Mandal DM, Mandal S. Honey: its medicinal property and antibacterial activity. *Asian Pac J Trop Biomed* 2011; 1: 154–160.
2. Hajar R. History of Medicine. *Heart Views* 2002; 3: 10. Available at: http://www.heartviews.org/article.asp?issn=1995-705X; year=2002; volume=3; issue=4; spage=10; epage=10; aulast=Hajar; aid=HeartViews_2002_3_4_10_64525. Accessed Jan 5, 2016.
3. Carnwath R, Graham EM, Reynolds K et al. The antimicrobial activity of honey against common equine wound bacterial isolates. *Vet J* 2014; 199: 110–114.

4. George NM, Cutting KF. Antibacterial honey: in-vitro activity against clinical isolates of MRSA, VRE, and other multi-resistant Gram-negative organisms. *Wounds* 2007; 19: 231–236.
5. Merckoll P, Jonassen TO, Vad ME et al. Bacteria, biofilm and honey: a study of the effects of honey on 'planktonic' and biofilm-embedded chronic wound bacteria. *Scand J Infect Dis* 2009; 41: 341–347.
6. Creemers T, Bosma JW. Honey based wound ointment for wound healing and skin disorders with animals. *Dier en Arts (Anim and Vet)* 2006; 1–4. Available at: www.bfactory.nl/artikelen/Vetramil%20publication%20english.pdf. Accessed Jan 5, 2016.
7. Stobberingh EE, Vandersanden G. The anti-bacterial activity of honey-based ointment against antibiotic resistant *Staph. aureus* and *Ps. aeruginosa* (in-vitro, clinical isolates). *L-Mesitran® Clinical Data File* 2010; 4–8. Available at: [http://www.klinion.nl/files/files/Stobberingh%20EE%20-%20Activity%20of%20L-Mesitran%20Ointment%20and%20Soft%20against%20\(antibiotic%20resistant\)%20bacteriae%20-%20December%202010.pdf](http://www.klinion.nl/files/files/Stobberingh%20EE%20-%20Activity%20of%20L-Mesitran%20Ointment%20and%20Soft%20against%20(antibiotic%20resistant)%20bacteriae%20-%20December%202010.pdf). Accessed Jan 5, 2016.
8. Aron M, Akinpelu V, Dorion D et al. Otologic safety of manuka honey. *J Otolaryngol Head Neck Surg* 2012; 41(Suppl.1): S21–S30.
9. Aron M, Akinpelu OV, Gasbarrino K et al. Safety of transtympanic application of 4% manuka honey in a chinchilla animal model. *Eur Arch Otorhinolaryngol* 2015; 272: 537–542.
10. Guardabassi L, Schwarz S, Lloyd DH. Pet animals as reservoirs of antimicrobial-resistant bacteria. *J Antimicrob Chemother* 2004; 54: 321–332.
11. Pedersen K, Pedersen K, Jensen H et al. Occurrence of antimicrobial resistance in bacteria from diagnostic samples from dogs. *J Antimicrob Chemother* 2007; 60: 775–781.
12. Rougier S, Borell D, Pheulpin S et al. A comparative study of two antimicrobial/anti-inflammatory formulations in the treatment of canine otitis externa. *Vet Dermatol* 2005; 16: 299–307.
13. Rosser EJ. Causes of otitis externa. *Vet Clin North Am Small Anim Pract* 2004; 34: 459–468.
14. University of Glasgow School of Veterinary Medicine website. Short Form of the Glasgow Composite Measure Pain Scale. Available at: <http://www.gla.ac.uk/schools/vet/sah/services/anaesthesiaandpainmanagement>. Accessed on Oct 5, 2014.
15. Nuttall T, Bensignor E. A pilot study to develop an objective clinical score for canine otitis externa. *Vet Dermatol* 2014; 25: 530–537, e91–2.
16. Ginel PJ, Lucena R, Rodriguez JC et al. A semiquantitative cytological evaluation of normal and pathological samples from the external ear canal of normal and pathological samples from the external ear canal of dogs and cats. *Vet Dermatol* 2002; 13: 151–156.
17. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals: Second Informational Supplement. CLSI document VET01-S2. Wayne, PA: CLSI, 2003.
18. NF EN 1040: Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of bactericidal activity-based antiseptics and chemical disinfectants – Test methods and requirements (Phase 1) (Classification index: T 72-152) (April 2006) [Translated from the French]. Available at: http://standards.cen.eu/dyn/www/f?p=204:110:0:::FSP_PROJECT,FSP_ORG_ID:24648,6197&cs=1EB2972DA121C88E0B4A406B8C4F33242. Accessed Jan 5, 2016.
19. Malayeri HZ, Jamshidi S, Salehi TZ. Identification and antimicrobial susceptibility patterns bacteria causing otitis externa in dogs. *Vet Res Commun* 2010; 35: 435–444.
20. Hariharan H, Coles M, Poole D et al. Update on antimicrobial susceptibilities of bacterial isolates from canine and feline otitis externa. *Can Vet J* 2006; 47: 253–255.

Supporting Information

Additional Supporting Information may be found in the online version of this article.

Figure S1. Owner-assessed VAS scores over time.

Résumé

Contexte – La haute prévalence de la résistance antimicrobienne des pathogènes auriculaires a créé un besoin de traitements alternatifs des otites externes (OE). La médecine basée sur les preuves suggère que le MGH (medical grade honey) peut être efficace contre les pathogènes résistants.

Hypothèses/Objectifs – L'efficacité d'un composé commercial de MGH a été évaluée dans une étude clinique ouverte. Nous supposons qu'il s'agit d'une alternative utile aux traitements conventionnels.

Sujets – Des chiens de propriétaires ($n = 15$) avec un diagnostic confirmé d'OE infectieuse ont été inclus dans cette étude pilote.

Méthodes – Les chiens ont reçus du MGH (1 mL par jour par oreille) jusqu'à guérison ou pour un maximum de 21 jours. L'évaluation était basée sur les scores cliniques hebdomadaires, l'évolution cytologique et l'estimation du prurit par les propriétaires. Les écouvillons étaient soumis pour culture et test de sensibilité. L'activité biocide de MGH a été évalué contre es souches bactériennes isolées.

Résultats – Le MGH a permis une progression clinique rapide avec 70% de chiens guéris entre 7 et 14 jours et plus de 90% à jour 21. Il y avait une diminution du score clinique au long de la durée de, l'essai ($P < 0.001$) et l'estimation du score de prurit par les propriétaires a également diminué significativement ($P < 0.05$). Les tests *in vitro* de l'activité biocide de MGH ont montré une activité contre toutes les bactéries isolées y compris les souches résistantes à la méticilline de *Staphylococcus pseudintermedius* (MRSP) et les autres espèces de bactéries résistantes.

Conclusion et importance clinique – Le MGH a été efficace à la fois sur les données cliniques et de laboratoire, démontrant ainsi son intérêt dans les traitements alternatifs des OE canines.

Resumen

Introducción – la alta prevalencia de resistencia antimicrobiana con patógenos del oído ha creado la necesidad de terapias alternativas para los tratamientos de la otitis externa (OE). Las evidencias sugieren que la miel de grado médico (MGH) puede ser efectiva contra los patógenos resistentes a algunos antibióticos.

Hipótesis/Objetivos – la eficacia de un compuesto comercial con miel de grado médico fue evaluada en ensayo clínico abierto. Nuestra hipótesis era que sería una alternativa efectiva a los tratamientos convencionales.

Animales – perros de propietarios privados (n = 15) con diagnóstico confirmado de otitis externa infecciosa fueron incluidos en este estudio piloto.

Métodos – a los perros se les recetó miel de grado médico (1 ml al día por cada oído) hasta que se obtuvo la cura, o durante un máximo de 21 días. La evaluación se basó en valoración clínica semanal, progresión citológica, y prurito evaluado por los propietarios. Se remitieron muestras de hisopos para cultivo y pruebas de susceptibilidad. La miel de grado médico fue probada por su actividad biocida contra los aislados bacterianos.

Resultados – la aplicación de miel de grado médico produjo una mejora clínica rápida, con 70% de los perros obteniendo cura completa entre los días 7 y 14, y más de un 90% obteniendo resolución para el día 21. Hubo una disminución en los valores clínicos durante la duración de la prueba ($P < 0,001$) y el prurito evaluado por los propietarios también disminuyó significativamente ($P < 0,05$). Los ensayos *in vitro* de la actividad y biocida de la miel de grado médico mostraron actividad frente a los aislados bacterianos, incluyendo cepas resistentes a la meticilina de *Staphylococcus pseudintermedius* (MRSP) y otras especies de bacterias resistentes a fármacos.

Conclusión e importancia clínica – la miel de grado médico tuvo éxito tanto en las pruebas clínicas como laboratoriales, demostrando su potencial para convertirse en un tratamiento alternativo para la otitis externa canina.

Zusammenfassung

Hintergrund – Die hohe Prävalenz von antimikrobieller Resistenz bei pathogenen Keimen in den Ohren hat den Bedarf an alternativen Behandlungsmöglichkeiten bei Otitis externa (OE) notwendig gemacht. Es gibt Evidenz, dass medizinischer Honig (MGH) wirksam gegenüber Medikamenten-resistenten pathogenen Keimen sein kann.

Hypothese/Ziele – Die Wirksamkeit einer kommerziellen MGH Verbindung wurde in einer offenen klinischen Studie untersucht. Wir stellten die Hypothese auf, dass es sich dabei um eine effektive Alternative zu konventionellen Behandlungen handeln könnte.

Tiere – Hunde in Privatbesitz (n=15) mit einer bestätigten Diagnose einer infektiösen OE wurde in die Pilotstudie aufgenommen.

Methoden – Den Hunden wurde MGH (1ml pro Ohr täglich) bis zur Heilung oder für maximal 21d verschrieben. Die Evaluierung basierte auf wöchentlicher klinischer Beurteilung, zytologischem Fortschritt und der Beurteilung des Juckreizes durch die BesitzerInnen. Es wurden Tupfer zur Bakterienkultur und zum Antibiogramm eingeschickt. Der MGH wurde auf seine biozide Wirksamkeit gegenüber den bakteriellen Isolaten getestet.

Ergebnisse – Der medizinische Honig bewirkte einen raschen klinischen Fortschritt, wobei bei 70% der Hunde eine klinische Heilung zwischen den Tagen 7 und 14 auftrat und zum Tag 21 über 90% ausgeheilt waren. Es gab eine Verminderung der klinischen Werte während dem gesamten Versuch ($P < 0,001$) und der durch die BesitzerInnen beurteilte Juckreiz nahm ebenfalls signifikant ab ($P < 0,05$). *In vitro* Tests zur bioziden Aktivität des MGH zeigten Wirksamkeit gegenüber allen bakteriellen Isolaten, inklusive Methicillin-resistenten Stämmen von *Staphylococcus pseudintermedius* (MRSP) und anderen Spezies von Medikamenten-resistenten Bakterien.

Schlussfolgerung und klinische Bedeutung – Medizinischer Honig war sowohl im klinischen wie auch im Labor Setting erfolgreich, was sein Potential als alternative Behandlung der OE des Hundes zeigte.

要約

背景 – 高率に認められる耳の病原体の抗菌剤耐性により、外耳炎(OE)の代替療法が必要となっている。医療用蜂蜜(MGH)は薬剤耐性病原体に対して効果がある可能性を示す証拠が示唆されている。

仮説/目的 – 市販のMGH化合物の効果是非盲検臨床試験にて評価した。筆者らは、この化合物が従来の治療法の効果的な代替療法になると仮説した。

供与動物 – 感染性OEの診断が確認された飼い犬(n=15)をこの予備研究に組み入れた。

方法 – イヌに、MGH(片耳につき1日1mL)を治癒するまで、もしくは最大21日間処方した。評価は、週に1度の臨床スコア、細胞学的な進展、および飼い主が評価したそう痒を元に行った。スワブ材料を培養および感受性検査に提出した。分離した細菌に対する殺菌活性についてMGHを検査した。

結果 – 医療用蜂蜜は、70%のイヌが7-14日目の間に臨床的に治癒、90%以上が21日までに症状が消失するという迅速な臨床的改善を示した。試験期間を通して、臨床スコアは減少し($P < 0.001$)、飼い主が評価したそう痒も有意に減少した($P < 0.05$)。MGHの殺菌活性の*In vitro*分析は、メチシリン耐性の*Staphylococcus pseudintermedius* (MRSP)および他の薬剤耐性細菌を含むすべての分離菌に対して活性を示した。

結論および臨床的な重要性 – 医療用蜂蜜は臨床および実験の両方において成果を示した。それゆえイヌのOEの代替療法になる可能性が示された。

摘要

背景 — 由于耳道致病菌耐药性的流行率增加,外耳炎(OE)迫切需要寻找替代疗法。有证据显示医疗级蜂蜜(MGH)对耐药致病菌可能有效。

假设/目的 — 通过开放性的临床试验,评估商品化医疗级蜂蜜混合物的疗效。我们假设其将成为常规治疗的有效替代品。

动物 — 本次预备实验中,使用的是确诊为感染性外耳炎的家养犬($n = 15$)。

方法 — 按规定给予犬MGH(1 mL 每耳每日)直至痊愈,或最多不超过21天。基于每周临床评分、细胞学连续监测和动物主人对犬的瘙痒评分进行评估。耳道拭子取样,送检进行培养和药敏试验。检测MGH对细菌菌落的杀灭能力。

结果 — 医疗级蜂蜜临床疗效快速,70%犬在7-14天内实现临床治愈,并且90%犬在21天内治愈。整个实验($P < 0.001$)过程中临床评分有所下降,并且动物主人给出的瘙痒评分明显降低($P < 0.05$)。体外试验显示,MGH对所有细菌菌落均有杀菌活性,包括甲氧西林耐药的假中间型葡萄球菌和其他多种耐药菌。

总结和临床意义 — 医疗级蜂蜜在临床和实验室试验中均获得成功,结论证明其可能成为治疗犬OE的替代疗法。