

Co-reactivity between related and unrelated environmental allergens in equine allergen-specific IgE serology testing in the UK

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Background – Identification of environmental allergens in horses with allergic disease facilitates allergen avoidance and targeted immunotherapy.

Hypothesis/Objectives – To evaluate allergenic co-reactivity between 44 environmental allergens.

Animals – Horses with suspected allergic disease ($n = 344$) whose sera were submitted for environmental allergen testing.

Methods and materials – Allergen-specific IgE serological assays were performed using 44 allergens divided into six taxonomically related groups: house dust/storage mites, moulds, insects, grass, tree and weed pollens. Using pairwise comparisons, odds ratios (ORs) were calculated for each environmental pair to determine if there was increased or decreased likelihood of a positive result for one allergen, given a positive result to another. The OR significance was set (using Holm–Bonferroni correction) at $P < 0.00006$ for all horses ($n = 344$) and $P < 0.00005$ for horses with at least one positive reaction ($n = 239$). Using one-way ANOVA with Tukey's *post hoc* tests (significance at $P < 0.05$), differences in mean log e ORs between three groups, taxonomically related allergens with a statistically significant association (related-associated), related allergens lacking a significant association (related-nonassociated) and unrelated allergens were tested.

Results – Statistically significant associations were found between both related and unrelated allergen pairs, the former being more frequent. For all horses ($n = 344$) and horses with at least one positive reaction ($n = 239$), co-reactivity ranged from 100% (grasses) to 0% (moulds). The weeds group was exceptional in having more co-reactions with another group (grasses).

Conclusions and clinical importance – Co-reactivity was shown within and between certain related allergen groups. Further studies are required to determine whether this is the result of antigenic cross-reactivity.

Introduction

Equine allergic diseases can affect both the skin and the respiratory tract and include insect bite hypersensitivity (IBH), atopic dermatitis (AD), food allergy and asthma.¹ Horses frequently suffer from a combination of allergic conditions and consequently detection of all contributing allergenic stimuli is important for successful management.² A provisional diagnosis of allergic disease is made firstly by the systematic ruling-out of other conditions.³ Subsequently, causative allergens may be identified by an enzyme-linked immunosorbent assay (ELISA) for allergen-specific immunoglobulin (Ig)E and or intradermal tests (IDT).^{4–8} Correlation of these results with the clinical history is critical regardless of the method of allergen

identification.⁹ Management strategies can include allergen avoidance, where practical, or allergen-specific immunotherapy (ASIT) which, in a limited number of studies, has been found to be beneficial in 60–80% of horses.^{2,3,7}

Precise identification of the causative allergen(s) is essential; where structural similarities exist between allergens they may be recognized as the same (i.e. as cross-reactive).¹⁰ This may be suspected when pairs or groups of allergens are significantly co-positive (or co-reactive). Statistical pairwise analysis can be used to demonstrate co-reactivity between allergens identified by either IDT or serology.^{10,11}

One canine study addressing this issue looked at IDT results from 268 dogs diagnosed with AD.¹² Pairwise analysis was used to evaluate 30 comparisons within five distinct subsets of allergens. No significant differences were found between concurrent positive reactions to closely related and unrelated plant allergens. A second canine study looked at 25 pairwise comparisons of 22 related and unrelated airborne allergens, using 1,000 IDT results from atopic dogs.¹³ The results found a significantly higher mean log odds ratio (OR) between the

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closely related allergens, when compared to the nonrelated allergens. It was concluded that this had resulted from either co-sensitization or cross-reactivity. However, two other findings in the study led to the overall conclusion that cross-reactivity could not be established. The first was that there also were significant differences in the mean log OR between most of the groups which were comprised of nonrelated allergen pairs. The second was that pairwise analysis revealed a large number of dogs (30–50%) with positive reactions to the allergens evaluated, which lacked concurrent positive reactions to related allergens.

A further study of IDT results for 53 environmental allergens in 651 atopic dogs involved pairwise analysis within and between related and unrelated allergen groups, with the related groups defined as house dust/storage mites, epidermals/fibres, moulds, insects, tree, weed and grass pollens.¹⁰ The ORs, 95% confidence intervals (CIs) and statistical significance were calculated, and it was shown that results for allergens within each group were statistically associated, with the exception of cotton, cockroach, red clover, grain smut and *Penicillium*. This relationship also extended beyond the immediate family groups to plant-derived allergens, with 94% of the results between the tree, weed and grass pollen allergens found to be significantly associated, with the exceptions of red clover and cotton. In contrast, few allergens from unrelated groups were found to be significantly associated.

Published data on co-reactivity between, or within, equine environmental allergen groups, is less extensive and focussed on insects.^{5,14–21} The current study investigated co-reactivity based on the results of IgE serology to 44 environmental allergens belonging to allergen groups that are routinely employed when investigating cases of suspected allergic disease in horses. It is the first critical step enabling subsequent studies to determine whether any demonstrable co-reactivity is the result of cross-reactivity. In turn, this can potentially lead to more effective allergen avoidance and/or ASIT.

Methods and materials

Serum samples

Pre-existing, anonymized data from 344 serological tests were evaluated retrospectively for this observational cohort study. Sera were submitted to the Avacta Animal Health laboratory, Wetherby, UK, for commercial testing between March 2016 and July 2018.

ELISA for allergen-specific IgE

Allergens employed

Allergens (n = 44), selected by likely environmental exposure (or a closely related species depending on commercial availability), were purchased from Stallergenes Greer (Lenoir, NC, USA) or Squarix GmbH (Marl, Germany).

Detection reagent

The detection reagent used in the assay was a monoclonal antibody specific for horse IgE (University of Bristol, UK) as described previously.²²

Coating of the plates

Allergens were diluted in 0.05 M carbonate/bicarbonate buffer, pH 9.6, pipetted at predetermined saturating levels onto 96-well microtitre plates (Microlon 600, Greiner; Kremsmunster, Austria) and

incubated at 2–8°C overnight. Plates were washed three times with tris-buffered saline (TBS) containing 0.05% Tween-20 (TBST) and blocked with TBS containing 0.5% sucrose and 0.5% polyvinylpyrrolidone 10 (PVP10; blocking solution) for 2 h at ambient temperature. After removal of the blocking solution, plates were dried at 37°C for 2 h and stored at 2–8°C sealed in foil pouches with desiccant until required.

Performance of the assay

Equine serum samples were diluted 1 in 10 in TBST. Standards employed were serial three-fold dilutions of a pool of horse sera containing a high level of anti-*Dermatophagoides farinae* – specific IgE. Undiluted, this pool was assigned a value of 25,000 arbitrary units (AUs). Dilutions of the standard (1.23–100 AU) and samples were applied to the allergen-coated wells and incubated at 2–8°C overnight. Plates were washed three times with TBST and incubated with alkaline phosphatase (AP)-labelled anti-horse IgE (University of Bristol, UK, conjugated with Lightning Link AP, Expedeon, Cambridge, UK) for 2 h at ambient temperature. After a final six washes with TBST, plates were developed with AP substrate (pNPP, Surmodics Inc.; Eden Prairie, MN, USA) at 2–8°C overnight. The ELISA plates were brought to room temperature and the reaction was stopped with 1 M sodium hydroxide solution. Absorbances were determined at 405 nm using a microplate reader (BioTek; Winooski, VT, USA) within 10 min.

Data interpretation

The anti-IgE reagent lacked detectable reactivity against equine IgG on wells coated with $\leq 10 \mu\text{g/mL}$. Inter-assay variation for all sera tested was <20% and intra-assay variation was <20%. The absorbance readings were subjected to blank subtraction and the standards data fitted to a 4-PL (parameter logistic) curve fit. For each of the samples, the absorbance value read at 405 nm was interpolated against the standard curve. A value of ≥ 5 AU was classed as positive.

Statistical analysis

The allergens were separated into six taxonomically related groups (house dust/storage mites, tree pollens, grass pollens, weed pollens, insects and moulds). Contingency tables were generated from the positive and negative serological reactions for all allergens within the related groups and also between allergens from unrelated groups, to enable pairwise comparisons (n = 946). The ORs, 95% CIs and statistical significance was calculated for each allergen pair using the statistical package R [R Core Team (2018): A language and environment for statistical computing, R Foundation for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org/>]. Separate analyses were undertaken for the total population (n = 344) and for horses with at least one positive IgE reaction (n = 239), hereafter known as 'positive reactors'. The Holm–Bonferroni method was used to calculate a corrected P-value, adjusting the commonly used value for significance of 0.05. This is essential due to multiple comparisons being made, to avoid the problem whereby statistically unlikely events become likely when many statistical comparisons are performed. The P-value in this work means the probability of a result given no association between two allergens. However, at $P < 0.05$, only 20 comparisons would be needed to see such an unlikely outcome. Given that hundreds of such comparisons are made, a P-value adjustment is essential. The Holm–Bonferroni correction was used, which orders the P-values from contingency tables (generated via a Fisher exact test) and compares them with a list of increasing adjusted P-values. The first P-value that is larger than the adjusted P-value is the adjusted significance level. The calculated values for the Holm–Bonferroni were $P < 0.00006$ for all horses (n = 344) and $P < 0.00005$ for the positive reactors (n = 239).

The mean log e ORs were calculated to show the associations between three groups: taxonomically related allergens with a statistically significant association (related-associated), related allergens with a nonstatistically significant association (related-nonassociated) and unrelated allergens. One-way ANOVAs with Tukey's *post hoc* tests (significance at $P < 0.05$) were used to test for differences

between mean log e ORs between the three groups as employed in previous co-reactivity studies.^{10,11} This method requires the underlying data to be normally distributed, which was confirmed by visually checking a quartile-quartile plot and the plot of the three distributions.

Ethical approval

Consent was obtained on the laboratory submission form covering the collection, storage and subsequent publication of summarized (anonymized) data. The study was approved by the Nottingham Trent University ethics committee (reference number ARE810).

Results

Incidence of reactions

Of the 344 horses, 239 (69%) had a positive IgE reaction to one or more allergens (Table 1). Of these positive reactors (n = 239), 119 (50%) and 60 (25%) had a positive IgE reaction to 1–4 and 5–8 allergens, respectively. This equates to 35% and 17% of all horses tested (n = 344). Reactions to 8–12 allergens and >12 allergens were less frequent. The median number of reactions was 4.5. The five most prevalent reactions were to *Dermatophagoides farinae*, *Tabanus* spp., dock, *Culicoides nubeculosus* and *D. pteronyssinus* (Figure 1).

Pairwise analysis

The results of the pairwise analysis for all horses (n = 344) are displayed as a correlation plot in Figure 2, and for the positive reactors (n = 239) in Figure 3.

For both cohorts statistically significant associations were found between both related and unrelated allergen pairs. However, associations were more frequent between related pairs, with 47% and 41% of allergens co-reacting compared to 14% and 6% of unrelated allergens for all horses (n = 344) and positive reactors (n = 239), respectively. For all horses (n = 344) the ORs ranged from 0.225 (CI 0.0133–3.90) to 664 (CI 82.8–5324) ($P = 0.22$ to $P < 0.0001$) and for the positive reactors (n = 239) from 0.140 (CI 0.008–2.42) to 454 (CI 56.6–3645) ($P = 0.12$ to $P < 0.0001$). Such ORs should be interpreted with respect to the increased or decreased likelihood of a horse having a positive result for one allergen given a positive result to another. For example, the smallest OR seen, 0.22, was for privet and *Acarus siro*. This OR below 1 suggests a negative correlation and can be interpreted as saying that if a horse is positive to privet, it is 78% less likely to be positive to *A. siro*. At the other extreme, the largest OR seen, 664, was for nettle and privet. This suggests that if a horse is positive to nettle it is 664 times more likely to be positive to privet.

The percentage of allergens which were shown to co-react both within and between each of the six

taxonomically related groups is shown in Table 2. Grass pollens were 100% co-reactive in both cohorts. For all horses (n = 344) and positive reactors (n = 239), the percentage of co-reactivity within each of the other five taxonomically related groups was (respectively) 73% and 67% for mites, 49% and 42% for weeds, 33% and 20% for insects, 29% and 18% for trees and 0% for both in the case of moulds. For both cohorts, the weeds group was exceptional in being the only group where more co-reactions were seen with another group (grasses). For both the cohort consisting of all horses (n = 344) and cohort of positive reactors (n = 239), the mean (SD) log e ORs of the related/associated allergen pairs [3.49 (0.73)] and [3.10 (0.72)], respectively, were significantly greater ($P < 0.05$) than all unrelated allergen pairs [1.52 (1.10)] and [1.04 (1.12)] (Table 3; Figure 4).

Discussion

In this study significant associations were found between both related and unrelated equine allergen pairs. The co-reactions were, however, significantly more frequent between related pairs of allergens than unrelated pairs, which is in agreement with a canine study of a similar number of pairwise comparisons both within and between groups.¹⁰ The result differs from earlier canine studies which were of limited value due to the small number of comparisons made both within, and between, different groups of allergens.^{12,13} This was likely to have limited the ability to detect association patterns.

Over two-thirds (69%) of the 344 horses showed a positive IgE reaction to one or more of the 44 environmental allergens. Samples were submitted from veterinary practices as part of the investigation of the potentially allergic horse and inclusion criteria did not require a definite diagnosis of allergy. It is thus possible that the 31% lacking reactivity were suffering from conditions unrelated to allergy or, alternatively, were reactive to allergens that were not included in the testing panel. It also is possible that their disease was immunologically mediated but that IgE was not involved. However, the existence of atopic-like dermatitis, which in dogs is defined as "an inflammatory and pruritic dermatitis with clinical features identical to those seen in canine AD in which an IgE response to environmental or other allergens cannot be documented"²³ has yet to be demonstrated in horses. The administration of drugs that effect immunoglobulin production cannot be ruled out either, although the laboratory does provide guidance on recommended withdrawal times before sampling. Pairwise comparisons for horses with at least one positive IgE reaction were included to establish whether these factors could have skewed the

Table 1. Incidence of IgE reactions in 344 horses to environmental allergens

IgE reaction	Number of horses (n = 344)	Percentage of all horses (n = 344)	Percentage out of all positive reactors (n = 239)
Negative to all allergens	105	30.5	
Positive to ≥1 allergen	239	69.5	
Positive to 1–4 allergens	119	34.6	49.8
Positive to 5–8 allergens	60	17.4	25.1
Positive to 8–12 allergens	31	9.0	13.0
Positive to >12 allergens	29	8.4	12.1

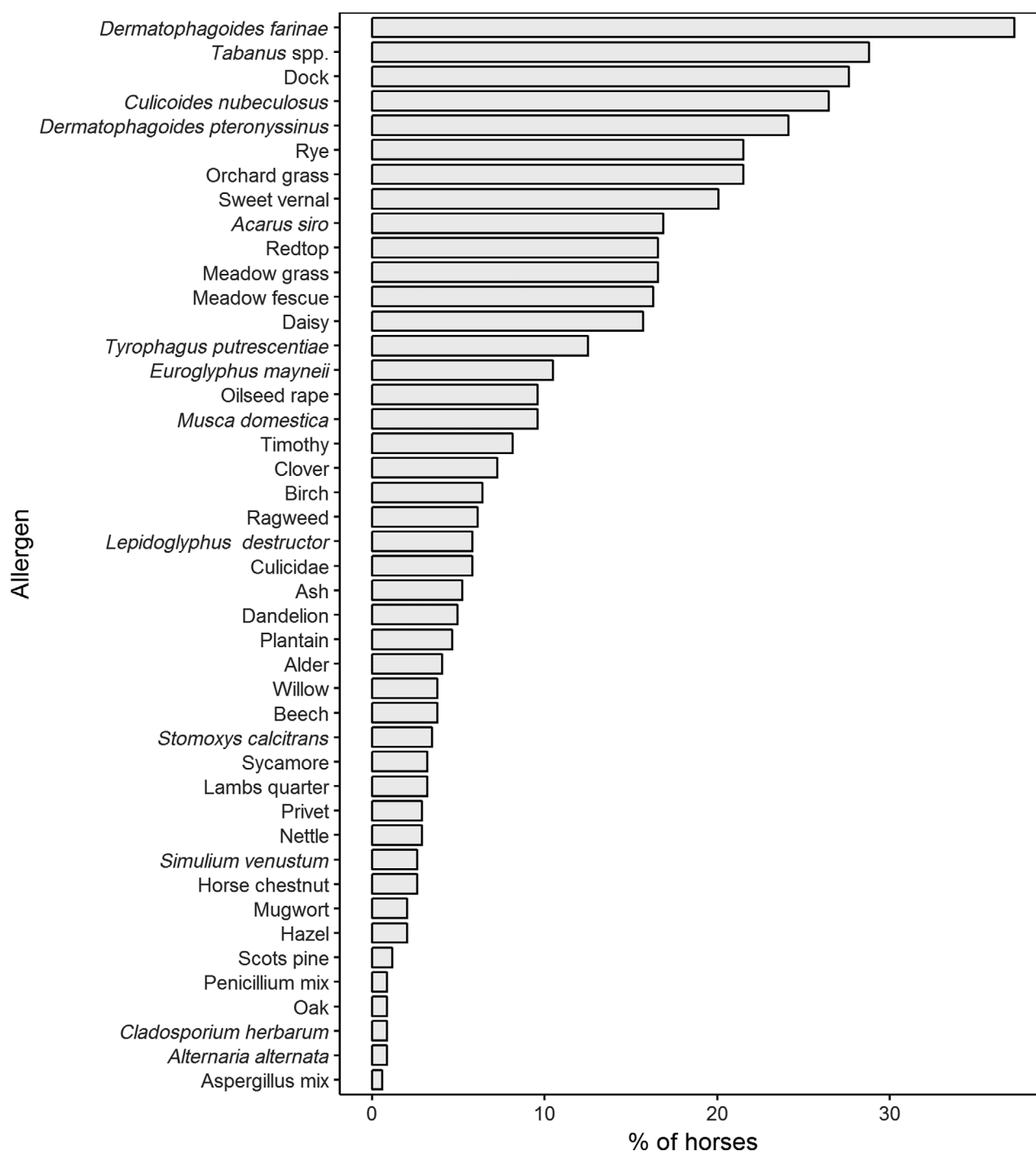


Figure 1. The frequency of IgE reactions to 44 environmental allergens in all horses (n = 344) with suspected allergic disease.

results.¹¹ The conclusion for both the all horses data (n = 344) and the positive reactors (n = 239) was, however, the same; that co-reactions were significantly more frequent between related pairs of allergens than unrelated pairs.

Dermatophagoides farinae was the most frequently recorded positive reaction in this study with *D. pteronyssinus* the fifth most common. Both dust mites have been reported as common household environmental allergens with established allergenic significance in canine AD.²⁴ An Australian study demonstrated the presence of both dust and storage mites in horse rugs,²⁵ but neither *D. farinae* nor *D. pteronyssinus* were

identified within the equine stabling environment in the United States.²⁶ In two UK studies, *D. farinae* was found only rarely within the household environment with the apparent marked sensitization attributed to likely cross-reactivity,^{27,28} which was conclusively shown in a study conducted in Japan.²⁹ Further work is needed to establish the prevalence of these mites within the UK equine environment, although significant levels of co-reactivity within the mites group was shown in this study. For the cohort containing all horses (n = 344), with the exception of *Lepidoglyphus destructor*, all mites within this group (*D. farinae*, *D. pteronyssinus*, *Euroglyphus maynei*, *A. siro* and *Tyrophagus putrescentiae*) co-reacted. This

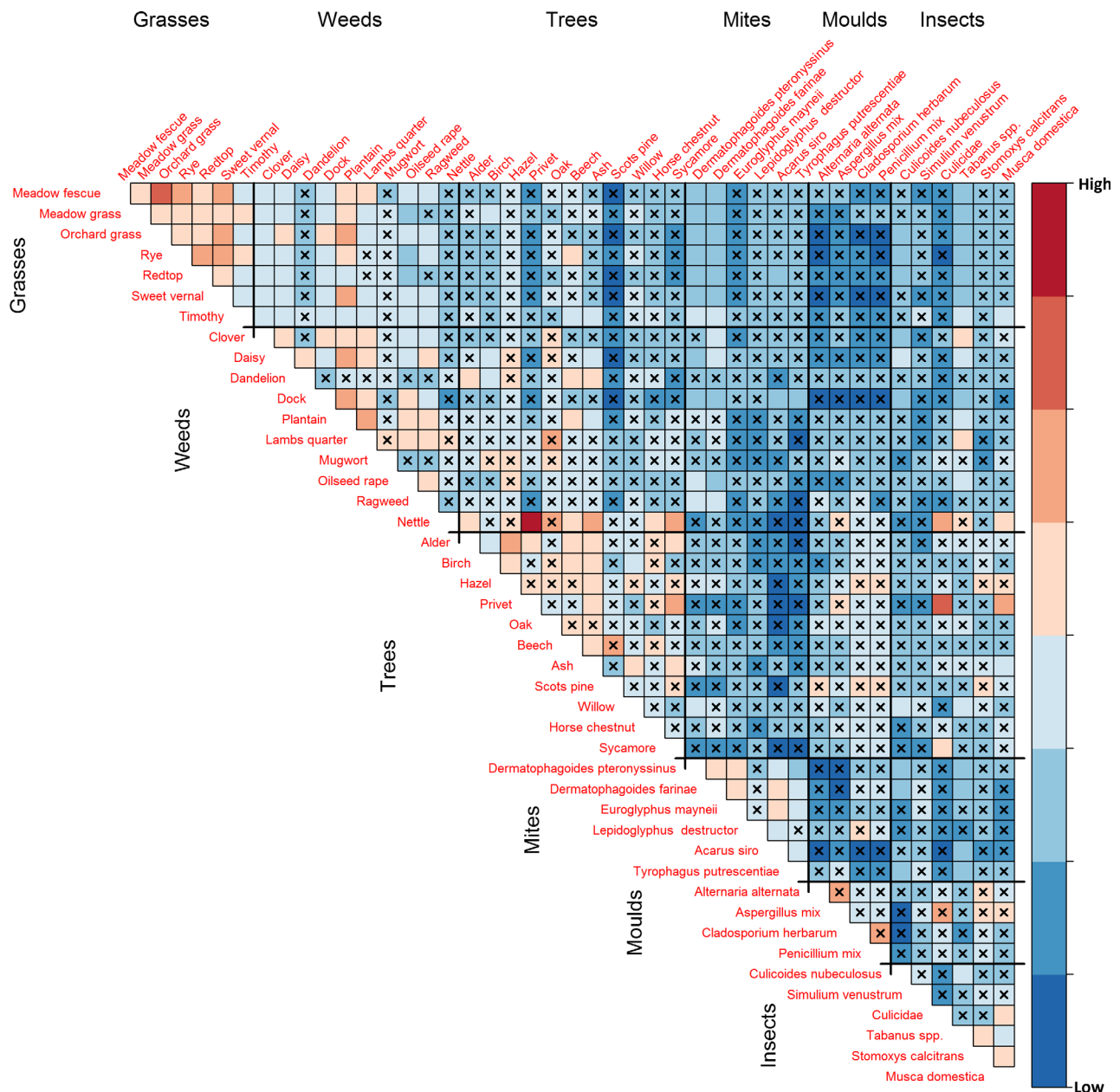


Figure 2. A visual representation of the odds ratio (OR) results with adjusted Holm–Bonferroni significance for each allergen pairwise analysis for all horses ($n = 344$).

Colours are used for the scale with red being a high OR and blue being a low OR. Due to the presence of a small number of very high ORs the values were logged before attributing colours in order to better differentiate the lower OR values from the higher ones. Values which had a P -value greater than the adjusted Holm–Bonferroni significance value are crossed out because they are therefore deemed statistically nonsignificant.

correlates with other cross-inhibition studies which have found extensive cross-reactivity between house dust and storage mites.^{24,30}

In the current study, marked differences in the level of co-reactivity within each of the six taxonomically related groups were found. For the cohort containing all horses ($n = 344$) the majority of allergens within the grasses, weeds and mites groups were significantly associated with the exceptions of dandelion, mugwort, nettle and *L. destructor*. Co-reactivity for the majority of allergens within these three groups also was found in the previously mentioned canine study, in which a statistical association was found within all of the other taxonomically related groups that they investigated and also across all

plant-derived allergens.¹⁰ This is in contrast with the current study, although co-reactivity was shown by the majority of the weed and grass allergens for the all horse cohort ($n = 344$).

Grass pollens were found to be the most co-reactive group with 100% co-reactivity in both cohorts, whereas the majority of tree allergens in this study did not show statistical correlation with weeds or grasses. This agrees with a finding that grass pollens are more cross-reactive than tree pollens in man.³¹ The potential impact of cross-reactive carbohydrate determinants (CCDs), which may result in an IgE response and therefore contribute to false positive results, has been linked particularly with grass pollens.^{32–34} A canine study looking at sera from 38 dogs

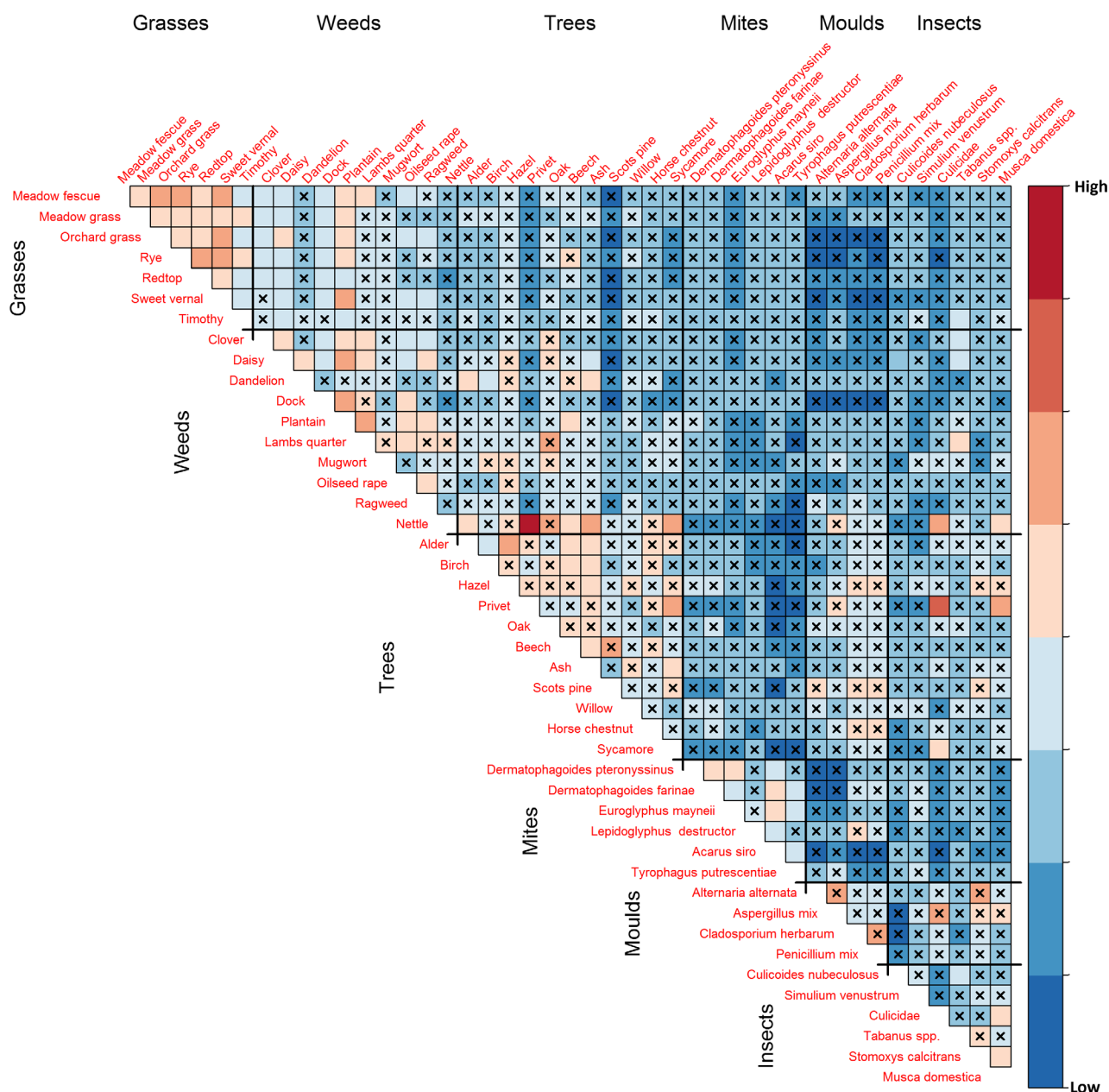


Figure 3. A visual representation of the odds ratio (OR) results with adjusted Holm–Bonferroni significance for each allergen pairwise analysis for all horses with at least one positive reaction ($n = 239$).

Colours are used for the scale with red being a high OR and blue being a low OR. Due to the presence of a small number of very high ORs the values were logged before attributing colours in order to better differentiate the lower OR values from the higher ones. Values which had a P -value greater than the adjusted Holm–Bonferroni significance value are crossed out because they are therefore deemed statistically nonsignificant.

diagnosed with AD, detected anti-CCD IgE in 24% of those tested.³⁴ All dogs with anti-CCD IgE had strong serological reactivity to grass pollens which is in agreement with previous human studies. In a second study using a CCD inhibitor to investigate sera from 60 dogs, the greatest levels of inhibition were found in those dogs which had shown positive IgE reactivity to all grasses.³² The role of CCD-specific IgE is unknown but is believed to be nonpathogenic.³⁴

No statistically significant co-reactivity was found either within or between the mould group in this study although the frequency of IgE reactivity was very low. However, it is noteworthy that in a study on sera from horses with what was previously termed recurrent airway obstruction

(RAO), reactivity was more common to recombinant allergens than to crude allergens such as were used in the current study.^{35,36} It also has been suggested that IgE plays a role only in a subset of RAO horses which have a genetic predisposition.^{5,37} Furthermore, considerations such as seasonality, climatic variation and environmental conditions (i.e. stabled, kept at grass or combination of both, whether or not exposed to dried forage) were not factored into the evaluation of these results. Each of these could have impacted on the level of allergen exposure and therefore the likelihood of reactivity and co-reactivity being demonstrated. A Polish study investigating environmental allergen-specific IgE responses in healthy horses found marked seasonal variation in reactivity

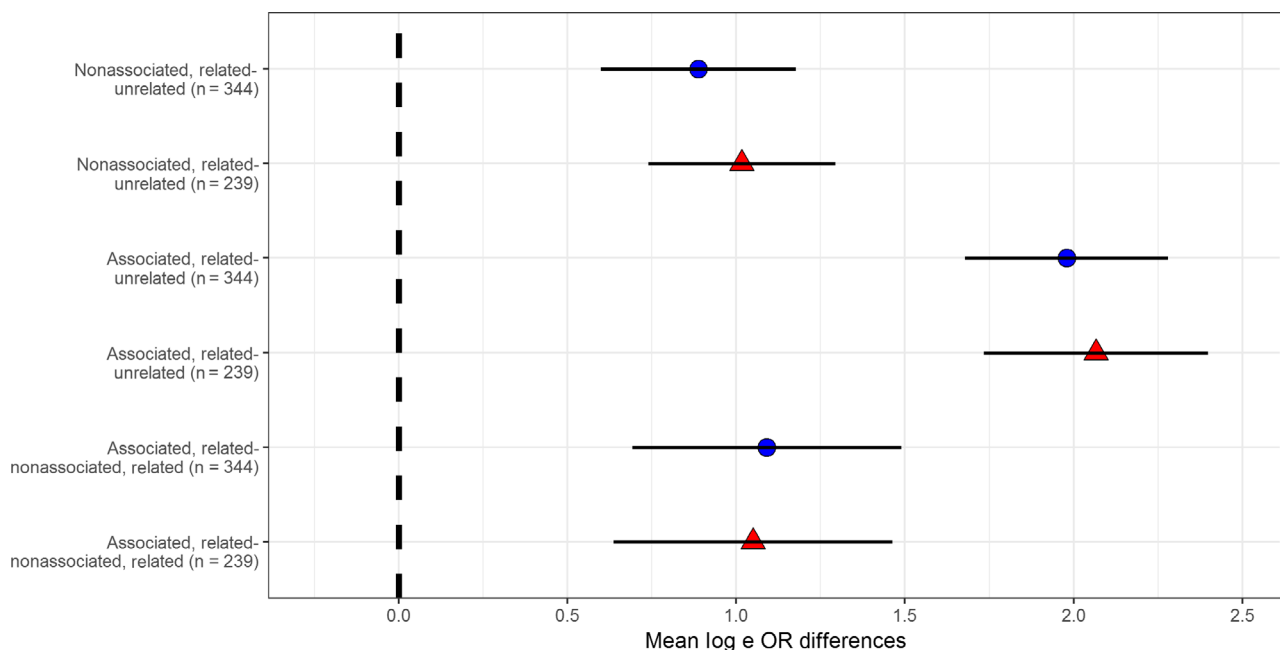
Table 2. The percentage of allergens co-reacting within and between taxonomically related groups

		All horses (n = 344)					
		Grasses	Weeds	Trees	Mites	Moulds	Insects
Positive reactors (n=239)	Grasses	100.00 100.00	64.30	5.20	35.70	0.00	26.20
	Weeds	44.30	48.90 42.20	12.70	18.30	0.00	18.30
	Trees	0.00	9.10	29.10 18.20	1.50	0.00	9.10
	Mites	0.00	0.00	0.00	73.30 66.70	0.00	19.40
	Moulds	0.00	0.00	0.00	0.00	0.00 0.00	0.00
	Insects	2.40	10.00	4.50	0.00	0.00	33.30 20.00

Values in bold show the percentage of co-reactivity within each group of related allergens, for both all horses (n = 344, upper value) and positive reactors (n = 239, lower value).

Table 3. The mean, SD and number of cases for log e odds ratios between the three groups: taxonomically related/associated allergens, related/nonassociated allergens and unrelated allergens

	All horses (n = 344)			Positive reactors (n = 239)		
	Associated - Related	Nonassociated - Related	Unrelated	Associated - Related	Nonassociated - Related	Unrelated
Mean	3.49	2.40	1.52	3.10	2.05	1.04
SD	0.73	0.93	1.10	0.72	0.95	1.12
n	75.00	82.00	789.00	63.00	94.00	789.00

**Figure 4.** Plot of the mean log e odds ratio (OR) differences between three groups; taxonomically related/associated allergens, related/nonassociated allergens and unrelated allergens.

The circle and triangle represent the mean value for all horses (n = 344) and the positive reactors (n = 239), respectively, and the horizontal line from this point shows the 95% CI. The dotted line indicates the point of zero difference between mean values.

levels.³⁸ A similar seasonal effect was not, however, demonstrated in horses with IBH³⁹, leading to the hypothesis that in allergic horse's the plasma cells may continuously produce IgE without B-cell reactivation.³⁸

Although positive reactions to insect allergens were common (particularly to *Tabanus* spp. and *Culicoides*

spp.) co-reactivity was infrequent. Notably, significant co-reactivity between *Culicoides* spp. and *Simulium* spp. was absent, which is in contrast to earlier studies demonstrating cross-reactivity between the two.²¹ Employing recombinant techniques, a salivary gland antigen belonging to the 5-like protein family was identified in both

Culicoides spp. and *Simulium* spp. which was responsible for both *in vitro* and *in vivo* cross-reactivity in a proportion of horses affected with hypersensitivity to *Culicoides* spp.^{5,14–21} In the current study, the OR results between the two species for the all horses data ($n = 344$) and positive reactors data ($n = 239$) were 10.46 and 6.08, respectively, but when adjusted by the Holm–Bonferroni method both were statistically nonsignificant. However, reactions to *Simulium* spp. were rare (nine of 344, or 2.62%) which limits the likelihood of demonstrating co-reactivity. This could reflect a low density of *Simulium* spp. in the environment of the sampled population or a lower sensitivity of the assay due to the use of whole-body extract as opposed to recombinant allergens.⁴⁰ Additionally, limited commercial availability of *Simulium* spp. allergen necessitated the use of *S. venustum* as opposed to *S. vittatum* as used in the studies noted previously.^{19–21} Of additional interest is that whilst *Culicoides* spp. was the fourth most prevalent allergen in this study, with 26% (91 of 344) of all horses demonstrating a hypersensitivity response, only six horses reacted to this antigen alone. This equates to 2% of all horses tested (six of 344) or 7% of those horses showing *Culicoides* spp. hypersensitivity (six of 91). This suggests the possibility that hypersensitivity to additional allergens may contribute to the clinical signs seen in horses diagnosed as suffering from *Culicoides* spp. hypersensitivity, which is an important consideration in their management.

In conclusion, this study found statistical association in both related and unrelated equine environmental allergen pairs. The co-reactions were significantly more frequent between related than unrelated allergen pairs. Further work using cross-inhibition ELISAs is required to establish whether this co-reactivity was due to co-sensitization (separate IgE-binding epitopes) or to antigenic cross-reactivity (shared IgE-binding epitopes).¹¹ Any cross-reacting proteins can then subsequently be identified by Western blotting and mass spectrometry.⁴¹ Improving knowledge of cross-reactivity between allergens is of clinical importance for optimizing strategies for allergen avoidance (where practical) and/or ASIT.

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Résumé

Contexte – L'identification d'allergènes environnementaux chez le cheval allergique facilite l'éviction et l'immunothérapie ciblée.

Hypothèses/Objectifs – Evaluer la co-réactivité allergénique entre 44 allergènes environnementaux.

Sujets – Des chevaux suspects d'allergie (n=344) dont les sera ont été soumis pour tests d'allergènes environnementaux.

Matériels et méthodes – Les tests sérologiques d'IgE spécifiques d'allergènes ont été réalisés à l'aide de 44 allergènes divisés en six groupes de taxonomie reliée : acariens de la poussière de maison/stockage, moisissures, insectes, pollens d'herbe, d'arbre et de graminées. A l'aide de comparaisons paires, les odds ratios (Ors) ont été calculés pour chaque paire environnementale pour déterminer si il y avait une augmentation ou diminution de la probabilité d'un résultat positif pour un allergène donnant un résultat positif pour un autre. L'importance de l'OR a été définie (à l'aide d'une correction Holm-Bonferroni) à $P < 0.00006$ pour tous les chevaux (n=344) et $P < 0.00005$ pour les chevaux avec au moins une réaction positive (n=239). A l'aide d'ANOVA unidirectionnelle avec tests post hoc de Turkey (significatif à $P < 0.05$), les différences de log e moyen des ORs entre les trois groupes, les allergènes reliés par taxonomie avec une association statistiquement significative (apparenté-associé), les allergènes reliés manquant une association significative (reliés-non associés) et les allergènes non reliés ont été testés.

Résultats – Des associations statistiquement significatives ont été trouvées entre les paires d'allergènes liés et non liés, ces derniers étaient plus fréquents. Pour tous les chevaux (n= 344) et les chevaux avec au moins une réaction positive (n=239), la co-réactivité évoluait de 100% (herbes) à 0% (moisissures). Le groupe des graminées présentait exceptionnellement des co-réactions avec le groupe des herbacées.

Conclusions et importance clinique – La co-réactivité a été démontrée en intra et intergroupes d'allergènes. D'autres études sont nécessaires pour déterminer si ceci résulte d'une réaction antigénique croisée.

Resumen

Introducción – la identificación de alérgenos ambientales en caballos con enfermedad alérgica facilita la exposición a alérgenos y la inmunoterapia dirigida.

Hipótesis/Objetivos – Evaluar la co-reactividad alérgica entre 44 alérgenos ambientales.

Animales – caballos con sospecha de enfermedad alérgica (n = 344) cuyos sueros fueron sometidos a pruebas de alérgenos ambientales.

Métodos y materiales – se realizaron ensayos serológicos IgE específicos para alérgenos utilizando 44 alérgenos divididos en seis grupos taxonómicamente relacionados: ácaros del polvo doméstico/de almacenamiento, mohos, insectos, hierba, árboles y pólenes de malezas. Utilizando comparaciones pareadas, se calcularon las relaciones de probabilidad (OR) para cada par ambiental para determinar si había una probabilidad aumentada o disminuida de un resultado positivo para un alérgeno, dado un resultado positivo para el otro. La significación de OR se estableció (usando la corrección de Holm-Bonferroni) en $P < 0,00006$ para todos los caballos ($n = 344$) y $P < 0,00005$ para caballos con al menos una reacción positiva ($n = 239$). Se probaron usando ANOVA unidireccional con las pruebas post hoc de Tukey (significancia en $P < 0,05$), diferencias en el logaritmo en base e (ln) de OR promedio entre tres grupos, alérgenos relacionados taxonómicamente con una asociación estadísticamente significativa (relacionada-asociada), alérgenos relacionados que carecen de una asociación significativa (relacionada-no asociada) y alérgenos no relacionados.

Resultados – se encontraron asociaciones estadísticamente significativas entre pares de alérgenos relacionados y no relacionados, siendo el primero más frecuente. Para todos los caballos ($n = 344$) y los caballos con al menos una reacción positiva ($n = 239$), la co-reactividad varió de 100% (hierbas) a 0% (mohos). El grupo de malezas fue excepcional al tener más co-reacciones con otro grupo (hierbas).

Conclusiones e importancia clínica – la co-reactividad se mostró dentro de grupos y entre ciertos grupos de alérgenos relacionados. Se requieren más estudios para determinar si este es el resultado de reactividad cruzada antigénica.

Zusammenfassung

Hintergrund – Die Identifizierung von Umweltallergenen bei Pferden mit allergischer Erkrankung ermöglicht eine Allergenvermeidung und eine gezielte Immuntherapie.

Hypothese/Ziele – Eine Evaluierung der allergenen Co-Reaktivität zwischen 44 Umweltallergenen.

Tiere – Pferde mit Verdacht auf allergische Erkrankung ($n = 344$), deren Sera zum Austesten einer Umweltallergie eingesendet worden waren.

Methoden und Materialien – Es wurden Allergen-spezifische IgE serologische Assays mittels 44 Allergenen, aufgeteilt in sechs taxonomisch verwandte Gruppen, durchgeführt: Hausstaub/Futtermittelmilben, Schimmelpilze, Insekten, Gras-, Baum- und Unkrautpollen. Bei Verwendung von paarweisen Vergleichen wurde die Odds Ratio (das Quotenverhältnis; ORs) für jedes Umweltpaar kalkuliert, um festzustellen, ob eine erhöhte oder verminderte Wahrscheinlichkeit für ein positives Ergebnis eines einzelnen Allergens bestand, wenn für das andere ein positives Ergebnis vorlag. Die OR Signifikanz wurde (mittels Holm-Bonferroni Korrektur) bei $P < 0,00006$ für alle Pferde ($n = 344$) und $P < 0,00005$ für Pferde mit mindestens einer positiven Reaktion ($n = 239$) festgelegt. Mittels One way ANOVA mit Tukey's post hoc Tests (Signifikanz bei $P < 0,05$) wurden Unterschiede im Mean log e ORs zwischen drei Gruppen, nämlich taxonomisch verwandte Allergene mit einem statistisch signifikanten Zusammenhang (verwandt-assoziert), verwandte Allergene, denen ein signifikanter Zusammenhang fehlte (verwandt-nicht-assoziert) und unverwandte Allergene getestet.

Ergebnisse – Statistisch signifikante Zusammenhänge wurden zwischen verwandten und auch nicht verwandten Allergenpaaren gefunden, wobei erstere häufiger betroffen waren. Bei allen Pferden ($n = 344$) und bei Pferden mit wenigstens einer positiven Reaktion ($n = 239$), reichte die Co-Reaktion von 100% (Gräser) bis 0% (Schimmelpilze). Die Unkräutergruppe war herausragend, da sie mehr Co-Reaktionen aufwies wie eine andere Gruppe (Gräser).

Schlussfolgerungen und klinische Bedeutung – Co-Reaktivität wurde innerhalb und zwischen einigen verwandten Allergengruppen gezeigt. Weitere Studien sind nötig, um herauszufinden, ob es sich dabei um eine antigene Kreuz-Reaktivität handelte.

要約

背景 – アレルギー性疾患を有する馬の環境アレルゲンの同定は、アレルゲン回避および標的免疫療法を円滑にする。

仮説/目的 – 本研究の目的は、44種類の環境アレルゲン間のアレルギー反応を評価することである。

被験動物 – アレルギー性疾患が疑われる馬($n = 344$)。その血清を環境アレルゲン検査に提出した。

材料と方法 – アレルゲン特異的IgE血清学的アッセイを、分類学的に関連する6つのグループ(ハウスダスト/貯蔵ダニ、カビ、昆虫、草、木および雑草の花粉)に分類した44アレルゲンを使用して実行した。対比較を使用して、各環境ペアのオッズ比(OR)を計算し、別のアレルゲンに対して陽性結果を得た場合、あるアレルゲンに対する陽性結果の可能性が増加または減少したかどうかを判断した。ORの有意性を、Holm-Bonferroni補正を使用して、すべての馬は $P < 0.00006$ ($n = 344$)、少なくとも1つの陽性反応を示す馬($n = 239$)は $P < 0.00005$ に設定した。Tukeyの事後検定($P < 0.05$ での有意性)を用いた一元配置分散分析を使用して、3グループ間(分類学的に関連した、統計学的に有意な関連性を持つアレルゲン(related-associated)、分類学的に関連した、統計学的に有意な関連性のないアレルゲン(related-nonassociated)および無関係のアレルゲンの平均log e ORの差をテストした。

結果 – 分類学的に関連したアレルゲンペアおよび無関係なアレルゲンペアの両方で統計学的に有意な関

連性を検出し、前者はより頻繁であった。すべての馬($n = 344$)および少なくとも1つの陽性反応($n = 239$)を持つ馬では、共反応性は100%(草)から0%(カビ)の範囲であった。雑草グループは、別のグループ(草)とのより多くの共反応を持つという点で例外的であった。

結論および臨床的重要性 – 特定の分類学的に関連したアレルゲングループ内およびグループ間で共反応性を示した。これが抗原交差反応の結果であるかどうかを判断するには、さらなる研究が必要である。

摘要

背景 – 鉴定过敏马的环境过敏原有助于避开过敏原和开展靶向免疫治疗。

假设/目标 – 评估44种环境过敏原之间的共反应性。

动物 – 对怀疑过敏马($n = 344$)的血清进行环境过敏原测试。

方法和材料 – 使用44种过敏原进行过敏原特异性IgE血清学检测,分为六个分类相关组:屋尘螨/仓储螨、霉菌、昆虫、草、树和杂草花粉。通过两两比较,计算每个环境对的优势比(ORs),以确定一种过敏原的阳性结果,是否会增加或减弱另一种过敏原阳性结果。设定显著OR值(使用Holm-Bonferroni校正),所有马($n = 344$)为 $P < 0.00006$,至少一项过敏原阳性($n = 239$)的马为 $P < 0.00005$ 。在单因素方差分析后用Tukey检验(显著性 $P < 0.05$),依据统计学分类为关联显著的相关过敏原(相关)、关联不显著过敏原(相关-非关联)和不相关过敏原,检验这三组间平均 $\log_e$ OR差异。

结果 – 在相关和不相关的过敏原对之间,发现统计学关联显著,前者更明显。对于所有马($n = 344$)和至少一项阳性反应($n = 239$)的马,共反应范围从100%(草)至0%(霉菌)不等。杂草组与另一组(草)共反应更强。

结论和临床意义 – 在某些相关过敏原组内和组间显示出共反应性。需要进一步研究来确定这是否是抗原交叉反应的结果。

Resumo

Contexto – A identificação de alérgenos ambientais em cavalos com doenças alérgicas facilita o afastamento de alérgenos e a imunoterapia específica.

Hipótese/Objetivos – Avaliar co-reatividade alérgica entre 44 alérgenos ambientais.

Animais – Cavalos com suspeita de doença alérgica ($n = 344$) cujo soro foi testado para alérgenos ambientais.

Métodos e materiais – Foram realizados ensaios sorológicos para IgE alérgeno-específica com 44 alérgenos divididos em quatro grupos relacionados taxonômicamente: ácaros da poeira doméstica/de estocagem, bolores, insetos, gramíneas, pólenes de árvores e herbáceas. Utilizando comparações pareadas, calculou-se odds ratios (ORs) para cada par ambiental a fim de se determinar se havia probabilidade aumentada ou diminuída de se apresentar um resultado positivo para um alérgeno, no caso de positividade ao outro. A significância do OR foi configurada (utilizando a correção de Holm-Bonferroni) como $P < 0.00006$ para todos os cavalos ($n = 344$) e $P < 0.00005$ para os cavalos com ao menos uma reação positiva ($n = 239$). Utilizando a ANOVA unidirecional e os testes post hoc de Tukey (significância de $P < 0.05$), testou-se as diferenças nos logaritmos médios e ORs entre os três grupos, alérgenos taxonômicamente relacionados com uma associação estatisticamente significativa (relacionados e associados), alérgenos relacionados sem relação significativa (relacionados não associados) e alérgenos não relacionados.

Resultados – Associações estatisticamente significativas foram observadas entre pares de alérgenos relacionados e não relacionados, o primeiro sendo mais frequente. Para todos os cavalos ($n = 344$) e cavalos com ao menos uma reação positiva ($n = 239$), a reação cruzada variou entre 100% (gramíneas) e 0% (bolores). O grupo das herbáceas foi uma exceção por apresentar mais reação cruzada com outro grupo (gramíneas).

Conclusões e importância clínica – Observou-se co-reatividade dentro e entre certos grupos de alérgenos relacionados. Mais estudos são necessários para determinar se isso é devido à reação cruzada alérgica.