

Equine intradermal test threshold concentrations for house dust mite and storage mite allergens and identification of stable acari fauna

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Background – House dust mite (HDM) and storage mite (SM) stable fauna and their associated equine intradermal test (IDT) threshold concentrations (TCs) for the midwestern region of the USA are unknown.

Hypothesis/Objectives – To determine IDT TCs and serum IgE concentrations for two HDM and three SM species in clinically normal horses over two seasons, and to identify the mite taxa and habitats in a stable.

Animals – Thirty-eight clinically normal horses.

Methods – Threshold concentrations for HDMs and SMs were determined using IDT subjective measurements and a statistical model. An enzyme-linked immunosorbent assay was used to quantify serum IgE concentrations for the same mite species. A modified flotation method was used to identify morphologically HDMs and SMs.

Results – Subjective IDT TCs were as follows: 1:80,000 w/v for *Dermatophagoides farinae* in both seasons; 1:80,000 w/v in spring and 1:160,000 w/v in late summer for *Dermatophagoides pteronyssinus*; 1:40,000 w/v in spring and 1:20,000 w/v in late summer for *Acarus siro*; 1:20,000 w/v for *Lepidoglyphus destructor* in both seasons; and 1:20,000 w/v in spring and 1:10,000 w/v in late summer for *Tyrophagus putrescentiae*. Statistically significant associations for increased serum IgE and a positive IDT reaction were evident for *D. farinae* in the spring and *D. pteronyssinus* in both seasons. One mite from all four genera specific to this study was identified; however, two HDM and *A. siro* species were not detected.

Conclusions and clinical importance – This study established HDM and SM IDT dilution concentrations for the horses in this region. Exposure to diverse acaridae fauna may contribute to the pathogenesis of equine allergic disease.

Introduction

Intradermal (IDT) and serological allergen testing (SAT) are used to identify potential environmental allergic triggers in atopic patients.^{1–6} The results of these tests help form the rationale for the therapeutic plan. The reliability of the IDT and the resultant reactions are dependent on many factors. False-positive reactions have been reported in healthy horses, suggesting possible subclinical hypersensitivity, previous exposure without disease, irritant reactions or contamination of allergens with micro-organisms, or may be the result of ectoparasitism known to produce IgE that can cross-react with house dust mite (HDM) or storage mite (SM) allergens.^{4,7–11} False-negative reactions can be a consequence of using allergen

concentrations that are too low, testing off-season or the administration to the animal of medications that are known to inhibit IgE reactions.⁴ Limited data are available regarding SAT in horses. In previous studies that compared SAT in healthy horses and clinically diseased horses affected by atopy, insect hypersensitivity or recurrent airway obstruction, no significant difference was found between IgE levels in the two groups for the majority of specific allergens.^{12–15} When compared with the gold standard of IDT, the sensitivity and specificity of the SATs were often low or unpredictable.^{13,14}

Choosing specific allergens for inclusion in the IDT is of utmost importance in detecting the correct environmental sensitizers. Selection is based on the regional distribution of pollens, moulds, insects and mites.¹⁶ By convention, allergen concentrations used for IDTs are determined from threshold concentrations (TCs). The TC is determined to distinguish between the concentration of allergen that produces irritant reactions versus the concentration that produces allergic reactions in patients with hypersensitivity. Threshold concentrations for the IDT are established by subjectively and objectively scored reactions produced from the injection of serial dilutions of allergens. Ideally, TCs should be determined in animals

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that are not diseased and have been raised and maintained in sterile, allergen-free environments. These animals would not have IDT reactions that represent subclinical disease, because there has been no previous sensitization. Any IDT reaction in this population would represent irritant reactions. Although raising horses in specific pathogen-free conditions has been done, this is extremely costly. Threshold concentrations have been arbitrarily defined as the concentration where <10% of nondiseased animals have a positive reaction.^{2,9} It is proposed that if a greater percentage of healthy animals react to the allergen concentration used, then the reaction should be considered a false-positive result because the allergen was acting as an irritant.^{1,2}

Allergen concentrations used for equine IDTs are commonly extrapolated from the human, canine or feline literature. Equine studies for TCs are limited, both in number and in scope. Five studies have examined IDT TCs in horses.^{9,10,17–19} Three studies examined TCs of insect extracts^{10,18,19} and the other two reported the seasonal variation of TCs for pollen, insect, mould and mite extracts.^{9,17} In one study, the TCs of two HDM and one storage mite SM extract could not be determined owing to excessive reactivity at the lowest TC used.⁹

Worldwide, HDMs represent one of the most common environmental allergens and are known to be significant contributors to the antigenic burdens in atopic dermatitis, allergic rhinitis and asthma.^{1,20–23} House dust mites are found in homes with carpeting, upholstered furniture and mattresses; they feed primarily on epidermal scale.^{23,24} Experimentally, HDMs have also survived by feeding on equine keratinocytes *in vitro*.²⁵ The ambient relative humidity limits their survival and is the key factor that determines where these mites can survive and live. The cold hardiness of the HDM *Dermatophagoides farinae* is impressive, because it can survive for 7 days at -18°C ; thus, it is likely to survive in barns during the midwestern USA winter months. Three species of HDM have been found previously in midwestern USA homes.²⁶ *Dermatophagoides farinae* was the most prevalent species, followed by *Dermatophagoides pteronyssinus* and *Euroglyphus maynei*. Storage mites are found in grains such as oats, corn and hay, as well as in facilities that manufacture and store grains. *Acarus siro*, *Tyrophagus putrescentiae*, *Lepidoglyphus destructor* and *Glycyphagus domesticus* are the most common species found in cereals, flour and baking mixes in homes in the UK.²⁷ In the temperate seasonal climates of North America, the prevalence of live mites fluctuates seasonally. Highest live-mite levels occur during the humid summer months, when the indoor relative humidity conditions for mite survival and breeding are most favourable. Live-mite levels drop during the late autumn months. The residue of dead mites and the level of mite allergen produced show similar seasonal trends that parallel the live-mite densities.²³

Relatively little information has been reported regarding the potential of HDMs and SMs to act as allergens in equine allergic disease. The first report of SMs in association with equine chronic bronchitis examined serum IgE antibodies detected by enzyme-linked immunosorbent assay (ELISA) in both normal horses and horses diagnosed with chronic bronchitis.¹² Wallace

*et al.*²⁸ evaluated horse rugs and saddle blankets for the presence of HDMs; *Dermatophagoides* mites were found via a modified flotation technique in eight of the 16 rugs and blankets sampled.²⁸ A European study evaluated mites in the equine environment, but no species identification was performed.²⁹ In The Netherlands, hay from dairy farms had 10 times as many SMs as grain and three times as many SMs as straw.³⁰ *Lepidoglyphus destructor* was the dominant mite in grain and straw, while *A. siro* was the dominant mite in hay.³⁰ Based on evidence from other species and limited information in horses, it is probable that HDMs and SMs thrive in the hay, grain, barn dust and bedding of horses and may play an important role in the pathogenesis of equine allergic disease. As such, a significant need exists to identify and determine the prevalence of the HDM and SM fauna of the stable. Furthermore, TCs for selected HDM and SM allergen extracts need to be established in nonallergic horses so that IDT results from a horse with allergic disease can be interpreted. We propose that the stable environment provides a nourishing habitat to maintain a viable HDM and SM population year-round. Furthermore, we hypothesize that HDM and SM allergen intradermal TCs can be determined and will vary with the season and that SAT results will not significantly correlate with the IDT TCs of any mite species. The aims of this study were to determine the IDT TCs of horses without allergic disease to HDMs and SMs during the spring and late summer, to quantify seasonal serum specific HDM and SM IgE concentrations in healthy horses and to characterize the mite fauna of a stable over three seasons.

Materials and methods

Study design

All phases of the experimental design of the study were approved by the Ohio State University's Institutional Animal Care and Use Committee. The study was a prospective study.

Horses

The horses used in this study were donated to the Ohio State University's Veterinary Medical Center and were housed at the same farm. All horses had lived at the farm for at least 12 months, if not longer. Horses were kept primarily on pasture, with access to a shelter. The shelters were cleaned weekly by removal of all bedding and replacement with fresh shavings and/or straw. Biannual disinfection was performed after removal of bedding by spraying hard surfaces with a solution that has antimicrobial, fungicidal and virucidal properties (0.39% Quat-Stat SC™; Betco Corporation, Toledo, OH, USA). Free-choice mixed grass hay, pasture, salt and mineral blocks and water *ad libitum* were provided. Each pasture contained three to 15 horses that had access to either a three-sided run-in shed or to an open metal barn. One run-in shed contained an attached wooden barn with three box stalls. The bedding in each enclosure contained mixed hardwood sawdust, straw or a combination of the two. Community feeders within each pasture and/or barn contained mixed-grass, round-baled hay.

Thirty-eight clinically normal horses were used for this study. Inclusion criteria required no previous history of seasonal or perennial pruritus, recurrent urticaria or respiratory disease. The horses had not received any medications that could influence the results of IgE serology or intradermal testing for at least 60 days prior to study commencement or during the duration of the study. These medications included topical, oral or injectable glucocorticoids,

nonsteroidal anti-inflammatories, antihistamines or supplements in which the exact ingredients could not be determined. Additionally, these horses had never presented with clinical signs of dermatological disease nor had an IDT. Horses received regular rotation anthelmintic treatment.

Allergens for intradermal testing

A total of five mite allergen extracts were used for testing and included two species of HDMs (*D. farinae* and *D. pteronyssinus*) and three species of SMS (*A. siro*, *L. destructor* and *T. putrescentiae*). Extracts were made from semi-purified whole-body mite preparations that were purchased as 10 mL glass vials of a 1:100 w/v aqueous solution with 0.4% phenol added as an antimicrobial preservative (Greer Laboratories, Lenoir, NC, USA). Allergens and dilutions were stored in glass vials at 4°C and removed 10 min before the IDT was performed. One vial of each mite extract was used for all allergen dilutions and all IDTs. Allergen concentrations were derived from a pilot study that commenced 2 weeks prior to the start of the reported study. The pilot study tested 10 serial dilutions for each mite species in five normal horses. The results from the pilot study are not included in the presented data.

For the reported study, five to six serial dilutions of each mite allergen extract were made using 0.4% phenolated saline (Greer Laboratories) as sterile diluents. Histamine phosphate (Histatrol®; Alk Abelló, Port Washington, NY, USA), 0.275 mg/mL at a concentration of 1:100,000 w/v, and 0.4% phenolated saline were used as positive and negative controls, respectively. Mite allergen extract IDT concentrations used were as follows: for *D. pteronyssinus* and *D. farinae*, 1:20,000 w/v (25 PNU), 1:40,000 w/v (12.5 PNU), 1:80,000 w/v (6.25 PNU), 1:160,000 w/v (3.125 PNU) and 1:320,000 w/v (1.563 PNU); and for *A. siro*, *L. destructor* and *T. putrescentiae*, 1:5000 w/v (100 PNU), 1:10,000 w/v (50 PNU), 1:20,000 w/v (25 PNU), 1:40,000 w/v (12.5 PNU), 1:80,000 w/v (6.25 PNU) and 1:160,000 w/v (3.125 PNU). The dilutions were made the day before the testing period. A total of 30 intradermal injections were administered for each test per horse; 28 injections were the allergens.

Intradermal testing

The intradermal tests were conducted during two consecutive seasons, early April (spring) and August (late summer). Seven to eight horses were tested per day over a successive 5 day period in the spring and again in the late summer. The horses were kept in the same environment for the duration of the study. Fractious horses were sedated with 0.01–0.02 mg/kg detomidine hydrochloride (Dormosedan®; Pfizer, New York, NY, USA) intravenously via jugular venipuncture 5 min prior to testing. A square test site area, measuring approximately 20 cm × 20 cm, was made on the centre of the left or right lateral aspect of the neck by gently clipping the hair with a number 40 blade (Oster Power Pro Ultra Cordless Clipper Kit, McMinnville, TN, USA). A waterproof permanent marker was used to create a testing template, which consisted of two horizontal rows of 10 dots. Intradermal injections of control solutions and allergens were made above and below the dots. Syringes were preloaded with 0.1 mL of the each individual allergen to avoid under- or overinjection. One investigator performed all intradermal injections for all horses. A volume of 0.1 mL of each allergen extract was injected intradermally to form a visible bleb using a 27 gauge (0.5 mm) needle and 1 mL syringe. Needles on testing syringes were changed between horses to prevent the transfer of any infectious agent. Each skin test site was evaluated subjectively by a single investigator at 15 min for both seasons. The reactions were subjectively scored using a scale of 0 (negative) to 4+ based on wheal size, erythema and turgidity. A 4+ score was comparable to the positive histamine control, whereas a 0 score corresponded to the negative saline control. Subjective scores of ≥2+ were considered positive for the purpose of TC determination. The subjective TC was determined based on the highest concentration of a mite allergen used in the IDT where ≤10% of horses had a subjective reaction of ≥2+ at 15 min. Concentrations of allergens used that caused >10% of horses to have positive subjective 15 min reactions were considered probable irritant reactions. Following IDT,

~3 mL of a 1% hydrocortisone leave-on lotion (Resicort®; Virbac Animal Health, Fort Worth, TX, USA) was applied to the test site.

Allergen-specific IgE serum testing

Serum from each horse was evaluated for IgE concentrations to *D. farinae*, *D. pteronyssinus*, *A. siro*, *L. destructor* and *T. putrescentiae* mites. A 10 mL aliquot of blood was obtained for serum IgE evaluation prior to the IDT. Blood was collected via jugular venipuncture, using a BD Vacutainer® (BD, Franklin Lakes, NJ, USA), 20 gauge needle and holder, into a 10 mL no-additive, silicone-coated interior serum tube (BD). Serum was separated by centrifugation for 10 min at 748 g and stored at –80°C. Samples were shipped overnight on dry ice to the diagnostic laboratory (Heska, Loveland, CO, USA) at the end of each testing season. Analysis of equine serum-specific IgE was performed using a commercially available allergen-specific IgE ELISA test (Heska ALLERCEPT® ELISA, Loveland, CO, USA) performed in CMG-HESKA® Laboratories. Briefly, allergen-specific IgE is detected using a biotinylated recombinant form of the extracellular part of the α-chain of the human high-affinity IgE receptor (FcεRIα).^{31,32} The assay used diluted horse serum and streptavidin–alkaline phosphatase reagents for detection of the FcεRIα. The allergens used to coat the wells of the assay plate were obtained from HDM and SM extracts manufactured by CMG-HESKA® Laboratories. Positive and negative controls used for each assay are proprietary. The test results were expressed in ELISA antibody (EA) units based on optical density values, with results ≥150 EA units considered positive. Assays were performed simultaneously on one plate to decrease interassay variability for each season. The ELISA was performed blinded to the IDT results.

Mite sampling

A 133-acre farm located in the midwestern USA was selected for mite analysis. This farm serves as a teaching and research farm for large farm animals, mostly horses but also cattle. The horses used in this study reside at the farm. Passive mite sampling was conducted over three consecutive seasons (spring, late summer and winter). Sampling was conducted from the same locations at the farm each season by the same individual. Mite numbers and identification analysis were evaluated from contents obtained from nine different locations on the farm. These sites were as follows: (i) a wooden community feeder measuring approximately 6 m × 0.45 m × 0.25 m at palpation stalls, which always contained a small amount of corn, grain and mixed grass hay, where mares could go and come freely; (ii) a community run-in shed attached to a pasture used to house mares with bedding that consisted of straw, mixed grass hay, dirt and mixed organic debris; (iii) individual stall, which housed one horse for 12–18 h a day with bedding that contained mixed organic debris, mixed grass hay, shavings and dirt; (iv) a bucket that sat in the barn and had a continual supply of contained cracked corn; (v) a large round grass hay bale stored in a three-sided outdoor shed with no horses; (vi) a large square bale of mixed grass hay stored in a three-sided outdoor shed with no horses; (vii) a pine wood sawdust pile stored in a barn; (viii) a hay feeder that contained mixed-grass, round-baled hay that had sawdust and mixed organic debris and housed a large group of geldings that could freely go out to pasture; and (ix) an uncovered hay feeder in pasture that contained mixed-grass, round-baled hay, mixed organic debris and dirt and two geldings. Approximately 300 g of mixed materials from each testing site was collected, labelled and secured within two polyethylene, sealed bags. Samples were stored at –20°C prior to being shipped to The Food and Environment Research Agency (Sand Hutton, York, UK). Relative humidity and temperature were recorded at each location daily over 4 days consecutively, including immediately prior to sample collection. A hand-held RH300 Dual Input Hygro-Thermometer Psychrometer® (Extech Instruments, Nashua, NH, USA) was used to take the measurements.

Mite counts and identification

Mites were extracted from the samples by using a modified flotation technique.³³ Briefly, the test sample was combined with 100 mL of

deionized water in a beaker. Subsequently, 20 mL of ethanol, 20 mL of 35% hydrochloric acid and 20 mL of saturated sodium chloride solution were added to the beaker and then transferred to a 750 mL flotation flask. The combined solution was mechanically mixed while 10 mL of ethylene blue stain was added to dye the aqueous phase and any vegetative material. From a bottom inlet, kerosene was added to the flotation flask and allowed to permeate through the aqueous portion. As a result of the lipophilic properties of the mites, they adhered to the kerosene material and were carried to an interface portion near the top of the flask. After decanting the kerosene layer with mites over filter paper, methylene blue was added to the filter paper and it was examined under a low-powered stereomicroscope for morphological identification by professional acarologists.

Statistical analysis

Threshold concentrations were determined based on the highest concentration of a mite allergen tested where $\leq 10\%$ of horses had a subjective reaction of $\geq 2+$ at 15 min. Specifically, TCs were determined for each of the five mite species (*D. farinae*, *D. pteronyssinus*, *A. siro*, *T. putrescentiae* and *L. destructor*) for two seasons (spring and late summer). McNemar's test was used to compare the subjective reaction of $\geq 2+$ at 15 min between the two seasons. McNemar's test was also used to compare allergen-specific IgE serum dichotomized at ≥ 150 EA units to the subjective reaction of $\geq 2+$ at 15 min. A random-effects logistic regression model was generated to define the exact TCs for each of the five mite species in both seasons. The model used the seasonal IDT data from the 38 horses and the same parameters used for the determination of the subjective TCs. The subjective reaction $\geq 2+$ (yes versus no) was regressed on mite allergen concentration, season and the interaction of concentration and season. If the interaction term was not significant, it was dropped from the model. After fitting each regression model, nonlinear contrast statements were used to estimate the 10% TC for each mite species and season and for the difference in the 10% TC across seasons. The resulting *P*-values from McNemar's test and the regression analysis were adjusted using Holm's procedure to conserve the overall type 1 error at 0.05 due to the multiple testing. All analyses were run using STATA 12.1 (Stata Corporation, College Station, TX, USA). A *P*-value of ≤ 0.05 was used to determine statistical significance.

Results

Horses

The study population included 13 mares and 25 geldings, ranging from 4 to 26 years old, with a median age of 12 years. The age and breed of one gelding were unknown. Breeds represented were 13 Thoroughbreds, 11 American Quarter Horses, six Warmbloods, five Standardbreds, one Appaloosa and one American Saddlebred. The same population of horses was used in each season.

Intradermal testing

During both seasons, all horses exhibited an adequate subjective reaction graded as a 4+ to the positive histamine control and had subjective reactions graded as a 0 to the negative saline control. In this population of horses, positive wheals ($\geq 2+$) at 15 min were found at more dilute allergen concentrations despite having a negative subjective ($< 2+$) reaction at the higher concentration for the mite tested. For the spring, this occurred at 17 mite concentration sites on 14 different horses, while it occurred at eight mite concentration sites on eight horses for the late summer. Of the horses affected, three had similar results in both seasons. Two of these three horses were affected for the same mite species in both seasons, although the

concentrations that caused these discordant results were different between the two seasons. These untoward reactions could not be linked consistently to a specific mite extract or dilution. However, positive reactions to *A. siro* at lower (i.e. weaker) concentrations with negative subjective reactions at higher concentrations occurred in seven horses in the spring, which was the greatest number of these results for any species of mites in any season.

Subjective 15 min intradermal threshold concentrations

The spring subjective TCs for both HDMs (*D. farinae* and *D. pteronyssinus*) were 1:80,000 w/v. The spring subjective TCs for SMs were 1:40,000 w/v for *A. siro* and 1:20,000 w/v for both *L. destructor* and *T. putrescentiae*. The late summer subjective TCs were unchanged for *D. farinae* and *L. destructor*; however, the TC for *D. pteronyssinus* changed to a more dilute concentration. The TC dilutions for *A. siro* and *T. putrescentiae* increased in potency. The changes in the mite subjective TCs between the two seasons were not statistically significant (Table 1).

Exact 15 min intradermal threshold concentrations

In addition to the determination of the subjective TCs, a random-effects logistic regression model was used to estimate the exact 10% TCs for each of the five mite species in both seasons. A significant difference was found for the *D. pteronyssinus* allergen TCs needed for $\leq 10\%$ of normal horses to react in spring versus late summer (Table 2). The mite with the most variance between seasons was *D. pteronyssinus*, whereas *T. putrescentiae* had the least amount of variance (Figure 1). When considering how the exact model TCs fit within the scope of the determined subjective TCs, all subjective TCs were within the 95% confidence interval (95% CI) of the exact TCs with the exception of *T. putrescentiae* and *D. pteronyssinus* in the spring (Table 2). The exact probabilities from the logistic regression model can be used to extrapolate the mite allergen concentration needed to obtain a TC percentage different from 10%. For example, if the

Table 1. Comparison of spring and late summer subjective intradermal test threshold concentrations

Mite species	Spring concentration (w/v)	Late summer concentration (w/v)	<i>P</i> -Value*
<i>Dermatophagoides farinae</i>	1:80,000	1:80,000	0.655
<i>Dermatophagoides pteronyssinus</i>	1:80,000	1:160,000	—†
<i>Acarus siro</i>	1:40,000	1:20,000	0.157
<i>Lepidoglyphus destructor</i>	1:20,000	1:20,000	0.564
<i>Tyrophagus putrescentiae</i>	1:20,000	1:10,000	0.654

Concentrations are expressed as weight/volume (w/v).

*The *P*-values were not adjusted using Holm's procedure because there were no statistically significant comparisons.

†Could not be estimated because all observations were 'no' for both seasons.

Table 2. Comparison of spring and late summer statistically derived exact intradermal threshold concentrations

Mite species	Spring		Late summer		Change (from spring to late summer)	
	Concentration (95% CI) [$\times 100,000^{-1}$]	Concentration ratio (95% CI) (w/v)	Concentration (95% CI) [$\times 100,000^{-1}$]	Concentration ratio (95% CI) (w/v)	Estimate (95% CI) [$\times 100,000^{-1}$]	P-Value*
<i>D. farinae</i>	2.37 (1.55–3.20)	1:42,195 (1:31,250–1:64,516)	1.67 (1.14–2.19)	1:59,880 (1:45,662–1:87,719)	0.71 (–0.02 to 1.43)	0.223
<i>D. pteronyssinus</i>	4.73 (3.42–6.04)	1:21,140 (1:16,556–1:29,239)	2.10 (1.02–3.19)	1:47,620 (1:31,347–1:98,039)	2.63 (1.45–3.81)	<0.001
<i>A. siro</i>	6.68 (4.69–8.68)	1:14,970 (1:11,520–1:21,000)	10.24 (6.11–14.4)	1:9765 (1:6944–1:16,366)	–3.56 (–7.62 to 0.49)	0.255
<i>L. destructor</i>	7.97 (5.77–10.17)	1:12,550 (1:9832–1:17,331)	7.63 (5.56–9.71)	1:13,105 (1:10,298–1:17,985)	0.34 (–1.47 to 2.14)	0.714
<i>T. putrescentiae</i>	15.1 (10.6–19.6)	1:6620 (1:5102–1:9433)	15.0 (11.2–18.7)	1:6665 (1:5347–1:8928)	0.16 (–4.11 to 4.43)	0.942

Concentrations are expressed as weight/volume (w/v). Abbreviation: CI, confidence interval.
* P-Values are adjusted using Holm's procedure to conserve the overall type 1 error rate at 0.05.

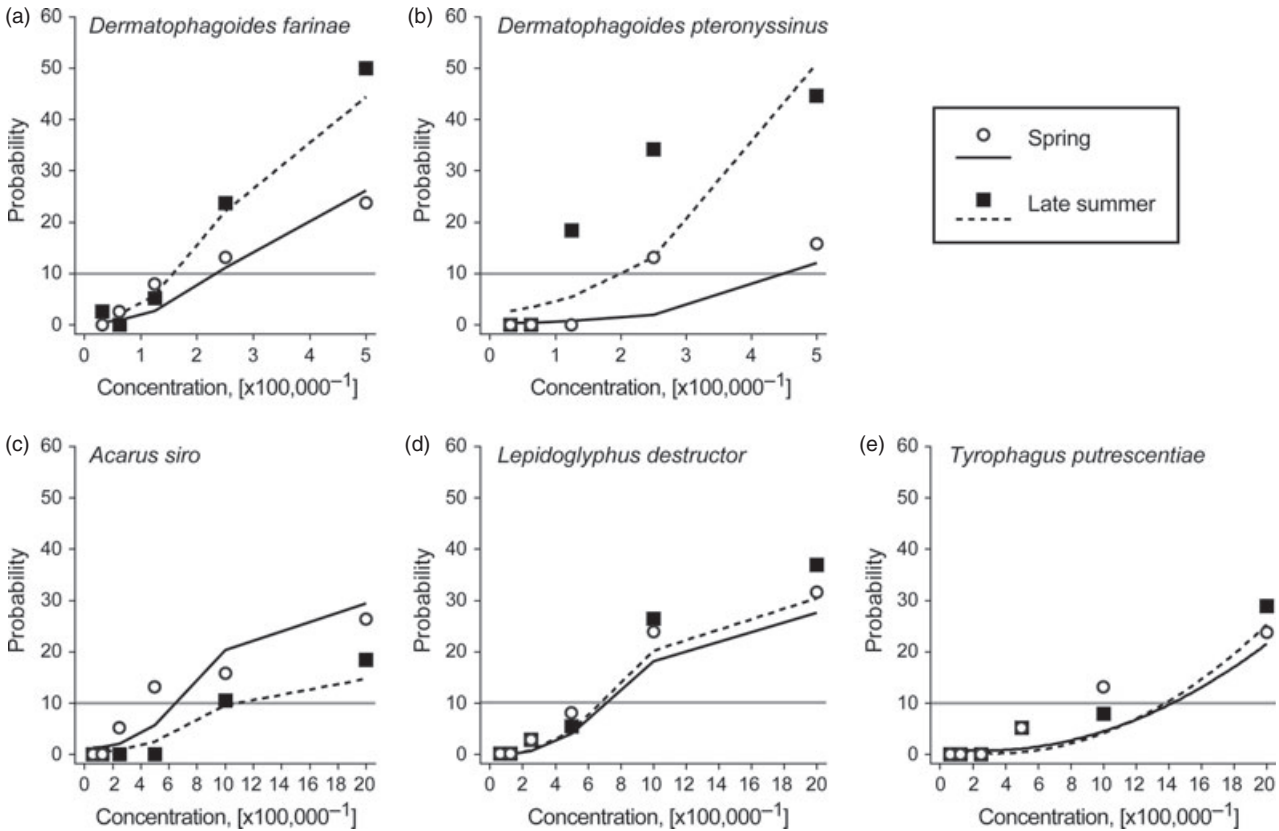


Figure 1. Probability representative of the percentage of clinically normal horses with a positive (2+) intradermal reaction. Concentration was measured as weight/volume (w/v).

desired percentage of normal horses that had a positive ($\geq 2+$) reactions was $\leq 1\%$ for *D. farinae* in the late summer, then the TC would be approximately $0.25 \times 100,000^{-1}$, which is then converted to a ratio, and a 1:400,000 w/v concentration of allergen should be used for the IDT.³⁴

Allergen-specific IgE serum testing

Positive IgE EA units to HDMs and SMs ranged from 150 to 1170. In both seasons, at least one horse had a positive serum IgE result for each HDM or SM evaluated. Negative IgE results for all species of mites occurred in 55%

(21 of 38) and 66% (25 of 38) of the horses in the spring and late summer, respectively. When considering the percentage of horses in the spring that had multiple positive serum IgE results, reactions to two mite species were the most common, occurring in 18% (seven of 38) of the horses, whereas 5% (two of 38) of the horses had positive IgE results to three mite species and 5% (two of 38) were considered to have significant IgE levels for four species of mites. In the late summer, the percentage of horses with positive serum IgE results for two mite species was the most common, occurring in 13% (five of 38) of the horses, whereas 5% (two of 38) of the horses had

positive IgE results to three mite species and only 3% (one of 38) were considered to have significant IgE results for four species of mites.

There was no significant difference in the percentage of horses with positive serum IgE results in late summer versus those reported in the spring. Across the two seasons, only eight horses had positive measurable IgE concentrations in the spring for the same mite species in the late summer. All other positive IgE concentrations for a specific mite species were found in horses with a negative serum IgE level in the other season. In both seasons, *D. farinae* was the species with the most positive horses, with a mean IgE of 260 EA (range 28–323 EA) for spring and 275 EA (range 2–671 EA) for late summer. The overall results for mite allergen-specific serum IgE concentrations in spring and late summer are listed in Table 3. When considering the subjectively determined IDT TCs, significant associations between positive serum IgE results with a positive IDT reaction were found for *D. farinae* in the spring and for *D. pteronyssinus* in both seasons (Table 4). In contrast, when using horses with positive reactions ($\geq 2+$) at concentrations above the exact IDT TCs, a significant positive association between a positive IgE result and a ($\geq 2+$) positive IDT reaction was found only for *A. siro* in the late summer (Table 4).

Table 3. Percentage of horses with positive seasonal allergen-specific serum IgE

Mite species	Horses with IgE ≥ 150 EA in spring	Horses with IgE ≥ 150 EA in late summer
<i>D. farinae</i>	37% (14 of 38)	24% (9 of 38)
<i>D. pteronyssinus</i>	18% (7 of 38)	18% (7 of 38)
<i>A. siro</i>	11% (4 of 38)	8% (3 of 38)
<i>L. destructor</i>	3% (1 of 38)	3% (1 of 38)
<i>T. putrescentiae</i>	16% (6 of 38)	18% (7 of 38)

Serum IgE levels are expressed as ELISA antibody (EA) units. Allergen-specific serum IgE results are considered positive at ≥ 150 EA.

Table 4. Comparison of subjective and exact intradermal threshold concentrations with positive allergen-specific serum IgE (≥ 150 EA)

Mite species	Spring concentration (w/v)	Spring P-value*	Late summer concentration (w/v)	Late summer P-value*
Subjective intradermal threshold concentrations				
<i>D. farinae</i>	1:80,000	0.012	1:80,000	0.139
<i>D. pteronyssinus</i>	1:80,000	0.033	1:160,000	0.041
<i>A. siro</i>	1:40,000	0.414	1:20,000	0.472
<i>L. destructor</i>	1:20,000	0.635	1:20,000	0.564
<i>T. putrescentiae</i>	1:20,000	0.472	1:10,000	0.412
Exact intradermal threshold concentration				
<i>D. farinae</i>	1:42,195	0.063	1:58,880	>0.99
<i>D. pteronyssinus</i>	1:21,140	0.063	1:47,620	0.130
<i>A. siro</i>	1:14,970	0.081	1:9765	0.013
<i>L. destructor</i>	1:12,550	0.225	1:13,105	0.739
<i>T. putrescentiae</i>	1:6620	0.331	1:6665	0.617

Concentrations are expressed as weight/volume (w/v). Serum IgE levels are expressed as ELISA antibody (EA) units. Allergen-specific serum IgE results are considered positive at ≥ 150 EA.

*P-Values are adjusted using Holm's procedure to conserve the overall type 1 error rate at 0.05.

Mite identification, counts, locations, temperature and relative humidity

The morphological analysis for mites in the environment on this farm revealed a rich Astigmata and Prostigmata fauna. Mites of the suborder Astigmata belonging to 30 different genera were identified. Pyroglyphida species were rare, whereas Chortoglyphidae (nonpyroglyphidae) were common. A total of 3332 mites were collected over the three seasons. Of these, 20% (660 of 3332) were of the four genera relevant to this study. Spring was the only season in which two of the five species and all four genera of mites were identified (Table 5). When considering the seasonal numbers of mites in four genera of this study, the winter mite collection was the greatest at 281, followed by the spring collection with 232 mites, and finally, the summer analysis had 78 mites.

Throughout all seasons, the SM fauna was dominated by the abundance of *Tyrophagus*. This SM genera was the most abundant in the winter and least numerous in the late summer. A total of 211 *T. putrescentiae* mites were identified and represented 6% (211 of 3332) of all mites collected and 32% (211 of 660) of the four genera SMs and HDMs used for IDT TC determination. *Tyrophagus sylvestris* was the most plentiful *Tyrophagus* species identified. This species accounted for 192 mites obtained in the winter from the stall that housed a stallion for at least 16 h a day for the last 2 years. The greatest number of *T. putrescentiae* were found in the spring in the straw bedding, which was the location with the second greatest population of mites collected from one area. The sites sampled revealed that the *Lepidoglyphus* fauna was the only other mite genera present during all three seasons. *Lepidoglyphus* had the greatest number of mites found in the spring. *Lepidoglyphus destructor* represented 1.9% (63 of 3332) of all the mites identified and 9.5% (63 of 660) of the four SMs and HDMs evaluated for TCs. *Tyrophagus* and *Lepidoglyphus* were the only genera present in the late summer.

The *Acarus* fauna of *A. farris*, *A. immobilis* and *A. nidicolous* were found in the spring and winter only. These species were identified in different seasons in the stall where the horse was present for most of the day [location (iii)]. *Acarus siro* was not found. Additionally, *Dermatophagoides* genera was nearly absent in samples collected from this rural environment. *Dermatophagoides farinae* or *D. pteronyssinus* species were not collected in any of the three seasons; however, one *D. microceras* was identified in the long communal wooden feeding trough [location (ii)] that had sparse remnants of cracked corn, fine grain dust, oat hulls and hay particles.

The highest infestation of mites was found in the individual stall, which housed one horse 12–18 h a day [location (iii)]. The stall had a total of 43 mites in the spring, 60 mites in the late summer and 233 mites in the winter. The only other sites in which mites were found in all three seasons was the sawdust pile stored in the barn. The bucket that had a continual supply of cracked corn was the only site sampled that did not contain any of the five mite species over the three seasons. All other locations contained at least one mite species in at least one season (Table 5).

Table 5. Mite genera and number in nine stable locations

Location	Spring	Late summer	Winter
(i) Feeder bin in indoor mare run-in shed	10 <i>Tyrophagus putrescentiae</i> 1 <i>Dermatophagoides microceras</i>	None	None
(ii) Straw bedding in indoor mare run-in	88 <i>T. putrescentiae</i> 4 <i>Lepidoglyphus destructor</i>	None	None
(iii) Wood shavings inside stall housing horse for 12–18 h/day	43 <i>Acarus farris</i>	60 <i>T. putrescentiae</i>	192 <i>Tyrophagus sylvestris</i> 41 <i>Acarus immobilis</i>
(iv) Corn bucket inside barn	None	None	None
(v) Round hay bales in three-sided shed	35 <i>Tyrophagus neiswanderi</i>	None	25 <i>T. putrescentiae</i> 11 <i>L. destructor</i>
(vi) Square hay bales in three-sided shed	29 <i>L. destructor</i>	None	3 <i>L. destructor</i> 2 <i>A. farris</i>
(vii) Sawdust pile stored inside barn	2 damaged <i>Tyrophagus</i> sp.	12 <i>L. destructor</i>	4 <i>L. destructor</i> 2 <i>T. putrescentiae</i> 1 <i>Acarus nidicolous</i>
(viii) Hay feeder inside gelding barn	20 <i>T. putrescentiae</i>	None	None
(ix) Hay feeder in outside front pasture	None	6 <i>T. putrescentiae</i>	None

Locations are given as the corresponding number as well as a description of the location site. The number of mites, with corresponding genus and, when applicable, species found at each location during each season, is provided.

The average relative humidity and temperature for each site over each season varied in range (Table 6).

Discussion

In order to define allergen test performance optimally, a method should be reproducible and validated. Evaluation of physiological responses during direct allergen challenge in healthy animals without allergic disease remains an appropriate method to help define the significance of intradermal reactivity when known concentrations of allergens are used. This study determined the ideal equine IDT cut-off threshold values for two HDM and three SM allergen dilution concentrations for an immediate type I reaction. Two evaluation methods (subjective and statistical model) were used in order to ensure that the recommended IDT mite allergen concentrations would be reliable for distinguishing intradermal reactions that represent true sensitization in clinically affected horses rather than irritant or false-positive reactions.

Current recommendations for equine IDT HDM testing concentrations are 60–250 PNU/mL, which represents dilutions of 1:2000–1:8333 w/v, respectively.^{4,16} References for equine IDT SM testing concentrations as determined by subjective TCs are limited and reported as 1000 Noon units/mL for *T. putrescentiae* and *A. siro* for horses in The Netherlands, and 1:10,000 w/v for

L. destructor and *T. putrescentiae* for horses in Australia.^{9,17} In the study by Baxter *et al.*,⁹ the TCs of *D. farinae*, *D. pteronyssinus* and *A. siro* were not determined owing to excessive reactivity at the least potent dilutions used, which were 1:30,000 w/v for *D. pteronyssinus*, 1:12,000 w/v for *D. farinae* and 1:10,000 w/v for *A. siro*. In the present study, the subjective TCs for *T. putrescentiae* of 1:10,000 w/v for late summer did corroborate the recommended TCs for this mite as reported by Baxter *et al.*⁹ The subjectively determined TC for *L. destructor* of 1:20,000 w/v in this study was only one serial dilution less than what had been reported previously. In our study, the subjective TC for *A. siro* was the most dilute for the SMs ranging from 1:40,000 to 1:20,000 w/v; therefore it was not surprising that when 1:10,000 w/v was used, the TC could not be determined.

The subjective TCs for both HDMs were very dilute, at 1:80,000 w/v for *D. farinae* and 1:80,000–1:160,000 w/v for *D. pteronyssinus*. Although it was expected that more dilute TCs for HDMs compared with the TCs for SMs would be found based on our pilot study and previous studies, what was surprising was the apparent lack of exposure of this population to HDMs in this agricultural setting. The reactivity to HDM is likely to reflect cross-reactivity among mite allergens that are taxonomically related. Cross-reactivity between allergens may cause 'covariation of sensitization', i.e. a higher observed frequency of sensitization to two or more allergens than the expected frequency. Sensitization to several HDM and SM species exists in mite-allergic humans and dogs.³⁵ The presence of multiple mite hypersensitivities often reflects the likeness of mite allergens, which share similar IgE binding epitopes, and therefore, may be a cause of clinically reported cross-reactivity. Humans allergic to *Dermatophagoides* spp. may experience allergic symptoms when exposed to molluscs, crustaceans and multiple other insects. This cross-reactivity is reported to be due to the group 10 muscle allergen tropomyosin, present in mites and various insects (chironomids, mosquito and cockroach), snails and shrimp, as well as a 25 kDa allergen present in several arthropod groups.³⁶ The Chironomidae (commonly known as midges) is a group of

Table 6. Average relative humidity and temperature across three seasons

	Season		
	Spring	Late summer	Winter
Average relative humidity (%)	43.5	48.7	47.4
Average temperature	20.0°C (68.0°F)	26.4°C (79.5°F)	0.44°C (32.8°F)

Temperature was measured in degrees Celsius. Average relative humidity and temperature were measured using a RH300 Dual Input Hygro-Thermometer Psychrometer® (Extech Instruments, Nashua, NH, USA) over 4 days at nine locations during three seasons.

diverse flies with over 10,000 species worldwide. This group of horses would have had continual exposure to chironomids. Collectively, the evidence of the exposure of the horse to the enormous range of insects and mites in its environment, the identified low TCs to *D. farinae* and *D. pteronyssinus*, the statistically significant associations between positive serum IgE results with a positive IDT reaction ($\geq 2+$) to *D. farinae* in the spring and *D. pteronyssinus* in both seasons, and finally, the lack of *Dermatophagoides* in the environment would all support subclinical sensitization to allergens that cross-react with this genera. Recently, Liao *et al.*,³⁷ reported that 97% of humans with rhinitis who were sensitized to *T. putrescentiae* were also sensitive to *D. pteronyssinus* and, using the IgE-binding inhibition analysis, found complete absorption of IgE binding activity by *D. pteronyssinus*, which indicated that *T. putrescentiae* hypersensitivity was due to cross-reactivity, not dual sensitization.

Although cross-reactivity is a possible mechanism for the reactivity to very dilute HDM concentrations, HDM extracts have microbial contaminants. The microbial compounds lipopolysaccharide and β -glucan can be detected routinely in HDM extracts obtained from whole-mite cultures or mite bodies.³⁸ In addition, chitin and fungal cell walls are also in mite extracts.³⁹ Whether or not these microbial compounds are derived from endosymbionts and/or stable contaminants in mite culture remains to be addressed fully. Data have suggested that *Dermatophagoides* carry endosymbiotic bacteria that produce bacterial lipopeptides as well as having ecological relationships with xerophilic fungi, such as *Aspergillus penicillidies* conidia, that have been identified in their digestive system and faecal pellets. Moreover, yeast enters the composition of the mite growth medium. With such a diverse composition of inflammatory mediators and sensitizers in these extracts, it is impossible to discount the potential contribution of these to the low *D. farinae* and *D. pteronyssinus* TCs detected in the horses.

Positive IDT reactions were observed at more dilute concentrations for some mite species, while negative results were found at higher concentrations. To the authors' knowledge, this has not been reported previously. While the exact cause of this occurrence is unknown, potential explanations may include subjective observer error or traumatic injection at the individual intradermal site, which may have caused false-positive reactions. In the future, having multiple investigators read any reaction site and averaging the results may decrease intra-observer error, but may also lead to interobserver error. Another possible explanation would have been contamination with irritants, precipitates or proteases of the vial(s) containing the more dilute concentrations. Only three horses had these discordant results for both seasons, and of the three, only two had them for similar mite concentrations in both spring and late summer. There may have been some unidentified variation in the cutaneous sensitization of these animals, such as a focal increase in mast cells or epidermal Langerhans cells in the area of skin tested, that may have led to these results.

Investigation of seasonal TC determination was carried out to determine whether a concentration–response

relationship between the level of exposure to HDMs and SMs and cutaneous sensitization was present in this cohort of normal horses. The prevalence of allergic signs during annually recurring periods remains the standard best indicator of potential importance and reflects the dose–response relationship where the degree of clinical sensitivity varies with the environmental allergen concentration. Allergen load may also influence the development of serum specific IgE. Seasonal variation in the subjective TCs for the mites did exist, with changes representing one serial dilution for *D. pteronyssinus*, *T. putrescentiae* and *A. siro*; however, the differences were not statistically significant. It must be recognized that the magnitude of mite allergen concentration alone does not denote clinical relevance, because low levels of some mite genera may be more sensitizing than higher levels of others. The determined TCs and the very low prevalence of HDMs in this study would seem to suggest that horses are sensitized to one or more of these mites at very low levels that do not provoke clinical signs.

There is no universally accepted or defined percentage of normal animals that should have negative intradermal reactions in order to prevent the interpretation of reactions as false positives. Therefore, we developed a random-effects logistic regression model that can be used by the clinician to prevent testing with mite allergen concentrations above the threshold for a type I-mediated reaction. The overall outcome of using extrapolated allergen concentrations from this model will be to prevent 'irritant' or false-positive reactions and allow detection of true sensitization in affected horses. When comparing the exact TCs from the model with those found with subjective reading of the IDT, the determined subjective TCs were predominately within the 95% CI for the model; however, two TCs were not included within the CI. This is due to the limited number of serial titrations used. For example, the subjective TC for *D. farinae* in the spring was determined to be 1:80,000 w/v, while the exact statistical model TC was 1:42,195 w/v with a 95% CI of 1:31,250–1:64,516 w/v. Our study found that >10% of the normal horses reacted at 1:40,000 w/v, but not at the next weaker serial dilution of 1:80,000 w/v. Therefore, the model predicts that the TC lies somewhere between 1:40,000 and 1:80,000 w/v. If the testing panel had included concentrations of 1:50,000 or 1:60,000 w/v then our subjective TC may have been within the exact CI. Given that this is a statistical model, complete agreement between it and the subjective findings could not be achieved. A potential reason for the exceptions of *T. putrescentiae* and *D. pteronyssinus* in the spring could be due to the presence of outliers on the statistical model, which may have skewed the logistic regression line and caused a stronger statistically exact TC.

Concordance between the IDT and *in vitro* specific IgE assay in this study was dependent on the mite species evaluated. Positive allergen-specific serum IgE for mites was present in 45% of the horses in spring and 34% of the horses in late summer. Positive allergen-specific IgE would indicate the presence of subclinical sensitization, which would make any corresponding positive IDT reaction likely to be a true positive and not an irritant reaction,

because these should not be IgE mediated. However, the presence of a positive IDT reaction below the TC with a negative serum allergen-specific IgE result would be likely to indicate a false positive for that horse on the IDT. The greatest percentage of positive serum IgE results were for the HDMs, supporting the likelihood of subclinical sensitization due to cross-reactivity in this population. In order to determine the IgE cross-reactivity between *A. siro*, *L. destructor*, *T. putrescentiae*, *D. farinae* and *D. pteronyssinus*, a sensitized population to each mite would need to be identified, serum collected and then IgE immunoassay and immunoblotting inhibition experiments performed.

An aim of this study was to identify the mite fauna from various areas of a typical farm. The intent was twofold. First, as North American inhalant and cutaneous allergens are demographically, botanically and ecologically diverse, it is important to ensure that key allergens used for the IDT reflect the range of potential mite antigens that could act as environmental sensitizers. Inclusion of regional specific allergens maximizes the patient's chances of benefiting from allergen-specific immunotherapy. Second, knowledge of the local indigenous mite fauna can be used to help provide recommendations that would limit environmental exposure as well as pinpoint potential sources of infestation before allowing the infestation to spread. Currently, there are no studies that have identified and quantified the mite fauna in the rural horse farm environment of the USA. The morphological identification of mites over the three seasons was very successful, not only due to the method of collection used but also because the identification was performed by an experienced acarologist who can reliably identify partial and intact mites. No commercially available detection method developed to date has been able consistently to detect mixed mites in samples. Our study identified all four genera of mites used for determination of TCs; however, the different species of *Acarus* and paucity of *Dermatophagoides* detected suggests a possible reconsideration of relevant allergen extracts for use in the horse.

The *A. siro* mite was not part of this stable's microenvironment. It is known as the flour mite and is an economically important pest of stored food products that has been reported to cause lesions and digestive ulcers in livestock, but it is considered primarily an indoor species occurring in storage areas.^{40–42} The *Acarus* fauna of *A. farris*, *A. immobilis* and *A. nidicolous* were identified; *A. farris* and *A. immobilis* occur primarily in outside environments, such as the nests of birds and mammals, bat roosts, grass and on farm products, such as vegetables.⁴² *Acarus farris* and *L. destructor* dominate the fauna of 1-year-old hay in New Zealand.⁴³ They occur together in hay samples in such a way that *A. farris* is the most numerous in the most humid hay samples, whereas *L. destructor* takes advantage of the drier part of the hay. This relationship was found in one of our square hay bale samples in the winter, although the numbers were low. This may be due to the presence of the predator mite *Cheyletus eruditus*, which is a stylet feeder that gradually replaces these two species in hay. This sample did have several *C. eruditus* identified and over 200+ Tarsonemidae, which are known predatory mites that feed on other mites, mycelia

of fungi, algal bodies and higher plants (data not shown). Interestingly, freshly cut hay in the field was not found to contain any recognizable numbers of SMs; however, upon arrival in the barn hay becomes infested from old hay remaining there.⁴³ The maximal concentration of mites found in 1-year-old hay is 50,000 live mites/kg. Thereafter, the density slowly decreases but the mites never die out; 29-year-old hay still holds live mites.

Mites in the *Tyrophagus* genera were found in multiple locations across all three seasons in this study.⁴⁴ *Tyrophagus putrescentiae*, a mould mite, is a major pest in stored products worldwide as well as a known cause of allergic asthma and rhinitis.^{45–47} Products such as wheat, flour, cheese and cereal, as well as stored dry dog food, have been found to contain *T. putrescentiae* mites.^{45,48} The ideal survival conditions have been found to be 10.0–34.8°C for temperature and approximately 90% for relative humidity.^{44,49,50} Allergies induced by *T. putrescentiae* are mostly reported in farmers and food industry workers, which correlates to the presence of antigen for the mite species found in cattle farms and farmers' living quarters in Germany.^{46,51} In the present study, *T. putrescentiae* mites were found in multiple horse feeders, hay, straw bedding and sawdust in spring, late summer and winter. In addition to the aforementioned species, two other *Tyrophagus* mites, *T. neiswanderi* in the spring and *T. sylvester* in the winter, were also detected. Although little information is available on these two species, *T. neiswanderi* has been reported to have a similar optimal temperature to *T. putrescentiae* (7.39–31.7°C), and *T. sylvester* mites have been detected in the outdoor urban environment in Poland.^{52,53} For the time periods sampled in this study, the relative humidity was not ideal for the *T. putrescentiae* mites, but the average temperatures in spring and late summer were within the optimal range for both *T. putrescentiae* and *T. neiswanderi*. The lower than preferred temperatures in the winter may explain the decrease in mite numbers of both species during this study period.

The SM *L. destructor* is a major source of mite allergy in European rural environments, but it also causes allergy in urban populations around the world. On the island of Gotland, Sweden, five genera of SMs were detected on 16 farms, but the different mite species were not represented on all farms. *Lepidoglyphus destructor* was the dominating species on 13 of 16 farms.⁵⁴ The authors of earlier studies showed that *L. destructor* was the most important allergen causing symptoms from both upper and lower airways among farmers.⁵⁵ The most abundant aeroallergens found in air of commercial dairy barns, near Cooperstown (NY, USA) and Rochester (MN, USA), were *Aspergillus fumigatus* followed by *L. destructor*.⁵⁶ *Lepidoglyphus destructor* occurs in a greater range of conditions, with periodic outbreaks affected by the abundance of field fungi; therefore, this species has been used as a biomarker for grain spoilage.^{57,58} The described pattern of *L. destructor* fluctuation of large numbers of this mite developing in the late spring and early summer was verified in our study.⁵⁹

The bucket that contained the continual supply of cracked corn was the only site sampled that never contained any of the four genera of mites that were evalu-

ated for IDT in this study over the three seasons. However, the corn bucket was not mite free, because during the spring collection two different SM genera were identified and included 71 *Aeroglyphus robustus*, a SM that commonly occurs after grain has been stored for 2 years, as well as 15 *Glycyphagus domesticus*, which is phylogenetically closely related to *L. destructor* (data not shown). *Glycyphagus domesticus* was reported to be the second most common SM found in German farms, infesting 108 of 121 farms.⁶⁰

This was an exploratory study to identify mite genera and species and their habitat. Sinha and Wallace⁶¹ have determined that the following three factors regulate acarine numbers: (i) basic determinants (moisture, temperature, food and intrinsic rate of increase in species); (ii) influencers (seed cracks, microflora, predator mites and dockage); and (iii) seasonal and cyclic regulators (seasonality in temperature and several density-dependent factors). To develop a better understanding of determinants and main species of the equine mite fauna for this region, samples from additional locations and different types of farms over all four seasons are needed. Additionally, detection of the presence of a mite's specific allergen, if known and diagnostic reagents are available, in the environment would be of use to distinguish the level of allergen present versus the number of mites present.

There are recognized limitations of this study. The first limitation is that the horses were not specific pathogen free, and therefore, they were likely to have developed cutaneous sensitization from continuous exposure to insects and mites. This exposure may make the determined TCs less potent than if they had been assessed in horses that had always lived in a sterile environment. The second limitation is that the same population was used for the determination of TCs in both seasons. Iatrogenic exposure to mite allergen extracts in spring could have the potential to sensitize the horses to the mites, influencing the late summer TCs. Given that the TCs did not change significantly over the two seasons, it is unlikely that this low level of allergen concentration prejudiced the late summer TC results. Finally, the population of horses and farm used in this study were located in a single region. Geographical differences may yield different mite populations or various TCs.

In conclusion, our findings would suggest that the equine IDT panel of allergens may not need to include *D. farinae*, *D. pteronyssinus* and *A. siro* but rather the mites more commonly found in the stable microenvironment. Additionally, the apparent mite cross-reactivity in this population would advocate for the use of purified mite allergens to increase the specificity of the equine IDT. Exact dilution concentrations for the SM allergens relevant to this population would be in the range of 1:12,550–1:13,100 w/v for *L. destructor*, with a dilution of 1:6600 w/v for *T. putrescentiae*. Future investigations should be conducted not only across different regions of the country to determine the regional differences in TCs for HDMs and SMs, but also during all four seasons. Finally, it is recognized that there is an inherent risk of basing TCs purely on results in clinically normal horses, because the 'irritant' TC may deviate significantly from the concentration identified in hypersensitive horses. As

such, additional studies are needed in horses with recognized allergic disease to SMs to identify the allergen detection TCs.

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Résumé

Contexte – La composition des acariens de poussière (HDM) et de stockage (SM) dans une écurie et les concentrations seuils (TCs) des tests intradermiques (IDT) correspondants pour la région du Midwest des USA sont inconnus.

Hypothèses/Objectifs – Déterminer les TC d'IDT et les concentrations sériques d'IgE pour deux HDM et trois espèces de SM chez des chevaux cliniquement sains sur deux saisons et identifier les taxons et les habitats d'acarien dans une écurie.

Sujets – Trente huit chevaux cliniquement sains.

Méthodes – Les concentrations seuils pour HDMs et SMs étaient déterminées par des mesures subjectives d'IDT et un modèle statistique. Un test ELISA a été utilisé pour quantifier les concentrations sériques d'IgE pour les mêmes espèces d'acarien. Une méthode de flottaison modifiée a été utilisée pour déterminer la morphologie des HDMs et SMs.

Résultats – Les TCs d'IDT subjectifs étaient tels que: 1:80,000 w/v pour *Dermatophagoides farinae* pour les deux saisons; 1:80,000 w/v au printemps et 1:160,000 w/v en fin d'été pour *Dermatophagoides pteronyssinus*; 1:40,000 w/v au printemps et 1:20,000 w/v en fin d'été pour *Acarus siro*; 1:20,000 w/v pour *Lepidoglyphus destructor* pour les deux saisons; et 1:20,000 w/v au printemps et 1:10,000 w/v en fin d'été pour *Tyrophagus putrescentiae*. Les associations statistiquement significatives pour les augmentations d'IgE sériques et une réaction IDT positive étaient évidentes pour *D. farinae* au printemps et *D. pteronyssinus* pour les deux saisons. Un acarien des quatre genres spécifiques à cette étude était identifié; cependant, deux espèces de HDM et *A. siro* n'ont pas été identifiés.

Conclusions et importance clinique – Cette étude met en évidence des concentrations diluées d'IDT pour HDM et SM chez les chevaux dans cette région. L'exposition à une faune d'acarien variée pourrait contribuer à la pathogénie des allergies chez le cheval.

Resumen

Introducción – Se desconoce si existe presencia estable de los ácaros del polvo casero (HDM) y de almacén (SM) y la concentración adecuada (TC) para su prueba intradérmica (IDT) asociada en la región del medio-Oeste de los EEUU.

Hipótesis/Objetivos – Determinar la IDT TC y la concentración de IgE en suero para dos especies de HDM y tres especies de SM en caballos clínicamente normales en dos estaciones del año, e identificar la taxonomía y hábitat de los ácaros en un establo.

Animales – Treinta y ocho caballos clínicamente sanos.

Métodos – Se determinó la concentración idónea para IDT de HDM y SM subjetivamente y utilizando un modelo estadístico. Se utilizó una prueba de enzimo-inmunoensayo para cuantificar la concentración de IgE en suero para las mismas especies de ácaros. Un método de flotación modificado se utilizó para identificar morfológicamente HDM y SMs.

Resultados – los valores subjetivos de IDT TCs fueron los siguientes: 1:80000 p/v para *Dermatophagoides farinae* en ambas estaciones; 1:80000 p/v en primavera y 1:160000 p/v en verano tardío para *Dermatophagoides pteronyssinus*; 1:40000 w/v en primavera y 1:20000 p/v en verano tardío para *Acarus siro*; 1:20000 p/v para *Lepidoglyphus destructor* en ambas estaciones; y 1:20000 p/v en primavera y 1:10000 p/v en verano tardío para *Tyrophagus putrescentiae*. Hubo asociación evidente y estadísticamente significativa para la IgE aumentada en suero y reacción IDT positiva para *D. farinae* en primavera y *D. pteronyssinus* en ambas estaciones. Se identificó un acaro en los cuatro géneros específicos de este estudio; sin embargo dos HDM y *A. siro* no fueron detectados.

Conclusiones e importancia clínica – este estudio establece las concentraciones de diluciones de HDM y SM para IDT para caballos en esta región. La exposición a diversa fauna de ácaros puede contribuir a la patogénesis de la alergia equina.

Zusammenfassung

Hintergrund – Die stabile Fauna an Hausstaubmilben (HDM) und Futtermittelmilben (SM) und ihre entsprechenden Intradermaltest (IDT) Schwellenwertkonzentrationen (TCs) für den Mittelwesten der USA sind unbekannt.

Hypothese/Ziele – Das Ziel war eine Bestimmung der IDT TCs und der Serum IgE Konzentrationen für zwei HDM und drei SM Spezies in klinisch normalen Pferden im Verlauf zweier Saisons durchzuführen und die Systematik der Milben und ihre Lebensräume in Stallungen zu identifizieren.

Tiere – achtunddreißig klinisch normale Pferde.

Methoden – Die Schwellenwertkonzentrationen für HDMs und SMs wurden mittels subjektiven Messungen im IDT und mit Hilfe eines statistischen Modells bestimmt. Ein ELISA wurde verwendet, um die Serum IgE Konzentrationen derselben Milbenspezies zu quantifizieren. Eine modifizierte Flotationsmethode wurde verwendet, um die HDMs und SMs morphologisch zu identifizieren.

Ergebnisse – Die Ergebnisse der subjektiven IDT TCs waren folgendermaßen: 1:80.000 w/v *Dermatophagoides farinae* in beiden Saisonen; 1:80.000 w/v im Frühling und 1:160.000 w/v im Spätsommer für *Dermatophagoides pteronyssinus*; 1:40.000 w/v im Frühling und 1:20.000 w/v im Spätsommer für *Acarus siro*; 1:20.000 w/v für *Lepidoglyphus destructor* in beiden Saisonen; und 1:20.000 w/v im Frühling und 1:10.000 w/v im Spätsommer für *Tyrophagus putrescentiae*. Ein statistisch signifikanter Zusammenhang zwischen erhöhtem Serum IgE und einer positiven IDT Reaktion waren für *D. farinae* im Frühling und *D. pteronyssinus* in beiden Saisonen evident. Eine Milbe aller vier Genera, die für diese Studie interessant waren, wurde identifiziert; zwei HDM und *A. siro* Spezies wurden jedoch nicht entdeckt.

Schlussfolgerungen und klinische Bedeutung – In dieser Studie wurden die HDM und SM IDT Verdünnungskonzentrationen für die Pferde dieser Region bestimmt. Die Exposition zu diversen Akaridenfauna könnte zur Pathogenese der allergischen Erkrankung des Pferdes beitragen.

要約

背景 – アメリカ中西部でのハウスダストマイト (HDM) および貯蔵ダニ (SM) の安定した動物相ならびにそれらに関連するウマの皮内試験 (IDT) 開始濃度 (TC) は知られていない。

仮説/目的 – 臨床的に正常なウマで2つの季節以上における2種類のHDMならびに3つのSM種に対するIDT TCおよび血清IgE濃度を決定すること、およびダニ分類とダニの安定した生息場所を特定すること。

供与動物 – 38頭の臨床的に正常なウマ

方法 – HDMおよびSMの開始濃度をIDT主観的な測定および統計模型を用いて決定した。酵素結合免疫吸着測定法を同種のダニに対する血清IgE濃度を定量する為に使用した。修正した浮遊法をHDMおよびSMの形態学的な同定のために使用した。

結果 – 主観的IDT TCは以下であった。両方の季節において *Dermatophagoides farinae* に対して1:80,000 w/v、*Dermatophagoides pteronyssinus*に対して春は1:80,000 w/vで晩夏では1:160,000、*Acarus siro*に対して春は1:40,000 w/vで晩夏では1:20,000 w/v、*Lepidoglyphus destructor*に対して両方の季節で1:20,000 w/v、*Tyrophagus putrescentiae*に対して春は1:20,000 w/vで晩夏は1:10,000であった。増加した血清IgEおよび陽性IDT反応における統計学的な有意な関係は春における *D. farinae* および両方の季節における *D. pteronyssinus* で証明された。4つのすべての属でこの研究に特異的な1種類のダニが特定されたが、2種類のHDMおよび *A. siro* 種では検出できなかった。

結論および臨床的な重要性 – この研究はこの地域にいるウマに対するHDMおよびSM IDT希釈濃度を実証した。様々なコナダニ科動物相への暴露はウマのアレルギー性疾患の原因の1つとなるかもしれない。

摘要

背景 – 在美国中西部，屋尘螨 (HDM) 和储藏螨 (SM) 在马厩的种类，以及相应的马皮内实验 (IDT) 的阈浓度 (TCs) 均未知。

假设/目的 – 选择跨过两个季节的健康马匹，确定其体内两种HDM和三种SM的IDT TCs和血清IgE浓度，并鉴定该螨的亚群和马厩栖息地。

动物 – 38只健康马匹。

方法 – HDMs和SMs的阈浓度通过IDT主观测量和统计模型确定。酶联免疫吸附试验被用来量化同种螨虫的血清IgE浓度。改进的漂浮离心法被用来鉴定HDMs和SMs形态。

结果 – 主观的IDT TCs如下：1:80,000 w/v的美洲尘螨存在于两个季节；欧洲尘螨1:80,000 w/v在春季、1:160,000 w/v在夏末；粗脚粉螨1:40,000 w/v 在春季、1:20,000 w/v 在夏末；害嗜鳞螨1:20,000 w/v在两个季节；腐食酪螨1:20,000 w/v 在春季、1:10,000 w/v在夏末。美洲尘螨在春季和欧洲尘螨升高的血清IgE和IDT阳性反应，在两个季节统计学上显著相关。这项研究认为虫体来自所有四个种属；然而两种HDM和粗脚粉螨未被检出。

总结与临床意义 – 本次研究确定了该地区马HDM和SM的IDT稀释浓度。列出了不同的螨类种群，有助于了解马过敏症的发病机理。