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Discrepancies in the bilateral intradermal test and serum tests in atopic horses

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Background – In equine atopic patients intradermal testing (IDT) and immunoglobulin (Ig)E serology are used frequently. There is little evidence regarding the reproducibility of the IDT and IgE serology in horses.

Objectives – To compare the results of a simultaneously performed IDT on the left and right side of the neck in atopic horses, and to compare these results with allergen-specific IgE serology.

Animals – Ten equine patients from a university hospital population with chronic urticaria and/or pruritus.

Methods and materials – The IDT was performed using 16 allergens and the results were evaluated after 30 min, 1, 4 and 24 h. Thirteen allergens also were analysed in duplicate with two monoclonal allergen-specific IgE enzyme-linked immunosorbent assays (ELISA).

Results – Good agreement (Kappa > 0.6) between left and right IDT was found only for *Dermatophagoides farinae, Lepidoglyphus destructor,* birch pollen mixture and perennial rye at 30 min, birch pollen mixture at 1 h, and *Acarus siro* and nettle and common mugwort mixture at 4 h. The bilateral comparison of the other allergens and even the same allergens at other time points showed little or no concordance between left and right IDT. The interlaboratory comparison between both ELISAs, and the comparison between the ELISAs and IDT, showed a good agreement for two of 13 allergens: *D. farinae* and *Dermatophagoides pteronyssinus*.

Conclusions and clinical importance – Based on these preliminary data, IDT and IgE serological test results should be interpreted with great care and further studies are needed to indicate the clinical relevance of these findings.

Introduction

Equine atopic dermatitis (AD) is considered to be an immunoglobulin (Ig)E-mediated hypersensitivity reaction triggered by environmental allergens such as pollens of trees, weeds and grasses, fungal spores, and house dust and storage mites.[1,2] The disease is characterized clinically by seasonal or nonseasonal pruritus, and/or chronic, recurrent urticaria, and, sometimes, respiratory disorders.[2] In atopic horses, the face, pinnae, ventral thorax, abdomen, inguinal region and limbs are reported to be the most affected cutaneous regions.[1,2]

Equine AD is diagnosed by combination of a compatible history, clinical signs and the exclusion of infectious or other noninfectious causes of pruritus or urticaria.[1] Unlike canine AD, the equine condition may not always be chronic and life-long.[3]

The commonly accepted, and preferred, method of identifying allergens in atopic horses is an intradermal test (IDT).[1,4] Healthy (disease-free) horses as well as atopic horses could react positively to the allergens used in an IDT. This might relate to insufficient data in regard to the allergen-specific threshold concentrations.[5–7] However, atopic horses show positive reactions more frequently in IDT than healthy horses.[5] Serological IgE assays are considered less reliable or even completely unreliable for identifying allergens.[1] Studies in horses have shown either a high variability between different serological assays[8] or only slight agreement between the IDT and the IgE serology.[9] Nevertheless, IDT in combination with an allergen-specific IgE serological assay is frequently used by veterinary surgeons to select allergens to define avoidance strategies or to construct allergen-specific immunotherapy (ASIT); in studies ASIT is reported to have a success rate of 64–84%.[3,10,11]

Atopic dermatitis can be hard to manage effectively solely with symptomatic medication and, therefore, it can be the cause of serious welfare compromise and impaired athletic performance.[1,12]

The objectives of the present study were to determine the reproducibility of the IDT by performing a simultaneous bilateral intradermal test, and to determine the concordance between the intradermal test results and two serological IgE enzyme-linked immunosorbent assays

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(ELISAs) performed by two separate commercial laboratories. It was hypothesized that in the same horse similar reactions to IDT were triggered on both sides of the neck at the same time point.

Methods and materials

All procedures were approved by the Ethical Committee of the Utrecht University (10801-2016-1). All owners signed a written consent for approval of the study.

Animals and inclusion criteria

A total of 10 horses (two mares, seven geldings and one stallion) with a history of chronic pruritus and/or chronic, recurrent urticaria were enrolled onto the study. All horses (six Dutch warmblood horses, one Icelandic horse, one Haflinger, one Welsh cob and one cross-breed), with a mean age of 13 years, were referral patients.

Atopic dermatitis was diagnosed by excluding ectoparasite infestations and microbial infections, along with signalment. All horses showed clinical signs especially during the winter season except for one case diagnosed with insect bite hypersensitivity. Any medications that could influence the results of the IDT or IgE serology, including systemic and topical glucocorticoids were withdrawn for ≥14 days before the study.

Allergens for the intradermal test

A total of 16 aluminum-precipitated allergens or allergen mixtures were used for the IDT. These included the grasses, trees, weeds, fungal spores and mites commonly found in the region. Extracts from all allergens were delivered as 3 mL glass vials in an aqueous solution with 0.4% phenol (ArtuVet Animal Health B.V.; Lelystad, the Netherlands). The concentration of the allergens used was 1,000 protein nitrogen units (PNU) for pollen and maize antigen, 100 PNU for mite antigen and 100 μg/mL for fungal spore antigen. All allergens were registered for intracutaneous use in dogs. Allergens were stored in the original vials at 4 C. All allergens and controls were transferred to room temperature 10 min before the test. A positive control solution of 1:1,000 w/v dilution of histamine was used and as a negative control an isotonic phosphate buffered solution with 0.4% phenol was used (ArtuVet Animal Health B.V.).

Intradermal testing

All IDTs were conducted in the winter season. During the 24 h test, nine horses were stabled at the premises of Utrecht University and one horse was tested at home. Ten minutes before the IDT was carried out, jugular blood samples were collected into 5 mL vacutainer serum tubes. Three anxious horses were lightly sedated during the IDT injection phase with intravenous detomidine hydrochloride 0.01 mg/kg (Domosedan 10 mg/mL, Orion Corporation; Espoo, Finland) 5 min before testing. A rectangular test area on both sides of the neck was carefully clipped. A waterproof permanent marker pen was used to indicate 18 injection sites on each side: three horizontal rows of six sites each with a minimum of 3 cm space between the sites (see Figure 1). Intradermal injections were placed in the skin under the pen marks. Individual 1 mL syringes were preloaded with ≥0.2 mL of each of the control solutions and individual allergens. A 0.1 mL volume of each extract was injected i.d. using a 25Gx5/8 needle to form a visible bleb. Intradermal injections were consistently applied in the same order. To minimize injection technique variability, the same investigator performed all intradermal injections in all horses. The investigator reading the IDT was not informed of the specific allergens at each injection site.

All test locations were evaluated at four different time points: 30 min, 1, 4 and 24 h after injection. The reactions were scored subjectively for turgidity using a scale of – to +++ according to Lebis and colleagues:[6] 0, no palpable reaction; +/–, very flat reaction with a badly defined outline; +, reaction with just palpable thickness; ++, reaction with obvious thickness; and +++, reaction with same thickness as histamine reference. The reactions also were scored objectively by measuring the mean diameter of the reaction (mm) with a ruler. A reaction to an allergen was considered positive when the diameter of any wheal was equal to or greater than the mean diameter between the negative control and the positive control being measured at 30 min and 1 h, and the turgidity was ≥+. At 4 and 24 h only a subjective score could be used because the positive histamine control was no longer visible. Therefore, only a turgidity score of ≥++ was considered positive at 4 and 24 h, and the diameter of this reaction should be larger than the mean diameter of the positive and negative control at the 30 min time point.

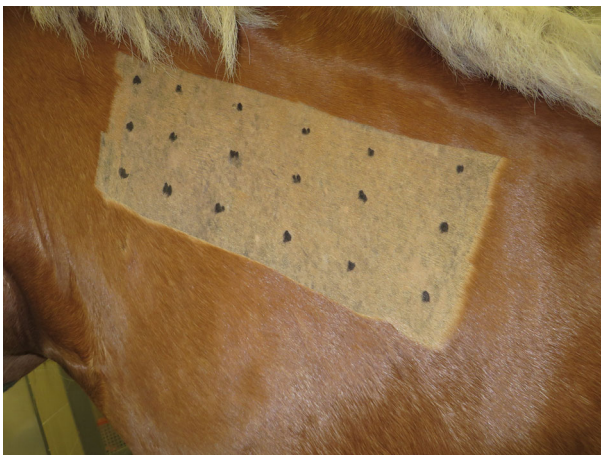

Figure 1. Before the start of the intradermal test, indelible marks were placed on the neck to identify the sites of injection of allergens.

Allergen-specific IgE serology

Serum from each horse was separated by centrifugation for 10 min at 1,000***g*** and four samples per horse were stored at −70°C. Samples were numbered randomly and shipped to two diagnostic laboratories, Laboratory A (LA) and LB. Two duplicate serum samples were sent to each laboratory for ELISA testing to show intraassay variability. The ELISA was performed blinded to the IDT results. Both laboratories used an ELISA technique for their assay: LA used a monoclonal antibody cocktail-based ELISA (macELISA) for detection of allergen-specific IgE (Aller-g-Complete, Greer laboratories; Lenoir, NC, USA)[13] and LB used an ELISA based on a specific single monoclonal anti-IgE antibody (smELISA) which was generated using equine recombinant IgE.[14] Semi-quantitative allergen-specific IgE levels reported by the laboratories were interpreted according to the EAU (ELISA Absorbance Unit) for LA and optical density (OD) for LB. A positive result was defined as >300 EAU in LA and >200 OD in LB.

Statistical analysis

All statistical analyses were performed with Prism v8.2.0 for Windows (GraphPad Software; San Diego, CA, USA, www.graphpad.com). All data were checked for normality with a normal probability plot. The Spearman rank correlation test (instead of the Pearson rank correlation test) was used when a nonlinear relationship was expected.

For intraassay analysis of the bilateral IDT at each time point, Spearman rank correlation and Cohen's kappa test of concordance were used.[15,16] To calculate the Spearman rank correlation, the original wheal diameters were divided by the mean diameter of the negative and positive control of the same side. For the kappa test, the combination of wheal diameter and turgidity was assessed.

For intralaboratory analysis of the duplicate blood samples (LA: EAU and LB: OD), both Pearson rank correlation and the kappa test of concordance were used. Logarithmically transformed data of macELISA and smELISA were used to calculate the Pearson rank correlation. For interlaboratory comparison of the two ELISAs, both Pearson rank correlation test and the kappa test of concordance were used. The average of both duplicate ELISAs was transformed logarithmically before calculating the Pearson rank correlation. Average

duplicate ELISA values >300 EAU in LA and >200 OD in LB were regarded as positive in the kappa test of concordance.

In order to quantify the agreement between the IDT (30 min, 1 and 4 h) and the two ELISAs, Spearman rank correlation and the kappa test of concordance were used. For the Spearman rank correlation, first, the average values of the duplicate IDT were calculated and corrected with the mean diameter of the negative and the positive control. Secondly, the average values of the duplicates of the logarithmically transformed data of macELISA and smELISA were calculated. Consecutively, the average values of duplicate IDT and duplicate ELISAs were compared using the Spearman rank correlation test. For the kappa test of concordance between duplicate IDT and ELISAs, average duplicate ELISA values >300 EAU in LA and >200 OD in LB were regarded as positive. Average duplicate IDT values greater than the average diameter of the negative and the positive control were regarded as positive.

A correlation coefficient $r > 0.8$ and a P-value < 0.001 were considered indicative of a good correlation. Kappa values (κ) of positive and negative results were calculated by using the formula $\kappa = \sum a - \sum ef / N - \sum ef$, where $\sum a$ is the sum of the agreements between both tests, $\sum ef$ is the expected frequency of these agreements occurring by chance and N is the number of horses being compared. Values of κ were interpreted as follows; ≤0.20, poor; 0.21–0.40, fair; 0.41–0.60, moderate; 0.61–0.80, good; and 0.81–1.00, excellent (in accordance with a previous published study).[17]

Results

Positive and negative controls

All horses showed a positive response to histamine on both sides of the neck at 30 min, with a mean difference between right and left of 1 mm. At 1 h the positive control still was clearly visible and the mean decreased by 1 mm. A broad range in the positive control diameter was seen after 4 h even in the same horse as illustrated in Table 1. Almost all positive controls were 0 mm at 24 h, except in one horse where the positive control was 12 mm on the left side only. The mean diameter of the negative control was <4 mm at all time points.

Bilateral IDT results

A total of 320 IDT allergen injections were administered. Considering all four time points, these produced a total of 140 positive reactions. Of all positive reactions, 37% were positive on both sides (52 injection sites) and 63% were positive on one side only (88 injection sites). The overall difference in wheal diameter between left and right sides is displayed in Table 2.

No correlation in wheal diameter between the left- and right-side IDT was found for almost all allergens at all time points except for *Dermatophagoides farinae* at 1 h (Table 3). Good to excellent agreement (κ) between positive and negative results at 30 min was found for four of 16 allergens: *Lepidoglyphus destructor*, *D. farinae*, perennial rye and birch pollen mixture. At 1 h, good agreement (κ) was found for one of 16 allergens (birch pollen mixture); at 4 h, good κ was found for two of 16 allergens (*Acarus siro* and the common mugwort and nettle mixture). At 24 h after the intradermal injections, no bilateral positive reactions were noticed. When considering all time points, the highest numbers of bilateral positive reactions were noticed for *D. farinae* (five of 10 horses) followed by perennial rye (three of 10 horses) and *Dermatophagoides pteronyssinus*, birch pollen mixture and pellitory-of-the-wall (two of 10 horses).

Intra- and interlaboratory analysis of macELISA and smELISA

The intra- and interlaboratory correlation and κ of macELISA (LA) and smELISA (LB) are illustrated in Table 4. To assess intralaboratory test variability, two serum samples from the same horse were analysed in duplicate in LA and LB.

In LA a good intralaboratory correlation in EAU of 11 of 14 allergens was found, except for perennial rye, timothy grass and fungal spores. Good to excellent intralaboratory agreement in κ between positive and negative results in LA was found for nine of 14 allergens. For three allergens (*Tyrophagus putrescentiae*, timothy grass and fungal spores) it was not possible to calculate the κ agreement due to complete negative results of the duplicate blood samples.

In LB a good intralaboratory correlation in OD for six of 14 allergens was found. A difference in intralaboratory correlation in LB was found for *L. destructor*, *Acarus siro*, perennial rye, timothy grass, birch pollen mixture, pellitory-of-the-wall, English plantain and fungal spores. Good to excellent intralaboratory κ agreement in LB was found for five of 14 allergens. For nine of 14 allergens it was not possible to calculate the κ agreement due to complete negative results of the duplicate blood samples.

The interlaboratory analysis between the macELISA and smELISA showed both a good correlation and an excellent κ for two of 13 allergens: *D. farinae* and *D. pteronyssinus* (Table 4). As far as negative and positive results were concerned, intralaboratory differences of LA were 2.9%, intralaboratory differences of LB were 1.4%, and interlaboratory differences between macELISA and smELISA were 8.6%

Interassay analysis of IDT versus macELISA and smELISA

No correlation was found between the macELISA and IDT and smELISA and IDT at all time points for all allergens. Good to excellent κ agreement of the positive and negative outcomes of macELISA versus IDT was found for *D. farinae* (30 min and 1 h IDT), *D. pteronyssinus* (30 min, 1 and 4 h IDT) and perennial rye (4 h IDT). Good to excellent κ agreement of smELISA versus IDT was

Table 1. Range, mean and standard deviation (SD) (mm) of wheal size of the positive and negative controls

Time point	Positive control range	Positive control mean	SD	Negative control range	Negative control mean	SD
30 min	14–18	16	2	0–10	4	4
1 h	12–18	15	2	0–10	3	4
4 h	0–18	10	6	0	0	2
24 h	0–12	1	3	0	0	0

Table 2. Summary of duplicate intradermal test results

	Bilateral different results		Bilateral positive results	
Time point	Number of positive reactions (n)	Mean difference in mm (range)	Number of positive reactions (n)	Mean difference in mm (range)
30 min	23	6.6 (1–18)	10	3.4 (1–7)
1 h	40	6.2 (0–14)	12	2.0 (1–5)
4 h	24	4.3 (0–20)	30	2.8 (0–15)
24 h	1	10 (10)	0	-

Bilateral different results: the right side showed a positive reaction and the left side showed a negative reaction or *vice versa*.
Bilateral positive results: both sides showed a positive reaction.

Table 3. Duplicate intradermal test results at 30 min, 1 and 4 h. Spearman rank correlation for comparison of the wheal diameters and Kappa (κ) for comparison of positive and negative results

Allergens		30 min Intraassay correlation (*r*)	30 min Intraassay κ	1 h Intraassay correlation (*r*)	1 h Intraassay κ	4 h Intraassay correlation (*r*)	4 h Intraassay κ
Mites	*Tyrophagus putrescentiae*	0.22	0	0.18	0	0.61	0
	Lepidoglyphus destructor	0.26	0.6**	0.35	0.2	0.27	−0.1
	Acarus siro	0.03	0	0.61	0	0.85	0.6**
	Dermatophagoides farinae	0.55	1**	0.91*	0.5	−0.13	0.4
	Dermatophagoides pteronyssinus	0.60	0.4	0.35	0.4	0.50	0.4
Grasses	*Lolium perenne* (perennial rye)	0.33	1**	−0.71	0	−0.18	0.5
	Phleum pratense (timothy grass)	0.05	0	−0.38	0	0.22	neg
	Poa pratensis (Kentucky blue grass)	0.24	0	0.21	0	0.46	neg
Trees	Birch, alder and hazel mixture	0.30	0.6**	0.27	0.6**	0.41	0.4
Weeds	*Parietaria officinalis* (pellitory-of-the-wall)	0.21	0	0.19	0	0.12	0.2
	Chenopodium album (lamb's quarters)	0.56	0	0.51	0.3	−0.32	neg
	Plantago lanceolate (English plantain)	−0.22	0	0.031	−0.2	0.43	neg
	A combination of *Artemisia vulgaris* (common mugwort) & *Urtica dioica* (nettle)	0.09	−0.2	−0.18	−0.2	−0.09	0.7**
	Rumex acetosella (sheep sorrel)	0.40	0	0.09	−0.2	−0.20	0
Fungal spores	A combination of *Alternaria alternata, Aspergillus fumigatus* and *Cladosporium herbarum*	0.48	neg	0.18	0	0.46	neg
Grain	*Zea mays* (maize)	0.17	−0.2	0.34	0	−0.20	−0.1

*Good correlation (> 0.8, $P < 0.001$);
**κ ≥ 0.6; neg, all negative.

Table 4. Intra- and interlaboratory comparison of immunoglobulin (Ig)E serological results of Laboratory A [LA: monoclonal antibody cocktail-based enzyme-linked immunosorbent assay(ELISA)] and Laboratory B (LB: single monoclonal ELISA)

Allergens		LA Intraassay correlation (*r*)	LA Intraassay κ	LB Intraassay correlation (*r*)	LB Intraassay κ	LA/LB Interassay correlation (*r*)	LA/LB Interassay κ
Mites	*Tyrophagus putrescentiae*	0.94*	neg	0.85*	1**	–0.79	0
	Lepidoglyphus destructor	0.83*	0	0.05	neg	–0.09	neg
	Acarus siro	0.93*	1**	0.72	0.6**	–0.82	–0.1
	Dermatophagoides farinae	0.98*	0.7**	0.97*	1**	0.89*	1**
	Dermatophagoides pteronyssinus	0.99*	1**	0.94*	1**	0.96*	1**
Grasses	*Lolium perenne* (perennial rye)	–0.15	1**	0.86	neg	0	0
	Phleum pratense (timothy grass)	0.57	neg	0.71	neg	–0.19	neg
	Poa pratensis (Kentucky blue grass)	0.93*	0	n.m.	n.m.	n.m.	n.m.
Trees	Birch, alder and hazel mixture	0.98*	1**	0.66	neg	0	0
Weeds	*Parietaria officinalis* (pellitory-of-the-wall)	0.94*	1**	0.09	neg	–0.5	0
	Chenopodium album (lamb's quarters)	0.98*	1**	0.89*	neg	0.19	0
	Plantago lanceolate (English plantain)	0.98*	0.6**	0.15	neg	0.21	0
	Artemisia vulgaris (common mugwort)	0.94*	1**	0.95*	neg	–0.53	0
	Rumex acetosella (sheep sorrel)	n.m.	n.m.	0.91*	1**	n.m.	n.m.
Fungal spores	A combination of *Alternaria alternata, Aspergillus fumigatus and Cladosporium herbarum*	0.46	neg	–0.01	neg	–0.30	neg

Pearson rank correlation for comparison of numerical values [ELISA Absorbance Units (EAU) and optical density (OD)] and Kappa (κ) for comparison of positive and negative results.
*Good correlation (> 0.8, $P < 0.001$).
**κ ≥ 0.6; n.m., allergen not measured; neg, all negative.

Table 5. Comparison of the intradermal test at 30 min and IgE serology results of Laboratory A [LA: monoclonal antibody cocktail-based enzyme-linked immunosorbent assay(ELISA)] and Laboratory B (LB: single monoclonal ELISA)

Allergens		LA/IDT Interassay correlation (*r*)	LA/IDT Interassay κ	LB/IDT Interassay correlation (*r*)	LB/IDT Interassay κ
Mites	*Tyrophagus putrescentiae*	0.09	neg	0.03	0
	Lepidoglyphus destructor	−0.02	0	−0.18	0
	Acarus siro	0.15	0	−0.25	0
	Dermatophagoides farinae	0.59	0.6**	0.66	0.6**
	Dermatophagoides pteronyssinus	0.63	0.6**	0.47	0.6**
Grasses	*Lolium perenne* (perennial rye)	0.10	−0.1	−0.1	0
	Phleum pratense (timothy grass)	0.33	neg	−0.28	neg
	Poa pratensis (Kentucky blue grass)	0.20	neg	n.m.	n.m.
Trees	*Betula* (birch)	0.35	−0.1	−0.12	0
Weeds	*Parietaria officinalis* (pellitory-of-the-wall)	0.23	−0.1	−0.32	0
	Chenopodium album (lamb's quarters)	−0.15	−0.1	−0.56	0
	Plantago lanceolate (English plantain)	0.21	0	0.33	neg
	Artemisia vulgaris (common mugwort)	−0.04	−0.1	0.2	0
	Rumex acetosella (sheep sorrel)	n.m.	n.m.	−0.32	0
Fungal spore	*Alternaria alternata*	0.21	neg	0.06	neg

Spearman rank correlation for comparison of numerical values and Kappa (κ) for comparison of positive and negative results.
*Good correlation ($r > 0.8$, $P < 0.001$).
**$\kappa \geq 0.6$; n.m., allergen not measured; neg, all negative.

found for *D. farinae* (30 min and 1 h IDT) and *D. pteronyssinus* (30 min, 1 and 4 h IDT). Interassay correlation between IDT at 30 min and macELISA and smELISA are illustrated in Table 5.

Discussion

To the best of the authors' knowledge, this is the first study assessing the agreement between IDT performed on the left side and right side in atopic horses. As our study was exploratory, only a small number of horses were included and, therefore, the results should be interpreted with caution. However, the outcome of the bilateral IDT was surprising considering the extent of the differences between the left and right sides at the same time points. Previous studies in horses have shown that the repeatability of the IDT (20 min after injection) was poor when the IDT was performed with an interval of four to five months in the same horse.[6] Furthermore, multiple studies over the years have shown that even healthy horses can react differently with intradermal tests, which is one of the reasons why the determination of thresholds for allergens is so difficult in horses.[5,18–20] However, from human studies, it is known that the repeatability of a skin prick test for birch pollen, grass pollen, *D. farinae* and *D. pteronyssinus* is high in symptomatic allergic individuals.[21] Based on our results, only the IDT results of *D. farinae*, *L. destructor*, perennial rye and birch pollen mixture can be reliably assessed at 30 min. It is interesting to note that two of these allergens overlap with the allergens showing a good correlation in atopic people.[21] The highest number of positive IDT reactions were noticed for *D. farinae* which is in agreement with previous studies.[3,6] House dust mite antigens can be found in horse rugs and because all these horses were ridden, they would most likely have been in contact with the *D. farinae* antigen.[22]

However, several weaknesses of the performed IDT must be acknowledged. Technical errors could have occurred when performing the IDT because the volume and the depth of the intradermal allergen injection could have varied. It was attempted to minimize this by filling each syringe with a specified volume for each allergen. Technical errors also were minimized by always using the same batch, the same type of syringe and needle, and by ensuring that all injections were administered by the same experienced investigator.

Randomization of the allergens per injection site was not performed. However, the investigator administering and reading the IDT was blinded to the specific allergens per injection site. Therefore, interpretation biases were assumed to be minimal.

Three anxious horses were lightly sedated with detomidine hydrochloride and this sedation might have influenced the IDT outcome. However, previous studies confirmed clear positive IDT reactions to histamine and allergens in horsed sedated with detomidine hydrochloride.[23] In atopic dogs, the use of α_2-adrenoceptor agonists during IDT is, also, regarded as a good sedative for facilitating intradermal testing with minimal to no effect on the outcome of the IDT.[24] Especially because the sedated horses showed clear bilateral positive reactions to histamine and showed positive reactions to allergens in the IDT, it was concluded that the influence of sedation was, at most, minimal.

A lack of knowledge regarding the correct threshold concentrations of the used allergens in horses in this study could explain false positive or false negative responses to the IDT. However, to the best of the authors' knowledge, threshold concentrations are not established in Europe. In Australia, however, one study suggested the use of >1,000 PNU/mL for pollen and fungal spore allergens.[19] Pollen allergen extracts of 1,000 PNU/mL were used in this study and no bilateral positive reactions were identified at 30 min and 1 h for two of the tested grasses and all five weeds, indicating that for these allergens irritant reactions might not play a role in our study. Recommended threshold concentrations for IDT allergen solutions of dust mites in horses are variable.[19,23] In the present study we used the recommended allergen concentration for atopic dogs for the

dust and storage mites owing to a lack of conclusive evidence for the best concentration of mite allergen extract in horses. This concentration might be too high, although if that was the case, bilaterally different results would not have been expected.

The intralaboratory variability of the macELISA and smELISA was evaluated by sending duplicate blood samples to each laboratory. The macELISA showed a good correlation and κ for the majority of the allergens and can, therefore, be regarded as an ELISA with a good intralaboratory reproducibility. The smELISA showed a good correlation for the minority of the allergens. In particular, although nine of 14 allergens showed a negative OD (OD < 200) in combination with a low correlation, it might be possible that the smELISA only shows a rather high variability with OD < 200. Larger studies are indicated to support this hypothesis. However, in atopic dogs, it is already known that another IgE serological test, the FcεRIα-based ELISA, showed prominent variability with low OD values around the cut-off point.[25]

The interlaboratory variability correlation of the macELISA and smELISA was good for only two of 13 allergens: *D. farinae* and *D. pteronyssinus*. In atopic horses, an interlaboratory comparison of these two commercially available tests has never been performed before as far as the authors are aware. Due mainly to time and financial constraints, some equine practitioners prefer to use only the serological IgE assay to identify allergens for immunotherapy. Based on our report, the result of an *in vitro* serological IgE assay is highly dependent on the specific test chosen and therefore immunotherapy recommendations also will show undesirable high variability for storage mites, grass, tree and weed pollen, and fungal spores.

This study described a high interassay variability between the IDT and macELISA IgE serology and IDT and smELISA IgE serology, for storage mites, grass, weed and tree pollen. Previous studies in horses, investigating the agreement between IDT and serological IgE tests, have already shown little or no concordance between the tests beyond chance for the majority of allergens.[8,9] As an example, one study reported as in this report, no agreement for birch pollen, perennial rye, *T. putrescentiae, A. siro*, English plantain, Kentucky blue grass, common mugwort and fungal spores, when comparing IgE serology with the IDT in horses with skin hypersensitivities.[9] Another study compared three different serological IgE tests with the IDT and found that none of their *in vitro* assays of allergen-specific IgE showed good correlation with the IDT, although the FcεRI-based ELISA showed a significantly better correlation than the other two serological IgE serological tests.[8] Retrospectively, it would have been of value to include the FcεRIα-based ELISA in our interlaboratory analysis to assess if this test showed a better interlaboratory correlation between the macELISA and smELISA and between the results of the IDT; another study reported a specificity > 89% for both house dust mites in atopic dogs in a comparison of the IDT and a FcεRIα-based ELISA.[26]

To summarize, the results presented herein demonstrate that in atopic horses the results of a bilateral intradermal test showed rather high variability. In addition, high variability was also demonstrated between the two investigated *in vitro* IgE serological tests and, moreover, between the IgE serological tests and the IDT at all time points. Some veterinarians dealing with equine cases use the IDT, mostly in combination with an *in vitro* IgE serological test, to identify allergens for avoidance or to compose ASIT in horses already clinically diagnosed as atopic. As always, evaluation of results should be performed in association with the history, clinical signs, exposure to allergens and elimination of other causes of pruritus. However, the noted high variability in both the IDT and the macELISA and smELISA could highly influence ASIT recommendations. In conclusion, our results show that there is a need for better reliability of both IDT and IgE serological tests. Randomized, independent, large-scale, peer-reviewed studies are needed to assess the variability of the IDT and *in vitro* equine IgE serological tests to provide evidence-based guidelines for equine intradermal and IgE serological testing in atopic horses.

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Résumé

Contexte – Chez les chevaux atopiques, les tests intradermiques (IDR) et la sérologie immunoglobuline (Ig)E sont souvent utilisés. Il y a peu de preuve concernant la reproductibilité des IDT et de la sérologie IgE chez le cheval.

Objectifs – Comparer les résultats d'IDR réalisés simultanément sur les faces droite et gauche du cou de chevaux atopiques et comparer ces résultats avec une sérologie IgE spécifique d'allergènes.

Sujets – Dix chevaux d'un hôpital universitaire avec urticaire chronique et/ou prurit.

Matériels et méthodes – Les IDR ont été réalisés avec 16 allergènes et les résultats ont été évalués après 30 min, 1, 4 et 24h. Treize allergènes ont aussi été analysés en duplicata avec deux tests ELISA IgE monoclonaux spécifiques d'allergènes.

Résultats – Une bonne concordance (Kappa > 0.6) entre les IDR droit et gauche a été trouvée pour *Dermatophagoides farinae*, *Lepidoglyphus destructor*, le pollen de bouleau et le seigle vivace à 30 min, le mélange de pollen de bouleau à 1h, et *Acarus siro*, l'ortie et l'armoise à 4 h. La comparaison bilatérale des autres allergènes et aussi, des mêmes allergènes à d'autres moments ont montré peu ou pas de concordance entre les IDR droit et gauche. La comparaison inter laboratoire entre les deux tests ELISA et la comparaison entre les ELISA et les IDR, a montré une bonne concordance pour deux des 13 allergènes : *D. farinae* and *Dermatophagoides pteronyssinus*.

Conclusions et importance clinique – A partir de ces données préliminaires, les IDR et les tests sérologiques d'IgE devraient être interprétés avec précaution et des études supplémentaires sont nécessaires pour indiquer la signification clinique de ces données.

Resumen

Introducción – en pacientes atópicos equinos, las pruebas intradérmicas (IDT) y la serología de inmunoglobulina (Ig) E se usan con frecuencia. Hay poca evidencia con respecto a la reproducibilidad de IDT y serología de IgE en caballos.

Objetivos – comparar los resultados de una IDT realizada simultáneamente en el lado izquierdo y derecho del cuello en caballos atópicos, y comparar estos resultados con la serología de IgE específica para alérgenos.

Animales – diez pacientes equinos de una población de un hospital universitario con urticaria crónica y/o prurito.

Métodos y materiales – la IDT se realizó con 16 alérgenos y los resultados se evaluaron después de 30 min, 1, 4 y 24 h. También se analizaron trece alérgenos por duplicado en pruebas con dos anticuerpos monoclonales para IgE específicos de alergeno inmunoabsorbidos y ligados a enzima (ELISA).

Resultados – se observó buena concordancia (Kappa> 0,6) entre IDT izquierda y derecha solo para *Dermatophagoides farinae*, *Lepidoglyphus destructor*, mezcla de polen de abedul y centeno perenne a los 30 min, mezcla de polen de abedul a 1 h, y *Acarus siro* y mezcla de ortiga y ajenjo común a las 4 h. La comparación bilateral de los otros alérgenos e incluso de los mismos alérgenos en otros puntos temporales mostró poca o ninguna concordancia entre la IDT izquierda y derecha. La comparación entre laboratorios entre ambos ELISA, y la comparación entre los ELISA y la IDT, mostró una buena correlación para dos de los 13 alérgenos: *D. farinae* y *Dermatophagoides pteronyssinus*.

Conclusiones e importancia clínica – en base a estos datos preliminares, los resultados de las pruebas serológicas IDT e IgE deben interpretarse con gran cuidado y se necesitan más estudios para indicar la relevancia clínica de estos hallazgos.

Zusammenfassung
Hintergrund – Bei Pferdepatienten mit Atopie wird häufig der Intradermaltest (IDT) und die Immunglobulin (Ig) E Serologie eingesetzt. Es gibt nur wenig Evidenz in Bezug auf die Reproduzierbarkeit des IDT und der IgE Serologie bei Pferden.
Ziele – Ein Vergleich der Ergebnisse simultan durchgeführter IDTs an der linken und an der rechten Seite des Nackens von atopischen Pferden, sowie ein Vergleich dieser Ergebnisse mit der Allergen-spezifischen IgE Serologie.
Tiere – Es wurden 10 Pferde der Population einer Universitätsklinik mit chronischen Urtikaria und/oder Pruritus verwendet.
Methoden und Materialien – Der IDT wurde unter Verwendung von 16 Allergenen durchgeführt und die Ergebnisse nach 30 Minuten, 1, 4 und 24h evaluiert. Dreizehn Allergene wurden im Duplikat mit zwei monoklonalen Allergen-spezifischen IgE mittels Enzyme-linked-immunosorbent Assays (ELISA) analysiert.
Ergebnisse – Es wurde nur eine gute Übereinstimmung (Kappa > 0,6) zwischen linkem und rechtem IDT bei *Dermatophagoides farinae*, *Lepidoglyphus destructor*, Birkenpollenmix und ganzjährigem Roggen nach 30 Minuten, Birkenpollenmix nach 1 h und *Acarus siro* und Nesseln sowie Gemeinem Beifuß Mix nach 4 h gefunden. Der bilaterale Vergleich der anderen Allergene und selbst dieselben Allergene zu anderen Zeitpunkten zeigten geringe oder keine Übereinstimmung zwischen linkem und rechtem IDT. Der Inter-Labor Vergleich zwischen den ELISAs und dem IDT zeigte eine gute Übereinstimmung für zwei der 3 Allergene: *D. farinae* und *Dermatophagoides pteronyssinus*.
Schlussfolgerungen und klinische Bedeutung – Die Ergebnisse des IDT und der IgE Serologie sollten, basierend auf diesen vorläufigen Daten, mit großer Sorgfalt interpretiert werden und es sind weitere Studien nötig, um die klinische Relevanz dieser Ergebnisse zu zeigen.

要約
背景 – アトピー馬では皮内検査（IDT）および免疫グロブリン（Ig）E血清学が頻繁に使用される。馬におけるIDTおよびIgE血清学の再現性に関する証拠はほとんどない。
目的 – 本研究の目的は、アトピー馬の首の左右で同時に行われたIDT結果を比較し、これらの結果をアレルゲン特異的IgE血清学と比較することであった。
被験動物 – 慢性蕁麻疹および/または瘙痒症を有する大学病院の10頭の馬。
材料と方法 – IDTは16のアレルゲンを使用して実行され、結果は30分、1、4、24時間後に評価された。13種類のアレルゲンも、2つのモノクローナルアレルゲン特異的IgE酵素免疫測定法（ELISA）によって2重に解析した。
結果 – 左右IDT間の良好な一致（カッパ> 0.6）は、30分後のDermatophagoides farinae、Lepidoglyphus destructor、シラカバ花粉混合物および多年生ライ麦で、1時間後のシラカバ花粉混合物、そして4時間後のアシブトコナダニ、イラクサおよびヨモギ混合物でのみ認められた。他のアレルゲンと他の時点での同じアレルゲンの両側間比較では、左右のIDT間にほとんどまたはまったく一致が見られなかった。両ELISA間の実験室間比較、そしてELISAおよびIDT間の比較は、13種類のアレルゲンのうち2つ（D. farinaeおよびDermatophagoides pteronyssinus）について良好な一致を示した。
結論と臨床的重要性 – これらの予備データに基づいて、IDTおよびIgE血清学的検査結果は細心の注意を払って解釈されるべきであり、これらの発見の臨床的関連性を示すためにさらなる研究が必要である。

摘要
背景 — 皮内试验(IDT)和免疫球蛋白 (Ig)E血清学试验经常用于异位性患马。关于马的IDT和IgE血清学试验结果重复性的证据很少。
目的 — 比较同时对异位性患马颈部左侧和右侧进行IDT的结果，并将这些结果与过敏原特异性IgE血清学试验进行比较。
动物 — 来自大学医院的10例患有慢性荨麻疹和/或瘙痒的马。
方法和材料 — 使用16种过敏原进行IDT，并在30min、1、4和24h后评价结果。还使用两种单克隆过敏原特异性IgE酶联免疫吸附试验 (ELISA)对13种过敏原进行重复性分析。
结果 — 左右IDT一致性良好(Kappa>0.6)仅见于30min的粉尘螨、鳞翅目昆虫、桦树花粉混合物和多年生黑麦，1h的桦树花粉混合物，4h的粗脚粉螨和荨麻以及普通艾蒿混合物，其他过敏原甚至相同过敏原在其他时间点的双侧比较显示左右IDT之间几乎不一致。两种ELISA的实验室间比较，以及ELISA与IDT之间的比较，显示在13种过敏原中的2种：粉尘螨和屋尘螨有很好的一致性。
结论和临床重要性 — 基于这些初步数据，应谨慎判断IDT和IgE血清学检测结果，需要进一步研究来表明这些结果的临床相关性。

Resumo
Contexto – Em pacientes equinos atópicos, os testes intradérmico (IDT) e sorológico (pesquisa de IgE)

são utilizados com frequência. Há poucas evidências sobre a concordância do IDT e da sorologia em cavalos.
Objetivos – Comparar os resultados de IDT realizado simultaneamente no lado esquerdo e direito do pescoço em cavalos atópicos e comparar esses resultados com a sorologia para IgE alérgenos-específica.
Animais – Dez pacientes equinos oriundos da população de um hospital universitário apresentando urticária crônica e/ou prurido.
Métodos e materiais – O IDT foi realizado utilizando 16 alérgenos e os resultados foram avaliados após 30 min, 1, 4 e 24 h. Treze alérgenos foram também analisados em duplicata por dois ensaios de imunoenzimáticos com anticorpos IgE alérgenos-específicas monoclonais (ELISA).
Resultados – Observou-se boa concordância (Kappa> 0,6) entre IDT esquerda e direita apenas para *Dermatophagoides farinae, Lepidoglyphus destructor,* mix de pólen de bétula e centeio perene aos 30 min, mix de pólen de bétula à 1 h, e *Acarus siro* e urtiga e mix de artemísia às 4 h. A comparação bilateral dos outros alérgenos e até dos mesmos alérgenos em outros tempos experimentais mostrou pouca ou nenhuma concordância entre o IDT esquerdo e direito. A comparação interlaboratorial entre os dois ELISA, e a comparação entre os ELISA e o IDT, mostrou uma boa concordância para dois dos 13 alérgenos: *D. farinae e Dermatophagoides pteronyssinus*.
Conclusões e importância clínica – Com base nesses dados preliminares, os resultados dos testes sorológico e IDT devem ser interpretados com muito cuidado e são necessários mais estudos para indicar a relevância clínica desses achados.