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Induction of atopic dermatitis by epicutaneous sensitisation with *Dermatophagoides farinae* in nongenetically predisposed Beagle dogs

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Abstract: This study was performed to induce atopic dermatitis (AD) using nongenetically predisposed Beagle dogs. Five healthy Beagle dogs were used. Twice weekly for 12 weeks, the dogs were painted on the axillae and groin with a solution of *Dermatophagoides farinae* (*D. farinae*). Each dog was thereafter placed in a cage where a house dust mite (HDM) solution was applied on the bottom of the cage. The dog remained in the cage for 3 h daily for 3 consecutive days for the environmental exposure to HDM. Serum samples were collected at 0 week and 6 weeks after sensitisation, and at 0 h and 72 h after the environmental exposure. During the environmental exposure, skin biopsies were obtained at 0 h, 36 h, and 72 hours. After the first environmental exposure, no dog had any marked clinical sign. An additional sensitisation was subsequently administered for 10–13 weeks. Three of the five dogs developed pruritic dermatitis with skin lesions after the second exposure. The histopathology of the lesions revealed severe infiltration of inflammatory cells and dermal oedema. The levels of *D. farinae*-specific IgE were also elevated. This study demonstrated that AD could be induced by epicutaneous sensitisation with HDM in nongenetically predisposed dogs.

Keywords: animal model; atopy; *D. farinae*; house dust mite; IgE

Atopic dermatitis (AD) is a common manifestation of allergic dermatitis in human and veterinary medicine (Leung et al. 2003; Pucheu-Haston et al. 2008). To identify the cause and pathophysiologic mechanisms of AD, animal models of AD have been developed, especially mouse models (Yamamoto et al. 2007). Investigators have suggested that mouse models such as the NC/Nga mouse or hapten-induced mouse are useful for elucidating the pathogenesis of AD and for evaluating therapeutic agents (Shiohara et al. 2004; Yamamoto et al. 2007). However, mouse models have significant disadvantages with regard to the similarity of their disease to naturally occurring human and canine AD (Yamamoto et al. 2007). Therefore, much effort has been devoted to establish a canine model of AD (Olivry and Baumer 2015) because dogs with AD naturally develop pruritic dermatitis which is clinically similar to that in human AD (Marsella and Olivry 2003; Santoro and Marsella 2014).

Colonies of high-immunoglobulin E (IgE)-producing dogs, which genetically produce high

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levels of IgE antibodies against allergens (de Weck et al. 1997), have recently been used to develop models of AD. The development of these dogs was based on the hypothesis that canine AD is cutaneous hypersensitivity associated with elevated levels of an allergen-specific IgE (Pucheu-Haston et al. 2008; Marsella et al. 2012). The IgE antibodies become elevated in these dogs when they are exposed to an allergen over time. In some studies (Marsella et al. 2006a; Pucheu-Haston et al. 2008), dermatitis that clinically, histologically, and immunologically resembled canine AD was induced by using high-IgE-producing dogs that had been epicutaneously sensitised to house dust mites (HDMs) in their early weeks of life. They all developed skin lesions and pruritus after environmental exposure to HDM.

Despite several attempts in studies (Schwartzman et al. 1983; Egli et al. 2002) to develop models of AD by using high-IgE-producing dogs, investigators have failed to induce pruritic dermatitis. In these studies, high-IgE-producing dogs were sensitised to several allergens, but only mild clinical signs occurred. A difference in the routes of the allergen exposure between the successful and failed studies may have affected the results, although unknown genetic factors may have also had an effect on the results. These results suggested that no evidence exists indicating that the capability to produce high levels of allergen-specific IgE is directly relevant to the clinical signs of AD.

To our knowledge, there is little information regarding the sensitisation of nongenetically predisposed dogs to HDM. For this reason, we aimed to sensitise normal dogs that were not genetically predisposed to produce high IgE, and to evaluate them as a possible model for AD.

MATERIAL AND METHODS

Study design

This study consisted of two protocols. The first protocol was the sensitisation of nongenetically predisposed dogs to HDM. The second protocol was the environmental exposure of these dogs to HDM.

The present study was approved by the Institutional Animal Care and Use Committee (CBNUA-1267-19-02) of the Laboratory Animal Research Center of Chungbuk National University.

Animals

Five laboratory Beagle dogs (two intact males and three intact females; DooYeol Biotech, Seoul, Republic of Korea) were used. They were not siblings and their age was 6.7 ± 0.75 weeks old [presented as the mean ± the standard deviation (SD)]. An identification (ID) number was allocated to each dog in order. All the dogs and their family were healthy without a history of AD. They had no signs of dermatologic problems on the physical and dermatologic examinations. They were screened for congenital systemic diseases by means of a complete blood count and serum chemistry analysis. They did not receive any drugs or additional treatment before the experiment. The dogs were fed twice daily with a commercial dry food (Natural Balance; Natural Balance Pet Foods Inc., Los Angeles, CA, USA), and fresh water was supplied continuously during the experimental period.

Experimental agent

The HDM, *Dermatophagoides farinae* (*D. farinae*), extract was purchased from Greer Laboratories [RMB83M; mite dust (*D. farinae*); Lenoir, NC, USA]. It contains a powder consisting of a pure culture of *D. farinae*. The HDM extract was stored at –20 °C. Exposure to room temperature was minimised to maintain the expiration date.

Sensitisation protocol

Before beginning the sensitisation protocol, all the dogs were intradermally tested with *D. farinae* (Greer Laboratories, Lenoir, NC, USA), *Dermatophagoides pteronyssinus* (Greer Laboratories, Lenoir, NC, USA), histamine (ALK-Abelló Inc., Port Washington, NY, USA), and saline to check for a negative reaction to HDM. To sensitise normal dogs, a modified sensitisation method was used, based on the method in a previous study (Marsella and Saridomichelakis 2010), in which AD was induced in high-IgE-producing dogs. A solution containing 120 mg of the HDM extract mixed with 0.3 ml of sterile saline was prepared. This solution was painted twice weekly for 12 weeks onto the axillae and groin by using a soft brush. Thirteen weeks after sensitisation, the intradermal skin test

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was repeated in the dogs by using the same allergens. Blood samples were collected at 0 week and 6 weeks for measuring the total IgE and *D. farinae*-specific IgE antibodies.

Environmental exposure protocol

The environmental exposure method used in this study was based on the method described in a previous study (Marsella et al. 2006a). The solution used for the environmental exposure was prepared. It contained 50 mg of the HDM extract mixed with 1.6 ml of phosphate buffered saline (Sigma-Aldrich Co., St. Louis, MO, USA). The solution was applied to the bottom of a traveling cage (45 cm × 66 cm × 46 cm) at 0 h, 24 h, and 48 hours. Each dog was placed individually in the cage and remained in the cage for 3 h daily for 3 consecutive days. At 0 h, 7 h, 31 h, 55 h, and 72 h, the dogs were examined for scoring of the Canine Atopic Dermatitis Extent and Severity Index (CADESI)-4 (Olivry et al. 2014). Skin biopsies were obtained at 0 h, 36 h, and 72 h for a histopathologic assessment, and blood samples were collected at 0 h and 72 h for measurements of the concentration of the total IgE and *D. farinae*-specific IgE antibodies. If changes were not detected in the second intradermal skin test and the first environmental exposure, then the HDM sensitisation was continued until the dog had a positive intradermal reaction with or without pruritus and skin lesions, and the second environmental exposure was performed immediately after the identification of a positive intradermal skin test. The evaluation procedures of the second environmental exposure were the same as those used for the first procedure, but the skin biopsies were executed only at 36 h and 72 hours.

Clinical evaluation

The extent and severity of the skin lesions were evaluated, based on the CADESI-4 scores (Olivry et al. 2014).

In total, 20 parts of the body were scored by evaluating the sum of three items (i.e., erythema, lichenification, and excoriations/alopecia) on a scale of "none" (score 0), "mild" (score 1), "moderate" (score 2), and "severe" (score 3). The maximal score was 180.

Histopathology

Skin biopsies were collected from representative lesions in all the dogs during the environmental exposures. If no skin lesion was detected, then the samples for the biopsy were obtained from the sites where the HDM solution had been applied (i.e., the axillae and groin). The biopsy samples were fixed in neutral buffered 10% formalin, embedded in paraffin, routinely sectioned, and stained with haematoxylin and eosin for the histopathologic evaluation. The severity of the infiltration of the inflammatory cells was assessed by enumerating the cells in the epidermis and dermis of five random fields by using a microscope (Eclipse Ci; Nikon, Tokyo, Japan) at 10 × 40 magnification. In addition, the area of the intercellular oedema was measured in the dermis of the same five fields by using morphometric software (ImageJ; National Institutes of Health, Bethesda, MD, USA). The intercellular oedematous area is presented as a percentage of the total area of the microscopic field.

Serology

All the serum samples obtained at 0 week and 6 weeks during the sensitisation period and at 0 h and 72 h during the exposure period were used for measuring the total IgE and *D. farinae*-specific IgE concentrations. The total IgE concentrations were analysed using a canine-specific enzyme-linked immunosorbent assay kit (Immunology Consultants Laboratory, Inc., Portland, OR, USA), based on the manufacturer's instructions. The concentrations of the *D. farinae*-specific IgE were measured using a multiple allergen simultaneous test (Roboscreen; MEDIWISS Analytic GmbH, Moers, Germany).

Statistical analysis

The data were analysed using GraphPad Prism v7 software (GraphPad Software Inc., San Diego, CA, USA). The CADESI-4 scores were analysed by employing the Friedman test within dogs for changes over time, and when an overall significance was identified, Dunn's multiple-comparison post hoc test was used to determine whether differences existed. The histopathologic assessment scores were analysed by using the Kruskal-Wallis test to detect

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significant differences between groups of five random microscopic fields at each time point in a dog. On detecting a significant difference, Dunn's multiple-comparison post hoc test was then conducted. Tukey's multiple comparisons test was also used to compare the histopathologic assessment scores of each dog at the same time points. All the values are expressed as the mean ± SD. The differences were significant at values of $P < 0.05$.

RESULTS

Clinical evaluation

Pruritic dermatitis was not detected in any dog after the first sensitisation period. The dogs had a negative intradermal reaction to HDM. After the first environmental exposure, no remarkable clinical sign manifested and the CADESI-4 scores were not significantly increased in any dog (Figure 1). An additional HDM sensitisation was subsequently conducted until significant clinical signs or a positive intradermal reaction to HDM occurred. After the additional sensitisation, three dogs (ID-1, ID-2, and ID-4) had a positive intradermal reaction with or without pruritus and skin lesions. The periods of additional sensitisation were 13 weeks, 10 weeks, and 12 weeks in the dogs ID-1, ID-2, and ID-4, respectively.

During the second environmental exposure, they developed severe pruritus within 7 h of the HDM exposure. Itching occurred on the groin, flanks, and lateral thigh regions. Severe clinical signs includ-

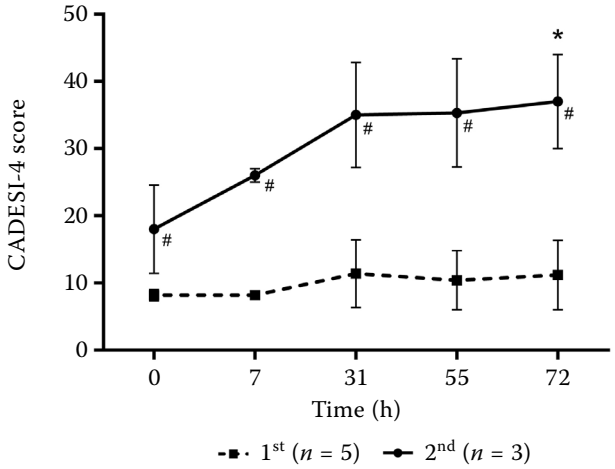

Figure 1. Changes in the mean CADESI-4 scores in the sensitised dogs after the environmental exposures
The scores of the five dogs did not significantly increase over time when the first environmental exposure was conducted after the first 12-week sensitisation period ($P > 0.05$). Three of the five dogs subsequently had a positive intradermal reaction to HDM after an additional sensitisation of 10–13 weeks. All the mean CADESI-4 scores of the second environmental exposure are significantly higher than those of the first environmental exposure for each time point ($P < 0.05$). After the second environmental exposure, the scores of the three dogs gradually increased over time. The values are presented as the mean ± SD
1st = first environmental exposure; 2nd = second environmental exposure; CADESI-4 = Canine Atopic Dermatitis Extent and Severity Index-4; HDM = house dust mite; SD = standard deviation
*$P < 0.05$ compared to 0 h; #$P < 0.05$ compared to the first environmental exposure

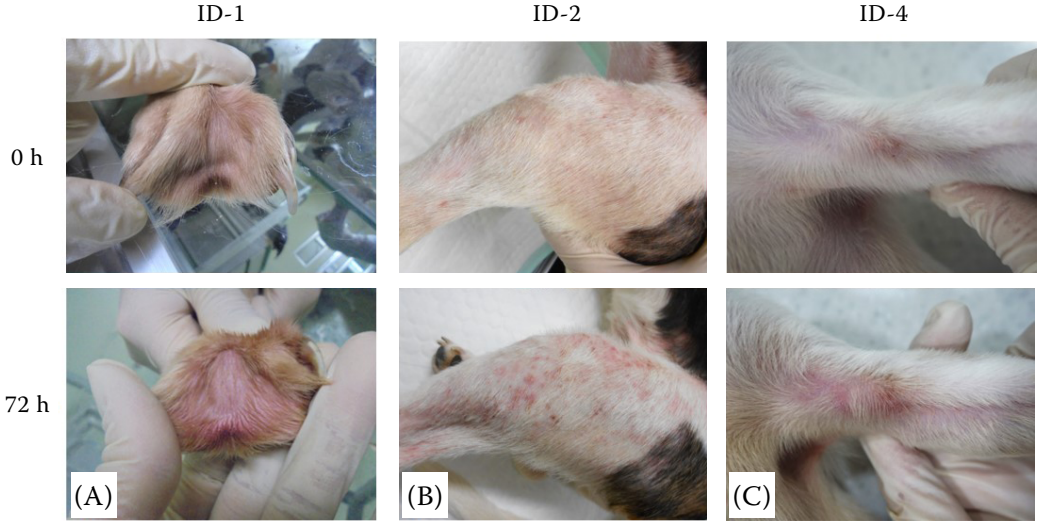

Figure 2. Representative skin lesions in the dogs ID-1 (A), ID-2 (B), and ID-4 (C) after the second environmental exposure
Compared to 0 h, severe clinical signs such as erythema, papules and alopecia are visible at 72 hours

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ing papules, erythema, and excoriation were noted (Figure 2). These signs persisted after the exposure was discontinued. The mean CADESI-4 scores of the second environmental exposure were significantly increased from the scores after the first environmental exposure ($P < 0.05$). The scores of the three dogs also gradually increased over time during the second environmental exposure, and showed a significant increase at 72 h, compared to 0 h ($P < 0.05$) (Figure 1).

Histopathology

After the first environmental exposure, the histopathologic findings of all the dogs revealed no signif-

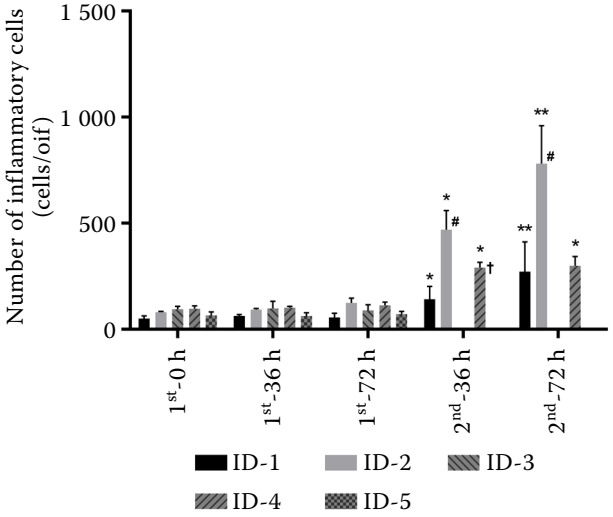

Figure 3. Changes in the mean number of the skin inflammatory cells after the environmental exposures

In all the dogs, the number of cells did not change after the first exposure ($P > 0.05$). The second environmental exposure was not administered in two dogs (ID-3 and ID-5), which had a negative intradermal reaction to HDM. Compared to the first environmental exposure, the infiltrated cells significantly increased in three dogs (ID-1, ID-2, and ID-4) after the second environmental exposure ($P < 0.05$). The mean number of inflammatory cells are significantly higher in ID-2 than in ID-1 and ID-4 at 36 h and 72 h after the second exposure ($P < 0.05$). The mean number of inflammatory cells are significantly higher in ID-4 than in ID-1 at 36 h after the second exposure ($P < 0.05$). The values are presented as the mean ± SD

1^{st} = first environmental exposure; 2^{nd} = second environmental exposure; HDM = house dust mite; SD = standard deviation
*$P < 0.05$ compared to 1^{st}-0 h; **$P < 0.01$ compared to 1^{st}-0 h; $^{\#}P < 0.05$ compared to ID-1 and ID-4 at the same time; $^{\dagger}P < 0.05$ compared to ID-1 at the same time

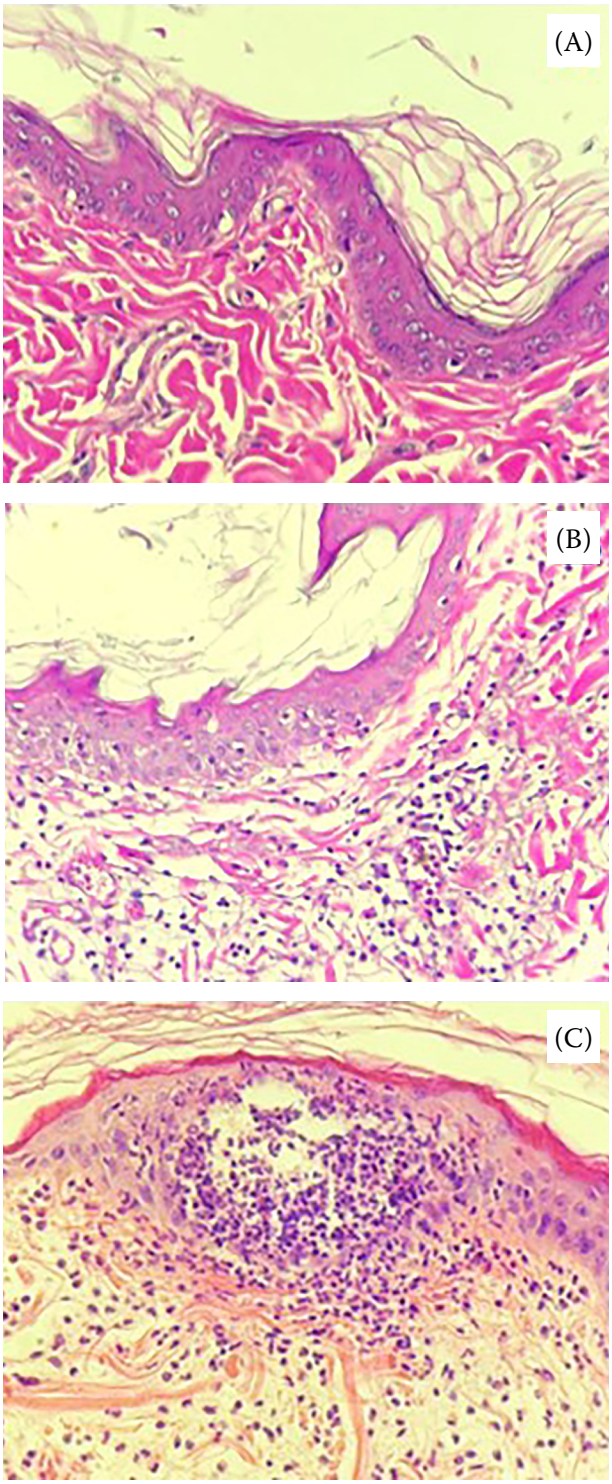

Figure 4. Representative histopathology images taken 72 h after the first exposure [ID-3 (A)] and the second exposure [ID-1 (B) and ID-2 (C)]

Compared to dog ID-3, dogs ID-1 and ID-2 have epidermal hyperplasia and severe dermal oedema. Dog ID-2 has a remarkable abscess formation and inflammatory cell recruitment (haematoxylin and eosin stain; magnification × 400)

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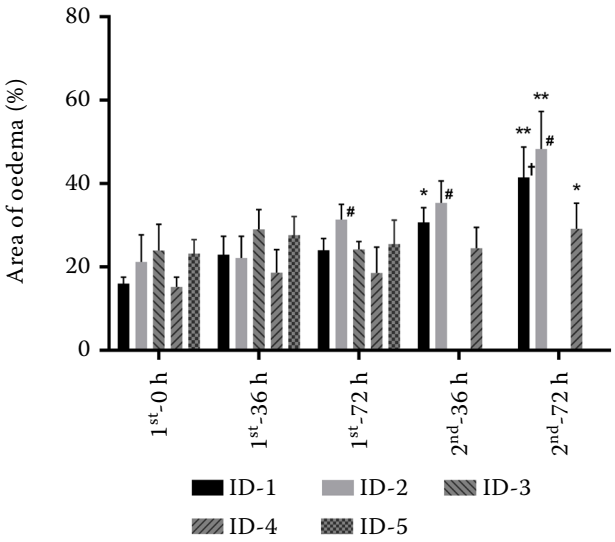

Figure 5. Changes in the mean areas of the intercellular oedema of the skin after the environmental exposures

In all the dogs, the degree of the oedema did not change after the first exposure ($P > 0.05$). The second environmental exposure was not administered in two dogs (ID-3 and ID-5), which had a negative intradermal reaction to HDM. Compared to the first environmental exposure, the areas of the oedema significantly increased in three dogs (ID-1, ID-2, and ID-4), after the second environmental exposure ($P < 0.05$). The mean areas of the intercellular oedema are significantly higher in ID-2 than in ID-4 at 36 h and 72 h after the second exposure, respectively ($P < 0.05$). The mean areas of the intercellular oedema are significantly higher in ID-1 than in ID-4 at 72 h after the second exposure ($P < 0.05$). The values are presented as the means ± SD

1^{st} = first environmental exposure; 2^{nd} = second environmental exposure; HDM = house dust mite; SD = standard deviation

$^{*}P < 0.05$ compared to 1^{st}-0 h; $^{**}P < 0.01$ compared to 1^{st}-0 h; $^{\#}P < 0.05$ compared to ID-4 at the same time; $^{\dagger}P < 0.05$ compared to ID-4 at the same time

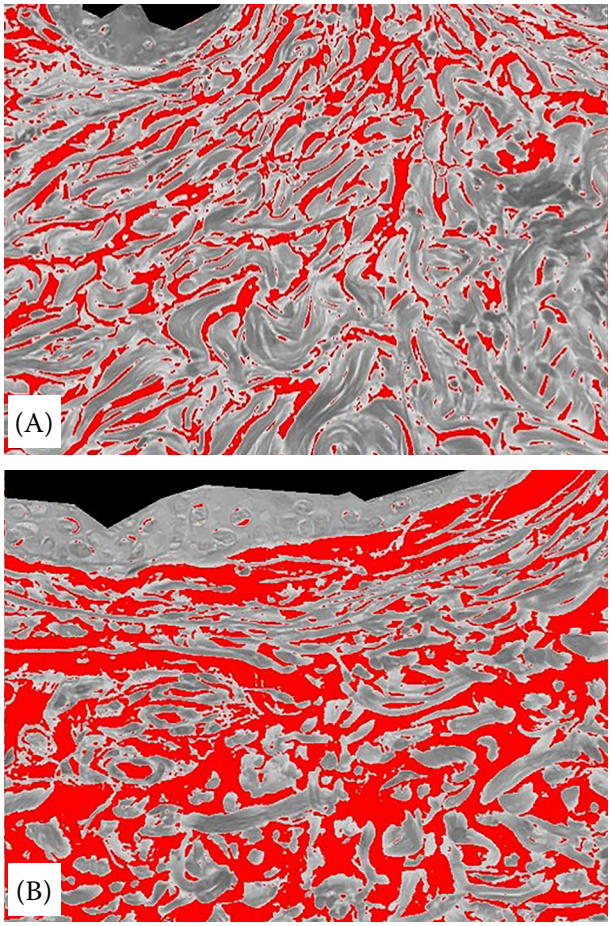

Figure 6. Images of the intercellular oedema of the biopsy samples obtained from dog ID-4 after the environmental exposures

Measured using ImageJ software (National Institutes of Health, Bethesda, MD, USA). The red areas indicate the oedematous changes. Compared to 0 h after the first environmental exposure (A), an abnormal amount of fluid has accumulated at 72 h after the second exposure (B) (haematoxylin and eosin stain; magnification × 400)

icant number of inflammatory cells at any time point. A few microscopic fields revealed a mild increase in the cellularity, compared with the other fields. However, this finding was histologically insufficient to show severe inflammatory dermatitis. However, the inflammatory cells in the positive intradermal reaction group (i.e., ID-1, ID-2, and ID-4) significantly and exponentially increased after the second environmental exposure ($P = 0.000\,6$) (Figure 3). The representative lesions at 72 h were characterised by a severe and diffuse infiltration of the inflammatory cells, compared to the infiltration at 0 h (Figure 4). This infiltrate was especially concentrated in the epidermis and superficial dermis. A mixture of neutrophils and mononuclear cells was noted.

The percentage of the areas with intercellular oedema in all the dogs was not significantly increased after the first environmental exposure. However, the mean percentage of the areas of the intercellular oedema in the positive intradermal reaction group was significantly increased after the second environmental exposure, compared to the percentage after the first environmental exposure ($P = 0.000\,4$) (Figure 5).

Remarkable intercellular oedematous changes with a severe infiltrate of inflammatory cells were detected in the dermis at 72 h, compared to at 0 h (Figure 6).

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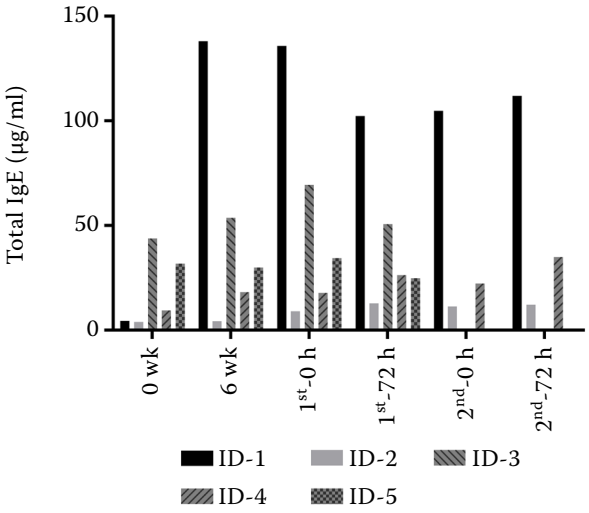

Figure 7. Changes in the total serum IgE level after sensitisation and the environmental exposures
Compared to the IgE value at 0 week, the IgE level is remarkably elevated in dog ID-1 at 6 weeks after sensitisation. However, the other four dogs do not have noticeable changes. All the dogs had similarly inconsistent changes or little changes between the before and after the first and second environmental exposures.
0 wk = 0 week after the sensitisation; 1st = first environmental exposure; 2nd = second environmental exposure; 6 wk = 6 weeks after the sensitisation; IgE = immunoglobulin E

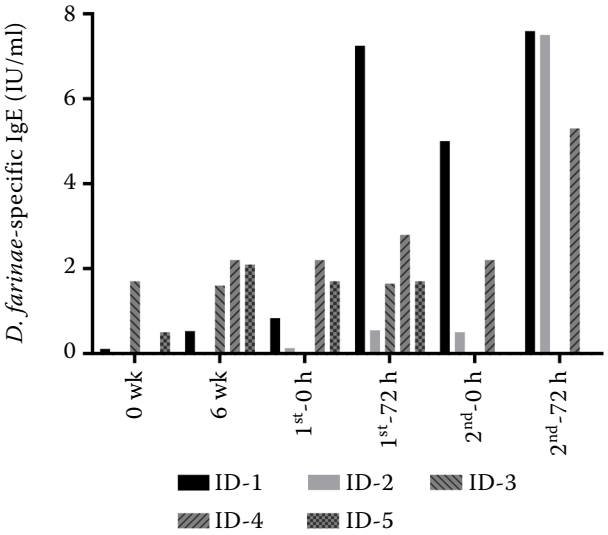

Figure 8. Changes in the serum *D. farinae*-specific IgE level after sensitisation and the environmental exposures
In the sensitisation period, the levels of *D. farinae*-specific IgE are elevated in dogs ID-1, ID-4, and ID-5 between 0 week and 6 weeks. Among the five dogs that underwent the first environmental exposure, the IgE level is elevated in dogs ID-1, ID-2, ID-3, and ID-4 between the before and after exposure. In three dogs (ID-1, ID-2, and ID-4) that underwent the second exposure, the IgE level is remarkably elevated in all the dogs between the before and after exposure
0 wk = 0 week after the sensitisation; 1st = first environmental exposure; 2nd = second environmental exposure; 6 wk = 6 weeks after the sensitisation; *D. farinae* = *Dermatophagoides farinae*; IgE = immunoglobulin E

Serology

The serum concentrations of the total IgE from most dogs revealed no significant difference at any time point (Figure 7).

In one dog (ID-1), the serum IgE levels were higher than the baseline level (0 week) at 6 weeks after beginning the sensitisation; however, no remarkable change was detected during the environmental exposures. However, the *D. farinae*-specific IgE was increased in the positive intradermal reaction group (i.e., dogs ID-1, ID-2, and ID-4) after the first and second exposures (Figure 8).

DISCUSSION

In this study, we aimed to sensitise normal dogs (which were not genetically predisposed to produce high IgE levels) to HDM, and to evaluate them as a possible model for AD. We found that, similar to the AD model in high-IgE-producing dogs, pruritic dermatitis was induced in the nongenetically predisposed Beagle dogs. Before initiating the sensitisation, the five dogs had a negative intradermal reaction to HDM; however, three dogs had a positive intradermal reaction after the HDM sensitisation. In addition, environmental exposure to *D. farinae* induced pruritic dermatitis, which was comparable with AD.

In most cases of canine AD, an allergen-specific IgE may be involved in the pathophysiology (Marsella et al. 2012). The allergen-specific IgE affected the extrinsic form of AD, defined as a genetically predisposed inflammatory and pruritic allergic skin disease with characteristic clinical features associated with IgE antibodies directed against environmental allergens (Akdis and Akdis 2003; Halliwell 2006). In the present study, the levels of the *D. farinae*-specific IgE were relatively higher in the positive reaction group than in the negative reaction group animals, especially at 72 h

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after the second environmental exposure. The clinical signs were most severe at the same time, which indicated that the levels of the allergen-specific IgE were somewhat proportional to the clinical signs. By contrast, the levels of the total serum IgE in the positive reaction group were not higher than those in the negative reaction group. In particular, the levels of the total serum IgE were not increased in the positive reaction group, although the dogs had severe clinical signs. An exception was the dog ID-1, which had remarkably high levels of the total serum IgE; its clinical scores were relatively lower than those of the other two dogs in the positive reaction group. In one study (Pucheu-Haston et al. 2008), the levels of the *D. farinae*-specific IgE were likewise markedly elevated, whereas the total serum IgE levels decreased over time. Moreover, in one report (Hill et al. 1995), the total serum IgE levels were not significantly different between normal dogs and atopic dogs. Therefore, not all serum IgE are pathogenic, and the allergen-specific IgE response may not be directly related to the induction of the total serum IgE antibody production.

The findings of a former study suggested no significant difference in the levels of the serum IgE between normal dogs and atopic dogs in early life (Pucheu-Haston et al. 2008). In the present study, the levels of the allergen-specific IgE between the positive and negative reaction groups were also not significantly different in the early weeks of the study. The levels of the allergen-specific IgE could reflect the clinical signs of AD in this study; however, the levels of serum specific IgE may not be used as a predictor of the early stages of AD.

The epicutaneous route was used to sensitise the dogs to HDM in this study. Many routes of allergen exposure such as inhalation, oral administration, and epicutaneous exposure have been applied for canine allergic diseases (Hirshman et al. 1980; Rhodes et al. 1987; Redman et al. 2001; Zosky and Sly 2007). However, research on the routes of allergen exposure for AD more recently has revealed that the epicutaneous route was the most important route in perpetuating the clinical signs of AD (Olivry et al. 1997; Marsella et al. 2006b; Marsella and Girolomoni 2009). The clinical scores (based on CADESI) of the epicutaneous exposure are greater than those of the other routes such as oral or inhalation exposure (Marsella et al. 2006b). Epicutaneous sensitisation allows allergens to penetrate the skin for a deleterious effect on the impaired skin barrier (Olivry and Hill 2001; Pucheu-Haston et al. 2008). Repeated epicutaneous sensitisation induces a T helper type 2 response and triggers the development of clinical signs (Nuttall et al. 2002; Marsella et al. 2006b; Yamamoto et al. 2007; Pucheu-Haston et al. 2008).

There are other factors which affect the induction of AD models. An intact skin barrier has an important role in preventing penetration of foreign substances into the body and excessive water loss from skin (van Smeden and Bouwstra 2016). There is growing evidence that the *stratum corneum* provides a skin barrier function against environmental allergens (van Smeden and Bouwstra 2016). There was an apparent difference between dogs in which an epicutaneous route was used with or without tape stripping (Olivry et al. 2011), showing that the *stratum corneum* removal facilitated sensitisation to HDM in the experimental AD models. These findings suggested the role of the *stratum corneum* as a barrier disturbing sensitisation to allergens.

Other factors that might have an impact on the clinical signs in an AD model during environmental exposure are the dose and source of the HDM (Marsella et al. 2006a), although these factors were kept consistent in the present study. The clinical severity is known to be dose-dependent and associated with the number of allergenic contacts (Marsella et al. 2006a). Whereas the clinical signs were not significantly different between the low dose (0.5 mg per dog, one time) group and the control group, the medium-dose (5 mg per dog, three times) and high-dose (50 mg per dog, three times) groups showed significant differences compared to the control group (Marsella et al. 2006a).

Young dogs (aged 6.7 ± 0.75 weeks old) were used in the current study because the sensitisation age is also important to induce canine AD models. Many studies have induced AD using young dogs (Marsella et al. 2006a; Marsella et al. 2006b; Pucheu-Haston et al. 2008; Marsella and Saridomichelakis 2010), because the capacity of the high IgE producing gene is achieved only under early (within the first week after birth) and repeated allergenic contact (de Weck et al. 1997). If the allergen exposure occurs early in life, specific IgE against the allergen is produced. The IgE will maintain a high concentration as long as exposure to the allergen occurs once every other month. However, Schwab-Richards et al. (2014) successfully developed AD models using dogs older than one year (ranging from 1 to 4 years). Therefore, this result suggested

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that there are many factors that affect the induction of AD models besides the age, such as the sensitising route, capacity to produce high IgE, source and dose of the HDM, and various factors still unknown.

In general, the limbs, ventral aspect of the abdomen, and perioral skin are the parts of the body most affected by AD. In the present study, clinical lesions were likewise predominantly on the caudal aspect of the carpus and tarsus, inguinal area, and the ventral aspect of the tail in the dogs that had a positive reaction intradermally.

In the current study, the clinical signs and positive intradermal reaction took longer to manifest in the dogs that were successfully sensitised to HDM, compared to the time to manifest in high-IgE producing dogs in a previous study (Pucheu-Haston et al. 2008). Twelve weeks may be insufficient to induce the clinical signs of AD in nongenetically predisposed Beagle dogs. Although the allergen sensitisation was conducted substantially before the environmental exposure, the nongenetically predisposed Beagle dogs seemed to require more time to develop the full range of allergen hypersensitivities (Hillier and DeBoer 2001) compared to high-IgE-producing dogs.

Not all the dogs in the current study were successfully sensitised to HDM. After the dogs underwent the environmental exposure, marked clinical signs and infiltration of inflammatory cells in the histopathological examinations were not observed in two of the five dogs. To date, trials have also been unsuccessful in developing canine models of AD by using high-IgE-producing dogs (Schwartzman et al. 1983; Egli et al. 2002). Therefore, some unknown genetic factors, besides the capacity to produce high IgE, may trigger the clinical signs of AD. Canine AD was recently defined as a multifaceted disease that is determined by a combination of genetic and environmental factors (de Weck et al. 1997). Both factors are important in causing AD (Bizikova et al. 2015). Under the same environmental conditions, some genetic mutations such as loss-of-function mutations in the filaggrin gene may play a key role in inducing the clinical signs of AD (Irvine et al. 2011).

There were many limitations to the present study. The main limitation was that the number of dogs was too small to yield significant results. Therefore, further studies with a larger number of dogs are needed to show accurate induction rates in nongenetically predisposed Beagle dogs, compared to the rate in high-IgE-producing dogs. Additionally, a comparison of these dogs with a control group of animals that are not sensitised to HDM is also essential. Special stains such as toluidine blue or Luna's stain need to be used to detect eosinophils or mast cells in the histopathology. Immunohistochemical examinations for IgE^+ dendritic cells, $CD3^+$ mononuclear cells, or $CD1c^+$ Langerhans cells is also essential. Comparing the induction rate by the various allergen sensitisation routes in normal dogs is also necessary. Further studies are needed to determine unknown genetic factors that affect the pathogenesis of AD.

In conclusion, this present study demonstrated that repeated epicutaneous sensitisation with HDM can induce AD in nongenetically predisposed Beagle dogs, regardless of the property for the production of high IgE. Therefore, normal dogs can be a canine model of AD that is not interrupted by a condition associated with a high level of allergen-specific IgE. These animals will also be a useful tool for testing the efficacy of pharmacotherapeutic interventions and performing advanced experiments that are limited to privately owned dogs.

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Conflict of interest

The authors declare no conflict of interest.

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