

Allergen-specific immunotherapy in horses with insect bite hypersensitivity: a double-blind, randomized, placebo-controlled study

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Background – Insect bite hypersensitivity (IBH) is a common cause of pruritus in horses, but there are few controlled studies on the efficacy of allergen-specific immunotherapy (ASIT). Atopic dermatitis and IBH can present with overlapping clinical signs; multiple insect and environmental allergens could be indicated in these horses to achieve effective hyposensitization. Although the success of ASIT using *Culicoides* spp. whole-body extracts is controversial, there are no controlled studies published that clearly show benefit from this form of therapy.

Hypothesis/Objectives – The objective was to evaluate the efficacy of ASIT in horses with IBH using commercially available extracts and tests.

Animals – Twenty horses with seasonal pruritus and positive intradermal reactions to a whole *Culicoides* extract.

Methods – An enzyme-linked immunosorbent assay test (Allercept®) was used to detect concurrent allergen-specific IgE for other insects and environmental allergens. The ASIT was formulated by adding the relevant serologically positive allergens to the *Culicoides* extract. After randomization, 10 horses received ASIT and the rest a placebo solution. Clinical response was assessed every 4 months during 1 year using a clinical scoring system based on the severity of four clinical signs at 10 different body regions. Horses were not stabled and, to minimize dropouts, an insect repellent was used weekly in both groups.

Results – Differences in clinical scores between groups were nonsignificant at any re-evaluation, while both groups improved to a similar extent, probably due to the insecticide treatment.

Conclusions and clinical importance – Using commercially available extracts and tests, we could not demonstrate a beneficial effect of 1 year multiple ASIT in nonstabled horses with IBH.

Introduction

Insect bite hypersensitivity (IBH) is the most common allergic skin disease of horses and manifests as recurrent seasonal pruritus caused by the bites of insects, especially of the genus *Culicoides*. The condition has been reported worldwide, and affected horses commonly show pruritus and crusts, first located at the mane and base of the tail, then progressing to the rump and dorsal midline. The face, dorsal aspect of the pinna, neck and shoulders can also be involved. Self-trauma and chronicity lead to erosions and ulcers, hair loss, lichenification and

pigmentary disturbances.^{1,2} At present, the definitive diagnosis is based on the history (seasonality of the condition and recurrence in sequential years), physical examination (specific clinical signs), ruling out other conditions causing pruritus and a positive response to insect control and therapy.³ Histopathological findings in skin biopsies or intradermal tests are not considered necessary for the diagnosis of IBH.⁴

Allergen-specific immunotherapy (ASIT) is used in human and small animal dermatology. In equine atopic dermatitis (AD), there are several open studies that have shown encouraging results using whole-allergen extracts.^{5–7} Conversely, there are few studies published on the efficacy of ASIT for horses with IBH, and the results using whole-body extracts of *Culicoides* spp. are contradictory. In an open study, 10 horses with IBH received weekly injections of *Culicoides* antigen administered with an adjuvant composed of a nonpathogenic mycobacterial cell wall fraction; nine of the 10 horses improved clinically after the first year of treatment, and eight horses were treated with a maintenance dose during

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a second year. Three of these eight horses were completely free of clinical signs, three showed much less severe clinical signs and two showed moderate reduction in clinical signs compared with untreated years.⁸ In contrast, a randomized, double-blinded, placebo-controlled clinical study did not show any improvement in the clinical signs of IBH-affected horses over a 6 month period.⁹

Equine AD is another potential cause of seasonal pruritus, and both equine AD and IBH are associated with allergen-specific IgE (to environmental allergens or biting insects, respectively).¹⁰ The disease may be seasonal or nonseasonal, depending on the allergens involved, and affected horses commonly show pruritus involving the face, distal legs or trunk during noninsect seasons or year round.¹¹ Equine AD and IBH can present with overlapping clinical signs and, depending on the geographical location, severe and more chronic IBH cases display lesions not only during the active period of the biting insects but year-round dermatitis, like some horses with AD.¹ Exclusion of AD in a horse with confirmed IBH is also hindered by the lack of reliable diagnostic tests to identify hypersensitization to environmental allergens by intradermal or serological testing methods. Many IBH-affected horses present positive results to environmental allergens in both skin and serological tests. Although the relevance of these reactions is controversial, some horses may have IBH and concurrent AD.¹

Several serology tests are available for the detection of allergen-specific IgE in the horse. An extensive study concluded that the equine Allercept® (Heska Corp., Boulder, CO, USA) test had a fair correlation with the intradermal test, thus representing a possible alternative to intradermal tests for the identification of hypersensitivity.¹² The use of serology in the horse has many advantages in comparison to skin tests, but the initial positive results of the test Allercept® could not be confirmed by a second study, in which sera from healthy and allergic horses were analysed blindly; the authors concluded that the test was not suitable as a tool for establishing a diagnosis of IBH or equine AD.¹⁰ As in dogs or cats, the results of intradermal and serological tests in horses should only be used to help formulate ASIT.

Currently, ASIT for IBH and equine AD is offered by many commercial laboratories and there is a strong demand from general practitioners and horse owners for this type of therapy, because measures to avoid or reduce exposure to insect bites may be difficult to implement. In this clinical context, a 3 month placebo-controlled study compared ASIT using whole-body insect extracts with ASIT containing insect and environmental allergens in horses affected by IBH and AD.¹³ Although differences between groups were not significant, there were significant differences within groups, and some horses showed an objective clinical improvement especially when whole-body insect allergens were used. Since that report in 1998, no further controlled studies have been published regarding ASIT in IBH-affected horses using either insect extract or multiple insect and environmental allergens in the same ASIT. As the placebo-controlled phase of the previously cited study lasted only 3 months, our hypothesis was that a similar but longer study would be necessary to establish the efficacy of

ASIT in horses with IBH. In order to achieve this goal, we selected horses with a clinical diagnosis of IBH and positive intradermal reactions to a *Culicoides nubeculosus* whole-body extract. Hypersensitivities to other insects and environmental allergens were investigated serologically, and the response to multiple ASIT was evaluated after 1 year of therapy.

Materials and methods

Animals and inclusion criteria

A total of 20 purebred Spanish horses (10 mares and 10 stallions; mean age, 5 years) from two different studs were included in the study. Horses were included if they had seasonal pruritus in two consecutive insect seasons, typical signs of IBH that improved when stabled and a positive skin test result against a whole extract prepared from *C. nubeculosus* (Greer Laboratories, Lenoir, NC, USA). The threshold concentration for the extract was 1 µg/mL as determined in 10 normal horses following criteria previously used to establish threshold concentrations in canine IDT.¹⁴ As recommended by several authors,^{4,15} only immediate reactions at 15–30 min were evaluated; diameters were calculated by objective measurement (in millimetres) of the wheal reaction determined as the average of two perpendicular axes. Subjective evaluation was not performed. Only horses showing clearly positive intradermal responses were included in the study.

To look for hypersensitivity to other insects and environmental allergens, sera from the horses were analysed with the use of Equine Allercept® (Heska Corp.), a noncompetitive, solid-phase enzyme-linked immunosorbent assay (ELISA) test designed to detect the presence of allergen-specific IgE in sera using the recombinant α -chain of the high-affinity IgE receptor (Fc ϵ R1 α).

Treatment

Horses were assigned to two groups, those being treated with ASIT and those receiving a placebo. Allergen-specific immunotherapy was formulated using alum-precipitated, adsorbed allergens prepared by a commercial laboratory (Univet, Barcelona, Spain) that performed the serological tests. The treatment group received multiple ASIT with *C. nubeculosus* antigen and relevant ELISA-positive allergens. Allergens were prepared in three 10-fold dilution vials, with a maximal concentration of 1×10^{-3} mg/mL protein in vial 3, following the standard procedure recommended by the manufacturing laboratory. Increasing volumes from vials 1 and 2 were administered subcutaneously every week until vial two administration was finished (9th week). Injections were then administered every 3 weeks four times until reaching the maximal dose of allergen to be administered (0.8 mL from vial 3; 21st week). For the rest of the treatment period, injections were administered monthly. To guarantee blinding for the owner and the clinicians evaluating the clinical response, both placebo and allergen-specific solutions were prepared to appear similar in consistency and dispensed in identically labelled vials. Horses were not stabled and, for humanitarian reasons and to be able to extend the time of the clinical trial, a 5% nonresidual cypermethrin formulation (Barricade®; Pfizer S.L.U. Alcobendas, Madrid, Spain) was used once weekly as a spray in both groups during the study. Based on our local clinical experience, this treatment would not induce clinical remission in nonstabled horses and was necessary to minimize dropouts. No other treatments were allowed.

Randomization was restricted, allocating 10 horses to each group stratified by sex, stud and disease severity, so that each group included five mares and five stallions, five horses from each herd of horses and a closely balanced clinical score between groups.

Assessment of clinical scores

To estimate the severity of the clinical signs and the treatment efficacy in the studied horses, clinician investigators used a lesional and pruritus scoring system based on the severity (0, none; 1–2, mild; 3–4, moderate; and 5–6, severe) of pruritus, alopecia, excoriations and skin thickening at 10 different body regions (head, right neck, left

neck, forelimbs, hindlimbs, dorsum, rump and mane, abdomen, axillae and perineal region). Therefore, the maximal clinical score achievable was 60 units for each of the four clinical signs evaluated, with a maximal total clinical score of 240 units. Horses were visited monthly, but evaluations were performed at the beginning of the treatment and every 4 months thereafter during 1 year. If a horse needed to be stabled, it was evaluated at that point in time, and the recorded clinical score carried forward to the next evaluation. There were no significant differences in clinical scores between groups at the start of the study, which was initiated in mid-January of 2008.

Outcome measures

The primary end-point variable was clinical improvement as measured by the clinical scores associated with ASIT and insect repellent administration. The tolerability of the treatment and adverse effects were also recorded.

Statistical analysis

Clinical scores were compared between groups and within groups at each re-evaluation time point. The distribution of the variable in all data columns was analysed by the Kolmogorov–Smirnov normality test. Mean clinical scores were compared by one-way repeated-measures ANOVA with Tukey's multiple comparisons *post hoc* test. All statistical calculations were performed using the Prism 5.01 software for windows (GraphPad Software, San Diego, CA, USA). The statistical power of the sample size was determined after the experiment was completed (Statmate 1.00; GraphPad Software).

Results

The distribution of lesions in the studied horses was mainly dorsal, involving, by decreasing frequency, the tail, rump, mane, dorsal midline and face. The abdomen and distal extremities were rarely involved. Seventeen of 20 horses had positive results in the ELISA test, and 12 of 20 horses were positive for *Culicoides*-specific serum IgE antibodies, which represented a 60% positive correlation between intradermal and ELISA tests for *Culicoides* antigen. The three horses with no positive ELISA results showed classical signs of IBH and had strongly positive intradermal reactions to the whole *Culicoides* extract.

Insects and mites were the most common positive serology test results. Positive reactions to pollens and especially moulds were less frequent (Table 1). More severely affected horses had the highest number of ELISA-positive results, but linear correlation with basal clinical scores was statistically not significant ($P > 0.6$). In

the treatment group, ASIT was formulated with a maximum of six allergens for treatment, combining *Culicoides* and the more relevant ELISA-positive allergens. Final ASIT composition included only *Culicoides* antigen in one horse, while six horses received only arthropod antigens and three horses received arthropod antigens plus another antigen (Table 2).

After stratified randomization, initial clinical scores were similar for both groups (Table 3). At each re-evaluation, clinical scores improved progressively, with a transient worsening at the third evaluation in September. Scores of both groups grossly overlapped during the year, with differences between mean clinical scores always being less than eight units. The statistical analysis comparing groups confirmed that differences in mean clinical scores at each time point were nonsignificant. In contrast, mean clinical scores within each group at each re-evaluation time point were significantly reduced in both groups compared with pretreatment clinical scores (Table 3); however, at the end of the trial no horse from either group was clinically normal.

Four horses from the placebo group and three horses from the treatment group had to be stabled during periods of time ranging from 1 to 3 weeks. Immunotherapy was well tolerated in all horses. No adverse reactions of any type were recorded. Once the study was completed, we calculated that sample size provided a 95% statistical power to detect a difference between mean clinical scores of 30.42 units, which represented approximately 32% of baseline clinical scores; smaller differences would not be considered statistically significant.

Discussion

At the end of the study, mean clinical scores at each re-evaluation time point were not significantly different between groups, and a similar number of horses from each group had to be stabled temporarily when the owners considered clinical signs unacceptable. Therefore, in this study, using commercially available extracts and tests, we could not demonstrate a significant clinical response to 1 year of ASIT in horses with IBH. Both groups showed a similar reduction in clinical scores that was significant at the end of the study, although all horses still showed clinical signs of IBH. This significant

Table 1. Allergens included in the equine enzyme-linked immunosorbent assay Allercept® and results for the 20 horses with seasonal pruritus in the study

Insects	(+)	Pollens	(+)	Fungi	(+)
<i>Tabanus</i>	13	<i>Phleum</i> sp.	3	<i>Alternaria alternata</i>	2
<i>Culicoides</i>	12	<i>Dactylis glomerata</i>	4	<i>Rizhopus nigricans</i>	0
<i>Simulium</i> spp.	12	<i>Secale cereale</i>	5	<i>Ustilago tritici</i>	0
Mosquito	10	<i>Rumex acetosella</i>	7	<i>Fusarium culmorum</i>	2
<i>Blatella germanica</i>	9	<i>Plantain</i> sp.	4	<i>Penicillium notatum</i>	0
<i>Stomoxys calcitrans</i>	1	<i>Urtica</i> sp.	1	<i>Helminthosporium halodes</i>	0
Mites		<i>Chenopodium album</i>	1	<i>Aspergillus fumigatus</i>	0
<i>Dermatophagoides farinae</i>	9	<i>Artemisia glutinosa</i>	0	<i>Cladosporium herbatum</i>	0
<i>Dermatophagoides pteronyssinus</i>	8	<i>Fagus sylvatica</i>	7	<i>Epicoccum nigrum purpurascens</i>	0
<i>Tyrophagus putrescentiae</i>	7	<i>Corylus avellana</i>	3		
<i>Acarus siro</i>	3	<i>Betula</i> sp.	2		
		<i>Salix</i> sp.	2		

Symbol: (+), number of horses positive to each allergen.

Table 2. Allergens selected for the allergen-specific immunotherapy in the treatment group

Horse no.	Allergens selected for allergen-specific immunotherapy
1	<i>Culicoides</i> ; <i>Tabanus</i> ; <i>D. farinae</i> ; <i>T. putrescentiae</i> ; <i>Rumex acetosella</i>
2	<i>Culicoides</i> ; <i>Tabanus</i> ; <i>T. putrescentiae</i>
3	<i>Culicoides</i>
4	<i>Culicoides</i> ; <i>T. putrescentiae</i> ; <i>Simulium</i> sp.; <i>D. pteronyssinus</i>
5	<i>Culicoides</i> ; <i>Tabanus</i> ; Mosquito; <i>D. farinae</i> ; <i>Simulium</i> sp.
6	<i>Culicoides</i> ; <i>Tabanus</i> ; Mosquito
7	<i>Culicoides</i> ; <i>Tabanus</i> ; Mosquito
8	<i>Culicoides</i> ; <i>Tabanus</i> ; Mosquito; <i>Rumex acetosella</i>
9	<i>Culicoides</i> ; Mosquito; <i>D. farinae</i> ; <i>A. siro</i>
10	<i>Culicoides</i> ; <i>Tabanus</i> ; <i>D. farinae</i> ; <i>T. putrescentiae</i> ; <i>A. siro</i> ; <i>Plantain</i> sp.

Abbreviations: *A. siro*, *Acarus siro*; *D. farinae*, *Dermatophagoides farinae*; and *T. putrescentiae*, *Tyrophagus putrescentiae*.

improvement was more probably related to the use of the weekly insecticide rather than ASIT.

Clinical studies with privately owned horses usually have limitations. In the present study, one concern is that a strong repellent action could have resulted in high placebo effect and masked the potential response to ASIT. Although we cannot exclude this interference, we consider it unlikely unless the beneficial effect of ASIT was small. It has to be taken into account that, in spite of the insecticide treatment, final mean clinical scores showed a <50% reduction from baseline values in both groups; also, clinical scores in spring and summer were high, and some horses even had to be stabled. The duration of the study justified the use of active measures to control insect exposure in the placebo group to avoid dropouts, but we purposely used an insecticide with no residual activity to minimize the treatment effect. In our experience, the use of insect repellents in nonstabled horses, as the only form of therapy, rarely results in good control of clinical signs. Moreover, a recent report evaluating the repellent effect of a similar insecticide (permethrin, 3.6%) found that it did not cause a significant reduction in the numbers of feeding *Culicoides*.¹⁶ Thus, we hypothesized that the administration of this insect repellent would not produce a strong enough effect to mask a clinically significant benefit from ASIT administration in the treated group. A noninferiority trial with a proven effective intervention is indicated when the duration of the study justifies the need for an active control of the placebo group.¹⁷ This was impossible to accomplish when designing our study, because we were not aware of any study reporting

such proven effective therapy in equine IBH using a validated or comparable severity scale index. Also, as any potential ASIT effect was expected to progress slowly, a measure to partly control the exposure to insect bites was considered unavoidable, and the owners had to be given the option to stable the horses if they considered clinical signs unacceptable.

In veterinary medicine, ASIT is a well-established therapy in the management of canine AD, and several open studies suggest that it is also useful in the management of equine AD;⁵ however, the usefulness of ASIT in equine IBH is controversial because few controlled studies are available.¹ One hypothesis is that the success of specific immunotherapy for IBH could be increased with the use of pure salivary gland proteins instead of *Culicoides* whole-body extracts. However, *Culicoides* whole-body extracts are the only antigen source readily available, and previous studies have shown that these contain a wide range of IgE-binding antigens and that allergic horses show positive reactions to these antigens.¹⁸ Also, a recent study found *C. obsoletus* whole-body extracts useful to support a clinical diagnosis of equine insect hypersensitivity,³ and in small animal and equine ASIT for AD, the use of whole-allergen extracts is a generalized practice. More recently, a prospective evaluation of ASIT in horses with AD found a significant clinical benefit in nine horses treated for 24 months.⁶ Thus, until pure salivary antigens are commercially available, the investigation of ASIT using whole-insect extracts will have clinical interest. In this respect, it is surprising that we could find only two previous controlled and double-blind studies about the use of ASIT in horses with IBH, and both of them were performed more than 15 years ago. The study of Barbet *et al.*⁹ evaluated ASIT in 14 horses with IBH during a 6 month trial. No other allergens were used in the ASIT because all horses were IDT negative to other insects and moulds, but they were not tested for pollens. Based on clinical scores, there was no difference between horses receiving the *Culicoides* antigen and control horses; however, untreated pollen hypersensitivity or the rather short trial period could have lowered ASIT efficacy.

In a second placebo-controlled report,¹³ insect hyposensitization was compared with multiple insect and environmental allergen hyposensitization in a group of horses with clinical IBH and AD. The placebo-controlled part of this study lasted only 3 months, and differences between groups were not significant. However, when the horses were evaluated within their group, the improvement was significant only in the treatment

Table 3. Descriptive statistics of clinical scores in the placebo (P) and treatment (T) groups

	January		May		September		December	
	P-0	T-0	P-1	T-1	P-2	T-2	P-3	T-3
Mean	74.4	74.8	54	47.6*	56.6	58	26.8**	34.8***
SD	16.46	33.15	11.08	16.27	14.85	25.35	25.98	23.31
Minimum	48	40	40	24	38	20	12	20
Median	76	60	52	44	55	55	38	20
Maximum	96	132	78	84	84	104	94	90

* $P \leq 0.05$, ** $P \leq 0.01$ and *** $P \leq 0.001$, statistical significance of mean clinical scores at each re-evaluation compared with pretreatment clinical scores within group. All results were normally distributed (Kolmogorov–Smirnov normality test); $n = 20$.

groups. The author concluded that some horses benefit through intradermal testing and hyposensitization, particularly those horses in which insect allergens were used.¹³ In our study, there was no significant difference between groups after a 12 month trial. Although the time to maximal clinical benefit before determining treatment efficacy of ASIT is variable, most studies in dogs using aqueous and alum-precipitated allergens report a time to improvement of <9 months.⁵ In horses with AD, a beneficial response to ASIT may be seen within 2–3 months of treatment, and the maximal benefit is expected to be achieved after 1 year.¹⁹ However, a recent prospective, uncontrolled study in horses with AD receiving ASIT reported that clinical improvement was evident before completing 12 months of therapy but continued in the next 12 months.⁶ Thus, a longer duration of ASIT would have been desirable, but due to a lack of perceived clinical efficacy the horse owners were not willing to maintain ASIT administration.

Other factors concerning the study design that could have contributed to an apparent lack of success were sample size and the clinical assessment scale used. Sample size was small but similar to many previous studies on ASIT in equine IBH or AD. Once the study was completed, we determined that sample size provided a 95% statistical power to detect a 32% or higher change from baseline values. A clinical improvement of <30% would not be clinically significant or regarded as acceptable for most owners.²⁰ Concerning the clinical scoring scale, it has been pointed out that unvalidated scales might not be precise enough to detect a small treatment effect, or they might not be reproducible enough to permit the detection of treatment efficacy.¹⁷ At the time of performing the study, validated scales were not available for the clinical assessment of horses with IBH or AD. Our scale was similar to those used in the assessment of clinical signs in canine AD and was considered reproducible by the evaluators before initiating the study. Although we could not find evidence for a clear tendency towards better final clinical scores in the treatment group, it cannot be excluded that a potential small beneficial effect of ASIT could be masked by the factors previously discussed.

In horses, the use of serology has many advantages compared with skin testing. Several specific serological tests are available for the detection of allergen-specific IgE in the horse. A previous extensive study concluded that the equine Allercept® (Heska Corp.) had a fair correlation with the intradermal test,¹² thus representing a possible alternative to intradermal tests for the identification of hypersensitivity. The initial positive results of the test Allercept® could not be confirmed by a second study, in which sera from healthy or allergic horses were analysed blind; the authors concluded that the test was not suitable as a tool for establishing a diagnosis of IBH or equine AD.¹⁰ This conclusion is well established in small animals, where results of intradermal and serological tests have minor diagnostic value but are used to formulate ASIT. Thus, controversies regarding the identification of allergen-specific IgE in the horse are not very different from those maintained in small animal AD, where ASIT is a well-accepted mode of therapy. Also, some horses have

been reported to have IBH and AD.¹ In addition to the positive intradermal reaction to whole *Culicoides* extract, most horses included in the study (17 of 20) had one or more positive ELISA results, and the inclusion of not only insect antigens but all relevant positive serological results had the potential to increase the clinical response to ASIT. The ELISA method could have produced a significant number of positive results that did not represent a true clinical sensitization, but this is a problem also encountered with the intradermal test.^{21–23} Although we cannot know how many of these reactions were relevant, we consider it unlikely that their inclusion in the ASIT formulation had a negative effect on the clinical response compared with the use of only *Culicoides* ASIT.

In this study, the administration of ASIT with whole *Culicoides* antigen, combined with other insect and environmental allergens identified by ELISA, failed to provide a significant improvement in nonstabled horses with *Culicoides* hypersensitivity compared with a placebo group. Although several limitations of the study design, such as sample size, time of follow-up and the use of insect repellents could have negatively influenced the final outcome, not even a slight tendency to better clinical scores was observed in the treated group. In conclusion, although a small beneficial effect of ASIT could have gone undetected, the results of this controlled study seem to indicate that using commercially available extracts and tests, 1 year multiple ASIT has a limited clinical value in nonstabled horses with IBH.

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Résumé

Contexte – L'hypersensibilité aux piqûres d'insectes (IBH) est une cause fréquente de prurit chez le cheval. Il existe cependant peu d'études contrôlées sur l'efficacité de l'immunothérapie spécifique d'allergènes (ASIT). La dermatite atopique et l'IBH peuvent présenter des signes cliniques communs; plusieurs allergènes d'insectes et de l'environnement pourraient être indiqués chez ces chevaux pour réaliser une hyposensibilisation efficace. Bien que le succès des ASIT à l'aide d'extraits de corps entier de *Culicoides* spp. soit controversé, il n'y a aucune étude publiée qui montre clairement les effets de cette forme de traitement.

Hypothèses/Objectifs – L'objectif était d'évaluer l'efficacité de l'ASIT chez le cheval atteint d'IBH à l'aide de tests et d'extraits disponibles dans le commerce.

Sujets – Vingt chevaux présentant un prurit saisonnier et des intradermoréactions positives à un extrait de *Culicoides*, corps entier.

Méthodes – Un test ELISA (Allercept®) a été utilisé pour détecter les IgE spécifiques d'allergènes environnementaux et les autres insectes. L'ASIT a été formulée en ajoutant les allergènes positifs significatifs à l'extrait de *Culicoides*. Après randomisation, 10 chevaux ont reçus l'ASIT et les autres, une solution placebo. La réponse clinique a été évaluée tous les 4 mois pendant 1 an à l'aide d'un système de notation clinique basé sur la sévérité de quatre signes cliniques sur 10 zones corporelles différentes. Les chevaux étaient au pré et, pour minimiser les sorties de l'étude, un répulsif d'insectes a été appliqué chaque semaine dans les deux groupes.

Résultats – Les différences de scores cliniques entre les groupes étaient non-significatives à chaque évaluation, les deux groupes s'améliorant de façon identique, probablement grâce au traitement insecticide.

Conclusions et importance clinique – En utilisant des tests et des extraits disponibles dans le commerce, nous n'avons pas pu démontrer d'effet bénéfique d'une ASIT multiple d'un an sur des chevaux au pré atteints d'IBH.

Resumen

Introducción – la hipersensibilidad a la picadura de insectos (IBH) es una causa frecuente de prurito en caballos, pero hay pocos estudios controlados acerca de la eficacia de la inmunoterapia específica de alérgeno (ASIT). La dermatitis atópica y la IBH pueden presentarse con signos clínicos parecidos; alérgenos múltiples de insectos y ambientales pueden estar indicados en estos caballos para obtener una inmunoterapia más efectiva. Aunque el éxito de ASIT utilizando extractos totales de *Culicoides* spp. es controvertido, no existen estudios controlados publicados que muestren un claro beneficio de esta terapia.

Hipótesis/Objetivos – el objetivo fue evaluar la eficacia de ASIT en caballos con IBH utilizando extractos y pruebas comerciales.

Animales – veinte caballos con prurito estacional y reacciones positivas intradérmicas a un extracto total de *Culicoides*.

Métodos – una prueba de enzoinmunoensayo (Allercept®) fue utilizada para detectar la presencia simultánea del IgE específica de alérgeno para otros insectos y alérgenos ambientales. La ASIT fue formulada añadiendo los alérgenos positivos relevantes a nivel serológico al extracto de *Culicoides*. Tras distribución

al azar, 10 caballos recibieron ASIT y el resto solución placebo. La respuesta clínica fue valorada cada cuatro meses durante un año utilizando un sistema de puntuación basado en la severidad de cuatro signos clínicos en 10 regiones diferentes del cuerpo. Los caballos no fueron estabulados y, para minimizar pruebas incompletas, se utilizó un repelente de insectos en ambos grupos.

Resultados – las diferencias en las puntuaciones clínicas entre grupos no fueron significativas en ninguna reevaluación, mientras que ambos grupos mejoraron al mismo nivel, probablemente debido al tratamiento insecticida.

Conclusiones e importancia clínica – utilizando extractos y pruebas comerciales no pudimos demostrar un efecto beneficioso en un año de múltiples ASIT en caballos no estabulados con IBH.

Zusammenfassung

Hintergrund – Insektenstich Hypersensitivität (IBH) ist eine häufige Ursache für Juckreiz bei Pferden, aber es gibt nur wenige kontrollierte Studien bezüglich der Wirksamkeit Allergen-spezifischer Immuntherapie (ASIT). Atopische Dermatitis und IBH können mit überlappender Symptomatik vorkommen; multiple Insekten- und Umweltallergene könnten bei diesen Pferden nötig sein, um eine wirksame Hyposensibilisierung zu erzielen. Obwohl der Erfolg von ASIT mittels *Culicoides* spp. Ganzkörperextrakten kontroversiell ist, sind keine kontrollierten Studien publiziert, die einen Nutzen dieser Therapieform klar zeigen.

Hypothese/Ziele – Das Ziel war es, die Wirksamkeit von ASIT bei Pferden mit IBH mittels kommerziell erhältlichen Extrakten und Tests zu evaluieren.

Tiere – Zwanzig Pferde mit saisonalem Juckreiz und positiven Intradermaltestreaktionen auf *Culicoides* Ganzkörperextrakt.

Methoden – Ein Enzym-linked Immunosorbent Assay Tests (Allercept®) wurde verwendet, um gleichzeitige Allergen-spezifische IgE auf Insekten und Umweltallergene zu bestimmen. Die ASIT wurde formuliert, indem die relevanten serologisch positiven Allergene zu den *Culicoides* Extrakten hinzugefügt wurden. Nach einer Randomisierung erhielten 10 Pferde ASIT und der Rest eine Placebolösung. Die klinische Antwort wurde während des ersten Jahres alle 4 Monate mit einem klinischen Beurteilungsschema, welches auf vier klinischen Zeichen an 10 verschiedenen Körperstellen beruhte, beurteilt. Die Pferde waren nicht aufgestellt, und um Ausfälle zu minimieren, wurde wöchentlich ein Insektenrepellent in beiden Gruppen verwendet.

Ergebnisse – Bei der Reevaluierung waren die Unterschiede der klinischen Werte zwischen den Gruppen nicht signifikant verschieden, während beide Gruppen eine Besserung in gewissem Ausmaß zeigten, möglicherweise durch die Behandlung mit einem Insektizid.

Schlussfolgerungen und klinische Bedeutung – Wir konnten mit der Verwendung kommerziell erhältlicher Extrakte und Tests mit multiplen ASIT bei nicht aufgestellten Pferden mit IBH nach einem Jahr keine Verbesserung zeigen.

要約

背景 – 昆虫刺傷過敏症 (IBH) はウマにおいて一般的なそう痒の原因となるが、アレルゲン特異的免疫療法 (ASIT) の効果における対照研究はほとんどない。アトピー性皮膚炎およびIBHは重複する臨床症状を示す可能性があり、複数の昆虫および環境抗原がそれらのウマにおいて効果的な減感作療法が成功する可能性がある。*Culicoides* spp. 全体の抽出液を用いたASITの成功は議論の余地があるが、この治療形態の利点を明確に示す対照研究は報告されていない。

仮説/目的 – 目的は市販されている抽出液と検査を用いて、IBHを示すウマにASITの効果を評価すること。

供与動物 – 季節性にそう痒を示し、皮内反応で*Culicoides*全体抽出物に陽性反応を示す20頭のウマ

方法 – 酵素免疫測定法検査 (Allercept®) を、その他の昆虫や環境中アレルゲンに対してアレルゲン特異的IgEの検出のために使用した。ASITは関連する血清学的な陽性アレルゲンを*Culicoides*抽出液に加えることによって調剤した。ランダム化した後、10頭のウマはASITを与えられ、その他はプラセボ液を与えられた。臨床的な反応を、10ヶ所の身体の部分における4つの臨床症状の重症度に基づいた臨床的なスコアシステムを用いて1年の間、4ヶ月ごとに評価した。ウマは厩舎ではなく管理され、脱落を最小にするため、両方のグループに毎週昆虫忌避剤を用いた。

結果 – グループ間における臨床スコアでの差はいずれの再評価でも有意差はなく、その一方でおそらく殺虫剤での治療によって、両方のグループで同程度に改善していた。

結論および臨床的な重要性 – 市販で入手可能な抽出液と検査を用いて、筆者らはIBHを示す厩舎にいないウマにおける1年の複合的なASITで有益な効果を実証することができなかった。

摘要

背景 – 昆虫叮咬 (IBH) 是马常见的瘙痒原因, 但是抗原特异性免疫治疗 (ASIT) 控制此病的研究很少。异位性皮炎和IBH的临床症状有交叉; 昆虫和环境混合抗原可能带给马有效的脱敏治疗。有研究发现使用库蠓整体萃取物有效, 但由于实验无对照组, 无法显示明确的有效性而存在争议。

假说/目的 – 使用商业萃取物和试验, 评估ASIT对马IBH的效果。

动物 – 20匹马患有季节性瘙痒, 而且对库蠓萃取物皮内实验反应阳性

方法 – 使用酶联免疫吸附试验 (Allercept®), 测试其他昆虫和环境抗原的抗原特异性IgE。使用血清学阳性抗原并加入库蠓萃取物作为ASIT组合。随机分组, 10匹马接受ASIT, 其他使用安慰剂注射液。为期一年每4个月评估一次, 用临床评分体系评估4种临床症状和10个身体部位。马匹没有限制在马厩, 为了控制实验动物流失, 两组均每周一次使用昆虫趋避药物。

结果 – 每次2组的临床评分均没有显著差异, 两组的临床改善程度相同, 可能归功于杀昆虫药的使用。结论和临结论和临床价值-使用商业萃取物和试验, 对于患有IBH的散养马匹进行组合ASIT, 没有显示临床效果。