UNIVERSITI PUTRA MALAYSIA

RETROSPECTIVE SURVEY ON THE COMMON ALLERGEN SPECIFIC IgE IN PET DOGS IN MALAYSIA

LEE WEN HAI

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A project paper submitted to the
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It is hereby certified that we have read this project paper entitled "**RETROSPECTIVE SURVEY ON THE COMMON ALLERGEN SPECIFIC IgE IN PET DOGS IN MALAYSIA"** by Lee Wen Hai and in our opinion it is satisfactory in terms of scope, quality and presentation as partial fulfillment of the requirement for the course VPD 4999-Final Year Project.

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Specially dedicated to my family, friends, and the special one that I love

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LIST OF ABBREVIATIONS

IgE	immunoglobulin E
HDM	house dust mite
IDT	intradermal test
SAT	serum allergen test
AD	atopic dermatitis
FAD	food allergic dermatitis
ALD	atopic-like dermatitis
RAST	radio allergosorbent
ELISA	enzyme-linked immunosorbent assay
TEWL	transepidermal water loss
APT	atopy patch test
FLG	Filaggrin
AMP	antimicrobial peptide
mRNA	messenger ribonucleic acid
IL-31	interleukin-31
M-W test	Mann-Whitney test
SPSS	Statistical Program for Social Science
≤	less than and equal to
>	more than
%	percentage

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ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD-4999-Projek

KAJIAN RETROSPEKTIF MENGENAI IgE ALERGEN BIASA TERTENTU DI KALANGAN ANJING KESAYANGAN DI MALAYSIA

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Alergen merupakan suatu bahan, ianya boleh terdiri daripada protein ataupun bukan protein, yang mampu menyebabkan alahan atau hypersensitiviti tertentu yang biasa dikenali sebagai alahan. Alergen boleh diklasifikasikan kepada dua sumber, iaitu sumber alam sekitar dan sumber makanan. Di kalangan anjing, alergen biasa yang bersumber alam sekitar seperti hama

debu rumah telah dilaporkan sebagai punca utama yang menyebabkan canine atopik dermatitis di luar Negara. Walau bagaimanapun, pada masa ini, tiada sebarang kajian mengenai IgE alergen biasa tertentu di kalangan anjing kesayangan yang dijangkiti gejala atopic di Malaysia. Objektif kajian ini adalah untuk menentukan IgE alergen biasa tertentu di kalangan anjing kesayangan di Malaysia.

Kajian retrospektif telah dijalankan menurut rekod yang terdapat dalam 70 kes anjing yang telah menjalankan ujian "*Canine 62 Allergen Specific IgE Serological Test*", oleh EBS, Taiwan. Sampel darah telah diambil oleh pelbagai doktor haiwan kesayangan di seluruh Malaysia daripada Novemeber 2012 hingga ke Discember 2015 untuk menjalankan ujian serologi. Informasi tentang baka anjing, umur semasa sampel diambil, jantina, sejarah (kegunaan ubat mengandungi glukokortikoid, rawatan ectoparasite, lokasi luka serta jenis luka) telah direkodkan. Keputusan ujian serologi telah diklasifikasikan dalam "Trace", "Mild", "Moderate" dan "Severe". Analisis statistic dengan mengunakan kaedah Mann-Whitney dan Chi-square untuk mengenalpasti perkaian tererti ($p<0.05$) di antara faktor tahap IgE dengan klinikal parameter.

Majoriti anjing yang terlibat dalam kajian ini adalah baka size kecil dan serdahana (64%), melebihi umur 3 tahun (42%) dan jantan (59%). Perkaitan tererti ($p= 0.006$) di antara alergen alam sekitar dengan alergen makanan. Tiga jenis alergen alam sekitar yang mencatatkan rekod positive tertinggi ialah hama (52.86%), kulat (38.57%) dan debunga (32.86%). Daripada kalangan 5 jenis hama, *Dermatophagoides farinae* merupakan alergen yang paling penting (25% anjing diklasifikasikan sebagai "Moderate" hingga "Severe". Selain itu, *Candida albicans* (kulat)

dan *Cynodon dactylon* (debunga) juga merupakan salah satu alergen yang penting di dalam kohort anjing ini. Kesimpulannya, kajian ini menunjukan bahawa alergen alam sekitar terutamanya hama debu rumah merupakan punca utama yang menyebabkan tahap IgE tertentu meningkat di kalangan anjing kesayangan di Malaysia.

Kata Kunci: *Dermatophagoides farinae*, IgE allergen tertentu, alergen alam sekitar, anjing, Malaysia

ABSTRACT

An abstract of the project paper presented to Faculty of Veterinary Medicine in partial fulfillment of the course VPD 4999 – Project

RETROSPECTIVE SURVEY ON THE COMMON ALLERGEN SPECIFIC IgE IN PET DOGS IN MALAYSIA

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Allergen is a substance, protein or non-protein, capable of inducing allergy or specific hypersensitivity which commonly term as allergy. It can be further classified into environmental or food source of allergens. In dogs, common environmental source of allergen such as house dust mite has been reported to be the common cause of canine atopic dermatitis in other countries. However, to date, there has not been any published studies on the common allergen specific IgE in pet dogs with atopy-like symptoms in Malaysia. The objective of this study is to determine the common allergen specific IgE among pet dogs with allergies symptoms in Malaysia.

A retrospective study was conducted on records of 70 pet dogs suspected with allergy based on clinical symptoms, lesion types and localization that were tested for "*Canine 62 Allergen Specific IgE Serological Test*", by EBS, Taiwan. Blood sample was submitted for

serology testing by various small animal clinic practitioners in Malaysia from November 2012 to December 2015. Information such as breed, age of sample collection, sex, history (prior use of glucocorticoid, ectoparasitic treatment, lesion localization and types of lesions) were recorded. The serology data was classified into "Trace (<500)", "Mild (500-2000)", "Moderate (2000-8000)" and Severe (> 8000)". Statistical analysis such as Mann-Whitney and Chi-square tests were carried out where appropriate to determine the significant difference and association of IgE measurements with clinical parameters ($p<0.05$). Tests were performed using SPSS version 23.

Majority of the dogs included in this study were medium-small breed (64%), >3 years old (42%) and male (59%). There was a significant difference ($p=0.006$) between the environmental allergens as compared to food source. The three specific environmental allergens that caused the highest positive rates are mites (52.9%), fungi (38.6%) and pollen (32.9%). Among the five groups of mites, the *Dermatophagoides farinae* appeared to be the most important allergen (28.5% of dogs classified as "Moderate-Severe"). The *Candida albicans* (fungi) (30 %) and *Cynodon dactylon* (pollen) (25.7 %) appear to contribute as other important allergens among the dogs in this cohort. In conclusion, this study showed that environmental allergen especially house dust mite is the major cause of elevated specific IgE in pet dogs with allergy in Malaysia.

Keywords: *Dermatophagoides farinae,* IgE, allergen, canine, Malaysia

1.0 INTRODUCTION

Allergen is a substance, protein or non-protein, capable of inducing allergy or specific hypersensitivity which commonly termed as allergy (Studdert *et al*., 2012). Almost every substance in the environment can be allergen, including pollen, spores of mold, food preservatives, drugs and others (Studdert *et al*., 2012). It can normally come from environmental or food sources.

Allergens from food sources are common causes for allergies and food insensitivities and sometimes adverse reactions in selected individuals (Taylor, 1987) (Taylor *et al*., 2001). Naturally occurring proteins in the foods are can cause abnormal immune response which further lead to food allergy (Bush *et al*., 1996). Food allergy can be further divided into two categories which are immediate hypersensitivity and delayed hypersensitivity reactions (Lemke *et al*., 1994). In immediate hypersensitivity reactions, the symptoms occur in minutes or hours after the consumption of the food material whereby in delayed hypersensitivity the symptoms only begin 24 hours or longer after ingestion of the food (Hefle *et al*., 1996, Lemke *et al*., 1994). The most common food allergy reported in dogs is beef (Lieberman *et al*., 2011).

Environmental sources of allergens are such as pollen, house dust mite, fungi, bacteria and others. Environmental allergens are also responsible for the cause of canine and human atopic dermatitis (Haliwell, 2009; Olivry *et al*., 2011; Marsella *et al*., 2012; Hensel *et al*., 2015).

Canine atopic dermatitis is a common dermatosis of dogs defined as a genetically-predisposed inflammatory and pruritic skin disease with characteristic clinical features that it

is associated with elevation in specific IgE antibodies, which are most commonly due to environmental allergens (Halliwell, 2006; Olivry *et al*., 2011; Marsella *et al*., 2012; Hensel *et al*., 2015). Although the above definition covers many aspect of the clinical condition of the disease, however there is no pathognomonic lesion of this disease and can be easily confused with 'atopic like dermatitis' possibly due to various factors and secondary conditions which can occur concurrently and complicate the presenting features of the disease (Olivry *et al*., 2011; Marsella *et al*., 2012; Hensel *et al*., 2015).

Aside from genetic predisposition, a number of environmental triggers that can cause atopic dermatitis in dogs and humans have been shown in some of the studies (Kim *et al*., 2010). Comparatively, in humans, house dust mite (HDM), cockroaches, fur from cats and dogs, pollen, and mold were identified as important causative allergens (Weiss *et al*., 1998; Omenaas *et al*., 1995; Nelson, 2000). Studies that have been conducted on the risk factors for canine atopic dermatitis on the past ten years have also shown that HDM and house dust are the common allergens in dogs (Park *et al*., 2000; Zur *et al*., 2002). However, there are no such reports on the common allergen specific IgE which causes atopic dermatitis in dogs in Malaysia.

Currently, the most common diagnostic methods for identifying the various types of allergens in atopic dogs are by using allergen specific IgE serological testing and the traditional intradermal test (IDT) (Wilemse, 1986; Codner *et al*., 1993; Kim *et al*., 2010). Although IDT is considered the preferred diagnostic method, serum allergen test (SAT) had several advantages over IDT such as no sedation is required, less traumatic as no repeated injections are required, more convenient with regards to no clipping of fur needed and lower risk of drugs (glucocorticoid/ anti-pruritis medications) interfering with the test results

(Hensel *et al*., 2015). The IDT often more time consuming, invasive and required longer time to elicit an immune response as compared to the allergen specific IgE serological testing (SAT) which is much easier, not affected by glucocorticoid and histamine therapy and rapid diagnostic time (Scott *et al*., 2001, Olivry *et al*., 2001). However, both IDT and SAT are still lacking standardization and it is suspected that false positive and false negative results do occur (Scott *et al*., 2001, Olivry *et al*., 2001; Hensel *et al*., 2015). It is also estimated that between 10 to 30% of dogs with a clinically confirmed canine AD may give negative result on IDT (Hillier *et al*., 2001; Hensel *et al*., 2012).

Many studies of canine atopic dermatitis allergen distribution have been conducted over the past 20 years, but within the past five years' studies on the topic have been rare (Kim *et al*., 2010). In addition, comparative research concerning common canine atopic dermatitis allergens in Asia has been lacking (Kim *et al*., 2010). Therefore, the objective of this study is to determine the common allergens specific IgE from all the 70 pets dog samples in Malaysia. The current study is conducted by analyzing the "Canine 62 allergen specific IgE serological test" reports of 70 domestic dogs with allergy from various small animal clinics in Malaysia between 2012 till 2015. It is hypothesized that, environmental allergens such as HDM and pollen are the most significant allergens in dog's population in Malaysia.

2.0 LITERATURE REVIEW

2.1 Allergen and Allergy

Allergen is a substance, protein or non-protein, capable of inducing allergy or specific hypersensitivity which commonly termed as allergy (Studdert *et al*., 2012). Almost every substance in the environment can be allergen, example including plant pollen, spores of mold, food preservatives, drugs and others (Studdert *et al*., 2012). It can originate from environmental source or food sources.

Food sources of allergen can cause food allergy/food sensitivities. It is an individualistic adverse reaction to foods (Taylor, 1987). Adverse food reaction can be further classified in to IgE and non IgE-mediated primary immunological sensitivities, non immunological food intolerances, and secondary sensitivities (Taylor *et al*., 2001). True food allergies are due to the triggered abnormal immune response to certain naturally occurring proteins in the foods (Lemke and Taylor, 1994; Bush and Hefle, 1996). True food allergies can further be divided into two main categories which are immediate and delayed hypersensitivity reactions (Lemke and Taylor, 1994). In immediate hypersensitivity reactions, symptoms begin to develop within minutes to hour after ingestion of a particular protein while delayed hypersensitivity symptoms will only develop 24 hours of longer upon ingestion of the allergen (Lemke and Taylor, 1994). In human, the most common food that trigger allergy are cow's milk, chicken egg and peanut; while other less common food allergens include soy, wheat, fish, and shellfish (Sicherer *et al*., 2010; Wang *et al*., 2011). A recent review on common food allergen that caused cutaneous adverse food reaction in dogs from Australia, Europe and North America reported that the common food sources are beef (34%), dairy product (17%), chicken (15%), wheat (13%) and lamb (14.5%)

(Mueller *et al*., 2016). Other less commonly reported food allergens include soy (6%), corn (4%), egg (4%), pork (2%), fish (X%) and rice (2%) (Mueller *et al*., 2016).

Sources of allergens from the environment include pollen, house dust and dust mites; fungi, bacteria (*Staphylococcus aureus*) and others. In human, the common environmental source of allergen that cause human AD are house dust mites, cockroaches, fur from pets such as cats and dogs, pollen, and mold (Omenass *et al*., 1995; Weiss *et al*., 1998; Nelson, 2000). Studies on canine AD suggested that the common environmental allergens include house dust and house dust mite (HDM) (Park *et. al*., 2000; Zur *et. al*., 2002).

2.2 Canine Atopic Dermatitis

Allergic dermatitis is an allergic skin disease in dogs which is caused by immunological hypersensitivity to common substances in the environment or through ingestion. Canine atopic dermatitis (AD) was originally thought to be of inhalant allergic dermatitis (Nuttal *et al*., 2013). Further understanding of the epidemiology, immunology and clinical signs lead to a further change in its definition by the International Task Force On Canine Atopic Dermatitis, as a genetically-predisposed inflammatory and pruritic skin disease with characteristic clinical features that it is associated with IgE antibodies, which are most commonly due to environmental allergens (Halliwell, 2006; Olivry *et al*., 2011; Marsella *et al*., 2012; Hensel *et al*., 2015). However, canine AD is also recognized as a complex multifactorial disease which actually does not have a pathognomonic clinical sign that enable definitive diagnosis be made based on history and physical examination alone (Hensel *et al*., 2015; Nuttal *et al*. 2013). Multiple factors such as intrinsic genetics (breed-associated phenotype) for example West Highland White Terrier, type and extend of lesions (localized or generalized), duration of symptom persistence or stage of the disease (acute versus chronic), the presence of secondary microbial infections, immune

dysregulation, allergic sensitization and skin barrier defect (Hensel *et al*., 2015; Nuttal *et al*. 2013). Atopic dermatitis is common in both dogs and human whereby it affect up to 30% of the population. (Santoro *et al*., 2012).

A similar condition with canine AD called "atopic-like dermatitis" (ALD) has been described where it can be easily confused with canine AD and should be recognized and differentiated from canine AD (Olivry *et al*., 2011; Nuttal *et al*., 2013). Even though ALD is clinically identical to canine AD, but IgE response to environmental or other allergens cannot be detected on serological analysis. (Hensel *et at*., 2015; Nuttal *et al*., 2013). However, in a recent study suggest that ALD has been associated with a lymphocyte-mediated adverse reaction to allergen from food sources (Hensel *et at*., 2015).

2.3 Clinical Signs & Diagnosis of Canine Atopic Dermatitis

Most of the study and journal article accepted that history and physical examination findings are important in the diagnosis of canine AD (Olivry *et al*., 2011; Nuttal *et al*., 2015; Hensel *et al*., 2015). The primary skin lesions usually consist of erythematous macules, patches and small papules (Olivry *et al*., 2011). However, most of the patient will be presented with lesions secondary to self trauma such as excoriations, self-induced alopecia, lichenification and hyperpigmentation (Halliwell *et al*., 2006; Olivry *et al*., 2007; Olivry *et al*., 2011). The distributions of the clinical signs is variable and dependent on the chronicity of the disease, but most of the clinical lesions are present on the face, ventral neck, ear pinnae, axillae, groin, abdomen, perineum, ventral tail as well as flexural and medial aspect of extremities (Olivry *et al*., 2011; Nodtvedt *et al*., 2007).

In order to diagnose canine AD, a concise history of the dog which include age of onset, breed, familial predisposition, seasonality, "*pruritus sine material*" at onset and efficacy of

previous treatment should be obtained (Hilllier *et al*., 2001; Halliwell *et al*., 2006; Olivry *et al*., 2007). Around 42 – 75% of the canine AD has seasonal onset of clinical signs with approximately 80% occur in spring or summer and lesser extent during winter and autumn (Hilllier *et al*., 2001; Halliwell *et al*., 2006; Olivry *et al*., 2007). Pruritus must be present and if absent, a diagnosis of atopy can be ruled out. Almost 61% of the affected dog exhibit "*pruritus sine material*", 43% with concurrent otitis externa, 78% responsive to glucocorticoids therapy and up to 82% have a history of spending most of the time indoors (Hilllier *et al*., 2001; Halliwell *et al*., 2006; Olivry *et al*., 2007).

A study reported that 66% of dogs with canine AD have concurrent infections; 33% infected with Malassezia and 50% present with otitis externa (Nodtvedt *et al*., 2007). However, most of these clinical signs are not specific for canine AD while the recognition of distribution of clinical signs would be more helpful (Nodtvedt *et al*., 2007). Unfortunately, all these areas are rarely simultaneously affected in the same individual, except in chronic cases (Nodtvedt *et al*., 2007).

The flow to assist in diagnosis of canine AD will be based on the following three approaches:

- Rule out other skin diseases that have clinical sign similar to canine AD, such as flea allergy dermatitis, ectoparasitic infestation (sarcoptic mange), demodicosis, dermatophytosis and cutaneous lymphoma
- Detailed interpretation of the historical and clinical features of the condition according to the "Favrot's criteria" which was published in 2010 (Table 1), whereby at least 5 out of 8 criteria should be present in the dog in order a diagnosis of canine AD can be achieved

- Perform an allergy test either an assessment of skin reactivity by Intradermal testing (IDT), or detection of serum IgE against specific allergens (SAT)

Table 1: Favrot's criterias to diagnose canine AD.

Favrot's criteria (Favrot *et al*., 2009)
• Age at onset <3 years old • Mostly indoor • Corticosteroid-responsive pruritus • Chronic or recurrent yeast infections • Affected front feet • Affected ear pinnae • Non-affected ear margins • Non-affected dorso-lumbar area

The study also showed that the sensitivity of using Favrot's criteria are 85% and specificity are 79% if 5 criteria are fulfilled while the sensitivity drops to 58% and specificity increases to 88% if 6 criteria are fulfilled (Hensel *et al*., 2015; Nuttall *et al*., 2013; Favrot *et al*., 2009). As an acceptable standard, a case could only be considered as canine AD if at least 5 out of 8 of the criteria is fulfilled because this is set of diagnostic criteria that currently agreed and with 5 criteria or more fulllfied, it had a high specificity which can reduced false positive.

2.4 Allergen Specific IgE Serological Testing

Allergen specific IgE testing is a serological allergy test conducted by collecting serum in order to detect the presence of allergen specific antibodies in canine serum samples, either by radio allergosorbent (RAST) or enzyme-linked immunosorbent assay (ELISA) (PARK *et al*., 2000; Kang *et al*., 2014). The advantages of using SAT are the lack of risk, discomfort and invasive to the patient, allowed for quantitative analysis of the results and able to performed on patients with widespread cutaneous inflammation (PARK *et al*., 2000). The early development of SAT assay utilized affinity-purified polyclonal anti-IgE antibodies, which is not sensitive enough

to replace IDT. Later, the development of monoclonal anti-IgE antibodies facilitated better detection of the causative allergens for more accurate immunotherapy (PARK *et al*., 2000). It is reported that SAT demonstrated sensitivity from 16.7% to 68. 2% while specificity from 94.9% to 100% (PARK *et al*., 2000). This is a major problem in SAT which has a high specificity and low sensitivity (PARK *et al*., 2000). One of the major reason for the discrepancy between IDT and SAT is the non standardization of the allergens used in IDT and there is absent of a standard method that accurately measures the biologic potency of allergen extract (PARK *et al*., 2000). The interpretation of both IDT and SAT results should be based on the development of clinical hypersensitivity upon exposure to the allergen (PARK *et al*., 2000). Furthermore, it is also questionable whether it is justifiable to use the SAT studies to identify allergens for immunotherapy due to the low sensitivity of the test (PARK *et al*., 2000). However, a study in Japan on canine AD concluded that there is no significant difference in response between dogs administered with a specific allergen immunotherapy identified by IDT as compared to SAT. This study also suggests that SAT could be effectively used to select a particular allergen for immunotherapy (PARK *et al*., 2000).

2.5 Allergy In Humans

Dogs are common pet animals and share similar environment as humans. Both can be subjected to similar allergens especially from the environmental sources an d to a lesser extent from food.

In human, environmental, occupational allergy and respiratory allergy that derived from airbone allergen (Aeroallergen) has been numerously reported as the most significant cause of allergic rhinitis and asthma in Malaysia.(Lim *et al*., 2015; Mahram *et al*., 2013; David *et al*., 2010). According to world health survey 2010, in Malaysia alone, the prevalence of doctor-

diagnosed adult asthma to be 5.2%, wheezing among adults 7.55% and allergic rhinitis 7.1% (Katelaris *et al*., 2011; To *et al*., 2012). While other studies in Asia country, the reported prevalence of doctor-diagnosed allergic rhinitis among children and adults is 2.5% in Philipines and 12.3% in Vietnam (Katelaris *et al*., 2011). Moreover, there is large variation of asthma among adults between Asian countries with the prevalence ranging from 0.7 to 11.9% (Song *et al*., 2014). The large variation in the prevalence rate of asthma and rhinitis could indicate that the various climatic difference across difference country in Asia (Lim *et al*., 2015). It was also speculated that the prevalence of asthma and allergic rhinitis in Asia country could be due to the increasing trend in pet ownership.

2.6 Comparison Between Allergies In Human and Dogs

Animal models have contributed greatly to the expansion of knowledge in the field of atopic dermatitis (Marsella *et al*., 2009). For example, canine pruritic dermatitis is clinically and immunologically similar to human atopic dermatitis (Marsella *et al*., 2009) (Table 2).

Table 2: The similarities and differences between canine and human AD(adapted from Helton *et al*., 1987; Willemse, 1988).

Characteristics	Canine AD	Human AD
Prevalence in general population (%)	10-15	5-20 of children
Genetically inherited	+	+
Age of onset (years)	1-3	<1-5
Skin areas affected	Face, skin folds	Face, skin folds
Spongiotic dermatitis	+	+
Skin-infiltrating eosinophils	+	±
Skin infiltration by IgE+CD1c+dendritic cells	+	+
Pruritus	Severe	Severe
Skin xerosis	+	+
Increased TEWL	+	+
Decreased epidermal Filaggrin	+	+
Higher skin colonization by *Staphylococcus aureus*	+	+
Th2-dominated immune response	+	+
APT	+	+

IgE-specific resposes (%)	80	55-90
Rhinitis and conjunctivitis (%)	<5	35
Asthma (%)	<5	30
Atopic march	No	Yes

*AD, atopic dermatitis; APT, atopy patch test; TEWL, transepidermal water loss

Other than that, the similarities between canine and human AD is not limited to clinical signs only (Marsella *et al*., 2009). Histologically, the lesions are characterized by spongiotic dermatitis with a mononuclear infiltrate, accumulation of epidermal and dermal IgE+CD1c+ cells, and epidermal eosinophil microaggregates that consistent with the importance of epidermal allergen contact (Olivry *et al*., 1997; Hill *et al*., 2001; Olivry and Hill, 2001).

2.7 Biomarkers of Canine Atopic Dermatitis

Studies have shown that Filaggrin (FLG) is a key protein for skin barrier formation formation (Kanda *et al*., 2013). This protein is translated from a large precursor protein, proFLG, which consists of tandem FLG monomer repeats (Kanda *et al*., 2013). Each of the FLG monomer repeat is then processed to generate the FLG monomer in keratohyalin granule in the stratum granulosum of the epidermis (Kanda *et al*., 2013). A strong association between FLG nonsense and frameshift gene mutations and AD has been reported in both human and canine populations (Sandilands *et al*., 2007). It has been hypothesized that the loss of skin barrier functions and the loss of FLG monomers contribute to the risk of human AD however, it is not certain in other species including dogs (Brown *et al*., 2012).

There were few studies on the possible involvement of antimicrobial peptide (AMP) in the pathogenesis of AD in dogs and the result was similar to human studies (Santoro *et al*., 2012). It showed that there was increased mRNA expression of selected canine peptides such as cBD1, cBD1-like, cBD3-like and cCath in lesional and non-lesional skin layers in dogs (Van *et*

al., 2009; Santoro *et al*., 2011). Other important AMP such as ß-defensins and cathelicidins had increase epidermal expression in canine AD(Santoro *et al*., 2011).

Interleukin-31 (IL-31) is a recently identified cytokine implicated in pruritic skin conditions such as human AD (Gonzales *et al*., 2013). Studies showed that 57% of the dogs diagnosed with naturally occurring AD has elevated skin IL-31 level. (Gonzales *et al*., 2013). IL-31 was not detected in healthy dogs (without pruritis or skin diseases) and in dogs with pruritis especially in flea allergies or in dogs sensitized to the house dust mite (HDM) allergen *Dermatophagoides farinae* (Gonzales *et al*., 2013). IL-31 may be dysregulated in dogs with AD and contribute to the pathology of this chronic allergy skin disease (Gonzales *et al*., 2013).

3.0 MATERIALS AND METHODS

3.1 Data Collection

A retrospective survey was performed on the records provided by Rhone Ma (M) Sdn. Bhd. The records include reports of "*Canine 62 Allergen Specific IgE Serological Test*" that was performed on suspected AD dogs throughout West Malaysia for a period of 4 years from 2012 till 2015. All the data are then screened and important relevant information was noted for case signalment, history, clinical presentation and the result of the "*Canine 62 Allergen Specific IgE Serological Test*".

Data and signalment that retrieve for the study include the date of sample received by pharmaceutical company, age, breed and gender. Historical data obtained include previous food history such as previous food ingredients, management (indoor or outdoor), and first appearance of clinical symptoms whether is more than 3 years old or less than 3 years old, corticosteroid-responsive pruritus and pruritus sine material at onset (generalized itching in the absence of visible skin rashes). Other than that, historical data regarding the lesion of the sample upon presentation at various clinics also has been recorded such as whether the sample has affected front feet, affected ear pinnae, non-affected ear margins and non-affected dorso-lumbar region. All the sample were presumably submitted for IgE analysis based on suspicious of canine AD.

3.2 Questionnaire and Telephone Interview

A questionnaire was prepared for the veterinarians. The questionnaires were either sent thru email or was used in phone interviews to various participating small animal clinics to obtain additional information that was missed during submission.

3.3 Data Tabulation and Statistical Analysis

All data were tabulated in Microsoft Excel spreadsheet and transferred to the SPSS version 23 spreadsheet for further analysis such as analyze for descriptive statistic (median, mean, mode) (where applicable) and Tests of Normality using Shapiro-Wilk. $P<0.05$ rejected the null hypothesis when the distribution of data is not normally distributed. After performing normality test on the sets of data (allergen group, breed and age), additional analytical test was performed by selecting non-parametric test in the SPSS to carry out further analysis to test for significant of association by using Chi-square test for comparison between allergen groups, with breed, age and sex. While Mann-Whitney (M-W test) test are conducted in order to identify the significant difference between the environmental and food source allergens. Other than that, M-W test was also performed to test the significant difference between mites' and pollen allergen groups. The 62 tested allergens were first categorized into environmental and food source allergen. Environmental allergen subgroup consists of mites, fungi, insect, pollen hair and epithelial while food source pollen subgroup consist of milk/egg, meat, fruit, seafood, vegetables, yeasts, grains, beans, nuts and flax. The 62 allergen also further classified into either positive or negative. The criteria for categorization of the 62 tested allergens into positive and negative is by intensity of a specific allergen to be 2000 and above will be positive and those below 2000 will be negative. Statistical significance is recorded at $p<0.05$, whereby the null hypothesis will be rejected indicate that the data is statistically significant. All statistical analysis was conducted using the IBM SPSS (Statistical Program for Social Science) software version 23.0. Selected factors that used to determine the association test were allergen group, breed, age and sex.

4.0 RESULTS

4.1 Demographics

From September 2012 till November 2015, there were a total of 70 dogs' samples send to conduct the "*Canine 62 Allergen Specific IgE Serological Test*" by various small animal clinic located throughout Malaysia.

The number of cases each year ranging from 11 to 26 cases whereby the highest cases attain at the year 2013(n=26) and there was a drastic increase in the amount of sample sent for the serological test from year 2012(n=11) to year 2013(n=26). The frequency and percentage of the sample sent each year from year 2012 till 2015 is shown in Table 3. Figure 1.

Table 3: The frequency and percentage of sample sent for the serological test from year 2012 till 2015.

No.	Years	Frequency (n)	Percentage (%)
1	2012	11	15.71
2	2013	26	37.14
3	2014	15	21.43
4	2015	18	25.71
	Total	70	100.00

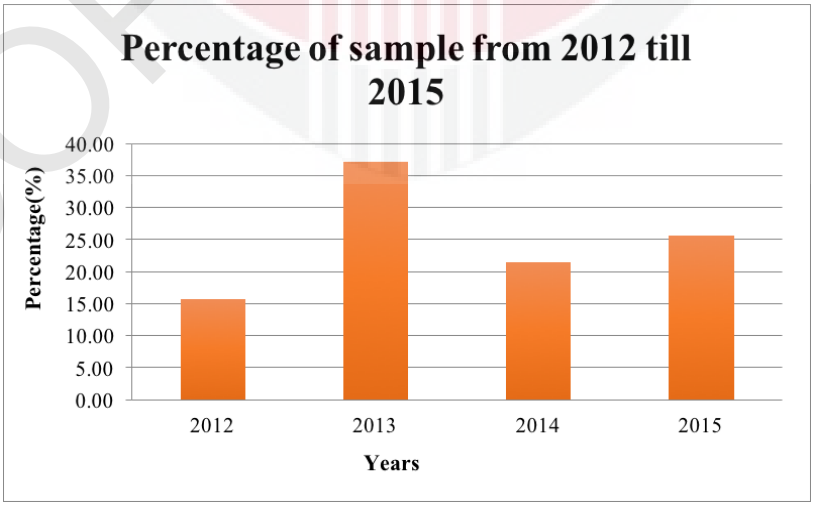

Figure 1: The percentage of sample sent for the serological test from year 2012 till 2015.

Among all the 70 samples, it is submitted by various small animal clinician throughout various state in Malaysia from the year 2012 till 2015. From our entire sample, majority is from Selangor (58.57%), followed by Johor (14.29%), Penang (12.86%), Kuala Lumpur (5.71%), Malacca (4.29%), Perak (2.86%) and Sarawak (1.43%). Figure 2 shows the distribution of dogs from different states in peninsular Malaysia.

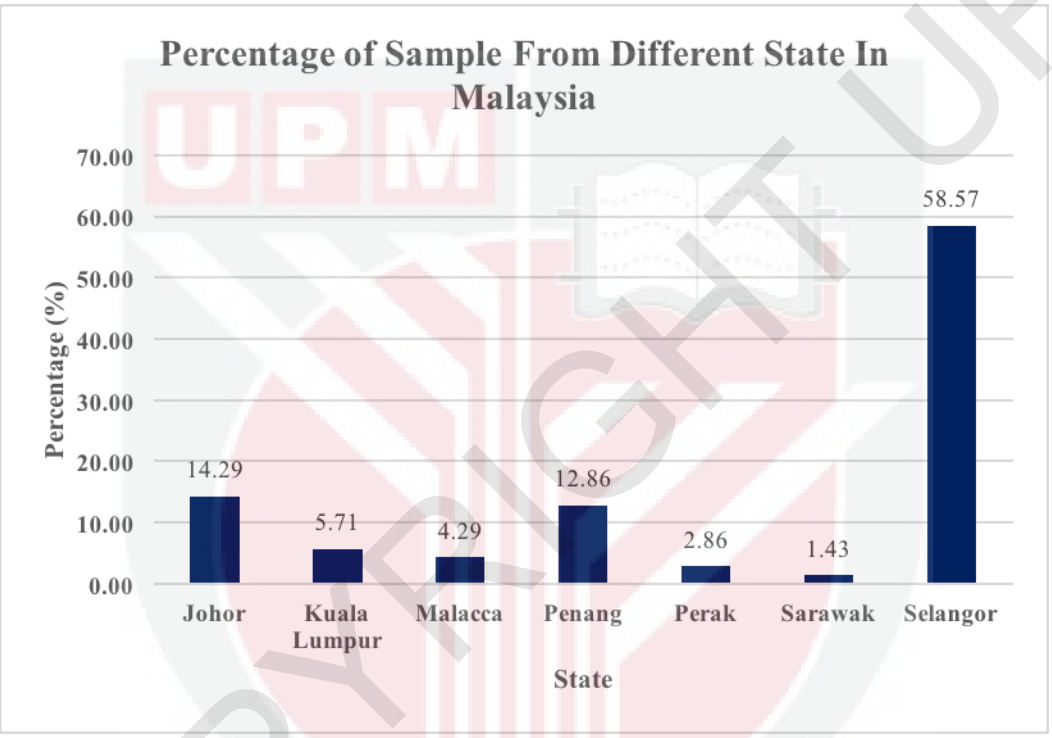

Figure 2: The distribution of samples from different states in Malaysia.

Among all the 70 samples submitted, the dogs were categorized based on breed and body size: giant/large breed or medium/small breed. Local dogs were considered medium sized. Whereby medium/small breed accounted for the majority, 64.29% (n=45) of the total samples. The dogs were further classified based on breed type include: pedigree 84.29% (n= 59), mixed breed 10% (n=7) and local breed 5.71% (n=4). The detailed number of dogs from each particular breed is shown in Table 4. Most of our samples are from Shih Tzu breed (n=10), followed by poodle(n=7), mixed breed(n=7), golden retriever (n=6), and mongrel (n=4).

The age of dogs are shown in Appendix A. The dogs were categorized into two large groups which are: below and equal to 3 years old (n=28, 40%) and animals more than 3 years old (n= 42, 60%). There were 41 males (58.6%) and 29 females (41.4%).

The number of dogs that fulfilled at least 5 out of the 8 Favrot's criteria , 10% (n=7) and 90% (n= 63).

Table 4: Frequency and percentage of the sample categories by every particular breed.

No.	Breed	Frequency	Percentage (%)
1	Cocker Spaniel	3	4.29
2	Beagle	3	4.29
3	Bulldog	3	4.29
5	Dachshunds	1	1.43
6	German shepherd	3	4.29
7	Golden Retriever	6	8.57
8	Golden Retriever Cross	1	1.43
9	Great Dane	2	2.86
10	Labrador Retriever	1	1.43
11	Labrador Retriever Cross	1	1.43
12	Maltese	1	1.43
14	Mini Schnauzer	1	1.43
15	Mixed Breed	7	10.00
16	Mongrel	4	5.71
17	Pekingese	1	1.43
18	Pomeranian	1	1.43
19	Poodle	7	10.00
20	Poodle Cross	1	1.43
21	Pug	2	2.86
22	Rottweiler	2	2.86
23	Scottish Terrier	1	1.43
24	Shih Tzu	10	14.29
25	Silky Terrier	2	2.86
26	Spitz Mix	1	1.43
27	Terrier Mix	1	1.43
28	Toy poodle	2	2.86
29	Bull Mastiff	1	1.43
30	Siberian Husky	1	1.43
	Total	70	100.00

4.2 Positive Rates From Serological Testing

The 62 specific allergens and their respective groupings and positive rates were calculated. The calculation of positive rate for each allergen is by whether the sample is positive for that particular allergen while the group positive rate is calculated based on the sample have at least one positive to that particular allergen group will be counted (Table 5).

Table 5: Allergen groups and their corresponding positive rate from the sample data.

Allergen Groups	Allergen Extracts	Positive Rate	Percentage (%)	Group Positive Rate	Percentage (%)
Mites	*Blomia tropicals*	*13/70*	18.57	*37/70*	*52.86*
	D. pteronyssinus	*19/70*	27.14		
	D. farinae	*20/70*	28.57		
	Acarus siro	*12/70*	*17.14*		
	Tyrophagus putrescentiae	*17/70*	*24.29*		
Fungi	*Cladosporium herbarum*	*2/70*	*2.86*	*27/70*	*38.57*
	Candida albicans	*21/70*	*30.00*		
	Alternaria alternata	*6/70*	*8.57*		
	Penicillium notatum	*1/70*	*1.43*		
	Aspergillus fumigatus	*4/70*	*5.71*		
	Malassezia pachydermatis	*3/70*	*4.29*		
	Staphylococcus aureus	*5/70*	*7.14*		
Insecta	Black Ant	1/70	1.43	14/70	20.00
	Tick	3/70	4.29		
	Flea	0/70	0.00		
	Culex pipiens	*9/70*	*12.86*		
	Aedes communis	*11/70*	*15.71*		
	Blatella germanica	*1/70*	*1.43*		
Pollen	*Eucalyptus globules*	*2/70*	*2.86*	*23/70*	*32.86*
	Poa pratensis	*16/70*	*22.86*		
	Amaranthus retroflexus	*10/70*	*14.29*		
	Rumex acetosella	*11/70*	*15.71*		
	Ambrosia artemisii	*7/70*	*10.00*		
	Acacia spp.	*4/70*	*5.71*		
	Cynodon dactylon	*18/70*	*25.71*		
Hair and	Mixed Feathers	0/70	0.00	2/70	2.86

Epithelial	Human Dander	0/70	0.00		
	Cow Epithelia	0/70	0.00		
	Goat Epithelia	2/70	2.86		
	Cat Hair	0/70	0.00		
Milk/Egg	Cheese	2/70	2.86	8/70	11.43
	Egg White	4/70	5.71		
	Egg Yolk	1/70	1.43		
	Milk	3/70	4.29		
Meat	Duck	2/70	2.86	8/70	11.43
	Lamb	2/70	2.86		
	Beef	0/70	0.00		
	Chicken	3/70	4.29		
	Turkey	3/70	4.29		
	Pork	2/70	2.86		
Fruit	Watermelon	6/70	8.57	10/70	14.29
	Cantaloupe	0/70	0.00		
	Pear	2/70	2.86		
	Mandarin Orange	2/70	2.86		
	Orange	3/70	4.29		
Seafood	Crab	9/70	12.86	20/70	28.57
	Shrimp	2/70	2.86		
	Mackerel	3/70	4.29		
	Sardine	9/70	12.86		
	Anchovy	5/70	7.14		
	Codfish	3/70	4.29		
	Salmon	7/70	10.00		
Vegetable	Carrot	5/70	7.14	11/70	15.71
	Corn	9/70	12.86		
	Potato	5/70	7.14		
Yeasts	Baker's Yeast	0/70	0.00	6/70	8.57
	Brewer's Yeast	6/70	8.57		
Grains, Beans and Nuts	Soybean	6/70	8.57	11/70	15.71
	Peanut	5/70	7.14		
	Rice	2/70	2.86		
	Wheat	6/70	8.57		
Flax	Flax	7/70	10.00	7/70	10.00

4.3 Analysis On Allergens Based On Group Positive Rates

4.3.1 Comparison Between Environmental and Food Allergen

The p-values of tests for significance (Mann-Whitney) upon comparing the sum of environmental allergen with food source allergen positive rates. There was a significant elevation (P value=0.006) in specific IgE levels in the environmental allergens collectively as compared to food source allergens..

4.3.2 Comparison Between Mites and Fungi Allergen Subgroups

There was no statistically significant difference (P=0.091) between the mite allergen subgroup IgE levels as compared to the pollen allergens subgroups. Both mite and fungi allergens are equally important in causing elevated specific IgE in dogs in cohort.

4.3.3 Between Mites and Pollen Allergen Subgroup

Dogs in this study have significantly increased IgE levels for mite allergens (P=0.017) as compared to pollen. Allergens from mites have a significant role in the pathophysiology of allergy in dogs in this cohort.

4.4 Analysis Of Association Between Difference Factors and Allergens Subgroups

4.4.1 Association Between Breed and Allergens Subgroups

There was no significant association when comparing positive test results between giant/ large with medium/ small breeds and the 13 allergens subgroups (Table 6).

Table 6: Chi-square analysis p value for significant of association between breeds and 13 allergens subgroups.

Allergen Group	**Breed**	**Test result**		***p* value**
		Positive (n)	**Negative (n)**	
Mites	Giant/Large	13	12	*p*=0.915
	Medium/Small	24	21	
Fungi	Giant/Large	10	15	*p*=0.855
	Medium/Small	17	28	
Insecta	Giant/Large	3	22	*p*=0.212
	Medium/Small	11	34	
Pollen	Giant/Large	9	16	*p*=0.676
	Medium/Small	14	31	
Hair and	Giant/Large	0	25	*p*=0.285
Epithelial	Medium/Small	2	43	
Milk/Egg	Giant/Large	3	22	*p*=0.911
	Medium/Small	5	40	
Meat	Giant/Large	3	22	*p*=0.911
	Medium/Small	5	40	
Fruit	Giant/Large	4	21	*p*=0.760
	Medium/Small	6	39	
Seafood	Giant/Large	6	19	*p*=0.528
	Medium/Small	14	31	
Vegetable	Giant/Large	3	22	*p*=0.524
	Medium/Small	8	37	
Yeasts	Giant/Large	1	24	*p*=0.309
	Medium/Small	5	40	
Grains, Beans and	Giant/Large	4	21	*p*=0.961
Nuts	Medium/Small	7	38	
Flax	Giant/Large	1	24	*p*=0.212
	Medium/Small	6	39	

4.4.2 Association Between Age and Allergens Subgroups

Chi-square analysis revealed that there was no significant of association between age groups and allergen subgroup (Table 7).

Table 7: Chi-square analysis p value for significant of association between age groups and 13 allergens subgroups.

Allergen Group	**Age group**	**Test result**		***p* value**
		Positive (n)	**Negative (n)**	
Mites	≤ 3 years old	14	14	*p*=0.696
	> 3 years old	23	19	
Fungi	≤ 3 years old	11	17	*p*=0.920
	> 3 years old	16	26	
Insecta	≤ 3 years old	7	21	*p*=0.393
	> 3 years old	7	35	
Pollen	≤ 3 years old	8	20	*p*=0.533
	> 3 years old	15	27	
Hair and	≤ 3 years old	1	27	*p*=0.770
Epithelial	> 3 years old	1	41	
Milk/Egg	≤ 3 years old	4	24	*p*=0.540
	> 3 years old	4	38	
Meat	≤ 3 years old	4	24	*p*=0.540
	> 3 years old	4	38	
Fruit	≤ 3 years old	4	24	*p*=1.000
	> 3 years old	6	36	
Seafood	≤ 3 years old	10	18	*p*=0.280
	> 3 years old	10	32	
Vegetable	≤ 3 years old	6	22	*p*=0.283
	> 3 years old	5	37	
Yeasts	≤ 3 years old	2	26	*p*=0.727
	> 3 years old	4	38	
Grains, Beans and	≤ 3 years old	5	23	*p*=0.688
Nuts	> 3 years old	6	36	
Flax	≤ 3 years old	3	25	*p*=0.871
	> 3 years old	4	38	

4.4.3 Association Between Sex and Allergens Subgroups

Data analysis resulted in statistically non significant association between sex and allergen subgroup (Table 8).

Table 8: Chi-square analysis p value for significant of association between sex and 13 allergens subgroups.

Allergen Group	Breed	Test result		*p* value
		Positive (n)	Negative (n)	
Mites	Male	21	20	*p*=0.744
	Female	16	13	
Fungi	Male	15	26	*p*=0.685
	Female	12	17	
Insecta	Male	10	31	*p*=0.275
	Female	4	25	
Pollen	Male	13	28	*p*=0.808
	Female	10	19	
Hair and	Male	1	40	*p*=0.803
Epithelial	Female	1	28	
Milk/Egg	Male	3	38	*p*=0.199
	Female	5	24	
Meat	Male	4	37	*p*=0.601
	Female	4	25	
Fruit	Male	6	35	*p*=0.921
	Female	4	25	
Seafood	Male	11	30	*p*=0.701
	Female	9	20	
Vegetable	Male	6	35	*p*=0.768
	Female	5	24	
Yeasts	Male	2	39	*p*=0.189
	Female	4	25	
Grains, Beans and	Male	7	34	*p*=0.710
Nuts	Female	4	25	
Flax	Male	4	37	*p*=0.936
	Female	3	26	

5.0 DISCUSSION

In this study, 70 pet dogs serum sample submitted by various small animal practitioners from various small animals clinics/ hospitals for "*Canine 62 Allergen Specific IgE Serological Test*" from year 2012 till 2015 in Malaysia. The serum samples were collected from pets dog presented to various small animal clinics/ hospital and submission of the serum sample for conducting the IgE analysis is presumably based on the suspicion of canine AD by the small animal practitioners. However, the detailed history of food elimination trial could not be obtain in most of the sample. Other important medical history such as breed, sex, age and serological test result were recorded.

From the serological test result, the 62 allergens are classified into various allergen subgroups such as mites, fungi, pollen, insecta, hair and epithelial, milk/egg, meat, fruit, seafood, vegetables, yeasts, grains, beans and nuts as well as flax. Furthermore, all of these 13 allergen subgroup is further categories into two major groups which is environmental allergens (30 allergens) and food source allergens (32 allergens).

Among all the 13 allergens groups, mites' allergen subgroup has the highest percentage of group positive rate which is around 52.86%. Which means that 52.83% of the dogs involved in this study had a positive result to at least one of the five mite allergens subgroups as shown in table 5.

This is followed by the fungi allergen subgroups which are 38.57% and 32.86% for the pollen allergen subgroups. All of these three allergens subgroups are derived from environmental allergen. This is similar to some studies suggesting that environmental allergens were responsible for the cause of canine and human atopic dermatitis (Haliwell, 2009; Olivry *et al*., 2011; Marsella *et al*., 2012; Hensel *et al*., 2015). Among the mites allergen subgroups, house

dust mite *Dermatophagoides farinea (D. farinae)* having the highest positive rate which is 28.57% followed by another house dust mite *Dermatophagoides pteronyssinus(D. pteronyssinus)* which is 27.14%. This is similar to some studies showing that HDM was one of the risk factor for developing canine atopic dermatitis as well as human atopic dermatitis in Europe.(Omenass *et al*., 1995; Weiss *et al*., 1998; Nelson, 2000; Park et al., 2000; Zur et al., 2002). Moreover, other studies also show that 60 to 90% of cases had positive skin test or specific serum IgE level for mite allergen (DeBoer *et al*., 1989; Zur *et al*., 2002). However, there was one study show that 58% of clinically healthy dogs had a positive skin test reaction to house dust mite (Codner *et al*., 1995).

Another similar study was conducted in Taiwan whereby 54% of the dogs included in the analysis had a positive result to at least one of the five mites' species (Tsai *et al*., 2012). This study was very similar result with the current survey which is 52.83%. Furthermore, one study in Taiwan show that D. pteronyssinus was the most common allergen which had a positive rate of 29.5% compared to another study in Korea whereby the most common house dust mite allergen was *D. farinae* that accounted for 63% positive rate (Youn *et al*, 2002). The difference in positive rate can be explained by the difference in climate and habitat for the two species of mites (Tsai *et al*., 2012). However, the study show that *D. farinae* are common in dryer area such as Korea with an average humidity of 53% while *D. pteronysinnus* is more common in tropic and subtropical area like Taiwan with average humidity of 82% (Tsai *et al*., 2012). This is difference from our current study whereby *D. farinae* is more common (28.57%) versus *D pteronyssinnus* (27.14%) even though Malaysia is a tropical country with an average humidity of 80-90% which is very similar to Taiwan. However, statistically it showed no significant difference between *D. farinae and D pteronyssinnus* means that both are equally important. Another explanation to the

result of this study is that it had been reported that mixed type of house dust mite allergen will have a higher false positive reaction (Bond *et al*., 1996). There was also reported in one of the study showing that 60 to 90% of canine AD cases had immunological response to mites (Tsai *et al*.,2012).

In human, *D. pteronyssinus* is the species most commonly involved in human allergy in many countries including Malaysia (Mueller *et al*., 2000; Mahram *et al*., 2013; Lim *et al*., 2015). *D. pteronyssinus* required higher humidity and not able to survive extended dry period as well as *D. farinae,* which is a dominant species in some countries such as Canada and United States (NMueller *et al*., 2000). However, in dogs, *D. farinae* was reported as a more frequent cause of allergic skin disease and one study found out that 34% of dogs positive for *D. farinae* while only 14% positive to *D. pteronyssinus* (Mueller *et al*., 2000). The aforementioned study have a similar result as this present study whereby *D.farinae* had a higher positive rate compared to *D. pteronyssinus.* In Malaysia, the reported prevalence of house dust mite allergy, to be 50.3% for *D. pteronyssinus,* 49% for *D. farinae* among office workers (Lim *et al*., 2015). This showed that in human, *D. pteronyssinus* is the most commonly involved in house dust mite allergy as compared to dogs.

A total of 38.7% of the dogs in this cohort have elevated IgE in at least one of the 7 fungi allergens. There appears no significant difference when comparing IgE measurements when exposed to mites and fungi allergens, suggesting that both allergens are equally important to pet dogs' population in Malaysia. Among the various fungi allergens tested in this study, *Candida albicans* has the highest positive rate, which is 30% positive rate. Another similar study also gives a result of positive rate for fungi of 35.4% and particularly *Candida albicans* has a positive rate of 16.13% (reference). One study conducted in Korea showed that, 33.3% of positive rate

among all the sample was positive to *Candida albicans* (Kang *et al*., 2014). However, in Korea study regarding the common sensitization rate for *Pullularia* and *Alternata* was 44.8% and 39.6% which is higher than *Candida albicans* in our study (Kang *et al*., 2014).

However, according to other previous reports, sensitivity of the allergen-specific IgE serological test to fungi was lower than the intra dermal skin test and serology result are less reliable for detecting fungal hypersensitivity (Mueller *et al*., 1999; Foster *et al*., 2003). Furthermore, another different study conducted in Seoul, Korea also reported positive rate of 67.3% for mold which is higher than house dust mite (49.1%) (Kim *et al*, 2011). The difference in positive rates among the fungi allergens across different countries in the world probably due to the different climatic condition and lifestyle of the pets. In particular, molds and pollens were affected by temperature and humidity (Beggs *et al*., 2004). In Korea, due to the changing of lifestyle and most of the pets were kept indoor which cause outdoor allergens such as trees, weeds and grasses were found to be less positive reaction (Kim *et al*, 2011).

In Japan, HDM and Japanese cedar pollen were the most common allergen to dogs and human in that particular region (Masuda *et al*., 2000). However, there was no allergen have yet been reported which is unique to Malaysia that affect both dogs and human. This is similar to Korea whereby further studies may uncover specific regional differences (Kim *et al*, 2011).

From the above all the studies, we can conclude that molds and pollen allergen are widely difference in their positive rate across difference region and generalization of result from different region is generally very difficult and inaccurate.

In human, outdoor molds such as *Alternaria alternate* was the most important cause of asthma than pollen (Peyton *et al*., 2001; Peden *et al*., 2010; Mahram *et al*., 2013). One studies showed that, 26.7% human sample were positive for *Alternaria* fungus (Mahram *et al*., 2013).

Other important species include *Penicllium* and *Aspergillus* which were often present in greater amount indoor than in outdoor (Peyton *et al*., 2001). From our study result, the case positive rate for *Alternaria* (8.57%), *Penicillium* (1.43%) and *Aspergillus* (5.71%) which is not that important when compare with Candida albicans (30%) in dogs. This show that dogs and human have different allergy level towards fungal allergen.

Furthermore, for pollen allergen subgroup, the group positive rate is 32.86% meaning that around 32.86% of the total sample population is positive to at least one of the pollen allergen subgroup. And within the pollen allergen subgroup, *Cynodon dactylon* (Bermuda grass) which has the highest positive rate (25.71%). One of the study showed that grass pollen was one of the main pollen that causes allergy in animals in most of the countries (Noli *et al*., 2014). Furthermore, cross-sensitization was very common (Noli *et al*., 2014). One study conducted in Europe showed that dog can be in contact with these pollen even out of the pollination season because the pollens were produce during pollination season and can be on the ground for whole year round (Noli *et al*., 2014). Furthermore, this study showed that indoor environment can also be rich in grass pollen allergens whole year round (Noli *et al*., 2014). Hence, grass pollens can be considered as non-seasonal allergens in some atopic dog (Noli *et al*., 2014). This study suggests that the high positive rate in *Cynodon dactylon* could be due to the fact that the pollen is present all year round at both outdoor and indoor irrespective of the pollination season which was usually from March to September.

However, another study in Korea showed that pollen allergens does not cause significant problem and suggested that it maybe due to most of the sample dog population were from the indoor and not expose to the pollen (Kim *et al*., 2011). The study in Taiwan showed that highest sample positive rate for pollen in sample collected in spring (Tsai *et al*., 2012). From most of the

study, significance and the important of pollen groups of allergen cannot be generalized and applied from one region to another as it is varying widely across different region during every different season, temperature and climatic condition (Kim *et al*., 2011; Tsai *et al*., 2012; Noli *et al*., 2014).

In human, pollen was considered as one of the most abundant and inevitable elements in allergic rhinitis and asthma (Peden *et al*., 2010; Mahram *et al*, 2013). In human, the most important pollen allergen groups were ragweed and the increase prevalence of allergy rhinitis reported are due to changes in global climate and global warming which promote the growth of certain plant that cause the increase prevalence of pollen induced allergy rhinitis (Peden *et al*., 2010; Mahram *et al*, 2013).

On the other hand, for comparing association between breed and allergen group, statistical analysis showed that there is no significant association between breed and any allergen group indicate that all breeds are having the similar risk factor to contact any of the 62 test allergen and cause elevation in specific IgE level. This could be due to the fact that the sample sizes are too small (n=70) as compare to other studies. In other study, breed predisposition are well known but are varies from one part of the world to another (Haliwell *et al*., 2009; Nuttall *et al*., 2013). However, most of the common breed affected include Labrador, golden retriever, West highland white terriers, English Springer spaniels, Chinese SharPei, Bull Terriers, Bichon Fries and Tibetan Terriers while mixed breed dogs has a lower prevalence (Nuttall *et al*., 2013). For example, in Switzerland, West Highland White Terriers, Boxers, French Bulldogs, Hungarian Vizslas. Bull Terriers, Rhodesian Ridgebacks and Basset Hounds (Picco *et al*., 2008). While in Britain, reported breed were Labrador and Golden retriever (Nuttall *et al*., 2013). These two studies showed that the breed affected is varies across different region.

One study in Taiwan showed that most of the breed presented were mixed breeds, Golden Retrievers and Maltese (Tsai *et al*., 2012). While two study conducted in Korea showed that most of the breed included in the studies were from small breed such as Maltese, Shih Tzu, Miniature pinscher and medium breed such as Cocker Spaniel (Kim *et al*., 2011; Kang *et al*., 2014). In our study, most of the sample are from medium/small breed (n=45) while Shih Tzu (n=10), mixed breed (n=7), Poodle(n=7), Golden Retriever(n=6) and others. It is clear that the breed predisposition varies across different geographical region. However, from our sample, it suggests that it is more similar to other study population conducted in Asian countries such as Korea and Taiwan rather than Europe or America as most of the sample population are from medium/small breed.

Other than that, there is no significant association between age and allergen group and this indicate that age is not the risk factor in this study. The chances of getting at least one positive results from the 62 tested allergen in the two age group show no difference statistically. This is largely due to most of the sample are submitted by various clinician from different small animal clinic throughout Malaysia whereby most of the time the reported age is during the sample collection/submission and not the actual age in which the first onset of clinical sign. The data regarding the first age of the onset of clinical sign could not be trace due to various factor and the fact that most of the pet owners might change clinician gradually before conducting this serological test which make it almost impossible to obtain the exact age of first clinical presentation. One study in Taiwan also demonstrated that there was no significant association between breed and risk factor in canine atopic dermatitis due to lack of age information regarding the first age of clinical onset (Tsai *et al*., 2012).

Moreover, there is also no significant association between sex and allergen group and this indicate that sex is not the risk factor in this study. The chances of getting at least one positive results from the 62 tested allergen in the two sex group show no difference statistically. In other studies, inconsistent result regarding sex as a risk factor in canine atopic dermatitis. Some of the studies reported predisposition for male, female or neither sex (Griffin *et al*., 2001). However, another study conducted using large sample size of 843 canine atopic dermatitis dogs reported that there was no sex predilection as a whole population (Favrot *et al*., 2009; Picco *et al*., 2008). There was also study that show that sex predisposition was detected in some breeds such as golden retriever or Labrador retriever (more female) or Boxer (more male) (Favrot *et al*., 2009).

In this study, food allergen accounted for 32 allergens out of the 62 allergens which is about 52% of the total tested allergen. However, most of the group positive rate is lower than 20% as compared to environmental allergen which is more than 30%. Only seafood allergen subgroups have achieved a group positive rate of 28.57% with the crab and sardine allergen have a positive rate of 12.86% respectively which is the highest positive rate in seafood allergen subgroups. Food allergic dermatitis (FAD) was the most common form of canine allergic dermatitis in most country followed by CAD (Tsai *et al*., 2012). CAD and FAD occur together in 35-70% of all allergic dermatitis (Youn *et al*., 2002). However, in human, the different cut off value exists among different food items with a very wide range (Tsai *et al*., 2012). Since the cut off value for the food source allergens in this study are all the same, hence it might under/over estimated the result. More reliable methods for the diagnosis of FAD as suggested by previous study include the use of dietary elimination challenge trials (Tsai *et al*., 2012). The result of food source allergen in this serological test can only be used as a quick screening test or side information for the owner, but did not stand on itself as a diagnosis (Verlinden *et al*, 2006).

While for other tested allergen subgroup such as insect had a group positive rate of 20% whereby *Aedes communis* and *Culex pipens* both has the high positive rate which is 15.71% and 12.86% respectively probably due to the fact that Malaysia is a tropical country and mosquitoes are breeding and presence all year round. While for the tick and flea allergen in this subgroup had a low positive rate as well which is 4.29% and 0%. This could be due to all of the sample had proper ectoparasite control or treatment before conducting this test. Whereas for hair and epithelial allergen subgroup, the group positive rate is only 2.86% and this suggested that human and animal sources (goat and cow epithelial) is not the factor which contribute to canine AD in our sample.

6.0 CONCLUSION

In conclusion, allergens from the environmental source play a more significant role as compared to food source in development of allergy among the dogs in this cohort. House dust mites contribute to elevation in IgE levels in almost 52.85% of dogs. *Dermatophagoides farinae* appear to be the most important mite allergen with 28.57% positive rate in these dogs. There was no significant association between the types of allergens with breed, age and sex. A prospective trial on immunotherapy against house dust mites may be useful among dogs in Malaysia.

7.0 RECOMMENDATIONS

For recommendation, a more detail prospective study regarding the canine atopic dermatitis can be conducted with a full and proper history, fulfilled at least 5 of the Favrot's criteria, proper dietary elimination and challenge trial as well as ruling out the other differential diagnosis in order to make the result of the study more reliable and accurate. Furthermore, prospective study comparing the findings on common allergen in canine atopic dermatitis dog using intradermal skin testing with serological method and immunotherapy respond after allergen identification can be done.

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APPENDIX A

Data for all the information on dogs analyzed in this study

No.	Breed	Age	Sex	Date Received	Date of Report	State
1	Poodle X Shih Tzu	10y	M	13/09/2012	19/09/2012	Selangor
2	English Cocker Spaniel	10y	F	25/02/2015	03/03/2015	Selangor
3	Bull Dog	1y	M	01/03/2013	07/03/2013	Selangor
4	Rottweiler	1y	M	11/03/2013	15/03/2013	Penang
5	Mongrel	1y	M	14/06/2013	20/06/2013	Penang
6	Golden Retriever	1y	M	06/12/2013	12/12/2013	Penang
7	Pug	1y	M	05/06/2015	11/06/2015	Kuala Lumpur
8	Great Dane	1y11m	M	20/08/2013	26/08/2013	Johor
9	Golden Retriever	1y2m	F	21/11/2014	27/11/2014	Malacca
10	Poodle	1y3m	M	11/03/2013	15/03/2013	Penang
11	Rottweiler	1y5m	M	10/01/2013	16/01/2013	Selangor
12	Golden Retriever	1y5m	M	01/04/2013	08/04/2013	Johor
13	Shih Tzu	1y9m	F	18/11/2014	24/11/2014	Selangor
14	Mixed Breed	2y	CM	13/09/2012	19/09/2012	Selangor
15	Poodle	2y	F	03/12/2012	07/12/2012	Selangor
16	Mini Schnauzer	2Y	M	24/01/2013	30/01/2013	Selangor
17	Beagle	2y	M	18/07/2013	24/07/2013	Penang
18	Dachshunds	2y	F	30/10/2013	05/11/2013	Selangor
19	Mixed Breed	2y	M	13/12/2013	19/12/2013	Selangor
20	Golden Retriever	2y	M	09/07/2014	15/07/2014	Selangor
21	Toy Poodle	2y	F	30/09/2015	05/10/2015	Sarawak
22	Toy Poodle	2y11m	SF	10/09/2012	14/09/2012	Johor
23	Mixed Breed	3-4y	F	17/10/2014	23/10/2014	Perak
24	Pug	3y	M	11/03/2013	15/03/2013	Selangor
25	Golden Retriever	3y	F	21/08/2014	27/08/2014	Selangor
26	Mongrel	3y	SF	03/12/2014	09/12/2014	Johor
27	Poodle	3y	M	28/01/2015	03/02/2015	Selangor
28	Shih Tzu	3y	F	20/03/2015	26/03/2015	Selangor
29	Poodle	3y	M	07/04/2015	13/04/2015	Selangor
30	Siberian Husky	3Y	M	-	-	Selangor
31	Beagle	3y11m	F	29/05/2013	04/06/2013	Johor
32	Maltese	3Y6m	F	27/02/2014	06/03/2014	Selangor
33	Shih Tzu	4y	M	14/01/2013	18/01/2013	Selangor
34	Mongrel	4y	CM	01/03/2013	07/03/2013	Selangor

35	English Bulldog	4y	M	22/07/2013	26/07/2013	Selangor
36	Poodle	4y	M	04/07/2014	10/07/2014	Selangor
37	Golden Retriever	4y	F	07/01/2015	13/01/2015	Selangor
38	Mixed breed	4y	M	12/10/2015	15/10/2015	Malacca
39	Golden Retriever	4Y	SF	09/09/2015	15/09/2015	Selangor
40	Labrador Retriever	4y10m	M	10/09/2012	14/09/2012	Johor
41	Poodle	4Y2m	SF	30/09/2015	05/10/2015	Malacca
42	Maltese x Poodle	4y6m	SF	05/11/2014	11/11/2014	Kuala Lumpur
43	Scottish Terrier	4y7m	M	21/11/2014	27/11/2014	Johor
44	Shih Tzu	5y	CM	05/10/2012	11/10/2012	Selangor
45	Golden Retriever Cross	5y	M	26/11/2012	30/11/2012	Selangor
46	Silky Terrier	5y	F	18/07/2013	24/07/2013	Penang
47	Bull Mastiff	5y+	SF	29/04/2015	05/05/2015	Selangor
48	Labrador Cross	5y3m	CM	30/01/2015	05/02/2015	Selangor
49	Spitz Cross	6y	SF	13/09/2012	19/09/2012	Selangor
50	Great Dane	6y	SF	13/09/2012	19/09/2012	Selangor
51	Mixed Breed	6y	F	01/11/2012	07/11/2012	Penang
52	Pekingese	6y	CM	25/03/2015	31/03/2015	Selangor
53	Poodle Cross	6y8m	F	18/08/2014	22/08/2014	Selangor
54	American Cocker Spaniel	7y	M	02/01/2013	08/01/2013	Selangor
55	Poodle	7y	M	24/01/2013	30/01/2013	Selangor
56	Shih Tzu	7y	M	01/08/2013	07/08/2013	Penang
57	German Shepherd	7y	F	23/09/2013	27/09/2013	Johor
58	German Shepherd	7y	M	10/06/2015	16/06/2015	Selangor
59	Cocker Spaniel	7y9m	SF	21/11/2014	27/11/2014	Kuala Lumpur
60	Terrier Mixed	8y	M	13/09/2012	19/09/2012	Selangor
61	Silky Terrier	8y	F	11/03/2013	15/03/2013	Penang
62	German shepherd	8y	M	29/04/2013	03/05/2013	Selangor
63	Shih Tzu	8y	M	23/05/2013	29/05/2013	Selangor
64	Shih Tzu	8y	F	13/11/2014	19/11/2014	Johor
65	Bulldog Cross	8y	M	25/02/2015	03/03/2015	Selangor
66	Shih Tzu	8y	M	27/03/2015	01/04/2015	Perak
67	Mongrel	9m	SF	06/11/2015	12/11/2015	Johor
68	Pomeranian	9Y	M	03/09/2013	09/09/2013	Selangor
69	Shih Tzu	9y	M	16/06/2014	20/06/2014	Selangor
70	Beagle	9y	SF	10/12/2014	16/12/2014	Kuala Lumpur

APPENDIX B

Data on types of diet given to the dogs prior to the serology testing.

No.	Breed	Diet
1	Poodle X Shih Tzu	Mutton, Hills z/d diet
2	English Cocker Spaniel	Chicken, Potato, Royal Canin Hypoallergic
3	Bull Dog	Salmon
4	Rottweiler	Chicken, Pork, Mutton, Egg, Rice, Milk
5	Mongrel	Chicken, Pork, Mutton, Cheese, Egg, Salmon, Rice, Bread
6	Golden Retriever	Chicken, Beef, Pork, Salmon, Rice, Potato
7	Pug	Milk, Commercial dog food, Lamb, Rice, Brown rice, Chicken
8	Great Dane	Chicken
9	Golden Retriever	Chicken, Pork, Egg, Salmon, Potato, Yeast, Venison, pumpkin, apple
10	Poodle	Chicken, Beef, Pork, Mutton, Cheese, Salmon, Rice, Milk, vegetables
11	Rottweiler	Chicken, Mutton, Salmon, Rice
12	Golden Retriever	Beef, Pork, Mutton, Cheese, Salmon, Yogurt
13	Shih Tzu	Chicken, Beef, Rice
14	Mixed Breed	Mutton, Rice
15	Poodle	Beef, Potato, Yeast
16	Mini Schnauzer	Vegetarian
17	Beagle	Chicken
18	Dachshunds	Chicken, Milk
19	Mixed Breed	Salmon
20	Golden Retriever	Hills z/d diet
21	Toy Poodle	Chicken, Egg, Rice, Vegetable, Fruits
22	Toy Poodle	Hills z/d diet
23	Mixed Breed	Royal Canine Hypoallergic
24	Pug	Chicken, Salmon, treats, homecook food
25	Golden Retriever	Rice, Milk, Potato, Homecook food, Fruits
26	Mongrel	Codfish
27	Poodle	Chicken, Beef,
28	Shih Tzu	Chicken, Duck, Royal Canin Sensitivity, Royal Canin Control
29	Poodle	-
30	Siberian Husky	Chicken
31	Beagle	Chicken, Rice, Hypoallergenic diet dog food for 2 weeks
32	Maltese	Chicken, Beef, Pork, Cheese, Egg, Salmon, Rice, Potato, Table Scraps
33	Shih Tzu	Salmon

34	Mongrel	Chicken, Beef
35	English Bulldog	Salmon, Rice, Carrot
36	Poodle	Salmon
37	Golden Retriever	Salmon
38	Mixed breed	Chicken, Beef, Prok, Mutton, Cheese, Egg, Salmon, Rice, Wheat, Milk, Soya Sauce
39	Golden Retriever	Salmon, BARF
40	Labrador Retriever	Chicken, Mutton, Rice
41	Poodle	Chicken, Beef, Pork, Mutton, Egg, Salmon, Rice, Wheat, Milk, Potato, Pumpkin, Carrot, Cabbage
42	Maltese x Poodle	Chicken
43	Scottish Terrier	Pork, Egg, Rice, Milk, Potato, Yeast, Bread, Cookies
44	Shih Tzu	Hills z/d diet
45	Golden Retriever Cross	Chicken, Salmon
46	Silky Terrier	Chicken, Beef, Cheese, Salmon, Yeast, Papaya, Grape, Apple, Carrot, Butter
47	Bull Mastiff	Chicken, Beef, Prok, Mutton, Egg, Rice, Wheat, Potato, Pear, Bread, Apple
48	Labrador Cross	Royal Canine Hypoallergic
49	Spitz Cross	Mutton, Rice
50	Great Dane	Mutton, Rice, Salmon
51	Mixed Breed	Chicken, Beef, Pork, Mutton, Egg, Salmon, Milk, Potato, Unknown
52	Pekingese	Royal Canin Hypoallergic diet
53	Poodle Cross	Chicken, Beef, Rice
54	American Cocker Spaniel	Chicken, Pork, Salmon, Rice, Brown Rice
55	Poodle	Salmon, Potato
56	Shih Tzu	-
57	German Shepherd	Unknown
58	German Shepherd	-
59	Cocker Spaniel	Fish, vegetarian (2-4years)
60	Terrier Mixed	Chicken, Pork, Rice
61	Silky Terrier	Chicken, Rice, Marigold extract, vegetable fibre, Rice, Maize floor, Maze gluten, egg, Fructo-oligo-saccharides
62	German shepherd	Salmon, Rice
63	Shih Tzu	Pork, Potato
64	Shih Tzu	Chicken, Pork, Mutton, Cheese, Egg, Salmon, Rice, Milk, Potato, Yeast, Hill's z/d, Origin Ocean fish, Acana, Blackwood 3000
65	Bulldog Cross	Salmon, Potato, Hill's d/d diet
66	Shih Tzu	Royal Canin Hypoallergic diet
67	Mongrel	Chicken, Salmon, Rice, Potato
68	Pomeranian	Royal Canine Hypoallergic
69	Shih Tzu	White fish
70	Beagle	Chicken, Beef, Pork, Mutton,, Raw meat, Mackerel

APPENDIX C

Data on management and clinical signs observed in the dogs

No.	Breed	onset of sign <3yr	living indoors	Glucocorticoid-responsive pruritus	Pruritus sine with material at onset	Affected front feet	Affected ear pinnae	Non-affected ear margins	Non-affected dorso-lumbar area
1	Poodle X Shih Tzu			Y		Y			
2	English Cocker Spaniel		Y			Y		Y	Y
3	Bull Dog								
4	Rottweiler	Y		Y			Y		
5	Mongrel	Y							
6	Golden Retriever	Y					Y		
7	Pug								
8	Great Dane	Y		Y		Y	Y		
9	Golden Retriever	Y	Y		Y	Y			
10	Poodle	Y	Y	Y			Y		
11	Rottweiler	Y					Y	Y	
12	Golden Retriever			Y					Y
13	Shih Tzu		Y	Y		Y	Y		
14	Mixed Breed	Y		Y		Y			
15	Poodle	Y			Y				
16	Mini Schnauzer	Y							Y
17	Beagle	Y							
18	Dachshunds			Y					
19	Mixed Breed	Y	Y	Y			Y		
20	Golden Retriever	Y		Y					
21	Toy Poodle	Y	Y	Y					
22	Toy Poodle	Y		Y		Y	Y		Y
23	Mixed Breed			Y					
24	Pug	Y	Y			Y			
25	Golden Retriever	Y	Y					Y	Y
26	Mongrel	Y		Y		Y	Y		
27	Poodle	Y	Y	Y					
28	Shih Tzu	Y		Y		Y			Y
29	Poodle	Y		Y		Y			
30	Siberian Husky	Y				Y			
31	Beagle	Y		Y		Y		Y	
32	Maltese	Y							
33	Shih Tzu	Y	Y	Y		Y			

34	Mongrel				Y			Y	
35	English Bulldog	Y	Y	Y		Y			
36	Poodle	Y		Y					
37	Golden Retriever	Y		Y			Y		
38	Mixed breed			Y		Y			Y
39	Golden Retriever	Y	Y		Y	Y	Y	Y	Y
40	Labrador Retriever	Y	Y	Y		Y	Y	Y	Y
41	Poodle		Y	Y					
42	Maltese x Poodle		Y			Y			
43	Scottish Terrier		Y			Y	Y		
44	Shih Tzu		Y						
45	Golden Retriever Cross			Y					Y
46	Silky Terrier	Y							
47	Bull Mastiff	Y				Y	Y		
48	Labrador Cross	Y	Y	Y				Y	Y
49	Spitz Cross	Y							Y
50	Great Dane							Y	
51	Mixed Breed	Y	Y						
52	Pekingese	Y	Y	Y		Y	Y		Y
53	Poodle Cross		Y	Y		Y			
54	American Cocker Spaniel					Y			
55	Poodle						Y		
56	Shih Tzu						Y		Y
57	German Shepherd								
58	German Shepherd	Y		Y				Y	
59	Cocker Spaniel	Y	Y					Y	
60	Terrier Mixed	Y		Y		Y			
61	Silky Terrier	Y	Y	Y		Y	Y		
62	German shepherd			Y		Y		Y	
63	Shih Tzu			Y			Y		
64	Shih Tzu	Y	Y				Y		
65	Bulldog Cross	Y				Y			
66	Shih Tzu		Y			Y			
67	Mongrel		Y				Y		
68	Pomeranian		Y					Y	
69	Shih Tzu			Y					
70	Beagle		N				Y		

Y: yes

N: no

APPENDIX D

Data on "*Canine 62 Allergen Specific IgE Serological Test"* quantitative result on mites allergen subgroup.

No.	Breed	*Blomia tropicals*	*D. pteronyssinus*	*D. farinae*	*Acarus siro*	*Tyrophagus putrescentiae*
1	Poodle X Shih Tzu	696	684	1009	300	691
2	English Cocker Spaniel	1474	2412	549	678	3844
3	Bull Dog	0	2130	0	872	0
4	Rottweiler	721	51	1364	1634	1926
5	Mongrel	0	0	442	0	0
6	Golden Retriever	0	620	299	93	1145
7	Pug	1031	3408	5336	423	3907
8	Great Dane	403	0	436	1072	2958
9	Golden Retriever	0	1584	0	0	0
10	Poodle	0	0	0	0	0
11	Rottweiler	0	1510	0	0	489
12	Golden Retriever	3625	957	3346	2135	2192
13	Shih Tzu	0	0	600	0	0
14	Mixed Breed	954	868	923	639	980
15	Poodle	393	352	699	0	0
16	Mini Schnauzer	102	0	0	0	0
17	Beagle	15	0	0	2234	3619
18	Dachshunds	398	4034	295	0	366
19	Mixed Breed	0	0	0	0	0
20	Golden Retriever	4373	0	0	602	449
21	Toy Poodle	2566	5061	0	1162	4311
22	Toy Poodle	458	5046	2439	342	0
23	Mixed Breed	1208	3999	1999	867	20
24	Pug	2028	0	5315	1590	316
25	Golden Retriever	10279	2832	9498	3477	2923
26	Mongrel	642	0	0	808	1846
27	Poodle	0	0	820	860	0
28	Shih Tzu	0	1377	6360	2196	1789
29	Poodle	0	5413	259	0	0
30	Siberian Husky	528	0	2668	1180	0
31	Beagle	0	0	612	73	0

32	Maltese	390	362	547	567	574
33	Shih Tzu	5228	1845	16430	2670	28467
34	Mongrel	1591	0	0	0	0
35	English Bulldog	4431	2839	7764	2078	2189
36	Poodle	1045	176	1912	2500	1991
37	Golden Retriever	369	524	307	359	373
38	Mixed breed	1236	1627	1023	0	0
39	Golden Retriever	1759	439	2516	835	1923
40	Labrador Retriever	1093	0	5983	0	3346
41	Poodle	3792	2324	766	0	56
42	Maltese x Poodle	2034	0	5345	1754	3073
43	Scottish Terrier	2499	7078	139	1856	8066
44	Shih Tzu	0	4650	1559	772	0
45	Golden Retriever Cross	2357	6503	3568	2240	5331
46	Silky Terrier	3366	2741	4339	4394	6022
47	Bull Mastiff	1907	1607	1798	565	934
48	Labrador Cross	664	1601	764	96	1387
49	Spitz Cross	222	271	0	34	0
50	Great Dane	1885	0	4306	416	3634
51	Mixed Breed	108	338	0	3357	907
52	Pekingese	1649	814	2258	1285	1494
53	Poodle Cross	877	0	2618	672	872
54	American Cocker Spaniel	678	240	116	181	264
55	Poodle	679	691	664	361	458
56	Shih Tzu	460	457	549	308	232
57	German Shepherd	0	0	0	0	0
58	German Shepherd	707	7396	218	1568	5635
59	Cocker Spaniel	1688	2788	10034	4263	5110
60	Terrier Mixed	547	15	312	297	573
61	Silky Terrier	222	692	1781	248	0
62	German shepherd	0	231	583	1607	1353
63	Shih Tzu	5149	1839	400	11969	1666
64	Shih Tzu	1411	4129	2385	630	841
65	Bulldog Cross	704	3701	479	395	1434
66	Shih Tzu	94	0	925	543	45
67	Mongrel	0	0	0	0	0
68	Pomeranian	1425	349	2145	713	304
69	Shih Tzu	158	1047	5	247	0
70	Beagle	325	1781	708	131	567

APPENDIX E

Data on "*Canine 62 Allergen Specific IgE Serological Test"* quantitative result on fungi and bacteria allergen subgroup.

No.	Breed	*Cladosporium herbarum*	*Candida albicans*	*Alternaria alternata*	*Penicillium notatum*	*Aspergillus fumigatus*	*Malassezia pachydermatis*	*Staphylococcus aureus*
1	Poodle X Shih Tzu	245	940	664	370	389	594	561
2	English Cocker Spaniel	203	4506	1123	49	151	532	2194
3	Bull Dog	0	1344	0	0	1919	0	794
4	Rottweiler	2819	6686	3950	274	0	919	414
5	Mongrel	0	0	374	0	0	0	78
6	Golden Retriever	0	0	741	92	0	860	484
7	Pug	399	0	0	251	0	284	0
8	Great Dane	890	4149	654	1068	2198	1364	0
9	Golden Retriever	72	4303	598	0	0	755	0
10	Poodle	520	1706	156	308	65	847	0
11	Rottweiler	1600	2146	386	572	221	1763	0
12	Golden Retriever	218	1095	396	180	90	276	660
13	Shih Tzu	0	0	0	0	0	0	0
14	Mixed Breed	547	1918	954	724	532	891	657
15	Poodle	394	0	0	421	1159	734	415
16	Mini Schnauzer	0	0	0	0	0	0	288
17	Beagle	0	0	0	0	0	915	0
18	Dachshunds	1248	4215	1927	392	1447	2209	3349
19	Mixed Breed	0	0	0	0	0	306	0
20	Golden Retriever	492	0	0	540	0	0	0
21	Toy Poodle	1336	162	2966	457	0	21	482
22	Toy Poodle	0	404	395	764	1854	0	521
23	Mixed Breed	149	0	942	406	0	1481	2874
24	Pug	3820	2654	6961	141	0	993	0
25	Golden Retriever	106	2288	562	131	384	73	961
26	Mongrel	0	0	0	119	163	0	492
27	Poodle	0	0	0	0	0	0	0
28	Shih Tzu	0	5500	0	1095	0	259	4
29	Poodle	0	2310	0	0	590	0	0
30	Siberian Husky	0	0	0	0	0	0	463

31	Beagle	0	0	0	0	0	0	0
32	Maltese	518	35	210	237	307	264	655
33	Shih Tzu	0	86	421	0	0	240	4752
34	Mongrel	1569	0	1018	0	0	564	1001
35	English Bulldog	67	1017	135	651	1610	272	1525
36	Poodle	156	3141	84	121	0	675	469
37	Golden Retriever	114	0	167	273	307	0	302
38	Mixed breed	896	2235	1890	167	0	1019	0
39	Golden Retriever	16	135	116	0	0	286	212
40	Labrador Retriever	0	2492	0	0	0	316	0
41	Poodle	0	4494	0	0	0	0	1465
42	Maltese x Poodle	0	0	0	868	0	0	1097
43	Scottish Terrier	1341	21649	5144	2051	6288	5977	3834
44	Shih Tzu	0	0	2246	0	0	0	1344
45	Golden Retriever Cross	932	1451	5422	731	1089	213	1493
46	Silky Terrier	160	3992	1380	746	0	1695	0
47	Bull Mastiff	184	1521	433	261	1268	700	0
48	Labrador Cross	390	2091	632	180	108	1372	435
49	Spitz Cross	156	599	196	122	0	220	0
50	Great Dane	0	0	0	0	0	0	0
51	Mixed Breed	108	552	111	0	475	45	0
52	Pekingese	818	1259	745	916	675	1010	816
53	Poodle Cross	0	0	0	136	0	538	599
54	American Cocker Spaniel	100	60	6	0	0	194	0
55	Poodle	33	0	234	498	472	0	115
56	Shih Tzu	572	1311	732	625	413	864	617
57	German Shepherd	413	1052	0	192	0	1171	0
58	German Shepherd	0	0	0	0	0	0	5
59	Cocker Spaniel	0	8032	0	0	0	702	232
60	Terrier Mixed	184	0	273	231	0	820	459
61	Silky Terrier	0	1067	0	0	0	0	214
62	German shepherd	307	775	368	0	0	0	0
63	Shih Tzu	0	879	0	128	2054	0	0
64	Shih Tzu	41	3057	451	0	0	480	930
65	Bulldog Cross	476	6271	378	792	3123	2080	285
66	Shih Tzu	0	0	0	0	0	0	0
67	Mongrel	0	0	0	0	0	545	0
68	Pomeranian	1103	2608	197	868	1033	480	0
69	Shih Tzu	0	0	260	0	0	224	263
70	Beagle	49	593	385	5	51	205	482

APPENDIX F

Data on "*Canine 62 Allergen Specific IgE Serological Test"* quantitative result on insecta allergen subgroup.

No.	Breed	Black Ant	Tick	Flea	*Culex pipiens*	*Aedes communis*	*Blatella germanica*
1	Poodle X Shih Tzu	372	230	293	69	165	508
2	English Cocker Spaniel	1017	819	161	254	741	952
3	Bull Dog	138	157	544	4117	6196	0
4	Rottweiler	1677	0	453	220	439	1173
5	Mongrel	0	0	0	0	0	333
6	Golden Retriever	1746	252	81	0	16	0
7	Pug	0	13	243	259	0	876
8	Great Dane	167	3	348	295	278	307
9	Golden Retriever	0	0	0	0	1367	0
10	Poodle	0	0	9	0	0	0
11	Rottweiler	85	0	61	0	0	0
12	Golden Retriever	459	423	144	52	107	295
13	Shih Tzu	0	0	273	0	0	0
14	Mixed Breed	383	208	494	748	698	323
15	Poodle	370	446	136	0	0	0
16	Mini Schnauzer	0	250	324	0	0	90
17	Beagle	0	0	108	0	929	0
18	Dachshunds	535	1196	214	373	694	356
19	Mixed Breed	177	0	0	461	0	0
20	Golden Retriever	0	0	0	0	0	208
21	Toy Poodle	1610	1197	475	0	462	152
22	Toy Poodle	364	1769	84	8339	5170	150
23	Mixed Breed	926	13	204	1995	3615	1302
24	Pug	0	0	0	0	0	0
25	Golden Retriever	557	896	564	647	1068	469
26	Mongrel	0	0	189	0	0	0
27	Poodle	0	0	0	0	0	0
28	Shih Tzu	10	0	0	0	0	0
29	Poodle	0	2035	0	3503	5813	0
30	Siberian Husky	88	0	1089	0	0	0
31	Beagle	0	0	0	1704	0	0
32	Maltese	562	140	650	527	423	505

33	Shih Tzu	1420	3256	0	85	682	1983
34	Mongrel	1546	291	261	0	0	0
35	English Bulldog	640	826	593	1010	1188	417
36	Poodle	0	0	13	0	0	0
37	Golden Retriever	532	300	367	2149	1112	235
38	Mixed breed	0	0	0	0	0	0
39	Golden Retriever	221	0	78	43	147	55
40	Labrador Retriever	0	205	0	11592	4632	0
41	Poodle	645	0	131	1865	2671	0
42	Maltese x Poodle	82	0	332	0	0	0
43	Scottish Terrier	969	2488	393	14748	6537	2576
44	Shih Tzu	1243	0	0	0	1208	1673
45	Golden Retriever Cross	872	287	0	142	0	0
46	Silky Terrier	0	1526	0	23938	20548	0
47	Bull Mastiff	395	32	5	156	763	555
48	Labrador Cross	326	97	22	195	248	364
49	Spitz Cross	174	0	53	3	188	1092
50	Great Dane	0	0	0	0	0	0
51	Mixed Breed	360	33	256	0	161	198
52	Pekingese	955	147	852	868	706	439
53	Poodle Cross	2676	0	0	0	0	0
54	American Cocker Spaniel	135	115	303	401	550	518
55	Poodle	467	437	400	15	0	98
56	Shih Tzu	766	245	366	797	744	642
57	German Shepherd	0	92	284	0	0	91
58	German Shepherd	361	0	0	9583	9514	419
59	Cocker Spaniel	0	0	0	0	0	155
60	Terrier Mixed	246	51	335	249	432	6
61	Silky Terrier	0	0	70	847	778	174
62	German shepherd	961	0	0	0	0	0
63	Shih Tzu	0	992	478	0	3078	0
64	Shih Tzu	733	1383	214	657	780	691
65	Bulldog Cross	901	464	0	12960	11734	54
66	Shih Tzu	0	154	33	98	533	0
67	Mongrel	0	0	0	0	0	105
68	Pomeranian	1487	0	142	0	0	155
69	Shih Tzu	833	620	151	445	515	453
70	Beagle	534	0	196	198	418	314

APPENDIX G

Data on "*Canine 62 Allergen Specific IgE Serological Test"* quantitative result on pollen allergen subgroup.

No.	Breed	*Eucalyptus globules*	*Poa pratensis*	*Amaranthus retroflexus*	*Rumex acetosella*	*Ambrosia artemisii*	*Acacia spp.*	*Cynodon dactylon*
1	Poodle X Shih Tzu	380	1002	677	879	450	415	1034
2	English Cocker Spaniel	465	716	1382	995	1090	926	1710
3	Bull Dog	318	1840	817	398	0	171	3074
4	Rottweiler	1892	8951	8801	5107	4023	3636	13675
5	Mongrel	0	0	212	0	0	457	222
6	Golden Retriever	0	442	201	0	31	588	778
7	Pug	1002	0	1782	0	63	2068	0
8	Great Dane	920	0	923	15	115	872	1031
9	Golden Retriever	0	4127	1788	2699	1117	1046	6615
10	Poodle	88	0	0	14	0	0	163
11	Rottweiler	73	109	53	0	198	0	0
12	Golden Retriever	305	2229	1040	2460	818	423	2658
13	Shih Tzu	288	0	0	38	0	0	447
14	Mixed Breed	442	1273	909	956	411	528	2720
15	Poodle	0	0	83	340	0	0	246
16	Mini Schnauzer	0	0	0	0	152	846	0
17	Beagle	0	0	95	0	0	0	0
18	Dachshunds	510	7139	3417	4535	2484	845	10543
19	Mixed Breed	152	6732	2473	4112	1723	1334	17872
20	Golden Retriever	88	290	655	0	725	0	1943
21	Toy Poodle	757	212	722	0	774	493	141
22	Toy Poodle	137	140	284	0	77	351	172
23	Mixed Breed	216	265	0	0	0	878	1
24	Pug	710	322	1347	788	1621	1174	1576
25	Golden Retriever	739	11555	7852	7608	1890	1163	17447
26	Mongrel	246	7254	1107	4826	3278	971	17020
27	Poodle	0	0	0	0	0	0	0
28	Shih Tzu	307	0	0	0	0	0	0
29	Poodle	0	3879	327	6904	166	0	15389
30	Siberian Husky	320	0	0	0	704	408	0

31	Beagle	283	0	0	153	0	0	0
32	Maltese	403	194	436	559	482	621	599
33	Shih Tzu	2711	33560	20080	15811	11353	3921	33462
34	Mongrel	1219	20593	10837	7779	6505	3153	12102
35	English Bulldog	218	1453	908	1066	577	324	1248
36	Poodle	432	771	279	25	159	645	597
37	Golden Retriever	385	437	154	282	255	406	307
38	Mixed breed	1769	1071	885	698	797	1069	918
39	Golden Retriever	234	305	84	438	1068	197	243
40	Labrador Retriever	0	0	0	0	0	0	0
41	Poodle	41	20	0	0	0	0	0
42	Maltese x Poodle	0	229	136	290	0	347	1170
43	Scottish Terrier	406	1342	5044	476	777	1487	613
44	Shih Tzu	0	0	0	167	1261	1315	0
45	Golden Retriever Cross	2052	2254	0	570	435	1007	1588
46	Silky Terrier	175	423	6416	634	526	77	1917
47	Bull Mastiff	197	745	470	182	290	388	419
48	Labrador Cross	125	349	307	69	110	265	151
49	Spitz Cross	69	94	7	130	182	266	166
50	Great Dane	0	1279	265	55	0	0	1343
51	Mixed Breed	92	521	140	130	369	387	321
52	Pekingese	783	621	909	624	482	204	590
53	Poodle Cross	720	3252	357	766	780	1513	3121
54	American Cocker Spaniel	122	116	119	811	56	279	126
55	Poodle	183	337	275	158	258	254	583
56	Shih Tzu	406	648	532	498	430	517	684
57	German Shepherd	0	0	0	0	94	536	96
58	German Shepherd	0	830	891	55	0	0	1620
59	Cocker Spaniel	0	300	0	0	0	0	2130
60	Terrier Mixed	159	189	0	155	0	377	172
61	Silky Terrier	1	0	0	445	0	0	185
62	German shepherd	0	1501	0	0	0	0	728
63	Shih Tzu	331	1191	2796	553	2473	502	2420
64	Shih Tzu	547	3897	2625	5053	2306	1026	6536
65	Bulldog Cross	312	3090	1438	1086	286	0	4818
66	Shih Tzu	0	0	330	0	300	0	0
67	Mongrel	159	0	0	100	0	0	0
68	Pomeranian	124	2426	447	625	834	678	675
69	Shih Tzu	245	2008	1195	885	1049	452	2697
70	Beagle	112	845	380	384	212	322	1999

APPENDIX H

Data on "*Canine 62 Allergen Specific IgE Serological Test"* quantitative result on hair and epithelial allergen subgroup.

No.	Breed	Mixed Feathers	Human Dander	Cow Epithelia	Goat Epithelia	Cat Hair
1	Poodle X Shih Tzu	455	278	306	606	297
2	English Cocker Spaniel	624	295	215	215	382
3	Bull Dog	0	0	0	189	189
4	Rottweiler	687	50	458	147	1142
5	Mongrel	0	0	0	0	0
6	Golden Retriever	94	5	0	0	84
7	Pug	0	0	0	0	0
8	Great Dane	0	103	0	193	615
9	Golden Retriever	0	0	0	0	0
10	Poodle	0	32	0	0	228
11	Rottweiler	0	35	0	1	27
12	Golden Retriever	157	153	45	1061	27
13	Shih Tzu	0	0	0	0	198
14	Mixed Breed	296	368	0	0	406
15	Poodle	0	0	0	0	133
16	Mini Schnauzer	0	558	0	607	97
17	Beagle	0	0	0	0	0
18	Dachshunds	394	123	64	473	96
19	Mixed Breed	0	0	695	0	0
20	Golden Retriever	20	173	0	0	118
21	Toy Poodle	854	478	448	619	200
22	Toy Poodle	130	4	0	0	0
23	Mixed Breed	56	0	0	0	0
24	Pug	0	115	0	0	0
25	Golden Retriever	199	181	102	164	325
26	Mongrel	274	0	0	0	40
27	Poodle	0	0	0	1825	0
28	Shih Tzu	0	0	0	0	158
29	Poodle	0	0	0	0	0
30	Siberian Husky	0	0	0	0	235
31	Beagle	31	0	0	295	32

32	Maltese	429	441	678	276	451
33	Shih Tzu	0	48	0	0	925
34	Mongrel	74	417	0	0	529
35	English Bulldog	313	417	20	898	183
36	Poodle	0	0	238	112	0
37	Golden Retriever	267	209	329	496	254
38	Mixed breed	0	0	0	190	174
39	Golden Retriever	139	234	90	402	155
40	Labrador Retriever	0	0	0	0	5
41	Poodle	0	274	0	0	0
42	Maltese x Poodle	0	0	0	4763	45
43	Scottish Terrier	1119	962	596	4135	491
44	Shih Tzu	0	0	0	0	0
45	Golden Retriever Cross	120	0	0	0	1138
46	Silky Terrier	0	621	0	1323	49
47	Bull Mastiff	212	0	170	152	55
48	Labrador Cross	213	94	92	191	11
49	Spitz Cross	200	155	56	227	52
50	Great Dane	0	0	0	0	0
51	Mixed Breed	146	149	35	416	43
52	Pekingese	380	526	0	88	603
53	Poodle Cross	0	37	0	839	0
54	American Cocker Spaniel	286	64	165	493	143
55	Poodle	235	10	356	563	318
56	Shih Tzu	299	412	398	128	235
57	German Shepherd	214	0	913	245	478
58	German Shepherd	0	133	592	376	0
59	Cocker Spaniel	45	0	0	0	680
60	Terrier Mixed	0	155	234	0	111
61	Silky Terrier	0	0	0	1018	49
62	German shepherd	0	0	0	0	1603
63	Shih Tzu	117	101	0	0	242
64	Shih Tzu	1041	442	332	385	305
65	Bulldog Cross	334	60	0	0	0
66	Shih Tzu	105	0	0	0	0
67	Mongrel	0	0	0	0	0
68	Pomeranian	111	17	0	151	294
69	Shih Tzu	540	183	1052	0	32
70	Beagle	270	318	97	185	51

APPENDIX I

Data on "*Canine 62 Allergen Specific IgE Serological Test"* quantitative result on milk/egg allergen subgroup.

No.	Breed	Cheese	Egg White	Egg Yolk	Milk
1	Poodle X Shih Tzu	318	484	310	260
2	English Cocker Spaniel	324	2638	1256	49
3	Bull Dog	0	0	0	0
4	Rottweiler	221	108	0	0
5	Mongrel	0	0	360	51
6	Golden Retriever	0	406	0	0
7	Pug	0	0	0	0
8	Great Dane	38	0	0	0
9	Golden Retriever	0	0	0	60
10	Poodle	0	0	0	0
11	Rottweiler	0	0	0	0
12	Golden Retriever	28	257	0	20
13	Shih Tzu	50	810	0	0
14	Mixed Breed	491	506	368	416
15	Poodle	12	0	0	0
16	Mini Schnauzer	0	0	0	0
17	Beagle	0	0	0	0
18	Dachshunds	119	231	0	40
19	Mixed Breed	0	0	0	0
20	Golden Retriever	584	1553	0	385
21	Toy Poodle	156	3186	810	154
22	Toy Poodle	1053	248	529	183
23	Mixed Breed	1482	3423	674	2017
24	Pug	0	0	0	0
25	Golden Retriever	393	69	604	156
26	Mongrel	0	226	1118	0
27	Poodle	0	0	0	0
28	Shih Tzu	0	0	0	0
29	Poodle	215	345	0	0
30	Siberian Husky	925	0	0	5431
31	Beagle	0	0	0	0

32	Maltese	504	554	364	336
33	Shih Tzu	0	288	0	0
34	Mongrel	143	0	0	0
35	English Bulldog	45	178	39	174
36	Poodle	0	192	152	971
37	Golden Retriever	338	779	550	339
38	Mixed breed	0	0	0	0
39	Golden Retriever	18	25	0	116
40	Labrador Retriever	0	0	0	0
41	Poodle	0	0	0	0
42	Maltese x Poodle	0	0	0	0
43	Scottish Terrier	145	626	24533	0
44	Shih Tzu	0	5052	0	0
45	Golden Retriever Cross	549	86	316	444
46	Silky Terrier	4159	1048	400	480
47	Bull Mastiff	0	401	0	0
48	Labrador Cross	2	93	0	0
49	Spitz Cross	6	164	139	0
50	Great Dane	0	0	0	0
51	Mixed Breed	138	160	0	0
52	Pekingese	663	429	303	724
53	Poodle Cross	0	952	1178	0
54	American Cocker Spaniel	138	126	0	0
55	Poodle	138	400	195	197
56	Shih Tzu	377	366	391	96
57	German Shepherd	0	0	852	0
58	German Shepherd	1789	0	150	184
59	Cocker Spaniel	0	0	0	0
60	Terrier Mixed	0	0	604	0
61	Silky Terrier	299	0	43	254
62	German shepherd	0	0	0	0
63	Shih Tzu	111	0	0	401
64	Shih Tzu	57	186	0	53
65	Bulldog Cross	3435	500	386	2321
66	Shih Tzu	14	0	0	58
67	Mongrel	0	1717	0	0
68	Pomeranian	23	0	0	0
69	Shih Tzu	118	266	0	0
70	Beagle	470	756	0	0

APPENDIX J

Data on "*Canine 62 Allergen Specific IgE Serological Test*" quantitative result on meat allergen subgroup.

No.	Breed	Duck	Lamb	Beef	Chicken	Turkey	Pork
1	Poodle X Shih Tzu	794	562	673	538	540	601
2	English Cocker Spaniel	1325	1043	667	1517	1899	1043
3	Bull Dog	1467	1153	0	0	709	1259
4	Rottweiler	1105	0	730	271	3184	2061
5	Mongrel	0	0	390	0	0	97
6	Golden Retriever	217	0	0	0	69	371
7	Pug	164	0	512	0	67	0
8	Great Dane	0	0	332	0	155	34
9	Golden Retriever	0	0	0	0	0	0
10	Poodle	0	0	204	0	0	0
11	Rottweiler	0	0	360	294	1338	0
12	Golden Retriever	412	734	304	460	373	375
13	Shih Tzu	0	0	0	121	0	0
14	Mixed Breed	634	428	540	476	477	860
15	Poodle	0	0	0	790	186	286
16	Mini Schnauzer	299	457	278	0	228	735
17	Beagle	0	0	0	0	0	0
18	Dachshunds	820	485	543	640	810	689
19	Mixed Breed	0	7	0	0	0	11
20	Golden Retriever	0	0	726	0	96	0
21	Toy Poodle	1151	3031	1782	1895	26	1195
22	Toy Poodle	514	1136	0	276	0	406
23	Mixed Breed	2893	1384	1531	8613	1396	3198
24	Pug	0	0	0	0	0	0
25	Golden Retriever	75	130	160	115	97	156
26	Mongrel	385	0	0	1596	60	822
27	Poodle	0	0	0	0	0	0
28	Shih Tzu	119	0	0	0	0	65
29	Poodle	0	0	209	0	0	32
30	Siberian Husky	0	0	0	228	20	0
31	Beagle	64	0	0	676	700	0
32	Maltese	446	550	571	546	365	568

33	Shih Tzu	283	0	1226	1038	2078	7
34	Mongrel	0	0	0	0	0	1234
35	English Bulldog	447	999	493	286	287	875
36	Poodle	0	60	119	13	0	474
37	Golden Retriever	421	583	401	526	635	661
38	Mixed breed	0	0	510	309	745	0
39	Golden Retriever	133	306	217	161	98	80
40	Labrador Retriever	0	110	0	0	123	0
41	Poodle	0	0	0	0	271	0
42	Maltese x Poodle	504	0	0	0	36	0
43	Scottish Terrier	1742	2898	1775	36	665	903
44	Shih Tzu	0	0	0	1487	2574	758
45	Golden Retriever Cross	448	0	278	107	617	569
46	Silky Terrier	0	0	565	2899	198	1116
47	Bull Mastiff	357	303	493	495	591	418
48	Labrador Cross	337	325	331	617	483	426
49	Spitz Cross	285	239	109	275	309	244
50	Great Dane	0	0	0	0	0	0
51	Mixed Breed	98	139	156	205	233	211
52	Pekingese	899	801	801	560	583	954
53	Poodle Cross	214	0	0	716	926	22
54	American Cocker Spaniel	49	103	0	21	0	9
55	Poodle	447	469	365	361	328	492
56	Shih Tzu	539	496	542	455	522	626
57	German Shepherd	0	0	0	0	0	0
58	German Shepherd	257	1469	1338	748	50	792
59	Cocker Spaniel	0	0	0	0	0	0
60	Terrier Mixed	897	766	669	118	83	563
61	Silky Terrier	114	0	0	0	110	0
62	German shepherd	0	0	3	0	0	0
63	Shih Tzu	449	191	0	0	0	0
64	Shih Tzu	483	329	444	621	620	802
65	Bulldog Cross	2193	1236	369	2026	1255	411
66	Shih Tzu	0	0	50	0	0	0
67	Mongrel	0	0	171	0	166	0
68	Pomeranian	75	0	236	140	422	470
69	Shih Tzu	450	156	359	856	617	453
70	Beagle	561	390	307	913	923	923

APPENDIX K

Data on "*Canine 62 Allergen Specific IgE Serological Test"* quantitative result on fruit allergen subgroup.

No.	Breed	Watermelon	Cantaloupe	Pear	Mandarin Orange	Orange
1	Poodle X Shih Tzu	631	459	422	423	736
2	English Cocker Spaniel	741	487	909	882	359
3	Bull Dog	263	0	200	0	179
4	Rottweiler	3163	1085	1679	2612	1815
5	Mongrel	0	0	0	0	0
6	Golden Retriever	231	0	33	0	0
7	Pug	0	0	468	0	0
8	Great Dane	597	56	334	0	86
9	Golden Retriever	138	0	470	0	0
10	Poodle	0	81	0	0	0
11	Rottweiler	71	0	0	0	0
12	Golden Retriever	949	65	142	78	133
13	Shih Tzu	0	0	93	0	0
14	Mixed Breed	757	597	411	759	656
15	Poodle	0	71	135	0	0
16	Mini Schnauzer	441	78	0	0	417
17	Beagle	0	0	0	0	0
18	Dachshunds	4605	236	1081	366	325
19	Mixed Breed	1869	0	151	0	3286
20	Golden Retriever	126	119	42	0	0
21	Toy Poodle	314	203	361	0	191
22	Toy Poodle	0	120	45	236	491
23	Mixed Breed	0	106	0	0	856
24	Pug	0	178	154	0	331
25	Golden Retriever	6346	237	985	1530	195
26	Mongrel	1139	225	0	0	2244
27	Poodle	0	0	0	0	0
28	Shih Tzu	0	174	0	0	0
29	Poodle	401	0	0	0	0
30	Siberian Husky	139	0	0	0	0
31	Beagle	0	65	0	265	0
32	Maltese	260	484	540	627	450

33	Shih Tzu	4870	964	2235	1017	0
34	Mongrel	3162	801	2221	0	867
35	English Bulldog	1020	159	238	753	234
36	Poodle	326	112	87	0	179
37	Golden Retriever	288	342	443	481	480
38	Mixed breed	826	401	334	0	574
39	Golden Retriever	20	143	17	76	0
40	Labrador Retriever	0	0	0	0	0
41	Poodle	0	0	0	0	0
42	Maltese x Poodle	0	428	0	0	0
43	Scottish Terrier	1530	910	238	3226	700
44	Shih Tzu	223	0	0	23	0
45	Golden Retriever Cross	268	390	521	0	390
46	Silky Terrier	35	0	0	1798	66
47	Bull Mastiff	527	360	183	715	183
48	Labrador Cross	218	37	114	195	196
49	Spitz Cross	68	155	0	106	17
50	Great Dane	0	0	0	0	0
51	Mixed Breed	148	90	0	360	90
52	Pekingese	760	738	833	899	638
53	Poodle Cross	412	97	94	0	1172
54	American Cocker Spaniel	164	288	184	218	87
55	Poodle	123	340	223	251	408
56	Shih Tzu	332	326	310	651	446
57	German Shepherd	103	56	84	0	0
58	German Shepherd	0	142	0	228	0
59	Cocker Spaniel	0	0	0	0	0
60	Terrier Mixed	45	160	0	307	67
61	Silky Terrier	67	0	53	210	116
62	German shepherd	2379	0	109	971	191
63	Shih Tzu	277	176	608	571	880
64	Shih Tzu	1618	286	565	452	266
65	Bulldog Cross	703	371	202	509	2274
66	Shih Tzu	0	0	0	0	0
67	Mongrel	210	73	0	0	31
68	Pomeranian	560	0	0	0	0
69	Shih Tzu	1050	166	463	806	388
70	Beagle	474	75	64	175	106

APPENDIX L

Data on "*Canine 62 Allergen Specific IgE Serological Test"* quantitative result on seafood allergen subgroup.

No.	Breed	Crab	Shrimp	Mackerel	Sardine	Anchovy	Codfish	Salmon
1	Poodle X Shih Tzu	340	1081	601	317	470	606	365
2	English Cocker Spaniel	1053	958	1735	359	1529	607	840
3	Bull Dog	5	433	855	1733	0	432	3232
4	Rottweiler	6662	0	940	0	91	769	924
5	Mongrel	0	0	0	0	75	0	999
6	Golden Retriever	0	0	0	0	56	0	0
7	Pug	0	0	0	336	0	854	0
8	Great Dane	0	0	0	1590	0	193	491
9	Golden Retriever	0	184	1095	0	0	0	0
10	Poodle	0	0	470	0	0	17	0
11	Rottweiler	3142	218	2000	0	0	467	0
12	Golden Retriever	36	298	258	420	289	899	933
13	Shih Tzu	0	0	146	0	0	1691	41
14	Mixed Breed	497	467	922	648	367	1374	598
15	Poodle	91	322	372	0	222	0	188
16	Mini Schnauzer	0	410	164	0	30	0	30
17	Beagle	0	0	0	0	0	0	0
18	Dachshunds	97	460	368	334	304	350	128
19	Mixed Breed	0	0	0	0	0	0	0
20	Golden Retriever	0	131	260	0	595	115	544
21	Toy Poodle	2995	1285	1318	207	437	1377	2625
22	Toy Poodle	825	1567	229	2389	351	1028	0
23	Mixed Breed	4241	7762	3560	1742	595	4588	750
24	Pug	0	0	0	0	0	0	0
25	Golden Retriever	406	360	183	247	963	153	328
26	Mongrel	0	0	0	0	0	0	0
27	Poodle	0	0	0	0	105	0	163
28	Shih Tzu	0	0	155	0	0	0	0
29	Poodle	0	0	0	4449	24	0	0
30	Siberian Husky	0	0	0	0	0	0	0
31	Beagle	0	0	0	2552	212	0	2648
32	Maltese	180	184	148	206	291	167	283

33	Shih Tzu	278	1151	221	1894	2000	635	0
34	Mongrel	0	0	0	0	0	0	0
35	English Bulldog	205	646	289	176	2397	318	1116
36	Poodle	0	0	0	0	633	397	409
37	Golden Retriever	563	655	487	488	344	566	536
38	Mixed breed	0	0	1530	0	0	185	0
39	Golden Retriever	867	661	78	227	0	236	393
40	Labrador Retriever	3360	0	487	0	0	0	4074
41	Poodle	0	135	1383	1135	0	0	1007
42	Maltese x Poodle	0	0	1822	17608	61	0	17799
43	Scottish Terrier	2359	11324	2143	13101	73	2949	14868
44	Shih Tzu	0	0	849	0	2741	0	0
45	Golden Retriever Cross	2259	0	0	728	6772	148	253
46	Silky Terrier	2683	0	1537	15931	0	3028	445
47	Bull Mastiff	76	323	713	952	193	604	516
48	Labrador Cross	61	132	255	497	349	391	539
49	Spitz Cross	0	54	156	318	256	158	665
50	Great Dane	0	0	0	0	0	0	0
51	Mixed Breed	482	139	166	207	299	317	1220
52	Pekingese	137	598	583	854	740	666	757
53	Poodle Cross	0	1495	1224	0	6	247	822
54	American Cocker Spaniel	0	115	61	379	700	436	59
55	Poodle	1042	388	486	717	446	546	424
56	Shih Tzu	0	526	670	979	492	430	391
57	German Shepherd	0	0	0	0	34	0	0
58	German Shepherd	932	0	0	8519	637	158	413
59	Cocker Spaniel	0	0	0	0	0	0	0
60	Terrier Mixed	0	162	0	0	479	78	0
61	Silky Terrier	0	0	512	2123	965	0	3834
62	German shepherd	0	0	0	0	0	0	0
63	Shih Tzu	0	0	612	0	1275	0	0
64	Shih Tzu	194	119	729	1033	850	529	503
65	Bulldog Cross	2758	1005	166	12864	444	946	0
66	Shih Tzu	346	0	0	0	94	0	369
67	Mongrel	0	0	350	0	577	0	1087
68	Pomeranian	266	0	518	1269	6408	835	57
69	Shih Tzu	99	0	195	0	753	0	325
70	Beagle	161	220	581	582	0	478	313

APPENDIX M

Data on "*Canine 62 Allergen Specific IgE Serological Test"* quantitative result on vegetables and yeasts allergen subgroup.

No.	Breed	Carrot	Corn	Potato	Baker's Yeast	Brewer's Yeast
1	Poodle X Shih Tzu	526	593	505	253	605
2	English Cocker Spaniel	361	1644	191	261	895
3	Bull Dog	716	0	714	0	0
4	Rottweiler	7239	2693	6960	733	316
5	Mongrel	0	0	0	0	0
6	Golden Retriever	360	0	420	104	0
7	Pug	0	0	457	0	0
8	Great Dane	241	0	660	519	0
9	Golden Retriever	0	527	1345	0	0
10	Poodle	0	0	0	248	0
11	Rottweiler	0	0	0	128	0
12	Golden Retriever	367	457	635	208	103
13	Shih Tzu	135	0	671	227	0
14	Mixed Breed	375	776	629	651	674
15	Poodle	0	0	157	0	0
16	Mini Schnauzer	116	0	218	0	0
17	Beagle	0	0	312	0	428
18	Dachshunds	615	919	1075	249	2566
19	Mixed Breed	887	1048	1780	0	0
20	Golden Retriever	86	364	45	0	138
21	Toy Poodle	540	2428	425	0	144
22	Toy Poodle	0	0	102	722	195
23	Mixed Breed	1191	1261	543	1067	18
24	Pug	429	0	76	44	0
25	Golden Retriever	2192	383	4111	38	223
26	Mongrel	509	2545	3442	0	0
27	Poodle	0	0	0	0	0
28	Shih Tzu	0	0	397	0	0
29	Poodle	0	0	1992	0	531
30	Siberian Husky	0	0	590	103	0
31	Beagle	0	0	0	0	0
32	Maltese	262	400	321	408	292

33	Shih Tzu	3359	2205	10457	34	0
34	Mongrel	2569	2484	7472	0	0
35	English Bulldog	306	399	219	118	284
36	Poodle	385	0	326	0	0
37	Golden Retriever	138	110	243	422	138
38	Mixed breed	601	826	432	268	509
39	Golden Retriever	123	0	11	135	138
40	Labrador Retriever	0	0	0	0	0
41	Poodle	0	0	0	517	6122
42	Maltese x Poodle	0	0	319	0	0
43	Scottish Terrier	1563	3093	444	885	7933
44	Shih Tzu	0	0	0	202	2083
45	Golden Retriever Cross	128	382	0	317	2847
46	Silky Terrier	0	3147	124	0	2011
47	Bull Mastiff	733	590	105	92	355
48	Labrador Cross	172	436	20	228	1085
49	Spitz Cross	101	215	42	39	637
50	Great Dane	0	0	684	0	0
51	Mixed Breed	174	702	163	15	204
52	Pekingese	563	778	627	898	847
53	Poodle Cross	0	7	354	0	35
54	American Cocker Spaniel	89	374	11	54	15
55	Poodle	139	80	230	390	379
56	Shih Tzu	397	223	321	399	625
57	German Shepherd	0	0	290	0	0
58	German Shepherd	0	850	0	121	0
59	Cocker Spaniel	0	0	0	0	0
60	Terrier Mixed	0	100	0	126	0
61	Silky Terrier	0	0	356	100	0
62	German shepherd	2511	0	629	0	0
63	Shih Tzu	1646	824	422	81	836
64	Shih Tzu	528	1159	1443	78	986
65	Bulldog Cross	556	2707	770	273	878
66	Shih Tzu	0	0	0	0	0
67	Mongrel	7	0	674	0	0
68	Pomeranian	319	634	207	71	0
69	Shih Tzu	523	5646	305	124	374
70	Beagle	52	395	206	181	650

APPENDIX N

Data on "*Canine 62 Allergen Specific IgE Serological Test"* quantitative result on grains, beans, nuts and flax allergen subgroup.

No.	Breed	Soybean	Peanut	Rice	Wheat	Flax
1	Poodle X Shih Tzu	405	543	511	457	623
2	English Cocker Spaniel	1446	809	125	1516	1175
3	Bull Dog	172	187	0	3154	0
4	Rottweiler	2364	0	404	1617	0
5	Mongrel	88	0	80	0	518
6	Golden Retriever	0	464	0	0	0
7	Pug	25	1742	744	0	0
8	Great Dane	0	0	220	0	0
9	Golden Retriever	1163	757	172	0	0
10	Poodle	0	0	0	0	185
11	Rottweiler	204	699	0	0	301
12	Golden Retriever	279	301	25	739	311
13	Shih Tzu	0	0	399	0	0
14	Mixed Breed	490	532	967	229	788
15	Poodle	0	122	0	230	0
16	Mini Schnauzer	0	0	362	0	0
17	Beagle	0	0	0	0	0
18	Dachshunds	1064	720	445	1224	1102
19	Mixed Breed	70	2	865	707	0
20	Golden Retriever	617	847	0	989	0
21	Toy Poodle	2148	1666	230	2162	840
22	Toy Poodle	113	853	247	974	0
23	Mixed Breed	4904	9810	167	5899	6703
24	Pug	0	0	0	0	0
25	Golden Retriever	688	719	231	1001	409
26	Mongrel	0	0	487	0	0
27	Poodle	0	0	0	0	0
28	Shih Tzu	0	0	0	0	0
29	Poodle	0	0	94	0	342
30	Siberian Husky	0	0	0	0	0
31	Beagle	0	0	0	0	0
32	Maltese	356	172	344	148	192

33	Shih Tzu	2229	4133	2911	2898	2784
34	Mongrel	1708	6947	2779	0	0
35	English Bulldog	359	404	179	287	1114
36	Poodle	376	14	539	0	0
37	Golden Retriever	420	462	450	242	449
38	Mixed breed	452	2061	284	1486	499
39	Golden Retriever	53	239	0	478	61
40	Labrador Retriever	0	1369	0	2858	0
41	Poodle	0	0	0	0	1541
42	Maltese x Poodle	0	0	0	1998	0
43	Scottish Terrier	0	0	1353	2268	11231
44	Shih Tzu	2440	0	145	321	0
45	Golden Retriever Cross	0	0	1589	1595	0
46	Silky Terrier	147	0	0	421	3624
47	Bull Mastiff	309	233	387	227	861
48	Labrador Cross	1413	296	129	291	197
49	Spitz Cross	117	1083	0	39	0
350	Great Dane	0	1772	0	0	0
51	Mixed Breed	147	454	0	382	0
52	Pekingese	750	839	646	497	747
53	Poodle Cross	0	0	112	0	0
54	American Cocker Spaniel	114	123	131	130	319
55	Poodle	193	126	205	217	342
56	Shih Tzu	227	354	442	370	388
57	German Shepherd	0	0	56	0	0
58	German Shepherd	0	225	0	124	2747
59	Cocker Spaniel	0	0	290	1660	0
60	Terrier Mixed	0	282	210	184	0
61	Silky Terrier	0	0	0	0	0
62	German shepherd	0	0	0	0	0
63	Shih Tzu	0	0	110	0	2124
64	Shih Tzu	624	416	354	580	219
65	Bulldog Cross	3393	2554	1079	1982	4430
66	Shih Tzu	338	0	0	0	0
67	Mongrel	0	0	276	597	0
68	Pomeranian	101	488	461	696	0
69	Shih Tzu	1574	806	174	354	471
70	Beagle	200	202	38	0	0