

Determination of irritant threshold concentrations of multiple tree, grass, weed and mould allergens for intradermal testing of horses residing in the southern USA

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Background – Appropriate allergen threshold concentrations (TCs) for intradermal testing (IDT) have not been established in horses for many pollen and mould allergens.

Objectives – To determine the TCs in non-allergic horses and describe the frequency of late phase reactions for 26 allergens, including trees, grasses, weeds and moulds in horses residing in the southern United States.

Animals – Twenty four clinically normal horses in the southern United States.

Methods – Threshold concentrations for different allergens were determined using IDT subjective measurements at 30 minutes. Delayed reactions were evaluated at 4 and 24 h.

Results – Threshold concentrations (all PNU/mL) were established for eight tree allergens (black willow 1,000, box elder 1,000, live oak 1,000, pecan 2,000, white ash 4,000, red oak 4,000, red mulberry 2,000 and green ash 2,000); two grass allergens (Johnson grass 250 PNU/mL and Kentucky blue grass 500 PNU/mL); two weeds (carelessweed 1,000 PNU/mL, great ragweed 500 PNU/mL) and one mould (*Curvularia* 8,000 PNU/mL). The TC was not determined due to excessive reactivity at the lowest concentration tested (1,000 PNU/mL) for bahia and perennial rye grass. Eleven other allergens did not meet the criteria to establish a TC when evaluated at 30 min due to lack of positive reactions. Multiple allergens caused positive reactions in $\geq 10\%$ of horses at 4 h. Reactions at 24 h were rare with the exception of one horse.

Conclusions and clinical importance – This study identified intradermal TC for multiple pollen and mould allergens in horses. These values may prove useful for optimizing allergen concentrations for IDT of allergic horses.

Introduction

Atopic dermatitis (AD) is a heritable condition characterized by an abnormal IgE-mediated immune response to environmental allergens.^{1,2} Affected horses may be pruritic and develop secondary lesions such as alopecia, crusts, excoriation, erythema and urticaria; with the face, pinnae, ventrum and legs commonly affected.^{1,2} Depending on the environment and causative allergens, clinical signs may be seasonal or non-seasonal in nature.¹ Severely affected horses may even have loss of body condition or behavioural changes secondary to

the discomfort associated with unrelenting pruritus. Atopic dermatitis is generally considered to be an incurable disease and requires lifelong therapy for most animals.^{2–5}

Successful management of AD usually involves a multimodal approach with a combination of allergen avoidance, allergen specific immunotherapy (ASIT), topical therapy and systemic medications. Unfortunately, many of the medications used systemically and/or topically, such as glucocorticoids, may be associated with significant adverse reactions.^{5,6} Furthermore, with exception of ASIT, none of these approaches can directly modulate the immune response. For these reasons, ASIT is considered to be an important tool in the management of atopic dermatitis.^{2,3} The development of effective ASIT is influenced by the accuracy of the associated testing method. Several variables have been shown to contribute to discordant intradermal test (IDT) results in dogs and horses, including the use of inappropriate allergen concentrations, variability in allergen extracts obtained from different companies, contaminants, and differences in testing and interpretation between veterinarians.^{2,7–9} The development of standardized allergen concentrations for IDT will help to maximize the consistency (and hopefully

Abbreviations: ASIT, allergen specific immunotherapy; IDT, intradermal testing; TC, threshold concentrations.

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accuracy) of the results and could, potentially, improve treatment outcomes in horses treated with ASIT.

In addition to dermatological signs, horses can also develop respiratory issues that may be associated with environmental exposure to certain airborne allergens.^{10,11} In the southeastern United States, summer pasture-associated obstructive pulmonary disease (SPAOPD) is a condition seen in horses kept outdoors.¹² These horses have respiratory disease characterized by bronchospasm, excessive airway mucus production, coughing, exercise intolerance and dyspnoea.¹² Previous work has demonstrated a seasonal exacerbation of these clinical signs in affected horses, which has been associated with increased environmental levels of fungal spores and grass pollen.^{11,13} These findings suggest a possible role for environmental allergens in the pathogenesis of this condition, although there is conflicting evidence regarding the exact immunological mechanism involved in the mediation of these responses.^{2,14–20} Nonetheless, it is hoped that ASIT may eventually prove to be an effective method for the treatment of these disorders.¹¹

One limitation of IDT is that the concentrations of allergens to be injected must be optimized as the injection of overly-concentrated solutions may cause focal irritation. This irritation may incorrectly be assumed to represent a “true” positive reaction. There is little published information available regarding the most appropriate concentrations of allergens to be used for IDT in horses. There are only six published studies which have attempted to determine potentially irritating concentrations of allergens for intradermal injection in this species.^{21–26} Three of these six studies only evaluated insect allergens.^{23–25} In addition, only two of the studies were performed in North America and these were performed in northern parts of the country.^{21,23} Very limited data are available regarding possible irritant thresholds for other allergens (such as moulds) or grass allergens and none of these studies have been conducted in North America.²² Furthermore, there are no published data evaluating possible irritant concentrations for allergen testing in the subtropical climate of the southern United States. Animals in different geographic locations have different levels of overall environmental allergen exposure. This could potentially influence irritant concentration thresholds, as has been demonstrated in both horses and dogs, necessitating the need to individualize allergen tests regionally.^{7,21,27}

Historically, the recommended intradermal testing concentration for pollens in horses is 1,000 PNU/mL.² Studies in dogs suggest that concentrations as high as 1,750 PNU/mL for plant allergens may still fall below the threshold for irritant reactions.²⁸ The few studies that have been performed in horses suggest that these concentrations may also differ from the historical standard.^{21,22,24,29} Results suggest that, in some cases, higher allergen testing concentrations may be used without inducing an irritant reaction. The use of higher test concentrations may increase the sensitivity of IDT, but irritant concentration thresholds must be determined prior to doing so. In previous studies, threshold concentrations (TCs) have been defined as the highest concentration at which less than 10% of non-allergic animals will not develop a false-positive, or irritant, reaction.^{8,21,22} The

ideal allergen concentration for IDT would be the highest concentration under the threshold for creating irritant reactions, as to minimize both false-negative results in sensitized animals as well as false-positive results in non-allergic animals. Although the TC concept is generally applied to immediate (15–30 min after injection) reactions, it may be appropriate to determine the influence of allergen concentration on the development of delayed reactions (those occurring 4, 6 or 24 h after initial injection) because these later time points are also evaluated by some clinicians.

The primary objective of this prospective study was to determine the TCs in non-allergic horses for 26 allergens, including trees, grasses, weeds and moulds, that are commonly implicated amongst horses suffering from allergic disease in the southeastern United States. Injection sites were also evaluated at 4 and 24 h after IDT to better characterize the potential for irritant reactions at these time points. Finally, correlation analysis was performed to determine the association between objective and subjective scoring methods at the 30 min time point.

Materials and methods

Study design

All phases of the experimental design were approved by the Louisiana State University School of Veterinary Medicine Institutional Animal Care and Use Committee.

Horses

The Louisiana State University School of Veterinary Medicine Equine Health Studies Program maintains a herd of approximately 80 research horses. Housing of this herd is in accordance with the National Research Council's 1996 Guide for Care and Use of Laboratory Animals. Thirty five horses were selected from this colony based upon lack of clinical or historical evidence of allergic skin or respiratory disease, cutaneous bacterial infections or systemic disease. Horses that had received topical, oral or injectable glucocorticoids, non-steroidal anti-inflammatory drugs, antihistamines or supplements with unknown ingredients two months prior to or during the study period were excluded. All horses' physical examination findings prior to IDT were within normal limits. All horses had been vaccinated one month prior to the study and received regular rotation anthelmintic treatment. The horses were primarily kept on pasture, but were stalled during the course of the study.

Allergens for intradermal testing – Groups 1 and 2

A total of 26 allergens at various dilutions were used for IDT, including grasses, trees, weeds and moulds common in the southeastern United States (Table 1). All allergens were aqueous extracts obtained from Greer Laboratories (Lenoir, NC, USA). Three to five dilutions were prepared for each extract using phenolated saline sterile diluent one week prior to initiating the study. Individual syringes for each horse were drawn up one to two days before IDT was performed. Allergens and dilutions were stored at 4°C and kept chilled until 10 min prior to beginning the IDT.

Group 1 horses (pilot study)

A pilot study was performed on 11 of the 35 selected non-allergic horses. The initial allergen concentrations used for Group 1 were chosen based on current recommended testing concentrations by the manufacturer on the allergen package insert and the results of previous studies in horses and dogs (Table 1).²² Allergen concentrations were adjusted in Group 1 at three different points, resulting in four different iterations of the IDT, to aid in threshold determination. Group 1 results were used to create a single standardized IDT utilized across all 24 horses reported in the second phase of this study (Group 2).

Table 1. Common names, scientific names and range of testing concentrations for intradermal testing with allergens in (non-allergic) Group 1 and Group 2 horses.

	Group 1 testing concentrations (PNU/mL)	Group 2 testing concentrations (PNU/mL)
Trees		
Black walnut (<i>Juglans nigra</i>)	1,000–8,000	1,000–4,000
Black willow (<i>Salix nigra</i>)	1,000–8,000	500–2,000
Box elder (<i>Acer negundo</i>)	1,000–8,000	1,000–2,000
Live oak (<i>Quercus agrifolia</i>)	1,000–8,000	1,000–2,000
Pecan (<i>Carya illinoensis</i>)	1,000–8,000	1,000–4,000
Red cedar (<i>Juniperus virginiana</i>)	250–8,000	62.5–500
Red maple (<i>Acer rubrum</i>)	500–8,000	250–1,000
White ash (<i>Fraxinus americana</i>)	1,000–12,000	1,000–12,000
White hickory (<i>Quercus alba</i>)	1,000–12,000	1,000–4,000
Red oak (<i>Quercus rubra</i>)	1,000–8,000	500–8,000
Red mulberry (<i>Morus rubra</i>)	250–8,000	250–4,000
Green ash (<i>Fraxinus pennsylvanica</i>)	1,000–8,000	1,000–4,000
Grasses		
Bahia (<i>Paspalum notatum</i>)	1,000–8,000	1,000–4,000
Bermuda (<i>Cynodon dactylon</i>)	1,000–8,000	500–2,000
Johnson (<i>Sorghum halepense</i>)	1,000–8,000	250–500
Kentucky Blue (<i>Poa pratensis</i>)	1,000–8,000	250–2,000
Perennial Rye (<i>Lolium perenne</i>)	1,000–8,000	1,000–4,000
Weeds		
Carelessweed (<i>Amaranthus hybridus</i>)	250–8,000	250–2,000
Great (Giant) ragweed (<i>Ambrosia trifida</i>)	500–8,000	250–2,000
Rough pigweed (<i>Amaranthus retroflexus</i>)	500–8,000	250–1,000
Short ragweed (<i>Ambrosia artemisiifolia</i>)	500–16,000	500–20,000
Baccharis (<i>Baccharis spp.</i>)	250–8,000	250–500
Moulds		
<i>Alternaria tenuis</i>	1,000–8,000	1,000–12,000
<i>Aspergillus fumigatus</i>	1,000–8,000	1,000–2,000
<i>Cladosporium herbarum</i>	2,000–12,000	2,000–8,000
<i>Curvularia spicifera</i> (<i>Drechslera spicifera</i>)	1,000–12,000	1,000–12,000

Group 2 horses

Group 2 was made up of the remaining 24 non-allergic horses. Group 2 horses all received a single, standardized IDT (Table 1). The allergen concentrations utilized in the IDT for Group 2 were based on results from the pilot study performed on Group 1.

Intradermal testing – Groups 1 and 2

All intradermal tests in this study were performed during the months of November and December in a single calendar year. Three to six horses were tested per day on 11 different dates spanning a three week period. Each horse was lightly sedated with intravenous (i.v.) xylazine (0.4 mg/kg; XylaMed™; Bimeda-MTC, Cambridge, ON, Canada) 5–10 min prior to the procedure. All horses had the hair on both sides of the lateral neck shaved in a roughly 20 × 20 cm area using a number 40 clipper blade. Black or red waterproof markers, depending on the horse's coat colour, were used to create a testing grid consisting of three rows of ten dots, with injections to be administered above and below each dot. Each horse was injected intradermally with 0.1 mL of phenolated saline and with 0.1 mL of histamine (1:100,000 w:v) on each side of the neck to serve as the negative and positive controls, respectively. A volume of 0.1 mL of each allergen extract was injected intradermally to form a visible bleb using a 1 mL syringe with a new, permanently attached 27-gauge needle (BD, Franklin Lakes, NJ, USA). Each horse in Group 2 received 91 total injections (four control injections and 87 allergen injections of varying concentrations). All injections were performed by the same investigator. Following injection and the initial evaluation, horses were housed individually until the end of the study to prevent disruption of the injection sites.

All injection sites were examined at 30 min, 4 and 24 h after injection by the same investigator. At the 30 min and 4 h time points, the reactions were subjectively scored on a scale of 0 (negative) to 4+ based on wheal size, erythema and turgidity, through comparison with controls. A score of 0 was comparable to the score of the negative control, saline; a score of 4+ was comparable to the positive

control, histamine. Any score ≥ 2 was considered a positive reaction for the purposes of this study. At the 24 h time point a positive reaction was noted if any palpable wheal remained at a given injection site. In addition, the diameter of the cutaneous reactions was measured in two perpendicular directions at the 30 min and 4 h time points; these values were used to determine the surface area of each reaction (objective scoring). Objective measurements were not taken at 24 h due to the reactions being minimally visible and palpable.

Data analysis

The data analysis in this study is in reference to Group 2 horses, unless specifically stated otherwise. The percentage of horses with positive subjective outcomes (scores ≥ 2) to each allergen at a given concentration was calculated based on the total number of horses tested at that concentration. The TC was defined as the highest concentration of a specific allergen tested at which fewer than 10% of the horses had positive scores at the 30 min time point. Percentages of positive reactions were also calculated for delayed reactions at 4 and 24 h for each allergen at a given concentration; however, a TC was not specifically defined for late phase reactions.

All analyses were conducted with SAS® (version 9.4, SAS Institute Inc., Cary, NC, USA) statistical software. A Spearman (rank) correlation analysis was performed to calculate a correlation coefficient for the subjective score of positive outcomes (i.e. 2–4) and the calculated surface area based on the vertical and horizontal wheal diameters of each positive reaction. All analyses were considered significant at $P \leq 0.05$.

Results

Horses

The study population was composed of 25 geldings and 10 mares, ranging in age from 4 to 24 years with a median age of 9 years. There were four quarter horses and 31

Table 2. Threshold concentrations for intradermal testing with pollens and moulds when evaluated at 30 min and Group 2 allergens for which no threshold concentration could be determined.

Allergen	30 min		Pilot study	
	Threshold concentration (PNU/mL)	Group 2 positive reactions for threshold concentration	Group 1 concentration (PNU/mL)	Group 1 positive reactions
Trees				
Black willow	1,000	2/24 (8.33%)	—	—
Box elder	1,000	2/24 (8.33%)	—	—
Live oak	1,000	1/24 (4.17%)	—	—
Pecan	2,000	0/24 (0.00%)	—	—
White ash	4,000	1/24 (4.17%)	—	—
Red oak	4,000	2/24 (8.33%)	—	—
Red mulberry	2,000	0/24 (0.00%)	—	—
Green ash	2,000	0/24 (0.00%)	—	—
Black walnut	>4,000	2/24 (8.33%)	8,000	4/11 (36.36%)
Red cedar	>500	1/24 (4.17%)	1,000	3/11 (27.27%)
Red maple	>1,000	1/24 (4.17%)	2,000	4/5 (80.00%)
White hickory	>4,000	2/24 (8.33%)	8,000	3/11 (27.27%)
Grasses				
Bahia	<1,000	3/24 (12.5%)	—	—
Johnson	250	2/24 (8.33%)	—	—
Kentucky blue	500	2/24 (8.33%)	—	—
Perennial rye	<1,000	3/24 (12.5%)	—	—
Bermuda	>2,000	2/24 (8.33%)	8,000	4/11 (36.36%)
Weeds				
Carelessweed	1,000	2/24 (8.33%)	—	—
Great ragweed	500	1/24 (4.17%)	—	—
Rough pigweed	>1,000	2/24 (8.33%)	2,000	4/11 (36.36%)
Short ragweed	>2,000	1/24 (4.17%)	4,000	3/11 (27.27%)
Baccharis	>500	2/24 (8.33%)	1,000	5/11 (45.45%)
Moulds				
<i>Curvularia</i>	8,000	1/24 (4.17%)	—	—
<i>Alternaria</i>	>12,000	2/24 (8.33%)	—	—
<i>Aspergillus</i>	>2,000	1/24 (4.17%)	4,000	3/11 (27.27%)
<i>Cladosporium</i>	>8,000	2/24 (8.33%)	12,000	3/11 (27.27%)

Threshold concentrations were defined as the highest concentration where <10% of non-allergic horses had 'positive' reactions (i.e. subjective scores ≥ 2). Data from Group 1 is provided to allow speculation of TC based on the next highest testing concentration from Group 1 for those allergens that did not meet the criteria to define a TC in test Group 2. Group 1 and 2 positive reactions are presented as the number of horses with a positive reaction/total number of horses tested for that concentration (percentage positive). *Alternaria* was not tested at higher concentrations in Group 1 than Group 2.

PNU/mL, protein nitrogen units/millilitre.

thoroughbreds. Of these horses, 11 were used in the preliminary study as part of Group 1 and the remaining 24 were used in Group 2.

Intradermal testing

All horses produced an adequate subjective response to the positive histamine controls (graded as a 4+) and an appropriate minimal response to the negative saline controls (graded as a 0).

Threshold concentrations at 30 min as determined by subjective scores

Using a cut off of <10% positive reactivity in non-allergic horses, the TC was established for eight tree allergens. Black willow, box elder and live oak had TCs of 1,000 PNU/mL; pecan, red mulberry and green ash had TCs of 2,000 PNU/mL; white ash and red oak had TCs of 4,000 PNU/mL (Table 2). The TC was established for two grass allergens, with Johnson grass at 250 PNU/mL and Kentucky blue grass at 500 PNU/mL (Table 2). The TC was established for two weeds with carelessweed at 1,000 PNU/mL and great ragweed at 500 PNU/mL

(Table 2). The TC was established for one mould with *Curvularia* at 8,000 PNU/mL (Table 2).

For two grass allergens (bahia and perennial rye), more than 10% of horses developed injection site reactions even at the lowest concentration tested of 1,000 PNU/mL (Table 2). Therefore, the TC for these allergens is likely to be less than 1,000 PNU/mL. For 11 allergens (black walnut, red cedar, red maple, white hickory, Bermuda grass, rough pigweed, short ragweed, *Baccharis*, *Alternaria*, *Aspergillus* and *Cladosporium*) a TC could not be determined because fewer than 10% of horses developed injection site reactions to the highest tested concentration of allergen. These concentrations had been chosen based upon the results of the Group 1 pilot study. Therefore, the TC for these allergens is likely somewhere lower than the irritant concentration determined in Group 1, but higher than that tested in Group 2 (Table 2).

Four hour reactions

At four hours, $\geq 10\%$ of non-allergic horses had positive reactions (subjective scores ≥ 2) to six tree allergens (black willow and box elder at 1,000 PNU/mL; white ash

and red oak at 8,000 PNU/mL; red mulberry at 2,000 PNU/mL and green ash at 4,000 PNU/mL), three grass allergens (bahia grass at 4,000 PNU/mL, Johnson grass at 500 PNU/mL and perennial rye grass at 1,000 PNU/mL) and one weed allergen (carelessweed at 1,000 PNU/mL). For all other allergens, fewer than 10% of horses in Group 2 demonstrated late phase reactions at any concentration. Discordant results (in which a positive four hour reaction was noted at a particular concentration but not at one or more higher concentrations) were noted in ten horses involving nine allergen extracts (white ash, black willow, *Curvularia*, box elder, red cedar, Kentucky blue grass, perennial rye grass, carelessweed and red maple).

Twenty four hour reactions

Of the 24 horses tested, 18 had palpable areas of induration present at the site of at least one histamine injection site at 24 h. The presence of induration at allergen injection sites was highly variable. Only nine horses demonstrated reactions at allergen injection sites at 24 h. A total of 31 individual sites could be palpated, with more than half (17/31) of these coming from a single horse. Thirteen different allergens were associated with 24 h delayed reactions at varying concentrations (Table 3). Discordant results (in which a 24 h reaction was noted at a given concentration for an allergen, but not in one or more of the higher concentrations) were noted in three horses involving three allergens (*Alternaria*, Bahia, White Ash).

Correlation of subjective and objective scoring methods

Black willow exhibited strong correlation between the calculated area of the skin reaction and the subjective score with a correlation coefficient >0.75 ($P = 0.0001$). Seven allergens (red mulberry ($P = 0.0026$), bahia grass ($P = 0.0003$), Johnson grass ($P = 0.0524$), Kentucky blue grass ($P = 0.0183$), perennial rye grass ($P = 0.0003$), carelessweed ($P = 0.0081$) and *Alternaria* ($P = 0.0222$)) displayed good correlation between the two variables with a correlation coefficient between 0.5–0.75. A correlation could not be calculated for all allergens, due in large part to the relatively small number of overall positive reactions.

Discussion

By utilizing the 10% cut off for irritant reactions in non-allergic animals for a given allergen, the present study established TCs for 13 allergens when evaluated at 30 min following IDT. A TC was established for eight of the 12 tree pollens evaluated, including black willow, box elder, live oak, pecan, white ash, red oak, red mulberry and green ash (Table 2). Five of these TCs ranged from 2,000 to 4,000 PNU/mL, which is more concentrated than the typically recommended testing concentration of 1,000 PNU/mL. Of the four trees for which a TC could not be determined due to lack of reactivity, three were evaluated at concentrations ranging from 1,000 to 4,000 PNU/mL, which again suggests the TCs for these allergens are $>1,000$ PNU/mL. Only one tree, red cedar, had results suggesting a TC lower than current

recommendations (Table 2). Similar results were reported in an Australian study, although different trees were evaluated.²² The results from both of these studies suggest that the use of higher IDT concentrations may be possible or preferable for some tree allergens, potentially increasing the sensitivity of the diagnostic tool. This interpretation would be consistent with the results of a similar study performed in non-allergic dogs in the United States, which also demonstrated TCs higher than 1,000 PNU/ml for many of the same trees evaluated in the present study, including green ash, box elder, red maple, red mulberry, live oak, red cedar and black willow.⁸

In contrast to the tree pollens (for which TCs could be established for most of the extracts), excessive reactivity was demonstrated to four of the five tested grasses (bahia, Johnson, Kentucky blue and perennial rye) with greater than 10% of the horses exhibiting reactions to allergen concentrations less than or equal to the current recommended testing concentration of 1,000 PNU/mL. The reason for the sensitivity to these allergens at such low testing concentrations may be related to a heavy environmental exposure to these plants in this geographic area, and/or may be indicative of subclinical sensitization. Nonetheless, excessive reactivity was not observed for all of the grasses, as a TC could not be determined for Bermuda grass at even the highest tested concentration of 2,000 PNU/mL (Table 2). These findings are in contrast to those obtained in another study that determined TC for the grass allergens paspalum, couch, oat and plantain during multiple seasons in Australia. In that study, TC for all four grasses were greater than 1,000 PNU/mL.²² Along similar lines another study demonstrated canine TCs well above 1,000 PNU/ml for the five grass pollens evaluated in the current study.⁸ The results of the current study would suggest that intradermal reactivity to some grass pollens should be evaluated at lower concentrations than the typically recommended 1,000 PNU/ml, depending upon the horse's environment and location. These results further suggest that separate determination of TCs might be needed to be performed for different geographic regions, as well as for different allergens in those regions.

Threshold concentrations were established for two of the five weeds (carelessweed and great ragweed) evaluated in this study. The TC for carelessweed was 1,000 PNU/mL, which corresponds with current testing recommendations, while great ragweed had a lower TC at 500 PNU/mL. The other three weeds evaluated (rough pigweed, short ragweed and baccharis) did not cause reactions in enough horses to firmly establish a TC at the highest tested concentrations of 1,000 PNU/mL, 2,000 PNU/mL and 500 PNU/mL, respectively. These concentrations were chosen based upon reactivity displayed by the horses in Group 1 (Table 2). This information suggests that the TC for rough pigweed may lie slightly above the recommended testing concentration of 1,000 PNU/mL. One study found the TC for short ragweed to be $>6,000$ PNU/mL.²² Based on pilot study findings, the TC for short ragweed in the present study may fall between the 2,000 PNU/mL and 4,000 PNU/mL (Table 2). These studies were performed in different climates on different continents and these results support further investigation into the effect of these variables on

Table 3. Allergens with positive intradermal reactions at 24 h in healthy horses.

Allergen	Number of positive reactions	Concentration range (PNU/mL)
Trees		
Black willow	1	2,000
Pecan	2	4,000
White ash	1	8,000
Red oak	1	8,000
Grasses		
Bahia	4	1,000–4,000
Kentucky blue	2	1,000–2,000
Perennial rye	5	1,000–4,000
Weeds		
Carelessweed	1	2,000
Short ragweed	2	1,000–2,000
Great ragweed	1	2,000
Moulds		
<i>Alternaria</i>	6	4,000–12,000
<i>Aspergillus</i>	4	1,000–2,000
<i>Curvularia</i>	1	12,000

PNU/mL, protein nitrogen units/millilitre.

TCs. *Baccharis*, however, may have a threshold concentration greater than 500 PNU/mL but potentially less than 1,000 PNU/mL (Table 2). The results of the present study suggest that TCs may vary widely for different weed pollens and that individual investigation of specific allergens is warranted.

The TC for the mould allergen *Curvularia* was established at 8,000 PNU/mL, which is eight times higher than the current recommendation of 1,000 PNU/mL. The TC for *Alternaria*, *Aspergillus* and *Cladosporium* could not be determined in this study because the highest concentrations tested (12,000 PNU/mL, 2,000 PNU/mL and 8,000 PNU/mL, respectively) failed to cause $\geq 10\%$ of non-allergic horses to react. The TCs found in the present study were all greater than those established in a previous study performed in Australia (*Alternaria* 4,000 PNU/mL, *Aspergillus* 1,000 PNU/mL, *Cladosporium* >6,000 PNU/mL).²² Taken together, the results of both studies demonstrate that TCs for at least some moulds are higher than current recommendations.

In addition to immediate wheal and flare responses, intradermal allergen injection may also be associated with the development of focal induration and/or swelling, hours to days later. These late phase reactions are thought to occur due to infiltration of inflammatory cells in response to signals from mast cell chemoattractants released during the first six hours, leading to an initial influx of eosinophils, basophils and neutrophils, and later by mononuclear cells.³⁰ In addition to immediate wheal and flare responses, some practitioners evaluate late phase reactions as a means of detecting allergen specific reactivity. Thus, a secondary objective of this study was to determine if injection of any of the allergens was associated with late phase irritant-mediated reactions that might mimic allergic reactions. Of those allergens with noted positive reactions at four hours, black willow, box elder, red mulberry and carelessweed developed reactions at the same concentration established to be the 30-min TC. Late phase responses for white ash, red oak,

green ash, bahia, Johnson and perennial rye were observed at higher concentrations than the corresponding 30-min TC. These results suggest that delayed reactions can be seen in non-allergic horses when injected with allergens at, or higher than, the threshold concentration. Thus, their interpretation clinically can be difficult. In contrast, injection of moulds was not associated with the development of four hour reactions in >10% of the horses in this study. This is consistent with the results of previous studies performed in allergic and healthy horses, in which late-phase reactivity to mould injection was not observed.^{20,22} A previous study in horses found that the presence of delayed reactions were clearly correlated with the concentration of the allergen being tested.²² This was not necessarily true in our study, due to the presence of discordant results associated with nine different allergen extracts at 4 h and three extracts at 24 h.

When assessing the delayed reactions at 4 and 24 h, discordant results, in which positive reactions occurred at a given concentration for a particular allergen but failed to produce a positive reaction at a higher concentration of the same allergen, were rarely noted. The horses for which this occurred at 4 h did not experience the same phenomenon at 24 h and vice versa. White ash was the only allergen associated with discordant results at both 4 and 24 h. A similar finding was documented for immediate IDT reactions observed at 30 min in a previous study evaluating house dust mites.²¹ The reason for these apparent discrepancies is unclear, but may include observer error or traumatic injections. Also, contamination of the syringe contents may have resulted in irritant reactions.²¹ It is possible that positive late phase reactions to allergens that did not display positive reactivity in non-allergic horses may be meaningful; however, since there is limited literature available and clinical significance of late phase reactions in horses is poorly understood, these results should be interpreted with caution.²²

In this study subjective scoring was used as the primary means of defining a positive reaction for analysis because subjective scoring is the primary means of IDT interpretation in most of the current literature assessing TCs in animal species, as well as in clinical practice.^{7,8,22,26,28} However, the use of objective data has the potential to better standardize the scoring methods of IDT by providing more homogenous interpretation criteria, decreasing variability in IDT evaluation by a single practitioner and minimizing discordant results achieved by different veterinarians. In this study, most allergens with an adequate number of positive reactions to assess had a good to strong correlation between the subjective and objective scoring data. The small number of correlations reported was largely related to the fact that testing was performed in non-allergic horses equating to a relatively small number of positive reactions overall. The lack of a perfectly linear relationship between area and the subjective score may also be explained by the fact that multiple criteria contribute to a positive subjective score. While the diameter/area of the reaction is an important parameter to consider, most examiners also assess the degree of erythema and turgidity of the reaction site, which would not necessarily contribute to a reaction site's surface area. One previous anecdotal report in dogs

found that the number of positive reactions increased by 20–30% when subjective scoring was taken into consideration.⁷ One must also consider that the current study was performed in non-allergic horses, in which most of the “positive” reactions described are assumed to be irritant reactions. Correlation data might improve in clinically hypersensitive horses. While objective measurements add value to IDT scoring, they are unlikely to replace subjective scoring altogether due to the many variables and nuances that go into deeming an injection reaction positive. Both objective and subjective data should be considered in light of one another and IDT should be performed and assessed by a clinician that is experienced and trained in the technique.

The limitations of this study include the relatively small sample size and lack of breed diversity. Although none of the horses included in this study had any history of skin or respiratory disease, it is possible that some horses may have suffered from sub-clinical sensitization and produced true positive results to some injections, artificially lowering the TCs. For example, it was noted that one horse in particular was responsible for approximately 55% (17/31) of the palpable reactions at 24 h. Ideally, non-allergic animals used for TC determination would have been raised in allergen-free environments to guarantee the absence of sub-clinical allergic disease; unfortunately, this is not practical in most cases.²¹ In addition, all of the horses were from a single region of the country. While the TCs defined for allergens in this paper may not always translate to horses in other regions, by using a single-sourced herd, the accuracy of the results is likely maximized for horses living in the southeastern United States.

In conclusion, the current study determined TC for tree, grass and weed allergens which were above, equal to and below the current testing concentration recommendation of 1,000 PNU/mL. Based on these results, a generalization about ideal allergen concentrations for IDT within a single category (i.e. trees) or between groups of plant pollens (i.e. trees and grasses) cannot be formed. Assumptions about ideal testing concentrations based on data from other species, or even other allergens in the same species, may lead to false positive reactions and inaccurate test results. The differences in findings between similar studies emphasize the importance of establishing TCs for individual pollens within species of interest and ideally also for specific geographic regions. These data may aid in the development of guidelines for allergen testing concentrations in this region as well as for studies to examine additional allergens.

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Résumé

Contexte – Les concentrations seuils (TCs) d'allergène adaptées aux tests intradermiques (IDT) n'ont pas été déterminées chez le cheval pour de nombreux allergènes de pollens et moisissures.

Objectifs – Déterminer les TCs chez les chevaux non-allergiques et décrire la fréquence des réactions retardées pour 26 allergènes dont les arbres, les herbes, les graminées et les moisissures chez des chevaux vivant au sud des Etats Unis.

Sujets – Vingt quatre chevaux cliniquement sains du sud des Etats Unis.

Méthodes – Les concentrations seuils des différents allergènes ont été déterminées par mesure subjective des IDT à 30 minutes. Les réactions retardées ont été évaluées à 4 et 24h.

Résultats – Les concentrations seuils (PNU/mL) ont été déterminées pour huit des allergènes d'arbre (saule noir 1,000, chêne vert 1,000, érable 1,000, pacanier 2,000, frêne blanc 4,000, chêne rouge 4,000, mûrier rouge 2,000 et frêne vert 2,000); deux allergènes de graminées (Johnson grass 250 PNU/mL et Kentucky blue grass 500 PNU/mL); deux herbes (carelessweed 1,000 PNU/mL, great ragweed 500 PNU/mL) et une moisissure (*Curvularia* 8,000 PNU/mL). La TC n'a pas été déterminée à cause de la grande réactivité aux faibles concentrations testées (1,000 PNU/mL) pour la bahia et la ray-grass vivace. Onze autres allergènes n'ont pas rempli les critères pour la détermination d'une TC lorsqu'évaluée à 30 min en raison d'un manque de réaction positive. De nombreux allergènes ont causé des réactions positives pour $\geq 10\%$ des chevaux à 4h. Les réactions à 24h étaient rares à l'exception d'un cheval.

Conclusions et importance clinique – Cette étude a identifié les TC intradermiques pour de nombreux pollen et moisissures chez le cheval. Ces valeurs peuvent être utiles pour optimiser les concentrations d'allergènes pour les IDT des chevaux allergiques.

Resumen

Introducción – No se han establecido concentraciones umbral de alérgenos (TCs) apropiadas en el uso de pruebas intradérmicas (IDT) en caballos para muchos alérgenos de polen y moho.

Objetivos – Determinar los TCs en caballos no alérgicos y describir la frecuencia de reacciones en fase tardía de 26 alérgenos, incluidos árboles, hierbas, malas hierbas y mohos en caballos que residen en la zona Sur de los Estados Unidos.

Animales – Veinticuatro caballos clínicamente normales del Sur de los Estados Unidos.

Métodos – Se determinaron las concentraciones umbral para diferentes alérgenos usando mediciones subjetivas de IDT a los 30 minutos. Las reacciones tardías se evaluaron a las 4 y 24 h.

Resultados – Se establecieron concentraciones umbral (todas en PNU/mL) para ocho alérgenos arbóreos (sauce negro 1.000, arce negundo 1000, roble perenne 1.000, pecán 2.000, fresno blanco 4.000, roble rojo 2.000 y fresno verde 2.000); dos alérgenos herbáceos (hierba de Johnson 250 PNU/mL y hierba azul de Kentucky 500 PNU/mL); dos malas hierbas (*Amaranthus palmeri* 1000 PNU/mL, *Ambrosia trifida* 500 PNU/mL) y un moho (*Curvularia* 8.000 PNU / mL). El TC no se determinó debido a la reactividad excesiva con la concentración más baja ensayada (1.000 PNU/mL) para pasto bahia y *Lolium perenne*. Otros once alérgenos no cumplieron con los criterios para establecer un TC cuando se evaluaron a los 30 min debido a la falta de reacciones positivas. Muchos alérgenos causaron reacciones positivas en $\geq 10\%$ de los caballos a las 4 h. Las reacciones a las 24 h fueron raras con la excepción de un caballo.

Conclusiones y importancia clínica – Este estudio identificó TCs intradérmicas para múltiples alérgenos de polen y moho en caballos. Estos valores pueden resultar útiles para optimizar las concentraciones de alérgenos para pruebas intradérmicas en caballos alérgicos.

Zusammenfassung

Hintergrund – Für Pferde sind sinnvolle Allergenschwellenkonzentrationen (TCs) für Intradermaltests (IDT) für viele Pollen und Schimmelpilze nicht etabliert.

Ziele – Eine Bestimmung der TCs bei nicht-allergischen Pferden und die Beschreibung der Frequenz von Spätreaktionen auf 26 Allergene, zu denen Bäume, Gräser, Kräuter und Schimmel zählten. Das alles wurde bei Pferden, die in den südlichen Staaten der Vereinigten Staaten beheimatet waren, durchgeführt.

Tiere – Vierundzwanzig klinisch völlig normale Pferde in den südlichen Staaten der Vereinigten Staaten.

Ergebnisse – Schwellenkonzentrationen (alle in PNU/mL) wurden für acht Baumallergene (Schwarze Weide 1.000, Eschenahorn 1.000, Virginiaeiche 1.000, Pekannussbaum 2.000, Weißesche 4.000, Roteiche 4.000, Rote Maulbeere 2.000, Grünesche 2.000; für zwei Grassallergene (Aleppohirse 250 PNU/mL und Wiesenrispengras 500 PNU/mL); zwei Unkräuter (Palmer's Fuchsschwanz 1.000 PNU/mL, Traubenkraut 500 PNU/mL) und einen Schimmelpilz (*Curvularia* 8.000 PNU/mL) festgelegt. Die TC wurde aufgrund exzessiver Reaktionen bereits auf dem niedrigst getesteten Konzentrationslevel (1.000 PNU/mL) für Bahia und Weidelgras nicht bestimmt. Elf andere Allergene erfüllten die Kriterien nicht, um eine TC zu etablieren, da bei einer Evaluierung alle 30 Minuten keine positiven Reaktionen auftraten. Viele Allergene zeigten bei $\geq 10\%$ der Pferde positive Reaktionen nach 4h. Außer bei einem Pferd traten nach 24h keine Reaktionen auf.

Schlussfolgerungen und klinische Bedeutung – Diese Studie identifizierte eine intradermale TC für zahlreiche Pollen und Schimmelpilzallergene bei Pferden. Diese Werte könnten sich bei der Optimierung der Allergenkonzentrationen des IDTs für allergische Pferde als hilfreich erweisen.

要約

背景 – 多くの花粉およびカビアレルゲンについて、馬の皮内試験(IDT)のための適切なアレルゲン閾値濃度(TC)は確立されていない。

目的 – 非アレルギー性馬を用いてTCを決定し、アメリカ合衆国南部に居住する馬の樹木、草、雑草およびカビを含む26種のアレルゲンの遅延型反応の頻度を記述すること。

供与動物 – アメリカ合衆国南部に居住する24頭の臨床的に正常な馬。

方法 – 各アレルゲンの閾値濃度は、IDT実施30分後の主観測定値を用いて決定した。遅延型反応は4時間後および24時間後に評価した。

結果 – 8つの樹木(black willow 1,000, box elder 1,000, ライブオーク1,000, ペカン2,000, white ash 4,000, レッドオーク4,000, 赤桑2,000およびグリーンアッシュ2,000;すべてPNU/mL)、2つの草(ジョンソングラス 250 PNU/mLおよびケンタッキーブルーグラス 500 PNU/mL)、2つの雑草(careless weed 1,000 PNU/mL, great ragweed 500 PNU/mL)および1つのカビ(*Curvularia* 8,000 PNU / mL)において、アレルゲンの閾値濃度を確立した。パビアおよび多年生ライグラスでは、試験した最も低い濃度(1,000 PNU/mL)でも過剰反応性を起こしたために、TCは決定されなかった。その他の11のアレルゲンについては、30分後の評価時点で陽性反応が欠如していたためにTCを確立する基準を満たさなかった。複数のアレルゲンが、4時間後に10%以上の馬で陽性反応を引き起こした。24時間後での反応はまれで、1頭のみでしか認められなかった。

結論および臨床的な重要性 – 本研究は、複数の花粉およびカビのアレルゲンに対する馬の皮内TCを確立した。これらの値は、アレルギー性馬のIDTにおけるアレルゲン濃度を最適化するのに有用であると考えられる。

摘要

背景 – 目前,还没有建立适用于马皮内试验(IDT)的合理过敏原阈值浓度(TCs),例如许多花粉和霉菌过敏原。

目的 – 为了确定生活在美国南部的无过敏性马的TCs,并且描述26种过敏原的延迟反应,包括树木、青草、杂草和霉菌。

动物 – 选取二十四匹位于美国南部的,临床检查正常的马。

方法 – 采用IDT,通过在30分钟内进行主观测量,来确定不同过敏原的阈值浓度。在第4h和24h时评估延迟反应。

结果 – 确定以下几种过敏原的阈值浓度(所有单位均为PNU/mL),八种树木(黑柳1000,桤木1000,榲桲1000,美洲山核桃2000,美国白蜡树4000,红橡木4000,美洲红桑2000和美国红桤2000),两种青草过敏原(约翰逊草250PNU/mL和肯塔基蓝草500PNU/mL),两种杂草(粗心杂草1000 PNU/mL,三裂豚草500 PNU/mL)和一种霉菌(弯孢霉属8000 PNU/mL)。由于巴伊亚和多年生黑麦草的最低测试浓度(1,000 PNU/mL)都导致过度反应,因此未能确定其阈值浓度。由于缺乏阳性反应,另外11种过敏原在30分钟评估时不符合建立TC的标准。多数过敏原在4h内引起 $\geq 10\%$ 的马发生阳性反应。24h反应很罕见,仅见于一匹马。

总结与临床意义 – 本研究确定了马对多种花粉和霉菌过敏原的皮内TC,这些数值可能有助于优化过敏马匹在进行IDT时的过敏原浓度。

Resumo

Contexto – Os limiares irritativos (LIs) para teste intradérmico (IDT) não foram ainda estabelecidos para equinos para diversos alérgenos de pólen e bolores.

Objetivos – Determinar os LIs em cavalos não alérgicos e descrever a frequência de reações tardias para 26 alérgenos, incluindo árvores, gramíneas, ervas e leveduras em equinos residentes do sul dos Estados Unidos.

Animais – Vinte e quatro cavalos clinicamente normais do sul dos Estados Unidos.

Métodos – O limiar irritativo para os diversos alérgenos foi determinado pela mensuração subjetiva do IDT após 30 minutos. Reações tardias foram avaliadas em 4 e em 24 horas.

Resultados – Os limiares irritativos (todos em PNU/mL) foram estabelecidos para oito alérgenos de árvores (salgueiro preto 1,000, box elder 1,000, carvalho 1,000, nogueira 2,000, freixo 4,000, carvalho vermelho 4,000, pé de amora 2,000 e freixo verde 2,000); dois alérgenos de gramíneas (*Johnson grass* 250 PNU/mL e *Kentucky blue grass* 500 PNU/mL); duas ervas (*careless weed* 1,000 PNU/ mL, *great ragweed* 500 PNU/

mL) e um bolor (*Curvularia* 8 PNU/mL). O LI não pode ser determinado devido à irritabilidade excessiva na menor concentração testada (1,000 PNU/mL) para as gramas bahia e azevém perene. Onze outros alérgenos não atingiram os critérios para estabelecer um LI quando avaliados em 30 min devido à ausência de reações positivas. Múltiplos alérgenos causaram reações positivas em $\geq 10\%$ dos cavalos em 4 horas. As reações após 24 horas foram raras, com exceção de um animal.

Conclusões e importância clínica – Este estudo identificou o LI intradérmico para múltiplos alérgenos de pólen e bolores em equinos. Estes valores podem ser úteis para a otimização da concentração alergênica no IDT de equinos alérgicos.