Comparison of the intradermal irritant threshold concentrations of nine allergens from two different manufacturers in clinically nonallergic dogs in the USA

Desirae A. Foust-Wheatcraft*†, Darin L. Dell*, Wayne S. Rosenkrantz† and Craig E. Griffin‡

*Animal Dermatology Clinic, 3901 East 82nd Street, Indianapolis, IN 46240, USA
†Animal Dermatology Clinic, 2965 Edinger Avenue, Tustin, CA 92780, USA
‡Animal Dermatology Clinic, 5610 Kearny Mesa Road, San Diego, CA 92111, USA

Correspondence: Desirae Foust-Wheatcraft, Animal Dermatology Clinic, 3901 East 82nd Street, Indianapolis, IN 46240, USA. E-mail: dfoust-wheatcraft@adcmg.com

Background – The intradermal irritant threshold concentration for many allergens is unknown.

Objective – To determine the intradermal irritant threshold concentration (ITC) of nine allergens from two different manufacturers.

Animals – Twenty privately owned clinically nonallergic dogs.

Methods – Alternaria, cat dander, Dermatophagoides farinae, Chenopodium album (lamb’s quarter), Xanthium strumarium (cocklebur), Prosopis glandulosa (mesquite), Morus alba (white mulberry), Cynodon dactylon (Bermuda grass) and Phleum pretense (Timothy grass) from two manufacturers (ALK; Round Rock, TX, USA and Greer/C226 Laboratories; Lenoir, NC, USA) were injected intradermally at two dilutions and at 15 and 30 min evaluated subjectively (1–4) and objectively (horizontal wheal diameter) by two blinded investigators. A subjective score of 3 or 4 by either investigator at either timed reading was considered positive. If both concentrations resulted in positive reactions, two additional dilutions were performed. The ITC was defined as the lowest tested concentration that elicited a positive reaction in ≥ 10% of animals.

Results – The ITCs were Alternaria > 2,000 PNU/mL; cat dander 750 PNU/mL (ALK) and 2,000 PNU/mL (Greer®); D. farinae < 1:10,000 w/v; C. album < 6,000 PNU/mL; X. strumarium < 6,000 PNU/mL; P. glandulosa < 500 PNU/mL; M. alba < 6,000 PNU/mL; C. dactylon < 10,000 PNU/mL (ALK) and < 6,000 PNU/mL (Greer®); and P. pretense < 6,000 PNU/mL.

Conclusions and clinical significance – There were significant differences in subjective scoring and objective measurement between manufacturers for Alternaria, cat dander and P. pretense. Results revealed significant positive correlation between subjective scoring and objective measurement for each time, investigator and manufacturer separately.

Introduction

Canine atopic dermatitis (CAD) is a genetically predisposed inflammatory and pruritic allergic skin disease with characteristic clinical features associated with IgE most commonly directed against environmental allergens. In vivo allergen testing is performed to identify specific allergens to which a dog is sensitive. The standard testing concentrations for pollens and moulds is 1,000 PNU/mL. If allergens are injected at concentrations that are too high, an irritant reaction may be elicited resulting in a false positive reaction. Conversely, lack of reaction (false negative) may occur if allergens are injected at concentrations that are too low. Therefore, it is important to determine and use the optimal testing concentration of allergens in order to prevent false positive and false negative reactions.

Optimal testing concentrations of allergens can be determined through serial dilutions and finding the highest tested concentration that does not produce an irritant reaction in at least 90% of normal individuals. Previous studies have been performed to determine the irritant threshold concentration (ITC) for a variety of allergens in dogs. Unfortunately, these studies were unable to determine the exact ITC for many of the allergens tested at concentrations as high as 8,000 PNU/mL.

In the United States, there is more than one allergen extract manufacturer and these companies offer the same allergens. The comparative allergenicity and reactivity of these allergens during intradermal testing (IDT) have not been evaluated but could be determined through serial dilution testing in atopic dogs. Before this can be done, it is necessary to determine the ITC.
for similar allergenic extracts from different manufacturers. With the exception of two previous studies evaluating the response to intradermal dust mite allergens from multiple manufacturers in clinically nonallergic dogs,7,8 there is a lack of ITC studies evaluating a variety of allergenic extracts from multiple manufacturers. For this reason, and the fact that the exact ITC for many allergens is unknown, further evaluation of ITCs is warranted.

The primary objective of this study was to determine the ITC of nine allergens from two different manufacturers through IDT in clinically nonallergic dogs in the US. Additional objectives included comparison of the subjective scoring and objective measurements of the allergens based on manufacturer and to define a cut-off point for subjective scoring which consistently defines a positive reaction using blinded histamine and saline reactions.

Materials and methods
Study subjects and enrolment
This was a prospective, duplicate blinded study. Twenty privately owned, clinically nonallergic dogs were enrolled. The signalment and weight for each case is listed in Table S1. The mean age was 4.5 years (1–12 years) and the mean weight was 23.6 kg (13.5–40.5 kg).

In order to be enrolled in the study, dogs had to have been living in their current homes for the past year and have no prior treatment for pruritus or ear infection nor any sign of gastrointestinal distress (vomiting, diarrhea, flatulence) as determined by the owner via a validated questionnaire.9 The level of pruritus for each dog was assessed by the owner via a pruritus Visual Analog Scale (PVAS).10 whose scores must have been two or lower to be included in the study. All owners signed an informed consent form prior to enrolment in the study; under US Federal Law intradermal testing of allergens from multiple manufacturers in clinically nonallergic dogs,7,8 there is a lack of ITC studies evaluating a similar allergenic extracts from different manufacturers for intradermal testing of healthy dogs is permitted because it is deemed to be a procedure that is noninvasive, is performed in a humane fashion and is within the bounds of general veterinary practice.

Allergens
The allergens selected were a representation of different allergen groups and included Alternaria, cat dander, Dermatophagoides farinae, Xanthium strumarium (cocklebur), Chenopodium album (lamb’s quarter), Prosopis glandulosa (mesquite), Morus alba (white mulberry), Cynodon dactylon (Bermuda grass) and Phleum pretense (Timothy grass). These nine matching aqueous allergen solutions obtained from ALK (Round Rock, TX, USA) and Greer® Laboratories Inc. (Lenoir, NC, USA) were supplied as a weight to volume (w/v) concentration. The stock solutions were stored in their original glass vials at 3°C upon receipt. Multiple lots of stock solution were used to perform the study and were replaced every 12 months. A designated, nonblinded veterinary technician (NBVT) was responsible for dilution of the allergens. All dilutions were stored in glass vials at 3°C and discarded after 30 days. The two initial testing concentrations of each allergen are listed in Table 1. After dilution, the NBVT randomly assigned each allergen along with a blinded saline and histamine (0.005 mg/mL) a letter A to K for each manufacturer set. Tuberculin syringes with 27 gauge needles were individually filled with each dilution prior to IDT.

Intradermal testing
Prior to testing, each set of allergens from ALK and Greer® were randomly assigned as Set 1 and Set 2 for each patient by the NBVT. All intradermal injections were performed by the NBVT and evaluated by two investigators (DFW and DDI). In preparation for testing, an intravenous catheter was aseptically placed in the cephalic vein (either right or left) in order to administer dexmedetomidine 375 µg/mL intravenously (Dexdormitor®, Zoets; Kalamazoo, MI, USA) for sedation. After placement in lateral recumbency, a patch of hair was gently clipped on the lateral aspect of the thorax. Skin test sites were marked with a permanent pen. A total volume of 0.06 mL of known saline and histamine was injected intradermally to the left of the blinded A–K samples so the investigators could use these as negative and positive controls. The concentration of 0.005 mg/mL for histamine was utilized as this is the standard concentration used in the authors’ practices. The same volume of the initial concentration for each allergen was injected intradermally in duplicate for each set. Immediately afterward, a higher concentration of the nine allergens and the same concentration of saline and histamine were injected beneath the rows of the initial concentration sites (Figure S1).

The first investigator subjectively evaluated each site on a 1–4 scale and objectively evaluated each test site by measuring the horizontal diameter in millimetres at 15 and 30 min. The second investigator evaluated the test sites in the same manner immediately following the first investigator. A subjective score of 3 or 4 by either investigator at either the 15 or 30 min reading was considered to be positive. If the initial duplicate and higher concentration reactions were all positive by either investigator, then two lower dilutions of the allergen were intradermally injected by the NBVT. In order to keep the investigators blinded for subsequent testing, every blinded histamine or saline that met the positive criteria was also injected two more times at their initial concentration. The dilution concentrations of each allergen are listed in Table 1.

A new known saline and histamine control were injected intradermally for each manufacturer set prior to injection of the dilution series. Each dilution series was evaluated in the same manner by both investigators as previously described. Once the test was finished, each test site was covered with a thin layer of gentamicin sulfate and betamethasone spray (GentaSpray™ topical spray, Henry Schein® Animal Health; Dublin, OH, USA) and the dog received an equal volume of atipamezole (Antisedan®, Zoets; Kalamazoo, MI, USA) intramuscularly for reversal of sedation. If possible, a t-shirt was placed on the dog to cover the test site.

### Table 1. Intradermal allergen concentrations used for initial testing and serial dilutions.

<table>
<thead>
<tr>
<th>Allergen</th>
<th>Initial concentration</th>
<th>Higher concentration</th>
<th>Dilution 1</th>
<th>Dilution 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xanthium strumarium (cocklebur)</td>
<td>10,000</td>
<td>12,000</td>
<td>8,000</td>
<td>6,000</td>
</tr>
<tr>
<td>Phleum pretense (Timothy grass)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morus alba (white mulberry)</td>
<td>1,000</td>
<td>2,000</td>
<td>750</td>
<td>500</td>
</tr>
<tr>
<td>Alternaria</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cynodon dactylon (Bermuda grass)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cat Dander</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chenopodium album (lamb’s quarter)</td>
<td>1:1,000</td>
<td>1:100</td>
<td>1:5,000</td>
<td>1:10,000</td>
</tr>
<tr>
<td>Dermatophagoides farinae</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All concentrations are expressed in PNU/mL except for D. farinae which is expressed in w/v.

© 2017 ESVD and ACVD, Veterinary Dermatology, 28, 564–e136.
**Determination of irritant threshold concentration**

Irritant threshold concentration was defined as the lowest tested concentration to which ≥10% of the test subjects reacted, as assessed by at least one investigator, at any timed reading.

**Statistical analyses**

All analyses were performed using SAS v8.4 (SAS Institute, Cary, NC, USA) with the exception of Lin’s concordance correlation coefficient which was performed using the epirR (v0.9-69, 2015) package in R (R Core Team. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria, 2015). Spearman’s correlation analysis was used to test for correlation between subjective and objective measurements at the initial concentration for each time, investigator and manufacturer separately.

A linear mixed-effects model was used to test for differences in objective values between manufacturers for each allergen separately at the initial concentration. The full linear mixed model included fixed factors for investigator, time and manufacturer, and all two- and three-way interactions, and a random intercept for each wheal.

A generalized linear mixed model (GLMM) was used to examine differences in subjective values between manufacturers for each allergen separately and between investigators for all allergens except histamine and saline controls pooled at the initial concentration. The model to test for manufacturer differences included fixed factors for investigator, time and allergen, and all two- and three-way interactions and a random factor of wheal. The full model to test for investigator differences included fixed factors for investigator, time and allergen, and all two-, three- and four-way interactions, and a random factor of wheal. A multinomial distribution with a cumulative logit link function and an independent correlation structure was used for all GLMM models.

Lin’s concordance correlation coefficients were calculated to quantify agreement between manufacturer subjective values and objective measurements for each investigator and time separately at the initial concentration. Cochran’s Q test was used to compare percentages of blinded saline and histamine samples that were correctly identified as negative or positive, respectively, between certain cut-off points.

**Results**

**Irritant threshold concentration**

The ITC for each allergen is listed in Table 2.

**Correlation between subjective scoring and objective measurements**

The correlation coefficient between subjective scoring and objective measurements at the initial concentration ranged from 0.62 to 0.85 (Table 3). There was significant positive correlation between subjective scoring and objective measurements for each time, investigator and manufacturer separately.

**Table 2.** Irritant threshold concentration (ITC) for intradermal allergens, based on manufacturer.

<table>
<thead>
<tr>
<th>Allergen</th>
<th>ALK</th>
<th>Greer®</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alternaria</td>
<td>&gt;2,000</td>
<td>&gt;2,000</td>
</tr>
<tr>
<td>Cynodon dactylon (Bermuda grass)</td>
<td>&lt;10,000</td>
<td>&lt;6,000</td>
</tr>
<tr>
<td>Cat dander</td>
<td>750</td>
<td>2,000</td>
</tr>
<tr>
<td>Xanthium strumarium (cocklebur)</td>
<td>&lt;6,000</td>
<td>&lt;6,000</td>
</tr>
<tr>
<td>Dermatophagoides farinae</td>
<td>&lt;1:10,000</td>
<td>&lt;1:10,000</td>
</tr>
<tr>
<td>Chenopodium album (lamb’s quarter)</td>
<td>&lt;8,000</td>
<td>&lt;8,000</td>
</tr>
<tr>
<td>Prosopis glandulosa (mesquite)</td>
<td>&lt;500</td>
<td>&lt;500</td>
</tr>
<tr>
<td>Phleum pretense (Timothy grass)</td>
<td>&lt;6,000</td>
<td>&lt;6,000</td>
</tr>
<tr>
<td>Morus alba (white mulberry)</td>
<td>&lt;8,000</td>
<td>&lt;8,000</td>
</tr>
</tbody>
</table>

All ITCs are expressed in PNU/mL except *D. farinae* which is expressed in w/v.

**Table 3.** Spearman correlation coefficients and *P*-values of subjective scoring versus objective measurements for intradermal reactions at the initial concentration for each time, observer and manufacturer.

<table>
<thead>
<tr>
<th>Time</th>
<th>Observer</th>
<th>Manufacturer</th>
<th><em>R</em></th>
<th><em>P</em>-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>1</td>
<td>ALK</td>
<td>0.77</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>15</td>
<td>1</td>
<td>Greer®</td>
<td>0.69</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>15</td>
<td>2</td>
<td>ALK</td>
<td>0.85</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>15</td>
<td>2</td>
<td>Greer®</td>
<td>0.71</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>30</td>
<td>1</td>
<td>ALK</td>
<td>0.62</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>30</td>
<td>1</td>
<td>Greer®</td>
<td>0.71</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>30</td>
<td>2</td>
<td>ALK</td>
<td>0.85</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>30</td>
<td>2</td>
<td>Greer®</td>
<td>0.75</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

**Comparison of subjective scoring and objective measurements between manufacturers for each allergen**

Both subjective scoring and objective measurement values showed significant differences between manufacturers for the following allergens: *Alternaria*, cat dander and *P. pretense* (subjective *P*-values: 0.0083, 0.0007 and 0.0273, respectively, objective *P*-values: 0.0004, 0.0005 and 0.0149, respectively).

**Concordance correlation coefficients for subjective scoring and objective measurements between manufacturers at initial concentration**

Lin’s concordance correlation coefficients for subjective scoring at each timed reading based on investigators are listed in Table 4 with poor agreement. Lin’s concordance correlation coefficients for objective measurement at each timed reading based on investigators are listed in Table 4 with poor agreement.

**Comparison of subjective scoring by observer for each allergen**

There were no significant differences between investigators for subjective scoring for overall allergens pooled (*P* = 0.6650).

**Determination of positive cut-off point for subjective scoring for histamine and saline controls**

The combined number of blinded histamine and saline reactions for both investigators broken down by subjective scoring at the 15 min reading are listed in Table 5. The combined number of blinded histamine and saline reactions for both investigators broken down by subjective scoring at the 30 min reading are listed in Table 5. Using Cochran’s *Q* test, a cut-off of 3 was significantly

**Table 4.** Lin’s concordance correlation coefficients (CCC) for objective and subjective scoring of initial concentration reactions based on investigators.

<table>
<thead>
<tr>
<th>Investigator</th>
<th>15 min objective reading</th>
<th>30 min objective reading</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.57 (0.50–0.64)</td>
<td>0.60 (0.54–0.67)</td>
</tr>
<tr>
<td>2</td>
<td>0.72 (0.67–0.77)</td>
<td>0.73 (0.68–0.77)</td>
</tr>
<tr>
<td>Investigator</td>
<td>15 min subjective reading</td>
<td>30 min subjective reading</td>
</tr>
<tr>
<td>1</td>
<td>0.78 (0.74–0.82)</td>
<td>0.79 (0.75–0.83)</td>
</tr>
<tr>
<td>2</td>
<td>0.72 (0.67–0.77)</td>
<td>0.71 (0.66–0.78)</td>
</tr>
</tbody>
</table>

CCC values <0.90 indicate poor agreement.
Irritant threshold concentrations in dogs

better at correctly identifying blinded histamine and saline as positive and negative reactions, respectively, at both the 15 and 30 min readings ($P < 0.0001$).

### Discussion

The exact ITC was determined for cat dander alone from both manufacturers in this study. The inability to determine the exact ITC for the remaining tested allergens is similar to previous ITC studies. One previous study used lower allergen testing concentrations (1750 PNU/mL for pollens and moulds) whereas a different study used lower allergen testing concentrations (1750 PNU/mL). In both studies, the exact ITC for used much higher allergen testing concentrations (as high as 8,000 PNU/mL). In both studies, the exact ITC for several tested allergens could not be determined as fewer than 10% of the tested dogs reacted positively to the highest tested concentration.

To the best of the authors’ knowledge, this is the first study that has attempted to determine the ITC for a variety of allergens from multiple allergen groups from two manufacturers. Four of the allergens in this study (C. dactylon, P. pretense, X. strumarium, C. album) also were used in a previous study. Using the same ≥10% cut-off for ITC determination, neither study was able to determine the exact ITC for these allergens. In the present study, the ITC for P. pretense, X. strumarium and C. album for both manufacturers and C. dactylon for Greer was <6,000 PNU/mL. This differs from previous results of >8,000 PNU/mL. The difference in histamine concentration used in each study may have contributed to the differences in results as the previous study used a histamine concentration of 0.01 mg/mL whereas the current study’s histamine concentration was 0.005 mg/mL. The histamine concentration utilized in this study was chosen because it is the standard concentration used in the authors’ practices.

Five of the allergens used in this study (C. dactylon, P. pretense, C. album, D. farinae and cat dander) were also used in another previous study. Again, both studies used the same ≥10% cut-off for ITC determination and neither study was able to determine the exact ITC of these allergens. The authors evaluated ITC at two different histamine concentrations (H1: 0.01 mg/mL and H2: 0.1 mg/mL) and found the ITC for C. dactylon, P. pretense and C. album to be the same (≥1,750 PNU/mL) when compared to the two different histamine control concentrations. This also was found for cat dander (≥1,250 PNU/mL). There was a difference in ITC for D. farinae based on the two histamine concentrations (H1: ≤1:12,000 w/v and H2: 1:12,000 w/v).

All three studies were unsuccessful at determining the exact ITC for many of the tested allergens as the current study was only able to determine the exact ITC for cat dander. The ITC of cat dander from Greer (2,000 PNU/mL) and the ITC of Alternaria (>2,000 PNU/mL) from the present study, in combination with the results of previous studies, suggest the current standard testing concentration of 1,000 PNU/mL for most allergens is likely to be too low. Using testing concentrations that are too low may result in false negative reactions in atopic dogs, thus decreasing the accuracy of IDT results. Further investigation is necessary in order to determine the exact ITC for commonly used allergens in IDT.

A previous study evaluating skin test reactivity of dust mites from two manufacturers found significant correlation and agreement when comparing batches of allergens from different manufacturers. Although the present study also had agreement with subjective scoring and objective measurements for D. farinae between manufacturers, significant differences in subjective scoring and objective measurements for Alternaria, cat dander and P. pretense were discovered between manufacturers. These differences may be attributed by variation in the sourcing of raw allergen, the process in which allergen extraction occurs between the two manufacturers, or individual investigator scoring. The sourcing of raw materials can differ geographically with climate and soil variations affecting pollen protein levels between manufacturers. Other external factors that can affect the composition of the allergen and differ between manufacturers include the extraction process (milling and defatting), extraction conditions (time, temperature, pH, extraction fluid composition, degree of wetting and mixing), post-extraction process (filtration) and storage.

Another variable which could also explain these differences may be related to variability of major allergens (MAs) which can change from lot to lot within the same manufacturer. The MAs are active ingredients in allergen extracts responsible for creating an allergic reaction in the majority of patients. Variability of MAs has been reported among multiple lots of different antigens over a 10 year period.

Allergens from two lots were used in this study for several of the allergens from both manufacturers over a 15 month period. The study by Plunkett et al. was over a much longer period of time which may have contributed to the variability of MAs observed in that study. Variability is unavoidable in clinical practice where allergen solutions are constantly utilized and reordered for testing and manufacturing of allergen-specific immunotherapy. Future ITC studies could attempt to avoid this variability by utilizing only one allergen stock solution for the entire study.

Historically, a subjective reading of ≥2 on a 0–4 scale for IDT has been considered potentially significant. In the present study, the authors used a 1–4 scale which is another grading scale commonly used by veterinarians. In the authors’ experience, there is more subjectivity for reactions graded a 2 between investigators with either scale. Therefore, a secondary goal of this study was to

<table>
<thead>
<tr>
<th>Allergen Frequency</th>
<th>Subjective 1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histamine</td>
<td>0</td>
<td>2</td>
<td>59</td>
<td>339</td>
<td>400</td>
</tr>
<tr>
<td>Saline</td>
<td>178</td>
<td>55</td>
<td>7</td>
<td>0</td>
<td>240</td>
</tr>
<tr>
<td>Total</td>
<td>178</td>
<td>57</td>
<td>66</td>
<td>339</td>
<td>640</td>
</tr>
<tr>
<td>30 min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histamine</td>
<td>1</td>
<td>6</td>
<td>42</td>
<td>351</td>
<td>400</td>
</tr>
<tr>
<td>Saline</td>
<td>204</td>
<td>36</td>
<td>0</td>
<td>0</td>
<td>240</td>
</tr>
<tr>
<td>Total</td>
<td>205</td>
<td>42</td>
<td>42</td>
<td>351</td>
<td>640</td>
</tr>
</tbody>
</table>

© 2017 ESVd and ACvd, Veterinary Dermatology, 28, 564–e136.
determine a cut-off point for subjective scoring which consistently defines a positive reaction using blinded histamine and saline reactions. Blinded histamine and saline reactions were not always correctly identified as positive or negative by the investigators during this study. For example, blinded saline was subjectively scored as a 3 in seven of 240 readings at the 15 min reading and blinded histamine was subjectively scored as a 2 in two of 400 readings at the 15 min reading and six of 400 at the 30 min reading. Possible causes for these results include variability in the depth at which histamine or saline was injected in the dermis and the amount injected at each site. Injecting controls and allergens too shallow or injecting larger amounts may result in false-positive reactions. The study was designed to limit this variation by having a single designated individual responsible for performing the IDT. The results of this study showed a statistically significant difference when using a cut-off of 3 or higher in correctly identifying blinded saline and histamine combined. Therefore, these results suggest that a subjective score of 3 or higher would be better at identifying a positive reaction when interpreting allergen reactions on IDT.

A weakness of this study was the small sample size compared to previous studies with the same goal of determining ITC. Although both the current study and previous studies excluded dogs with a history of pruritus or ear infection, the current study went one step further by also excluding dogs with gastrointestinal signs, eliminating dogs with possible concurrent adverse food reaction. A larger sample size would be ideal for future studies. However, despite having a smaller sample size, this study supports previous work suggesting that the recommended 1,000 PNU/mL testing concentration for most pollens is inappropriate. 5, 6

Determining criteria to correctly identify and remove all allergic dogs from a study population can be difficult. In this study, the authors included historical questions and physical examination in an attempt to define the dogs as likely healthy and nonallergic. There still was one subject (Dog 8) which reacted positively to seven of nine allergens. Given this dog’s young age, it is possible that it may develop clinical signs of atopic dermatitis in the future. If this dog’s results were to be removed from ITC determination, only one of seven ITC (P. pretense) would be affected. The ITC for P. pretense from ALK would have been adjusted from 308 to 306. Significant positive correlation between subjective scoring and objective measurement for each time, investigator and manufacturer separately was observed. Future studies are warranted to further compare reactivity to allergenic extract supplied by different manufacturers and to determine ideal testing concentrations for IDT.

Acknowledgements

The authors wish to thank ALK (Round Rock, TX, USA) for material support, Deborah Keys for her statistical support and Lisa Petty, Julie Valdez and Carly Schroeder for their technical assistance.

References

11. Greer®. Allergy Immunotherapy Compendium. Greer® Veterinary Allergy, Lenoir NC, 2014, pp. 48–49. Available from Greer, 639 Nuway Circle NE, PO Box 800, Lenoir, NC 28645, USA.

Supporting Information

Additional Supporting Information may be found in the online version of this article.

Figure S1. Photographic image portraying the intradermal test grid.

Table S1. Patient Information.
Contexte – La concentration intradérmique seuil pour de nombreux allergènes n’est pas connue.

Objectif – Déterminer la concentration seuil intradérmique irritante (ITC) de neufs allergènes de deux fabricants différents.

Sujets – Vingt chiens cliniquement non allergiques de propriétaires.

Méthodes – Alternaria, carottes de chat, Dermatophagoides farinae, Chenopodium album, Xanthium strumarium, Prosopis glandulosa, Morus alba, Cynodon dactylon et Phleum pretense de deux fabricants (ALK; Round Rock, TX, USA et Greer® Laboratoires; Lenoir, NC, USA) ont été injectés en intradermique à deux dilutions et évalués à 15 et 30 min subjectivement (1-4) et objectivement (diamètre horizontal de la plaque) par deux investigateurs en aveugle. Un score subjectif de 3 ou 4 par un investigateur à un moment donné était considéré comme positif; si deux concentrations résultats en des réactions positives, deux nouvelles dilutions étaient réalisées. L’ITC était définie comme la plus faible concentration entrainant une réaction positive pour ≥10% des animaux.

Résultats – Les ITC étaient Alternaria >2,000 PNU/mL; carotte de chat 750 PNU/mL (ALK) et 2,000 PNU/mL (Greer®); D. farinae <1:10,000 p/v; C. album <6,000 PNU/mL; X. strumarium <6,000 PNU/mL; P. glandulosa <500 PNU/mL; M. alba <6,000 PNU/mL; C. dactylon <10,000 PNU/mL (ALK) et <6,000 PNU/mL (Greer®); et P. pretense <6,000 PNU/mL.

Conclusions et signification clinique – Il y avait des différences significatives dans le scoring subjectif et objectif entre les fabricants pour Alternaria, les carottes de chat et P. pretense. Les résultats ont montré une corrélation positive entre le scoring subjectif et les mesures objectives pour chaque instant, investigateur et fabriquant séparément.

Resumen

Introducción – Se desconoce la concentración umbral irritante intradérmica para muchos alérgenos.

Objetivo – Determinar la concentración umbral irritante intradérmica (ITC) de nueve alérgenos de dos fabricantes diferentes.

Animales – Veinte perros clínicamente no alérgicos de propietarios privados.

Métodos – Se inyectaron por vía intradérmica a dos diluciones Alternaria, carótida de gato, Dermatophagoides farinae, Chenopodium album (aja), Xanthium strumarium, Prosopis glandulosa (mezquite dulce), Morus alba (morera), Cynodon dactylon (grama) y Phleum pretense de dos fabricantes (ALK; Round Rock, TX, EE.UU. y Greer® Laboratories, Lenoir, NC, EE.UU.) a evaluar subjetivamente a los 15 y 30 minutos (1-4) y objetivamente (diámetro horizontal de la papula) por dos investigadores que desconocían los alérgenos. Un valor subjetivo de 3 o 4 de cualquiera de los investigadores en cada tiempo de lectura se consideró positivo. Si ambas concentraciones dieron lugar a reacciones positivas, se realizaron dos diluciones adicionales. El ITC se definió como la concentración más baja probada que provocó una reacción positiva en ≥10% de los animales.

Resultados – Las ITC fueron Alternaria >2,000 PNU/mL; carótida de gato 750 PNU/mL (ALK) y 2,000 PNU/mL (Greer®); D. farinae <1:10,000 p/v; C. album <6,000 PNU/mL; X. strumarium <6,000 PNU/mL; P. glandulosa <500 PNU/mL; M. alba <6,000 PNU/mL; C. dactylon <10,000 PNU/mL (ALK) y <6,000 PNU/mL (Greer®); y P. pretense <6,000 PNU/mL.

Conclusiones y significado clínico – Hubo diferencias significativas en el valor subjetivo y la medición objetiva entre los fabricantes de Alternaria, carótida de gato y P. pretense. Los resultados revelaron una correlación positiva significativa entre el valor subjetivo y la medición objetiva para cada tiempo, investigador y fabricante por separado.

Zusammenfassung

Hintergrund – Die intradermale Reiz-auslösende Schwellenkonzentration ist für viele Allergene unbekannt.


Tiere – Zwarzig klinisch nicht allergische Hunde in Privatbesitz.

Methoden – Alternaria, Katzenschuppen, Dermatophagoides farinae, Chenopodium album (Weißer Gänsefuß), Xanthium strumarium (Gewöhnliche Spitzklette), Prosopis glanulosa (Mesquite), Morus alba (Weiße Maulbeere), Cynodon dactylon (Bermudagras) und Phleum pretense (Thymothyras) von zwei Herstellern (ALK; Round Rock, TX, USA und Greer® Laboratories; Lenoir, NC, USA) wurde intradermal in zwei Verdünnungen injiziert und nach 15 und 30 Minuten subjektiv (1-4) und objektiv (horizontaler Quaddeldurchmesser) von zwei geblindeten UntersucherInnen evaluiert. Ein Subjektivwert von 3 oder 4 bei beiden Untersucher/Innen zu beiden Zeitpunkten wurde als positiv betrachtet. Wenn beide Konzentrationen eine positive Reaktion auslösten wurden zwei weitere Verdünnungen durchgeführt. Die ITC wurde als die niedrigste getestete Konzentration, welche eine positive Reaktion bei ≥10% der Tiere auslöste, definiert.
Ergebnisse – Die ITCs waren: Alternaria > 2,000 PNU/mL; Katzenschuppen 750 PNU/mL und 2,000 PNU/mL (Greer®); D. farinace < 1:10.000 w/v; C. album < 6,000 PNU/mL; X. strumarium < 6,000 PNU/mL; P. glandulosa < 500 PNU/mL; M. alba < 6,000 PNU/mL; C. dactylon < 10,000 PNU/mL (ALK) und < 6,000 PNU/mL (Greer®); und P. pretense < 6,000 PNU/mL.


要約
背景 – 许多过敏原的皮内刺激阈值浓度是未知的。
目的 – 鉴定未被两个不同厂家的9种过敏原的皮内刺激阈值浓度(IcT)。
動物 – 20只临床非过敏性家犬。
方法 – 来自两个厂家(ALK: Round Rock, TX, USA and Greer® Laboratories; Lenoir, NC, USA)的9种过敏原分别来自链格孢菌、猫皮屑、粉螨、茎乳果(Bermuda)、苔藓(Alexander), 酸模属(酸模叶)、甘草(Baker's dactylium), 百合大草(狗牙根)和狗牙草(猫皮屑), 按两种稀释度进行皮内注射, 并由两名双盲调查者在15-30分钟内观测主要的(1-4)和客观的(水平风偏)反应。任何一名调查者在任何时刻的阅读中, 所得主观评分为3或4分, 则被认为是阳性。如果两种浓度均导致阳性反应, 则计算出两者浓度的平均值。IcT被定义为引起≥10%的动物产生阳性反应的最低测试浓度。结果9种过敏原的IcT分别为, 链格孢菌>2,000 PNU/mL, 猫皮屑<750 PNU/mL (ALK)和<2,000 PNU/mL (Greer®); 粉螨<1:10,000 w/v; 茎乳果<6,000 PNU/mL; 苔藓<6,000 PNU/mL; 酸模属<500 PNU/mL; 甘草<6,000 PNU/mL; 狗牙根<10,000 PNU/mL (ALK)和<6,000 PNU/mL (Greer®); 猫皮屑<6,000 PNU/mL。

c. Conclusions and Clinical Significance – 两个厂商的链格孢菌、猫皮屑和酸模属之间, 在主观评分和客观测量值上存在明显差异。结果显示, 对同一时间点、调查者和制造商, 主观评分与客观测量结果之间呈显著的正相关。

要约
背景 – 多数的敏感度的皮内刺激阈值浓度还不清楚。
目的 – 通过对2种不同的测试者的9种过敏原的皮内刺激阈值浓度 (IcT) 测定, 确定其阈值。
供与动物 – 感受性实验中, 未受过敏原刺激。
方法 – 2种不同的动物(ALK: 美国德克萨斯州拉伯克, Greer® Laboratories; 美国北卡罗来纳州里诺)的9种过敏原: 链格孢菌(Alternaria)、猫的皮屑、粉螨、茎乳果(Dermatophagoides farinae)、芝果(Cenopodium album)、酸模属(Xanthium strumarium)、酸模(Prospis glandulosa)、甘草(Morus alba)、豚草(Cynodon dactylon)以及其中的过敏性刺激(Phleum pretense)。方法为15-30分钟内进行皮内注射, 由两名盲法观察者在15-30分钟内进行主观的(1-4)和客观的(皮疹的严重程度)评价。若观察者中的任意一人认为该动物对过敏原反应, 则被定义为阳性反应。根据观察者分类, 每种过敏原的皮内刺激阈值浓度为10%的动物的皮内刺激阈值浓度。结果: IcT为>2,000 PNU/mL, 猫的皮屑<750 PNU/mL (ALK)和<2,000 PNU/mL (Greer®); D. farinae<1:10,000 w/v; C. album<6,000 PNU/mL; X. strumarium<6,000 PNU/mL; P. glandulosa<500 PNU/mL; M. alba<6,000 PNU/mL; C. dactylon<10,000 PNU/mL (ALK)和<6,000 PNU/mL (Greer®); P. pretense<6,000 PNU/mL。

結論和臨床意義 – 两个厂商的链格孢菌、猫皮屑和酸模属之间, 在主观评分和客观测量值上存在明显差异。结果显示, 对同一时间点、调查者和制造商, 主观评分与客观测量结果之间呈显著的正相关。

Resumo
Contexto – A concentração do limiar irritante intradérmico para diversos alérgenos é desconhecida.
Objetivo – Determinar a concentração do limiar irritante intradérmico (IcT) de nove alérgenos de dois fabricantes diferentes.
Animais – Vinte cães não alérgicos de proprietários.
Métodos – Alternaria, epitélio de gato, Dermatophagoides farinae, Chenopodium album, Xanthium strumarium, Prosopis glandulosa (mesquita), Morus alba, Cynodon dactylon (grama Bermida) e Phleum pretense (grama Timothy) de dois fabricantes (ALK: Round Rock, TX, USA e Greer® Laboratories; Lenoir, NC, USA) foram injetados por via intradérmica em duas diluições e avaliados subjetivamente (1-4) e objetivamente (diâmetro horizontal da pápula) nos tempos 15 e 30 minutos, por dois investigadores cegos. Um escor subjetivo de 3 ou 4 pelos dois investigadores em qualquer tempo, foi considerado positivo. Se as duas concentrações resultaram em reações positivas, duas diluições adicionais foram realizadas. O IcT foi definido como a dose mais baixa testada que provocou reações positivas em ≥10% dos animais.
Resultados – Os ITCs foram Alternaria >2,000 PNU/mL; epitélio de gato 750 PNU/mL (ALK) e 2,000 PNU/mL (Greer®); D. farinae<1:10,000 w/v; C. album<6,000 PNU/mL; X. strumarium<6,000 PNU/mL; P. glandulosa<500 PNU/mL; M. alba<6,000 PNU/mL; C. dactylon<10,000 PNU/mL (ALK) e <6,000 PNU/mL (Greer®); e P. pretense<6,000 PNU/mL.
Conclusões e importância clínica – Não houve alterações significativas no escore subjetivo e objetivo entre os fabricantes para *Alternaria*, epitélio de gato e *P. pretense*. Os resultados revelaram uma correlação positiva entre as mensurações subjetivas e objetivas para cada tempo, cada investigador e cada fabricante separadamente.