

Studies on the role of routes of allergen exposure in high IgE-producing beagle dogs sensitized to house dust mites

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What is known about the topic of this paper

- Epicutaneous route of allergen exposure plays a role in canine atopic dermatitis based on histopathological studies.
- House dust mite is an important allergen.

What this paper adds to the field of veterinary dermatology

- Distribution of lesions is not dependent on the route of allergen exposure.
 - Oral administration of environmental allergens such as house dust mites can induce atopic dermatitis lesions.
 - All routes of allergen exposure play an additive effect but epicutaneous route appears to be the most important one
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Abstract

The current study aimed to investigate the role played by oral, epicutaneous, and inhalation routes of exposure to house dust mites (HDM). The colony of high IgE-producing beagle dogs has been shown to develop pruritic dermatitis compatible with atopic dermatitis following environmental exposure (EE) to HDM. In crossover experiments, the response to EE was compared to two modified challenges, oral exposure (OE) and snood and muzzle exposure (SME). For OE, HDM were fed daily for 3 days. For SME, ingestion of allergen was prevented but there was inhalation and epicutaneous exposure to all body regions except to one ear. In all experiments, dogs were challenged for three consecutive days, and evaluated before, 6 h after exposure and daily thereafter, for 5 days. After a wash-out period, groups were crossed-over so that each dog was randomly challenged to all three protocols. Clinical scores were analysed using least squares analysis of variance. All dogs developed pruritic dermatitis regardless of the protocol. With OE, lesions developed

in the same body regions as with EE although scores were lower. This difference became more evident after the first 3 days when OE scores decreased and EE scores continued to increase. The scores of covered and uncovered ears did not differ with SME. Scores for the remainder of the body were significantly lower than for EE. The development of lesions on covered ears supports the importance of inhalation or a systemic reaction to epicutaneous exposure in other areas. It is concluded that all routes are important and have additive effects, that route of exposure does not determine the distribution of lesions and that continuous epicutaneous exposure probably plays the most important role.

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Introduction

Controversy exists regarding the routes of allergen exposure in canine atopic dermatitis (cAD).¹ Former studies have emphasized the inhalatory route of allergen exposure.² More recently, histopathological studies of canine atopic lesional skin³ and experiments using atopy patch testing^{4,5} have emphasized the epicutaneous route of allergen exposure. The epicutaneous route of exposure might help explain the distribution of lesions (contact areas such as muzzle, feet, and pinnae) observed in dogs with AD. This route has been well documented in human medicine^{6,7} and has been recently highlighted by the demonstration of a genetic basis for a defective barrier function in humans with AD.⁸

In a recently described model of cAD, high-IgE beagle dogs sensitized to house dust mites (HDM) at a young age developed dermatitis upon environmental exposure (EE) to HDM.⁹ In this method of challenge, liquid HDM was applied to the floor of kennels and dogs were allowed to be exposed by a combination of inhalatory, oral, and epicutaneous routes, mimicking exposure to allergens as it would occur in a natural setting. Lesions observed after EE involved mostly contact areas, such as the face, feet, ventral abdomen and pinnae, and consisted of pruritic erythematous macules and papules. Immunohistochemical evaluation of these lesions demonstrated local accumulation of dermal dendritic cells suggesting a role for epicutaneous capture of the allergen, as previously described in dogs with natural occurring AD.³ The purpose of the present study was to further evaluate the individual role played by oral, epicutaneous, and inhalatory routes of allergen exposure in an

experimental model of cAD and whether the distribution of lesions is linked to the route of allergen exposure.

Materials and methods

Animals

Six high-IgE Beagles, epicutaneously sensitized to HDM at a young age and known to develop dermatitis upon EE challenge with HDM⁹ and to have positive Atopy Patch Test (APT) reactions to HDM,¹⁰ were used. All procedures applied in this study were approved by the Institutional Animal Care and Use Committee of the University of Florida.

At the time of the experiments the dogs were 6 years old. Five were intact females and one was an intact male. Dogs were housed in individual cement runs in a research facility of the University of Florida. Runs were cleaned daily using high temperature and high pressure wash. Stuffed toys, carpets, soft bedding or anything that could trap dust were not allowed in the runs. Walls of the runs and filters were checked monthly to ensure the absence of HDM in the environment in between challenges. Results were always below the detection level of the test used (MITE-T-Fast™ Allergen Detection System, Aveho Biosciences, Monterey, TN, USA).

Experimental design

Using crossover experiments all dogs were randomly challenged by EE and two modified ways. Modified challenges included an oral exposure (OE) protocol and a snood and muzzle exposure (SME) protocol. All dogs were challenged by the three exposure protocols at different times.

In all experiments the dogs were challenged for three consecutive days. The dogs were evaluated and scored before and 6 h after each exposure and daily thereafter, for 5 days. At the end of the 5-day experiment, the dogs were bathed with a hypoallergenic shampoo (HydraPearls® shampoo, Evsco Pharmaceuticals, Buena, NJ, USA) and a leave-on hydrocortisone conditioner (Resi-CORT®, Virbac, Fort Worth, TX, USA) was applied if needed to resolve the inflammation, for a maximum of 5 days. After a wash-out period of 1 month, the dogs were crossed-over and switched to another challenge.

House dust mite preparation

For all experiments the same liquid HDM formulation was used. The HDM solution was prepared from a HDM culture (natural *Dermatophagoides farinae*, Greer Laboratories Inc, Lenoir, NC, USA), and mixed with filtered (0.2 µm syringe filter) PBS (pH = 7.2) to obtain a final concentration of 31 mg mL⁻¹. The solution was vortexed and sonicated prior to use. On each of the three challenge days, 1.6 mL (50 mg) of HDM solution was used per dog for all exposure types. The concentration and volume of HDM used was selected based on previous studies on EE with HDM.⁹

Plain environmental exposure

In the EE, liquid HDM (1.6 mL, 50 mg/challenge) was applied daily for 3 days on the floor of a plastic air kennel measuring 50.8 × 68.6 × 48.3 cm. Each dog was placed in the kennel for 3 h day⁻¹, during three consecutive days. The kennels were washed at the end of the last challenge.

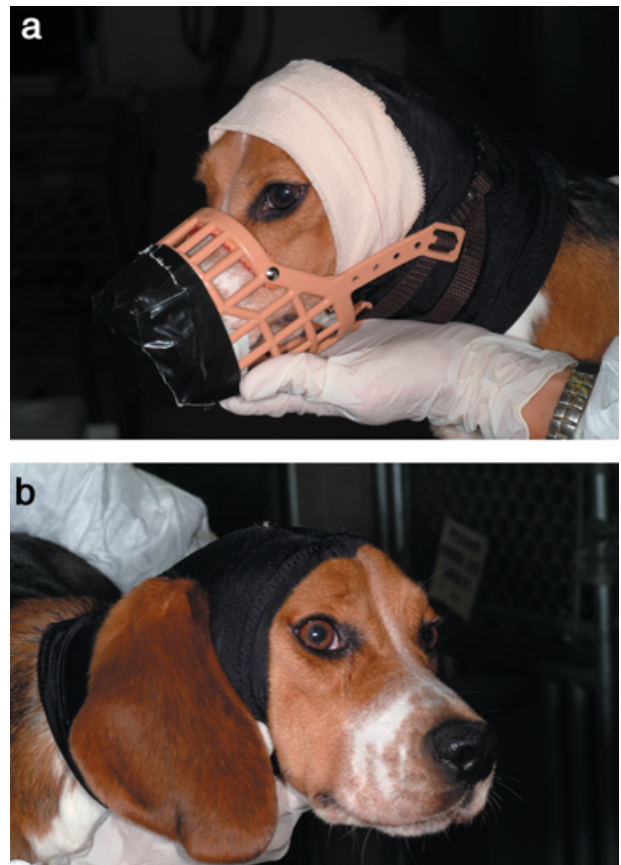


Figure 1. During the (a) snood and muzzle experiments, dogs wore a (b) modified type of tubular stockinet made of impermeable material that would allow one ear to be exposed while the other ear was covered. (a) The snood was secured by the use of Elastikon (Johnson & Johnson, New Brunswick, NJ, USA).

Oral exposure

In the OE, liquid HDM (1.6 mL, 50 mg/challenge) was mixed with canned food and fed to the dogs once daily for three consecutive days. The dose used for the oral challenge was the same as for other challenges to minimize confounding factors.

Snood and muzzle exposure

In the SME, liquid HDM (1.6 mL, 50 mg/challenge) was sprayed daily for 3 days on the floor of the kennels (same size kennels used for EE) but the dogs wore snoods to allow epicutaneous exposure of one ear only while the other ear was covered by impermeable material (Figure 1). They also wore muzzles while in the kennels (3 h day⁻¹, for three consecutive days). For the rest of the time, muzzles were replaced by E-collars to allow the dogs to eat and drink yet prevent grooming and the ingestion of HDM. Snoods were used for the entire duration of the challenge. This experiment was designed to allow HDM exposure through inhalation and epicutaneously albeit avoiding exposure to one ear.

Clinical scoring

Dogs were evaluated using a modified Canine Atopic Dermatitis Extent and Severity Index (mCADESI).¹¹ Briefly, the dog's body was divided into 50 small sections,

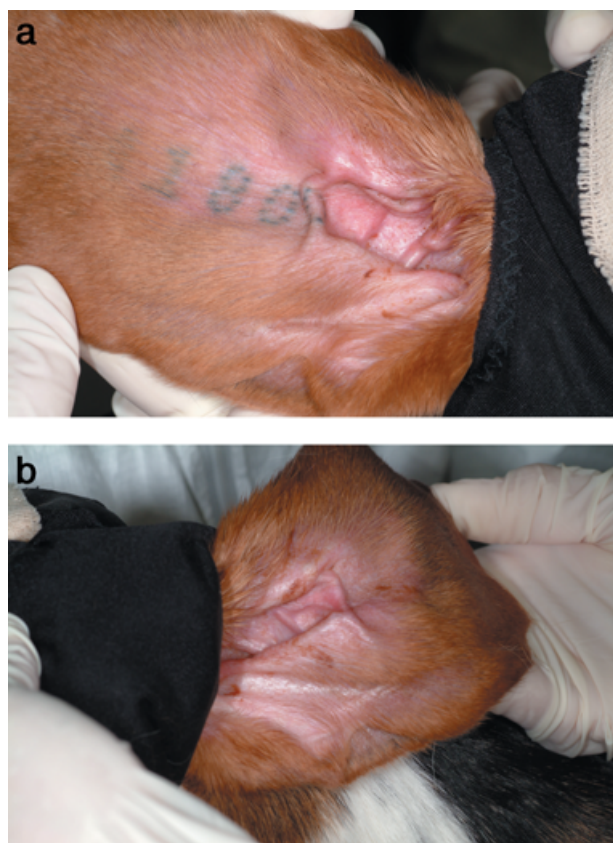


Figure 2. Ears of one of the dogs, 24 h after the beginning of the snood and muzzle exposure. No appreciable difference in the erythema between (a) exposed and (b) covered ears.

each of which received a score based on the clinical signs evaluated. The total score was calculated by adding the scores of all clinical signs and body sites. Total score was used in the statistical analysis. Clinical signs evaluated included diffuse erythema, erythematous macules, papules, excoriations and alopecia. The scoring system ranged from 0 (absent) to 3 (severe) for each site and each symptom. This scoring system had a maximum possible score of 150. Presence of pruritus was also noted.

Statistics

Clinical scores between types of exposure were compared using repeated-measure ANOVA with both exposure and time of evaluation as within-subject factors. When planned comparisons are reported, exposure differences at each evaluation time were analysed using paired Student's *t*-tests. All analyses were performed using the statistical software package SAS system for Windows version 9.0 (SAS Institute, Cary, NC, USA). A *P*-value < 0.05 was considered significant. All data in Figures 3, 4 and 6 are presented as mean $\pm$ SEM, unless otherwise indicated.

Results

Erythema, pruritus, and papules developed in all dogs regardless of the type of exposure. In all experiments the same body sites became lesional. They included the face (periocular, perilabial and preauricular), ear pinnae (con-

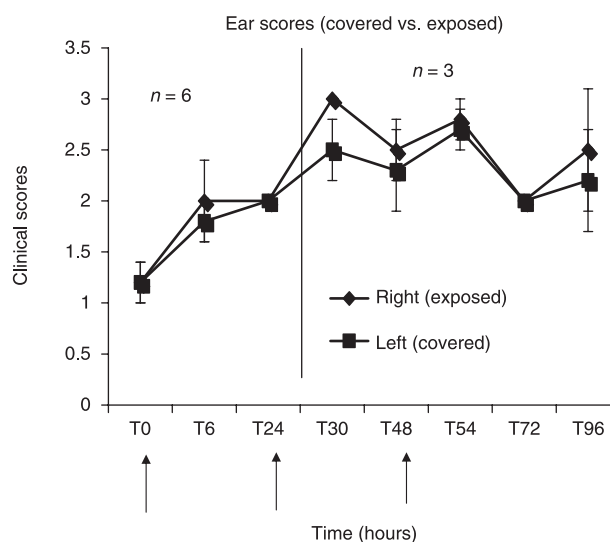


Figure 3. Clinical scores of the ears in the snood and muzzle experiment (SME). In this experiment the oral exposure was prevented and one ear was covered to prevent epicutaneous exposure while the other ear was left uncovered. This experiment was designed to evaluate the role of inhalatory route and epicutaneous route of allergen exposure. For each experiment dogs were challenged for three consecutive days (days of challenge are indicated by arrows) with 50 mg of house dust mites sprayed on the floor of their kennel. Figure shows means for all data points: when there were six observations, all six were used to calculate means and standard errors (SE) of the means (bars); when there were only three observations, only three observations were used to calculate means and SEs. The vertical line indicates the time at which three dogs removed their snoods and muzzle and only the data of the three dogs that still had the snoods and muzzles in place were included.

cave surface), feet (interdigital areas), inguinal area, ventral abdomen and axillae.

Snood and muzzle exposure vs. plain environmental exposure

Ear scores

All ears developed erythema starting 6 h after the challenge regardless whether they were covered or exposed (Figure 2a). Snoods and muzzles were lost in 3 dogs after the first 24 h; therefore, only three dogs were able to complete the SME experiment. Since there were missing observations at times 30 h through 96 h, data were analysed using only paired *t*-tests at each time point. There were no significant differences in mean clinical scores at any time point (*P*-values > 0.05) between covered and exposed ears. Scores appeared to increase after each exposure to HDM and then decrease 24 h after each challenge (Fig. 3). Peak values were reached at 30 h.

Body scores (ears excluded)

Due to the loss of the muzzles in three dogs and missing observations at times 30 h through 96 h, data relative to the body scores were analysed using only paired *t*-tests at each time point. The mean clinical scores of the EE tended to be higher than the ones of the SME (Figure 4) and such difference was statistically significant at 24 h and 48 h (*P* = 0.027 and *P* = 0.001, respectively). Peak values were reached at 72 h.

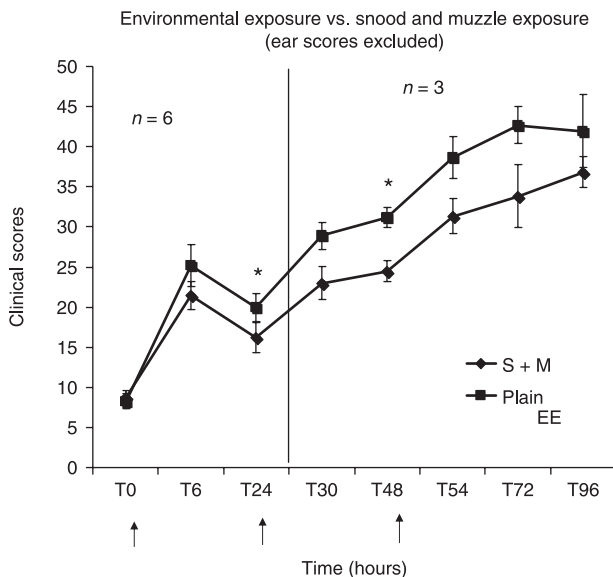


Figure 4. Clinical scores of the body, except for ears scores, in snood and muzzle exposure (SME) compared to the scores of plain environmental exposure (plain EE). In the SME experiment, oral exposure was prevented and one ear was covered to prevent epicutaneous exposure while the other ear was left uncovered. This experiment was designed to evaluate the role of inhalation allergen exposure and the effect of preventing oral exposure. In each experiment, dogs were challenged for three consecutive days (days of challenge are indicated by arrows) with 50 mg of house dust mites sprayed on the floor of their kennel. Figure shows means for all data points: when there were six observations, all six were used to calculate means and standard errors (SE) of the means (bars); when there were only three observations, only three observations were used to calculate means and SEs. The vertical line indicates the time at which three dogs removed their snoods and muzzle and only the data of the three dogs that still had the snoods and muzzles in place were included. Asterisk indicates times at which the difference in mean clinical scores between the two groups reached statistical significance.

Oral exposure vs. plain environmental exposure

All dogs developed erythema, pruritus and a mild to moderate papular eruption after oral administration of HDM (Figure 5a). The body sites that developed lesions after OE were the same as the ones of the EE (face, feet, ears and ventral abdomen). Mean clinical scores of OE peaked at 48 h (last challenge) and then slowly decreased over time. In contrast, mean scores of EE continued to increase after the last HDM exposure reaching a peak value at 72 h (Figure 6).

Data were analysed using two-exposure type $\times$ eight-time repeated measures ANOVA. Significant effect of exposure type ($P = 0.0031$) and time ($P < 0.0001$) and a significant exposure–time interaction ($P < 0.0001$) were detected. Additionally, paired t -tests showed that EE mean clinical scores were significantly higher than OE scores at 6, 54, 72 and 96 h (P -values < 0.005).

Discussion

In this study, pruritic dermatitis could be triggered by any route of allergen exposure, suggesting that all routes are contributory. The distribution of lesions appeared to be

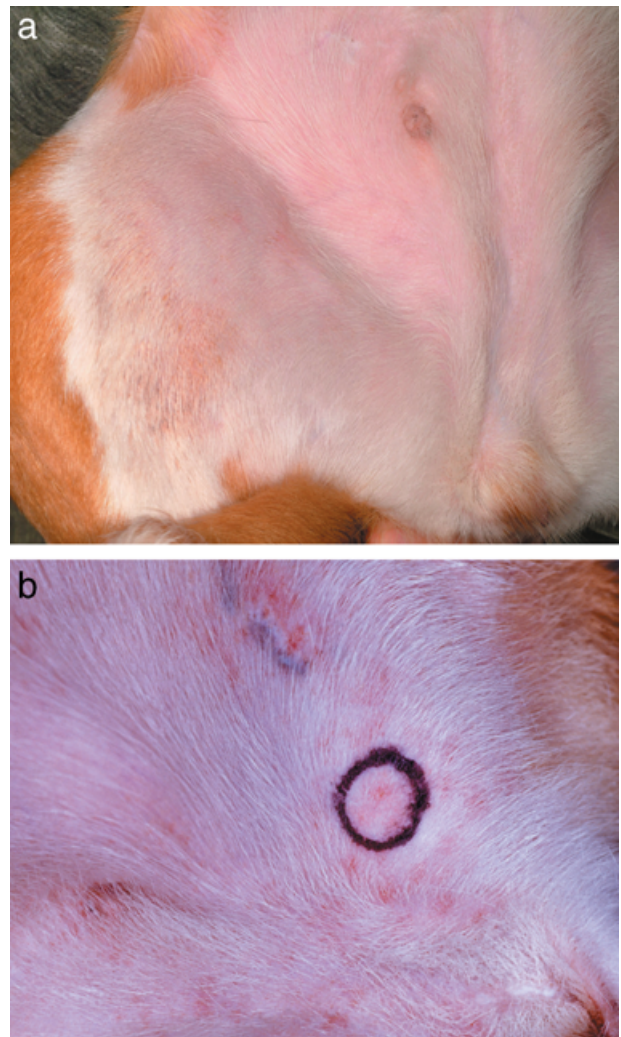


Figure 5. (a) Erythema on the ventral abdomen of a beagle 24 h after ingestion of 50 mg of HDM. A mild papular eruption was noticeable on the medial aspect of the rear leg. (b) Close up of the papular eruption developed 48 h after the beginning of the oral challenge.

independent of the route of exposure, suggesting that certain areas of the body seem to be 'preprogrammed' to develop inflammation upon allergen challenge once sensitization has occurred.

In SME, erythema developed in all ears, whether they were covered or not. Because of the low number of dogs and the loss of the snoods and muzzles in three dogs after the first 24 h, statistical conclusions are limited. A variety of snoods had been tried in pilot studies to find the snood model that would have the highest likelihood to stay in place. Unfortunately, some dogs managed to consistently remove muzzles and snoods regardless of the model used. Of the dogs in which the snoods and muzzles stayed in place, it can be speculated that either inhalation of the allergen or a systemic reaction following epicutaneous exposure to other parts of the body or both was responsible for the development of erythema in the covered ears.

The inhalatory route of allergen exposure was explored and emphasized in previous studies that attempted to develop a model for asthma using dogs.¹² In the early 40s, rhinitis, conjunctivitis, and asthma symptoms were

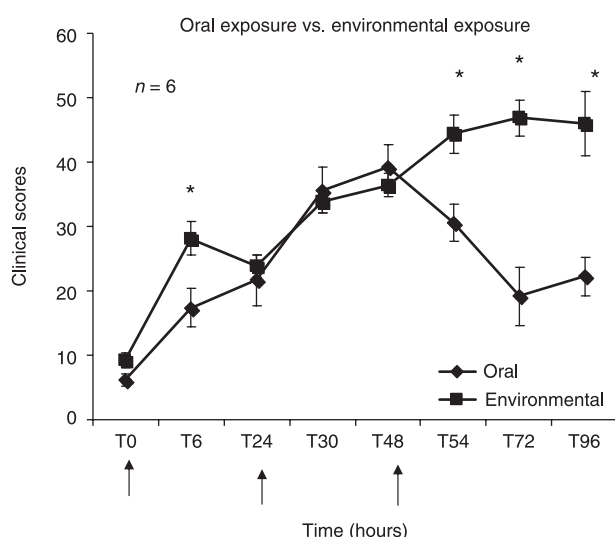


Figure 6. Clinical scores of the oral exposure (OE) compared to the plain environmental challenge (plain EE). With each experiment, dogs were challenged for three consecutive days (days of challenge are indicated by arrows) with 50 mg of HDM. Figure shows means and standard errors (SE) of the means (bars) for all data points. While the mean clinical scores in the oral challenge experiment decreased after discontinuation of challenge, in the environmental challenge they continued to increase despite discontinuation. Asterisk indicates times at which the difference in mean clinical scores between the two groups reached statistical significance.

induced by aerosolizing ragweed pollens in a dog with spontaneous allergic conjunctivitis and dermatitis.¹³ Additionally, beagle dogs experimentally sensitized to *Ascaris*, developed bronchial hyperreactivity and pulmonary inflammation after bronchial allergen challenge.¹⁴ In our experiments the allergen was not aerosolized in order to minimize irritation and more closely mimic a natural setting. Interestingly, only two of the dogs in this colony developed conjunctivitis with EE and none of them had ever developed respiratory signs. Although allergen inhalation may play some role in the canine disease, previous studies also demonstrated that it is not the single most important route of exposure. When atopic dogs were intranasally challenged with allergens, although respiratory signs developed in some dogs, worsening of skin lesions could not be induced in any of the atopic dogs.¹⁵

The possibility of a systemic reaction following epicutaneous exposure to the allergen in other body parts is therefore another intriguing explanation for the development of erythema in the covered ears. It is currently unknown whether in cAD the allergen may be captured in one part of the body and then migrate to 'preprogrammed' areas to trigger an inflammatory reaction. Whether the inflammation in the covered ear was induced by an allergen that physically migrated to that area or by selective homing of memory T cells in 'preprogrammed' sites will need to be evaluated in future studies.

An interesting finding of this study was that dermatitis clinically compatible with AD could be triggered by simple ingestion of the allergen. This route of exposure in cAD may indeed be more important than initially thought because of the grooming habits of our patients. To the best of the authors' knowledge, this is the first study

focusing on the role of ingestion of environmental allergens in cAD. Cross-reactivity between food and pollens has been extensively investigated in human medicine and is starting to be explored in veterinary medicine.¹⁶ However, ingestion of pollens or other environmental allergens had not been previously investigated in veterinary medicine. It is interesting to note that selective homing of allergen-specific T cells to lesional atopic areas has been demonstrated in humans with food allergies. Specifically, worsening of AD lesions can be induced after oral challenge with birch-related foods in patients allergic to birch.¹⁷ Thus, it is conceivable that HDM-specific memory T cells may be present in 'atopic sites' in the dogs used in the current study and that these cells may be responsible for the flaring up of lesions, regardless of the route of exposure to the allergen.

As the distribution of lesions was not determined by the route of exposure, a fascinating question arises regarding the anatomic sites of AD. These sites are strikingly similar between humans and dogs.¹⁸ While for dogs the epicutaneous route of allergen exposure has been traditionally used to help explain the anatomic predisposition of certain sites (e.g. walking on grass, sniffing the ground), this does not seem to apply as well when humans are considered. It could be speculated that lesional areas are sites where there is impaired sweat and/or abnormal barrier function potentially leading to increased allergen exposure and increased risk for sensitization. Growing evidence suggests alterations in skin pH and impairment in the sweat response and barrier function of the skin in human AD.¹⁹⁻²² The anomalous sweat response has been demonstrated to be attributable to an abnormal sudomotor axon reflex.²³ Differences in sweat response were found between lesional areas and non-lesional areas, potentially explaining the distribution of lesions in human AD.²³ Recently, the impaired barrier function has been linked to loss-of-function genetic variants in the gene encoding filaggrin, a key protein that facilitates terminal differentiation of the epidermis and the formation of the skin barrier.²⁴ Alterations in tissue kallikreins have also been considered to explain the impaired barrier function in human AD as kallikreins are essential for the regulation of serine protease activity in skin and for steady desquamation, sebum and sweat maturation.²⁵

Based on the results of this study, the epicutaneous route of allergen exposure appears to be the most clinically important as clinical scores continued to increase only in experiments where epicutaneous route was allowed. This was particularly noticeable when the clinical scores of the plain EE were compared to the OE. In the OE, scores peaked at 48 h and then slowly decreased, as allergen administration was not protracted beyond the first 3 days. In the EE, although no new exposure to the allergen was performed after the first 3 days, the persistence of the allergen on the skin (dogs were not bathed until the end of the week) was likely responsible for sustaining the inflammation and the worsening of clinical scores. These results, combined with the detection of HDM allergens on the skin and in the coat of dogs,²⁶ can explain how dogs with AD experience temporary relief with topical therapy and how this source of allergen may act as a reservoir for continual cutaneous inflammation.

In summary, all routes of allergen exposure (oral, inhalatory and epicutaneous) appear to play a role in triggering AD lesions in dogs sensitized to HDM, with epicutaneous being the most important one in the perpetuation of clinical signs. The distribution of lesions also appears to be independent of the route of exposure. Further studies are necessary to better elucidate the mechanisms involved in determining the anatomic distribution of AD lesions and the development of lesions in 'preprogrammed' atopic sites.

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Résumé Cette étude s'est intéressée aux différentes voies de pénétration (orale, épicutanée, respiratoire) des acariens des poussières (HDM). Une colonie de Beagle haut répondeur en IgE s'est révélée capable de développer une dermatite prurigineuse comparable avec une dermatite atopique quand stimulée par une exposition environnementale (EE) à HDM. Dans des essais en cross-over, la réponse à EE a été comparée à deux provocations modifiées, une exposition orale (OE) et une provocation nasale (SME). Pour OE, des HDM ont été ingérés quotidiennement pendant 3 jours. Pour SME, l'ingestion a été évitée, mais une inhalation et une exposition épicutanée à toutes les régions cutanées sauf une oreille. Dans tous les essais, les chiens ont été provoqués pendant trois jours consécutifs, et évalués avant, 6h après exposition, et tous les jours pendant 5 jours. Après une période de wash out, les groupes ont été échangés, afin que chaque chien subisse les trois protocoles. Les scores cliniques ont été évalués en utilisant une analyse de la variance. Tous les chiens développèrent une dermatite prurigineuse quelque soit le protocole. Avec OE, les lésions apparurent dans les mêmes zones que avec EE bien que les scores furent plus faibles. Cette différence

était plus marquée après les trois premiers jours, quand les scores d'OE ont diminué et que les scores d'EE continuèrent d'augmenter. Les scores entre les oreilles provoquées et non provoquées n'étaient pas différents pour SME. Les scores pour le reste du corps étaient plus faibles que pour EE. L'apparition de lésions sur les oreilles couvertes souligne l'importance de l'inhalation ou de la voie systémique en réaction à une provocation épicutanée dans d'autres zones. Nous concluons que toutes les voies de stimulation sont importantes et ont des effets qui s'additionnent, que la voie de stimulation n'est pas liée à la localisation des lésions et qu'une exposition épicutanée continue joue probablement le plus grand rôle.

Resumen En el presente estudio nos planteamos investigar el papel que juegan las rutas oral, epicutánea e inhalatoria en la exposición a los ácaros del polvo. La colonia de perros Beagle con producción de IgE elevada ha demostrado desarrollar dermatitis prurítica compatible con dermatitis atópica tras la exposición en el ambiente a ácaros del polvo. En experimentos cruzados, la respuesta a la exposición ambiental se comparó con dos desafíos modificados: la exposición oral, y la exposición en el hocico y a cofias. Para la vía oral los animales fueron alimentados diariamente por 3 días. Para la ruta hocico/cofia, se evitó la ingestión del alérgeno, pero se indujo inhalación y exposición epicutánea en todas las regiones anatómicas a excepción de una oreja. En todos los experimentos, los perros se desafiaron con el alérgeno durante 3 días consecutivos, y se evaluaron antes, 6 horas tras la primera exposición, y después a diario durante cinco días. Tras un periodo de descanso, los grupos se cruzaron de forma que cada perro se desafió al azar con los tres protocolos. Los valores clínicos se analizaron utilizando el ajuste mínimo cuadrático de la varianza. Todos los perros desarrollaron dermatitis prurítica independiente del protocolo. Con la exposición oral, las lesiones aparecieron en las mismas regiones corporales que tras la exposición ambiental, aunque los valores fueron menores. Esta diferencia fue más evidente tras los tres primeros días cuando los valores orales disminuyeron y los valores ambientales continuaron aumentando. Los valores en orejas cubiertas o descubiertas no fueron diferentes con el protocolo hocico/cofia. Los valores para el resto del cuerpo fueron significativamente menores que con el protocolo de exposición ambiental. El desarrollo de lesiones en orejas cubiertas indica la importancia de la inhalación o de una reacción sistémica a la exposición epicutánea en otras áreas. Concluimos que todas las rutas son importantes y tienen efectos aditivos, que la ruta de exposición no determina la distribución de las lesiones, y que la continua exposición epicutánea posiblemente juega el papel más importante.

Zusammenfassung Das Ziel der vorliegenden Studie war es, zu untersuchen, welche Rolle die Route der Exposition (oral, epikutan und Inhalation) zu Hausstaubmilben (HDM) spielt. Es konnte gezeigt werden, dass eine Kolonie von Beagles mit hoher IgE Produktion eine juckende Dermatitis entwickeln, welche mit atopischer Dermatitis vergleichbar ist, nachdem sie Hausstaubmilben in der Umwelt (EE) ausgesetzt worden waren. In Cross-over Experimenten wurde die Antwort auf EE mit zwei modifizierten Provokationen verglichen, nämlich der oralen Exposition (OE) und der Exposition über Schnauze und Maul (SME). Für OE wurden die HDM 3 Tage lang täglich gefüttert. Für SME wurde die orale Aufnahme von Allergenen verhindert, aber es bestand Inhalation und epikutane Exposition an allen Körperregionen außer an einem Ohr. In allen Experimenten wurden die Hunde für 3 aufeinanderfolgende Tage der Provokation ausgesetzt und vor und 6 Stunden nach der Exposition, sowie danach täglich 5 Tage lang, evaluiert. Nach einer Wash-out Phase, wurden die Gruppen überkreuzt, sodass jeder Hund dem Zufallsprinzip folgend mit allen drei Protokollen provoziert wurde. Die klinischen Veränderungen wurden mittels 'least squares analysis of variance' analysiert. Alle Hunde entwickelten unabhängig vom verwendeten Protokoll eine juckende Dermatitis. Bei OE entwickelten sich die Hautveränderungen in denselben Körperregionen wie mit EE, obwohl die Grade der Veränderungen geringer waren. Dieser Unterschied wurde nach den ersten 3 Tagen gravierender, da die OE Veränderungen abnahmen während die EE Veränderungen weiterhin zunahmen. Die Grade der Veränderungen bei bedeckten und unbedeckten Ohren waren bei SME nicht unterschiedlich. Für den Rest des Körpers waren die Grade der Veränderungen signifikant niedriger als bei EE. Die Entstehung von Läsionen an den bedeckten Ohren bestätigt die Bedeutung der Inhalation oder einer systemischen Reaktion auf die epikutane Exposition in anderen Regionen. Es wird daraus geschlossen, dass alle Routen wichtig sind und ein additiver Effekt besteht; dass die Route der Exposition die Verteilung der Läsionen nicht bestimmt und dass kontinuierliche epikutane Exposition möglicherweise die wichtigste Rolle spielt.