Comparison of intradermal and percutaneous testing to histamine, saline and nine allergens in healthy adult cats

Christina M Gentry and Linda Messinger

Department of Dermatology and Allergy, Veterinary Referral Center of Colorado, 3550 South Jason Street, Englewood, CO 80110, USA
Correspondence: Christina M Gentry, Veterinary Referral Center of Colorado, Department of Dermatology and Allergy, 3550 South Jason Street, Englewood, CO 80110, USA. E-mail: cgentry@vrcc.com

Background – Intradermal testing (IDT) in cats has potential limitations; this has led to an interest in novel testing methods. A pilot study demonstrated that healthy cats produced reliable percutaneous glycerinated (PG) histamine wheals, whereas percutaneously applied glycerosaline did not lead to wheal formation.

Hypothesis/Objective – The purpose of this study was to determine if percutaneously applied aqueous and glycerinated allergens would lead to irritant reactions in healthy cats.

Methods – Percutaneous testing (PCT) with both glycerinated and aqueous allergens and IDT were compared in twelve healthy cats. The lateral thorax was clipped and histamine, saline and nine allergens were tested in rows. Objective and subjective evaluations were performed at 15, 20 and 25 min, and 4 h. Results were evaluated as positive or negative at 15, 20, 25 min and 4 h.

Results – Skin test reactions for intradermal (ID) histamine wheals were larger when compared to PG and percutaneous aqueous (PA) at the immediate reading points (P < 0.05) subjectively and objectively; however, PG was not significantly different from ID when compared as either positive (2–4) or negative (0–1). PG histamine and allergen reactions, when present, were larger than equivalent PA reactions. PG and PA allergens did not cause irritant reactions at tested concentrations. Bassia scoparia (kochia), when tested at 1000 PNU/mL with IDT, was suspected to be an irritant.

Conclusions and Clinical Importance – Percutaneously (PCT) applied allergens did not cause irritant reactions in healthy cats. PG histamine wheals, although smaller than ID histamine wheals, were easily recognizable and PCT was simple to perform.

Introduction

Feline hypersensitivity disorder (HD), which includes eosinophilic granuloma complex, miliary dermatitis, self-induced alopecia, head and neck pruritus, urticaria, angioedema and allergic asthma, may be caused by flea allergy dermatitis, cutaneous adverse food reactions and hypersensitivity to environmental allergens.1–3 Environmental allergen-induced HD is diagnosed by clinical signs, history and exclusion of other potentially pruritic skin conditions, such as ectoparasitism and cutaneous adverse food reactions.3,4 Following a presumptive diagnosis of HD, identification of significant environmental allergens can be used to create allergen-specific immunotherapy, as well as to practice avoidance.4,5 Allergens can be identified using allergen-specific IgE serology, epicutaneous patch testing and intradermal testing.6,7 Intradermal testing (IDT) with aqueous allergens is routinely used to identify significant allergens for allergen-specific immunotherapy in dogs, cats and horses. There is general consensus that this method provides reliable results in the dog and horse with atopic dermatitis.6,8 Intradermal testing in the cat can provide variable and potentially false negative results.9,10 This has led to an increased interest in novel methods of reliably identifying significant environmental allergens in the cat.

A pilot study demonstrated that percutaneous testing (PCT) could be performed in sedated healthy cats. That study evaluated histamine, saline and glycerosaline reactions using two different skin picks at 15, 20 and 25 min.11 The GREER® Pick System (GREER®, Lenoir, NC, USA) produced reliable and well-demarcated wheals with 6 mg/mL glycerinated histamine as a positive control without creating wheals with the negative glycerosaline control. In contrast, the Duotip-test II pick® (Lincoln Diagnostics Inc.; Decatur, IL, USA) glycerinated histamine reactions were small and not significantly different from glycerosaline. Compared to the histamine wheals created by IDT, the histamine wheals made using the GREER® Pick® System were better demarcated, indicating that PCT may be used as an alternative to IDT in the cat.11

Our study compared PCT and IDT in healthy cats using nine allergens as well as histamine and saline controls. The primary goal was to determine if percutaneously applied aqueous and glycerinated allergens would create...
irritant reactions in healthy domestic cats using the GREER® Pick® System. We hypothesized that irritant reactions would not occur. Four secondary goals were to (i) confirm the reliability of control wheal formation from the pilot study, (ii) recommend a preferred PCT immediate reading time, (iii) determine if currently used IDT concentrations would cause irritant reactions in healthy cats and (iv) determine if subjective and objective data correlated.

Materials and methods

Cats

Twelve client-owned, adult cats without known or suspected hypersensitivities were enrolled. Cat owners provided informed written consent for their cats’ enrolment after reviewing the experimental design. Physical examination was performed to rule out any dermatological or other medical conditions. When available, prior medical records were reviewed to rule out pre-existing conditions that could interfere with test results or preclude sedation. To be included, cats must have been 1 year of age or greater, had no prior or current evidence of dermatological or other medical conditions and could not be currently receiving oral or topical medications other than routine heartworm and ectoparasite control. Heartworm and ectoparasite control were not mandated to be included in the study due to the low prevalence of heartworm disease and ectoparasites in cats in the authors’ region. There was an 8 week withdrawal for oral, topical and injectable glucocorticoids; an 8 week withdrawal for oral ciclosporin; and a 4 week withdrawal for topical or injectable glucocorticoids. Exclusion criteria included cats with previously diagnosed with HD, contact hypersensitivity and failure to meet any of the inclusion criteria listed.

Sedation

After physical examination, the cats were sedated with intramuscular dexmedetomidine (Dexdomitor®, Zoets; Exton, PA, USA) at a rate of 0.5 mg/kg. After testing was completed, the sedation was reversed with an epaxial muscle injection of atipamezole (Antisedan®, Zoets) at a dose equal to the dexmedetomidine used before. Cats were fasted for a minimum of 8 h prior to sedation.

Percutaneous and intradermal testing

A rectangle was clipped on the left lateral thorax and a permanent marker was used to numerically mark each of nine rows and dots indicated individual allergens and controls. Testing was performed in triplicate in each cat. The row numbers of percutaneous glycerinated (PG), percutaneous aqueous (PA) and ID 4 test sites were block randomized and the primary investigator (CMG) was blinded to the application sites. Percutaneous testing was performed with the GREER® Pick System®. Percutaneous application of both glycerinated and aqueous allergens were performed by holding the picks at an approximately 45° angle to the skin surface against the direction of hair growth. The picks, each containing 0.0035 ml of allergen or control, were pressed to the skin surface and twisted approximately 90° before removal.2,7 A new pick was used for each application of allergen or control.

Intradermal injections were performed with a volume of 0.05 mL of the allergens or controls through a 27 gauge needle. The positive control for PA and ID was sterile buffered 0.9% saline and the negative control for PG was 50% glycerol saline (GREER®). The nine tested allergens were: Dermatophagoides farinae, canine dander, Kentucky blue grass (Poa pratensis), Red cedar (Juniperus virginiana), eastern cottonwood (Populus deltoides), common cocklebur (Xanthium strumarium), kochia (Bassia scoparia), Pennicilium sp. and mosquito. The same manufacturer lot number of each allergen was used throughout the study.

The selected allergens were commonly positive on canine and feline IDT in the authors’ region and represented five distinct classes of allergens (house dust mite, dander, pollen, mould and insect). The tested concentrations of aqueous and glycerinated allergens are detailed in Table 1. Glycerinated D. farinae, P. pratensis and mosquito were either not commercially available or only available for clinicians treating human patients. For these allergens, the concentrated aqueous allergens were diluted 1:1 with 50% glycerin as suggested by the manufacturer (Tracy Burchette, GREER®, personal communication 2014).

Each PG, PA and ID site was evaluated at 15, 20 and 25 min, and approximately 4 h. Reaction grading of wheal size and potential flare (erythematous halo outside the boundary of the wheal), was evaluated subjectively and objectively at 15, 20 and 25 min as described previously.8,13 Subjective scoring was based on a 0–4 scale, where a 4 reaction was as strong as typical ID histamine and a 0 reaction was similar to most negative controls. Objective measurements were taken to the nearest millimetre by averaging the vertical and horizontal diameter of the wheal and potential flare. At approximately 4 h, reactions were graded subjectively as positive or negative. One cat was excluded from the 4 h evaluation time point due to fractious behaviour that did not allow further evaluation.

Statistical analysis

A repeated measures analysis (PROC Mixed) was used to detect differences between PA, ID and PG data points at 15, 20 and 25 min for subjective and objective data for histamine, saline and allergens. Individual time points were evaluated to determine the preferred reading time for PCT. These three immediate time points were combined into overall immediate time point data and evaluated in a similar way. A repeated measures analysis (PROC Mixed) was also used to detect reaction differences based on skin colour (grey, white or grey/white). An unstructured covariance structure was assumed. Multiple comparisons were made by Tukey’s test. P-values less than 0.05 were considered significant. A chi-square test was used to compare positive and negative reactions at the 4 h time point; chi-square and Fisher’s exact test analyses were performed to compare subjective reactions as either negative (0–1) or positive (2–4) at time points 15, 20 and 25 min. Statistical analyses were performed using SAS v9.2 (Cary, NC, USA).

Results

Cats

All twelve cats met the inclusion and exclusion criteria. They included four spayed females and eight neutered males. The age of the cats ranged from one to 10 years (mean 4.6 years) and weights ranged from 3.2 to 7.6 kg (mean 5.3 kg). Eight of the cats were domestic short hair and one each of the following breeds: domestic medium hair, domestic long hair, Scottish fold and Himalayan. Four cats had white skin, six cats had grey skin and two cats had mixed grey and white skin at the testing site.

Table 1. Testing concentrations of aqueous and glycerinated allergens used in the study

<table>
<thead>
<tr>
<th>Allergen</th>
<th>Dermatophagoides farinae</th>
<th>Canine dander</th>
<th>Poa pratensis</th>
<th>Juniperus virginiana</th>
<th>Populus deltoides</th>
<th>Xanthium strumarium</th>
<th>Bassia scoparia</th>
<th>Pennicilium sp.</th>
<th>Mosquito</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous</td>
<td>1/5,000 w/v</td>
<td>1000 PNU/mL</td>
<td>1000 PNU/mL</td>
<td>1000 PNU/mL</td>
<td>1000 PNU/mL</td>
<td>1000 PNU/mL</td>
<td>1000 PNU/mL</td>
<td>1000 PNU/mL</td>
<td>1000 PNU/mL</td>
</tr>
<tr>
<td>Glycerinated</td>
<td>1/200 w/v</td>
<td>1/20 w/v</td>
<td>20,000 PNU/mL</td>
<td>1/20 w/v</td>
<td>1/20 w/v</td>
<td>1/20 w/v</td>
<td>1/20 w/v</td>
<td>1/20 w/v</td>
<td>10,000 PNU/mL</td>
</tr>
</tbody>
</table>

© 2016 ESVD and ACVD, Veterinary Dermatology, 27, 370–e92. 371
ID versus PG immediate allergen reactions at individual and combined evaluation points

Objective data
Canine dander and *D. farinae* had significantly larger ID wheals overall when compared to PG wheals (*P* < 0.05). *Poa pratensis* and *Penicillium* sp. had statistically significantly larger ID wheals overall (*P* < 0.05) and *P. pratensis* ID wheals were also larger at the 15 min time point when compared to PG wheals (*P* < 0.05). *Bassia scoparia* and mosquito had larger ID wheals overall and at each of the immediate evaluation points (*P* < 0.05; Table 2). There were no significant differences between PG and ID reactions for *J. virginiana*, *P. deltoides* and *X. strumarium* overall, or at individual time points (Figures S3, S4). No flare reactions were noted for any ID or PG allergens.

Subjective data
*D. farinae* had larger ID wheals overall when compared to PG wheals; however, these differences were not statistically significant at the 20 min time point (*P* < 0.05; Table 2). *Poa pratensis* ID wheals were larger when compared to PG wheals overall and at the 15 min evaluation point (*P* < 0.05). *Penicillium* sp. ID wheals were larger than PG wheals overall, but not at individual evaluation points (*P* < 0.05; Table 2). *Bassia scoparia* and mosquito ID had larger wheals overall and at each of the immediate evaluation points (*P* < 0.05; Table 2). There were no significant differences between PG and ID reactions for *J. virginiana*, *P. deltoides* and *X. strumarium* overall, or at individual time points (Figures S3, S4).

ID versus PG comparison as positive or negative
The data for ID and PG were evaluated at 15, 20 and 25 min as either negative (a subjective score of 0–1) or positive (a subjective score of 2–4) as this most closely mimics current clinical practice for determining significance of reactions. The data were evaluated at 4 h in a similar way. There were significantly more positive reactions for ID when compared to PG for *B. scoparia* and mosquito at time points 15, 20 and 25 min (*P* < 0.05). At 4 h, PG histamine had significantly more positive reactions than ID histamine (*P* < 0.05) and were no differences between ID and PG for the nine allergens.

PA immediate allergen reactions at individual and combined evaluation points
Most PA allergen immediate reactions were graded subjectively as 0 (data not shown). Subjectively, PG reactions of the combined immediate reading points were always greater than PA; however, this difference was statistically significant only for canine dander (*P* < 0.05) and *B. scoparia* (*P* < 0.05) overall, there was also a statistically significant difference for *B. scoparia* at the 15 min evaluation point (*P* < 0.05). Objectively, PG reactions were greater than PA for *B. scoparia* overall (*P* < 0.05) and at the 15 min evaluation point (*P* < 0.05; Figures S5, S6). Similar to PG, ID reactions were always greater than PA allergen reactions. No flare reactions were observed for any PA allergens.

Evaluation of controls

Histamine
Wheals from ID histamine were significantly larger than the wheals from PG and PA overall (combined immediate time points) and at each individual immediate time point when evaluated both subjectively and objectively (*P* < 0.05; Table 3, Figure S7). PG histamine wheals were significantly larger than wheals created by PA histamine overall and at each individual time point when evaluated both subjectively and objectively (*P* < 0.05; Table 3). PG histamine wheals were significantly larger at 25 min when compared subjectively to PG wheals at 15 min (*P* < 0.05). PG histamine wheals at the 20 and 25 min evaluation points were not significantly different.

Saline and glycerosaline
Wheals from ID saline were significantly larger both subjectively and objectively when compared to PG glycerosaline wheals overall and at the 15 min evaluation point (*P* < 0.05) and PA saline wheals overall and at the 15 min evaluation point (*P* < 0.05). There was no statistical difference between ID, PG and PA saline wheals when compared subjectively to PG wheals at 15 min (*P* < 0.05). PG histamine wheals at 20 and 25 min evaluation points were not significantly different.

### Table 2. Comparison of intradermal (ID) and percutaneous glycerinated (PG) allergens at the 20 min evaluation point

<table>
<thead>
<tr>
<th>Allergen</th>
<th>Subjective score range 0–4 (mean)</th>
<th>Wheal size range (mean) (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Dermatophagoides farinae</em> (ID)</td>
<td>0–4 (0.39)</td>
<td>0.0–0.9 (0.08)</td>
</tr>
<tr>
<td><em>D. farinae</em> (PG)</td>
<td>0–2 (0.11)</td>
<td>0.0–0.5 (0.03)</td>
</tr>
<tr>
<td>Canine dander (ID)</td>
<td>0–2 (0.17)</td>
<td>0.0–0.75 (0.07)</td>
</tr>
<tr>
<td>Canine dander (PG)</td>
<td>0–2 (0.14)</td>
<td>0.0–0.45 (0.03)</td>
</tr>
<tr>
<td><em>Poa pratensis</em> (ID)</td>
<td>0–2 (0.17)</td>
<td>0.0–0.5 (0.04)</td>
</tr>
<tr>
<td><em>P. pratensis</em> (PG)</td>
<td>0–1 (0.03)</td>
<td>0.0–0.2 (0.01)</td>
</tr>
<tr>
<td><em>Juniperus virginiana</em> (ID)</td>
<td>0–0 (0)</td>
<td>0.0 (0.0)</td>
</tr>
<tr>
<td><em>J. virginiana</em> (PG)</td>
<td>0–1 (0.03)</td>
<td>0.0–0.2 (0.03)</td>
</tr>
<tr>
<td><em>Populus deltoides</em> (ID)</td>
<td>0–2 (0.06)</td>
<td>0.0–0.40 (0.01)</td>
</tr>
<tr>
<td>P. deltoides (PG)</td>
<td>0–0 (0)</td>
<td>0.0 (0.0)</td>
</tr>
<tr>
<td><em>Xanthium strumarium</em> (ID)</td>
<td>0–2 (0.06)</td>
<td>0.0–0.4 (0.01)</td>
</tr>
<tr>
<td>X. strumarium (PG)</td>
<td>0–0 (0)</td>
<td>0.0 (0.0)</td>
</tr>
<tr>
<td><em>Bassia scoparia</em> (ID)</td>
<td>0–3 (1.25)*</td>
<td>0.0–0.55 (0.3)*</td>
</tr>
<tr>
<td><em>B. scoparia</em> (PG)</td>
<td>0–2 (0.31)*</td>
<td>0.0–0.4 (0.07)*</td>
</tr>
<tr>
<td><em>Penicillium</em> sp. (ID)</td>
<td>0–2 (0.08)</td>
<td>0.0–0.65 (0.03)</td>
</tr>
<tr>
<td><em>Penicillium</em> sp. (PG)</td>
<td>0–1 (0.03)</td>
<td>0.0–0.25 (0.01)</td>
</tr>
<tr>
<td>Mosquito (ID)</td>
<td>0–3 (0.94)*</td>
<td>0.0–0.8 (0.24)*</td>
</tr>
<tr>
<td>Mosquito (PG)</td>
<td>0–2 (0.17)*</td>
<td>0.0–0.35 (0.04)*</td>
</tr>
</tbody>
</table>

*Indicates significance with *P* < 0.05 with either subjective or objective evaluation between testing methods.

### Table 3. Comparison of intradermal (ID), percutaneous glycerinated (PG) and percutaneous aqueous (PA) histamine and saline at the 20 min evaluation point

<table>
<thead>
<tr>
<th>Control</th>
<th>Subjective score range 0–4 (mean)</th>
<th>Wheal size range (mean) (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histamine (ID)</td>
<td>3–4 (3.83)*</td>
<td>0.55–1.15 (0.87)*</td>
</tr>
<tr>
<td>Histamine (PG)</td>
<td>1–4 (2.86)*</td>
<td>0.2–1.2 (0.66)*</td>
</tr>
<tr>
<td>Histamine (PA)</td>
<td>0–2 (0.60)*</td>
<td>0.0–0.45 (0.15)*</td>
</tr>
<tr>
<td>Saline (ID)</td>
<td>0–2 (0.11)</td>
<td>0.0–0.7 (0.04)</td>
</tr>
<tr>
<td>Saline (PG)</td>
<td>0–1 (0.03)</td>
<td>0.0–0.25 (0.01)</td>
</tr>
<tr>
<td>Saline (PA)</td>
<td>0–0 (0)</td>
<td>0.0–0.0 (0.0)</td>
</tr>
</tbody>
</table>

*Indicates significance with *P* < 0.05 with either subjective or objective evaluation between testing methods.

evaluated at the 20 and 25 min evaluation points (Table 3, Figure S8). No flare reactions were observed for histamine, saline or glycerosaline for ID, PA or PG.

Evaluation of reactions based on cat skin colour
There was a significant difference both subjectively and objectively overall for *Penicillium* sp. when comparing white skin to grey or mixed colour skin ($P < 0.05$). Otherwise, there was no difference in PG, PA and ID reactions with the different skin colours.

Correlation of subjective and objective data
Subjective and objective data were compared for correlation with near complete agreement of pooled cat data between ID and PG. There was a single discrepancy of the pooled cat data between ID and PG wheal size for canine dander with the combined immediate time points; it was significant during objective evaluation ($P < 0.05$), but not during subjective evaluation ($P = 0.450$). There was a single discrepancy for the comparison of PA and PG for canine dander for the combined immediate reaction time data, which was significant subjectively ($P < 0.05$) but not objectively ($P = 0.1909$). There were two discrepancies between PA and ID. The first was for *D. farinae* at the 15 min reading point, which was significant subjectively ($P < 0.05$) but not objectively ($P = 0.0503$); the second was for canine dander at the 15 min reading point, which was significant objectively ($P < 0.05$) but not subjectively ($P = 0.124$).

Discussion
The primary goal of this study was to determine if percutaneously applied allergen extracts previously used in the identification of allergen-specific hypersensitivities would create irritant reactions in healthy adult cats. In this study we found that PA and PG allergens did not lead to irritant reactions. PG and ID histamine provided reliable controls in this study, whereas PA histamine did not. Based on the larger wheal size at the 20 and 25 min evaluation points for PG, the 20 min evaluation point is recommended and subjective evaluation can be used. The current IDT concentrations of *B. scoparia* and mosquito may be irritants and these testing concentrations may need to be re-evaluated.

The majority of the percutaneously applied allergen wheals at the 20 min time point were graded as 0–1, which in a clinical setting would be considered insignificant (data not shown for PA at 20 mins). Overall, PA and PG allergens created smaller or similar sized wheals when compared to ID allergen wheals. These data suggest that these nine allergens do not cause irritant reactions in healthy adult cats when applied percutaneously at the tested concentrations. An increase in sample size may have detected additional differences between testing methods.

The allergens chosen were from multiple allergen classes including: mites, dander, grass pollen, weed pollen, tree pollen, mould and insects, to encompass the majority of allergen groups tested on a routine basis. It is speculated that other allergens from these same classes would not create irritant reactions in healthy cats when tested at the manufacturer’s suggested concentrations. This speculation is supported by the lack of irritant reactions to 10 mg/mL concentration of house dust mite, storage mite and polllens applied to the inner thigh of healthy adult dogs using pick lancets (Entaco Ltd; Worcestershire, UK).$^{14}$ In the same study, 19 of 34 (56%) of the atopic dogs had one or more positive allergen reactions.$^{14}$ However, it is not known if the allergens used in that study were glycerinated or aqueous.

Percutaneously applied glycerosaline and aqueous saline did not lead to irritant reactions consistent with the previous studies of cat and human PCT.$^{11,15}$ Likewise, a PCT study, albeit in abstract form, reported on a canine model where there were no wheal reactions with glycerosaline when applied to both the lateral thorax and inner thigh.$^{16}$ Based on these previous studies and the current study, glycerosaline can be used as a percutaneous negative control in canine and feline patients.

In a previous study, ID histamine wheals were slightly larger in size than percutaneous 6 mg/mL glycerinated histamine wheals, although the latter were noted to be firmer and easier to read.$^{11}$ The groups were not compared statistically. In the current study, the PG and ID wheals were compared statistically, with the ID histamine wheals being consistently larger. Similar to the previous study, the PG histamine wheals were well demarcated and easy to evaluate.$^{11}$ A canine study also found that percutaneously applied 5 mg/mL histamine created well-demarcated wheals.$^{14}$ PA histamine wheals were poorly visible and PA testing with 0.0275 mg/mL aqueous histamine is therefore not recommended.

The PA allergens were lower in concentration compared to PG. PA allergens were the same concentration as the ID allergens, but the volume applied was smaller. Therefore, the combination of low volume and more diluted concentration may have led to the weaker PA wheals when compared to ID and PG wheals. This concentration was chosen for convenience as allergens are already diluted for intradermal testing in this manner. However, it is unclear if the stock concentrated solutions of aqueous histamine and aqueous allergens would produce reliable reactions without causing irritant reactions.

With the exception of *B. scoparia* and mosquito, the majority of ID reactions were graded as 0–1. There were multiple reactions graded as 2–3 for *B. scoparia* and mosquito using IDT. The cause for these positive reactions may include a subclinical hypersensitivity or irritant reaction. Previous studies identified that clinically normal animals may have positive reactions on both intradermal and serum allergen tests, which is why these tests cannot be substituted for a thorough history and clinical signs in the diagnosis of HD.$^{3,4}$ As many of the study cats were young to middle-aged, it is possible that these patients may develop HD in the future, making the positive reactions potentially clinically relevant. Another likely explanation is that the currently tested concentration of *B. scoparia* at 1000 PNU/mL is an irritant because 10 of 12 cats had two or greater subjective reactions to this allergen. There have been minimal published data on allergen testing concentrations in the cat, with a previous study evaluating 10 allergens in healthy cats and cats with environmentally induced HD.$^{10}$ In that study, *B. scoparia* and mosquito
were not evaluated. It is likely that this tested concentration is a true irritant or the currently used lot number of *B. scoparia* was more concentrated than previous lot numbers. A previous study has shown that allergen concentrations may vary between batches.17

For mosquito, 5 of 12 cats had ID reactions graded as 2–3. Similar to *B. scoparia*, the tested concentration of mosquito may represent an irritant reaction or a more concentrated lot of allergen. Interestingly, four of five cats with a reaction to mosquito had a history of spending time outdoors, whereas the remainder of the cats in the study (7 of 12) did not. This may support the theory that cats with outdoor exposure may be subclinically hypersensitized to mosquito.

The 25 min reading produced larger wheals for PG histamine compared to the 15 min reading point. There was no significant difference between the 20 and 25 min time points. As the 20 min reading points were similar to 25 min time point, the 20 min time point is recommended to reduce the length of sedation in cats as well as technician time. This is similar to previous findings where 20 min was the recommended reading time because larger histamine wheals were produced at 20 and 25 min compared to the 15 min time point.11 Also, similar to the pilot study there was good to excellent correlation for subjective and objective grading, suggesting that subjective grading can be used for PCT in cats.11

Skin colour did not create significant differences in subjective or objective wheal size for the controls or eight allergens in the current study, with only *Penicillium sp* having larger wheals in white-skinned cats. The lack of significant differences with histamine and almost all allergens may be in contrast to what can be seen in human PCT, where darker skin colour PCT are typically more difficult to evaluate subjectively when compared to lighter skin colour PCT wheals (Raweewan Hoontrakoon, personal communication 2014).

Ideally, the same concentration of glycerinated allergens would have been used for all allergens. However, this was not possible as the *D. farinae*, *P. pratensis* and mosquito allergens used in this study were not available. In these cases the manufacturer had recommended a dilution of the concentrated allergen 1:1 with glycerin. As the goal of this study was to determine if PG allergens would lead to irritant reactions, we did not attempt to determine the optimal PG allergen concentration for future testing.

In conclusion, the selected percutaneous aqueous and percutaneous glycerinated allergens did not cause irritant reactions in healthy adult cats. Percutaneously applied glycerinated histamine and glycerosaline provide reliable controls. Subjective grading is reliable for further feline PCT studies and the 20 min reading point is recommended. Additional studies will be needed to determine the ideal testing concentrations for both percutaneous glycerinated and percutaneous aqueous allergens. In addition, ideal IDT concentrations may need to be evaluated for mosquito and *B. scoparia*. Future goals include comparing complete percutaneous glycerinated and ID allergen tests in cats with environmental hypersensitivities and the response to allergen-specific immunotherapy based on PCT.

Acknowledgements

The authors would like to thank Miriam Cabrera and Genna Nault for technical assistance and Deborah Keys for statistical assistance.

References


2. Reineri C. Advances in the understanding of pathogenesis, and diagnostics and therapeutics for feline allergic asthma. *Vet J* 2011; 190: 28–33


Supporting Information

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

Figure S1. Comparison of mosquito objective wheal size in cm for intradermal (ID), percutaneous aqueous (PA)
and percutaneous glycerinated (PG) testing. The 36 data points for each group represent the data performed in triplicate in the 12 study cats at the 20 min reading point. Means are shown as solid lines.

**Figure S2.** Comparison of mosquito subjective wheal size (0–4) for intradermal (ID), percutaneous aqueous (PA) and percutaneous glycerinated (PG) testing. The 36 data points for each group represent the data performed in triplicate in the 12 study cats at the 20 min reading point. Means are shown as solid lines.

**Figure S3.** Comparison of *P. deltoides* objective wheal size in cm for intradermal (ID), percutaneous aqueous (PA) and percutaneous glycerinated (PG) testing. The 36 data points for each group represent the data performed in triplicate in the 12 study cats at the 20 min reading point. Means are shown as solid lines.

**Figure S4.** Comparison of *P. deltoides* subjective wheal size (0–4) for intradermal (ID), percutaneous aqueous (PA) and percutaneous glycerinated (PG) testing. The 36 data points for each group represent the data performed in triplicate in the 12 study cats at the 20 min reading point. Means are shown as solid lines.

**Figure S5.** Comparison of *B. scaparia* objective wheal size in cm for intradermal (ID), percutaneous aqueous (PA) and percutaneous glycerinated (PG) testing. The 36 data points for each group represent the data performed in triplicate in the 12 study cats at the 20 min reading point. Means are shown as solid lines.
Zusammenfassung
Hintergrund – Der Intradermatest (IDT) bei Katzen weist möglicherweise Limitierungen auf; das hat dazu geführt, dass Interesse an neuen Testmethoden entstand. Eine Pilotstudie zeigte gesunde Katzen, die verlässliche percutane glyzerinierte (PG) Histaminreaktionen produzierten, während percutan applizierte glyzerinierte Kochsalzlösung zu keiner Quaddelbildung führte.

Hypothesen/Ziele – Das Ziel dieser Studie war es zu untersuchen, ob percutan applizierte wässrige und glyzerinierte Allergene zu irritierenden Reaktionen bei gesunden Katzen führen würden.


Ergebnisse – Die Hauttestreaktionen der intradermalen (ID) Histaminquaddeln waren sowohl subjektiv als auch objektiv bei den unmittelbaren Beurteilungszeitpunkten (P<0,05) stärker im Vergleich zu den PG und percutanen wässrigen (PA); PG war jedoch nicht signifikant verschieden vom IDT, wenn sie entweder als positiv (2–4) oder negativ (0–1) beurteilt wurden. Histamin- und Allergenreaktionen waren, wenn sie vorhanden waren, größer als die entsprechenden PA-Reaktionen. PG und PA Allergene verursachten bei dem getesteten Konzentrationen keine irritierenden Reaktionen. PG und PA Allergene verursachten bei den untersuchten Testkonzentrationen keine irritierenden Reaktionen. Bassia scoparia (kochia) wurde bei einer Konzentration von 1000 PNU/mL mittels IDT als irritierend verdächtig.

Schlussfolgerung und klinische Bedeutung – Percutan applizierte Allergene verursachten bei gesunden Katzen keine irritierenden Reaktionen. PG Histamin Quadten waren zwar kleiner wie die ID Histaminquaddeln, waren aber einfach erkennbar und PCT war einfach in der Durchführung.