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Final report

A NOVEL PREVENTION STRATEGY TO IMPROVE WELFARE OF HORSES AFFECTED BY INSECT BITE HYPERSENSITIVITY

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Part 1: Detailed summary

Bakgrund: Sommareksem (Insect bite hypersensitivity, (IBH)) är en allvarlig kronisk hudinflammation som orsakas av en överkänslighet mot saliv från bitande insekter, framför allt svidknott (*Culicoides*). Sjukdomen är vanligt förekommande samt medicinskt svårbehandlad. Trots att IBH tydligt påverkar hästens välbefinnande har det saknats studier kring om hur stort lidande sjukdomen medför drabbade hästar. Dessutom finns det endast få studier angående metoder för att förhindra knottangrepp och därmed förebygga sjukdomen.
Syfte: Studien skall dokumentera hur sommareksem påverkar hästens välbefinnande samt utvärdera en ny profylaktisk metod för att förhindra knottangrepp. I studien undersöktes om naturliga giftfria insektsrepellerande doftämnen kan användas som ett miljövänligt kompletterande skydd till traditionella profylaktiska metoder för att förhindra knottangrepp.
Studiens genomförande: Totalt inkluderades 30 privatägda hästar till studien varav 14 var friska kontrollhästar och 16 hästar med historia av IBH. I studien inkluderades enbart IBH hästar som visade kliniska symptom trots insatta profylaktiska åtgärder (t.ex. eksemtäcke). Hästarnas kliniska symptom och beteende dokumenterades under två sommarsäsonger (April-Oktober). Studien var planerad att genomföras som en ”prospektiv cross-over” och ”case-control” studie, dvs man inkluderar friska kontrollhästar men IBH drabbade hästar är även sina egna kontroller (med eller utan insektsrepellerande halsband). Tyvärr såldes eller avlivades nästan alla IBH hästar (7 av 8) som inte fick halsband med repellerande doftämnen under första sommaren. Hästarna slet även sönder behållarna för doftämnen i halsbanden. Följande sommar användes inte halsband utan alla IBH hästar (pga bortfall år 1) fick insektsrepellerande doftämnen som placerades i små tvättpåsar som syddes fast i eksem/flugtäcken vid nackregionen och nära svansen. Rörelseaktiveten hos hästar med sommareksem jämfördes med friska hästar med hjälp av IceTag accelerometrar. Hypoteserna var att eksemhästar har en högre rörelseaktivitet, en kortare liggtid och en högre frekvens av utförda kli-beteenden jämfört med kontrollerna. Rörelseaktiveten dokumenterades med IceTags i en vecka under betesperioden. Direktobservationer av beteendet utfördes en gång på morgonen och en gång på kvällen i 60 minuter för varje häst. Kliniska symptom på inflammation i huden (tex hårlöshet, sår etc)

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graderades under vår och höst. Utöver detta togs även hudbiopsier från IBH hästar vid samma tillfällen. Dessa biopsier graderades sedan i avseende av tecken för allergisk inflammation.
Resultat och slutsatser: Resultat från denna studie visar att sommareksem påverkar hästarnas välbefinnande samt att djurägare valde att sälja eller avliva hästar med IBH trots att man kan förebygga sjukdomen. Trots att beteendet hos de drabbade hästarna statistiskt inte skiljde sig från kontrollerna under observationstiden, fick majoriteten av de drabbade hästar tydliga hudförändringar pga klåda. Utöver detta visade data från direktobservationerna att alla hästar utförde fler kli-beteenden på kvällen jämfört med morgonen. Sammanfattningsvis kan även kortvariga kli-beteenden orsaka tydliga hudförändringar hos IBH hästar och dessa hästar bör skyddas även mot kortvarig exponering för svidknott samt skyddas extra mot insekter under kvällstid när hästarna utför fler kli-beteenden.
Effekten av insektsrepellerande doftämnen behöver studeras vidare pga bortfall av IBH hästar i denna studie. Eventuella skillnader i förekomsten av knott mellan olika år gör det även svårare att dra någon slutsats angående effekten av insektsrepellerande doftämnen. Teoretiskt sätt kan även det insektsrepellerande ämnet ha en profylaktisk effekt även för de friska kontrollerna i samma hage. Resultaten från studien visar dock att det repellerande doftämnet är ofarligt för både människor och djur och kan användas som ett miljövänligt kompletterande skydd till traditionella profylaktiska metoder för att förhindra knottangrepp och sannolikt förbättra välfärden hos hästar med sommareksem.

Part 2: Main report

Introduction

Insect bite hypersensitivity, summer eczema (IBH, “sweet itch”; Swedish: “man- och svansskorv” or “sommareksem”) is the most common chronic allergic skin disease in horses worldwide, and has a severe negative effect on the welfare of affected horses. The disease can affect all breeds, irrespectively of age and sex . The prevalence of IBH varies widely (3-60 %) and between breeds, in Sweden, the reported prevalence in these horses is 26-35 % [1]. The affected horses mainly react against antigens present in the saliva of *Culicoides* biting midges. The main clinical sign at onset is severe pruritus caused by hypersensitivity reactions against insect bites during the warmer months from spring to autumn, which reflects the active season for the biting insects . Typically, the clinical signs regress during the winter in the absence of exposure. The preferred feeding sites of the insects are the base of the mane, base of the tail, ear pinnae, intermandibular area and the ventral midline [1], correlating with the most prominent lesional areas. Crusted papules, lichenification and dermal oedema with skin folding as well as self-trauma induced fractured hairs (“rat tail”, “buzzed off mane”), excoriations with open wounds and crusting are typical clinical signs. In addition, pigmentation disturbances and secondary bacterial infections can occur. Horses may also show behavioural changes such as increased restlessness, depression, anxiety, nervousness and due to these, loss of weight. While the suffering and pain related to an open wound is relatively obvious, less is known about how itching contributes to decreased welfare in horses. The pain induced by scratching the skin suppresses the itch temporarily, but this behaviour may lead to decreased periods of rest and development of stress. Such periods may therefore be associated with increased overall movement activity, but this has never been studied in the IBH-affected horse. If movement activity is correlated with pruritus, this measure could be used in the monitoring of insect attacks or for example of the effect of treatment of IBH.
The current clinical recommended treatment of IBH is to avoid exposure to insect, especially *Culicoides* allergens. The most common method to achieve this and prevent disease in IBH-affected horses is by mechanical protection with full-body blankets or to keep affected horses in adequately closed stables. Prophylactic insect control protocols using insecticidal repellents including pyrethrins (permethrine, cypermethrine) can be partly effective. As those agents are

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toxic to aquatic organisms, a less environmentally problematic approach is to improve the efficacy of the mosquito traps, which are commercially available. This can be achieved by exploiting the semiochemicals, which are attractants used by the insect during the location and discrimination of a suitable host. Among these are non-host volatiles that have the potential to be used in repellent collars or placed on protective blankets to reduce the attraction of blood-feeding insects [2-4]. However, the eventual benefit of such use has never been tested in horses before, and the aim of this study was therefore to test a new strategy by using an insect repellent in order to reduce the number of insect bites from *Culicoides* and thus exposure to allergens in IBH horses. Furthermore, the aim was also to investigate the effect of IBH on movement activity and behaviour of the horse. The hypothesis was that IBH-affected horses have a higher movement activity and perform more itching behaviours compared to non IBH-affected controls.

Material and methods

2.1 Study plan

A prospective cross-over and case-control study was performed during two consecutive summers (2019-2020). The study plan was approved by Regional Ethical Review Board, Sweden (5.2.18-8707/14). Horses that participated in both 2019 and 2020 were located both summer seasons in the same geographical area. Each participating stable had one or more IBH-affected horse, and one non-affected control for each horse with IBH.

At the start of the study, information about the horses were collected using a questionnaire. From June until October 2019 randomly selected horses with IBH were equipped with a collar (Horsepol, Kobyłka, Poland) were a sachet with a formulation of a novel insect-repellent odour, i.e., a blend of four non-host volatile organic compounds identified in cattle [2-4] was placed. The collar was supposed to be kept on the horse all day and night except during exercise. As there were practical problems with the repellent collars during the first study year, in 2020, two sachets of the repellent formulation were placed in small synthethic mesh bags that were sutured onto the protective blankets (one in the neck region and the second close to the tail), and all IBH horses (n=8) were provided with the novel repellent. All farms participating in the study were provided with a Mosquito Magnet® trap in order to attract and capture host-seeking mosquitoes and *Culicoides*.

2.2 *Clinical assessment and biopsy collection*

IBH-affected horses were clinically examined, and the severity of the IBH was graded in total three times during the summer using an Equine Eczema Dermatitis Extent and Severity (EEDESI) score to grade skin lesions with a maximum total lesion score of 120. Control horses were also examined to ensure that they had no clinical signs of IBH. In 2019, clinical signs of IBH were assessed in 15 IBH-affected and 13 control horses (Table 1). Nine IBH-affected horses and eight controls were assessed in both 2019 and 2020 (Figure 1). In early and late summer skin biopsies were collected from horses with IBH. The biopsies were either collected from areas with typical skin lesions indicating IBH or from the base of the mane or tail when the skin appeared normal at clinical examination. Stain biopsies were blindly graded subjectively and relatively by Diplomate in Veterinary pathology regarding grade of inflammation.

2.3 Horses

In total, 30 convenience-sampled horses were included in the study, 16 with IBH and 14 controls with no clinical signs of IBH. (Table 1). The mean age of IBH-horses and controls at the start of the study was 14.6 years and 15.7 years, respectively. During the experiment, horses were fed, kept and treated as usual, and protective horse blankets and treatments were used for some of the horses.

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Table 1. Table 1. Information on age, breed and gender for horses with insect bite hypersensitivity (IBH) and control horses that participated in the study during at least one season.

Horse	Stable	IBH/Control	Age (years)	Breed	Gender
A[1]	1	IBH	17	KWPN	Mare
A[2]	1	Control	15	Friesian	Gelding
B[1]	2	IBH	13	Irish Cob	Gelding
B[2]	2	Control	18	Knabstrup	Gelding
C[1]	3	IBH	13	New Forest	Mare
C[2]	3	Control	9	Warmblood	Mare
D[1]	3	IBH	15	Shetland pony	Mare
D[2]	3	Control	26	Arabian	Gelding
E[1]	4	IBH	24	Shetland pony	Mare
E[2]	4	Control	21	Welsh Mountain	Gelding
F[1]	4	IBH	19	Dutch pony	Gelding
F[2]	4	Control	17	Shetland pony	Gelding
G1[1]	5	IBH	13	Mixed breed	Gelding
G2[1]	5	IBH	26	Dales pony	Mare
G3[1]	5	IBH	2	Dales pony	Mare
G[2]	5	Control	24	Coldblood trotter	Mare
H[1]	6	IBH	13	Mixed breed	Gelding
H[2]	6	Control	15	Mixed breed	Mare
I[1]	6	IBH	12	Connemara	Mare
I[2]	6	Control	13	Mixed breed	Mare
J[1]	6	IBH	16	Mixed breed	Mare
J[2]	6	Control	10	Mixed breed	Mare
K[1]	7	IBH	5	Polish pony	Gelding
K[2]	7	Control	8	Mixed breed	Gelding
L[1]	7	IBH	15	KWPN	Mare
L[2]	7	Control	11	Warmblood	Mare
M[1]	8	IBH	9	Icelandic horse	Mare
M[2]	8	Control	7	Icelandic horse	Mare
N[1]	8	IBH	22	Icelandic horse	Mare
N[2]	8	Control	27	Icelandic horse	Mare

[1] IBH affected horses, [2] Control horses

2.3 Movement activity

IceTag® accelerometers (IceRobotics Ltd, Edinburgh, UK) were placed on a randomly selected hindleg of the horse. The accelerometers measured motion index which is a proprietary measure of the overall activity of the animal measured in three dimensions, steps (n) and lying (t). The

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accelerometers measured the movement activity continuously for approximately seven days, between May and August for both controls and horses with IBH, during the two consecutive summers. All matched pairs (one IBH-affected horse and one control) were measured at the same time. In 2019, movement activity was measured in total 20 horses (11 IBH-affected horses and nine controls). In 2020, movement activity was measured in total 11 horses (six IBH-affected horses and five controls). In total five IBH-affected horses and four controls were included in the study during the two years. After the measurements, the IceTags® were read in an IceReader® (IceRobotics Ltd, Edinburgh, UK) and processed in the IceManager® software (IceRobotics Ltd, Edinburgh, UK). Data were exported to Microsoft® Excel 2016 (Microsoft, Redmond, WA, USA) and non-relevant time periods, e.g., during exercise, were removed.

2.4 Direct observations of behaviours

Direct observations using an ethogram of insect-repellent behaviours (modified from Hartmann et al., 2015, Table 2[5]) were performed on 11 horses, 6 IBH- affected horses, with an average age of 14 (range 2 to 26) years, and 5 controls, with an average age of 16 (range 7 to 24) years, at five different farms during the two consecutive summers. In the summer of 2019, direct observations were carried out on another six IBH-affected horses and five controls, in addition to the 11 same horses observed in both 2019 and 2020. The total number of horses (22) observed during 2019 were located at seven different farms. Observations were performed for 60 min, and in total two times per horse and documented using video recordings. One observation was performed during the evening and one during early morning. Behaviours performed by the horses were noted in a protocol and later exported to Microsoft® Excel 2016 (Microsoft, Redmond, WA, USA).

Table 2. Ethogram used for the behavioural observations.

Behaviour	***Description***
Rolling	Lying down and moving body side to side
Body shaking	Movements in the whole body at the same time, *e.g.*, when shaking off insects
Scratching with teeth (Grooming behaviour)	Scratching with teeth at any body part
Scratching with head (Grooming behaviour)	Scratching with head (often the side of the face) at any body part, usually on the front legs
Scratching with hind leg (Grooming behaviour)	Horse brings one hind leg to its head and scratches its head or neck with the hoof .
Lifting hind leg	Hind leg moves forcefully up and down, e.g., when shaking off insects or due to irritation of the leg pad
Biting on any body part	Horse is biting lightly with the teeth on any body part
Scratching against an object	Horse scratch itself on any body part against an object, e.g., a tree or a building

2.5 Data analysis and statistics

All statistical analyses were performed in SAS software (version 9.4; SAS Institute Inc., Cary NC, USA). (info). Separate analyses for itching behaviour and EEDESI score were performed for IBH-affected horses and controls for 2019, for horses that participated in the study in both 2019 and 2020. For the analysis of movement activity, data were analysed separately for each year and from data of horses that participated in both 2019 and 2020. Data of steps (n) and lying (t) are presented as root square data when analysing data from horses participating both 2019 and 2020. The significance level was set to $P<0.05$ and the tendency level to $P<0.1$. Data from direct observations and clinical assessments were root-squared transformed before statistical analyses in order to get a normal distribution of the residuals. The effect of IBH on the number

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of observed itching behaviours was analysed with a general linear mixed model (Proc Mixed). Stable, group (IBH affected or control), observation, time of day (morning or evening), weather (sunny, cloudy or rainy) and the use or not of protective horse blankets were included in the statistical model as fixed effects, and horse as a random effect. The effect of IBH on EEDESI score was analysed using a general linear mixed model (Proc Mixed) with stable, group (IBH-affected or control), time (early summer, summer or autumn) and the use or not of protective horse blankets as fixed effects, and horse as a random effect. When analysing the effect of season (early summer, summer or autumn) on scores given to IBH-affected horses, group was removed from the statistical model. A comparison of inflammation markers from biopsies taken 2019 and 2020, and between biopsies taken during early summer and autumn, were performed using a general mixed model (Proc Mixed). The model included time (early summer or autumn and 2019 or 2020), the use or not of protective horse blankets and the use or not of treatment for IBH as fixed effects, and horse as a random effect.A mixed model (Proc Mixed), including season (early summer or autumn), the use or not of protective horse blankets and the use or not of treatment for IBH as fixed effects, and horse as a random effect was used when analysing EEDESI scores and skin inflammation markers from biopsies. A general linear model (GLM) was used to investigate the effect of IBH on movement activity for horses participating in 2019, and a general linear mixed model for horses participating in both 2019 and 2020. The GLM included group (IBH-affected horse or control), stable, the use or not of protective horse blankets as fixed effects, and age as a continuous effect. An average per minute was calculated for movement activity and number of steps. Data from direct observations and clinical assessments are presented as median (range) and from IceTags® and biopsy assessments as least square mean (LSM) ± standard error (SE), if nothing else is stated.

Results and discussion

3.1 Horses included in the study

In total 11 IBH horses and four controls were withdrawn during the study period. For the IBH-affected horses, the main reason for this was euthanasia due to other diagnosis than IBH and that the horses were sold and moved from the stable during the study period. Seven (of totally eight) IBH-affected horses that did not carry the collar during summer 2019 were sold, euthanized or left the study before next season. Four of the IBH-affected horses that carried the insect-repellent collar during season 2019 were sold (n=3) or euthanized before spring 2020. Due to missing data and technical problem with collars 2019, the IBH-affected horses that carried an insect-repellent collar in 2019 could not be used as their own controls during summer 2020.

3.2 Itching behaviours

No difference in the number of total itching behaviours between IBH-affected horses and controls was observed ($P>0.5$). An effect of stable and time of the day was found, in which a higher number of itching behaviours was observed during the evenings compared to the mornings (median 10, range 0-43 *vs*. median 1, range 0-24, respectively, $P<0.05$). Year, weather and use of insect protective horse blankets did not affect the number of itching behaviours performed by the horses ($P>0.05$).

3.3 Movement activity

Mean motion index/min, mean number of steps/min and mean lying (t) did not differ between IBH-affected horses and controls during 2019 and 2020 (Table 3a, $P>0.05$). Stable had an effect on number of steps/min in 2019 and on motion index/min in 2020 (Table, $P<0.05$). When analysing data from both years, a tendency to a higher average motion index/min was found in IBH-horses compared to controls and in horses that used protective horse blankets ($P<0.1$).

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Mean steps (n)/min and mean lying (t) did not differ between IBH-affected horses and controls (Table 3b, P>0.05).

Table 3. Average movement activity (least square mean (LSM) ± standard error (SE)) measured by IceTag accelerometers (IceRobotics Ltd, Edinburgh, UK) over approximately seven days (a) during summer 2019 (nine insect bite hypersensitivity (IBH)-affected horses and eight controls) and summer 2020 (five IBH-affected horses and four controls), and**(b)** for horses participating both 2019 and 2020 (five IBH-affected horses and four controls).

3(a)

	Year	IBH-affected horses	Controls	Effect of group	Effect of stable	Effect of age
Motion index/min	2019	17.18 ± 2.37	19.31 ± 3.70	N.S	N.S	N.S
Steps (n/min)	2019	3 ± 0.4	3 ± 0.6	N.S	<0.05	N.S
Lying (min)	2019	506 ± 99	638 ± 168	N.S	<0.1	N.S
Motion index/min	2020	16.75 ± 1.30	15.10 ± 1.40	N.S	<0.05	N.S
Steps (n/min)	2020	3 ± 0.3	3 ± 0.3	N.S	N.S	N.S
Lying (min)[a]	2020	769 ± 212	869 ± 173	N.S	N.S	N.S

[a]Average total lying time (min) measured for approximately seven days

3(b)

	IBH-affected horses	Controls	Effect of group	Effect of stable	Effect of age	Effect of protective clothing	Effect of year
Motion index/min	21.55 ± 2.7	9.34 ± 3.06	P<0.1	P<0.05	N.S	P<0.1	N.S
Steps (n/min)[a]	3 (0-4)	3 (2-5)	N.S	P<0.05	N.S	N.S	N.S
Lying (min)[ab]	623 (337-1200)	810 (653-1273)	N.S	N.S	N.S	N.S	N.S

[a]Values shown median and range, [b]Average total lying time (min) measured for approximately seven days.
Horses that were studiedin both years had a mean daily lying time of 126 ± 46 (SD) min.

3.4 Clinical signs of allergic dermatitis

During 2019, IBH- affected horses had a higher overall score of clinical lesions associated with allergic dermatitis compared to the controls (median 6, range 1-35 *vs*. median 0, range 0-5, respectively, P<0.05). During the same year, IBH-affected horses received lower lesion scores in early summer compared to in the middle of the summer and autumn (P<0.05<9. IBH-affected horses received higher lesion scores during autumn 2019 compared to early summer and summer 2020 (Table 3, Figure 1, P<0.05). The same horses were given higher lesion scores during summer 2019 compared to early summer 2020. When including both years in the statistical model, lesion scores were affected by stable (P<0.05), but not of the use of insect protective horse blankets (P>0.05). Overall, horses received higher lesion scores in 2019 compared to 2020 (P<0.05).

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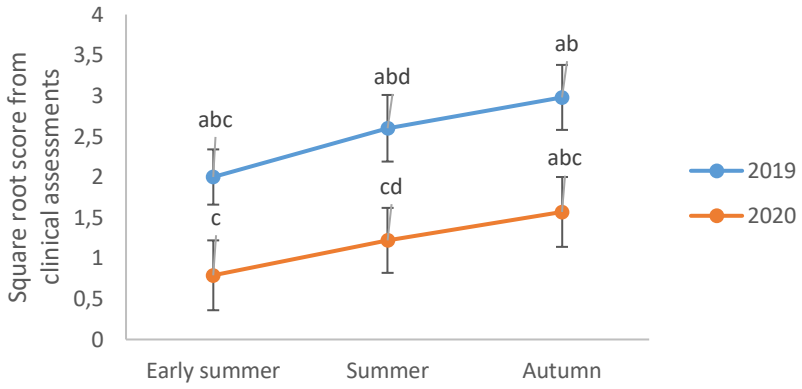

Figure 1. Scores in clinical assessments of the severity of clinical signs in nine horses affected by insect bite hypersensitivity (IBH) in early summer, summer and autumn 2019 and 2020. Values shown (square-root transformed) are least square mean ± standard error. Different letters (a-d) indicate significant differences between seasons. Overall, horses received higher scores in 2019 compared with 2020 (P<0.05).

Table 4. Scores recorded in clinical assessments (general linear mixed model (Proc Mixed)) of(a) 15 insect bite hypersensitivity (IBH)-affected horses in early summer, summer and autumn 2019 and (b) in nine IBH-affected horses in early summer, summer and autumn in both 2019 and 2020.

4(a)

Clinical Assessment	Score (mean ± SD)	Score (median and range)
Early summer 2019	5 ± 4^{a}	$3\ (1\text{-}14)^{a}$
Summer 2019	10 ± 7^{b}	$7\ (1\text{-}26)^{b}$
Autumn 2019	15 ± 11^{b}	$14\ (1\text{-}35)^{b}$

4(b)

Clinical Assessment	Score (mean ± SD)	Score (median and range)
Early summer 2019	4 ± 3^{abcd}	$3\ (2\text{-}12)^{abcd}$
Summer 2019	5 ± 3^{abc}	$6\ (1\text{-}8)^{abc}$
Autumn 2019	11 ± 11^{ab}	$8\ (1\text{-}29)^{ab}$
Early summer 2020	1 ± 1^{d}	$0\ (0\text{-}3)^{d}$
Summer 2020	3 ± 3^{cd}	$2\ (0\text{-}8)^{cd}$
Autumn 2020	3 ± 3^{abcd}	$3\ (0\text{-}8)^{abcd}$

Different superscripts indicate significant differences between seasons (P<0.05) analysed from square-root transformed data of scores from clinical assessments (not shown). For horses assessed in both 2019 and 2020, a higher total score was given to IBH-affected horses than to controls (P<0.05)

3.5 Skin biopsies

The total score from the biopsy assessment were higher in the autumn compared to early summer (Figure 2, P<0.05). Overall, no differences were found between scores in 2019 and 2020 (Figure 2, P>0.05). Horses that used protective horse blankets received higher scores (3± 0.5) compared to horses with no insect protective horse blankets (1.5±0.5, P<0.05).

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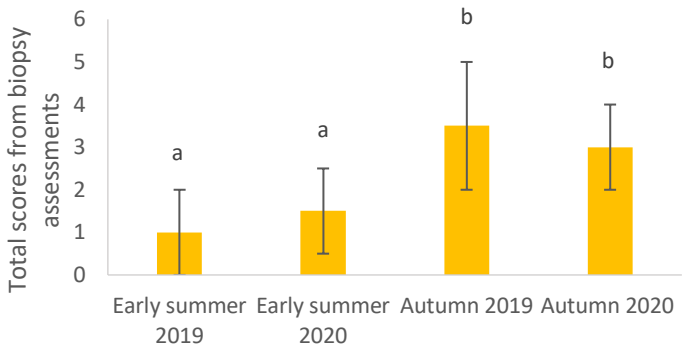

Figure 2. Total scores (least square mean ± standard error) of skin inflammation markers in biopsies collected from eight horses with insect bite hypersensitivity (IBH) in 2019 and 2020. Different letters (a, b) indicate significant differences between seasons (P<0.05)

In this study, IBH was not associated with increased movement activity and number of itching behaviours. Nevertheless, IBH-affected horses showed clinical lesions associated with scratching and the itching behaviours. One possible explanation is that the direct observation periods were too short. This is interesting, since observations were done in the periods when *Culiocoides* are most active (morning and evening). The horses performed more itching behaviour in evenings compared to mornings a finding that concurs with the results from enthomogical studies that showed that *Culicoides* are most active the first hours following sunset. This highlights the importance of adequate protection from *Culicoides* for affected horses during that period. There was also a great variation in the availability of physical objects to scratch against between different stables

According to the clinical assessments, more severe signs for IBH were recorded in 2019 compared to 2020. This may indicate a higher amount of *Culicoides* in 2019. When comparing skin inflammation markers from biopsies, no difference between the years was found. The difference in scores from the clinical assessments between the years can be due to a discrepancy in the scoring by the two assessors. However a recent study showed that different clinical scoring systems had excellent ability to correctly determine the severity of IBH in horses . Horses that used protective horse blankets had more markers for IBH according to the biopsy assessment, which may seem contradictory because, e.g., a blanket functioning as a mechanical protection from biting insects. However, the hypothesis is that horses with less severe signs of IBH did not use insect protective horse blankets and therefore received lower lesion scores. The number of horses that were excluded from the study indicate that there is a higher risk that horse owners chose to euthanize or sell an IBH-affected horse. There were no owner-reported reported changes in the horses' social interaction or any adverse skin reactions in horses that were equipped with the novel insect-repellent. Due to missing data and technical problems the prophylactic efficacy of seminochemicals against IBH symptoms could not be determined. Further studies are needed in order to evaluate the efficacy of semiochemicals to reduce allergen exposure and prevent clinical signs of IBH.

Conclusions

The results of this study demonstrate that even short periods of scratching can be associated to moderate/severe inflammatory skin lesions, and that horses display more itching during evenings. The findings of this study indicate promising preliminary results that the semiochemicals can be used as a safe, nontoxic prophylactic, environmental prophylactic strategy to potentially reduce allergen exposure and prevent signs of IBH. However, further studies are 505 needed to determine the efficacy of the semiochemicals in reducing allergen exposure 506 and preventing clinical signs of IBH.

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Relevance for the practical horse sector incl. recommendations

This study could not find any differences in movement activity and observed behaviour between IBH-affected horses and control horses. However, horses showed more itching behaviours during evenings compared to mornings and should therefore be stabled/get extra protection by e.g. insect-repellents and protective horse blankets during evenings when *Culicoides* are most active. The study indicates that even short periods of scratching are associated with moderate/severe inflammatory skin lesions. In order to increase the welfare of IBH-affected horses, even short-term exposure to *Culicoides* should be avoided.

References

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Part 3: Result dissemination

Scientific publications, *published*	**Söderroos, D, Ignell R, Haubro Andersen P, Bergvall K and Riihimäki, M. "**The effect of insect bite hypersensitivity on movement activity and behaviour of the horse" Published: 8 April 2023**.** Animals 2023, 13. https://doi.org/10.3390/ani13081283
Scientific publications, *submitted*	
Scientific publications, *manuscript*	
Conference publications/ presentations	**Oral Presentation:** "The effect of insect bite hypersensitivity on movement activity and behavior of the horse" D. Söderroos[1], P. Haubro Andersen[1]., R. Ignell[2] & M. Riihimäki[3]. International Conference: "On Horses", 11-13 November 2022 in Cáceres, Spain.
Other publications, *media etc.*	Populärvetenskaplig publikation: Ny metod att behandla sommareksem - med doftämnen. April 13, 2023, HästSverige, hemsida, samt sociala medier (facebook/instagram)
Oral communication, *to horse sector, students etc.*	Norsk Hestesenter, November 30, 2022 (oral web presentation): The effect of insect bite hypersensitivity on movement activity and behavior of the horse" D. Söderroos[1], P. Haubro Andersen[1]., R. Ignell[2] & M. Riihimäki[3]
Student theses	Marklund, Denise, 2019. *The effect of insect bite hypersensitivity on the movement activity and behavior of the horse*) Supervisor: Miia Riihimäki and Assistant supervisor: Pia Haubro Andersen, Department of Clinical Sciences, SLU.
Other	

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