

The healing effects of a topical phytogenic ointment on insect bite hypersensitivity lesions in horses

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ABSTRACT

Insect bite hypersensitivity (IBH) is the most common cause of pruritus in horses and is a serious welfare issue for affected animals. In this study, the effect of a topical phytogenic ointment on the healing of cutaneous lesions was investigated in a double-blind trial involving 26 horses with IBH. The number of lesions and their total surface area were recorded on days 0, 7, and 21 in horses treated for 3 weeks with either verum or placebo ointment. After unblinding of treatment assignment, the horses that had been treated with the placebo ointment received the verum preparation for an additional 3 weeks and the number of lesions and their total surface area were again recorded. This part of the study was not blinded. The number of lesions and the total surface area decreased in both treatment groups (no significant difference). Owners also scored the degree of discomfort suffered by their horses as a result of IBH lesions, and at the end of the 3-week period this score was significantly lower in the verum than in the placebo group ($P=0.04$). When placebo-treated horses subsequently received the verum ointment, their wound severity score also decreased significantly ($P<0.01$). Daily application of an ointment (verum or placebo) does not cure IBH, but use of the phytogenic ointment led to a decrease in the owner-assessed discomfort suffered by horses.

SAMENVATTING

De genezende werking van een topicale fyto-gene zalf op laesies ten gevolge van staart- en maneneceem bij paarden
 Staart-en-maneneceem (SME, Engels: Insect Bite Hypersensitivity, IBH) is de meest voorkomende oorzaak van jeuk bij paarden en het welzijn van SME lijdens kan ernstig aangetast zijn. In deze studie is het

effect van een topicale fyto-gene zalf op de genezing van huidlaesies onderzocht in een dubbelblinde proef met 26 paarden met SME. Het aantal laesies en het totale laesieoppervlak werden beoordeeld op dag 0, 7 en 21 bij paarden die gedurende drie weken werden behandeld met de verum- of placebozalf. Nadat bekend werd tot welke groep paarden behoorden, werden paarden die behandeld waren met de placebozalf, vervolgens drie weken behandeld met de verumzalf en het aantal laesies en hun oppervlak werden nogmaals beoordeeld. Dit deel van het onderzoek was niet geblindeerd. Het aantal laesies en het totale oppervlak nam in beide behandelgroepen af en er was geen significant verschil tussen de groepen. Eigenaren moesten ook aangeven hoeveel last hun paard had ten gevolge van hun laesies en aan het eind van de behandelperiode van drie weken was deze score significant lager in de verum- dan in de placebogroep ($P=0,04$). Toen de paarden die eerst behandeld waren met placebozalf, vervolgens de verumzalf kregen nam deze eigenarenscore eveneens significant af ($P<0,01$). Het dagelijks smeren van een zalf (verum of placebo) geneest SME niet, maar het gebruik van de fyto-gene zalf leidde tot een afname van het ongemak dat paarden ondervonden ten gevolge van hun laesies, zoals beoordeeld door de eigenaar.

INTRODUCTION

Insect bite hypersensitivity (IBH) is the most common cause of pruritus in horses, affecting equids in almost all parts of the world (9). IBH is an allergic reaction to the bite of various insects, most notably *Culicoides* species (10), and the number of horses affected varies considerably per breed as the susceptibility is considered to be (partly) genetically determined (11, 15). In the Netherlands, breeds most commonly affected include Icelandic and Friesian horses and Shetland ponies (18, 20). Van Grevenhof et al (2007) reported that 18% of Friesian mares and 8% of Shetland pony mares used for breeding in the Netherlands are affected by IBH.

Apart from a genetic predisposition, the development of the clinical signs of IBH requires the presence of the causative insects, mainly *Culicoides*. There are many different species of *Culicoides* (6), and different species probably play a role in different geographical areas (2, 5, 7, 12, 14, 17). In the Netherlands, the species that are trapped in the vicinity of horses are *C. obsoletus* (95%) and *C. pulicaris* (5%) (8, 19). *Culicoides* are poor fliers that require at least moderate temperatures and stagnant water to reproduce (3, 4, 22). This explains why *Culicoides* are present in huge numbers in some areas and absent in others, depending on the geographical and climatic conditions. In general, they thrive in warm weather, with little wind (or with

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vegetation to protect them), in the vicinity of stagnant water, and in areas with clay soil (20). When a susceptible horse is bitten by a *Culicoides* midge, intense pruritus follows, caused by a type I, IgE-mediated, hypersensitivity reaction (16). Scratching and rubbing causes secondary lesions, such as broken hairs, excoriations, exudation of serum, and patchy alopecia. When horses are affected chronically, scaling, hyperkeratosis, and transverse ridges can develop (14, 16). Signs develop where horses are bitten, with the mane and base of the tail being most commonly affected, although other regions, such as the ventral abdomen, may also be involved (1).

At present there is no cure for IBH and various strategies are used in an attempt to reduce the severity of symptoms. These include stabling horses when *Culicoides* are most active, the use of various rugs, the application of insecticides, and the administration of corticosteroids (13, 18). In addition, a vast array of topical preparations are used by owners to try to relieve signs in affected horses, mainly in an attempt to stop the vicious circle of events caused by pruritus leading to self-trauma causing more pruritus, etc.

In this study, a topical phytogenic ointment – i.e. an ointment containing plant-derived constituents – was tested on horses with skin lesions as a result of IBH. The hypothesis that was tested in a double-blind, placebo-controlled trial was that lesion healing would be accelerated following treatment with the ointment and, as a result, horses would suffer less discomfort (as judged subjectively by their owners).

MATERIALS AND METHODS

Twenty-eight horses started and 26 completed the trial. They had been selected because they had one or more open lesions as a result of rubbing because of pruritus due to IBH. Other topical medication had to be discontinued during the study and two horses (one from the verum group and one from the placebo group) were withdrawn from the trial because another product was used. The diagnosis of IBH was made on the basis of the seasonal occurrence and the typical lesion distribution. The trial was performed in September to October 2007 (8 horses) and June to September 2008 (18 horses).

An ointment containing a plant-derived compound (Phyto-treat-SEO[®], Alfasan, Woerden, the Netherlands) or a placebo ointment was applied to all lesions. The plant-derived compound – which does not show any structural resemblance to corticosteroids – was previously found to possess promising anti-inflammatory and wound-healing properties, affecting the function of polymorphonuclear leukocytes and proteolytic enzymes involved in remodelling of the extracellular matrix (personal communication S.B.A. Halkes). It was chemically modified in such a way that it can better penetrate the skin,

where it is converted back into the active principle (pro-drug concept) by enzymes in cells of the epidermal layer. The plant-derived compound was formulated in a polyethylene glycol-based ointment containing benzoic acid as preservative and a citric acid-citrate buffering system. The placebo consisted of the polyethylene glycol-based ointment only.

Before the start of the trial, all owners were asked what strategies they used to try to relieve symptoms due to IBH. While all topical medication had to be discontinued for the duration of the study, other treatments were allowed in an effort to keep other factors as constant as possible and so as not to discourage owners from entering their horses into the study. This meant that all horses were kept outside, at least during the day, and were thus exposed to *Culicoides* midges.

At the start of the trial (day 0), all lesions on each horse were counted, photographed, and the surface area calculated by tracing the circumference of the wound onto squared paper and counting the number of squares. In addition, owners were asked five questions concerning their horse and its lesions (table 1). Each of these questions could be scored with 0, 1, or 2, giving a total score between zero and 10, with zero representing no clinical signs and 10 the most severe signs.

After the initial examination of the lesions, owners were instructed to apply a thin layer of ointment to all open lesions once daily. Neither the owner nor the authors were aware of whether a particular horse was being treated with the ointment containing the test ingredients (verum) or the placebo ointment (placebo). Seven and 21 days after the start of treatment, all lesions were again counted (those present on day 0 and any new wounds), photographed, and the surface area measured. Also, on days 7, 14, and 21 owners were again asked to complete the questionnaires regarding symptom severity. The owners were also asked to assess the degree of discomfort suffered by their horse as a result of the lesions compared to the week before: less (0), the same (1), or more (2) and whether they believed they were applying the verum ointment or the placebo.

Following the assessment on day 21, it was revealed whether the horse had been treated with verum (n=12) or placebo (n=14) ointment. All horses that had received the placebo treatment were subsequently treated for an additional 3 weeks with the verum ointment (verum after placebo group, n=14). During this extra trial period, the owners again assessed their horse's behaviour and discomfort on days 28, 35, and 42, and the number and size of lesions on days 28 and 42 (i.e., 7 and 21 days after start of treatment with the verum ointment) were recorded. At the completion of each treatment period the tubes containing the ointment were returned and weighed, to determine the amount of ointment applied.

	no	sometimes/slightly	often/clearly
did you observe the horse rubbing?	0	1	2
is/are the lesion(s) red?	0	1	2
is/are the lesion(s) moist?	0	1	2
are there broken hairs?	0	1	2
is the skin thickened?	0	1	2

Table 1
Questions asked regarding the horses and their lesions.

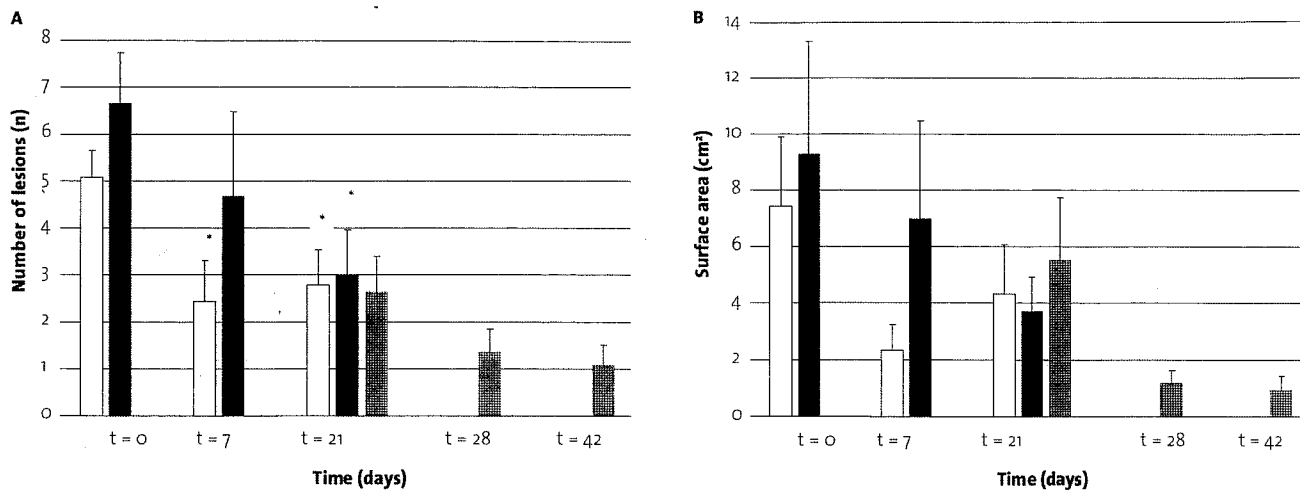


Figure 1
 The total number of lesions (A, mean + sem) and total lesion surface area (B, mean + sem) in the placebo (white), verum (black), and verum after placebo (grey) groups.
 * significantly ($P < 0.05$) different compared to $t=0$.

The experimental protocol was evaluated by Utrecht University's Animal Experimental Committee (DEC), but was not considered to constitute an animal experiment.

STATISTICAL ANALYSIS

Statistical analysis was performed using the SPSS software package (version 16). Normality of data was assessed using the Kolmogorov-Smirnov test. The data on lesion number and surface area were normally distributed, and the influence of time was investigated using a paired Student's t-test and the influence of treatment using an independent sample t-test. Data on the severity of symptoms and discomfort due to the lesions

were not normally distributed, and differences between groups were investigated using the Mann-Whitney U test. A P-value < 0.05 was considered to be statistically significant.

RESULTS

Horses

The age of the horses varied from 3 to 25 years (mean 12 years); the horses in the verum group ($n=12$, mean 14.3 years) were significantly ($p=0.04$) older than those in the placebo group ($n=14$, mean 9.8). All horses were kept outside during the day and most remained outside at night. The breeds included were Icelandic horses (19), Haflinger horses (2), Friesian horses (2),

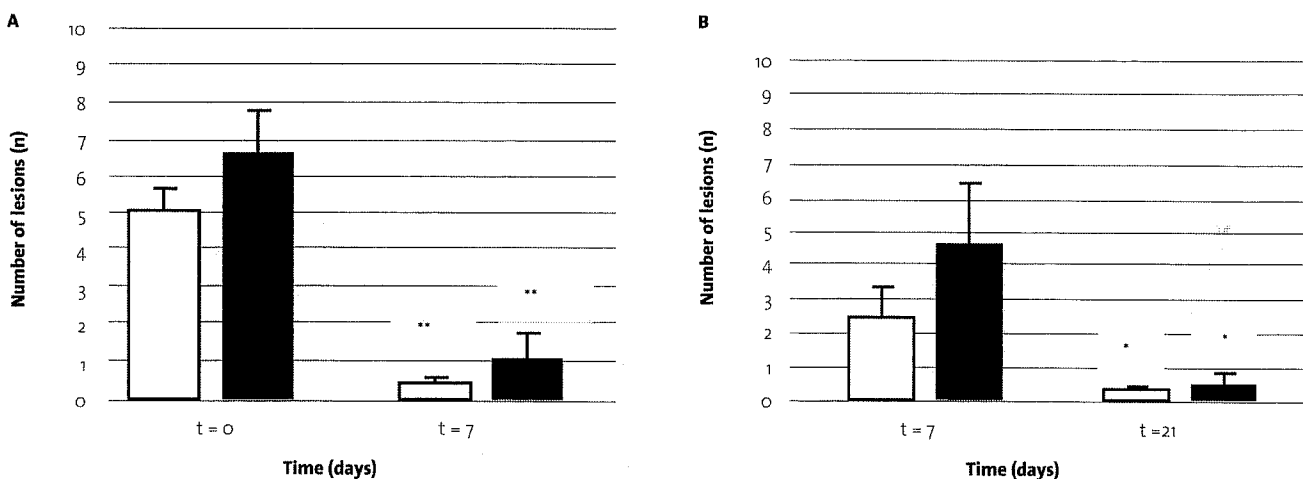


Figure 2
 A. The number of lesions (mean + sem) present at $t=0$ and the number of these lesions that were still present at $t=7$. B. The number (mean + sem) of new lesions present at $t=7$ and the mean number of these lesions that were still present at $t=21$, in the placebo (white) and verum (black) groups.
 ** significantly ($P < 0.01$) different compared to $t=0$
 * significantly ($P < 0.05$) different compared to $t=7$

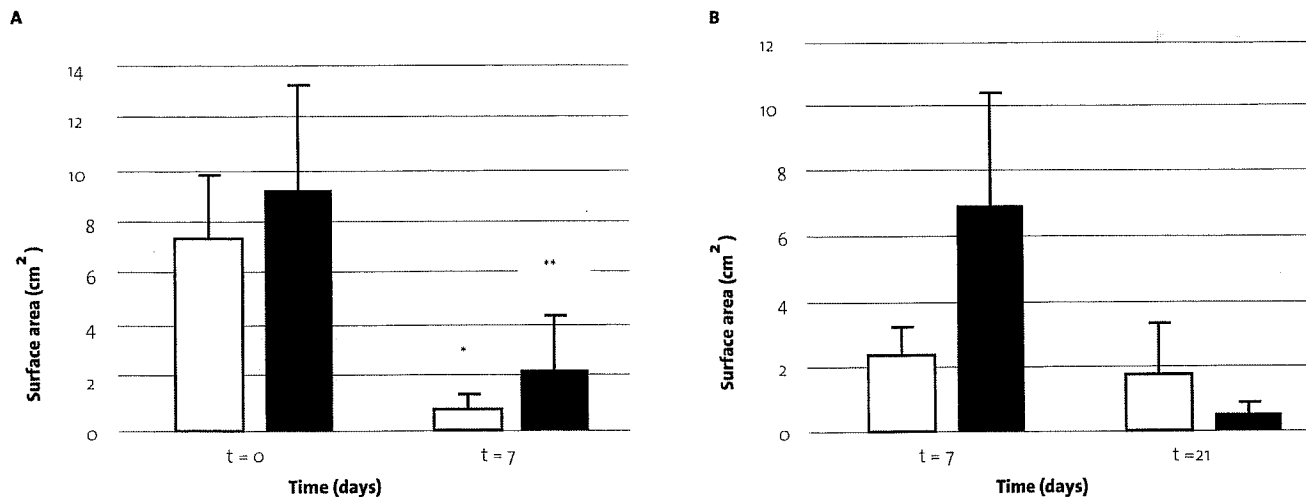


Figure 3

A. The surface area of lesions at t=0 (mean + sem) and of the same lesions at t=7. B. The surface area (mean + sem) of new lesions at t=7 and of these same lesions at t=21, in the placebo (white) and verum (black) groups.

** significantly ($P < 0.01$) different compared to t=0

* significantly ($P < 0.05$) different compared to t=0

Fjordic horse (1), Tinker (1), and Shetland pony (1). All owners used one or more strategies to reduce the severity of signs caused by IBH, including the use of rugs, stabling the horses at certain times of the day, moving the horse to a different location, insecticides, ointments, shampoos, medication, and homeopathy.

Total number of lesions and surface area

Although the verum-treated horses had more lesions and the total surface area affected was larger at the start of the study (t=0), this difference was not significant. The total number of lesions (figure 1A) in the verum group of horses was reduced at t=7 and further reduced at t=21; the difference from baseline at 3 weeks was significant. In the placebo group, there was a significant reduction in the total number of lesions at t=7 and the number of lesions remained more or less constant during the subsequent 2 weeks of the trial. Compared with the number of lesions at t=21 in the placebo group, there were fewer lesions at t=28 and t=42 in the verum after placebo group. The same trend was seen for the total lesion surface area, although these differences were not statistically significant (figure 1B).

Number and surface area of new lesions

Of the lesions present at t=0, the number was reduced by 84% (6.7 to 1.1, $P < 0.01$) at t=7 in the verum group and by 92% (5.1 to 0.4, $P < 0.01$) in the placebo group (figure 2A). Of the new lesions present at t=7, 89% (4.6 to 0.5, $P < 0.05$) and 88% (2.4 to 0.3, $P < 0.05$) had disappeared at t=21 in the verum and placebo groups respectively (figure 2B). There was no significant difference in the reduction in the number of lesions between the two groups.

The mean surface area of the lesions present at t=0 was reduced by 75% (9.3 to 2.3 cm², $P < 0.01$) at t=7 in the verum group and by 90% (7.4 to 0.8 cm², $P < 0.05$) in the placebo group (figure 3A). Of the new lesions present at t=7, the total surface area was reduced by 6.5 cm² (7.0 to 0.5 cm², 92%, NS) and 0.5

cm² (2.3 to 1.8 cm², 24%, NS) at t=21 in the verum and placebo groups respectively (figure 3B). The difference in the decrease in lesion surface area between the two groups was not statistically significant.

The amount of ointment applied (per 3 week period) was calculated by weighing the tubes before and after each treatment period. The most ointment was applied in the placebo group (mean 70 g/horse/3 weeks), less in the verum group (60 g/horse/3 weeks), and least in the verum after placebo group (57 g/horse/3 weeks). The differences between groups were not significant.

Both verum and placebo groups showed the same trend regarding the severity of IBH symptoms displayed: a slight reduction in the severity of symptoms initially and more-or-less constant severity after that (figure 4). In the verum after placebo group, there was a steady decrease in the severity of symptoms (figure 4), but differences were not statistically significant. At the start of the trial, the severity of symptoms was also assessed by the researcher: horses in the verum group were judged to have slightly more severe signs, although the difference between groups was not significant (data not shown).

Figure 5 shows the discomfort caused by the lesions compared to the week before. Compared to the start of the study, horses in the verum group displayed slightly more discomfort during the first week of the study, the level of discomfort remained the same during the second week, and improved during the last week. Horses in the placebo group showed a slight improvement after the first week, but deteriorated thereafter, with the difference between weeks 1 and 3 being significant. After the third week of treatment, the difference between the verum and placebo groups was also significant. When horses were treated with the verum ointment after 3 weeks of placebo treatment, the deterioration stopped and the degree of discomfort stabilized and then decreased during the third week of treatment. During the third week of verum treatment, the horses suffered less discomfort than during the third week of placebo treatment.

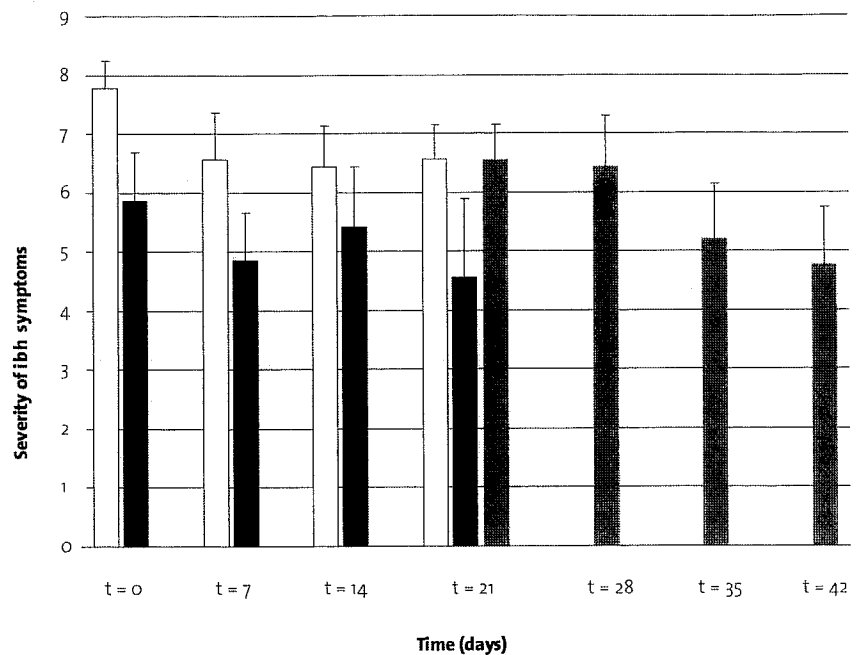


Figure 4
The severity of IBH symptoms (as scored by the owner, mean + sem, score 0-10) at different times during the study in the placebo (white), verum (black), and verum after placebo (grey) groups.

Owners were asked if they thought their horse was being treated with the verum ointment or the placebo ointment. At the end of the 3-week treatment period, 50% of owners in the verum group thought their horse was receiving the ointment with the active ingredient(s), compared to 33% in the placebo group.

DISCUSSION

The horses included in the present study showed the typical clinical signs associated with IBH and the diagnosis was based on these symptoms, in combination with the seasonal occurrence. The breeds included in the study were the breeds commonly affected by this condition in the Netherlands (18, 21). However, the breeds were not randomly selected as adverts were placed on the website of an Icelandic Horse club. One of the inclusion criteria for this trial was the presence of one or more skin lesions. It proved more difficult than expected to find horses meeting this prerequisite. Apparently, owners motivated to participate in such a study are often able to prevent the development of open lesions.

Horses in the verum group were significantly older than those of the placebo group. As treatment assignment was blinded, the age of the horses could not be controlled for. However, the severity of IBH tends to increase with increasing age (18, 21), and this might explain the greater number of lesions and the greater lesion surface area seen in the horses of the verum group, although the differences compared to the placebo group were not statistically significant. The severity of symptoms (as assessed by the owners) was not more severe in the verum group than in the placebo group, although horses in the verum group were judged to have slightly more severe symptoms by the researcher.

The ointment was used in conjunction with other strategies already used by owners, as would be the case when owners choose to treat their horse topically. This allowed better assessment of the efficacy of the ointment as all other factors were kept constant. If, for example, all horses usually covered

with rugs were kept outside without rugs during the trial, any change in the number and size of the lesions and the discomfort suffered would also be affected by that fact and not just attributable to the ointment applied.

The total number of lesions was reduced in both the verum and placebo groups, suggesting that regular application of an ointment, irrespective of whether the ointment has active ingredients or not, speeds the healing of lesions. The total number of lesions can be regarded as an indirect measure of horse-inflicted trauma, and the reduction suggests that application of a topical ointment may help relieve the severity of IBH in horses. Because both groups of horses were treated, it cannot be excluded that the number of lesions and lesion size would have improved without treatment. It would be difficult to form such a group as owners (especially those willing to participate in a research project) are reluctant to withhold treatment. Similarly, several owners indicated that they were not prepared to enrol their horses in the trial if they were to receive placebo after verum treatment. When horses were treated with the verum ointment after 3 weeks of placebo treatment, the number of lesions and total surface area were both reduced further. This could be due to an improved action of the verum ointment over the placebo or represent a continuation of the improvement that had already occurred during the 3-week placebo treatment period. The latter may be less likely given that horses suffered more discomfort from their wounds when treated with placebo and less when subsequently treated with the verum ointment. Another explanation for a reduction in the severity of clinical signs could be the seasonal occurrence of the disease, with symptom severity decreasing at the end of the 'IBH season'. However, as the horses were recruited into the study at different times of the year, this possibility can be ruled out.

It could be argued that the total number of lesions and total surface area are not a reliable measure of ointment efficacy, because the ointment cannot prevent the formation of new wounds. Therefore, the fate of wounds present at t=0 and t=7

was assessed at $t=7$ and $t=21$, respectively. Again, there was a reduction in lesion number in both groups during both the first week and the second and third weeks of the trial and the total surface area of these lesions was reduced significantly from $t=0$ to $t=7$. Although the verum ointment seemed to have a more pronounced effect during the second period than the placebo treatment, this difference was not significant.

The amount of ointment applied over the entire trial period was not significantly different between groups. It was not possible to relate the amount of ointment used to the number of lesions or lesion surface area as these were only measured at $t=0$, $t=7$, and $t=21$. The reason for determining the amount of ointment used was to ascertain whether any difference in therapeutic effect might be due to the amount of ointment being applied. However, the data showed that this was not the case.

The severity of IBH symptoms as assessed by the owner did not change significantly during the study period in any of the groups. This is not necessarily surprising as application of a topical ointment does not cure IBH and *Culicoides* will continue to bite the horses. The pruritus caused by these bites will lead to scratching and new lesions, which are all reflected in the total severity score. When the (perceived) discomfort caused by the lesions was assessed separately from the other IBH symptoms, there was a difference between verum and placebo treatments, in that the discomfort due to the lesions had increased significantly in the placebo group and decreased slightly in the verum group at 3 weeks. This suggests that the verum ointment had a soothing effect on the lesions, and the anti-inflammatory and wound healing properties of the plant-derived compound may have contributed to this effect. However, the factors influencing the healing process are complex and the ingredient (or even the mechanism) responsible for a positive effect cannot be determined from the present study. The results of this trial indicate

that it takes some time for this effect to become evident, and a significant difference compared to the placebo group was only detected after 3 weeks. In addition, the increase in discomfort suffered by horses in the placebo group occurred at the same time as the slight increase in lesion surface area (following the earlier reduction). Indirectly, any soothing effect would be expected to improve lesion healing, for if horses do not scratch/rub the lesion, healing will proceed more rapidly. As noted earlier, the positive effect of the verum ointment was not due to more ointment being applied, as in fact less ointment was used in this group of horses, which could also be seen as indirect proof of efficacy.

In conclusion, the topical application of an ointment led to a decrease in the number of lesions and their surface area in horses with IBH. The severity of IBH signs was not significantly influenced by treatment, but the degree of discomfort caused by the lesions (as judged by the owner) increased significantly in horses treated with the placebo ointment and decreased slightly (but non-significantly) when the verum ointment was used, with a significant treatment effect between groups after 3 weeks. The anti-inflammatory and wound-healing properties of the plant-derived compound in the verum ointment may have contributed to these effects. However, the exact mechanism of action cannot be determined from this study because of the complex pathophysiology of IBH.

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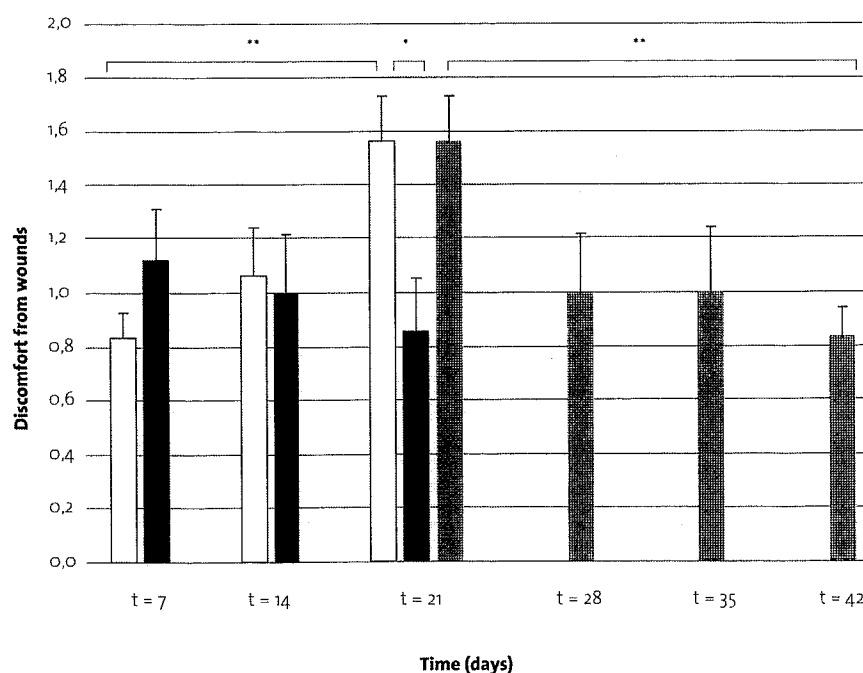


Figure 5
Discomfort suffered due to wounds compared to the week before (as scored by the owner, mean + sem, score 0-2) at different times during the study in the placebo (white), verum (black), and verum after placebo (grey) groups.

* $P < 0.05$

** $P < 0.01$

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