

Environmental and oral challenge with storage mites in beagles experimentally sensitized to *Dermatophagoides farinae*

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Abstract

This study aimed to investigate whether challenge with storage mites elicited flare ups of atopic dermatitis (AD) in *Dermatophagoides farinae* sensitized atopic Beagles housed in a house dust and storage mite-free environment. Atopic Beagles were environmentally challenged with 50 mg of *Tyrophagus putrescentiae* for three days in a row. Clinical signs were scored before, 6 h after each challenge and then every 24 h for a total of 5 days using a Canine Atopic Dermatitis Extent and Severity Index. Four healthy Beagles, negative on serology and intradermal testing for both house dust and storage mites, were used as controls and similarly challenged. A month after environmental challenge, the atopic Beagles were challenged by the oral route (50 mg of *T. putrescentiae* for three days in a row) and evaluated as described. Analyses of variance (ANOVAs) were used for comparisons between groups and types of challenges. All atopic Beagles developed erythematous pruritic lesions clinically compatible with AD on the face, pinnae, feet and ventral abdomen after both environmental and oral challenge. Control dogs did not develop dermatitis except for mild pinna erythema in one dog. In the environmental challenge, ANOVA showed a significant effect of time, group, and group x time interaction, with atopic Beagles showing significantly higher scores than the controls. There were no significant differences in clinical scores after oral and environmental challenge in the atopic group. Cross-reactivity between house dust and storage mites could therefore contribute to flare ups of AD in house dust mite allergic dogs.

Introduction

House dust mites, such as *Dermatophagoides farinae*, are with the most important environmental allergens in canine atopic dermatitis (AD).^{1,2} Positive intradermal tests and/or serology to storage mites, such as *Acarus siro*, *Tyrophagus putrescentiae*, and *Lepidoglyphus destructor*, are also frequently encountered.¹ However, the role of these mites remains difficult to understand since storage mites are rarely detected in the microenvironment of both healthy^{3,4} and atopic dogs.⁵ Simultaneous sensitization to two or more house dust and/or storage mites may occur in mite-sensitive dogs with AD,⁶ depending on the mite species involved.

A recently published study demonstrated extensive cross-reactivity among two house dust (*D. farinae*, *D. pteronyssinus*) and three storage mites (*A. siro*, *T. putrescentiae*, *L. destructor*) in a colony of atopic Beagles experimentally sensitized to *D. farinae* using intradermal testing and enzyme-linked immunosorbent assay (ELISA) serology, and ELISA cross-inhibition.⁷ This was suspected based on the results of Since no evidence of exposure to storage mites was found upon analysis of food and dust samples from the environment of these dogs, it was concluded that the positive results of intradermal testing and serology for storage mites were probably due to cross-reactivity and not due to concurrent sensitization. The clinical significance of this cross-reactivity is, however, unknown at this time and would be hard to investigate in privately owned patients since controlled allergen challenge may not be possible and past exposure to different mite species would remain obscure.

The availability of a colony of atopic Beagles, housed in a controlled environment with controlled allergen challenge, could help to elucidate the clinical relevance, if any, of allergen cross-reactivity. These dogs develop lesions typical of AD after both environmental and oral challenge with *D. farinae*.^{8,9} The purpose of this study was therefore to assess whether pruritic dermatitis clinically compatible with AD could be elicited by exposure to storage mites in *D. farinae* sensitized atopic dogs. The first step was to determine whether environmental exposure to storage mites would elicit clinical signs compatible with AD in the atopic dogs and for this purpose both normal and atopic dogs were similarly challenged. The atopic dogs were then further challenged to evaluate the impor-

tance of environmental and oral exposure. *T. putrescentiae* was selected for this study since it is the most likely species to be found in dry dog food,¹⁰ and in many reports of dogs with natural AD positive intradermal tests and serology to this mite are more frequent than those to the other storage mites.^{11,12}

Materials and methods

This study was designed as a prospective controlled study. All procedures were approved by the Institutional Animal Care Committee of the University of Florida.

Animals

Atopic Beagles (atopic dogs n = 13).

Thirteen atopic Beagles, epicutaneously sensitized to *D. farinae* at a young age and known to develop AD upon environmental exposure to *D. farinae* were used. Sensitization started when the dogs were 3 weeks old. A pure culture of *D. farinae* (RMB83M, Greer Laboratories, >99% pure whole bodied natural milled *D. farinae*). Allergenic analysis in an external laboratory (Indoor Biotechnologies, Charlottesville, VA, USA) confirmed that it contained only trace amounts of Der p 1 (2.32 µg/mL) compared to Der f 1 (1862 µg/mL). One gram of *D. farinae* culture was mixed with sterile saline (2.5 mL) to create a thick paste (400 mg/mL). Each puppy received 0.3 mL painted onto small areas of previously untreated skin on the axillae and groin using a soft brush twice weekly for a total of 12 weeks. Successful sensitization was demonstrated by: (1) development of lesions at the sites of application, (2) flare up of dermatitis clinically compatible with AD upon environmental allergen challenge,⁸ (3) positive intradermal skin test reactivity to *D. farinae* at 25PNU/mL (positive reaction defined as 2+ or higher on subjective evaluation, where 0 is the score of the saline injection and 4+ is the score of the histamine injection site), and (4) positive serology for *D. farinae* (>150 ELISA Antigen [EA] units on ALLERCEPT® Heska Corporation, Ft Collins, CO, USA). According to the manufacturer's recommendations for this serology testing, results higher than 150 EA are considered 'positive'.

The atopic dogs comprised six adults (4.75–5.5 years) and seven 5-month old puppies. There were three intact males and ten intact females. The last environmental challenge with *D. farinae* was performed 37–48 days before the current experiments. Sensitization was demonstrated by the development of clinical signs typical of canine AD accompanied by an increase of a Canine Atopic Dermatitis Extend and Severity Index (ICADESI), median increase = 35.1) after environmental exposure to 50 mg *D. farinae* allergen preparation, and by positive serology (>3,000 EA units on ALLERCEPT® Heska) for *D. farinae*. The atopic Beagles had no history of parasitic mite infestations or experimental exposure to other mites, with the exception of four intradermal injections with a 50/50 mixture of *D. farinae* and *D. pteronyssinus* in the adult Beagles only (Greer Laboratories Inc.).

Prior to the beginning of the experiments, all the dogs were intradermally tested with *D. farinae*, *D. pteronyssinus*, *A. siro*, *T. putrescentiae*, and *L. destructor* allergen extracts (Greer Laboratories Inc.) at three dilutions (1/5000, 1/25 000, and 1/100 000 w/v). There were variable positive reactions to all of them, depending on the allergen extract, concentration and the method of evaluation (subjective or objective).⁷ Co-sensitization occurred in >50% of dogs between *D. farinae* and *D. pteronyssinus*, between *D. farinae* and the three storage mites (*A. siro*, *T. putrescentiae* and *L. destructor*), between *D. pteronyssinus* and the three storage mites, as well as among the storage mites.⁷ Similarly, serology performed at two different laboratories (Heska Corporation and Greer Laboratories Inc.) was positive for all five mites.⁷ The frequency of co-sensitization ranged from 45 to 100% for all possible pairs of mites. ELISA cross-inhibition revealed that: (1) *D. farinae* was strong inhibitor of IgE reactivity against the remaining four mites (*D. pteronyssinus*, *A. siro*, *T. putrescentiae* and *L. destructor*); (2) *D. pteronyssinus* was a strong inhibitor of *L. destructor*; (3) *A. siro* was strong inhibitor of the remaining

four mites (*D. farinae*, *D. pteronyssinus*, *T. putrescentiae* and *L. destructor*); (4) *T. putrescentiae* was strong inhibitor of the remaining four mites (*D. farinae*, *D. pteronyssinus*, *A. siro* and *L. destructor*); and (5) *L. destructor* was not strong inhibitor of any mite tested.⁷

Normal Beagles (healthy controls; n = 4)

The control group was comprised of clinically healthy 4- to 5.5-month old laboratory Beagle puppies with no history of skin disease or experimental exposure to house dust or storage mites. They included three intact males and one intact female. These dogs had negative intradermal tests and serology for both house dust and storage mites.

Housing conditions

All dogs were housed in the same temperature and humidity controlled research facility at the University of Florida (temperature was set at 18 °C and the average humidity was 68%). The dogs were housed in individual cement runs that were cleaned daily using high temperature and high pressure washes. Stuffed toys, carpets, soft bedding or anything that could trap dust were not allowed in these runs. Walls and air filters were checked monthly to ensure the absence of mites in the environment in between challenges. Results were always below the detection level of the test (MITE-T-Fast™ Allergen Detection System; Aveho Biosciences, Monterey, TN, USA).

Dust was also collected using a 1440W vacuum cleaner from kennels (walls, ceiling, floor and air filters) and dry food containers. A sample of dry food was manually collected from the bottom of opened dry food bags. The dust and food samples were examined for the presence of mites after mechanical sieving followed by sodium chloride flotation.¹³ The guanine content was also assessed (Acarex® test; Lifestyle Vertiebsgesellschaft mbH, Wahlstedt, Germany). Three additional randomly selected household dust samples were simultaneously analyzed (one of them was contaminated with dry dog food particles to macroscopically resemble dry dog food samples) in a blinded fashion. Samples from the kennels, food containers and the dry food were negative for mites. In contrast, 2.6–119.6 mites/g of dust were found in the three household samples (including, in decreasing order of frequency, *D. pteronyssinus*, *Dermatophagoides* spp., *T. putrescentiae*, *Acaridae* and *D. farinae*). The Acarex® test was negative for all samples examined.⁷

Diet

All dogs were fed the same dry diet (Hill's Science Diet Adult Canine Maintenance dry) prior and during the study. The food was stored in a temperature and humidity controlled room, separate from where the animals were kept. The temperature of this room was 15 °C and average recorded humidity was 60%. Opened bags were stored in an enclosed container and used within 3 days due to the large number of dogs in the colony.

Allergen challenge

Environmental challenge

Both atopic and normal Beagles were environmentally challenged following the same protocol. For the environmental challenge, a water solution containing 50 mg of *T. putrescentiae* (Greer Laboratories Inc.) mixed with saline was placed on the floor of travelling kennels, where atopic and healthy Beagles were allowed to spend 3 h/day, 3 days in a row. With this method, epicutaneous as well as some oral exposure to the mite was achieved. This method of challenge has been described in detail previously.^{8,9} Dogs were evaluated and clinical signs scored at baseline, 6 h after the beginning of each challenge, and daily afterwards for a total of 5 days (0, 6, 24, 30, 48, 54, 72 and 96 h). After a 30-day wash-out period, the atopic dogs were additionally challenged by the oral route.

Oral challenge.

For the oral challenge a water solution containing 50 mg of *T. putrescentiae* mixed with saline was fed to the atopic Beagles with a small

amount of canned dog food (Hill's Science Diet Adult Savory Chicken Entrée), 3 days in a row. Dogs were evaluated using the same evaluation times as in the environmental challenge protocol.

Clinical evaluation

Dogs were evaluated using a modified Canine Atopic Dermatitis Extent and Severity Index (CADESI).¹⁴ Briefly, the dog's body was divided into small sections and each of them received a score based on the clinical signs evaluated. Total score was calculated by adding the scores of all body sites and was used in the statistical analysis. Clinical signs evaluated included diffuse erythema, erythematous macules, papules, excoriations, alopecia, and were scored from 0 (absent) to 3 (severe) for each site and each symptom. This version was modified from the original CADESI in the sense that lichenification was not evaluated. As this is a sign of chronic disease and this experiment aimed at evaluating more "acute" changes, it was decided not to score lichenification.

Statistics

Clinical scores were analyzed using repeated-measures analyses of variance (ANOVA). The method of allergen challenge (environmental or oral), the group (atopic or normal Beagles), and the time after challenge were evaluated as variable factors. For each time point, unpaired *t* tests were also used for comparison between the two methods of allergen challenge. All analyses were performed using the statistical software package SAS System for Windows version 9.0 (SAS Institute, Cary, NC, USA). A $P < 0.05$ was considered significant. All data are presented as mean $\pm$ standard error of the mean (SEM), unless otherwise indicated.

Results

All the atopic Beagles developed an erythematous pruritic dermatitis clinically compatible with AD after both environmental and oral challenge. In both cases lesions appeared over the face, pinnae, interdigital skin and ventral abdomen (Figures 1 and 2). Control dogs did not develop pruritus or dermatitis after environmental challenge, except for one dog that developed mild pinna erythema.

When mean CADESI scores after environmental challenge were compared between the two groups (Figure 3), ANOVA showed a significant main effect of group ($P < 0.0015$), time ($P < 0.001$) and a significant group $\times$ time interaction ($P < 0.0015$), with higher scores in the atopic group than the controls. Comparison of mean CADESI scores of the environmental and oral challenges in



Figure 1. Pinnal erythema in an atopic Beagle, 24 h after environmental exposure to *Tyrophagus putrescentiae*.



Figure 2. Erythematous ventral interdigital skin of an atopic Beagle, 48 h after oral exposure to *Tyrophagus putrescentiae*.

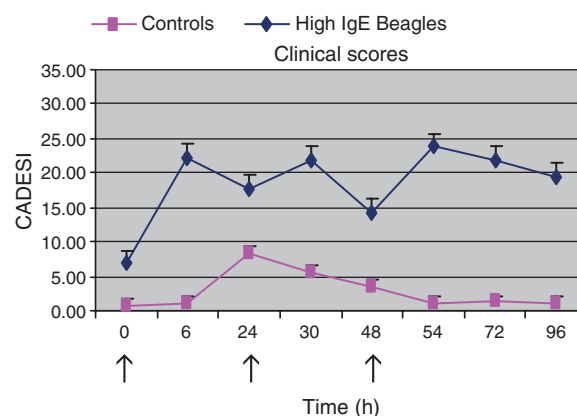


Figure 3. Means and standard error of the mean for the modified Canine Atopic Dermatitis Extent and Severity Index scores of both atopic and normal Beagles after environmental challenge with *Tyrophagus putrescentiae* for 3 days. Analyses of variance showed a significant main effect of group ($P < 0.0015$), time ($P < 0.001$), and group $\times$ time interaction ($P < 0.0015$), with increasing scores over time in the atopic Beagles but not in controls. Arrows indicate the times at which allergen challenge was done.

the atopic group (Figure 4), revealed a significant effect of time ($P < 0.0001$) and exposure $\times$ time ($P < 0.0001$), but not an effect of exposure. Unpaired *t* tests only showed significant differences between the types of exposure at 6 and 24 h, with environmental exposure yielding higher scores at 6 h ($P = 0.0003$) and oral exposure at 24 h ($P = 0.003$).

Discussion

This study shows that either environmental or oral challenge with the storage mite *T. putrescentiae* exacerbates AD in house dust mite (*D. farinae*) sensitized atopic Beagles. This indicates that cross-reactivity between house

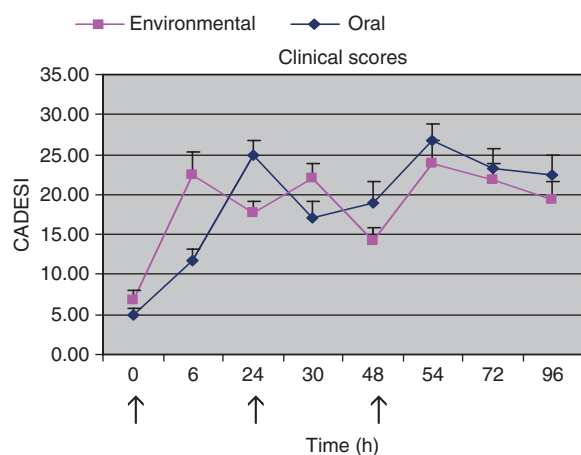


Figure 4. Means and standard error of the mean for the modified Canine Atopic Dermatitis Extent and Severity Index scores of atopic Beagles after oral and environmental challenge with *Tyrophagus putrescentiae* for 3 days. No significant effect of exposure was found, but significant effects of time and exposure $\times$ time were detected ($P < 0.0001$). Unpaired t tests showed that environmental and oral exposure were significantly different at 6 and 24 h. Arrows indicate the times at which allergen challenge was done.

dust and storage mites can be clinically relevant in atopic dogs.

Tyrophagus putrescentiae was used for this study positive skin test and serology results are frequently reported.¹⁵ Furthermore, when the atopic Beagles of this colony were intradermally tested with three dilutions of storage mite allergen extracts and evaluated objectively and subjectively by two investigators, a higher number of positive results to *T. putrescentiae* ($n = 64$) compared to *A. siro* ($n = 43$) and *L. destructor* ($n = 54$) were recorded.⁷ In addition, the frequency of positive serology to *T. putrescentiae* (84.6%) was equal to or higher than that for the other two storage mites, and ELISA cross-inhibition revealed that *T. putrescentiae* was a strong inhibitor of *D. farinae*.⁷ Thus, *T. putrescentiae* was considered a good candidate for this initial evaluation of the potential clinical relevance of cross-reactivity between house dust and storage mites.

There are some notable differences in the microclimate requirements and in the places where house dust and storage mites flourish. The ideal environmental humidity for storage mite proliferation ($>75\%$) is higher than that for house dust mites (60–75%).¹⁶ The former are usually associated with humid areas of households (kitchen, bathroom, larders, etc.) compared to house dust mites, which flourish in bedrooms, living room carpets and upholstered furniture.¹⁷ The evidence of *in vivo* and *in vitro* cross-reactivity from this and other trials suggests that environmental control for dust mites should not be restricted to mattresses and carpeting,¹⁸ but should also include storage mite preferred areas, even if atopic dogs are sensitive only to house dust mites. Another consideration is that limitation of house dust exposure by keeping an atopic dog strictly outdoors may not result in clinical benefit if the animal can be exposed to storage mites by having access to barns or other suitable habitats.¹⁹

It is interesting that oral and environmental exposure to storage mites overall appears to have equal clinical

significance. Statistically significant differences between the two routes of allergen exposure were detected only at two time points, with environmental exposure yielding higher scores at 6 h and oral exposure at 24 h. The reason for this is unknown and could reflect differences in immunopathogenesis or a statistical error. Environmental challenge of the same colony of dogs with *D. farinae*, in contrast, appears to be more important than the oral challenge,⁹ suggesting that the epicutaneous route of exposure may be crucial for house dust mite-related symptoms. A possible explanation for the difference may be the age of the dogs. The dogs in the *D. farinae* study⁹ were 6 years old while the present study included both adults and puppies (5 months old). Although the number of dogs is too small to make any analysis based on age, the immaturity of the digestive tract of puppies may have affected the response.

Storage mites are not typically present in commercial diets,⁴ but they have been found in pre-packaged dry dog foods, especially when they are stored in open bags.¹⁰ This may be a possible source of exposure that could lead to flare ups of AD in house dust mite-sensitive allergic dogs, similar to the situation in atopic humans.²⁰ These findings therefore emphasize the importance of controlling oral exposure of dust mite sensitive atopic dogs through proper storage of any open bag of dry dog food.

Another intriguing question is whether inclusion of just one mite could be sufficient for allergen-specific immunotherapy of dogs that are sensitive to multiple house dust and storage mite species. A recent randomized placebo controlled trial that included dogs allergic to *D. farinae* and at least one additional allergen found that *D. farinae* restricted immunotherapy was insufficient to control the disease and that the solution used for immunotherapy should be customized to include the remaining offending allergens.²¹ Since several dogs included in the study were allergic to just mites and not to any pollen allergens, it appears that it is still important to include all mites to increase the success of immunotherapy.

The protocol for the environmental and oral challenge in this study was the same as previously described.^{8,9} The environmental challenge has been designed to allow all possible routes of exposure, since the dogs were free to ingest, inhale or simply contact the allergen. The dose of 50 mg/dog/day was established in previous reports of house dust mite challenges.^{8,9} The amount of storage mites encountered in real life greatly varies, depending on the temperature and humidity and, in the case of dry dog foods, the environmental conditions and the duration of storage. It was recently reported that 200 mg of poorly stored food would contain as many as 181 mites.¹⁰ It was estimated that 50 mg of mite culture would contain at least 100 mites. Since a Beagle would eat 200 mg of dry dog food/day a dose of 50 mg of allergen for the challenges was considered representative of the amount of storage mite exposure due to poorly preserved diet. To control for the possibility that such a dose would be excessive or irritant, normal controls were exposed to the same dose. Since minimal reaction was detected in the

normal Beagles it was decided that the dose was not irritating.

There are several limitations to this study. The first is the small number of dogs used. Although it would have been desirable to enrol more animals, particularly in the control group, statistically significant differences were still evident. Another limitation is the fact that the control group only included young dogs and no adults while both age groups were present in the atopic group. It would have been more appropriate to have the control match the atopic group for age. Unfortunately, due to limitations in the number of normal Beagles housed in the same research facility only these dogs were available for the present experiment. Additionally, it would have been better to orally challenge the normal controls too. Unfortunately, these dogs belonged to another investigator and had just been enrolled in another long-term project at the time that the oral challenge was done. It is important to point out, however, that the primary purpose of this study was to assess whether challenge with storage mites in *D. farinae* sensitized dogs would lead to flare ups of AD. Once this was proven by the environmental challenge, the additional question was how the various routes of allergen challenge would relate to each other in the atopic group.

An important consideration is how AD in this experimental group of dogs relates to AD in dogs with naturally occurring disease, and whether such dogs would have a similar clinical response when challenged with storage mites. This particular colony of atopic Beagles has been demonstrated to suffer from a dermatitis that is clinically, immunologically and histologically similar to naturally occurring AD.⁸ Despite the limitations intrinsic to experimental models, they are nevertheless very useful in that allergen exposure and confounding factors can be tightly controlled.

Cross-reactivity between house dust and storage mites has been proved in atopic humans^{22–24} and dogs.^{7,25} Although no storage mites were found in the environment and in the food of these dogs, it cannot be excluded that, at some point in time, their commercial dog food contained storage mites, thus resulting in concurrent sensitization rather than in cross-reactivity. Although this possibility cannot be ruled out since food was not consistently evaluated for storage mites over time, it is considered unlikely due to the controlled environmental conditions of the research facility (enclosed, air conditioned) and the fact that food was promptly used (e.g. one new bag of food opened every third day).

In summary, flare ups of AD could be induced in house dust mite sensitized atopic Beagles when challenged both orally and environmentally with the storage mite *T. putrescentiae*. It is therefore likely that cross-reactivity between house dust and storage mites may be clinically relevant, and that exposure of house dust mite allergic dogs to storage mites may exacerbate their condition.

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References

- Hill PB, DeBoer DJ. The ACVD task force on canine atopic dermatitis (IV): environmental allergens. *Veterinary Immunology and Immunopathology* 2001; 81: 169–86.
- Nuttall TJ, Hill PB, Bensignor E, Willemse T. House dust and forage mite allergens and their role in human and canine atopic dermatitis. *Veterinary Dermatology* 2006; 17: 223–35.
- Jackson AP, Foster AP, Hart BJ, *et al.* Prevalence of house dust mites and *Dermatophagoides* group 1 antigens collected from bedding, skin and hair coat of dogs in south-west England. *Veterinary Dermatology* 2005; 16: 32–8.
- Henneveld K, Beck W, Mueller RS. Evaluation of storage mite contamination of dry dog food and the environment. *Veterinary Dermatology* 2006; 17: 209.
- Farmaki R, Saridomichelakis MN, Koutinas AF, *et al.* The presence of dust mite species in the environment of dust mite-sensitized atopic dogs. *Veterinary Dermatology* 2004; 15: 54.
- Arlian LG, Schumann RJ, Morgan MS, *et al.* Serum immunoglobulin E against storage mite allergens in dogs with atopic dermatitis. *American Journal of Veterinary Research* 2003; 64: 32–6.
- Saridomichelakis M, Marsella R, Lee K., *et al.* Assessment of cross-reactivity among five species of house dust and storage mites. *Veterinary Dermatology* 2008; 19: 67–76.
- Marsella R, Olivry T, Nicklin C, *et al.* Pilot investigations toward a model for Canine Atopic Dermatitis: Environmental challenges with house dust mites in high IgE producing Beagles, in mite hypersensitive dogs with natural occurring atopic dermatitis and in normal dogs. *Veterinary Dermatology* 2006; 17: 24–35.
- Marsella R, Nicklin C, Lopez J. Studies on the role of various routes of allergen exposure in high IgE producing Beagle dogs sensitized to house dust mites. *Veterinary Dermatology* 2006; 17: 306–12.
- Brazis P, Serra M, Sellés A, *et al.* Evaluation of storage mite contamination of commercial dry dog food. *Veterinary Dermatology* 2008; 19: 209–14.
- Kozak M, Chandoga P, Bilek J. Canine atopy in Slovakia. *Medycyna Weterinarna* 2002; 60: 34–7.
- Beco L, Fontaine J. Major aeroallergens in Belgium: regional influences. In: *Proceedings 2nd European Congress F.E.C.A.V.A.* Brussels, Belgium. 1995: 311–3.
- van Bronswijk JEMH. *Dermatophagoides pteronyssinus* (Trouessart, 1987) in mattress and floor dust in a temperate climate (Acari: Pyroglyphidae). *Journal of Medical Entomology* 1973; 10: 63–70.
- Olivry T, Rivierre C, Jackson HA, *et al.* Cyclosporine decreases skin lesions and pruritus in dogs with atopic dermatitis: a blinded randomized prednisolone-controlled trial. *Veterinary Dermatology* 2002; 13: 77–87.
- Mueller RS, Fieseler KV, Rosychuk RA, *et al.* Intradermal testing with the storage mite *Tyrophagus putrescentiae* in normal dogs and dogs with atopic dermatitis in Colorado. *Veterinary Dermatology* 2005; 16: 27–31.
- Wharton GW. House dust mites. *Journal of Medical Entomology* 1976; 12: 577–621.
- Warner A, Boström S, Möller C, Kjellman NI. Mite fauna in the home and sensitivity to house-dust and storage mites. *Allergy* 1999; 54: 681–90.
- Swinnen C, Vroom M. The clinical effect of environmental control of house dust mites in 60 house dust mite-sensitive dogs. *Veterinary Dermatology* 2004; 15: 31–6.
- Fernandez-Caldas E. Mite species of allergologic importance in Europe. *Allergy* 1997; 52: 383–7.
- Matsumoto T, Isano T, Hamaguchi M, *et al.* Systemic anaphylaxis after eating storage mite-contaminated food. *International Archives of Allergy and Immunology* 1996; 109: 197–200.
- Willemse T, Bardagi M, Carlotti DN, *et al.* *Dermatophagoides farinae*-specific immunotherapy in atopic dogs with hypersensitivity to multiple allergens: a randomised, double blind, placebo-controlled study. *Veterinary Journal* 2009; 180: 337–42.

22. Johansson E, Johansson SG, van Hage-Hamsten M. Allergenic characterization of *Acarus siro* and *Tyrophagus putrescentiae* and their cross-reactivity with *Lepidoglyphus destructor* and *Dermatophagoides pteronyssinus*. *Clinical and Experimental Allergy* 1994; 24: 743–51.
23. Munhbayarlah S, Park JW, Ko SH, *et al.* Identification of *Tyrophagus putrescentiae* allergens and evaluation of cross-reactivity with *Dermatophagoides pteronyssinus*. *Yonsei Medical Journal* 1998; 39: 109–15.
24. Park JW, Ko SH, Yong TS, *et al.* Cross-reactivity of *Tyrophagus putrescentiae* with *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* in urban areas. *Annals of Allergy, Asthma and Immunology* 1999; 83: 533–9.
25. Lee KW, Wheeling MS, Blankenship KD, *et al.* Prevalence of specific IgE to mite antigens in the serum of dogs in the USA. *Veterinary Dermatology* 2006; 17: 211.

Résumé Cette étude a pour but d'évaluer si les acariens de stockage peuvent provoquer des poussées de dermatite atopique (AD) chez des Beagles atopiques sensibilisés à *Dermatophagoides farinae* vivant dans un environnement ne contenant aucun acarien de stockage ou acarien de poussière de maison. Les Beagles atopiques ont été mis en présence de 50 mg de *Tyrophagus putrescentiae* dans l'environnement pendant trois jours. Les signes cliniques ont été notés avant, 6h après chaque exposition puis toutes les 24h pendant 5 jours à l'aide du CADESI (Canine Atopic Dermatitis Extent and Severity Index). Quatre Beagles sains, avec sérologies négatives et tests intradermiques négatifs pour les acariens de stockage et de poussière de maison ont été inclus comme groupe contrôle. Un mois après l'exposition environnementale, les chiens atopiques ont été exposés à 50mg de *T. putrescentiae* pendant 3 jours par voie orale puis évalués comme précédemment. Les analyses de variance (ANOVA) ont été utilisées pour comparer les groupes et les types d'exposition. Tous les Beagles atopiques ont développés des lésions érythémateuses prurigineuses cliniquement compatibles avec l'AD sur la face, les pavillons auriculaires, les pieds et l'abdomen ventral après exposition environnementale et orale. Les chiens contrôles n'ont pas développés de dermatite sauf un érythème auriculaire modéré dans un cas. Dans l'exposition environnementale, ANOVA a montré un effet significatif de la durée, du groupe et de l'interaction groupe et durée avec les chiens atopiques montrant des scores cliniques significativement plus élevés que les contrôles. Il n'y avait pas de différence significative dans les scores cliniques après exposition orale ou environnementale dans le groupe atopique. Une réactivité croisée entre les acariens de poussière de maison et les acariens de stockage pourrait contribuer aux poussées de dermatite atopique chez les chiens allergiques aux acariens de la poussière de maison.

Resumen Este estudio tuvo el propósito de investigar si la exposición ácaros de almacenamiento producía agravamiento en los signos dermatitis atópica (AD) en perros atópicos Beagle sensibilizados frente a *Dermatophagoides faringe*, y alojados en un ambiente libre de polvo y de ácaros de almacenamiento. Los perros Beagle atópicos fueron expuestos a 50 mg de *Tyrophagus putrescentiae* durante tres días seguidos. Los signos clínicos se evaluaron antes, seis horas después de la exposición y después cada 24 horas durante cinco días utilizando un índice de extensión y severidad de dermatitis atópica canina. Cuatro perros Beagle sanos, negativos en serología y prueba intradérmica para ácaros del polvo y de almacenamiento, fueron utilizados como controles y expuestos de forma similar. Un mes tras la exposición en el ambiente, los perros Beagle atópicos fueron expuestos por vía oral a los ácaros (50 mg de *T. putrescentiae* durante tres días seguidos) y evaluados como se ha descrito anteriormente. Se utilizaron análisis de varianza (ANOVA) para las comparaciones entre grupos y el tipo de exposición. Todos los perros Beagle atópicos desarrollaron lesiones eritematosas pruríticas clínicamente compatibles con AD en la cara, orejas, pezuñas y abdomen ventral tras ambas exposiciones en el ambiente y por vía oral. Los perros control no desarrollaron dermatitis a excepción de eritema ligero en la oreja en un perro. En la exposición en el ambiente, los valores de ANOVA mostraron un efecto significativo del tiempo, grupo e interacción grupo x tiempo, con los perros Beagle atópicos mostrando valores más elevados que los perros control. No hubo diferencias significativas en los valores clínicos tras la exposición oral y ambiental en el grupo atópico. Así pues una reactividad cruzada entre los ácaros del polvo y de almacenamiento puede contribuir a empeoramientos de los signos clínicos de AD en perros alérgicos al ácaro del polvo.

Zusammenfassung Diese Studie zielte darauf ab herauszufinden, ob eine Provokation mit Futtermittelmilben Schübe von atopischer Dermatitis (AD) bei mit *Dermatophagoides farinae* sensibilisierten atopischen Beagles, die in einer Hausstaub- und Futtermittelmilben-freien Umgebung untergebracht waren, auslöste. Die Umwelt-Provokation der atopischen Beagles erfolgte mit 50mg *Tyrophagus putrescentiae* an drei aufeinander folgenden Tagen. Die klinischen Zeichen wurden vor, 6 Stunden nach jeder Provokation und dann alle 24 Stunden für insgesamt 5 Tage mittels Canine Atopic Dermatitis Extent and Severity Index durchgeführt. Vier gesunde Beagles, die einen negativen serologischen und intradermalen Test für Hausstaub- und Futtermittelmilben aufwiesen, wurden als Kontrolltiere verwendet und es wurde in ähnlicher Weise eine Provokation durchgeführt. Einen Monat nach der Umgebungsprovokation, erfolgte bei den atopischen Beagles eine Provokation per os (50mg *Tyrophagus putrescentiae* an drei aufeinander folgenden Tagen) und wie beschrieben beurteilt. Varianzanalysen (ANOVAs) wurden verwendet, um die Gruppen und die Arten der Provokation miteinander zu vergleichen. Bei allen atopischen Beagles entstanden nach sowohl Umgebungs- als auch nach Provokation per os erythematöse, juckende Läsionen, die klinisch mit AD im Gesicht, in den Ohrmuscheln, den Füßen und am ventralen Abdomen vergleichbar waren. Bei den

Hunden der Kontrollgruppe entstand, außer einem milden Erythem in den Ohrmuscheln bei einem Hund, keine Dermatitis. Bei der Umgebungsprovokation zeigte die Varianzanalyse eine signifikante Auswirkung der Zeit, der Gruppe und der Interaktion Gruppe x Zeit, wobei die atopischen Beagles signifikant höhere Werte zeigten als die Hunde in der Kontrollgruppe. Es bestanden keine signifikanten Unterschiede bei den klinischen Werten nach der Provokation per os bzw. nach der Umgebungsprovokation in der atopischen Gruppe. Eine Kreuzreaktivität zwischen den Hausstaub- und den Futtermittelmilben könnte folglich zu Schüben von AD bei Hausstaubmilben-allergischen Hunden beitragen.