

Intradermal testing with the storage mite *Tyrophagus putrescentiae* in normal dogs and dogs with atopic dermatitis in Colorado

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Abstract The purpose of this study was to evaluate reactions to intradermal injections of *Tyrophagus putrescentiae* extract in healthy dogs and dogs with atopic dermatitis and to compare the prevalence of positive reactions in the two groups. Twenty-one healthy dogs and 26 atopic dogs were tested intradermally with *T. putrescentiae* extract at 1000, 500, 250, 125, 63, 32 and 16 PNU/mL. Reactions were evaluated objectively and subjectively. A Mann–Whitney test was used to determine differences in grade of reaction to storage mites between healthy dogs and dogs with atopic dermatitis. Positive reactions to storage mite extract were most common at 1000 PNU/mL with approximately one third of normal and atopic dogs showing a positive reaction to *T. putrescentiae*. There was no significant difference in the incidence of positive reactions between normal and atopic dogs for any of the *Tyrophagus* extract concentrations.

INTRODUCTION

Atopic dermatitis (AD) is a common disease in dogs¹ and intradermal testing (IDT) is frequently used to determine allergens involved in the pathogenesis.^{2,3} On the basis of this test, allergen-specific immunotherapy can be formulated.^{4–6} False-positive reactions may develop if the allergen extract used for IDT is too concentrated.^{7,8} Threshold concentrations may differ for various animal species and individual allergens.⁹ House dust mites have been considered important allergens in humans and dogs^{10–12} but their threshold concentrations have been reported to be lower than the threshold concentrations of other allergens in the dog.^{7,8} Storage mites are also considered relevant allergens in humans.^{13–15} In dogs, the involvement of storage mites in AD is a topic of ongoing research.^{16–18} The purpose of this study was to evaluate reactions to intradermal injections of *Tyrophagus putrescentiae* extract in healthy dogs and dogs with AD and to compare the prevalence of positive reactions in the two groups.

MATERIALS AND METHODS

The study protocol was approved by the Colorado State University Animal Care and Use Committee.

Twenty-one healthy dogs with no history or clinical signs of disease and 26 atopic dogs diagnosed by history, clinical examination,¹⁹ and ruling out differential diagnoses such as food adverse reaction or scabies, were included in the study. All atopic dogs had clinical signs all year round. None of the dogs had received oral or injectable glucocorticoids in the 4–6 weeks and topical glucocorticoids, fatty acids or antihistamines in the 2 weeks prior to IDT. Dogs were sedated with 0.4 mg/kg xylazine intravenously, a commonly used sedative not inhibiting skin test reactions.^{20,21} An area of $\approx 15 \times 10$ cm was clipped with a #40 blade. A permanent marker was used to mark the injection sites. A negative and a positive control and *T. putrescentiae* extract in various concentrations were injected intradermally at 0.05 mL with 26 G needles. The negative control was the diluent of the allergen extracts, a solution of 0.04% potassium phosphate, 0.11% sodium phosphate, 0.50% sodium chloride and 0.40% phenol (sterile diluent for allergenic extracts, Greer Laboratories, Lenoir, NC, USA), the positive control was histamine phosphate 0.275 mg/mL (Histatrol, Center Laboratories, Port Washington, NY, USA) diluted to 1:100 000. *T. putrescentiae* extracts (Center Laboratories) were used at concentrations of 1000, 500, 250, 125, 63, 32 and 15 protein nitrogen units (PNU)/mL. Reactions were evaluated objectively 15 and 25 min after injection by measuring the widest diameter of the wheal caused by the injection and subjectively by grading the reactions from 0 (the negative control) to 4 (the positive control or larger), based on diameter, induration and erythema of the wheals. The same clinician (RM) evaluated all intradermal tests. Positive reactions were defined as having a diameter of more than half the sum of the negative and the positive

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control (objective evaluation) or as a subjective score of 2 or more.^{2,3,9} In four normal dogs and one dog with AD, a blood sample was obtained by jugular venepuncture. Serum allergen-specific IgE against *T. putrescentiae* was determined with an enzyme-linked immunosorbent assay (ELISA) using a labelled form of the high-affinity Fc epsilon receptor alpha chain (Fcε RIα) by the Heska Corporation (Fort Collins, CO, USA). This test has been shown to be useful in the identification of offending allergens in canine AD.²² Absorbance values (EA units) were determined on a spectrophotometer. Positive and negative controls were well-pooled sera with known low and high concentrations to the house dust mite *Dermatophagoides farinae*.

Differences between the evaluations at 15 and 25 min were evaluated with a paired *t*-test. Positive reactions were determined for each concentration in each group and a Mann–Whitney test was used to determine differences in grade of reaction to storage mites between healthy dogs and dogs with AD. As the individual injections were not randomized, there was a possibility of detection bias from the clinician reading the intradermal test. Detection bias should lead to a different number of subjectively evaluated positive reactions. Objective and subjective positive and negative evaluations of intradermal injections for all concentrations of the storage mite were compared with a Fisher's exact test. A two-tailed *P*-value of < 0.05 was considered significant.

RESULTS

The mean age of the healthy dogs was 4 years with a range of 1–11 years. There were nine spayed females, six castrated males, five intact females and one intact male. In the atopic group there were 14 male dogs (11 of which were castrated) and 12 females (11 of which were spayed). The mean age in this group was 4.1 years with a range of 15 months to 8 years. Results of the IDT are outlined in Table 1. There was no significant difference between the evaluations of the negative and positive control and the individual concentrations of

T. putrescentiae at the two different time points, 15 and 25 min, with the exception of the evaluations after 15 min of reactions to *T. putrescentiae* objectively at 1000 PNU (*P* = 0.04) and subjectively at 500 PNU (*P* = 0.02) and 1000 PNU (*P* = 0.003) in the atopic group which were greater than the subsequent evaluations at 25 min. For all subsequent evaluations, the results obtained at 15 min were used. A number of healthy and atopic dogs showed positive reactions to intradermal injections of various concentrations of storage mite extract.

Allergen-specific IgE was determined in four normal dogs and one atopic dog. The atopic dog and one normal dog had high EA values of 4.2 and 4.166, respectively; both of these dogs reacted to intradermal injection of antigen at 32 and 16 PNU/mL, respectively. Two normal dogs did not show positive reactions after intradermal injection of *T. putrescentiae* at any concentration at any testing concentration, their EA values were 0.154 and 0.11, which was comparable with the negative control (0.103 EA). One dog had an EA value of 1.24 and reacted positive to intradermal injection of antigen at concentrations of 63 PNU/mL.

Comparing the objective and subjective positive reactions of normal vs. atopic dogs, there was no significant difference for any of the *T. putrescentiae* extract concentrations between the two groups. Comparing objective and subjective evaluations for all concentrations of *T. putrescentiae*, a mean of 12.3% more positive reactions with a range of 0–23% were detected with subjective evaluation. This was significant (*P* = 0.0008).

DISCUSSION

This study evaluated intradermal reactions to various concentrations of *T. putrescentiae* in normal and atopic dogs and found no difference in reactivity between the two groups. Storage mites belong to the family Glycyphagidae (*Glycophagus*, *Lepidoglyphus*) and Acaridae (*Tyrophagus*, *Acarus*).²³ They are free-living inhabitants of decaying organic matter and although they tolerate a wide range of temperatures,

Table 1. Results of intradermal testing with *Tyrophagus putrescentiae* extract in normal dogs and dogs with atopic dermatitis

Extract concentration (PNU/mL)	Healthy dogs (<i>n</i> = 21)				Atopic dogs (<i>n</i> = 26)			
	Objective		Subjective (≥ 2)		Objective		Subjective (≥ 2)	
	Positive reactions (%)	Mean diameter ± SD (mm)	Positive reactions (%)	Mean score ± SD	Positive reactions (%)	Mean diameter ± SD (mm)	Positive reactions (%)	Mean score ± SD
1000	8 (38)	12 ± 2	10 (48)	2.1 ± 1.5	6 (23)	12 ± 3	9 (35)	2.3 ± 1.3
500	8 (38)	12 ± 2	11 (52)	2.1 ± 1.5	6 (23)	11 ± 3	11 (42)	2.1 ± 1.2
250	6 (29)	11 ± 2	10 (48)	2.0 ± 1.4	3 (12)	10 ± 3	9 (35)	1.3 ± 1.2
125	6 (29)	11 ± 2	8 (38)	1.5 ± 1.6	4 (15)	10 ± 3	7 (27)	0.8 ± 1.1
63	5 (24)	10 ± 2	7 (33)	1.0 ± 1.4	1 (4)	9 ± 4	2 (8)	0.4 ± 0.7
32	2 (10)	10 ± 1	6 (29)	0.7 ± 1.1	1 (4)	8 ± 3	1 (4)	0.3 ± 0.7
16	1 (5%)	10 ± 1	3 (14)	0.4 ± 0.7	0 (0)	8 ± 4	0 (0)	0.2 ± 0.4

PNU, Protein Nitrogen Units; SD, standard deviation.

they prefer warm, moist environments.²⁴ A large body of literature suggesting a relationship between storage mites and allergic disease has developed in human medicine. Storage mite hypersensitivity based on IDT and serum testing for allergen-specific IgE has been recognized in humans with rhinitis, asthma and AD.^{25–27} Positive reactions of dogs with AD to intradermal injections with *T. putrescentiae*, *Glycophagus domesticus* and *Acarus siro* have been reported in various European countries.^{16,17,28} The percentage of positive reactions is similar to that found in our study, although different or undefined inclusion criteria, allergen extract concentrations and sources make direct comparison difficult. Hillier¹⁸ used the same allergen extract and found that > 80% of allergic dogs reacted to *T. putrescentiae* at 250 PNU/mL or higher. Hillier's numbers are much higher than the corresponding ones in our study, which may be due to different climatic conditions such as a higher humidity favouring higher numbers of storage mites in the environment with subsequent greater exposure of dogs.

Nesbitt *et al.*²⁹ estimated 20–30% more positive reactions with IDT on subjective compared with objective evaluation. This estimation was based on a comparison of the subjective evaluation of 135 intradermal tests and an objective evaluation of 100 intradermal tests performed by Scott.³⁰ In our study, objective and subjective evaluations were graded differently in 12.3% of the reactions. These differences are smaller than those mentioned by Nesbitt, but the same clinician evaluated the patients subjectively and objectively. However, a detection bias of the clinician evaluating the reactions subjectively was present, based on the significant increase of positive reactions compared with the objective evaluation. As comparisons between atopic and normal dogs using exclusively objective evaluations had the same results as those using exclusively subjective evaluations, this detection bias was not considered to be a confounding factor of the study.

In this study, there was no significant difference between the number of positive reactions 15 and 25 min after injection of the extract for most concentrations. In the differing groups, the majority of positive reactions were due to an increased number at 15 min that became negative after a further 10 min. This seems to support the (albeit unsubstantiated) general guidelines in veterinary medicine to evaluate IDT in the dog 15 min after injecting the allergen.⁹ Thus, positive reactions after 15 min were considered relevant and used as a basis for statistical evaluation.

House dust mites have been reported to cause a high number of positive reactions in normal dogs at concentrations classically used for IDT and thus lower concentrations were recommended.^{7,8} Storage mites and house dust mites share some antigens relevant in human medicine.^{31–33} Thus, it seems conceivable that the ideal concentration for IDT in dogs is lower with storage mites than that of many other allergens and is similar to house dust mites. In our study, a large number of healthy dogs and dogs with AD showed

positive reactions to intradermal injections with *T. putrescentiae* extract at 1000 PNU/mL. The extracts may be irritating and reactions may not, therefore, reflect true allergic responses. The second possible explanation for the positive reactions to the storage mite extracts is production of allergen-specific IgE against storage mite antigens with no associated clinical signs. Storage mites are widely distributed²⁴ and most dogs would be exposed to these antigens. Even in areas like Colorado where house dust mite concentrations are decreased due to the low environmental humidity, storage mites may be readily detected (C. McCall, pers. commun.). Serum allergen-specific IgE against *T. putrescentiae* was detected in all five dogs tested. The concentrations correlated well with the intradermal test reactivity, although the sample size was too small to determine a meaningful statistical correlation. These results are suggestive of intradermal test reactivity caused by cross-linking of allergen-specific IgE on the surface of cutaneous mast cells leading to subsequent degranulation and positive test reaction. They further emphasized the importance of achieving a diagnosis of canine AD prior to any attempt to identify offending allergens and to correlate the results of IDT or serum testing for allergen-specific IgE to the patient's environment and clinical history. Based on these findings, a specific concentration of storage mite extracts to avoid irritant reactions cannot be recommended.

Comparing the prevalence of positive reactions in healthy dogs and dogs with AD, there was no significant difference for any of the concentrations. Based on these findings, it is not possible to differentiate dogs with clinical signs due to storage mite hypersensitivity from dogs with no clinical signs but having positive intradermal reactions to *T. putrescentiae* extract due to storage mite exposure. These results are similar to the results of a study evaluating intradermal reactions and allergen-specific IgE against *Dermatophagoides farinae* in normal dogs and dogs with AD.¹² Although the atopic dogs had higher concentrations of IgE, the difference was not statistically significant. Similarly, most normal dogs had positive reactions to *D. farinae* extract used at a dilution of 1:10 000. Functional heterogeneity of IgE or heterogeneity of mast cell receptors for IgE was proposed as possible explanations. Demonstrated physicochemical heterogeneity of IgE and increased histamine release from basophils of atopic compared with normal dogs despite a similar cell content of total histamine are further evidence for this functional IgE heterogeneity.^{34,35} Further studies are needed to evaluate the clinical significance of storage mite allergens in canine atopic dermatitis.

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Résumé Le but de cette étude était d'évaluer les réactions à l'injection intradermique d'extraits *Tyrophagus (T.) putrescentiae* chez des chiens sains et des chiens souffrant de dermatite atopique, et de comparer la prévalence des réactions positives dans les deux groupes. Vingt et un chiens sains et vingt-six chiens présentant une dermatite atopique ont été testés par l'injection intradermique d'extraits de *T. putrescentiae* aux doses de 1000 protein nitrogen units (PNU)/ml, 500, 250, 125, 63, 32 et 16 PNU/ml. Les réactions ont été évaluées objectivement et subjectivement. Un test de Mann-Whitney a été utilisé pour déterminer des différences entre les réactions entre les chiens sains et les chiens à dermatite atopique. Les réactions positives étaient plus fréquentes à la concentration de 1000 PNU/ml avec environ un tiers des chiens sains et des chiens atopiques présentant une réaction à *T. putrescentiae*. Aucune différence significative n'a été observée pour l'incidence des réactions positives entre chiens sains et chiens atopiques, et ce pour toutes les concentrations testées.

Resumen El objetivo del presente estudio fue evaluar las reacciones a inoculaciones intradérmicas de extracto de *Tyrophagus (T.) putrescentiae* en perros sanos y en perros con dermatitis atópica y comparar la prevalencia de reacciones positivas en los dos grupos. Veintiún perros sanos y veintiséis perros atópicos fueron analizados mediante pruebas intradérmicas con extracto de *T. putrescentiae* a 1000 unidades de proteína nitrógeno (PNU)/ml, 500, 250, 125, 63, 32 y 16 PNU/ml. Las reacciones fueron evaluadas objetivamente y subjetivamente. Se utilizó una prueba Mann-Whitney para determinar las diferencias en el grado de reacción a los ácaros de depósito entre perros sanos y perros con dermatitis atópica. Las reacciones positivas al extracto de los ácaros de depósito fueron más frecuentes a 1000 PNU/ml con aproximadamente una tercera parte de los perros normales y atópicos mostrando una reacción positiva a *T. putrescentiae*. No había una diferencia significativa en la incidencia de reacciones positivas entre perros normales frente a perros atópicos para cualquiera de las concentraciones de extracto de *Tyrophagus*.

Zusammenfassung Ziel dieser Studie war es, Reaktionen auf intradermale Injektionen von *Tyrophagus (T.) putrescentiae*-Extrakten bei gesunden Hunden und Hunden mit atopischer Dermatitis zu beurteilen und die Prävalenz positiver Reaktionen in diesen zwei Gruppen zu vergleichen. Einundzwanzig gesunde Hunde und sechszwanzig atopische Hunde wurden mit *T. putrescentiae*-Extrakt in einer Konzentration von 1000 protein nitrogen units (PNU)/ml, 500, 250, 125, 63, 32 und 16 PNU/ml getestet. Die Reaktionen wurden objektiv und subjektiv beurteilt. Ein Mann-Whitney-Test wurde verwandt, um Unterschiede im Grad der Reaktion auf Vorratsmilben zwischen gesunden Hunden und Hunden mit atopischer Dermatitis zu bestimmen. Positive Reaktionen auf den Vorratsmilbenextrakt waren bei 1000 PNU/ml am häufigsten, wobei ungefähr ein Drittel der normalen und atopischen Hunde positive Reaktionen auf *T. putrescentiae* zeigte. Es gab keinen signifikanten Unterschied in der Inzidenz positiver Reaktionen zwischen normalen und atopischen Hunden bei irgendeiner Konzentration des *Tyrophagus*-Extraktes.