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# Prevalence of allergic sensitization to imported fire ants in children living in an endemic region of the southeastern United States

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**Background:** Imported fire ant (IFA) stings are common in children in the southeastern United States, but little is known about antibody responses to stings.

**Objectives:** To examine the prevalence of detectable IgE and IgG antibodies to IFA venom (IFAV) and to assess the frequency of IFA stings in children in an endemic region.

**Methods:** We measured IFAV specific IgE using the ImmunoCAP assay and IgG anti-IFAV using protein G solid-phase radioimmunoassay in 183 serum samples from children living in the southeastern United States. A questionnaire was used to collect information about insect stings and bites from 182 children in a general pediatric clinic.

**Results:** Serum IgE anti-IFAV was detected (0.1 kUa/L) in 7.1% of 0- to 1-year-olds, 57.1% of 2- to 5-year-olds, and 64.4% of 6- to 10-year-olds. The prevalence of IgG anti-IFAV antibodies increased from 11.9% in 0- to 1-year-olds to 97.5% in 11- to 20-year-olds. Of children in the pediatric clinic, almost 40% had been stung by an IFA in the past month, with 23.9% receiving more than 6 stings per month.

**Conclusions:** In endemic areas, the onset of sensitization (IgE positivity) to IFAV occurs in the first years of life, with more than half of the children demonstrating sensitization by 2 to 5 years of age. Exposure to IFAV as evidenced by a positive IgG anti-IFAV antibody increased with age, and by the second decade, more than 97% of children had detectable antibody. Children living in an endemic region frequently encounter IFAs and, when stung, often receive multiple stings.

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## INTRODUCTION

Recent estimates suggest that imported fire ants (IFAs) infest nearly 310 million acres in the southeastern United States. *Solenopsis invicta*, the red fire ant, is rapidly becoming the dominant ant species in this area. Recent reports of cross-breeding with local ant species has led to the fear that IFAs may expand their territory to include previously unaffected regions of the country, possibly to include more than 25% of the southern United States.<sup>1</sup> As the habitat of IFAs expands, the number of individuals exposed to IFA venom (IFAV) is expected to rise. It is, therefore, critical to gain a better understanding of exposure patterns and the extent of allergic sensitization to IFA so that allergists practicing in endemic areas can more accurately diagnose and effectively manage individuals with severe reactions after IFA stings.

An estimated 30% of individuals living in an IFA endemic region are stung by IFAs annually.<sup>2</sup> The most frequent encounters with IFAs occur in children during the summertime as they play outdoors.<sup>1</sup> Although much less frequent, stings

can also occur indoors, particularly during periods of weather extremes, when fire ants are forced indoors to find sources of food and water.<sup>3</sup>

Detectable IFAV specific IgE antibody is common in adults living in an IFA endemic region. Caplan et al<sup>4</sup> demonstrated that 17% of adults living in Augusta, Georgia, were sensitized to IFAs compared with 2% of the population living in a nonendemic region. In a pediatric population, 48% of children 7 to 18 years of age living in this same area were sensitized to IFAV.<sup>5</sup> However, the rate at which sensitization develops in young children living in IFA endemic regions has remained unexplored.

Given similar frequent exposures, we hypothesized that the specific antibody responses in children with natural exposure to IFAV would be similar to the response described by Peng et al<sup>6</sup> in children with repeated natural exposures to mosquito saliva. Peng et al observed that the most severe reactions to mosquito bites occurred in very young children and across time; with repeated bites, the incidence of serious reactions declined. When they examined mosquito saliva specific IgE and IgG levels in children exposed to mosquito bites, they observed an inverse correlation between age and mosquito saliva specific IgE and IgG levels. Mosquito saliva specific IgE and IgG levels peaked in the first year of life and then steadily declined throughout childhood, which they believed may represent “natural desensitization” to allergenic proteins in mosquito saliva.<sup>6</sup>

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We performed a 2-part study in different groups of children living in an IFA endemic region. To evaluate the antibody responses resulting from ongoing natural exposure to IFAV, we conducted a cross-sectional, blinded survey of serum samples obtained from children living in an IFA endemic region who are presumably receiving accidental, natural IFA stings. The aims of this part of the study were to evaluate the prevalence of sensitization to IFAV, defined by detectable IFAV specific IgE antibodies, and to examine the relative changes in the prevalence of IFAV specific IgE and IgG antibodies with age in these children. In the second part of the study, we used a questionnaire to collect information regarding the frequency of insect stings and bites, the types of insect most often identified as causing the bites or stings, and the severity of sting reactions in a second group of children residing in an IFA endemic area.

## METHODS

The Human Assurance Committee of the Medical College of Georgia approved all aspects of both parts of this study.

### *Antibody Response*

We conducted a blinded survey of discarded serum samples from individuals from birth to 20 years of age from a tertiary care center immunology laboratory located in an IFA endemic region. This laboratory does not perform specific IgE testing. The serum samples represented a convenience sample of the local pediatric population. The collection period was approximately September 2005 to March 2006. Owing to the limitations of the human rights approval, the only information about the serum donors were the age and sex of the child. Data on race were not available. Only 1 serum sample per child was accepted. After being obtained, the samples were stored at  $-20^{\circ}\text{C}$  until assayed.

The level of IgE anti-IFAV was measured using the UniCAP-100 instrument (Phadia Inc, Portage, Michigan) per the manufacturer's protocol. In a preliminary work<sup>5</sup> comparing the commercial whole-body extract IFA-ImmunoCAPs with Phadia custom-made, purified *S invicta* venom, ImmunoCAPs suggested better sensitivity using the IFAV-CAPs. Based on these preliminary findings, all IgE assays in this study were performed using the IFAV-CAPs. Phadia has recently released new assay software that allows calculation of the quantity of allergen specific IgE antibodies to a concentration of 0.1 kUa/L instead of the older cutoff value for detectable IgE of 0.35 kUa/L. Where appropriate, prevalence data using both cutoff values are shown.

The level of IgG anti-IFAV was measured using a protein G solid-phase radioimmunoassay (SPRIA), with purified *S invicta* venom as the allergosorbent. The IgG anti-IFAV SPRIA was developed and standardized as previously described for the detection of Hymenoptera venom specific IgG antibody except that protein G replaces the protein A detection protein.<sup>7,8</sup> The SPRIA is a noncompetitive radioimmunoassay used to detect IgG antibodies. Briefly, solid-phase antigen (purified *S invicta* venom) binds IFAV specific anti-

body, and, after a buffer wash, radiolabeled streptococcal protein G detects bound IgG antibody.<sup>7</sup> Levels of IgG anti-IFAV greater than 0.1  $\mu\text{g/mL}$  were considered positive.

### *Frequency of Stings*

Parents of children 1 to 18 years of age who were seen in general and adolescent pediatric clinics at the same tertiary care center from which the serum samples were obtained were asked by one of us (W.B.) to participate in an anonymous questionnaire. Although the parents were primarily asked the questions on the survey, the children may have been present during the interview and may have provided information. Parents who agreed to participate answered questions about their child's number and frequency of insect stings or bites in the previous month and during the past summer, the type of insects involved in the stings, and the severity of insect sting reactions. Because most parents and their children could not give precise numbers, their best estimates were grouped into 5 categories: 0, 1 to 5, 6 to 10, 11 to 20, and more than 20 stings or bites. The survey was performed during September and October. Information was collected on 182 patients. While collecting this information, the common term *ant bites* was assumed to mean the result of the combination of an ant biting and then stinging. We will henceforth only refer to these as ant stings in contrast to mosquito bites.

### *Statistical Analysis*

For this analysis, children in both portions of the study were placed into 1 of 4 age groups. The age groups were arbitrary but were based on assumptions about the frequency of various types of outdoor activities and the ability of children to avoid IFA contact at different ages. Group 1 consisted of children younger than 1 year, assuming that few children younger than 1 year would have much outdoor exposure on the ground. Group 2 consisted of 2- to 5-year-olds; group 3, 6- to 10-year-olds; and group 4, 11- to 20-year-olds. These groupings meant that there were no children in group 1 among those questioned about bites or stings. Because of the limited number of participants in each study group and the nonnormal distribution of the data, descriptive statistics, other than age, are given as medians. For the same reasons, all comparisons between groups were performed using nonparametric statistics and a software program (SigmaStat 3.5; Systat Software, Richmond, California). The Kruskal-Wallis test was used for comparing multiple groups, and the Dunn test for 2-way comparisons was used when the multiple-group comparisons were significant. Correlations were computed using the nonparametric Spearman rank order statistic. Statistical significance was assumed to be  $P < .05$ , and because we had determined the comparisons of interest a priori, no corrections were made for multiple tests.

## RESULTS

Serum samples for antibody testing were collected from 183 individuals (89 girls and 94 boys) ranging in age from 6 days to 20 years. Ninety children had detectable IgE anti-IFAV

(>0.1 kUa/L), and 113 had detectable IgG anti-IFAV. Table 1 gives the number of children in each of the 4 age groups, the prevalence of IgE anti-IFAV (using both the 0.1- and the 0.35-kUa/L cutoff values), and the prevalence of IgG anti-IFAV. Using either cutoff value, the prevalence of detectable IFAV IgE rapidly increased after 1 year of age. Using the 0.1-kUa/L value, the prevalence of sensitization was only 7.1% in group 1 and increased with age to 65.0% in group 4. Compared with IgE, the prevalence of IFAV specific IgG antibodies was greater in each age group, reaching 97.5% in 11- to 20-year-olds (group 4). In the entire group of 183 individuals, the presence of IFAV specific IgE and IgG antibodies was significantly correlated ( $r = 0.66$ ;  $P < .001$ ). The median values of IgE anti-IFAV and IgG anti-IFAV measured in the sera, as a function of the age groups, are presented in Figure 1. There were significant differences in the median values among the 4 age groups for IgE anti-IFAV and IgG anti-IFAV. The median values for IgE anti-IFAV were significantly different because of significant differences between group 1 and groups 3 and 4 ( $P < .001$ ). The median values for IgG anti-IFAV were significantly different between group 1 and groups 2, 3, and 4 ( $P < .001$ ). There were no significant differences when the prevalence of positive anti-IFAV IgE and IgG were compared between boys and girls for each age group (data not shown). Even at young ages, some children produced high levels of IFAV specific IgE. For example, the highest concentrations of IgE antibody measured in the different age groups were 4.04 kUa/L (<1 year), 189 kUa/L (1–2 years), 169 kUa/L (2–3 years), 46.7 kUa/L (3–4 years), and 9.12 kUa/L (4–5 years).

Questionnaire information about stings and bites was collected from 182 children (84 boys and 98 girls), with 89 children living in the city, 47 in suburban areas, and 46 in rural areas. Sixty-nine of the children were aged 1 to 5 years, 66 were aged 6 to 10 years, 33 were aged 11 to 15 years, and 14 were aged 16 to 18 years. As seen in Table 2, IFA stings were reported for 38.6% of the children during the previous month, with more than 6 stings reported for 23.9% of the children. Of the stings that occurred during the past month, 92.1% ( $n = 163$ ) were due to IFAs and 7.9% ( $n = 14$ ) were due to bees, hornets, or wasps. In comparison, mosquito and

other insect bites were reported for 97.3% of children in the previous month. Note that the reported number of IFA stings in Table 2 may not correspond with the total number of stings because some IFA stings may have been reported as bites. Children in rural areas had significantly more stings reported in the previous month than those living in other settings (Table 3). Only 6 children (3.3%) reported having been taken to a physician for treatment of a reaction after a sting, and only 4 of these 6 reactions (2.2%) were described as being severe. All 4 of the children with severe reactions to a sting or bite were reported to have been seen by an allergist. The insects responsible for the most severe sting reactions during the past summer are as follows: mosquitos in 112 children (62.6%), IFAs in 58 (32.4%), bees in 8 (4.5%), wasps in 1 (0.6%), and hornets in 0. Symptoms described in severe reactions were local swelling in 151 children, a large local reaction in 1, hives at the site in 5, generalized hives in 1, and breathing difficulties in 0.

## DISCUSSION

This is the first study, to our knowledge, that examines the prevalence of allergic sensitization to IFAV and the age at which sensitization develops in children living in the southeastern United States, where IFAs are endemic. We were surprised to find the high prevalence of sensitization to IFAV beginning at such early ages. These findings are in agreement with the previous studies by Caplan et al<sup>5</sup> showing the prevalence of IFA sensitization to be approximately 50% in 7- to 18-year-olds living in the same IFA endemic region. The prevalence of sensitization in children is substantially higher than the 17% prevalence previously reported in adults (average age, 47 years) from this same area. We found a higher prevalence of sensitization to IFAV than other researchers have reported for other common allergens in early childhood. As a comparison, the reported sensitization rate to milk was 2.5% in the first 2 years of life, and the overall prevalence of food allergy in children was approximately 6%.<sup>9</sup> The prevalence of sensitization to Hymenoptera venom in children has been estimated to be 3.7% in school-aged children living in Italy.<sup>10</sup> Among 4,992 boy scouts 11 to 16 years of age living in New England, the prevalence of bee sting allergy was reported to be 0.4% to 0.8%.<sup>8</sup> Therefore, the prevalence of IFAV sensitization in children is higher than that described for foods and other venoms in the same age groups. In contrast to IgE anti-IFAV, the prevalence of IgG anti-IFAV antibodies gradually increased from the younger to the older age groups until almost all the children had detectable IgG anti-IFAV antibodies. This indicates that by age 11 to 20 years, exposure to IFAV in the southeastern United States seems sufficient to induce an IgG immune response in almost all children.

This study also extends previous studies on the frequency of IFA stings in children. Almost 40% of children living in an endemic region reported an IFA sting during the previous month (August or September), with 92.1% of these stings due to IFAs. This is similar to results from a study<sup>9</sup> performed in

Table 1. Prevalence of IgE and IgG Anti-IFAV Antibodies in 183 Children by Age Group

| Age group, y | Children, No. | IgE anti-IFAV positive, No. (%) |                  | IgG anti-IFAV positive at >0.1 $\mu$ g/mL, No. (%) |
|--------------|---------------|---------------------------------|------------------|----------------------------------------------------|
|              |               | At >0.1 kUa/L                   | At >0.35 kUa/L   |                                                    |
| ≤1           | 42            | 3 (7.1)                         | 1 (2.4)          | 5 (11.9)                                           |
| 2–5          | 42            | 24 (57.1)                       | 15 (35.7)        | 24 (57.1)                                          |
| 6–10         | 59            | 38 (64.4)                       | 31 (52.5)        | 43 (72.9)                                          |
| 11–20        | 40            | 26 (65.0)                       | 23 (57.5)        | 39 (97.5)                                          |
| <b>Total</b> | <b>183</b>    | <b>91 (49.7)</b>                | <b>70 (38.3)</b> | <b>111 (60.7)</b>                                  |

Abbreviations: IFAV, imported fire ant venom.

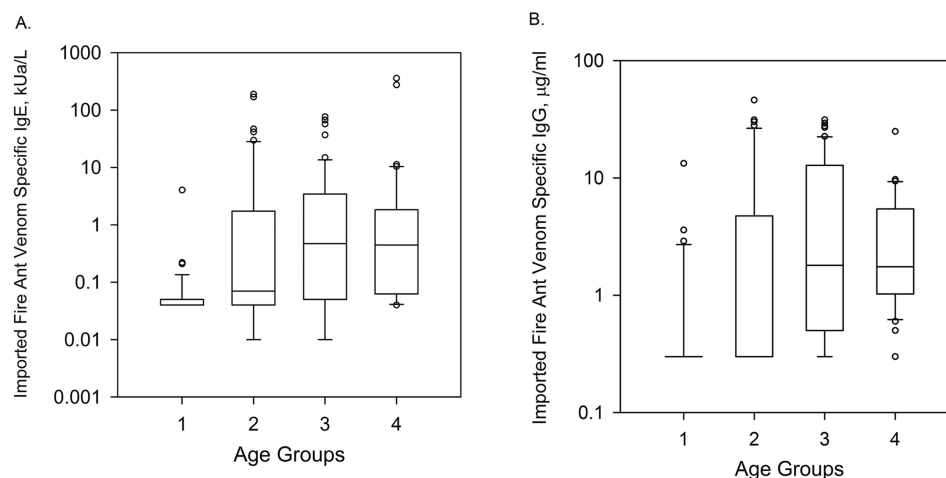


Figure 1. Imported fire ant (IFA) specific IgE and specific IgG antibody levels by age group. A, The median values (horizontal bars) for IgE anti-IFA venom were significantly different between group 1 and groups 3 and 4 ( $P < .001$ ). B, The median values (horizontal bars) for IgG anti-IFA venom were significantly different between group 1 and groups 2, 3, and 4 ( $P < .001$ ). Group 1 consisted of children younger than 1 year; group 2, 2- to 5-year-olds; group 3, 6- to 10-year-olds; and group 4, 11- to 20-year-olds. Boxes represent the 25th to 75th percentile; whiskers, the 5th to 95th percentile; circles, outliers.

Table 2. Questionnaire Information on Numbers of Insect Stings or Bites During the Previous Month in Children Aged 1 to 18 Years

|                                                         | Children, No. (%) |
|---------------------------------------------------------|-------------------|
| No. of insect stings in past month (n = 180)            |                   |
| 0                                                       | 109 (60.6)        |
| 1-5                                                     | 34 (18.9)         |
| 6-10                                                    | 24 (13.3)         |
| 11-20                                                   | 6 (3.3)           |
| >20                                                     | 7 (3.9)           |
| No. of insect bites in past month (n = 182)             |                   |
| 0                                                       | 5 (2.7)           |
| 1-5                                                     | 23 (12.6)         |
| 6-10                                                    | 58 (31.9)         |
| 11-20                                                   | 58 (31.9)         |
| >20                                                     | 38 (20.9)         |
| No. of imported fire ant stings in past month (n = 176) |                   |
| 0                                                       | 108 (61.4)        |
| 1-5                                                     | 26 (14.8)         |
| 6-10                                                    | 27 (15.3)         |
| 11-20                                                   | 11 (6.3)          |
| >20                                                     | 4 (2.3)           |

Louisiana that demonstrated an annual IFA sting attack rate of 29% between June and August. In children younger than 10 years, the sting attack rate was 55%. In a prospective study<sup>10</sup> of IFA-naïve adults who were visiting an IFA endemic region (San Antonio, Texas), 51% reported an IFA sting within the first 3 weeks of going to the area.

Multiple stings were commonly reported in the children in the present study, with 23.9% reporting more than 6 stings during the previous month. Because it is more common to be stung by multiple IFAs during 1 episode of encountering the insects, we presume that most of these multiple stings were

Table 3. Imported Fire Ant Stings in the Previous Month in Relationship to Where the Child Lives<sup>a</sup>

| Imported fire ant stings, No. | Child's residence, No. (%) <sup>b</sup> |           |           |
|-------------------------------|-----------------------------------------|-----------|-----------|
|                               | City                                    | Suburb    | Rural     |
| 0                             | 62 (35.2)                               | 24 (13.6) | 22 (12.5) |
| 1-5                           | 12 (6.8)                                | 9 (5.1)   | 5 (2.8)   |
| 6-10                          | 9 (5.1)                                 | 5 (2.8)   | 13 (7.4)  |
| 11-20                         | 0                                       | 7 (4.0)   | 4 (2.3)   |
| >20                           | 2 (1.1)                                 | 1 (0.6)   | 1 (0.6)   |

<sup>a</sup> The numbers of stings differ significantly by location of residence ( $\chi^2 = 23.75$ ;  $P = .003$ ).

<sup>b</sup> The percentages displayed are for all 176 children for whom information on both the number of imported fire ant stings and the setting in which they lived were available. One or both items were missing for 6 children.

probably due to IFAs. This study did not evaluate whether these stings occurred during a single stinging episode or during several separate episodes. Multiple stings by IFAs in elderly, bedridden patients living in nursing homes have been described.<sup>3,11</sup> Children may be equally vulnerable to multiple stings because they may not know that they should avoid stinging insects and they may not have the developmental skills necessary to remove a stinging insect. Children in rural areas had significantly more stings reported in the previous month than those living in either the suburbs or the city. We suspect that this may be because children living in rural locations are more likely to be outside in grassy areas where they will be exposed to IFAs or other stinging insects.

We found a low prevalence of severe reactions to insect stings, with 2.2% of children reporting a history of a severe reaction to a sting or bite. It has been estimated that potentially life-threatening reactions to insect stings occur in 0.4% to 0.8%

of children. In the present study, 32.4% of severe reactions were due to IFAs and 5.0% were due to bees, hornets, or wasps. However, only 1 patient reported generalized hives, and none of the patients reported breathing difficulties.<sup>12</sup> Past surveys have estimated that in the general population, 17% to 63% of all IFA stings result in a local sting reaction,<sup>2,13</sup> whereas anaphylaxis due to an IFA sting was estimated to occur in 6% to 16% of sensitized individuals.<sup>14,15</sup> In a survey of physicians practicing in 13 southern states during a single year, more than 20,000 individuals received medical treatment for an IFA sting reaction. Only 2% of these patients required treatment for anaphylaxis.<sup>16</sup> Accurate statistics on fatal anaphylaxis from IFA stings are difficult, if not impossible, to obtain because many states do not record this information in the state vital statistics database. In addition, reports of fatalities from anaphylaxis are gathered from a variety of sources, including the media and physician reports. Our clinical experience practicing in an IFA endemic region has been that there are far fewer cases of severe reactions to IFA stings than would be expected based on the high incidence of sensitization to IFAV demonstrated in this study. Because we did not see any IFA anaphylaxis during the period of serum sample collection, it is plausible to conclude that desensitization has occurred due to the frequent natural exposure to IFAV during childhood. The rapid development of specific IgG in this population would be consistent with this assumption.

There are several limitations to this study. The sample population is not a random sample of the local pediatric population. The testing for IgG anti-IFAV is not standardized, so this interpretation of the IgG anti-IFAV results required extrapolation from previous studies with Hymenoptera venom. Because of the anonymous nature of the children who provide serum samples for this antibody prevalence study, we could not correlate antibody levels with clinical reactions in specific patients. We also cannot exclude the possibility that the trends we observed were due to a small sample size or that the sample population is not truly representative of the local population because it is not a random sample. We are not aware of any cases of IFA anaphylaxis evaluated either in the outpatient or inpatient setting during the collection period, making it unlikely that any of the serum samples were from individuals with IFA anaphylaxis.

In summary, we demonstrated a high prevalence of sensitization to IFA in young children living in an IFA endemic region and that sensitization develops rapidly in the first few years of life. Until we know more about the natural history of IFA allergy, allergists practicing in an IFA endemic region need to be careful when interpreting results of IFAV specific IgE in children without a convincing history of anaphylaxis after an IFA sting. The prevalence of IgG anti-IFAV antibodies in the older age groups was greater than 97%, presumably because many children have frequent IFA stings, and, when stung, they often

receive multiple stings. Despite the high rate of sensitization to IFAV, the number of severe reactions to IFA stings seems to be low. More studies are needed to clarify the immunologic basis for this apparent dichotomy. These data may serve as a stimulus for future investigations.

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