Original Article

Children Monosensitized to Can f 5 Show Different Reactions to Male and Female Dog Allergen Extract Provocation: A Randomized Controlled Trial

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What is already known about this topic? Dog dander consists of several allergenic molecules including Can f 5, which is a protein expressed in the prostate of male dogs, suggesting that monosensitization to Can f 5 implies tolerance to female dogs.

What does this article add to our knowledge? This double-blind, randomized controlled trial shows that children monosensitized to Can f 5 react differently to male and female dog extract provocation using both skin prick test and conjunctival allergen provocation test, suggesting tolerance to female dogs.

How does this study impact current management guidelines? Patients allergic to dog suspected to react only to male dogs should be tested for dog components Can f 1 to 6 to establish whether they are monosensitized to Can f 5, in which case they can get a female dog.

BACKGROUND: Dog dander consists of several allergenic molecules including Can f 5, which is a protein expressed in the prostate of male dogs.
OBJECTIVE: To investigate whether children monosensitized to Can f 5 show different reactions to provocation tests with male versus female dog dander in a double-blind randomized clinical trial.
METHODS: Twenty-two children (15-18 years) with a history of dog sensitization were enrolled from the COpenhagen Prospective Studies on Asthma in Childhood$_{2000}$ mother-child cohort. Skin prick test, specific IgE levels to dog dander (e5), and dog components Can f 1, 2, 3, and 5 were first assessed. We subsequently performed skin prick test and conjunctival allergen provocation test using dog dander collected separately from male and female dogs.
RESULTS: Seven of the 22 children were monosensitized to Can f 5. Eight were sensitized to a mix of the dog components, and 7 were no longer sensitized to dog. Of the children monosensitized to Can f 5, all had a positive skin prick test result to male dog extract and 1 of 7 was also positive to female dog extract (P = .01). Furthermore, 5 of 7 had a positive conjunctival allergen provocation test result to male dog extract and 1 of 7 also reacted to the female dog extract (P = .03). There was no difference between reactions to male and female dog extract provocation in children sensitized to a mix of the dog components.
CONCLUSIONS: Children monosensitized to Can f 5 show different reactions to male and female dog extract provocation using both skin prick test and conjunctival allergen provocation test, suggesting tolerance to female dogs. © **2019 American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2020;■:■-■)**

Key words: *Allergy; Dog allergy; Can f 5; Male dog; Female dog; Monosensitization; Conjunctival allergen provocation; Children*

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Abbreviations used
CAP- Conjunctival allergen provocation
$COPSAC_{2000}$- COpenhagen Prospective Studies on Asthma in $Childhood_{2000}$
sIgE- Specific IgE
SPT- Skin prick test
TOSS- Total Ocular Symptom Score

INTRODUCTION

Dog dander is one of the most common causes of perennial respiratory allergy among children.[1] Exposure can cause allergic reactions such as rhinoconjunctivitis and asthma in sensitized people.[2,3] For the physician the most established diagnostic tools for allergic sensitization to dog are skin prick test (SPT) and measurement of specific IgE (sIgE) to dog dander extract, but the extracts vary in allergen content, which affects the reproducibility and reliability of the results.[4] Furthermore, the dog allergen consists of several allergenic molecules,[5] which can be used to refine the characterization of each individual's type of dog allergy.[6,7]

Molecularly based allergy diagnostics has introduced a new possibility to personalize allergy diagnostics. The most well-known dog allergens are the lipocalins Can f 1, 2, 4, and 6, which are present in dog hair, dander, saliva, and urine,[8-10] with Can f 1 being the major allergen detected in most patients sensitized to dog.[11] Can f 3 is a serum albumin, a minor allergen that is also present in dog hair, dander, and saliva.[12] Can f 5 has been identified as another major dog allergen, a prostatic kallikrein expressed in the prostate, and hence present only in male dogs.[13] The allergen is mainly found in the urine of male dogs, but also in extracts of dog hair and dander.[14] Can f 5 and its expression is reduced in neutered males.[13] There is no cross-reactivity between Can f 5 and the dog allergen components Can f 1, 2, or 3.[14] Another male-only dog allergen, Can f 7, has been identified.[15] It is an epididymal secretory protein present in only about 10% to 20% of patients allergic to dog,[15] and therefore not investigated further in previous studies.

Can f 5 IgE antibodies are present in approximately 70% of patients sensitized to dogs, and in between 30% and 60% of the patients, this is the only dog allergen that they are sensitized to.[14,16] This suggests that patients with dog allergy may react differently to male and female dogs, depending on their sensitization pattern. The commercially available extracts used for both sIgE and SPT are a mixture derived from male and female dogs of different breeds, leaving out the possibility of distinguishing a male-dog-only allergy. Therefore, there is a need to further explore the clinical relevance of sensitivity to dog allergen components.

In this randomized double-blind clinical trial, we aimed to compare children monosensitized to Can f 5 with children sensitized to a mix of the dog components, and investigate whether they react differently to SPT using male and female dog extract separately. In addition, we assessed allergic symptoms when children were exposed to male and female dog extract by conjunctival allergen provocation.

METHODS

Study design

A population of children (N = 33; age, 15-18 years) was recruited from the COpenhagen Prospective Studies on Asthma in $Childhood_{2000}$ ($COPSAC_{2000}$) cohort. The $COPSAC_{2000}$ is a single-center, prospective clinical mother-child cohort study previously described in detail.[17-19] The children were enrolled between March and August 2017. Inclusion criteria were a history of sensitization to dog dander verified on the basis of a positive SPT result (wheal diameter size $\geq$ 3 mm than the negative control), sIgE to dog dander greater than 0.35 kU_A/L, or both. The children were recruited regardless of their history of symptoms on contact with dogs. The exclusion criteria were uncontrolled asthma, daily need of antihistamine treatment, eye surgery within 6 months, any chronic disease of the eye, or ongoing or completed allergen immunotherapy to furry animals.

At the first visit, we collected a blood sample and performed SPT and conjunctival allergen provocation (CAP) test in the right eye. We did a double SPT on both volar forearms and applied a positive and negative control and the 3 extracts: dog (e5), male dog, and female dog (the latter 2 blinded for both investigator and child). The CAP test was done in the right eye with either the male or female extract (blinded for both investigator and child). Before application of the allergen, the left eye was used as control and installed with 1 drop of physiological sodium chloride (9 mg/mL). At least 1 week later, we saw the children for a second visit to complete the CAP test in the left eye using the other extract, and the right eye was used as control.

The study was registered at ClinicalTrials.gov with identifier NCT03097094.

Ethics

The Copenhagen Ethics Committee (HKF 01-289/96) and the Danish Data Protection Agency (2008-41-2434) approved the study, and oral and written informed consent was obtained from all participants and their parents at enrollment.

Randomization

A computerized randomization program (randomization.com) generated a list of random numbers prepared by an external investigator with no other involvement in the trial, and kept in a sealed envelope. A research assistant provided the investigator with either a male or female dog kit that included the extracts for the CAP. The male and female dog SPT and CAP extracts were contained in identical looking bottles marked with a number on them. The positive and negative controls and dog SPT (e5) were not blinded.

End points

Specific IgE. Levels of sIgE to dog dander and the dog components Can f 1 (lipocalin), Can f 2 (lipocalin), Can f 3 (albumin), and Can f 5 (arginine esterase, prostatic kallikrein) were determined using ImmunoCAP (Thermo Fisher Scientific, Uppsala, Sweden).[20] The children who were sensitized only to Can f 5, that is, monosensitized, are labeled *mono can f 5* throughout the study, the children sensitized to a mix of the dog components are labeled *mixed*, and the children no longer sensitized to dog are labeled *negative*.

Furthermore, we analyzed a full sensitization profile measuring allergic sensitization to 112 components using the ImmunoCAP Immuno Solid-phase Allergen Chip (Thermo Fisher).

Skin prick test. We performed an SPT using standard allergen extract to dog (ALK Abello, Soluprick SQ, Hørsholm, Denmark) and also, for the first time, we used dog dander collected separately from male and female golden retrievers according to standard extraction procedures for dander. We made a dilution of the extracts to perform SPT with a concentration of 25 mg/mL. Histamine

TABLE I. Baseline characteristics

Characteristic	Included (N = 22)	Mono Can f 5* (N = 7)	Mixed† (N = 8)	Negative e5‡ (N = 7)	Excluded (N = 11)
Sex: male	14 (64)	4 (57)	4 (50)	6 (86)	5 (45)
Age (y), mean ± SD	16.8 ± 0.9	16.5 ± 0.9	17.2 ± 1.0	16.7 ± 0.7	16.4 ± 1.0
Dog in home at birth	2 (9)	2 (29)	0	0	1 (9.1)
Dog in home current	3 (14)	0	1 (13)	2 (29)	6 (55)
Dog sIgE (e5), mean ± SD	2.8 ± 3.5	4.5 ± 3.5	3.5 ± 3.7	0.21 ± 0.1	—
Allergic rhinitis ever	20 (91)	6 (86)	8 (100)	6 (86)	11 (100)
Allergic rhinitis current	20 (91)	6 (86)	8 (100)	6 (86)	11 (100)
Asthma ever	10 (45)	2 (29)	5 (63)	3 (43)	7 (64)
Asthma current	9 (41)	2 (29)	5 (63)	2 (29)	4 (36)
Eczema ever	15 (68)	5 (71)	5 (63)	5 (71)	6 (55)
Eczema current	6 (27)	2 (29)	2 (25)	2 (29)	1 (9.1)
Older siblings	9 (41)	0	3 (38)	4 (57)	5 (45)

Values are n (%) unless otherwise indicated.
*The children who were monosensitized to Can f 5.
†The children sensitized to a mix of the dog components.
‡The children no longer sensitized to dog

dihydrochloride (10 mg/mL) and physiological sodium chloride (9 mg/mL) were used as positive and negative controls, respectively. We did a double SPT on both volar forearms and applied a positive and negative control and the 3 extracts—dog (e5), male dog, and female dog (the latter 2 blinded for both investigator and child)—and used a sterile lancet to prick through the droplets approximately 1 mm through the skin barrier. The reactions to the positive controls were read after 10 minutes, whereas the reactions to the allergens and negative controls were read after 15 minutes. The wheal diameters were measured as the average of the largest wheal diameter and its perpendicular. The final result used for analysis was an average of the 2 corresponding tests. A positive response was defined as greater than or equal to 2 mm than the negative control.

Conjunctival allergen provocation. We made dilutions of increasing strength, to perform CAP using the male and female dog extracts. CAP was done according to recommended guidelines.[21] Initially, we applied a droplet of extract with a concentration of 0.25 μg/mL, and every 15 minutes increased the concentration until a final concentration of 25 mg/mL. Droplets were applied in the inferior-external quadrant of the bulbar conjunctiva. During the first visit, the right eye was tested with either the male or female extract (blinded for both investigator and child). Before application of the allergen, the left eye was used as control and installed with 1 drop of physiological sodium chloride (9 mg/mL). On the second visit, minimum 1 week later, the left eye was tested with the other extract, and the right eye was used as control. A positive response was assessed according to the Total Ocular Symptom Score (TOSS),[21] evaluating itchiness (0-4), redness (0-3), and tearing (0-3) of the eye, but not evaluating chemosis, because this requires a slit-lamp examination. A total score of 4 was considered a positive response. The test was stopped when a positive response occurred or until the maximum dose was reached.

The children were instructed not to use antihistamines in any form within 72 hours of testing (the positive control was used as screening for use of antihistamines with longer half-life) as well as systemic corticosteroid or nasal or conjunctival corticosteroid 2 weeks before testing. Application of mild steroid creams (groups 1-2) on the arms was avoided 24 hours before testing. Stronger steroid creams (groups 3-4) were not used in our cohort.[22]

Statistical analysis

A paired *t* test was used for group comparisons between SPT wheal diameter size and CAP TOSS in the children monosensitized to Can f 5 and in the children sensitized to a mix of the dog components.

The results are presented as bar diagrams. Furthermore, a scatter box plot is used to present the difference between the SPT wheal diameter size to male dog extract and female dog extract. The CAP results are presented the same way. All statistical analyses were performed with Rstudio, version 1.1.442 (Rstudio, Inc, Boston, Mass). *P* values less than .05 were considered statistically significant.

RESULTS

Baseline characteristics

Of the 33 children recruited, 2 children did not meet the inclusion criteria, and 9 declined to participate after receiving detailed information about the project, resulting in 22 children included in the study. Seven of the 22 included children were monosensitized to Can f 5. Eight were sensitized to a mix of the dog components, and 7 were no longer sensitized to dog (for consort diagram, see Figure E1 in this article's Online Repository at www.jaci-inpractice.org). The mean age of the children was 16.8 years (range, 15-18 years), with the majority being boys (64%). Two of 22 had a dog at home at birth, and 3 of 22 were currently living in a home with a dog. Twenty of 22 had current allergic rhinitis, 9 of 22 had current asthma, and 6 of 22 had current eczema. The criteria for diagnosis of allergic rhinitis, asthma, and eczema in the $COPSAC_{2000}$ cohort are previously described in detail.[18,23]

The baseline characteristics of the children monosensitized to Can f 5, the children sensitized to a mix of the dog components, the children no longer sensitized to dog, and the excluded children are outlined in Table I.

SPT results

Of the Can f 5 monosensitized children, 7 of 7 had a positive SPT result to male dog extract and 1 of 7 was also positive to female dog extract. The average wheal diameter to the male dog extract was 3.6 mm in comparison to 1.3 mm to the female dog extract, mean difference = −2.36 (−4.03 to −0.69), $P = .01$

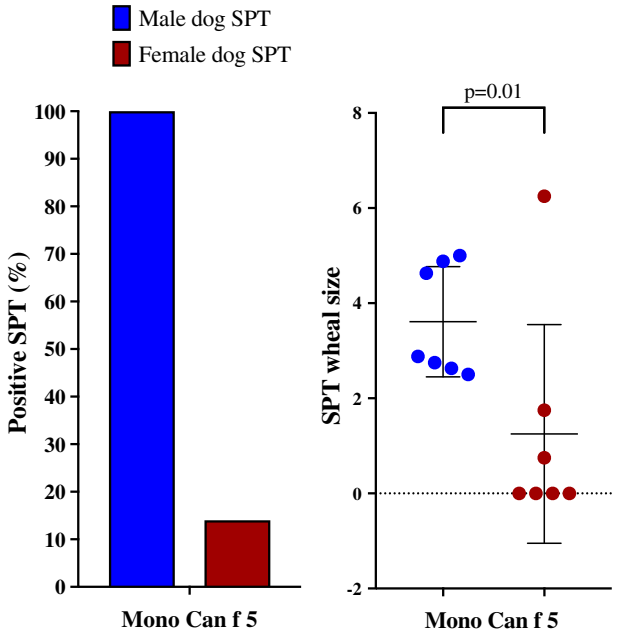

FIGURE 1. Comparison between reactions to male dog SPT and female dog SPT allergen extracts. Mono Can f 5 refers to the children who were sensitized only, that is, monosensitized, to Can f 5 of the dog components. The *P* value is from a *t* test.

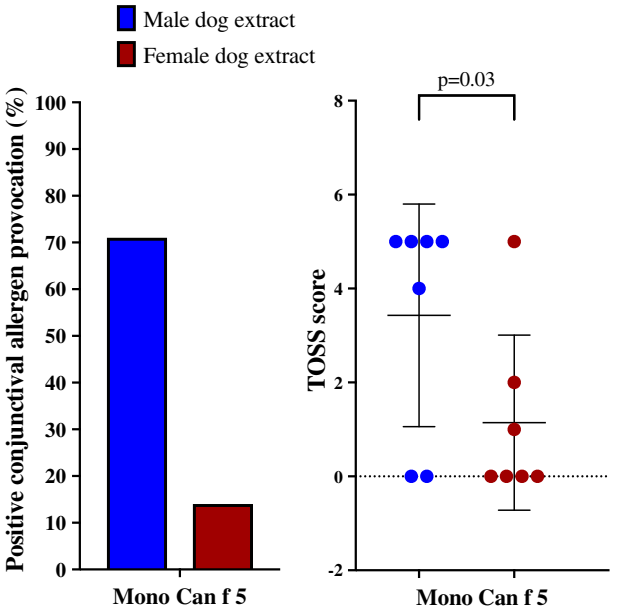

FIGURE 2. Comparison between CAP results to male dog allergen extracts and female dog allergen extracts. A TOSS of 4 was considered a positive response. Mono Can f 5 refers to the children who were sensitized only, that is, monosensitized, to Can f 5 of the dog components. The *P* value is from a *t* test.

(Figure 1). Of the children with a mixed sensitization pattern, 5 of 8 had a positive SPT result to male dog extract and 7 of 8 were positive to female dog extract. The average wheal diameter to the male dog extract was 2.7 mm in comparison to 3.0 mm to the female dog extract, mean difference = 0.26 (−0.84 to 1.36), $P = .59$.

CAP test

Of the Can f 5 monosensitized children, 5 of 7 had a positive CAP test result to male dog extract and 1 of 7 also reacted to the female dog extract. The average TOSS using the male dog extract was 3.4 in comparison to 1.1 using the female dog extract, mean difference = −2.29 (−4.33 to −0.24), $P = .03$ (Figure 2). Of the children with a mixed sensitization pattern, 6 of 8 had a positive CAP test result to male dog extract and 5 of 8 had a positive reaction to female dog extract. The average TOSS using the male dog extract was 4.9 in comparison to 3.6 using the female dog extract, mean difference = −1.25 (−3.81 to 1.31), $P = .29$.

Allergen profiles and sIgE levels to dog (e5)

The allergen profiles of the children were measured using the Immuno Solid-phase Allergen Chip test analyzing 112 components. The results from the food allergens and aeroallergens are listed in Table E1 in this article's Online Repository at www.jaci-inpractice.org, showing that all the children were sensitized to at least 2 other allergens than dog, regardless of their dog sensitization pattern. The sIgE levels to dog (e5) and Can f 5 using ImmunoCAP are outlined in Table E2 in this article's Online Repository at www.jaci-inpractice.org. There is a discrepancy between some of the e5 and the Can f 5 levels in the Can f 5 monosensitized children, which is most likely because the concentration of Can f 5 differs in the 2 extracts.

DISCUSSION

Primary findings

In this randomized double-blind controlled trial we examined the usefulness of molecularly based allergy diagnostics in children with suspected allergy to male dogs only. We analyzed sIgE levels to dog components Can f 1, 2, 3, and 5 and explored associations between reactions to male and female dog extracts in children monosensitized to Can f 5 and children with a mixed sensitization pattern. The Can f 5 monosensitized children had different reactions to male and female dog extract provocation using both SPT and CAP test, suggesting tolerance to female dogs. There was no difference between reactions to male and female dog extract provocation in children with a mixed sensitization pattern. This shows a distinct group of subjects sensitized to male but not female dogs, allergens from both of which are mixed in the currently available dog extracts.

Strengths and limitations

The theory of allergic sensitization to male dogs only has been hypothesized previously and demonstrated in case reports,[6,14] but this is the first time a randomized clinical trial has been carried out comparing reactions to male and female dog extract provocation in children monosensitized to Can f 5 and in children with a mixed sensitization pattern.

The study is strengthened by the fact that only 2 investigators from the COPSAC research unit were examining the children, ensuring the use of a consistent, standardized approach to examinations and diagnoses. Furthermore, this is the first time separate male and female dog extracts have been used in a clinical trial. Another strength is that both *in vitro* and *in vivo* tests including sIgE, SPT, and CAP tests were performed and evaluated in all patients according to current guidelines.[21]

The CAP test is of great importance in allergy diagnostics, because it directly shows the clinical relevance of the tested allergen for both the patient and the physician. However, the use of the CAP test also has its limitations, because the evaluation of chemosis requires a slit-lamp examination, which we did not perform.

The study is limited by the low numbers in each category of molecular sensitization pattern (Can f 5 monosensitization and mixed sensitization pattern). However, we assumed that all the children monosensitized to Can f 5 would react to the male SPT extract (99%) and very few (10%) would react to the female SPT extract. With these presumptions, we only needed 6 children monosensitized to Can f 5 to have a power of 80% to show a significantly different reaction pattern to male dog extract and female dog extract provocation at an alpha level of 5%. The power calculation is added to the Online Repository at www.jaci-inpractice.org. Another limitation is the missing examination of sensitization to 2 of the minor dog components, Can f 4 and Can f 6, which have recently been demonstrated as markers for clinically relevant dog allergy.[7] These components were not commercially available at the time the study was conducted, but are available for analysis today.

Interpretation

It has previously been reported that between 30% and 60% of patients allergic to dog are monosensitized to the Can f 5 allergen component.[5,16] These patients can be distinguished by using molecular allergy diagnostics or by using SPT extracts made exclusively from male or female dogs. Previously, there have been no studies investigating the reactions to male and female dogs in this subgroup of patients. We found that children monosensitized to the dog allergen Can f 5 showed different reactions to male and female dog extract provocation, suggesting tolerance to female dogs, which may be of importance for guiding the many dog-sensitized patients worldwide. Previous studies of the Can f 5 allergen have primarily focused on its cross-reactivity with human seminal plasma,[24-27] focusing on the rare cases of suspected semen allergy. More attention has recently been drawn to the perspective of a potential male-dog-only allergen, which we recently confirmed in a case report, showing that a patient with dog allergy monosensitized to Can f 5 was allergic only to male dogs.[6]

Other studies have demonstrated conflicting results when looking at populations monosensitized to Can f 5 and the association to clinical symptoms. Some have found negative nasal challenge results to dog dander extract in Can f 5 monosensitized children[7] and no relation to clinical symptoms,[28] whereas others found a strong association between severe, persistent rhinitis and the presence of elevated IgE to Can f 5, but did not specifically study subjects with Can f 5 monosensitization.[29,30] Furthermore, a strong independent relationship between the presence of IgE antibodies toward Can f 5 and asthma has been reported, but the study did not explore this relationship in the context of monosensitization.[31]

The commercially available dog dander extracts used for SPT and CAP test consist of a mixture derived from both male and female dogs of different breeds. In addition, the percentage of male and female dog content in these extracts may vary, and extracts from different batches can therefore give contradicting results in the same patient depending on their molecular allergen profile. This, together with the lack of attention to the sex of the dog, can explain the divergent results of the previous studies.

One of the children in our study, who we found to be monosensitized to Can f 5, showed reaction to the female dog extract using both SPT and CAP test, which is most likely because the child is sensitized to Can f 4 or 6, and therefore not truly Can f 5 monosensitized. To prove this hypothesis, we drew an extra blood sample from this child and measured sensitization to Can f 4 and Can f 6, which have become commercially available today. We found that he was in fact sensitized to Can f 4 (0.51 kU_A/L) and Can f 6 (10.5 kU_A/L), which means he is not monosensitized to Can f 5, and explains why he also reacted to the female dog extract. This was a post hoc analysis, and unfortunately not possible to carry out in all the participants. Figures 1 and 2 have been reconstructed not including this child and are available as Figures E2 and E3 in this article's Online Repository at www.jaci-inpractice.org.

Our study shows that patients allergic to dog who are truly monosensitized to Can f 5 are allergic only to male dogs, with no reaction to either SPT or CAP test using female dog extract and thereby not allergic to female dogs. Such precision medicine initiative in clinical allergy practice can pave the path for up to 60% of dog-allergic persons to get a female dog in their household.

To assess the diagnostic test performance of Can f 5, there is a need for studies in larger populations of children presenting for evaluation of dog allergy regardless of their dog sensitization history.

CONCLUSIONS

This double-blind, randomized controlled trial shows, for the first time, that children who are monosensitized to the dog allergen Can f 5 have tolerance to female dogs. We suggest that Can f 5 monosensitization and potentially male-dog-allergy only is first truly established when all the dog components have been explored (Can f 1-6[32]). This allows more precise diagnoses of allergy to dog.

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ONLINE REPOSITORY

METHODS

We made a power calculation on the basis of the following assumptions regarding the SPT results (dichotomous):

- Incidence of positive male SPT results in the children monosensitized to Can f 5: 99%.
- Incidence of positive female SPT results in the children monosensitized to Can f 5: 10%.
- Alpha: 0.05.
- Power: 80%.

Sample size: 6 children (mono Can f 5).

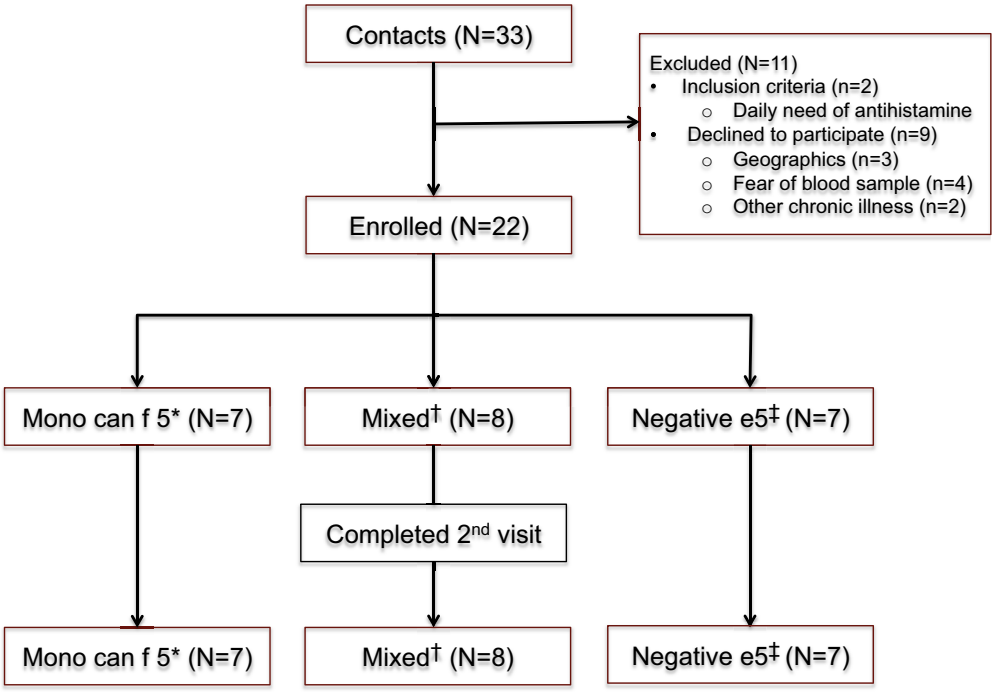

FIGURE E1. Consort diagram. *Children sensitized only, that is, monosensitized, to Can f 5 of the dog components. †Children sensitized to a mix of the dog components. ‡Children with a negative sIgE ($\leq$0.35 kU_A/L) to the dog extract e5.

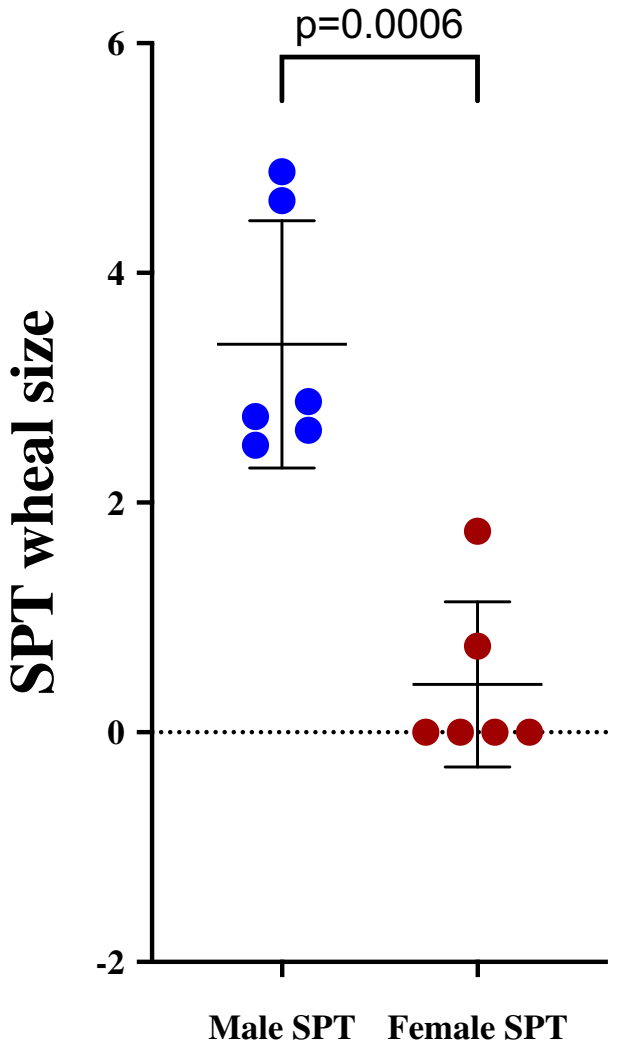

FIGURE E2. Post hoc analysis comparing reactions to male dog SPT and female dog SPT allergen extracts. Mono Can f 5 refers to the children who were sensitized only, that is, monosensitized, to Can f 5 of the dog components. The *P* value is from a *t* test.

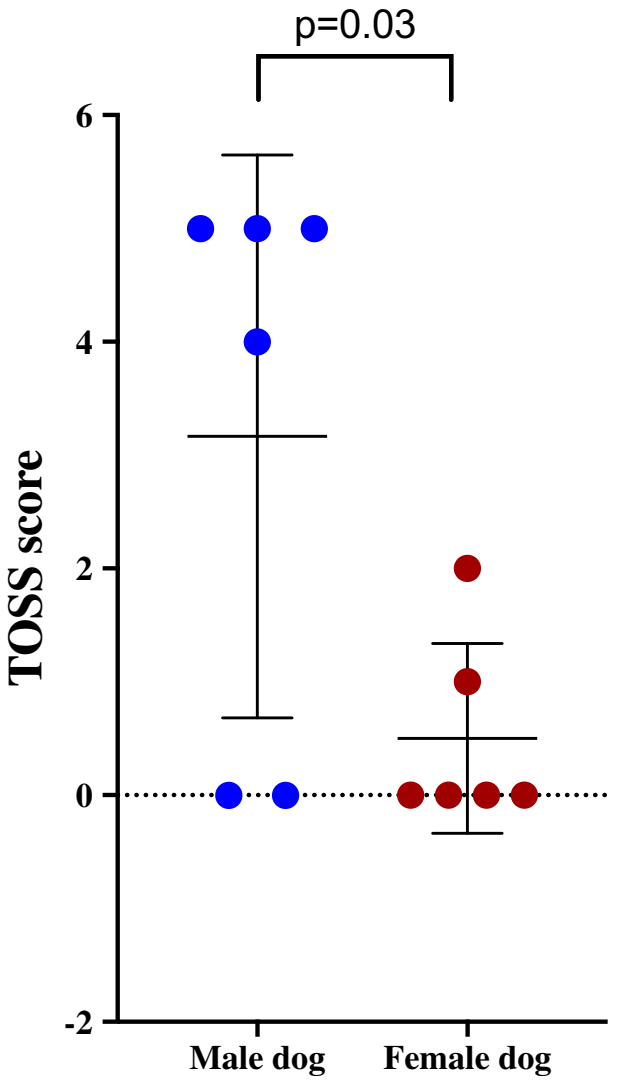

FIGURE E3. Post hoc analysis comparing the CAP test results to male dog allergen extracts and female dog allergen extracts. A TOSS of 4 was considered a positive response. Mono Can f 5 refers to the children who were sensitized only, that is, monosensitized, to Can f 5 of the dog components. The *P* value is from a *t* test.