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ORIGINAL ARTICLE

Essential oil spray reduces clinical signs of insect bite hypersensitivity in horses

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Objective To assess the efficacy of an herbal spray combining various essential oils, with a claim of mast cell stabilisation, antipruritic, anti-inflammatory, and insect repellent effects on the clinical presentation of insect bite hypersensitivity (IBH) in horses.

Design Double-blinded, placebo-controlled, randomised, cross-over clinical trial.

Methods Twenty adult horses with clinical IBH were treated with a daily application of herbal spray or placebo for 28 days in a randomised, cross-over fashion, separated by a>28-day washout period. Horses were examined and scored prior to and after the completion of each treatment. Histopathology was performed on four horses. Owners kept daily diaries of observations.

Results The herbal spray significantly reduced the severity of all assessed parameters (pruritus, excoriations, lichenification and alopecia; $P < 0.05$) compared with baseline values (pretreatment) and with placebo. Owners reported improvement of pruritus in 19/20 horses (95%) with complete resolution in 17 horses (85%) following treatment. Skin biopsies showed resolution of orthokeratosis in 4/4 horses, reduced thickness of the stratum spinosum in 2/4 horses and complete resolution of histopathological abnormalities in 1/4 horses after treatment, compared with either no change or deterioration of histopathologic lesions after placebo. No side effects were observed.

Conclusions The tested herbal spray may be an effective treatment for the management of equine IBH.

Keywords atopic dermatitis; *Culicoides* hypersensitivity; dermatology; Queensland itch; sweet itch

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Insect bite hypersensitivity (IBH; sweet itch; Queensland itch) is a seasonal allergic skin disease with a suggested genetic predisposition[1,2] and a reported incidence of 3% in the UK,[3] 37% in Germany[4] and up to 60% in Australia.[5] It is characterised by chronic hyper-responsiveness of the immune system; specifically, a type I and type IV hypersensitivity reaction to the saliva of *Culicoides* spp. (midges) and other insects, which manifests clinically as severe, unrelenting pruritus.[1,6] The presence of insect antigens triggers various host responses that play a role in resistance to ectoparasites, including activation of the inflammatory cascade and adaptive immunity.[6] Immune mechanisms orchestrated by mast cells and immunoglobulin E (IgE) lie at the core of these host responses.[7]

An association between the presence of IgE antibodies to *Culicoides* spp. salivary gland proteins and clinical signs of IBH has been well documented.[8-11] This IgE participates in the induction of mast cell degranulation,[6] which activates a broad range of inflammatory pathways and can influence pruritus responses indirectly. Histamine and serotonin released from mast cells can subsequently elicit pruritis by binding to H1, H4 or 5HT-2 receptors, respectively.[12,13] Via the induction of mast cell degranulation, IgE therefore provides a direct link between the immune response to *Culicoides* bites and the clinical signs of IBH in affected horses.

To date, efficacious treatment options for IBH remain elusive, with conflicting evidence and success rates <50% for hyposensitisation therapy and steroid-based immune-suppression.[1] Recently, a double-blinded, randomised, placebo-controlled study failed to show any benefit of allergen-specific immunotherapy.[14] Treatment strategies are mainly palliative and targeted at ameliorating clinical signs. Agents that prevent mediator release from mast cells (mast cell stabilisers), include a variety of natural, semi-synthetic and synthetic compounds and represent a possible means of treatment of IBH.[15] Management strategies are based on insect control and avoidance, which is difficult and impractical under common equine management and husbandry practices. These include frequent application of repellents and insecticides, rugs from ears to tail (which is problematic in hot climates), and the use of industrial fans. Thus, IBH results in major discomfort, compromised welfare and owner frustration. Furthermore, once hypersensitivity is induced, continuous or seasonal immunomodulatory treatment is required to maintain remission and delay progression. The use of immunomodulatory medications, of which steroids are the most frequently used in veterinary practice, can be associated with untoward effects.[1] Therefore, a disease-modifying therapeutic alternative with fewer or no side effects is desirable.

Essential oil extracts from plants such as *Cinnamonum camphora* (Camphor), *Cymbopogon citratus* (Lemongrass), *Litsea cubeba* (May Chang), *Mentha piperita* (Peppermint) and *Pogostemon cablin* (Patchouli) said to have immunomodulatory,[16] antihistamine,[17] antipruritic,[18] anti-inflammatory,[17,19,20] larvicidal and insect repellence effects,[21,22] as well as anti-allergy[23] and analgesic properties[23,24] that may be beneficial in the treatment of IBH.

The objective of the present study was to assess the efficacy of an herbal formulation on the clinical presentation of IBH-affected

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horses. Pilot data provided by the manufacturer (Red Healer PTY LTD, Murwillumbah, New South Wales, Australia), which was obtained through voluntary use of the formulation, suggest a positive effect of treatment. The manufacturer claims mast cell stabilisation, antipruritic, anti-inflammatory and insect repellent effects of the product.

Materials and methods

The project was approved by The University of Queensland Animal Ethics Committee (approval number: SVS/510/17) that monitors compliance with the Animal Welfare Act (2001) and The Australian Code of Practice for the care and use of animals for scientific purposes (current edition).

A double-blinded placebo-controlled clinical trial was conducted during summer in Queensland, Australia. The tested herbal formulation contained Peppermint, Lemongrass, May Chang, Camphor and Patchouli, in an emulsion of vegetable oil and water (Table 1). All ingredients in the product comply with the International Fragrance Association (http://www.ifraorg.org/) Standards, are registered in the Australian Register of Therapeutic Goods (https://www.tga.gov.au/), are registered as compliant with the Australian Pesticides and Veterinary Medicines Authority (APVMA; https://apvma.gov.au/) safety & toxicity guidelines, or are exempted from the requirements of APVMA approval for use in agricultural or veterinary chemical products.

Using pilot data for power calculation (β = 0.80) and to estimate time to reoccurrence of clinical signs following discontinuation of treatment, a required sample size ≥16 subjects, and a 4-week washout period were established. Allowing for an estimated 20% dropout during the trial, the desired total number of subjects to be enrolled was 20 horses. Horses were recruited from the local population via social media. Eligibility criteria included owner-identified IBH that had not been treated or managed in the past fortnight, absence of concurrent medical or dermatological conditions, and willingness to treat exclusively with the provided products and comply with all instructions for the duration of the trial (a questionnaire to determine that eligibility is available as Supplementary Information – S1).

Using a crossover design, with a 4-week washout period between treatments, horses were randomly assigned a de-identified formulation containing either the herbal formulation (*treatment*) or sham treatment. A formulation of similar organoleptic characteristics but without the active ingredients (*placebo*) was used (Table 1). Owners were provided with spray and roll-on applications of the assigned formulation and instructed to treat the affected areas of their horse(s) once daily for 28 days by spraying liberally (until fully covered by the product) on all active lesions (over all affected areas). The roll-on was provided for use on the face and head of non-compliant horses that did not tolerate spraying (i.e. head-shy horses). A booklet (Supplementary information – S2) for daily recording of treatment application, weekly subjective assessment of the effect of treatment on disease severity (better, unchanged or worse), and for recording personal observations was provided to each owner at the start of the trial. The severity of disease was subsequently categorised as IMPROVED (better) or NOT IMPROVED (unchanged or worse) for statistical analysis.

Table 1. Composition of the essential oil herbal formulation (treatment) and sham (placebo) sprays

Spray	Ingredient	Minimum content
	Scientific name (common name)	As %
Essential oil spray (treatment)	*Cymbopogon citratus* (Lemongrass)	1.5%
	Cinnamonum camphora (Camphor)	0.5%
	Litsea cubeba (May Chang)	3%
	Mentha piperita (Peppermint)	1.5%
	Pogostemon cablin (Patchouli)	1%
	Emulsifiers (main emulsifier: Polysorbate)	20%
	Vegetable oil	15%
	Water	q.s.
Sham spray (placebo)	Fragrances (main fragrance: Methyl Hexyl Keton)†	2%
	Emulsifiers (main emulsifier: Polysorbate)	20%
	Natural preservative	2%
	Vegetable oil	15%
	Water	q.s.

† The essential oil spray has a characteristic smell given by the multiple herbal products it contains; therefore, fragrances were added to the placebo.

Horses were examined by a veterinarian (either author AC, GC or KW) before and after each stage of the trial (days 0, 28, 56 and 84); the same veterinarian assessed the same horse on each occasion. At each veterinary examination, a complete general physical exam was performed, blood was collected via jugular venepuncture for complete blood count (CBC) and serum chemistry profile, photographs were taken, the IBH lesions were recorded on a dermatology assessment sheet designed for the study (Supplementary information – S3), and the owner's treatment record was inspected for completeness and scanned. Excoriations, lichenification and alopecia were graded separately by each of the two veterinarians examining each horse, based on a semi-quantitative scale of 0 to 5 using a grading system, where 0 = absent, 1 = minimal, 2 = mild, 3 = moderate, 4 = severe, and 5 = extreme (Supplementary information – S3). Four horses for which the owners gave written consent (H1, H2, H6 and H9) underwent skin biopsy sampling during each examination. Biopsies were collected with a 6-mm punch biopsy instrument from the left or right side of the neck, immediately ventral to the base of the mane and at the interface between IBH-affected and a healthy region of skin. Tissue samples were fixed in 10% neutral formalin and embedded into paraffin wax. Four-micrometre sections were

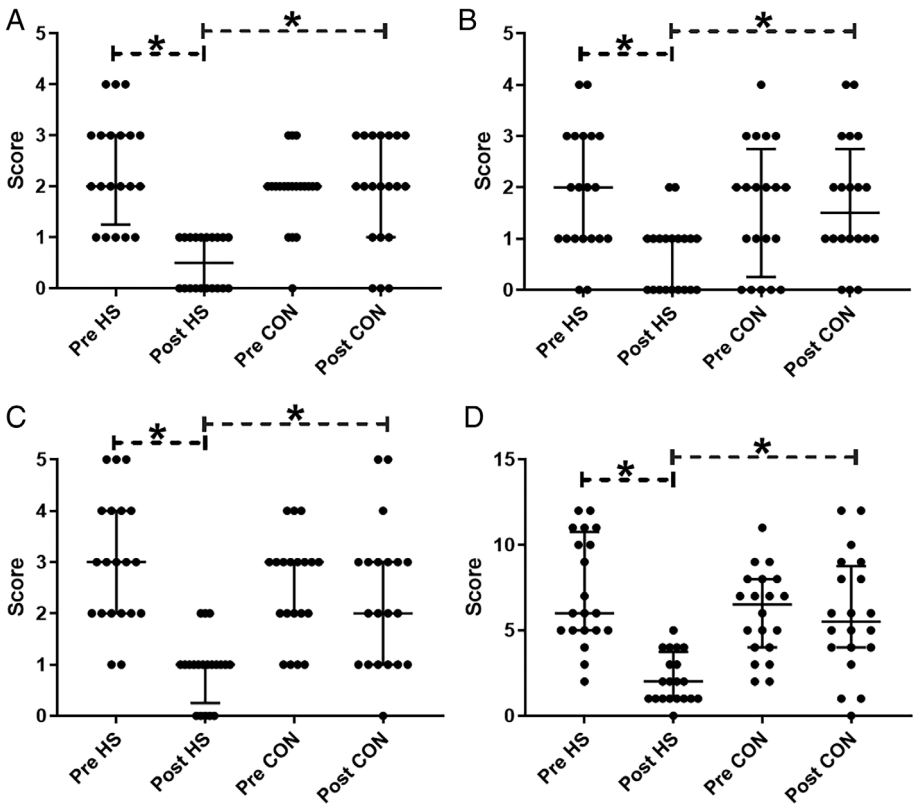

Figure 1. Severity of (A) excoriations, (B) lichenification, (C) alopecia and (D) sum of scores on a 0 to 5 grading scale where 0 = absent, 1 = minimal, 2 = mild, 3 = moderate, 4 = severe, and 5 = extreme in 20 adult horses previously diagnosed with insect bite hypersensitivity (IBH), recorded before (pre) and after (post) treatment with a herbal spray (HS) or placebo (CON). Dotted line with asterisk denotes statistically significant difference between groups ($P < 0.05$).

stained with haematoxylin and eosin and examined by a veterinary pathologist who also remained blinded to the treatment status of the horses.

Statistical analyses were performed using GraphPad Prism v7.00 for Windows (GraphPad Software®). Data were ordinal and hence treated as non-normally distributed. Wilcoxon signed-rank tests were used to compare the scores of each individual parameter and the total sum of all scores between treatment and control groups, and compared with baseline (pretreatment vs post-treatment). A Fisher's exact test was used to assess the association between the treatment and the presence or absence of pruritus, and the presence or absence of improvement in disease severity. Significance was set at $P < 0.05$.

Results

Twenty adult (8 mares; 12 geldings) privately owned horses and ponies of multiple breeds were enrolled in the trial. Ages ranged from 2 to 25 years (median 10 years [interquartile range, IQR = 4.5–13.8]). Nineteen horses (95%) completed the trial (i.e. remained enrolled until day 84). For the horse that did not continue until day 84, the owner requested re-examination and termination of the trial on day 80 due to worsening of clinical signs and severe pruritus leading to self-injury. After determining that this horse was receiving the placebo at the time of early withdrawal and because the dropout from trial occurred during the last week (i.e. 4 days early), the data from this horse were not excluded from the analysis. All horse owners were compliant with management and treatment instructions (data not shown). No adverse effects were noted.

Compared with baseline, veterinarian-assessed scores of all individual parameters as well as the total sum of all scores were significantly different ($P < 0.05$) for the treatment group, but did not reach significance for the placebo ($P \geq 0.49$) (Figure 1). Scores of all individual parameters (i.e. excoriations, lichenification and alopecia) as well as the total sum of all scores between groups (treatment vs placebo) were also significantly different ($P < 0.05$) (Figure 1). All 20 horses (100%) were described by the owner as pruritic prior to each phase of the trial. Owners' assessment of pruritus and disease severity following treatment is presented in Table 2. Upon re-examination following the washout period (prior to commencement of part 2), all horses displayed IBH lesions of similar severity to those observed prior to enrolment in the trial (before part 1).

Table 2. Owners' assessment of pruritus and disease severity after 28 days of daily application of an herbal spray treatment or placebo

	Pruritus		Severity	
	Yes	No	Improved	Not improved
Treatment	3 (15%)	17 (85%)	19 (95%)	1 (5%)[†]
Placebo	16 (80%)	4 (20%)	2 (10%)	18 (90%)[‡]

[†] Unchanged.
[‡] Worse 6 horses (30%); unchanged 12 horses (60%).

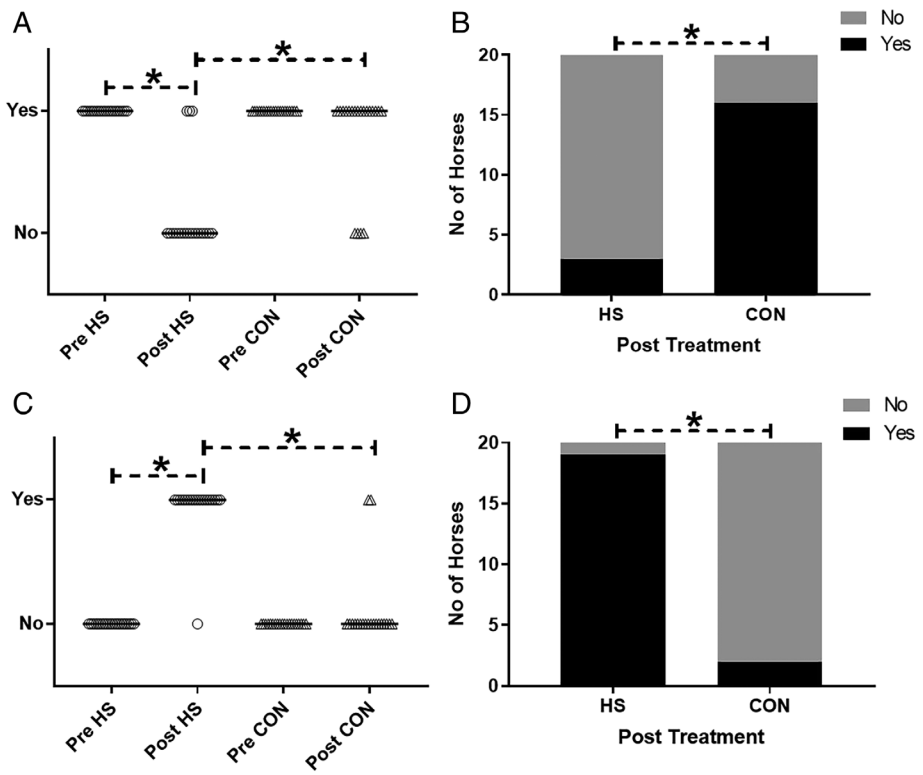

Figure 2. **Presence or absence of pruritus (A) or improvement in severity of clinical disease (C) as reported by the owner before (pre) and after (post) treatment with an herbal spray (HS) or placebo (CON). Note that all horses (20/20) had pruritus prior to both treatments (pre-HS and pre-CON), only 3/20 (15%) remained pruritic following treatment (post-HS), whereas 16/20 (80%) remained pruritic following placebo (post-CON). Contingency table depicting the total number of horses with (yes) and without (no) pruritus (B) and improvement in severity of clinical disease (D) after HS or CON treatment. Dotted line with asterisk denotes statistically significant difference between groups ($P < 0.05$).**

A significant association ($P < 0.01$) between treatment and the resolution of pruritus, as well as improvement in disease severity as reported by the owner at each examination was identified on contingency analysis (Figure 2). Subjectively, the improvement was readily apparent upon visual inspection, as exemplified by the photographic records from two enrolled horses showing resolution of alopecia and improvement in hair quality and colour following treatment (Figure 3). No association between placebo and change in pruritus or disease severity was found (Figure 2).

No abnormalities and no significant differences in the analysed CBC and serum chemistry parameters were identified at any time point (data not shown). Histopathological examination of baseline (day 0) skin biopsy specimens identified a mild perivascular lymphohistiocytic and eosinophilic dermatitis, with mild orthokeratotic hyperkeratosis and mild acanthosis (ca. 6–8 cells thick) in all four horses sampled. Compared with histopathological findings immediately before treatment, the orthokeratosis resolved in all four horses, and in two horses, a reduction in the acanthosis was apparent (ca. 4 cells thick). One horse had almost complete resolution of all histopathological abnormalities, with only mild acanthosis remaining. No improvement was apparent in any of the horses following placebo, and in addition to previously identified abnormalities, one horse developed spongiosis and an increase in thickness of the stratum spinosum (ca. 10 cells thick) was apparent in two horses (Figure 4).

Discussion

The present study demonstrates that application of the tested herbal spray resulted in a significant reduction in score severity for each

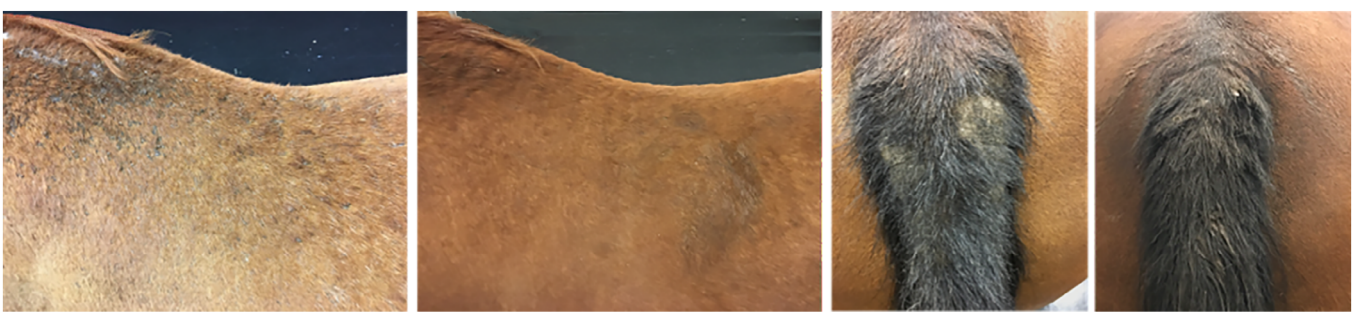

Figure 3. **Photographic record of the left withers and back, and tail base of two horses enrolled in the trial, before (left) and after (right) 4 weeks of treatment with a herbal spray formulation. Complete resolution of alopecia and improvement in hair quality and colour is apparent.**

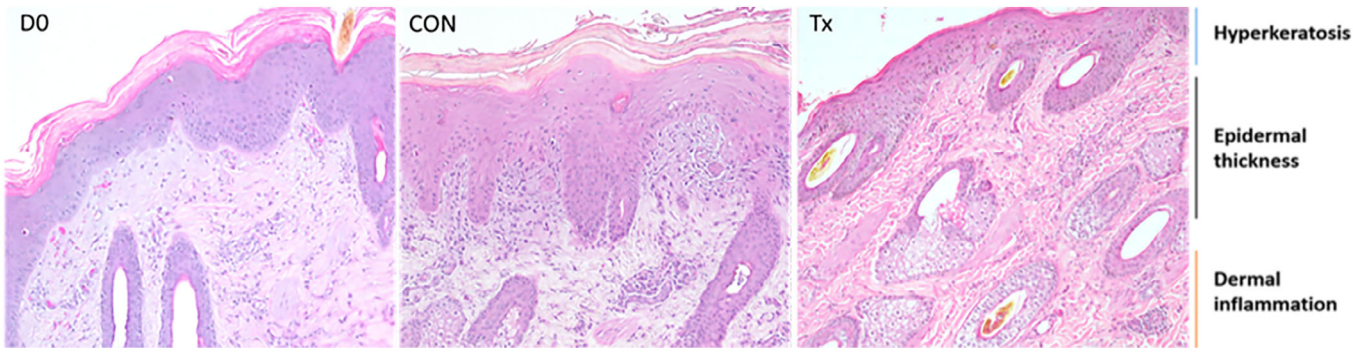

Figure 4. Microphotographs of skin biopsy specimens before (day 0: D0) and following treatment with the placebo (CON) and herbal spray (Tx). Hyperkeratosis, thickening of the epidermis and dermatitis on D0, persist following CON and decrease following Tx.

individual parameter assessed (i.e. excoriations, lichenification and alopecia), as well as for the sum of all scores when compared with both baseline values (pretreatment) and to placebo. Treatment was also significantly associated with the binary subjective measures of pruritus and clinical improvement. Improvement or partial resolution of the histopathological lesions was apparent in all four horses following treatment, with no improvement and even an apparent worsening of these lesions following placebo. These findings suggest that this product may be an effective alternative therapy in the treatment of equine IBH. No adverse reactions to treatment or untoward events (other than one horse worsening whilst being treated with placebo) were identified or reported by the horse owners, and no changes in blood parameters were observed.

The positive effect of the essential oils present in the tested herbal formulation (i.e. Camphor, Lemongrass, May Chang, Peppermint and Patchouli) in controlling the clinical signs of IBH-affected horses is likely the result of summation or even synergism of their well-characterised therapeutic properties.[16-21] These essential oils have been shown, both in vitro (cell culture and bioassays) and in vivo (topical application), to offer immunomodulatory,[16] antihistamine,[17] antipruritic,[18] anti-inflammatory,[17,19,20] larvicidal and insect repellence effects,[21,22] as well as anti-allergy[23] and analgesic properties[23,24] that may be beneficial in the treatment of IBH. However, establishing the individual effect and potential contribution to the overall treatment success of each separate ingredients of the tested spray was beyond the scope of this study. Furthermore, the cellular pathways on which these essential oils act to modulate the well characterised IgE antibody response and mast cell degranulation,[7] as well as the IgG antibodies which also occur in horses exposed to *Culicoides* spp.,[8,9,11] remain unknown. Interestingly, the antibody response to insect salivary antigens is biased towards an abundance of the IgG(T) subclass.[9] Both IgG(T) and IgE responses are a feature of a T-helper-2 (Th2) lymphocyte response. Recently, a clear IL-4 driven, type-2 skewing of the immune response upon intracutaneous *Culicoides* allergen injection in ponies with IBH was documented, while IBH-free ponies showed an IFNγ-driven, type-1 skewed immune response.[25] This likely explains why IBH is Th2-dominated. The development of such hypersensitivity reactions is characterised by antigen-specific (Th2) lymphocyte responses in susceptible individuals.[26,27] Modifying this Th2 bias, directing lymphocyte polarisation towards a Th1 response is thus a putative site for therapeutic intervention that could promote a decline in IBH allergen-specific IgE levels.[26] Disease-modifying therapeutic options with limited or no side effects should remain the long-term goal for the treatment of IBH.

Accurate dosage calculation for any therapeutic agent is of utmost importance for treatment success and avoidance of side effects. Dosage precision is a recognised source of variability when using topical formulations, where the dose accuracy is calculated as the quantity of therapeutic agent needed per diseased unit area (i.e. the affected surface) in order to achieve the desired effect.[28] Dosing topical formulations is further complicated by the need to use some topical medications such as steroids *sparingly*, due to potential side effects, while others, such as the spray tested in this study, are better applied *liberally*.[29] In addition to dose accuracy, ease of application to a horse is an important factor for treatment compliance and success.[30] In self-treating human subjects, sprays are considered appealing for multiple reasons, including ease of application. An example is the increased use of sunscreen sprays instead of lotions, with consumers feeling sprays are quick and easy to apply.[31] Overall, there is an increasing popularity of non-traditional vehicles such as sprays for topical therapy.[30] Thus, the tested essential oil formulation was designed as a spray, to facilitate application and secure treatment compliance. Though an exact dose was not prescribed, instructing to apply liberally, with indication of covering the lesions completely, has been shown to result in treatment success and application of adequate amounts of product in humans.[29] As an additional measure to enhance treatment compliance and horse cooperation, a roll-on presentation of the essential oil formulation was provided to facilitate product application in areas such as the head, where head-shy horses were likely to resist spraying.

The main limitations of this study were the small number of horses enrolled (n = 20) and that the horses were treated by their owners and remained at home in an uncontrolled environment for the duration of the study. However, a power calculation indicated the sample size to be adequate for a preliminary assessment of the efficacy of the tested product, and the main objective of the study was to evaluate changes in clinical parameters associated with IBH (pruritus, excoriations, lichenification and alopecia) following application of the product; thus maintaining the horses in their natural environment where IBH was occurring was considered desirable. An additional limitation of the study design is that, as it was targeted at assessing

changes in clinical signs, rather than the mechanism of action of the product and its effect on disease pathogenesis, the study does not determine whether the formulation had a direct effect on the immune system, or if it acted as a repellent or a mechanical barrier to prevent insect bites. This aspect may warrant further investigation to determine if the mechanism of action of the tested herbal spray is indeed related to the effects of the essential oils it contains. Despite these limitations, the herbal formulation resulted in clinical improvement in 19 out of the 20 horses and histopathological improvement in all 4 horses that were biopsied.

Altogether, the commercial herbal formulation appeared safe as it did not negatively impact or induce side effects in any of the treated animals. The findings of this trial suggest that the tested herbal formulation may be efficacious in the treatment of IBH in horses. It could mitigate the impact of this disease in the general horse population by decreasing the cost of management of IBH and reducing owner frustration due to treatment failure with currently available and expensive management strategies, which will benefit the equine industry as a whole. However, superiorities studies have are yet to be performed, and it therefore remains unknown whether this essential oil formulation is more effective than currently used treatment strategies.

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Conflicts of interest and sources of funding

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site: http://onlinelibrary.wiley.com/doi/10.1111/avj.12963/suppinfo.

Supplementary Information S1

Supplementary Information S2

Supplementary Information S3

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