Clinical relevance of intradermal test results in atopic dogs

DOI 10.1055/a-1584-4965
Tierärztliche Praxis Kleintiere/Heimtiere 2021; 49: 349–356

ISSN 1434-1239

Thieme

Clinical relevance of intradermal test results in atopic dogs

Klinische Relevanz von Intrakutantest-Ergebnissen bei atopischen Hunden

Authors

Stefanie Mallmann[1], Christoph Klinger[2], Janine Claßen[3], Iris Wagner[4], Andre Klima[5], Noemi Castelletti[5], Ralf S. Mueller[4]

Institutes

1 Small Animal Medicine Clinic, Centre for Clinical Veterinary Medicine, LMU Munich, Germany; present address: Finidore Manufaktur, Greifenberg, Germany
2 Small Animal Medicine Clinic, Centre for Clinical Veterinary Medicine, LMU Munich, Germany; present address: Tierklinik Stuttgart-Plieningen, Germany
3 Small Animal Medicine Clinic, Centre for Clinical Veterinary Medicine, LMU Munich, Germany; present address: Small Animal Clinic, Oberhaching, Germany
4 Small Animal Medicine Clinic, Centre for Clinical Veterinary Medicine, LMU Munich, Germany
5 Statistical consulting unit StaBLab, Department of Statistics, LMU Munich, Germany

Schlüsselwörter

Allergie, Atopie, kanin, Dermatitis, Intrakutantest, Juckreiz

Key words

Allergy, atopy, canine, dermatitis, intradermal testing, pruritus

received 06.12.2020
accepted 16.02.2021

Bibliografie

Tierarztl Prax Ausg K Kleintiere Heimtiere 2021; 49: 349–356
DOI 10.1055/a-1584-4965
ISSN 1434–1239

Georg Thieme Verlag KG, Rüdigerstraße 14,
70469 Stuttgart, Germany

Correspondence address

Ralf S. Mueller
Small Animal Medicine Clinic
Centre for Clinical Veterinary Medicine
LMU Munich
Veterinaerstraße 13
80539 Munich
Germany
r.mueller@medizinische-kleintierklinik.de

ZUSAMMENFASSUNG

Gegenstand und Ziel Die kanine atopische Dermatitis (AD) ist eine entzündliche und juckende Hautkrankheit, bei der in den meisten Fällen IgE-Antikörper gegen Umweltallergene auftreten. Bis heute stellt die Allergen-Immuntherapie (AIT) die einzige kausale Therapie dar. Am Krankheitsgeschehen beteiligte Allergene können durch Intrakutantests (IKT) oder Serumtests auf allergenspezifisches IgE identifiziert werden. Ziel dieser Studie war herauszufinden, ob positive IKT-Ergebnisse mit der Anamnese der atopischen Hunde korrelieren.

Material und Methoden Bei 48 atopischen Hunden, bei denen ein IKT durchgeführt wurde, füllten die Hundebesitzer einen detaillierten Fragebogen über den jahreszeitlichen Verlauf des Juckreizes ihrer Hunde aus. Die Antworten in den Fragebögen wurden in Beziehung zu den Resultaten des IKT gesetzt.

Ergebnisse Die häufigsten positiven Reaktionen wurden gegen Hausstaubmilben festgestellt (33,3–62,5 %). Die Prävalenz positiver Resultate gegen die getesteten Pollen von Bäumen, Gräsern und Kräutern lag zwischen 8,3 % und 25 %. Schimmelpilze und Epithelien führten bei 0–6,3 % zu positiven Reaktionen. Positive IKT-Ergebnisse gegen ganzjährige und saisonale Allergene korrelierten nicht mit dem Auftreten von Juckreiz.

Schlussfolgerung Die Bewertung von Reaktionen im IKT ist möglicherweise nicht die optimale Methode für die Bestimmung der klinisch relevanten Allergene bei kaniner AD.

Klinische Relevanz Die Ergebnisse dieser Studie bestätigen, dass bei der Auswahl von Allergenen für die AIT die klinische Anamnese zusätzlich zu den Ergebnissen des Allergietests berücksichtigt werden muss.

ABSTRACT

Topic and aims Canine atopic dermatitis (AD) is an inflammatory and pruritic skin disease and in most cases associated with IgE antibodies against environmental allergens. To date, the only causative therapeutic option is allergen immunotherapy (AIT). Offending allergens for AIT can be identified by intradermal testing (IDT) or serum allergen-specific IgE testing. The aim of the study was to evaluate positive IDT results considering the atopic dogs' clinical history.

Material and methods An IDT was performed on 48 atopic dogs and their owners completed a detailed questionnaire about the seasonal course of their pruritus. Results of IDT were correlated with the seasonal occurrence of pruritus.

Results The most common positive IDT reactions were observed to mite allergens (33.3–62.5 %). Prevalence of positive reactions to individual tree, grass and weed pollen ranged between 8.3 % and 25 %. Moulds and epithelial allergens produced positive reactions in only 0–6.3 %. A correlation between positive IDT reactions and course of pruritus could neither be found for perennial nor for seasonal allergens.

Conclusion The evaluation of IDT reactions may not be an optimal method for identification of clinically relevant allergens in canine AD.

Clinical relevance The results of this study emphasise the importance of considering clinical history in addition to allergy test results in the formulation of an allergen extract for desensitisation.

Introduction

Canine atopic dermatitis (AD) is an inflammatory and pruritic skin disease with characteristic clinical features. In most cases AD is associated with IgE antibodies against environmental allergens [1]. It was long considered a strictly IgE-mediated disease [2][3]. However, in some dogs with typical clinical signs of AD, no allergen-specific IgE antibodies can be detected. This phenomenon is called intrinsic AD or "canine atopic-like dermatitis" and indicates that there may be alternative pathogenic pathways leading to the development of clinical signs of AD [3][4].

To date there is no reliable diagnostic test for AD. The disease is diagnosed by a detailed history, careful clinical examination and excluding other differential diagnoses [5]. Once AD is diagnosed clinically and confirmed by allergy testing, it can be treated with allergen immunotherapy (AIT) or symptomatically with antipruritic drugs [6][7]. Symptomatic anti-inflammatory therapy may be associated with adverse effects (such as seen with glucocorticoids) and may have limited efficacy (such as with antihistamines or essential fatty acids) [6][7]. Other symptomatic therapies such as ciclosporin, oclacitinib or lokivetmab are safer options with a good efficacy, but are costly and do not change the course of the disease. AIT is a safe and effective treatment option and, due to its modification of the immune system, can alter the course of the disease. It has the potential to provide long-term remission of clinical signs [6][8][9].

Allergens included in the immunotherapy extract have to be identified, usually either by intradermal testing (IDT) or serum allergen-specific IgE testing (SAT) [8][9]. The success rate of AIT is similar with the 2 test methods [9] although IDT was (and for some still is) considered the 'gold standard' for years [10]. It is widely accepted, that not every positive reaction in IDT may be clinically relevant, however, there is no data about the prevalence of false-positive reactions and the correlation between test results and clinical history. The aim of this study was to correlate the individual clinical history of atopic dogs with their positive IDT reactions.

Materials and methods

Study design

The study was designed as a case-cohort study and approved by the ethics committee of the Centre for Clinical Veterinary Medicine, Faculty of Veterinary Medicine, LMU Munich (reference number: 48–18–05–2015).

Patient selection

Forty-eight client-owned dogs with diagnosed AD were included. The diagnosis of AD was made by clinical examination, history and ruling out differential diagnoses. Every patient underwent an elimination diet to evaluate the existence of concurrent food allergy. To minimize drug influence on test results, injectable glucocorticoids were withdrawn at least 6 weeks prior and oral glucocorticoids and ciclosporin at least 4 weeks prior to IDT. Oral antihistamines, topical glucocorticoids and oclacitinib had to be discontinued 2 weeks prior to IDT. Lokivetmab was not commercially available at the time.

Dogs were excluded from the study if they showed any health restrictions and sedation was not possible. They were also withdrawn if they displayed prominent skin lesions such as erythema, papules, pustules or crusts in the test area.

Clinical history

All owners completed a detailed questionnaire about the clinical history of their dogs and their dogs' environment (▶ **Fig. 1**). Pruritus was chosen as the parameter evaluating seasonality, because it is considered a cardinal sign of canine AD [11][12]. All dogs in the study population suffered from pruritus. The intensity of pruritus of each month was estimated retrospectively by the owners and graded as no (0), mild (1), moderate (2) or severe (4) pruritus. Months were grouped in seasons as follows: spring (February, March, April), summer (May, June, July, August), fall (September, October, November) and winter (December, January). The maximal pruritus of any month in a respective season was chosen as the representative value for this season.

Intradermal testing

Each dog was sedated with medetomidine hydrochloride (3–5 µg/kg i. v.). A test area of approximately 20 × 10 cm was clipped on the lateral thorax. The injection sites were marked with a permanent marker. IDT was performed with allergen extracts from an intradermal test set (Artu Biologicals Europe B. V.). Used allergen extracts are listed in ▶ **Table 1**.

Allergen solutions were injected intradermally using an insulin syringe. A positive and a negative control solution were injected before the first and after the last allergen injection. The diluent of the allergen solution (phosphate buffered saline with 0.47 % phenol) served as negative control, histamine in a concentration of 1:100,000 w/v as positive control. A volume of 0.1 ml of each allergen solution was typically injected. In dogs, where skin inflammation precluded the injection of the full amount (which is apparent with the first injection, the negative control), 0.05 ml were injected uniformly. Pollen allergens were tested at a concentration of 1000 noon units (NU)/ml, epithelia and mould allergens at a concentration of 100 µg/ml and mite allergens at a concentra-

Animal ID:	Age:	Breed:

Gender:

❑ Female ❑ Female neutered ❑ Male ❑ Male neutered

Was your animal obtained from a breeder? ❑ Yes ❑ No

What do you feed your dog?

Did your dog undergo an elimination diet? ❑ No ❑ Yes Result?

Besides the skin disease, are there any other known problems?

When did your dog's skin problems begin?

Which clinical signs does the dog show?

❑ Pruritus ❑ Erythema ❑ Scales ❑ Crusts ❑ Pustules ❑ Papules

❑ Alopecia ❑ Hyperpigmentation ❑ Dull coat ❑ Oily skin ❑ Lacrimation

Which body regions are affected? ❑ Head ❑ Ears ❑ Neck ❑ Back ❑ Axillae

❑ Ventrum ❑ Inguinal area ❑ Flanks ❑ Tail (-base) ❑ Paws

How severe is the pruritus?

no pruritus severe pruritus

In which months of the year was pruritus present?

												Severe pruritus
												Moderate pruritus
												Mild pruritus
Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	

Do you currently administer any medications to your dog? If so, which ones and at what dose?

When did your animal last receive one of the following medications?

❑ Cortisone: ❑ Antihistamines: ❑ Apoquel: ❑ Ear medications:

Where do you live? ❑ Urban (City,Town/Suburb) ❑ Rural (Village/Countryside)

Where is your dog most of the time? ❑ House/Appartment ❑ Garden/Yard

What flooring do you have in your appartment?

❑ Floor boards ❑ Tiles ❑ Carpet ❑ Linoleum

Where is the pruritus the most severe? ❑ Inside ❑ Outside

Which trees do you have in your immediate environment?

❑ Birch ❑ Beech ❑ Oak ❑ Poplar ❑ Pine ❑ Maple ❑ Walnut

❑ Linden ❑ Alder ❑ Willow

▶ **Fig. 1** Owner questionnaire. Source: © S. Mallmann.

▶ **Abb. 1** Fragebogen für die Hundebesitzer. Quelle: © S. Mallmann.

tion of 100 NU/ml. Skin reactions were evaluated 15 and 25 minutes after injection. They were graded with a subjective scoring method from 0 (equal to negative control) to 4 (equal to positive control) [10].

Statistical methods

For statistical analysis, allergens were grouped in seasonal allergens (tree, grass and weed pollen, *Cladosporium*, *Alternaria* and rape) and perennial allergens (mites, epithelia and *Aspergillus*). Dogs were first

▶ **Table 1** Common and latin names of allergens used in intradermal testing.

▶ **Tab. 1** Englische und lateinische Namen der im Intrakutantest verwendeten Allergene.

Common names	Latin names
Mites	
Grain mite	*Acarus siro*
House dust mite	*Dermatophagoides farinae*
House dust mite	*Dermatophagoides pteronyssinus*
Hay mite	*Lepidoglyphus destructor*
Mould mite	*Tyrophagus putrescentiae*
Pollen of crops	
Rape	*Brassica napus*
Weeds	
Common ragweed	*Ambrosia artemisiifolia* var. *elatior*
Common mugwort	*Artemisia vulgaris*
Lambs quarter	*Chenopodium album*
Pellitory	*Parietaria officinalis*
English plantain	*Plantago laceolata*
Sheep sorrel	*Rumex acetosella*
Goldenrod	*Solidago virgaurea*
Mugwort, nettle, dandelion, plantain mix	*Artemisia vulgaris, Urtica dioica, Taraxacum officinale, Plantago laceolata*
Trees	
Hazel, alder, birch mix	*Corylus avellana, Alnus glutinosa, Betula pendula*
Oak, beech, elm mix	*Quercus robur, Fagus sylvatica, Ulmus americana*
Hazel	*Corylus avellana*
Beech, European	*Fagus sylvatica*
Poplar	*Populus alba*
Grasses	
Cough grass	*Agropyron repens*
Redtop	*Agrostis gigantea*
Meadow foxtail	*Alopecurus pratensis*
Bermuda grass	*Cynodon dactylon*
Orchard grass	*Dactylis glomerata*
Velvet grass	*Holcus lanatus*
Rye grass	*Lolium perenne*
Blue grass, Kentucky	*Poa pratensis*
Moulds	
	Alternaria alternata
	Aspergillus fumigatus
	Cladosporium herbarum
Epithelia	
Sheep wool	*Ovis aries*
Duck, goose, chicken mix	*Anas platyrhynca, Anser anser, Pullus gallinaceus*

allocated to **group A** (non-seasonal pruritus) or **group B** (seasonal pruritus). In a second step, dogs with perennial pruritus and deterioration of pruritus in spring, summer and/or fall from group A as well as dogs from group B with seasonal pruritus were combined to **group C**, while dogs with perennial pruritus and a consistent level of pruritus throughout the year formed **group D**. Results of IDT were correlated with the seasonal occurrence of pruritus. For calculation of statistical significance, the Fisher's exact test was used. In 2 different analyses, reactions graded as ≥ 2 or ≥ 3 were considered positive, respectively. P values < 0.05 were considered significant. Statistical analysis was performed with RStudio (Version 3.3.1, RStudio, Inc.) and GraphPad Prism 6 (GraphPad Software Inc., San Diego, USA).

Results

Study participants

Twenty female dogs (12 of them were spayed) and 28 male dogs (12 of them were neutered) were included in the study. Their age ranged from 1 year to 11 years, with a mean of 3.75 years. Breeds were mixed-breeds (n = 11), Labrador Retriever (n = 6), French Bulldog (n = 5), Boxer (n = 4), Golden Retriever, Jack Russell Terrier, Pug (n = 2 each), German Shepherd, Bernese Mountain Dog, Australian Shepherd, Doberman, English Bulldog, English Setter, Eurasian, Goldendoodle, Havanese, Parson Russell Terrier, Rhodesian Ridge Back, Shiba Inu, Toy Poodle, Welsh Terrier, West Highland White Terrier and Yorkshire Terrier (n = 1 each). One third of the dogs were diagnosed as partially food allergic based on partial improvement on a diet, flare after rechallenge with the old food and subsequent improvement on the diet again.

Intradermal test reactions

Percentages of positive test reactions are outlined in ▶ **Table 2**. Positive IDT reactions against mite allergens were seen in most of the study participants, 79.2 % showed positive IDT reactions to at least one mite allergen. The most common positive reactions were observed to the storage mites *Tyrophagus putrescentiae* (62.5 %), *Acarus siro* (60.4 %) and *Lepidoglyphus destructor* (56.3 %). House dust mites *Dermatophagoides* (*D.*) *farinae* and *D. pteronyssinus* caused positive reactions in 52.1 % and 33.3 % of the patients, respectively. Meadow foxtail (*Alopecurus pratensis*) was the most common grass allergen and common ragweed (*Ambrosia artemisiifolia*) the most encountered weed allergen causing positive reactions in 25 % and 22.9 % of the dogs, respectively. The most common tree allergen was poplar (*Populus* spp.). Positive reactions to *Aspergillus* and *Alternaria* were only encountered in 3 and 2 dogs, respectively. No positive test reaction was observed for *Cladosporium*. Sheep wool and epithelia mixture were associated with a positive IDT reaction in one dog each.

Correlation of IDT reactions to the environment the dogs live in

Fifty-six percent of the owners and dogs lived in a rural environment, the remaining 44 % in an urban environment. Dogs kept in an urban environment showed strong reactions (grade ≥ 3) to seasonal allergens (38.1 %) more frequently than dogs in a rural environment (7.4 %). This difference was not statistically significant (p = 0.0798). The proportion of IDT reactions ≥ 2 was nearly equal in both groups (81 % in urban environment vs. 74.1 % in rural environment).

Correlation of IDT reactions to pruritus seasonality

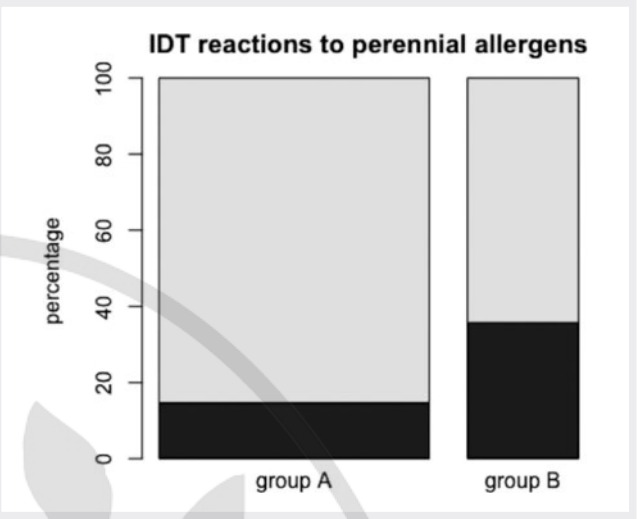
Pruritus was present in all 48 study participants. Most dogs (70.8 %) showed non-seasonal pruritus. In 48 % of those, the owners observed no variation in the intensity of pruritus throughout the year,

▶ **Table 2** Observed positive reactions in intradermal tests of 48 atopic dogs.

▶ **Tab. 2** Positive Reaktionen in Intrakutantests von 48 atopischen Hunden.

Allergen	Percentage of positive reactions	
	Grade ≥ 2	Grade ≥ 3
Epithelia	**4.2 %**	**0 %**
Sheep wool	2.1 %	0 %
Epithelia mixture I [1]	2.1 %	0 %
Moulds	**10.4 %**	**4.2 %**
Aspergillus	6.3 %	4.2 %
Alternaria	4.2 %	0 %
Cladosporium	0 %	0 %
Mites	**79.2 %**	**66.7 %**
Tyrophagus putrescentiae	62.5 %	35.4 %
Acarus siro	60.4 %	50.0 %
Lepidoglyphus destructor	56.3 %	33.3 %
Dermatophagoides farinae	52.1 %	41.7 %
Dermatophagoides pteronyssinus	33.3 %	10.4 %
Trees	**31.3 %**	**10.4 %**
Poplar	14.6 %	8.3 %
Tree pollen mixture I [2]	14.6 %	2.1 %
Tree pollen mixture II [3]	14.6 %	4.2 %
Beech	10.4 %	2.1 %
Hazel	8.3 %	4.2 %
Grasses	**52.1 %**	**14.6 %**
Meadow foxtail	25.0 %	10.4 %
Orchard grass	20.8 %	12.5 %
Cough grass	14.6 %	8.3 %
Rye grass	14.6 %	10.4 %
Blue grass, Kentucky	14.6 %	2.1 %
Bermuda grass	14.6 %	4.2 %
Velvet grass	14.6 %	10.4 %
Redtop	12.5 %	10.4 %
Weeds	**8.3 %**	**12.5 %**
Common ragweed	22.9 %	8.3 %
Weed pollen mixture [4]	22.9 %	4.2 %
English plantain	20.8 %	8.3 %
Pellitory	20.8 %	4.2 %
Common mugwort	18.8 %	8.3 %
Lambs quarter	14.6 %	8.3 %
Goldenrod	12.5 %	8.3 %
Sheep Sorrel	8.3 %	4.2 %
Pollen of crops		
Rape	16.7 %	4.2 %

[1] duck, goose, chicken; [2] hazel, alder, birch; [3] English oak, European beech, American elm; [4] common mugwort, stinging nettle, common dandelion, English plantain



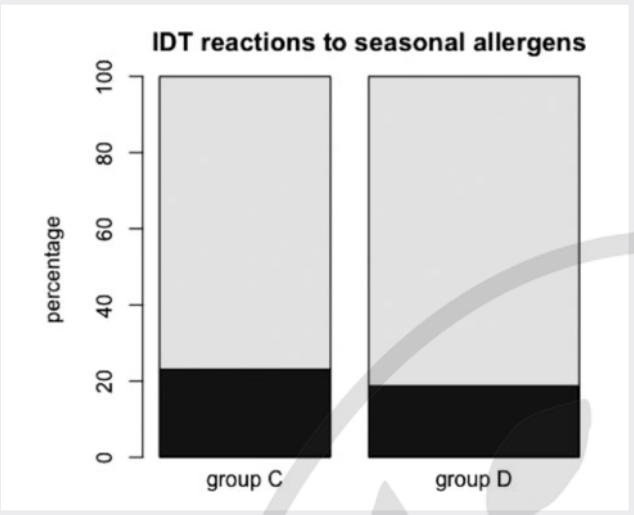
▶ **Fig. 2** Percentage of dogs with non-seasonal (group A) and with seasonal pruritus (group B) showing positive intradermal test (IDT) reactions to perennial allergens. The grey sector is the proportion of reactions ≥ 2, the black sector is the proportion of reactions < 2 (equaling negative results). The width of the bars reflects the size of groups. Source: © S. Mallmann.

▶ **Abb. 2** Hunde mit ganzjährigem (Gruppe A) und saisonalem Juckreiz (Gruppe B) mit positiven Reaktionen gegen ganzjährig auftretende Allergene. Der Anteil der Reaktionen ≥ 2 ist grau gefärbt, der Anteil der Reaktionen < 2 (also negativem Befund) schwarz. Die Breite der Blöcke spiegelt die Gruppengröße wider. Quelle: © S. Mallmann.

the dogs showed therefore no peak in their clinical course. The remaining 52 % showed worsening of pruritus in at least one season throughout the year.

The proportion of dogs with non-seasonal (group A) and seasonal (group B) pruritus showing positive IDT reactions to perennial allergens is illustrated in ▶ **Fig. 2**. Patients in group A displayed positive test reactions to perennial allergens in 85.3 % of cases when a positive reaction was defined as ≥ 2. Although participants in group B showed less positive reactions (64.3 %) to perennial allergens, this was not significant ($p = 0.1299$). When taking into account only the IDT reactions ≥ 3, a significant difference could also not be found between group A and B ($p = 0.3153$). The calculations were repeated with dogs only allergic to environmental allergies and excluding the dogs partially responding to an elimination diet, the results were similar.

The proportion of dogs with a seasonal peak in spring, summer and/or fall (group C) and with no peak (group D) of pruritus showing positive reactions to seasonal allergens is presented in ▶ **Fig. 3**. Positive IDT reactions to seasonal allergens were shown by 76.9 % of the dogs with a peak in spring, summer and/or fall. Dogs with a stable intensity of pruritus throughout the year exhibited a positive IDT reaction to seasonal allergens even more frequently (81.3 %, $p = 1.0$). To minimise the chance that owners missed a seasonal deterioration due to excessive pruritus, dogs with severe perennial pruritus were excluded from group D in a second comparison with the following result: Dogs with no peak of pruritus also showed more reactions ≤ 2 (25 %) to seasonal allergens, than patients with a peak in spring, summer and/or fall (15.4 %).



▶ **Fig. 3** Percentage of dogs with a seasonal peak in spring, summer and/or fall (group C) and with no peak (group D) of pruritus showing positive reactions to seasonal allergens. Only dogs with mild and moderate pruritus were taken into account in the group with no peak. The grey sector is the proportion of reactions ≥ 2, the black sector is the proportion of reactions < 2 (equaling negative results). The width of the bars reflects the size of the groups. Source: © S. Mallmann.

▶ **Abb. 3** Hunde mit saisonalem Juckreiz im Frühling, Sommer und/oder Herbst (Gruppe C) und ganzjährigem Juckreiz (Gruppe D) mit positiven Reaktionen gegen saisonale Allergene. Grau gefärbt der Anteil der Reaktionen ≥ 2, schwarz der Anteil der Reaktionen < 2 (also negativem Befund). Die Breite der Blöcke spiegelt die Gruppengröße wider. Quelle: © S. Mallmann.

Discussion

IDT reactions of 48 dogs with atopic dermatitis were correlated to the environment, the dogs lived in, and their clinical history. Dogs in an urban environment had more strongly positive reactions to pollen allergens than those living in a rural environment, there was no correlation between clinical course of the pruritus during the year and IDT results.

The determined prevalence of positive IDT results in the present study is similar to the findings in many other studies [13][14][15], although in our study the storage mite allergens *Acarus siro, Tyrophagus putrescentiae* and *Lepidoglyphus destructor* produced more positive reactions than the house dust mites *D. farinae* and *D. pteronyssinus*. This high prevalence of storage mite sensitization seems somewhat surprising, because in a study of Farmaki et al. [16] house dust mites were detected in between 35–64 % of the examined households, whereas storage mites were only found in 5–10 %. High amounts of storage mite allergens in normal households only occur in a humid or mouldy environment, which is uncommon in Germany. Furthermore, reproduction of storage mites in commercial dry dog food could not be detected in a previous study conducted in the same geographic area [17]. Saridomichelakis et al. [18] found in-vitro cross-reactivity between *D. farinae* and storage mites indicating that false-positive IDT reactions to storage mites occur in *D. farinae*-sensitized dogs. Alternatively, storage mite allergens may have a higher allergenic potential, so less amount of allergen may be sufficient to induce IgE formation.

Dogs living in an urban environment showed more severe IDT reactions to seasonal allergens than dogs living in a rural environment. In human medicine there is an urban-rural-gradient of allergic sensitization [19]. Similar findings in dogs would not be surprising, but in our study the difference between urban and rural held dogs was not statistically significant, may be due to a true lack of difference, may be due to the number of dogs included in the study. Alternatively, a poor correlation of IDT results and clinical course of allergy may be responsible for those results.

To evaluate the clinical relevance of positive IDT reactions, we attempted to correlate them to the patients' clinical history. As pruritus was present in all dogs and is considered a major symptom of AD [11][12], it was chosen as a marker for clinical relevance. There was no significant difference of positive reactions to perennial allergens between dogs with non-seasonal (group A) and with seasonal (group B) pruritus. In dogs with seasonal pruritus, these reactions represent either subclinical sensitizations or false-positive reactions. The latter can occur due to improper injection technique or allergen extracts injected at too high concentrations [10]. Both scenarios are rather unlikely, since IDT was performed by experienced dermatologists and the percentage of positive reactions for the individual allergen groups was similar to that reported in the literature. Nevertheless, allergen extracts are difficult to standardize and the actual allergen content can differ from batch to batch [10]. Since different threshold concentrations of mite allergens exist [20], it is conceivable that the actual concentration of mite allergen was too high and caused irritation. Since all dogs are exposed to house dust and storage mite allergens, it is conceivable that they develop subclinical sensitizations with clinically irrelevant allergen-specific IgE antibodies [10]. This may also explain the positive reactions seen in healthy dogs with IDT [21]. Specific IgE antibodies against cross-reactive carbohydrate determinates (CCDs) in allergen extracts may be responsible for false-positive reactions in in vitro allergy testing but seem not to influence IDT results [22]. Patch testing is considered an alternative testing method and used in research with humans [23] and dogs [24][25], but at this point it is too costly, laborious and time consuming in practice.

Similar results were observed when evaluating the dogs' reactions to seasonal allergens. Dogs showing clinical signs for seasonal allergens did not exhibit significantly more positive reactions to those allergens, rather dogs with the same pruritus throughout the year reacted slightly more often to seasonal allergens. Thus, IDT reactions may indicate either subclinical or clinically non-relevant sensitization for seasonal allergens in those patients. False-positive reactions seem unlikely due to the low irritant potential of pollen allergens [26]. However, the retrospective evaluation of pruritus seasonality relied on the owners' impression and memory which is a limitation of the study. Furthermore, pollen release varies from year to year, depending on the climatic conditions. Ideally, a prospective study would record pruritus and actual pollen counts for the last 2 years prior to testing and perform a correlation analysis with this data. However, few owners would be willing to participate in such a long-term study. Another limitation is that a third of the included dogs suffered from a concurrent adverse food reaction. Those dogs had undergone an elimination diet and were presumably stable on the current diet. Adverse food reactions can cause acute flares of AD as well as perennial pruritus [27]. However, we also per-

formed the statistical analysis without those dogs with similar results, therefore the concurrent adverse food reactions seemed to not influence our results. In addition, seasonal flares should not be influenced by the diet, as the diet did not change during the flares.

One possible explanation for the good response to AIT in the dog despite the lack of a correlation between skin test results and the patients' history as reported in this study could be the initiation of a non-specific immune response leading to a downregulation of the allergic type 2 response. Allergen immunotherapy with empirically chosen allergens has been reported as clinically effective in dogs with AD [28] as well as injections with bacterial oligodeoxynucleotides [29]. Those reports would support such a non-specific immune response in at least some of the patients.

The presented results demonstrate a poor correlation of positive IDT results and clinical history. This indicates that IDT may be a suboptimal tool for identification of offending allergens. In food allergic dogs, several studies showed, that the measurement of the lymphocyte proliferation response seems to be a more reliable method for identification of offending allergens than IgE measurement. Food allergy may be more lymphocyte-mediated than IgE-mediated [30]. As AD is not strictly an IgE-mediated disease, other pathogenic pathways may also play a major role [3][4]. This is emphasized by the observed changes during AIT in dogs and humans. A rapid improvement of clinical signs can often be observed despite an initial increase of allergen-specific IgE during the first months of AIT [31]. Other testing methods should be explored and may show a better performance than IDT.

CONCLUSION FOR PRACTICE

The results of this study demonstrated a poor correlation of IDT and clinical history. Intradermal testing may be a suboptimal tool for identification of relevant offending allergens. The findings of this study emphasise the need to consider historical information about the patient's pruritus in addition to the test results when choosing allergens for the desensitisation extract.

Conflict of interest

There is no direct conflict of interest, but Ralf Mueller has lectured for Nextmune and Artu Biologicals for the past 3 years, and has been supported in other allergy testing studies by Heska Laboratories and Nextmune.

References

[1] Olivry T, DeBoer DJ, Griffin CE et al. The ACVD task force on canine atopic dermatitis: forewords and lexicon. Vet Immunol Immunopathol 2001; 81: 143–146. doi:S0165242701003439 [pii]

[2] Marsella R, Sousa CA, Gonzales AJ et al. Current understanding of the pathophysiologic mechanisms of canine atopic dermatitis. J Am Vet Med Assoc 2012; 241: 194–207. doi:10.2460/javma.241.2.194

[3] Pucheu-Haston CM, Bizikova P, Eisenschenk MN et al. Review: The role of antibodies, autoantigens and food allergens in canine atopic dermatitis. Vet Dermatol 2015; 26: 115–e130. doi:10.1111/vde.12201

[4] Pucheu-Haston CM, Bizikova P, Marsella R et al. Review: Lymphocytes, cytokines, chemokines and the T-helper 1-T-helper 2 balance in canine atopic dermatitis. Vet Dermatol 2015; 26: 124–e132. doi:10.1111/vde.12205

[5] Hensel P, Santoro D, Favrot C et al. Canine atopic dermatitis: detailed guidelines for diagnosis and allergen identification. BMC Vet Res 2015; 11: 196. doi:10.1186/s12917-015-0515-5

[6] Olivry T, DeBoer DJ, Favrot C et al. Treatment of canine atopic dermatitis: 2015 updated guidelines from the International Committee on Allergic Diseases of Animals (ICADA). BMC Vet Res 2015; 11: 210. doi:10.1186/s12917-015-0514-6

[7] Olivry T, Foster AP, Mueller RS et al. Interventions for atopic dermatitis in dogs: a systematic review of randomized controlled trials. Vet Dermatol 2010; 21: 4–22. doi:10.1111/j.1365-3164.2009.00784.x

[8] Loewenstein C, Mueller RS. A review of allergen-specific immunotherapy in human and veterinary medicine. Vet Dermatol 2009; 20: 84–98. doi:10.1111/j.1365- 3164.2008.00727.x

[9] Mueller RS, Jensen-Jarolim E, Roth-Walter F et al. Allergen immunotherapy in people, dogs, cats and horses – differences, similarities and research needs. Allergy 2018; 73: 1989–1999. doi:10.1111/all.13464

[10] Hillier A, DeBoer DJ. The ACVD task force on canine atopic dermatitis (XVII): intradermal testing. Vet Immunol Immunopathol 2001; 81: 289–304. doi:10.1016/s0165-2427(01)00313-0

[11] Bizikova P, Santoro D, Marsella R et al. Review: Clinical and histological manifestations of canine atopic dermatitis. Vet Dermatol 2015; 26: 79–e24. doi:10.1111/vde.12196

[12] Favrot C, Steffan J, Seewald W et al. A prospective study on the clinical features of chronic canine atopic dermatitis and its diagnosis. Vet Dermatol 2010; 21: 23–31. doi:10.1111/j.1365-3164.2009.00758.x

[13] Mueller RS, Bettenay SV, Tideman L. Aero-allergens in canine atopic dermatitis in southeastern Australia based on 1000 intradermal skin tests. Aust Vet J 2000; 78: 392–399

[14] Nesbitt GH. Canine allergic inhalant dermatitis: a review of 230 cases. J Am Vet Med Assoc 1978; 172: 55–60

[15] Saridomichelakis MN, Koutinas AF, Gioulekas D et al. Canine atopic dermatitis in Greece: clinical observations and the prevalence of positive intradermal test reactions in 91 spontaneous cases. Vet Immunol Immunopathol 1999; 69: 61–73. doi:10.1016/s0165-2427(99)00040-9

[16] Farmaki R, Saridomichelakis MN, Leontides L et al. Dust mite species in the households of mite-sensitive dogs with atopic dermatitis. Vet Dermatol 2012; 23: 222–e245. doi:10.1111/j.1365-3164.2012.01052.x

[17] Henneveld K, Beck W, Mueller RS. Evaluierung von Vorratsmilben in kommerziellem Hundetrockenfutter und in der Umgebung sowie ihre Bedeutung in der Tiermedizin. Tierarztl Prax Ausg K Kleintiere Heimtiere 2007; 35 (5): 325–332. doi:10.1055/s-0038-1622643

[18] Saridomichelakis MN, Marsella R, Lee KW et al. Assessment of cross-reactivity among five species of house dust and storage mites. Vet Dermatol 2008; 19: 67–76. doi:10.1111/j.1365-3164.2008.00654.x

[19] Elholm G, Linneberg A, Husemoen LL et al. The Danish urban-rural gradient of allergic sensitization and disease in adults. Clin Exp Allergy 2016; 46: 103–111. doi:10.1111/cea.12583

[20] Hensel P, Austel M, Medleau L et al. Determination of threshold concentrations of allergens and evaluation of two different histamine concentrations in canine intradermal testing. Vet Dermatol 2004; 15: 304–308. doi:10.1111/j.1365-3164.2004.00400.x

[21] Mueller RS. Intradermale Reaktionen gegen die Vorratsmilbe Lepidoglyphus destructor bei normalen Hunden und Hunden mit atopischer Dermatitis. Kleintierprax 2011; 56: 5–10

[22] Gedon NKY, Boehm T, Klinger CJ et al. Agreement of serum allergen test results with unblocked and blocked IgE against cross-reactive carbohydrate determinants (CCD) and intradermal test results in atopic dogs. Vet Dermatol 2019; 30: 195–e161. doi:10.1111/vde.12742

[23] Rodrigues DF, Goulart EM. Patch-test results in children and adolescents: systematic review of a 15-year period. An Bras Dermatol 2016; 91: 64–72. doi:10.1590/abd1806-4841.20163927

[24] Marsella R, Nicklin C, Lopez J. Atopy patch test reactions in high-IgE beagles to different sources and concentrations of house dust mites. Vet Dermatol 2005; 16: 308–314. doi:10.1111/j.1365-3164.2005.00472.x

[25] Olivry T, Deangelo KB, Dunston SM et al. Patch testing of experimentally sensitized beagle dogs: development of a model for skin lesions of atopic dermatitis. Vet Dermatol 2006; 17: 95–102. doi:10.1111/j.1365-3164.2006.00502.x

[26] Bauer CL, Hensel P, Austel M et al. Determination of irritant threshold concentrations to weeds, trees and grasses through serial dilutions in intradermal testing on healthy clinically nonallergic dogs. Vet Dermatol 2010; 21: 192–197. doi:10.1111/j.1365-3164.2009.00797.x

[27] Picco F, Zini E, Nett C et al. A prospective study on canine atopic dermatitis and food-induced allergic dermatitis in Switzerland. Vet Dermatol 2008; 19: 150–155. doi:10.1111/j.1365-3164.2008.00669.x

[28] Plant JD, Neradilek MB. Effectiveness of regionally-specific immunotherapy for the management of canine atopic dermatitis. BMC Vet Res 2017; 13: 4. doi:10.1186/s12917-016-0917-z

[29] Wagner I, Geh KJ, Hubert M et al. Preliminary evaluation of cytosine-phosphate-guanine oligodeoxynucleotides bound to gelatine nanoparticles as immunotherapy for canine atopic dermatitis. Vet Rec 2017; 181 (5): 100–124. doi:10.1136/vr.104230

[30] Mueller RS, Olivry T. Critically appraised topic on adverse food reactions of companion animals (4): can we diagnose adverse food reactions in dogs and cats with in vivo or in vitro tests? BMC Vet Res 2017; 13: 275. doi:10.1186/s12917-017-1142-0

[31] Foster AP, Jackson HA, Stedman K et al. Serological responses to house dust mite antigens in atopic dogs while receiving allergen-specific immunotherapy. Vet Dermatol 2002; 13: 211–229