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Research paper

The detection of house dust mite *Dermatophagoides farinae*, Der f 2 and Zen-1 allergen-specific immunoglobulin E antibodies in dogs with atopic Dermatitis in Malaysia

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ABSTRACT

Canine atopic dermatitis (AD) is a chronic, inflammatory and pruritic allergic skin disease in dogs. House dust mites such as *Dermatophagoides farinae* are one of the known causative agents for the induction of canine AD worldwide. *D. farinae* protein Der f 2 is known as an important allergen involved in canine AD and recently, Zen-1 has also been identified as an allergenic protein. There is limited information on the prevalence and role of allergen sensitization to crude *D. farinae* extract (CDF), Der f 2 and Zen-1 among dogs diagnosed with AD in Malaysia. The aim of this study was to determine the proportion of CDF–, Der f 2– and Zen-1–specific reactive sera among dogs diagnosed with AD in Malaysia using an enzyme-linked immunosorbent assay (ELISA). Serum samples were collected from dogs diagnosed with AD from several veterinary clinics in Malaysia. The canine case records were retrieved and information on signalment, dermatological and non-dermatological histories, clinical presentation, food allergies, and exclusion of ectoparasitic, microbial and fungal skin infections were obtained through a survey form. All serum samples were evaluated to quantify the CDF–, Der f 2– and Zen-1–specific immunoglobulin E (IgE) levels. A total of 24.6%, 48.4% and 29.8% of dogs diagnosed with AD were positive for CDF–, Der f 2– and Zen-1–specific IgE, respectively. These results suggest that CDF–, Der f 2– and Zen-1 are important allergens that can contribute to AD in dogs in Malaysia, and serological testing can be performed to provide additional treatment options involving specific immunotherapies.

1. Introduction

Atopic dermatitis (AD) is a frequently observed chronic, recurrent, inflammatory and pruritic allergic skin disease in dogs with genetic predispositions that has been associated with the production of IgE antibodies in reaction towards environmental allergens (DeBoer and Hillier, 2001; Halliwell, 2006; Hill and DeBoer, 2001; Lian and Halliwell, 1998). Dogs with AD have various clinical symptoms depending on the stage of the disease (acute or chronic), genetic factors, severity of lesions and the presence of secondary infections, and it may be further complicated by other skin diseases with similar symptoms (Hensel et al., 2015). Canine AD is one of the more common dermatological problems in dogs, with a prevalence of 3.1% in 52 veterinary practices in the United States (Lund et al., 1999) and 4.8% in 20 small-animal general practices in England (Hill et al., 2006). A retrospective serological survey on common allergens among dogs with dermatological diseases in Malaysia revealed that 52.9% of dogs were seropositive for mites, suggesting the importance of mites as a major allergenic contributor in dogs in Malaysia (Lee et al., 2016).

The most common environmental factor causing IgE expression and associated with canine AD is house dust mites (HDMs), including *Dermatophagoides farinae* (Goicoa et al., 2008; Kang et al., 2014; Masuda et al., 2000; Nuttall et al., 2006; Zur et al., 2002). *D. farinae*, also known as the American HDM, can exhibit up to 34 groups of allergens, which have been studied and classified as Der f 1–4, 6–8, 10–11, 13–18, 20–37 and 39 according to the latest database update by the Allergen Nomenclature Subcommittee of the World Health Organization (WHO) and the International Union of Immunological Societies (IUIS) in May 2019. Several studies have identified high–molecular weight antigenic proteins Der f 15 and Der f 18 as important *D. farinae* allergens in atopic dogs (McCall et al., 2001; Nuttall et al., 2001; Weber et al., 2003). Der f 2 is a low–molecular weight antigenic protein and is reportedly a major allergen in canine AD caused by *D. farinae* in Japan (Masuda et al., 1999; Yamashita et al., 2002) but appears to be less

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important in other countries (Noli et al., 1996; Nuttall et al., 2001). Recently, Olivry et al. (2016) reported a new high–molecular weight *D. farinae* antigenic protein, Zen-1, as a major allergen that induces canine AD in North America and Europe.

According to the International Committee on Allergic Diseases of Animals (ICADA) guidelines, allergen-specific immunotherapy (ASIT) is considered a safe and specific method for alleviating the clinical symptoms of AD (Olivry et al., 2015). A retrospective survey by Carlotti et al. (2013) received positive feedback from owners who reported excellent to satisfactory resolution of clinical signs of pruritic skin lesions in 62.9% (n = 205) of their atopic dogs after receiving immunotherapy for at least a year. The aforementioned survey also reported that single-allergen immunotherapy was more effective compared to multi-allergen immunotherapy.

However, as with all similar therapies, correct diagnosis and identification of the causative allergen must be performed to determine the best treatment outcome. Intradermal skin testing (IDST) and allergen-specific IgE serology (ASIS) are the two recommended diagnostic methods used (Hensel et al., 2015) to determine the causative allergen that induces AD for dogs in Malaysia. lDST has been acknowledged as the gold standard for allergen identification; however, the procedures and results may be biased, and furthermore, requires sedation and shaving, the latter of which is not cosmetic, especially in small breeds (Hensel, 2012; Popiel and Cekiera, 2015). In recent years, ASIS has become an increasingly used alternative diagnostic method due to the convenience of obtaining routine serum from dogs, but again, results may vary due to non-standardization of assay testing between laboratories (Plant et al., 2014).

Due to the apparent geographic differences of *D. farinae* allergenic proteins as well as to determine the prevalence of specific allergens sensitization, this study examined crude *D. farinae* extract (CDF)–, Der f 2- and Zen-1– specific allergen sensitization in canine AD in Malaysia, via an enzyme-linked immunosorbent assay (ELISA) to determine the proportion of the three specific allergens reactive sera in pet dogs with AD.

2. Materials and methods

2.1. Length and locations of study

This study was performed between July 2014 and October 2015 at the University Veterinary Teaching Hospital of Universiti Putra Malaysia and at 14 small-animal veterinary practices in Peninsular Malaysia.

2.2. Criteria for animal selection

Regardless of breed, sex and age, a total of 62 client-owned dogs diagnosed with AD with at least 5 of the 8 clinical features of canine AD as described by Favrot et al. (2010) were included in this study. Ectoparasitic, bacterial or fungal cutaneous infections and food allergy reactions were ruled out prior to the diagnosis. Dogs with other causes of pruritus with cutaneous symptoms of superficial pyoderma, folliculitis and *Malassezia* yeast infection were excluded from the sample. A survey questionnaire to collect data on signalment, dermatological and non-dermatological histories and dermatological clinical presentations of the selected dog(s) was completed by each veterinarian. Clients who chose to participate in the study were asked for consent prior to the survey and the blood collection procedure.

2.3. Blood collection and serum storage

Blood samples were collected from the atopic dogs by the respective attending veterinarians consulting on the case for routine haematology and serum biochemistry laboratory diagnosis, and the remaining serum was stored for research purposes. The blood was kept at room temperature for 15–30 min before subjected for centrifugation at 1000–2,000x *g* for 10 min at 4 °C. The supernatant (serum) was transferred to a 2 mL microcentrifuge tube stored at −20 °C until used. Serum was also collected from apparently healthy dogs (undergoing elective procedures such as ovariohysterectomy or castration, with non-atopic dermatological conditions) for comparative purposes. Sera from three dogs (negative control) and three dogs sensitized to Zen 1, Der f 2 or CDF antigens (positive control) were obtained from Zenoaq.

2.4. Serological allergy testing

2.4.1. CDF and Der f 2–specific IgE detection

An assay for CDF and Der f 2–specific IgE measurement was performed according to previous studies (Moya et al., 2016; Yamashita et al., 2002) with some modifications. Briefly, CDF extract and lyophilized recombinant Der f 2 protein (Zenoaq/ Nippon Zenyaku Kogyo Co., Ltd., Japan) were diluted to a concentration of 50 µg/mL with sodium carbonate buffer (pH 9.6) to prepare the solid-phase antigen, and 100 µL of the resulting solution was added to each well of a 96-well flat-bottomed microtiter plate (TPP, Trasadingen, Switzerland). Following an incubation for 1 h at 37 °C, the solid-phase antigen was removed by wash buffer and 100 µL of ELISA buffer was added into each well for another 1 h of incubation as a blocking agent. A negative control and positive control (Zenoaq) (Moya et al., 2016) and test serum samples at 1:10 or 1:50 dilution and ELISA buffer alone (as a blank) were added to the appropriate wells in triplicate, and the plates were incubated for 1 h at 37 °C. After washing, the peroxidase-conjugated goat anti-dog IgE antibody (Bethyl Laboratories, Montgomery, Texas, United States) diluted up to 10,000-fold with ELISA buffer was added as the secondary antibody and incubated for 1 h at 37 °C. Colour development was initiated by adding 3,3′,5,5′-tetramethylbenzidine (TMB) substrate solution (Sigma-Aldrich, St. Louis, Missouri, United States) as instructed by the manufacturer, and the plate was protected from light for 30 min. The optical density (OD) was measured at 450 nm wavelength using a microtiter plate reader (Infinite M200 Pro, Tecan) after the enzyme reaction was stopped by the addition of 0.5 M sulfuric acid.

2.4.2. Zen-1–specific IgE detection

Microtiter plates were coated with 100 µL of native Zen-1 protein (Nippon Zenyaku Kogyo Co., Ltd., Japan) at 1 µg/mL in carbonate buffer (pH 9.6) and incubated overnight at 4 °C. After washing with PBST (PBS with 0.05% Tween 20), 100 µL of blocking buffer (25% Block Ace; DS Pharma Biomedical, Osaka, Japan) was added to the coated wells, followed by 1.5 h of incubation at 37 °C. Test serum samples, negative control and positive control were diluted 1:50 in blocking buffer and 100 µL of each including ELISA buffer alone acting as a blank (to account for background values) were added to the wells in duplicate and incubated further at 37 °C for 1.5 h. Next, 100 µL of HRP-conjugated anti-dog IgE monoclonal antibody (A40-125 P; Bethyl Laboratories Inc., Texas, USA) diluted to 1:2000 in blocking buffer was added to each well. After incubation at 37 °C for 1.5 h, the plates were washed with PBST, and 100 µL of TMB substrate solution (Sigma-Aldrich, St. Louis, Missouri, United States) was added to the wells for 20 min to initiate the enzyme–substrate reaction. Finally, the enzyme reaction was stopped by adding 50 µL of 2 N sulfuric acid, and the optical density at 450 nm wavelength was measured using a plate reader (BIO-RAD, model 680).

2.5. Measurement of IgE values

The average absorbance (optical density; OD values) of the 'blank' wells was subtracted from the OD value for each tested sample, including the negative and positive controls. The mean OD value of three wells per sample was used as the measurement value. The OD cut-off value was arbitrarily defined as two times the mean OD value of the

negative control. A sample with a mean OD value > OD cut-off value was considered ELISA-positive.

2.6. Statistical analysis

The frequency and overall percentage of CDF–, Der f 2– and Zen-1–specific IgE positive antibodies in the atopic dogs were determined.

3. Results

3.1. Dog signalment and clinical signs

In this study, sera was collected from 73 dogs in Malaysia. Among these dogs, 62 were diagnosed with AD (which fulfilled at least 5 out of 8 Favrot's criteria), 8 considered as "non-atopic" because although having 'atopic dermatitis' like symptoms they did not fulfil the Favrot's criteria for AD; and 3 dogs that are clinically healthy with no apparent dermatological lesions or complaints. The signalment, dermatological histories, and presentations of the 62 atopic dogs are presented in Table S1. Of the 62 dogs, 46 were purebred (74.2%) and 16 were mixed breed. The purebreds were Shih Tzu (n = 13), Poodle (n = 10), Golden Retriever (n = 3), Pug (n = 3), Beagle (n = 2), Bull Mastiff (n = 2), English Cocker Spaniel (n = 2), Pekingese (n = 2), Bulldog (n = 1), Chow Chow (n = 1), German Shepherd (n = 1), Jack Russell Terrier (n = 1), French bulldog (n = 1), Labrador (n = 1), Miniature Schnauzer (n = 1), Rottweiler (n = 1) and Siberian Husky (n = 1). Both sexes, female (n = 30) and male (n = 32), were almost equally represented, and 56.5% of the dogs had been neutered (n = 35). The atopic dogs were between 1–13 years of age, with 4–6 years being the most represented, while the average age of presentation to veterinarian was 6.4 years. Furthermore, 75.8% (n = 47) of the dogs had their first clinical signs of disease at under 3 years of age. Severe pruritus scores between 8 and 10 were recorded in 59.7% of the atopic dogs and 85.5% had a non-seasonal dermatological history. Skin lesions were mostly presented at the distal extremities, inguinal region, ears and face.

3.2. Fulfilment of Favrot's criteria in atopic dogs

The frequencies at which the atopic dogs (n = 62) met Favrot's criteria are listed in Table S2. More than 80% of the dogs fulfilled the criteria of dermatological skin conditions affecting the front feet (n = 56), living mostly indoors (n = 53) and skin lesions involving the ear pinnae (n = 51). More than 70% of the dogs had developed the onset of clinical signs of AD under 3 years of age (n = 47), pruritus that responded to glucocorticoid therapy (n = 46) and ear pinnae lesions not affecting the ear margins (n = 45). Up to 66.1% (n = 41) and 61.3% (n = 38) of the dogs had unaffected dorsolumbar areas and alesional pruritus at onset of disease, respectively.

3.3. ELISA and allergen-specific IgE detection

ELISA was performed on 73 serum samples which constitutes 62 atopic (that fulfilled the 5/8 Favrot's criteria), 8 "non-atopic" and 3 clinically healthy dogs from Malaysia. For the analysis, the three apparently healthy dogs with no dermatological lesions or clinical signs from Malaysia were excluded from being the "healthy control" dogs, since two out of the three dogs developed seropositive reaction (data not included) against all the three dust mite allergens; hence the values from negative and positive control dog sera from Japan (Zenoaq) were used for further analysis. A seropositive reaction against Der f 2–specific IgE was detected in highest proportion with 48.4% (30/62) of atopic dogs (Fig. 1a) as compared to CDF– and Zen-1–specific IgE-positive reactions which were only detected in 24.6% (14/57) and 29.8% (17/57) of atopic dogs, respectively (Fig. 1b and c). Data was not available for five dogs for CDF- and Zen-1-specific IgE detection due to exhaustion of sera after several assay optimization procedures performed, as these analyses were performed at a later time point. Seropositive reactions against Der f 2 – was detected in 50% (4/8) while both CDF– and Zen-1–specific IgE were found in 25% (2/8) of "non-atopic" dogs.

3.4. Fulfilment of Favrot's criteria in CDF– and Der f 2–seropositive atopic dogs

The frequencies at which the CDF–, Der f 2– and Zen-1–seropositive atopic dogs met Favrot's criteria are listed in Table 1. The top four most common criteria in the CDF–seropositive dogs were signs under the age of 3 years (13/14), living mostly indoors (12/14), affected front feet (11/14) and affected ear pinnae (11/14). Of the 30 Der f 2–seropositive dogs, the commonly recorded criteria were affected front feet (n = 27), living mostly indoors (n = 26), non-affected ear margins (n = 23) and non-affected dorsolumbar area (n = 23). Affected front feet, affected ear pinnae, and living mostly indoors were the three most common criteria for the Zen-1–seropositive atopic dogs.

4. Discussion

Diagnosing AD can be complex and challenging, and AD can be easily confused with other pruritic or atopic-like diseases. The ICADA guidelines (Hensel et al., 2015) place great importance on ensuring that all cutaneous diseases with similar and overlapping symptoms other than canine AD are first ruled out, cautioning meticulous historical investigation and identification of clinical conditions in dogs are needed based on Favrot's criteria. Furthermore, allergens suspected of inducing canine AD should be tested as candidates for ASIT. Due to the difficulties in eliciting a positive intradermal reaction or in identifying sensitizing allergen-specific IgE, test results may not always correlate with the clinical signs of canine AD (Lian and Halliwell, 1998; Mueller et al., 2016; Pucheu-Haston et al., 2015), with either IDST or ASIS being applied only after a clinical diagnosis of AD has been made.

The atopic dogs in this cohort were between six and seven years old upon AD diagnosis by the veterinarian. This is because most of the dogs were presented at a later time point for diagnosis upon exhaustion of all other diagnostic tests. However, more than 70% of them had the onset of clinical signs before the age of three years. The late definitive diagnosis could be attributed to poor compliance and financial constraints of their owners, which delayed ruling out all other diseases causing pruritus and atopic-like diseases in dogs, such as ectoparasitic diseases, bacterial and fungal infectious diseases and food allergies. Chronic and severe pruritus in dogs and the owner's poor understanding of AD may lead to frustration and impatience and decreases trust in veterinarians. This may cause the owner to seek immediate and more effective therapy from multiple veterinarians, which would then complicate the flow of ruling out other diseases that cause pruritus before the diagnosis of AD. An estimated minimum of 6.5 months is needed for a veterinarian to perform diagnosis before deciding on AD. For example, up to 12 weeks are needed to rule out ectoparasitic disease (Dryden et al., 2013), 8–12 weeks are required for food elimination trials and 10 weeks are required for food re-challenge (Rosser, 1993).

Shih Tzus and Poodles were over-represented purebreds in this study, and these small/toy breeds are often kept as house pets in Malaysia. In fact, 91.3% (21/23) of these breeds in our study were kept mostly indoors. There is a higher possibility that dogs kept indoors have higher exposure to HDM and their allergens. Breed predilection for AD in dogs may differ according to geographical location. A large study on 522 dogs from three countries (Australia, Germany, the United States) by Jaeger et al. (2010) revealed that Golden Retriever and German Shepherd were the breeds most commonly associated with AD, while a study from Switzerland found that West Highland White Terriers, Labradors, and Boxers were most commonly associated with AD (Picco et al., 2008). An older retrospective study from California by Zur et al. (2002) reported a similar observation, where Labrador Retriever, Golden Retriever, German Shepherd, Cocker Spaniel and West

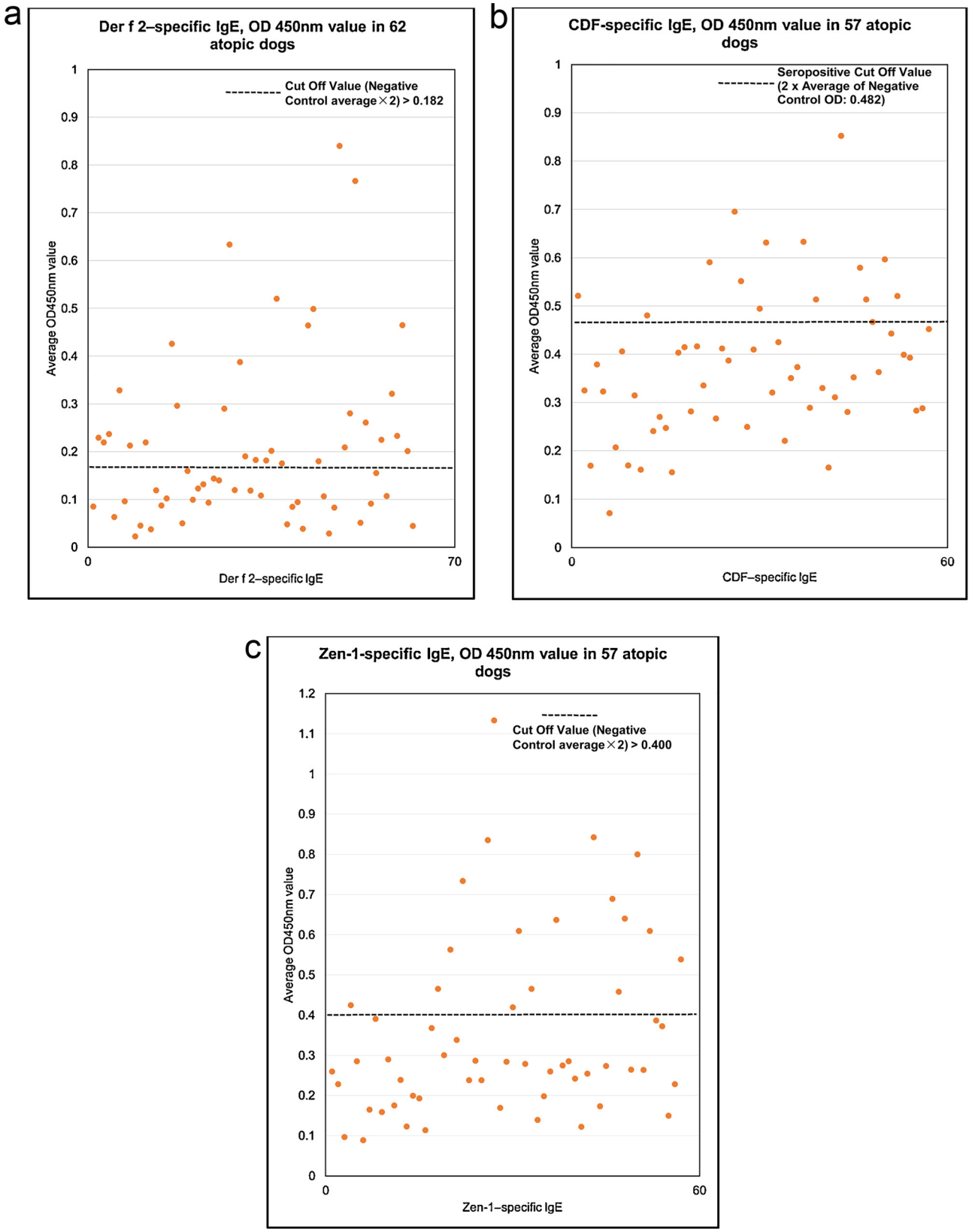

Fig. 1. a) Scatter plot graph of average OD 450 nm of Der f 2–specific Ig E in 62 atopic dogs. Der f 2–specific IgE positive reaction is indicated by $OD_{Average}$ 450 nm above cut off value (dotted line). b) Scatter plot graph of average OD 450 nm of CDF–specific Ig E in 57 atopic dogs. Der f–specific IgE positive reaction is indicated by $OD_{Average}$ 450 nm above cut off value (dotted line). c) Scatter plot graph of average OD 450 nm of Zen-1–specific Ig E in 57 atopic dogs. Zen-1–specific IgE positive reaction is indicated by $OD_{Average}$ 450 nm above cut off value (dotted line).

Table 1
Fulfilment of Favrot's criteria in atopic dogs with seropositive reaction against CDF, Der f 2 and Zen-1.

Favrot's 2010 criteria	CDF		Der f 2		Zen-1	
	Frequency (n = 14)	Percentage (%)	Frequency (n = 30)	Percentage (%)	Frequency (n = 17)	Percentage (%)
Affected front feet	11	78.6	27	90.0	14	82.4
Dog living mostly indoors	12	85.7	26	86.7	14	82.4
Not affected ear margins	10	71.4	23	76.7	13	76.5
Not affected dorso-lumbar area	10	71.4	23	76.7	9	52.9
Onset of signs under 3 years of age	13	92.9	22	73.0	13	76.5
Glucocorticoids responsive pruritus	10	71.4	22	73.0	13	76.5
Affected ear pinnae	11	78.6	20	66.7	14	82.4
Pruritus sine materia at onset	9	62.3	18	60.0	11	78.6

Highland White Terrier were the most commonly affected breeds. A review by Bizikova et al. (2015) suggested that AD is a heritable disease, where genetics play an important role in the pathogenesis of canine AD, as does exposure to environmental agents such as parasites, HDM and storage mites, mite allergen proteases and particulate matter or smoke, in canine AD disease development and progression.

Meeting 5 of 8 Favrot's criteria has 85% sensitivity and 79% specificity for distinguishing dogs with AD from dogs with chronic or recurrent pruritus without AD (Favrot et al., 2010). In the present study, 30 dogs, 14 dogs and 17 dogs with seropositive reactions to Der f 2–, CDF– and Zen-1–specific IgE, respectively, met the criteria of onset of signs below three years of age, living mostly indoors, pruritus resolved with glucocorticoids, and clinical presentations affecting the front feet and ear pinnae and not affecting the ear margins (Table 1). These are important clues in case histories and clinical features that should be taken into serious consideration by the veterinarian when considering the proper diagnosis for atopic dogs in Malaysia. To increase compliance and uninterrupted flow of diagnostic work-ups, the owner should be prepared and well-informed of the cost, time and rationale of the tests, treatment trials and therapies involved.

To the authors' knowledge, this is the first report of *D. farinae* specific allergen Der f2 and Zen-1 seropositive reactions in dogs diagnosed with AD in Malaysia. IgE seropositivity to Der f 2 was the highest with a frequency of 48.4% and thus, it is the most common HDM allergen associated with canine AD in our study. A similar observation was found in previous studies with a frequency of 43.8% (Masuda et al., 1999) and 74.4% in Japan (Yamashita et al., 2002). On the other hand, sensitization of Der f–specific IgE was much lower, in contrast to previous studies with a higher frequency between 37.5% and 71.0% (Kang et al., 2014; Masuda et al., 2000; Zur et al., 2002). As far as we know, the literature on Zen-1–specific IgE is limited. In our study, IgE seropositive reaction to Zen-1 was less significant in comparison to the study by Olivry et al. (2016), who found a high prevalence of 86% in American and European atopic dogs.

In this study, ELISA was performed in the laboratory to detect Der f–specific IgE spectrophotometrically, with a cut-off OD value set two times higher than that of the negative control mean OD value. Various studies have used ASIS using ELISA (Jackson et al., 2005; Kang et al., 2014; Moya et al., 2016; Raffan et al., 2005), and it is commercially available and used in many laboratories. However, Plant et al. (2014) reported disagreement on the interpretation of the results, which may affect the decision to pursue immunotherapy. A study using $Fc_{\varepsilon}RI\alpha$ receptor ELISA has shown that ASIS has high sensitivity (85–90%) but low specificity (25–50%) when run and compared concurrently with IDST (Popiel and Cekiera, 2015). Thus, IDST remains the preferred test in various studies (Chanthick et al., 2008; Kim et al., 2011; Sung and Huang, 2009) and is considered the gold standard to screen for causative allergens in sensitized atopic dogs. The immunodot assay had been used in earlier studies (Nuttall et al., 2001; Yamashita et al., 2002) in a laboratory setting to screen *Dermatophagoides* allergenic proteins in atopic dogs, but is now commercially available for clinic use to screen atopic dogs prior to performing a full panel of ASIS or IDST (Olivry and Paps, 2011).

Conventionally, oral therapies are the veterinarian's first line of treatment for dogs diagnosed with AD. Oclacitinib, a Janus kinase inhibitor, is effective for improving pruritus scores with rapid onset and minimal adverse effects such as gastrointestinal disturbances as compared to oral cyclosporine and oral glucocorticoids such as prednisolone (Cosgrove et al., 2013; Gadeyne et al., 2014; Little et al., 2015). However, such treatments appear to alleviate symptoms rather than treat the cause, and the symptoms return very soon after treatment is stopped. Alternatively, ASIT has become the latest trend for treating the root cause of allergens causing AD in dogs to improve clinical symptoms and stop disease progression (DeBoer, 2017). Typically, more than one causative allergen extract will be included in ASIT after IDST or ASIS is performed to identify the offending allergens. In a two-year study of ASIT with the customized use of multiple antigens, only 15.7% (18/117) of dogs with AD had complete remission and 60.7% of dogs (n = 71) improved clinically, but concurrent medications were still needed to different degrees (Schnabl et al., 2006). Carlotti et al. (2013) reported more significant successes in immunotherapy administration with one allergen compared to the inclusion of two or more allergens in the regimen. Hence, monoclonal immunotherapy has also been introduced recently. A pilot study on high-dose recombinant monoallergen subcutaneous immunotherapy cured atopic skin lesions in 5 out of 6 Der f 2–sensitized dogs (Olivry et al., 2017).

Sublingual immunotherapy (SLIT) and intra-lymphatic immunotherapy (ILIT) are two alternative therapies for cases of subcutaneous ASIT non-responding atopic dogs or for dogs that experience adverse events with injectable treatments. DeBoer et al. (2016) showed a reduction in dust mite–specific IgG levels and improvements in mean Canine Atopic Dermatitis Extent and Severity Index (CADESI)-03 scores after SLIT. The treatment is easily administered; the owners themselves can spray it on the dog's oral mucosa twice daily. ILIT was also proven effective in 20 dogs, showing a reduction in CADESI and pruritus scores without adverse events, but ultrasound-guided injection into the popliteal lymph nodes by veterinarians was still needed (Fischer et al., 2016).

In conclusion, we show that 24.6%, 48.4% and 29.8% of atopic dogs in Malaysia are sensitized to CDF, Der f 2 and Zen-1, respectively. A single-allergen specific immunotherapy may be recommended in Der f 2–seropositive dogs as part of the multi-modal treatment approach and management of canine AD to achieve a better quality of life for both dogs and owners.

Conflict of interest

Dr. T. Tsukui and Dr. R. Suzuki are employees of Nippon Zenyaku Kogyo Co., Ltd, who provided the negative and positive control sera from dogs is the manufacturer of Allermmune HDM, an immunotherapy for HDM-induced atopic dermatitis in dogs. However, they were not involved in the acquisition of samples and laboratory analysis which was done by CWY and GTS from Malaysia.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.vetimm.2019.05.002.

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