

Allergenicity of the cat flea (*Ctenocephalides felis felis*)

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Summary

Adult fleas, spent and unspent culture media were extracted and the radio-allergosorbent test (RAST) performed with sera of 48 cat flea skin test-positive individuals from the Tampa Bay area of Florida. Sixteen sera (33.6%) had a positive RAST to the cat flea extract prepared in our laboratory [1.7–11.4% of the total counts (TC) added]. Six of the 16 sera (12.5%) also contained specific IgE to allergens in the spent medium (0.8–3.3% TC). The allergen composition and strength were studied by RAST inhibition of two commercial cat flea extracts and compared with our in-house flea extract. The results demonstrated similar allergen compositions and different potencies. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of the in-house flea extract showed several Coomassie blue-stained bands (10–85 kD). SDS-PAGE immunoblots revealed five IgE-binding bands at 34, 35, 39, 54 and 60 kD. Flea allergens were quantified in eight house dust samples using RAST inhibition assays and expressed as RAST inhibition units; five of these samples contained detectable levels. Cat flea allergens may contribute to the allergenicity of house dust in areas of heavy flea infestation.

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Introduction

Fleas evolved at least 60 million years ago as specialized blood-sucking parasites. There are at least 2400 known species but fewer than 20 bite humans. Significant immunological crossreactivity has been demonstrated among flea species in sensitized guinea-pigs [1]. Fleas pierce the skin with a needle-like proboscis and feed directly from a capillary or a pool of extravasated blood [2]. They inject oral secretions which have anticoagulant, anaesthetic and antigenic activities. Papular urticaria to flea bites has been well documented [3] and is associated with two components of the oral secretions of the cat flea; component A has a molecular weight between 4 and 10 kD and component B is a fluorescent, aromatic compound with a molecular weight of less than 1 kD [4–10]. Attempts to desensitize humans to cat flea allergens have had variable success [11–18]. Specific IgE to flea antigens

has previously been detected in the serum of patients with suspected cat allergy, the majority of which bound IgE to a 30 kD component of the cat flea extract [19]. Rolfsen *et al.* [20] demonstrated by the radio-allergosorbent test (RAST), RAST inhibition and immunoblots that cat fleas are sources of important allergens and that sensitivity to cat flea could be masked by sensitivity to cat allergens. In their study, 9% of 70 patients with suspected cat allergy had specific serum IgE to cat flea but not to cat allergens. The specificity of IgE to cat flea allergens was also demonstrated by RAST inhibition. Positive skin test reactions to cat flea allergens were detected in 18% of patients evaluated for respiratory allergies in Tampa, Florida [20]. Some house dust samples collected in Tampa Bay area homes for identification and counting of house dust mites contained, along with a rich house dust mite fauna, live and dead fleas as well as larvae and eggs [21].

The purposes of this study were to measure specific IgE to cat flea in cat flea skin test-positive subjects, to examine the allergenic composition of two commercial cat flea extracts and our in-house extract by sodium dodecyl

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sulfate polyacrylamide gel electrophoresis (SDS-PAGE), immunoblots and RAST inhibition and to quantitate cat flea allergens in house dust samples.

Materials and methods

Skin testing and serum source

Skin tests, epicutaneous and intradermal, of atopic and non-atopic individuals from the Tampa Bay area were performed with a commercial cat flea extract (Greer Laboratories, Lenoir, NC, U.S.A.). This extract was prepared as a 1:100 w/v solution for prick skin testing and was further diluted to 1:500 w/v for intradermal skin testing. Reactions with a weal ≥ 3 mm in diameter and erythema ≥ 20 mm in diameter (2+ epicutaneous) or weal ≥ 8 mm in diameter (3+ intradermal) were considered positive.

Blood samples were obtained from the 48 cat flea skin test-positive individuals, from 198 cat flea skin test-negative individuals (skin test positive to other allergens) and 100 skin test-negative, non-atopic individuals.

Extraction of allergens

The in-house cat flea, spent medium (SM) and unspent medium (UM) extracts were provided by the U.S.D.A. (Gainesville, FL, U.S.A.). Immature fleas, harvested from cats with a hand-held vacuum cleaner, were cultured in sand, rodent chow and blood meal in a glass jar on an 18-inch high platform. Adult fleas were harvested from the bottom of the jar and were never exposed to cats. One and a half grams of cat fleas, SM and UM were extracted in 6 ml of a 0.2 M ammonium bicarbonate solution, pH 7.8. The supernatants were centrifuged at 10 000 *g*, dialysed against two changes of 5 l of distilled water, recentrifuged and preserved in 50% glycerin, yielding a 1:10 w/v extract.

Protein determinations

Protein concentrations were determined using the BCA protein assay kit (Pierce, Rockford, IL, U.S.A.) and fraction V bovine serum albumin (Pierce) as the standard [22].

RAST

In-house cat flea, SM and UM antigens were adsorbed to the surface of Immulon-4 microtitre Removawell strips (Dynatech Laboratories Inc., Alexandria, VA, U.S.A.) by adding 2 μ g of protein diluted in 100 μ l of 200 mM sodium bicarbonate buffer (pH 9.2) and incubated overnight at room temperature. The strips were then washed

three times with RAST wash (0.1 M potassium phosphate buffer, pH 7.4 and 0.01% Tween 20), incubated overnight with 100 μ l of sera, washed, incubated overnight with 100 μ l of ^{125} I-labelled anti-human IgE, diluted 1:2 (Pharmacia Diagnostic Division, Fairfield, NJ, U.S.A.), washed and individual wells counted for 1 min in a gamma counter. A RAST was considered positive when IgE binding was ≥ 4 times the negative control sera.

RAST inhibition

Crossreactivity between the in-house cat flea extract and SM, UM, commercial flea, cat, dog, cockroach and mite extracts was determined by comparing the ability of each extract to inhibit the binding of human anti-flea-specific IgE to the antigens on the solid phase. Plastic microtitre plates were coated with flea antigens as described above, washed three times in RAST wash and the inhibition assays conducted by placing 50 μ l aliquots of various concentrations of each extract in each well along with 50 μ l of the human flea RAST-positive serum. After incubation, the wells were washed with RAST wash. One hundred microlitres of radiolabelled anti-human IgE were added to each well. The plates were incubated, washed and each microtitre well counted in a gamma counter. The results were analysed by regression analysis and expressed as per cent inhibition.

Dust samples were collected for 2 min from a surface area of 2 m³ with a modified vacuum cleaner that incorporated a dust trap filter unit. The dust samples were extracted 1:20 w/v in 0.2 M ammonium bicarbonate and preserved in glycerin. Flea antigens in house dust extracts were quantitated by RAST inhibition. Fifty microlitres of each house dust extract were added to the allergen-adsorbed wells along with 50 μ l of flea RAST-positive serum pool. A standard curve was generated by adding serial dilutions of the in-house cat flea extract. The remainder of the assay was performed as for the RAST inhibition assays described above.

SDS-PAGE gels and immunoblots

SDS-PAGE was performed according to the method of Laemmli [23] in a vertical slab gel apparatus (Pharmacia LKB Biotechnology, Piscataway, NJ, U.S.A.). A 5–20% linear acrylamide gradient gel or 10% gel was used, incorporating 2.7% cross-linker, and 100 μ g of each sample was loaded per lane. Gels were electrophoresed at 30 mA/gel for approximately 4.5 hr. Following electrophoresis, a portion of the gel was removed for Coomassie blue staining and the remainder was cut into strips corresponding to the sample lanes and electroblotted to 0.1 μ m nitrocellulose (Sartorius Corp., Long Island,

Table 1. Skin test results to common aeroallergens in 48 cat flea skin test-positive individuals

Allergen	No. positive (%)
Flea	48 (100)
Dog	15 (31)
Cat	23 (48)
American cockroach	36 (75)
<i>Dermatophagoides pteronyssinus</i>	43 (90)
<i>Dermatophagoides farinae</i>	40 (83)
Trees	33 (69)
Grasses	31 (65)
Weeds	31 (65)
Moulds	24 (50)

NY, U.S.A.) using the continuous buffer system (48 mM Tris, 39 mM glycine, 0.0375% SDS, 20% methanol) and 0.8 mA/cm² for 90 min [24]. The efficiency of transfer from the blotted strips was determined by Coomassie blue staining. Molecular weights of the electrophoresed and the electroblotted proteins were determined using molecular weight markers (Bio-Rad Rockville Center, NY, U.S.A.) after staining with Amido Black and India ink, respectively. The nitrocellulose sheets containing the blotted samples were blocked for 4 hr with phosphate-buffered saline (PBS), 0.01% Tween 20 and 3% bovine serum albumin. The sheets were washed five times with PBS-Tween, cut into strips corresponding to the sample lanes and incubated, with gentle agitation, in cat flea RAST-positive sera or negative control sera diluted 1:3 in PBS-Tween. The strips were then washed, incubated overnight with 100 µl of ¹²⁵I-labelled anti-human IgE, washed, dried, mounted and autoradiographed for 72 hr with X-Omat AR film (Eastman Kodak, Rochester, NY, U.S.A.).

Native gels and immunoblotting

Native gels were prepared as above except that SDS was omitted from the tank buffer, electroblotting buffer and the sample treatment buffer. β-mercaptoethanol was also omitted from the treatment buffer and the samples were not boiled prior to loading.

Results

Protein concentrations in flea extracts

The in-house cat flea extract contained 2.25 mg/ml protein, the SM, 1.6 mg/ml and the UM, 2.5 mg/ml; the Greer extract (1:100 w/v) contained 2.1 mg/ml and the ALO extract (1:40 w/v), 1.6 mg/ml.

Table 2. Diagnosis, skin test and radio-allergosorbent test (RAST) results in 16 cat flea RAST-positive individuals

Serum no.	ST	Diagnosis	%TC cat flea	%TC SM
250	4+	R&C	11.4	0.1
208	4+	R&C	9.1	0.1
195	3+	R&C	7.3	1.7
10	4+	R&C	5.4	0.1
166	2+	A	3.8	0.8
207	4+	AR&C	3.2	0.1
9	4+	AR&C	2.8	0.1
107	4+	R&C	2.8	0.2
79	3+	R&C	2.8	1.2
140	4+	R&C	2.7	2.9
84	4+	R	2.6	0.3
76	3+	AR&C	2.4	3.3
3	4+	R&C	2.4	0.1
80	3+	AR&C	2.3	2.4
73	3+	R&C	1.7	0.1
19	4+	R&C	1.7	0.1

R&C, Rhinoconjunctivitis; A, asthma; R, rhinitis; AR&C, allergic rhinitis and conjunctivitis; TC, total count; SM, spent medium; ST, skin test.

Skin tests and RAST results

Serum was collected from 48 individuals with a positive skin test reaction to the Greer commercial cat flea extract. These individuals were also skin tested with a battery of common aeroallergens. All exhibited at least one positive skin test reaction in addition to cat flea (Table 1).

Sixteen of the 48 cat flea skin test-positive sera contained measurable specific IgE to cat flea allergens in the range 1.7–11.4% total count (TC). Six of these 16 individuals also bound IgE to SM in the range 0.8–3.3% TC (Table 2). One serum, used to compare RAST binding to the in-house, ALO and Greer cat flea extracts, bound 11.4%, 4.7% and 3.3% TC, respectively. Radio-allergo-sorbent tests with negative atopic and non-atopic sera did not bind IgE to the in-house cat flea, SM or UM extracts.

RAST inhibition and crossreactivity studies

Three different sera were used in the RAST inhibition assays. The potency and allergen composition of each flea extract were studied by RAST inhibition using the in-house cat flea extract in the solid phase. When the in-house cat flea extract was self-inhibited with 125–2250 ng/50 µl, the inhibition ranged from 20% to 90%. When inhibited with 80–1600 ng/50 µl of the ALO extract, the inhibition ranged from 22% to 78%. When inhibited with 105–2100 ng/50 µl of the Greer extract the inhibition

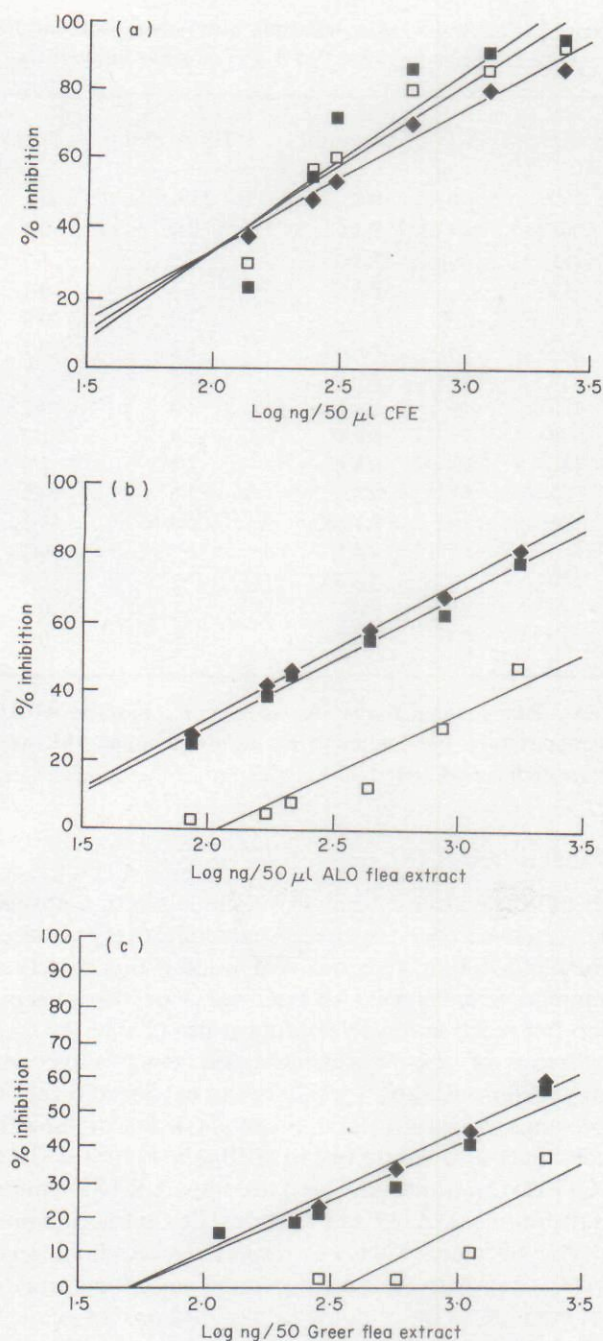


Fig. 1. Radio-allergosorbent test inhibition assays with the in-house cat flea extract (CFE) adsorbed to the solid phase. (a) Homologous inhibition; (b) inhibition performed with the ALO extract; (c) inhibition performed with the Greer extract. □, Serum 107; ◆, serum 195; ■, serum 208.

ranged from 13% to 56%. Spent medium, UM, cat, dog, cockroach and mite did not inhibit IgE binding to CFE adsorbed to the solid phase.

A similar allergen composition in the three cat flea extracts was indicated by the similarity of the slopes of the

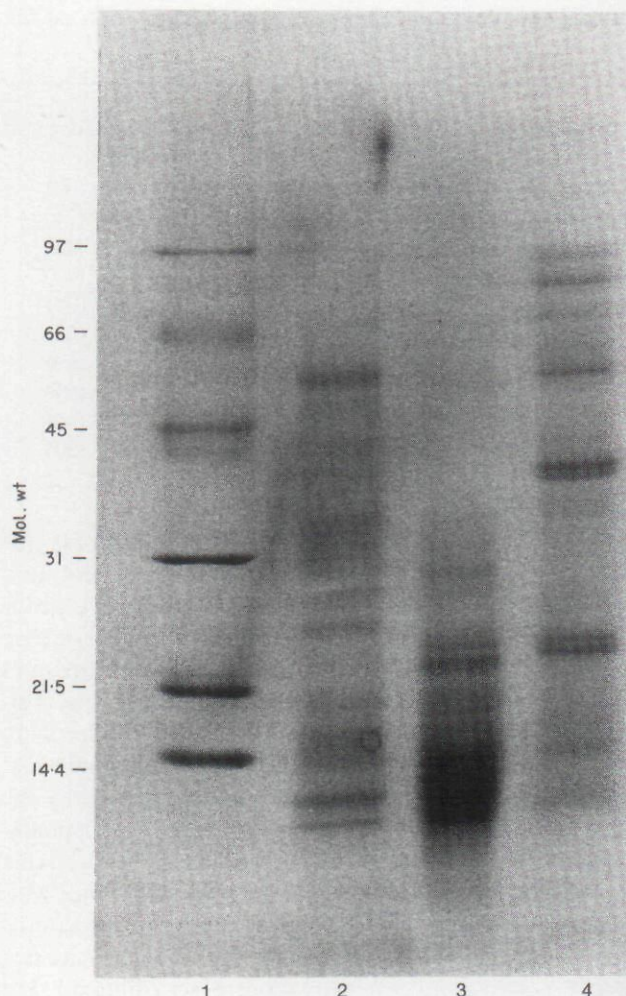


Fig. 2. Coomassie blue-stained SDS-PAGE of in-house extracts. Lane 1, Molecular weight standards; 2, in-house flea extract; 3, spent medium; 4, unspent medium.

inhibition curves generated by the three different sera (Fig. 1). Serum 107 recognized less allergenic determinants in the two commercial cat flea extracts than in the in-house preparation.

SDS-PAGE and immunoblots

SDS-PAGE of the in-house flea extract, SM and UM resulted in the separation of several proteins. The in-house cat flea extract showed bands in the range 85–10 kD, SM in the range 70–11 kD and UM in the range 91–15 kD (Fig. 2). Proteins in the commercial extracts migrated in the molecular weight range 14–90 kD. The Greer extract migrated as a low intensity, low molecular weight diffuse staining area (Fig. 3).

Autoradiography of electrophoresed and electroblotted in-house cat flea proteins was performed with five cat

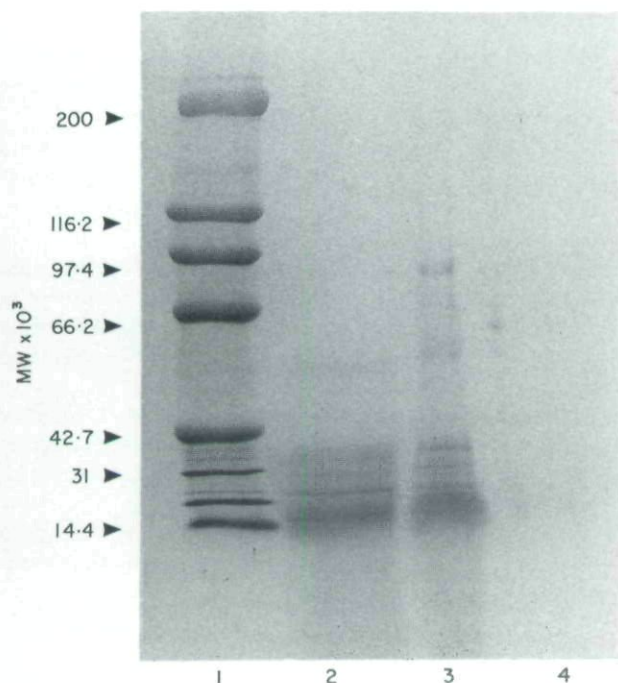
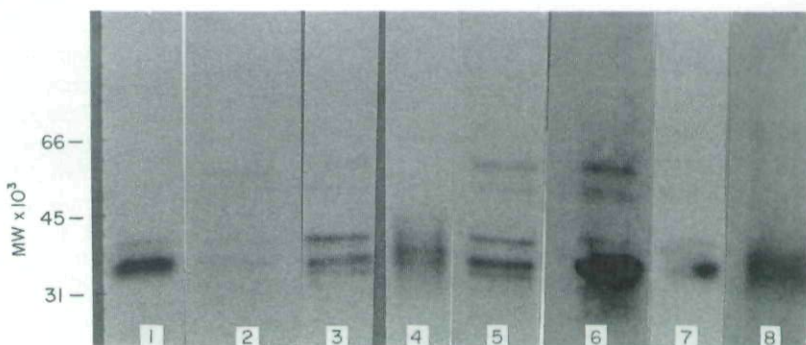


Fig. 3. Coomassie blue-stained SDS-PAGE of in-house and commercial flea extracts. Lane 1, Molecular weight standards; 2, in-house flea extract; 3, ALO flea extract; 4, Greer flea extract.

flea skin test- and RAST-positive sera and a pool of these sera. The five sera bound IgE to one or more bands at 34, 35, 39, 54 and 60 kD (Fig. 4).

Autoradiography of the SM extract with three SM RAST-positive sera and one SM RAST-positive serum pool resulted in IgE binding to four bands with molecular weights of 30, 45, 52 and 65 kD (Fig. 5). Autoradiography of the UM revealed no IgE binding. Incubation of the in-house cat flea, SM and UM immunoblots with atopic (negative skin test to cat flea) and non-atopic control sera did not show IgE binding bands on the autoradiographs.

Fig. 4. Autoradiograph of sodium dodecyl sulfate and native polyacrylamide gel electrophoresis immunoblots of in-house flea extract with cat flea skin test- and radio-allergosorbent test-positive sera. Lane 1, serum 107; 2, serum 10; 3, serum 250; 4, serum 107, native gel; 5, serum 97; 6, serum 195; 7, serum pool; 8, serum pool, native gel.



Quantitation of flea allergen in dust samples

Eight dust samples were analysed by RAST inhibition for their flea allergen content. Flea allergens were detected in five samples with a concentration range of 0.74–160 µg/g dust.

Discussion

Florida homes with domestic pets are periodically infested with large numbers of adults, larvae, pupae and eggs of the cat flea, *Ctenocephalides felis felis*. In a previous study we established the prevalence of positive skin tests to cat flea allergens among allergic individuals in the Tampa Bay area. Eighteen per cent of 100 consecutive patients evaluated for upper and lower allergic respiratory complaints had a positive skin test to a commercial cat flea extract [20]. In the present study, RAST inhibition assays demonstrated a similar allergenic composition in the three cat flea extracts studied and differences in their relative potency. The allergenicity of the SM extract was also examined. In-house flea extract and SM were not crossreactive by RAST inhibition, a finding suggesting that different antigenic proteins are expressed by the cat flea at different stages in its life cycle. This is supported by the observation that IgE binding to SM immunoblots differed significantly from binding to the in-house cat flea immunoblots. IgE binding bands to SM were detected at 29 and 65 kD while IgE binding to the in-house cat flea extract was detected at different molecular weights (immunoblots revealed most of the IgE binding directed to proteins in the molecular weight range 30–60 kD). Four intensely staining IgE bands were observed at 34, 35, 39 and 60 kD. Utilization of native gels changed this IgE binding pattern by failure to bind to the 54 and 60 kD bands. The lower molecular weight antigenic bands are monomeric. Similar IgE binding patterns have been observed with other insect species [25].

Components in the Greer flea extract failed to resolve clearly in SDS-PAGE gels. However, enough allergenic activity was present in this extract to identify flea skin test-

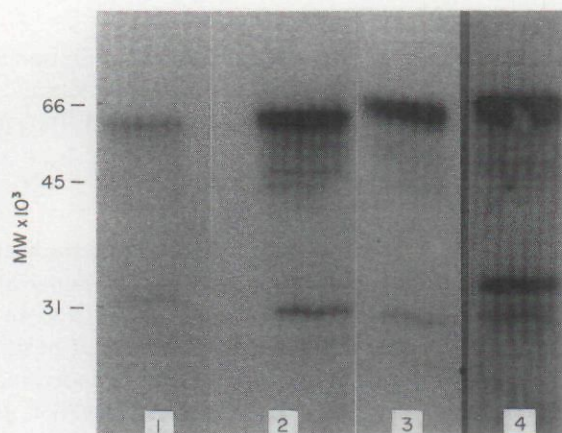


Fig. 5. Autoradiograph of SDS-PAGE gel of spent medium with SM radio-allergosorbent test-positive sera. Lane 1, serum 10; 2, serum 97; 3, serum 107; 4, serum pool.

positive subjects. It also showed a faint IgE binding band at approximately 34 kD, common to the other extracts. Preliminary studies using azocoll, a non-specific protease substrate, suggest that there is strong proteolytic activity in flea extract which could explain the poor electrophoretic resolution of some extracts (unpublished data).

The nature of the antigens associated with the cat flea and SM extracts remains to be characterized, but they are distinct from the salivary antigens described by previous workers. The present study failed to detect, with human serum and anti-human IgE, the two antigenic components of the oral secretions of the cat flea. Cat flea and SM allergens appear to be distinct from salivary antigens which suggests a mode of sensitization other than by bite, resembling sensitization to cockroach and other insects. Flea allergens are house dust constituents and may become airborne causing sensitization and then evoke symptoms in sensitized individuals. This is supported by the detection of flea allergens in house dust samples in variable quantities. Clinical manifestations of severe papular urticaria from flea bites were identified in some of the individuals with specific IgE to cat flea antigens, suggesting that these allergens may also be implicated in the pathogenesis of dermatological manifestations associated with flea bites.

We conclude that cat fleas and flea debris contribute to the complex allergenicity of house dust and that cat flea sensitivity deserves to be evaluated in areas of heavy flea infestation.

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