

Positive reactions to common allergens in 42 atopic dogs in Japan

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Abstract

Clinically important allergens for the diagnosis and treatment of atopic dermatitis vary geographically. In order to identify the most prevalent allergens in atopic dogs in Japan, 42 dogs with a clinical diagnosis of atopy were tested using both in vivo (intradermal skin test (IDST)) and in vitro (antigen-specific IgE assay) allergy tests. Allergens used for IDST included 26 allergen extracts from eight allergen groups: trees, weeds, grasses, house dust mites (HDM), molds, foods, epithelia, and arthropods. Immunodot assay was used to measure antigen-specific IgE against 24 allergens from these eight groups and against fish such as cod and sole. In the 42 dogs, the most common positive allergen reaction was to HDM on both IDST (29/42 dogs or 69%) and in vitro testing (23/42 or 54.8%). The second most frequent positive allergen reaction was to Japanese cedar pollen (21/42 or 50.0% for IDST and 7/42 or 16.7% for in vitro testing). In both tests, less than 20% of dogs had positive reactions to molds or foods. Positive reactions to cat epithelia were frequently found on IDST, but rarely found on in vitro testing. Agreement between the two tests was found in 26 instances: HDM (21 dogs), Japanese cedar pollen (five dogs) and wheat (one dog). In this study,

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the two most common allergens involved in atopic dermatitis in dogs in Japan were HDM and Japanese cedar pollen. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Canine atopic dermatitis is a common skin disease of dogs (Nesbitt et al., 1984; Willemse, 1986). Common clinical signs include a history of seasonal or nonseasonal pruritus, otitis externa, recurrent and chronic inflammatory dermatitis especially in the axillary, inguinal, and flexor skin surfaces, recurrent bacterial infections, face rubbing and/or foot licking and chewing (Nesbitt et al., 1984; Willemse, 1986). Reported allergens involved in the pathogenesis of canine atopy include house dust mites, plants, arthropods, epithelia, foods, and molds (Halliwell and Schwartzman, 1971; August, 1982; Jeffers et al., 1991; Rosser, 1993). Some allergens are ubiquitous in the environment (e.g. house dust); however, many other clinically important allergens vary with respect to season, climate, and/or geographic region. What is important in one geographic region or country, may not be important in another (Nesbitt et al., 1984; Sture et al., 1995).

In Japan, Japanese cedar pollen is known to be a common and important seasonal allergen in humans (Hashimoto et al., 1995) and monkey (Hashimoto et al., 1997). There is emerging evidence that this allergen may also be important in dogs. Recently, dogs were found to be sensitized to Japanese cedar pollen upon intradermal skin test (IDST) (Sasaki et al., 1995) and on in vitro serum testing (e.g. antigen-specific IgE test) (Sakaguchi et al., 1997). To date, there is little information on the prevalence of sensitization to Japanese cedar pollen in atopic dogs in Japan.

Diagnostic methods for identifying clinically important allergens in canine atopy have been rapidly improved in veterinary dermatology. IDST and in vitro antigen-specific IgE have recently been developed (August, 1982; Nesbitt et al., 1984; Sousa and Norton, 1990). IDSTs have been accepted as the 'gold standard' for the diagnosis and management of atopic skin disease because it is believed to most closely reflect the natural disease pathogenesis in the dog (Willemse, 1986; Codner and Lessard, 1993). Unfortunately, there are problems with false-positive reactions to the IDST because of cross-reactivity among allergens and possible contamination with histamine-like substances in the extracts (August, 1982; Codner and Tinker, 1995). False-negative reactions to the IDST are also problematic and can occur for a variety of reasons including poor injection technique, degradation of allergen solutions, immune status of the dog, and influences of medication (Nesbitt et al., 1984). Therefore, it is becoming a common practice to use both IDST and in vitro testing in the diagnosis and management of dogs with atopic dermatitis.

Topscreen and Immunodot (CMG Laboratories, Fribourg, Switzerland) have become available to veterinarians in Japan as screening test kits for detection of antigen-specific IgE in dogs (de Weck et al., 1998). Topscreen is used to screen for the presence of allergen specific IgE in an allergen group, e.g. mold mix. If a positive reaction is found within a major group, the Immunodot test is then used to identify the specific allergen(s).

These two tests screen for 24 allergens including indoor, outdoor, food, mold, and fish allergens. These tests are useful to confirm the presence of antigen-specific IgE, but a negative test result does not exclude the diagnosis of atopy because it is a less sensitive test than an IDST (de Weck et al., 1998). Since the concentration or amount of serum IgE can fluctuate, serum concentrations of IgE against seasonal allergens may not be detected year-round. Therefore, taking into account the advantages and disadvantages of the IDST and in vitro serum test, the optimum diagnostic approach may be to use both tests to identify clinically important allergens.

The purpose of the study reported here is to document the prevalence of positive allergen reactions on IDST and serum in vitro tests in 42 dogs with naturally occurring canine atopic dermatitis in Japan.

2. Materials and methods

2.1. Dogs

Based on Willemse's criteria for clinical diagnosis of canine atopic dermatitis, 42 dogs were diagnosed as having atopic dermatitis at the Veterinary Medical Center, the University of Tokyo, Japan. All the dogs had compatible historical and clinical findings of atopic dermatitis including seasonal or recurrent and chronic pruritus, otitis externa, and superficial pyoderma. Parasitic infestations (i.e. demodicosis, fleas, or scabies) and cutaneous infections (i.e. dermatophytosis and bacterial pyoderma) were ruled out as causes of pruritus. In particular, all dogs received appropriate antibacterial therapy to eliminate the possibility that the pruritus was caused by a superficial pyoderma.

2.2. Intradermal skin test (IDST)

Twenty-five allergen extracts for IDST were purchased from a commercial company (Greer Laboratories, Lenoir, NC) except for extract of Japanese cedar pollen which was prepared as reported previously (Yasueda et al., 1997). The extract of Japanese cedar pollen contains two major allergens, Cry j 1 and Cry j 2, which have been implicated in Japanese cedar pollinosis in humans (Yasueda et al., 1983) and monkeys (Hashimoto et al., 1997). There were eight groups of allergens used in this study: HDM (*Dermatophagoides pteronyssinus* and *D. farinae*), arthropods (cockroach, housefly, mosquito, etc.), cat epithelia, foods (wheat, rice, beef, etc.), molds (*Curvularia spicifera*, *Penicillium camemberti*, etc.), grasses (Kentucky blue, orchard, redtop, etc.), weeds (cocklebur, lambs quarter, rough pigweed, etc.), and trees (Japanese cedar, white ash, red birch, etc.) (Fig. 1). The allergen extracts were prepared and used according to manufacturer's instructions. Briefly, most of the allergen extracts were used at a concentration of 1000 PNU after being diluted with sterile diluent (0.9% sodium chloride and 0.4% phenol). The extracts of *D. pteronyssinus* and *D. farinae* were diluted to a concentration of 1:5000 w/v. The extract of Japanese cedar pollen was used at the concentration of 200 ng/ml. The diluent also was used as the negative control. Histamine phosphate (0.0275 mg/ml) was used as the positive control. The hair coat on the lateral

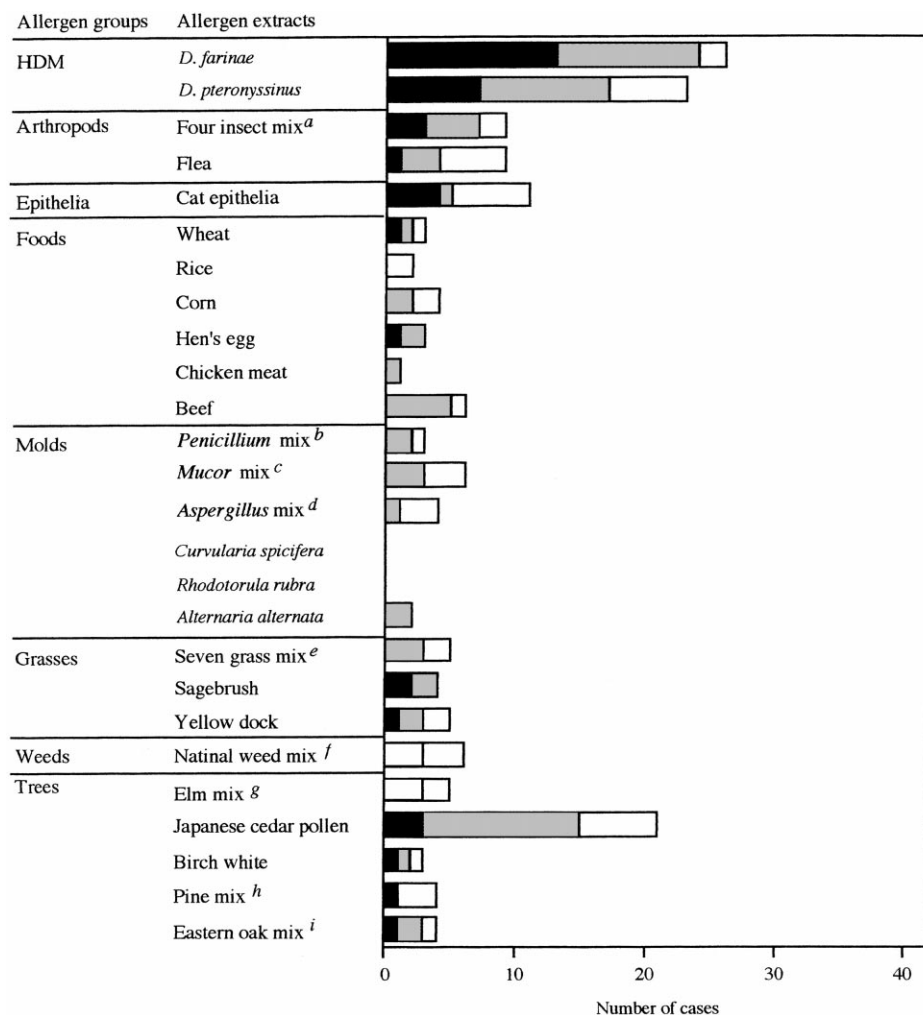


Fig. 1. Results of IDST in 42 dogs with atopic dermatitis. The IDST-positive reactions were classified into three grades as follows: (■) +++; (▒) ++; (□) +. (a) cockroach, housefly, mosquito, and moss. (b) *Penicillium camemberti*, *P. chrysogenum*, *P. digitatum*, *P. notatum*, and *P. roqueforti*. (c) *Mucor plumbeus* and *M. racemosus*. (d) *Aspergillus flavus*, *A. fumigates*, *A. glaucus*, *A. nidulans*, and *A. niger*. (e) Kentucky blue, orchard, redbud, timothy, sweet vernal, meadow fescue, and perennial rye. (f) cocklebur, lambs quarter, rough pigweed, giant ragweed, and short ragweed. (g) American and Chinese elm. (h) loblolly, white, and yellow pine. (i) white ash, American beech, red birch, shagbark hickory, sugar maple, red oak, sweet gum, and eastern sycamore.

thorax of the dog was clipped and approximately 0.05 ml of each allergen solution was injected intradermally using a skin test syringe with a 26-gauge needle without sedation. All IDSTs were performed by one clinician. The diameters of wheals were measured 15 to 20 min post injection. The test sites were graded as follows: +++, equal to or greater than the diameter of the positive control; ++, equal to or greater than the mean diameter

of the positive and negative controls; +, larger than the diameter of the negative control but small than the mean diameter of the positive and negative control; –, equal to or smaller than the diameter of the negative control.

2.3. Antigen-specific serum IgE test

Serum concentrations of antigen-specific IgE were measured using two commercially available test kits (Topscreen and Immunodot test kits, CMG Laboratories, Fribourg, Switzerland) as previously described (de Weck et al., 1998). The Topscreen test is a five spot screening test using mixtures of allergens such as outdoor, indoor, two food, and mold allergen groups. In contrast, the Immunodot test is used to detect individual antigen specific IgE (e.g. Japanese cedar pollen). In both of IDST and antigen-specific serum IgE test, reactivities to the same kinds of allergens (e.g. HDM, chicken egg, beef etc.) were examined in most instances, however, the allergen extracts used in these two tests were prepared by two different manufacturers, Greer Laboratories and CMG Laboratories, respectively. As an exception, the same allergen extract of Japanese cedar pollen prepared by Yasueda et al. (1997) was commonly used in both IDST and antigen-specific IgE test. Nitrocellulose strips each containing an allergen test mixture for the Topscreen or an individual allergen for Immunodot were prepared by a manufacturer (CMG Laboratories, Fribourg, Switzerland) and patient serum samples were spotted on individual nitrocellulose strips. After incubation, peroxidase conjugated monoclonal anti-canine IgE antibody and substrate solution were applied to the strips for colorimetric detection. The intensity of the color was measured with a densitometer. An optical density (O.D.) value greater than 1.0 in the Topscreen and an O.D. greater than 2.0 in the Immunodot were considered as positive for the allergen group or specific allergen, respectively. Topscreen was used to screen for the presence of IgE antibodies for a specific allergen group. If a positive reaction was detected, individual testing for specific allergens within a group was done using the Immunodot test. In this assay system, a total of 24 allergens belonging to nine allergen groups, HDM, fish, arthropods, epithelia, foods, molds, grasses, weeds, and trees were examined (Fig. 2). The number of the dogs that had positive reactions in this test was recorded.

3. Results

3.1. Dogs with atopic dermatitis

In this study, 35 of the 42 dogs were purebred and seven were mongrel dogs. The pure bred dog breeds represented in this study included Shiba Inu (4 cases), Shetland sheepdog (3), West Highland white terrier (3), American cocker spaniel (2), bichon frise (2), French bulldog (2), golden retriever (2), Great Pyreneese (2), pug (2), Akita (1), bearded collie (1), Dalmatian (1), Irish setter (1), Japanese terrier (1), Kai (1), Labrador retriever (1), Maltese (1), miniature dachshund (1), Old English sheepdog (1), Pomeranian (1), Siberian husky (1), Shih tzu (1). The age of onset of clinical signs ranged from 3 months to 9 years old with a median age of 1 year. In this study, 30 of the 42 dogs (71.0%) were

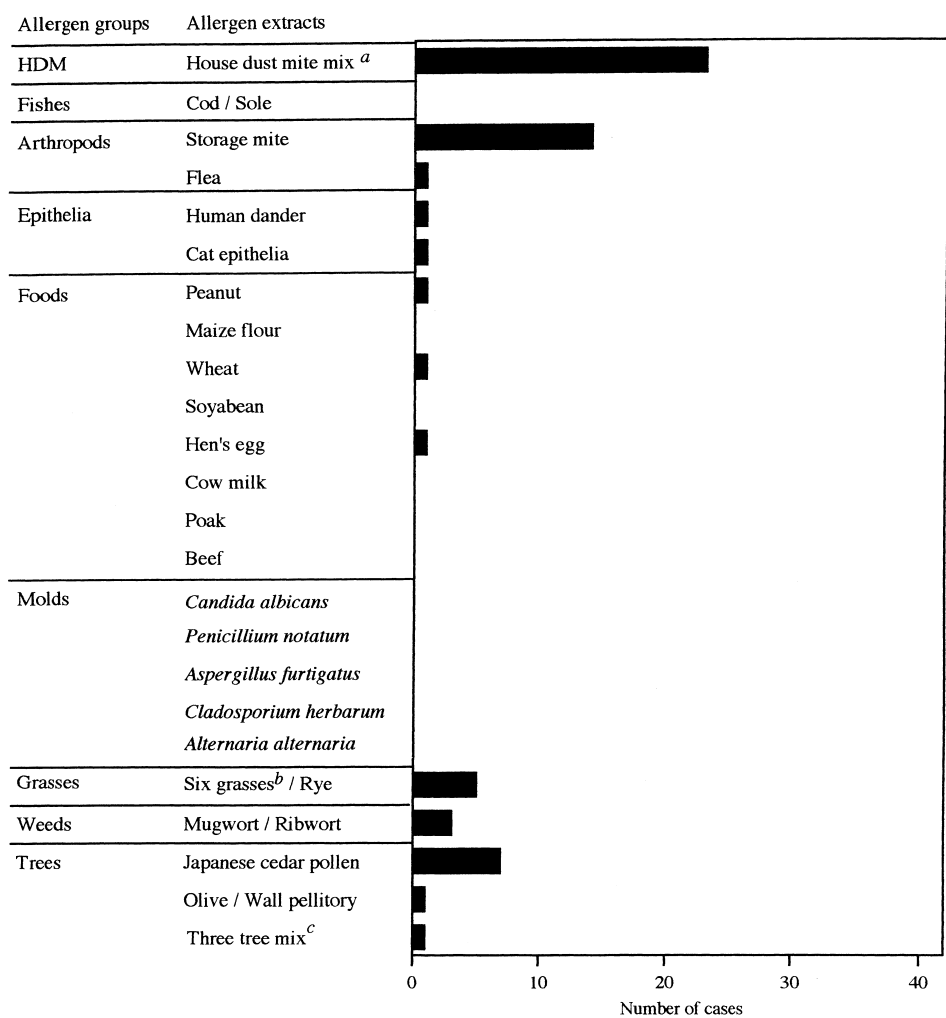


Fig. 2. Results of serum antigen-specific IgE test in 42 dogs with atopic dermatitis. (a) *Dermatophagoides farinae* and *D. pteronyssinus*. (b) cocksfoot, meadow fescur, perennial rye grass, timothy, Kentucky blue grass, velvet grass, and yorkshire. (c) birch, oak, and hazel.

less than 3 years old when they showed their first clinical symptoms. There was no evidence of a sexual predisposition with males and females being almost equally (male, 22 cases; female, 20 cases). Seasonality of the symptoms was reported in three dogs that had positive reactions to outdoor allergens on both IDST and antigen-specific IgE test.

3.2. IDST

The prevalence of positive IDST reactions is shown in Fig. 1. There were a large number of cases that had positive IDST reactions to indoor-allergen groups including

HDM, arthropods, and epithelia. The most common IDST allergen reaction was to HDM. Positive reactions to *Dermatophagoides farinae* and *D. pteronyssinus* were found in 64.3 and 54.8% of the atopic dogs, respectively. Nine dogs had positive reactions to either flea or the four insect mix, and all these dogs also had positive reactions to HDM. Eleven of the 42 atopic dogs (26.1%) showed positive reactions to cat epithelia, and 10/11 of these dogs also had positive reactions to HDM.

In the inhalant plant allergen groups (trees, weed, and grasses), reactions to Japanese cedar pollen were most common with 21 of 42 dogs (50.0%) having positive reactions. Six dogs had positive reactions to the national weed mix, but the sizes of the wheals were smaller than the positive control. Four to five dogs had positive reactions to seven grass mix, sagebrush, and yellow dock. In most instances, dogs with positive IDST reactions to the seven grass mix also had reactions to the weed mix.

Only a small number of dogs had positive reactions to mold or food (Fig. 1). Seven dogs had weak positive reactions to molds including *Penicillium* mix, *Mucor* mix, *Aspergillus* mix, and *Alternaria alternata*. None of the dogs in this study reacted to *Curvularia spicifera* or *Rhodotorula rubra*. With respect to the food allergens, two dogs had strong IDST reactions (+++) ($n=1$ wheat, $n=1$ chicken egg). Weak positive IDST reactions (+ and ++) were seen for other food allergens including wheat, rice, corn, hen's egg, chicken meat, and beef in a small number of dogs with atopic dermatitis. Five dogs had positive IDST reactions to beef antigen; beef was the most frequent allergen among the food ingredients examined in this assay.

3.3. Serum IgE specific to allergens

The results of the antigen-specific IgE test in the atopic dogs are shown in Fig. 2. Positive allergen reactions to HDM were the most commonly found reaction on serum in vitro testing (23 of 42 dogs or 54.4%). Positive reactions for antigen-specific IgE to storage mites were common (14/42 cases, 33.3%); however, all of these dogs also reacted to HDM. Of the 42 dogs, seven (16.7%) dogs had positive reactions to Japanese cedar pollen. Five of these seven dogs also had positive reactions to plant allergens such as grasses and weeds. All of the dogs with positive reactions to plant allergens also had positive reactions to Japanese cedar pollen. There were three dogs with positive reactions to food allergens (hen's eggs, peanut, and wheat, respectively). None of the dogs had positive reactions to mold allergens on this serum in vitro testing.

3.4. Comparison of IDST and specific IgE for allergens

The overall frequencies of positive reactions to allergens are shown in Fig. 3. In this study, the most common allergen reaction was to HDM for both the IDST and serum in vitro test. When the two systems were compared, positive reactions to HDM were more common on IDST ($n=29$) than on serum in vitro testing ($n=23$). With respect to dogs reactive to grass allergens, there was no difference between the two tests in detecting positive reactions. In the case of tree and weed allergens, more positive reactions were detected via IDST than with the serum in vitro testing. A relatively high percentage (11/42 case, 26.2%) of atopic dogs had positive reactions to cat epithelia on IDST; however,

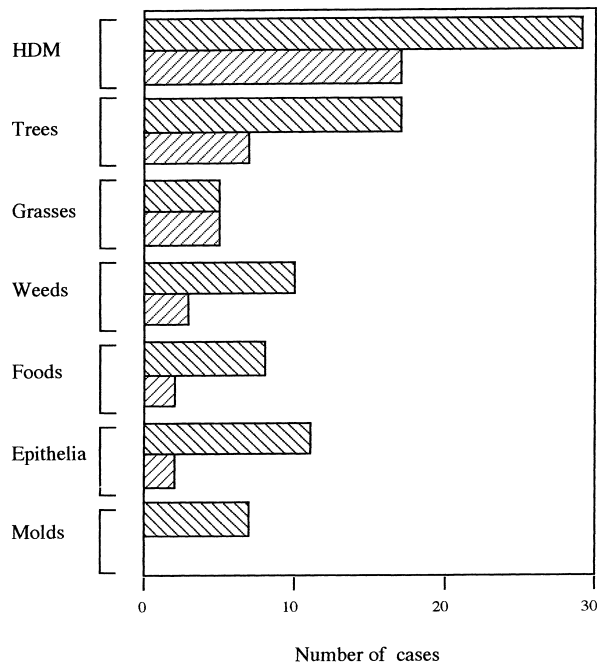


Fig. 3. Comparison of the results from IDST and serum antigen-specific IgE test in 42 dogs with atopic dermatitis. (▨) IDST-positive; (▩) antigen-specific IgE test-positive.

only one of these 11 dogs had a positive reaction on the serum in vitro test. Seven dogs (16.7%) had positive reactions for molds on IDST, but no dog had positive reaction to mold allergens on the serum in vitro testing. With respect to food allergens, eight dogs (19.0%) had positive reactions on IDST, but only two of these eight had positive reactions on serum in vitro testing. Overall, test results were identical on both tests in 26 dogs (HDM, $n=21$; Japanese cedar pollen, $n=4$; wheat, $n=1$).

4. Discussion

Dog breeds commonly reported to be predisposed to atopic dermatitis include the German shepherd, Irish setter, poodle, terrier breeds (West Highland white terrier, wire haired fox terrier, Cairn terrier, Scottish terrier, and Boston terrier), Lasa apso, Dalmatian, and miniature schnauzer (Halliwell and Schwartzman, 1971; Nesbitt, 1978; Scott, 1981). However, none of the studies to date report any predisposition of Japanese pure breeds to atopic dermatitis. In this study, we found a breed predisposition for atopy in Shiba Inu dogs ($n=4$). These findings were not surprising as they support our clinical impression of a predisposition for atopy in this breed. Larger surveys of the prevalence of atopy in dog breeds unique to Japan are needed. As allergy testing becomes more common place, it is very likely that many other breeds of dogs with a predisposition to atopy will be

identified. This information is important because of widespread introduction and cross breeding of 'new' breeds to other countries.

In the majority of dogs in this study, the onset of clinical signs occurred before the dog was 3 years of age. These findings were similar to those of other investigators. Willemse reported that atopic dermatitis appeared before 3 years of age (Willemse, 1986) in dogs he studied. In a large study of 449 dogs with atopy, 75.4% of atopic dogs developed clinical signs between 1 and 3 years of age (Carlotti and Costargent, 1992). The genetic predisposition to atopy is most likely unmasked by exposure to allergens at a very young age (de Weck, 1995). Therefore, the age of the first onset of clinical signs is a consistently useful piece of historical information in the diagnosis of canine atopy. In this study, we found no sexual predisposition for atopy, which is similar to the findings in other studies (Willemse et al., 1984; Carlotti and Costargent, 1992).

Based on the findings in this study, HDM is an important allergen in atopic dogs in Japan. In this study, we found a higher prevalence positive IDST reactions to *D. farinae* versus *D. pteronyssinus*. These findings are similar to those of a study in the United Kingdom where *D. farinae* also was found to be the major allergen in dogs with HDM allergies (Sture et al., 1995). Since *D. pteronyssinus* is both the predominant HDM in Japanese dwellings (Otani et al., 1984) and the predominant cause of allergic diseases in people in Japan (Yasueda et al., 1989), our data suggest that the high prevalence of positive reactions to *D. farinae* in these atopic dogs reflects specific immune responses rather than environmental exposure.

In this study, as in others, atopic dogs were found to have positive reactions to a number of outdoor allergens (trees, weeds, and grasses). We found that Japanese cedar pollen was a major outdoor plant allergen. This was not surprising because Japanese cedar pollen is one of the most problematic seasonal allergens in people in Japan and there are a large and increasing number of patients with Japanese cedar pollinosis (Inouye et al., 1986). Very recently, positive IDST (Sasaki et al., 1995) and positive antigen-specific IgE test (Sakaguchi et al., 1997) reactions for Japanese cedar pollen were reported in several dogs in Japan. In this study, 21 of 42 atopic dogs (50%) were found to have positive reactions on IDST. A lesser number of dogs were found to have positive reactions on serum in vitro testing. These results indicate that a large proportion of the population of atopic dogs in Japan are naturally sensitized to Japanese cedar pollen. In addition, identification of these dogs is best done through IDST. Although rhinitis and/or conjunctivitis are the major clinical signs in human Japanese cedar pollinosis, this was not the case in this study. In a previous study, we demonstrated that nasal discharge and sneezing in dogs naturally sensitized to Japanese cedar pollen can occur with provocative testing (Sasaki et al., 1995). The findings in this study show that Japanese cedar pollen is an important allergen in both dogs and people, similar to ragweed allergen in other geographic regions. As with other pollens, dogs sensitized to Japanese cedar pollen develop cutaneous allergies rather than allergic rhinitis as happens in people.

In this study, there was a small number of dogs with positive IDST reactions for mold allergens; none of the dogs had positive reactions to molds on in vitro testing. A previous report indicated that the percentages of positive IDST reactions for molds in atopic dogs varied among several areas in US; there was a higher percentage of positive reactions in North Carolina (eastern United States) than Illinois (midwestern United States) (Schick

and Fadok, 1986). It is unclear why these dogs in this study had such a low prevalence of positive reactions to mold allergens. The geographic variations in ambient temperature and humidity may partly explain variations in mold sensitivity in these dogs. This study was conducted from fall to winter in Tokyo where it is relatively warm. One explanation for the low number of positive reactions to mold could be a seasonal variation in allergen reactions as has been reported (Nesbitt et al., 1984). It will be necessary to repeat this study during different seasons to answer this question. Another explanation could be that there are geographic differences in the genetic gene pool and dogs in Japan are less likely to develop mold allergy.

In this study, only a small number of dogs had positive reactions to food allergens on either test. The findings in this study were lower than those of a previous study where 30% of 100 suspected allergic dogs were reactive to one or more food allergens on IDST (Kunkle and Horner, 1992). To date, the most reliable method for the diagnosis of a food allergies is response to restricted dietary trial and provocative testing (Rosser, 1993). IDST and antigen-specific IgE tests are not considered reliable diagnostic tools for identifying food allergic dogs (Jeffers et al., 1991; Kunkle and Horner, 1992). A dietary food trial was not done in dogs in this study, so we do not know how many of these dogs had concurrent food allergies or if the results of IDST and antigen specific testing were false positive or false negative reactions.

Cross-reactivity among different allergens must be taken into consideration when a dog shows multiple reactions within an allergen group. In this study, many dogs with positive reactions to Japanese cedar pollen also had positive reactions to other plant allergens. The results of the antigen specific IgE test also suggested a possible cross-reactivity between HDM and storage mites in this study. In people, it has been reported that similar epitopes are shared by tree pollens, such as birch, alder, hazel and hornbeam (Rohac et al., 1991). Cross-reactivity between flea and other insects on antigen-specific IgE testing has also been reported in dogs (Pucheu-Haston et al., 1996). Based upon previous studies, it is possible that some of the positive reactions found in this study may reflect cross-reactivity from similar or shared epitopes on the test allergens. In dogs showing reactions to multiple allergens on either IDST or on antigen-specific IgE testing, enzyme linked immunosorbant assays for individual allergens, antigen-specific lymphocyte blastogenesis, and histamine release tests may be helpful in differentiating between primary allergens and cross-reactive allergens.

There are advantages and disadvantages with both of the currently used diagnostic tests for canine atopy. In IDST, the major diagnostic problems are false positive (August, 1982; Codner and Tinker, 1995) and false negative reactions and/or problems with cross-reactivity among allergens (Nesbitt et al., 1984). On the other hand, a major problem with serum in vitro testing, particularly in this study, was high specificity and relatively low sensitivity (de Weck et al., 1998). Optimum diagnostic testing may be best achieved by using both tests simultaneously. In this study, we found agreement with respect to allergen reactions between both of these test in 26 of the 42 cases (61.9%). In the canine atopic dermatitis, identification of allergens can become very reliable when the results from IDST and antigen-specific IgE test are combined.

In summary, this study describes the prevalence of positive reactions to selected allergens in atopic dogs in Japan. The recognition of canine atopy as an important

problem in small animal practice is increasing. It is becoming obvious that recommendations for allergen selection need to be based upon regional testing. In this study, we demonstrated that Japanese cedar pollen, a geographically unique pollen, is an important allergen in the pathogenesis of canine atopy in dogs from Japan.

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