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Helminth infections and allergies in Ghana

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chapter 4

Peanut-specific IgE antibodies in asymptomatic Ghanaian children possibly caused by carbohydrate determinant cross-reactivity

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Abstract

Background: The prevalence of peanut allergy has increased in developed countries, but little is known about developing countries with high peanut consumption and widespread parasitic infections.

Objective: We sought to investigate peanut allergy in Ghana.

Methods: In a cross-sectional survey among Ghanaian schoolchildren (n = 1604), data were collected on reported adverse reactions to peanut, peanut sensitization (serum specific IgE and skin reactivity), consumption patterns, and parasitic infections. In a subset (n = 43) IgE against Ara h 1, 2, 3, and 9 as well as cross-reactive carbohydrate determinants (CCDs) was measured by using ImmunoCAP. Cross-reactivity and biological activity were investigated by means of ImmunoCAP inhibition and basophil histamine release, respectively.

Results: Adverse reactions to peanut were reported in 1.5%, skin prick test reactivity in 2.0%, and IgE sensitization (≥ 0.35 kU/L) in 17.5% of participants. Moreover, 92.4% of those IgE sensitized to peanut (≥ 0.35 kU/L) had negative peanut skin prick test responses. *Schistosoma haematobium* infection was positively associated with IgE sensitization (adjusted odds ratio, 2.29; 95% CI, 1.37-3.86). In the subset IgE titres to Ara h 1, 2, 3, and 9 were low (< 1.3 kU/L), except for 6 moderately strong reactions to Ara h 9. IgE against peanut was strongly correlated with IgE against CCDs ($r = 0.89$, $p < 0.001$) and could be almost completely inhibited by CCDs, as well as *S. haematobium* soluble egg antigen. Moreover, IgE to peanut showed poor biological activity.

Conclusions: Parasite-induced IgE against CCDs might account largely for high IgE levels to peanut in our study population of Ghanaian schoolchildren. No evidence of IgE-mediated peanut allergy was found.

Clinical Implications

Peanut-specific IgE antibodies in Ghana, a Sub-Saharan African country, show cross-reactivity with clinically irrelevant carbohydrate determinants and therefore may reduce the diagnostic value of this parameter in establishing peanut allergy.

Capsule Summary

In Ghana where peanut consumption is high and parasitic infections widespread, elevated peanut-specific IgE levels may primarily be due to cross-reactive carbohydrate determinants and may not result in skin reactivity or reported symptoms.

Key Words

Peanut allergy, skin prick testing, Immunoglobulin E, Sub-Saharan Africa, IgE cross-reactivity, cross-reactive carbohydrate determinants, helminth infections, basophil histamine release, EuroPrevall

Abbreviations

adjOR: Adjusted odds ratio

AWA: Adult worm antigen

BHR: Basophil histamine release

CCD: Cross-reactive carbohydrate determinant

CI: Confidence interval

CRD: Component-resolved diagnostics

SEA: Soluble egg antigen

SPT: Skin prick test

Introduction

Recent studies report a significant rise in the incidence of peanut allergy particularly in Europe and North America where self-reported peanut allergy is around 1% among individuals less than 18 years [1, 2]. According to a 5 year follow-up survey among children in Montréal, Canada, peanut allergy prevalence (confirmed by skin prick tests and oral food challenges) rose from 1.34% in 2000-2002 to 1.62% in 2005-2007 [3] while a population-based study conducted in Australia among infants aged 12 months found the prevalence of challenge-proven peanut allergy to be 3.0% [4].

Although extensive peanut allergy research has been conducted in Western countries, there are only a few published studies from other areas of the world where peanut consumption is high such as in South-East Asia. A population-based questionnaire survey in children 4-6 years as well as 14-16 years in two Asian populations indicates that self-reported adverse reactions to peanut in this region may vary between 0.43% and 0.64% [5]. Additionally, a food allergy study among children 6-11 years in China, India and Russia described peanut allergy to be uncommon in all three countries [6]. For Sub-Saharan Africa, no published data are available to date.

One reason proposed to explain the lower prevalence of allergic disorders in many developing countries is the possible suppressive role of chronic infections on the development of allergies [7]. Infections, especially parasitic ones, are highly prevalent in Africa, Asia and South America, particularly in rural areas or in poor sections of urban communities [8-10]. One mechanism by which helminth infections are believed to protect against allergies is by activating regulatory networks that involve the induction of regulatory T and B cells as well as the modulation of innate immune cells [11, 12]. Another mechanism of recent interest has been how cross-reactivity between parasite/helminth antigens and allergens may affect IgE sensitization patterns and their translation into clinical symptoms [13, 14].

As there is little information on peanut allergy in Sub-Saharan Africa and on associated risk factors, we set out to investigate the epidemiology of peanut allergy in schoolchildren in Ghana, a country where peanut consumption is estimated to be high. In 2009 alone, the per capita consumption of peanuts in Ghana was approximately 12 kg [15] compared to a per capita estimate of 6.6 kg for the United States in the same year [16]. Our objective was to identify factors associated with peanut sensitization and reported symptoms such as parasitic infections, peanut consumption patterns and peanut preparation methods. We also sought to characterize IgE reactivity to peanut in our population.

Methods

Study design and population

We conducted a cross-sectional study between March 2006 and March 2008 that was part of a larger investigation into allergic sensitization and parasitic infections in schoolchildren in Southern Ghana. This investigation was carried out within the

framework of the European Union-funded EuroPrevall [17] and GLOFAL [18] projects (see details in the supplementary material). Outcome parameters of interest were 1. reported adverse reactions to peanut and 2. peanut sensitization based on serum specific immunoglobulin E (IgE) levels and skin prick test (SPT) reactivity. The study was approved by the Noguchi Memorial Institute for Medical Research Institutional Review Board, Ghana (NMIMR-IRB CPN 012/04-05). Three districts in the Greater Accra Region were selected for the investigation. Within these districts, schools were randomly selected and approached to participate in the study (see sampling methodology in the supplementary material).

We recruited children aged between 5 and 16 years attending 6 rural and 3 urban schools. Approximately 35% (1714/4852) of all children attending targeted schools agreed to participate in the study (see Figure E1 in the supplementary material). The overall participation rate in the rural schools was 34.7% compared to 36.4% in the urban schools. There was no information available on non-participants. Of 1714 children enrolled, 59 subjects were in the end unavailable for data collection while 51 were excluded for being outside of the age-range (see Figure E2 in the supplementary material), leaving a total study population of 1604 children. Parameters measured were IgE serology (n=1328), skin prick test reactivity (n=1396), questionnaire (n=1372), urinary schistosomiasis (n=1537), intestinal helminths (n=1398) and malaria blood films (n=1468).

Component-resolved diagnostics (CRD) could only be performed for a maximum of 50 subjects due to budgetary limitations. Subjects for whom a sufficient serum volume (≥ 350 μ L) was available were included based on reported adverse reactions to peanut (n=8), peanut SPT positivity (n=15) and randomly selected subjects with IgE to peanut levels higher than 1.5 kU/L (n=15). This threshold was chosen to increase the sensitivity for measuring IgE against individual peanut allergens. Five randomly selected negative control subjects with no reported adverse reactions to peanut and no peanut sensitization were also included. Detailed selection procedure for the CRD subset can be found in the supplementary material.

Parasitological examinations

One stool sample per subject was collected for the detection of intestinal helminth eggs by the Kato-Katz technique [19] using 25 mg of stool. A urine sample was also collected to determine *S. haematobium* infection using the standard filtration method [20] in which 10 ml of urine is filtered through a nylon nucleopore filter (pore size, 12 μ m). For each subject, a small quantity of blood was collected to prepare a Giemsa-stained thick smear slide to detect malaria.

Questionnaire

A standard questionnaire (see thesis appendix) was administered to the parents or guardians of study subjects to collect information on demographic and socioeconomic parameters as well as information on established risk factors for the development

of allergy. Questions on the symptoms of adverse reactions to food were included in the questionnaire. These were adapted from the validated EuroPrevall survey questionnaire [21]. The questionnaire was administered by trained interviewers who were fluent in the local language of each participant. It was pre-tested in a pilot study under field conditions to ensure understanding and acceptability.

Skin prick testing

Skin prick test reactivity to a commercially available whole peanut extract (kindly provided by ALK-Abelló, Madrid, Spain) was assessed using the standard protocol [22, 23] as has been described in detail elsewhere [24]. We defined peanut SPT positivity as a mean wheal diameter ≥ 3 mm [25].

IgE antibody measurements

ImmunoCAP (Thermo Fisher Scientific, Uppsala, Sweden) measurements were carried out following the manufacturer's instructions. IgE to peanut was assessed in all participants and 0.35 kU/L was used as the sensitization cut-off. A cut-off of ≥ 15 kU/L, that is reported to have a positive predictive value of 95% for clinical peanut allergy [26], was also examined.

For the CRD subset ($n=43$), specific IgE to recombinant peanut allergens (rAra h 1, 2, 3 and 9), profilin (rPhl p 12) and to bromelain, a marker for cross-reactive carbohydrate determinants (CCD), was assessed by ImmunoCAP. Bet v 1 homologous Ara h 8 was excluded from the analysis because there is no exposure to Fagales tree pollen in Ghana.

IgE inhibition assays

Titred ImmunoCAP inhibition assays were conducted to establish the degree of cross-reactivity of peanut-specific IgE. To this end, 75 μ L of pooled serum comprised of equal volumes of 17 sera (all with peanut-specific IgE levels ≥ 5.5 kU/L and similar IgE responses to peanut as well as to bromelain) was mixed with 75 μ L of inhibitor. Inhibitors used were either bromelain, *Schistosoma haematobium* soluble egg antigen (SEA), *Schistosoma haematobium* adult worm antigen (AWA) or *Ascaris lumbricoides* antigen. For three subjects, two with high and one with low IgE titres to Ara h 9, individual sera were also tested by ImmunoCAP inhibition. Each serum pool (or individual sera) was pre-incubated with an inhibitor at room temperature for 1 hour. Subsequently, samples were analysed for peanut-specific IgE as described above. Results were expressed as percentages of an uninhibited control (phosphate buffered saline).

Basophil histamine release (BHR) assays

To assess the biological activity of peanut-specific IgE in our population, BHR assays were performed using stripped basophils from a non-allergic donor that were sensitized with sera of subjects selected from the CRD subset ($n=43$). Two sera with similar levels

of IgE against peanut and CCD were selected. In addition, two with higher IgE against peanut than against CCD in combination with high IgE against Ara h 9 were also evaluated (see full characteristics in Table E1 in the supplementary material). BHR assays were performed as has been described elsewhere [27, 28].

Statistical analysis

Analysis was carried out using STATA version 10 (StataCorp, Texas, USA). Urban-rural differences in subject characteristics as well as in peanut sensitization (IgE and SPT) and reported adverse reactions were examined by Pearson's χ^2 tests (with 1 degree of freedom). To assess factors associated with peanut sensitization (IgE and SPT) and reported adverse reactions, multivariable random effects logistic regression models were fitted that took into account possible correlation among observations within each school by modelling school as a random effect. This approach was used since children attending the same school were likely to share common characteristics as well as exposures. Models were adjusted for age, sex and urban-rural area (as a *priori* confounders) along with other variables significant from crude analysis.

Results

Characteristics of the study population

The characteristics of the study participants stratified by area are given in Table I. There were no significant differences in gender distribution and age-group comparing the two areas although urban children had a slightly higher median age. In addition, rural subjects had significantly more helminth infections and malaria.

Although peanut consumption was high in both areas, reported daily consumption was considerably higher among rural schoolchildren (36.2%) compared to their urban counterparts (9.8%). Furthermore, in the rural area, both "boiled only" and "roasted only" peanut preparation methods were reported more frequently than in the urban area where the combination of roasting and then boiling peanuts in soup preparation was more common. Topical exposure to peanut as assessed by the use of peanut oil as a skin ointment was higher in rural compared to urban schools.

Reported adverse reactions and sensitization (IgE and SPT) to peanut

Adverse reactions were reported in 1.5% (n=21/1372) of participants (see Table II) most of whom were rural schoolchildren. The distribution pattern of the characteristics of those reporting adverse reactions (see Table E2 in the supplementary material) did not differ significantly from the rest of the study population (statistical tests data not shown). About 67% of those reporting adverse reactions to peanut had gastrointestinal complaints and 43% had complaints described as itching of the mouth or difficulty swallowing. Only 4 out of 21 subjects reported a reaction time "within minutes" (see Table E3 in the supplementary material).

Table I. Characteristics of study population stratified by area

FACTOR		ALL n / N (%)	AREA		P-value #
			Rural n / N (%)	Urban n / N (%)	
Sex					
Males		757 / 1604 (47.2)	465 / 976 (47.6)	292 / 628 (46.5)	0.65
Females		847 / 1604 (52.8)	511 / 976 (52.4)	336 / 628 (53.5)	
Age					
<11 years or less		785 / 1604 (48.9)	496 / 976 (50.8)	289 / 628 (46.0)	0.06
≥more than 11years		819 / 1604 (51.1)	480 / 976 (49.2)	339 / 628 (54.0)	
Parasitic Infections					
Any intestinal helminth §	(positive)	248 / 1398 (17.7)	236 / 834 (28.3)	12 / 564 (2.1)	<0.001
<i>S. haematobium</i>	(positive)	103 / 1537 (6.7)	83 / 922 (9.0)	20 / 615 (3.3)	<0.001
<i>Plasmodium species</i> *	(positive)	349 / 1468 (23.8)	310 / 880 (35.2)	39 / 588 (6.6)	<0.001
Peanut Consumption					
Daily	(yes)	365 / 1372 (26.6)	316 / 874 (36.2)	49 / 498 (9.8)	<0.001
Weekly	(yes)	760 / 1372 (55.4)	438 / 874 (50.1)	322 / 498 (64.7)	<0.001
Monthly	(yes)	183 / 1372 (13.3)	70 / 874 (8.0)	113 / 498 (22.7)	<0.001
Every 6 months	(yes)	21 / 1372 (1.5)	12 / 874 (1.4)	9 / 498 (1.8)	0.52
Never	(yes)	35 / 1372 (2.6)	35 / 874 (4.0)	0 / 498 (0.0)	<0.001
Missing Consumption information		8 / 1372 (0.6)	3 / 874 (0.3)	5 / 498 (1.0)	
Exclusive Peanut Preparation Methods					
Boiled	ONLY (yes)	61 / 1372 (4.4)	56 / 874 (6.4)	5 / 498 (1.0)	<0.001
Fried	ONLY (yes)	19 / 1372 (1.4)	19 / 874 (2.2)	0 / 498 (0.0)	0.001
Roasted	ONLY (yes)	277 / 1372 (20.2)	276 / 874 (31.6)	1 / 498 (0.2)	<0.001
Other Peanut Preparation Methods					
Raw	(yes)	22 / 1372 (1.6)	3 / 874 (0.3)	19 / 498 (3.8)	<0.001
Peanut Oil**					
Use of peanut oil	(yes)	33 / 1370 (2.4)	32 / 872 (3.7)	1 / 498 (0.2)	<0.001

P-values were calculated by using Pearson's χ^2 test (1 degree of freedom). Values in boldface indicate significance.

§ Any intestinal helminth = *Ascaris lumbricoides*, hookworm (*Ancylostoma duodenale* or *Necator americanus*), *Trichuris trichiura* or *Schistosoma mansoni*.

* *Plasmodium species* = *Plasmodium falciparum* or *Plasmodium malariae* (the 2 malaria parasite species detected in our study population).

** Peanut oil use information missing for 2 participants.

The percentage of subjects with a positive peanut SPT was 2.0% (n=28/1396) and this was not significantly different between the two areas (see Table II). Positive wheal sizes for peanut ranged from 3.0 mm to 6.5 mm and did not vary between areas (data not shown).

Peanut IgE sensitization (≥ 0.35 kU/L) was observed in 17.5% (n=233/1328) of the study population with 23.6% of rural children being sensitized compared to 9.7% of

Table II. Prevalence of adverse reactions to peanut and peanut sensitization (SPT and IgE) stratified by area

FACTOR	ALL n / N (%)	AREA		P value #
		Rural n / N (%)	Urban n / N (%)	
Adverse reactions to food				
Any food	154 / 1372 (11.2)	115 / 874 (13.2)	39 / 498 (7.8)	0.003
Peanut	21 / 1372 (1.5)	18 / 874 (2.1)	3 / 498 (0.6)	0.035
Skin prick test reactivity				
Peanut Positive	28 / 1396 (2.0)	17 / 881 (1.9)	11 / 515 (2.1)	0.79
Peanut-specific IgE				
≥0.35 kU/L	233 / 1328 (17.5)	177 / 751 (23.6)	56 / 577 (9.7)	<0.001
≥15 kU/L	12 / 1328 (0.9)	8 / 751 (1.1)	4 / 577 (0.7)	0.48

P-values were calculated by using Pearson's χ^2 test (1 degree of freedom).
Values in boldface indicate significance.

urban participants ($p < 0.001$). However, 92.4% ($n=194/210$) of those IgE sensitized to peanut (≥ 0.35 kU/L) were peanut SPT negative. Interestingly, 0.9% ($n=12/1328$) of the study subjects were highly sensitized when using the IgE cut-off of ≥ 15 kU/L, which is reported to have a positive predictive value of 95% for clinical peanut allergy [26], but only 1 of them reported reactions. Figure 1 shows the overlap between the peanut-related outcomes for study subjects with complete allergy data (reported reactions, SPT and IgE). No individual was positive for all three parameters.

Factors associated with peanut sensitization (IgE and SPT) and reported adverse reactions to peanut

In multivariable analysis, area was strongly associated with peanut IgE sensitization ≥ 0.35 kU/L with urban subjects having a reduced odds of elevated IgE relative to their rural counterparts [adjOR= 0.41, 95% CI (0.25 - 0.67), $p < 0.001$] (see Table III). Being *S. haematobium* infected was also associated with peanut IgE sensitization [adjOR= 2.29, 95% CI (1.37 - 3.86), $p < 0.001$] while intestinal helminth infection was not.

Although the majority of peanut IgE sensitized individuals were not peanut SPT positive, almost all peanut SPT positive subjects were IgE sensitized. Thus, in multivariable analysis, IgE sensitization was associated with peanut SPT reactivity [adjOR= 17.09, 95%CI (6.30 – 46.36), $p < 0.01$]. In addition, while not observed in crude analysis, residing in the urban area was associated with a significantly higher chance of being SPT positive to peanut after adjusting for confounders (see Table III). No other factors, including helminth infection, had an effect on SPT to peanut (see Table III).

Data on peanut consumption and preparation methods as risk factors for peanut-related outcomes are shown in Table E4 (see supplementary material). 'Never' consuming peanuts, as a proxy for avoidance, was associated with reported symptoms [adjOR=5.40,

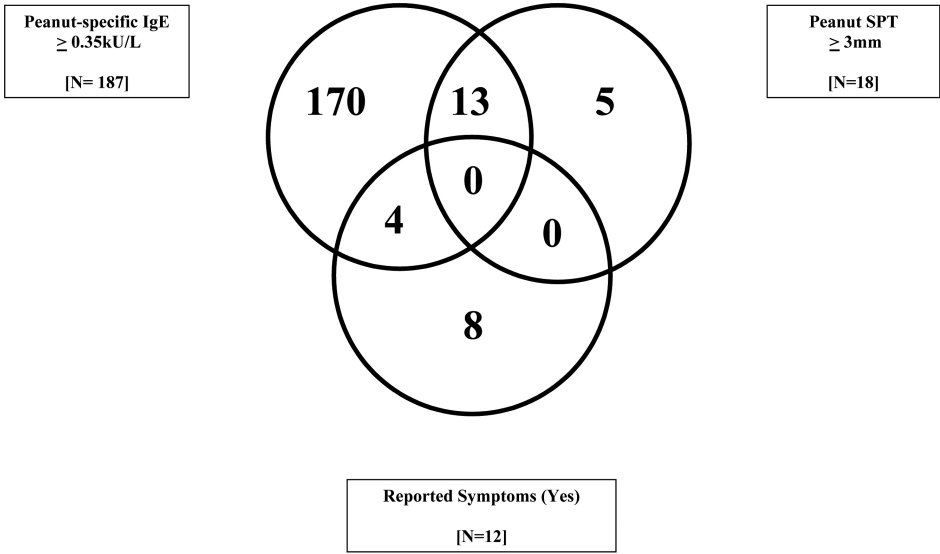


Figure 1: Overlap between peanut allergy outcomes
Overlap between reported adverse reactions to peanut and peanut sensitization (IgE levels and SPT responses) for subjects with complete data for allergy-related parameters. (n=1004).

95% CI (1.47 – 19.80), $p < 0.05$]. Raw peanut consumption was also linked to reported adverse reactions to peanut [adjOR=17.14, 95% CI (2.93 – 100.45), $p < 0.01$]. However, numbers were low as reflected in the wide confidence interval. All other factors, including helminth infection, were not significantly associated with reported adverse reactions to peanut (see Table III and Table E4 in the supplementary material).

Component-resolved IgE testing

Figure 2A shows the results of CRD performed in a subset (n=43) to better characterize peanut-specific IgE. Those with IgE to peanut >1.5 kU/L (median 12.5 kU/L) had high levels of IgE to CCD but low IgE responses (<1.3 kU/L) to rAra h 1-3 and rPhl p 12. A strong correlation was seen between peanut-specific IgE and CCD-specific IgE [$r = 0.89$, $p < 0.001$] (see Figure 2B). For some individuals, IgE against peanut was significantly higher than to CCD and in 6 of these, high titres of IgE to the lipid transfer protein rAra h 9 were observed (see Figure 2A). Of note, 4 out of 6 of these subjects were peanut SPT positive (see Table E1 in the supplementary material).

Inhibition of IgE binding to peanut by CCD and schistosome egg antigen

Titred CAP-inhibition assays demonstrated that binding of IgE from a serum pool of individuals (n=17) with similar IgE titres to peanut as to CCD was almost completely

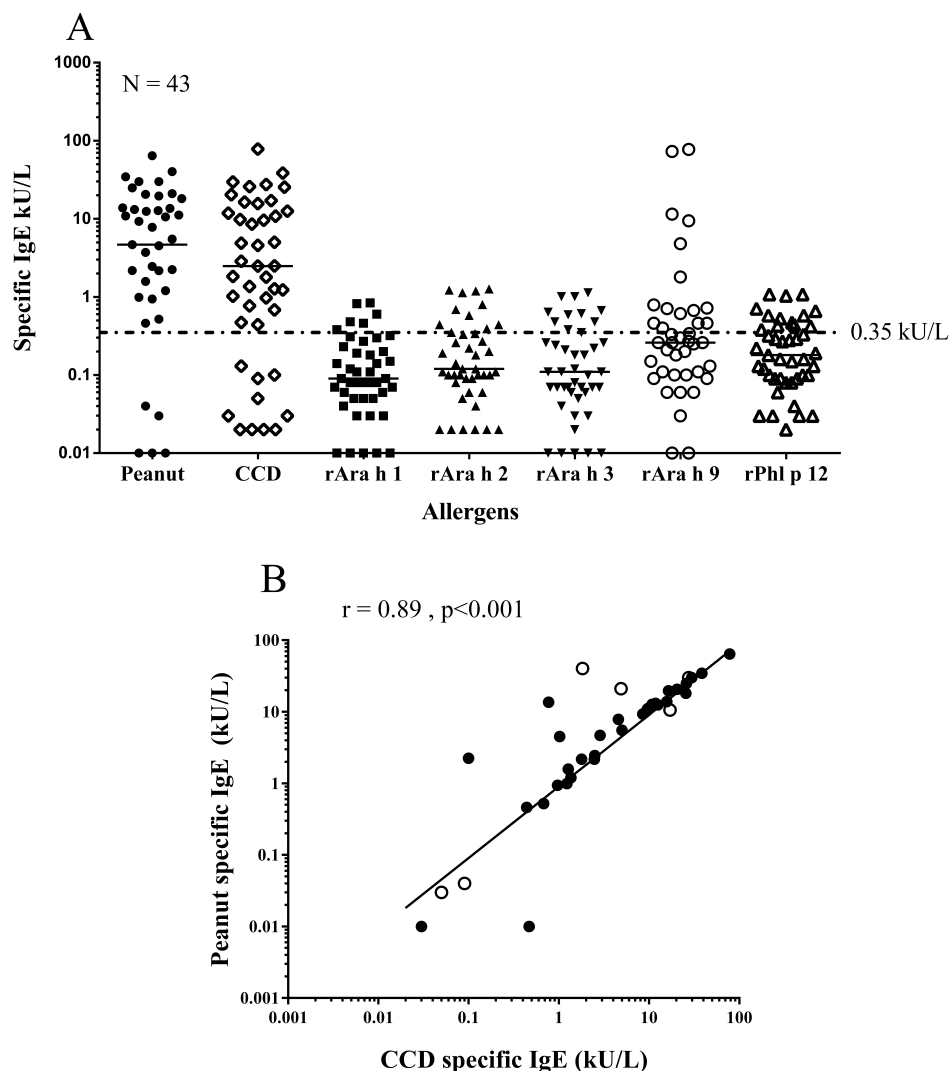


Figure 2: Characterization of specific IgE to peanut in a subset

[A] Measurement of specific IgE to whole peanut extract, recombinant peanut allergens, profilin and CCD marker bromelain in a subset ($n=43$). Median specific IgE levels are indicated by black lines. The dotted line shows IgE sensitization cut-off of 0.35 kU/L. **[B]** Correlation between peanut-specific IgE and CCD-specific IgE. Open circles (O) indicate subjects with IgE to rAra h 9 of greater than 1.5 kU/L.

inhibited by CCD as well as by *S. haematobium* SEA (see Figure 3). Individual inhibitions for two subjects with high IgE to peanut and to Ara h 9 as well as low IgE to CCD showed <10% inhibition by SEA (see Table E1 in the supplementary material). In addition, *S. haematobium* AWA and *A. lumbricoides* antigen did not inhibit binding significantly (data not shown).

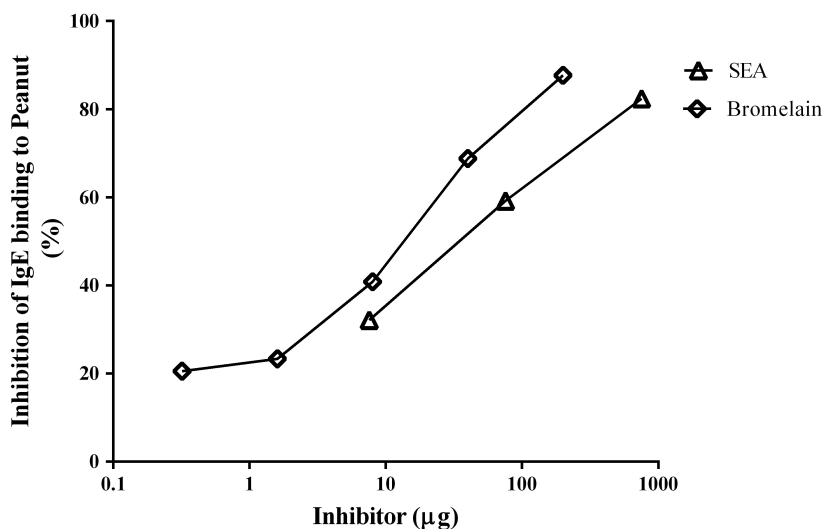


Figure 3: Inhibition of IgE binding to peanut

Inhibition of IgE binding to whole peanut by bromelain and *S. haematobium* soluble egg antigen (SEA) by using pooled sera ($n=17$). The figure shows that IgE binding to whole peanut extract was almost completely inhibited by bromelain (◇) and *S. haematobium* soluble egg antigen (Δ) respectively.

BHR Assays

Peanut extract induced little histamine release when basophils were sensitized with IgE from subjects with similar IgE reactivity to peanut as to CCD (see Figure 4A and 4B). For these individuals, the ability of *S. haematobium* SEA to induce histamine release was tested and only at a concentration of 10 μg/ml, release was observed. For two subjects with titres of IgE against Ara h 9 >70 kU/L (see Figure 4C and 4D), Ara h 9 induced significant histamine release starting at 10 pg/ml reaching maximum release at about 1 ng/ml while with peanut extract, release was seen starting from a concentration of 10 μg/ml.

Discussion

Our study is the first investigation of reported adverse reactions to peanut and peanut sensitization based on serum specific IgE as well as SPT reactivity in Sub-Saharan Africa among an unselected group of children. We confirmed that there was a high frequency of daily peanut consumption in Southern Ghana particularly among rural schoolchildren. We also observed an association between reported peanut adverse reactions and peanut avoidance. The percentage of reported peanut adverse reactions among schoolchildren in our survey was 1.5%. However, the majority of these reported reactions occurred within hours/days while IgE-mediated peanut allergy is typically associated with symptoms appearing within minutes or up to 2 hours [29].

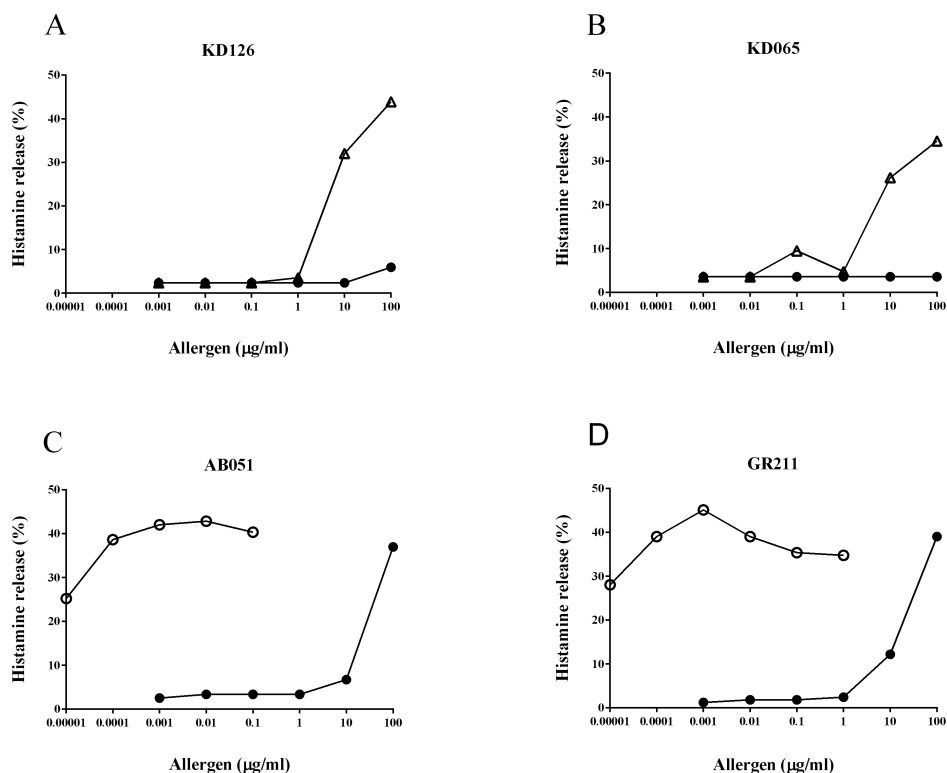


Figure 4: Basophil histamine release assays

Basophil histamine release assay results. Basophil histamine release induced by peanut extract (●), *S. haematobium* SEA (Δ) and Ara h 9 (○). [A] and [B] are results for two subjects with high IgE titres against peanut and CCD. [C] and [D] are results for two subjects with high IgE titres against peanut and Ara h 9 but low IgE titres against CCD.

Among study participants, 2.0% were peanut SPT positive. Although 17.5% of all subjects had elevated IgE to peanut (≥ 0.35 kU/L), 92.4% of these were peanut SPT negative. One explanation for the discrepancy between specific IgE and SPT could be the suppression of IgE induced inflammation by immunological regulatory networks [30] that might be operative during chronic helminth infections. However, we did not observe any association between helminth infection and SPT to peanut.

Notably, 12 out of 1328 participants had peanut-specific IgE levels ≥ 15 kU/L; a cut-off reported to have a positive predictive value of 95% for clinical peanut allergy in a European study population [26] but was virtually unaccompanied by reported symptoms in our study. This highlights the limitations in applying cut-off values determined in one population to other populations.

Analysis by component-resolved diagnostics in a subset indicated that the majority of those with high titres of IgE against peanut (median 12.5 kU/L) had low responses

(<1.3 kU/L) against the major peanut allergens (Ara h 1, 2 and 3) commonly associated with peanut allergy [31-33]. Recently, IgE responses to Ara h2 in particular, have been used to differentiate between clinical peanut allergy and asymptomatic peanut sensitization [34] as well as to improve diagnostic accuracy [35]. One study observed that a cut-off of IgE to rAra h2 >0.23 kU/L had a specificity of 97% and sensitivity of 93% among peanut allergic patients and controls in France [32]. Taken all together, sensitization to peanut storage proteins in Ghana appears weak and rare compared to European or US peanut allergic patients. The lack of clinical reactivity among study participants with elevated IgE responses to Ara h 2 would have to be explored further.

The most dominant molecular component recognized by IgE in peanut-sensitized subjects in our subset was CCD. A strong correlation was observed between IgE to peanut and to CCD. CCDs are N-glycans in plants and invertebrate glycoproteins that result in a high degree of cross-reactivity between pollen and foods [36]. CCDs have negligible *in vivo* biological activity as well as clinical relevance [37-39]. Grass pollen was found to be of minor importance in Ghanaian schoolchildren as was established in a pilot study preceding the present survey. In our study population, we observed that current *S. haematobium* infection was associated with elevated IgE to peanut. Moreover, among our subset, the results of the ImmunoCAP inhibition assays showed that plant-derived CCD (bromelain) inhibited IgE binding to peanut and that a *Schistosoma*-derived glycoprotein preparation was an equally potent inhibitor. These observations suggest that carbohydrate specific IgE is induced by glycoproteins from the eggs of *S. haematobium* that are different from but cross-reactive with those on bromelain. Interestingly, *Schistosoma* adult worm glycoproteins were not effective as inhibitors indicating the importance of stage-specific N-glycans in this cross-reactivity. The importance of cross-reactivity might also explain the residual effect for rural area on IgE to peanut, which was seen after adjusting for current *S. haematobium* infection. Past infections in individuals residing in the rural area might have led to cross-reactive IgE to peanut.

Interestingly, in the studied subset, IgE responses to Ara h 9 were elevated in 6 children with two having IgE titres >70 kU/L. Furthermore, IgE antibodies against Ara h 9 were biologically active at low allergen concentrations (pg/ng range) as determined by basophil histamine release assays. The observation that 4 out of 6 subjects with high IgE to Ara h 9 were peanut SPT positive is in line with these BHR results. However, none of these reported immediate adverse reactions to peanut. Altogether, the data suggest that sources other than CCD could contribute to elevated IgE to peanut extract. The origin of sensitization to this lipid transfer protein is unknown and whether a locally consumed fruit is at the basis of this sensitization, as is commonly reported in Europe in relation to peach [31, 40, 41], remains to be determined for Ghana.

Our study had a number of limitations such as a low participation rate but given our observation that IgE-mediated peanut allergy in Ghanaian schoolchildren is rare (if existing at all), it is unlikely that selection bias is affecting our findings in this respect. However, the borderline significant difference in age between rural and urban children

as well as the fact that the rural population is from areas that are endemic for helminth infections need to be taken into account when considering the generalizability of our findings. The absence of the gold standard for peanut allergy (oral food challenges) is another limitation but given that reported adverse reactions to peanut were largely not accompanied by immediate reactions, this is less likely to be an issue. An additional study weakness is the use of a questionnaire as a measurement tool for adverse reactions as well as other self-reported parameters. Furthermore, our school-based study design meant that children less than 5 years were excluded from the investigation which might bias the results by omitting an important age-group affected by peanut allergy. However, given the persistent nature of peanut allergy among most individuals, the effect of an older age cut-off of 5 years is likely to be minimal. The fact that CRD was conducted in a relatively small subset of our larger study population is another limitation although the subset did not differ from the wider study population on key demographic factors and parasitic infections.

Despite these limitations, our study provides new insights into the nature of peanut sensitization and reported adverse reactions to peanut in Ghana, a Sub-Saharan African country where peanut consumption is high but does not appear to translate into true peanut sensitization, let alone peanut allergy. Overall, our observations suggest that IgE-mediated peanut allergy in Ghanaian schoolchildren is rare. Among a subset, we found a role for N-glycans, particularly related to *Schistosoma*, in inducing cross-reactivity resulting in elevated IgE to peanut without skin reactivity or reported symptoms. This study once more highlights the poor biological activity of CCD-specific IgE. Interestingly, IgE to Ara h 9 demonstrated normal biological activity suggesting that lack of biological activity is not the only explanation for the lack of clinical peanut allergy. Future studies on the characteristics of cross-reactive IgEs and the pathways behind their development may be essential to the ongoing investigation of immune regulatory mechanisms in an effort to curtail strong allergic inflammation.

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Supplementary material

Study overview: EuroPrevall and GLOFAL

We conducted a cross-sectional investigation that was carried out within the framework of the European Union-funded EuroPrevall and Global View of Food Allergy (GLOFAL) projects. EuroPrevall was a multi-disciplinary project examining the prevalence, cost and basis of food allergy in Europe that ran between June 2005 and December 2009 [E1].

It involved the collaboration of 17 European Union member states as well as non-European partners [E2]. The main focus of the project was to explore the patterns and the prevalence of food allergies across Europe as well as to improve the diagnosis of food allergy. Within the frame of the EuroPrevall Project, the multi-centre GLOFAL initiative was formulated to provide insights from developing countries in Africa (Gabon and Ghana) as well as Asia (Indonesia). GLOFAL was a collaborative project between researchers in developing countries and Europe-based EuroPrevall partners [E3]. One of the specific objectives of the GLOFAL project was to generate novel insights into the interaction between food consumption, the immune system and the development of allergies. It was ultimately expected that the identification of risk factors for allergy in rapidly urbanizing countries may prevent allergy epidemics in these areas while the identification of protective factors may be useful in stemming the allergic march in more industrialized parts of the world [E4].

Sampling Methodology

For our investigation, a school-based design was deemed to be the most logistically feasible approach. To this end, urban and rural schools were targeted. Of particular interest were areas where parasitic infections were known to be prevalent and where no school-based mass deworming exercises had been conducted in recent years.

Out of the 10 administrative regions of Ghana, the Greater Accra Region was selected for the study. This was in part because the host institute for the research project, the Noguchi Memorial Institute for Medical Research was located in this region. In addition, areas within the Greater Accra region remain endemic for helminth infections and malaria. In 2006, the Greater Accra Region was comprised of 6 districts 2 of which were urban (Accra Metropolis and Tema Municipal Area). Out of the 4

remaining largely rural districts, we targeted ones where no district-wide school-based mass deworming program had taken place in recent years. Therefore, the Ga East and Dangme East districts were selected. Within both districts, one sub-district was randomly selected. Lists of all schools in the targeted sub-districts were obtained and each school approached about willingness to participate in the study.

For our urban schools, we selected one of the two urban districts of the Greater Accra Region, the Accra Metropolis. Out of the 6 sub-metros of Accra, two were selected. Within the two sub-metros, a list of all private and public schools with an enrolment greater than 200 that were also located within a 10 km radius of the host institute was generated. All schools were approached about willingness to participate in the study. After a school head agreed to participate, meetings were held where details of the study were verbally explained to parents with the aid of a powerpoint presentation. These meetings were conducted in the appropriate local language and information sheets distributed. Once a parent or guardian agreed to enrol their ward(s), signed or thumb-printed individual informed consent forms were obtained for each verbally assenting study subject. Schools where study enrolment was >30% were included in our analysis.

Selection of Component-Resolved Diagnostics (CRD) subset

Having observed that a significant proportion of study participants had elevated IgE to peanut without skin prick test reactivity and reported adverse reactions to peanut, component resolved diagnostics (CRD) was used to better characterize the following groups;

1. Individuals reporting adverse reactions to peanut
2. Peanut skin prick test positives
3. Those with elevated peanut-specific IgE
4. Negative controls

CRD could only be performed for a maximum of 50 subjects due to budgetary limitations.

In the database there were 21 subjects reporting adverse reactions. Of these, 12 subjects had sera samples that were used in the ImmunoCAP analysis. Of the 12 subjects who had sera, only 8 had sufficient volumes of sera ($\geq 350 \mu\text{L}$) available for CRD analysis.

Of the 28 subjects in the database who were skin prick test (SPT) positive to peanut, 22 had sera samples that were used in the ImmunoCAP analysis. Of these, 15 had sufficient sera ($\geq 350 \mu\text{L}$) left for CRD parameters.

In the database there were 97 subjects with IgE to peanut that was $\geq 1.5 \text{ kU/L}$. This threshold was chosen to increase the sensitivity for measuring IgE against individual peanut allergens. Of these, 67 had sufficient sera ($\geq 350 \mu\text{L}$) and 15 were selected randomly.

For controls, in the database there were 596 subjects with IgE to peanut $< 0.05 \text{ kU/L}$ who reported no symptoms and were peanut SPT negative. Out of these, 489 had sufficient sera and from these, 5 individuals were randomly selected. The total in

the CRD subset was 43 subjects. Figure E3 shows a flowchart of children selected for the CRD component of the study

Supplementary material references

- E1. Mills ENC, Mackie AR, Burney P, Beyer K, Frewer L, Madsen C, et al. The prevalence, cost and basis of food allergy across Europe. *Allergy* 2007; 62:717-22.
- E2. EuroPrevall: The Prevalence Cost & Basis of Food Allergy. Available from <http://www.europrevall.org>.
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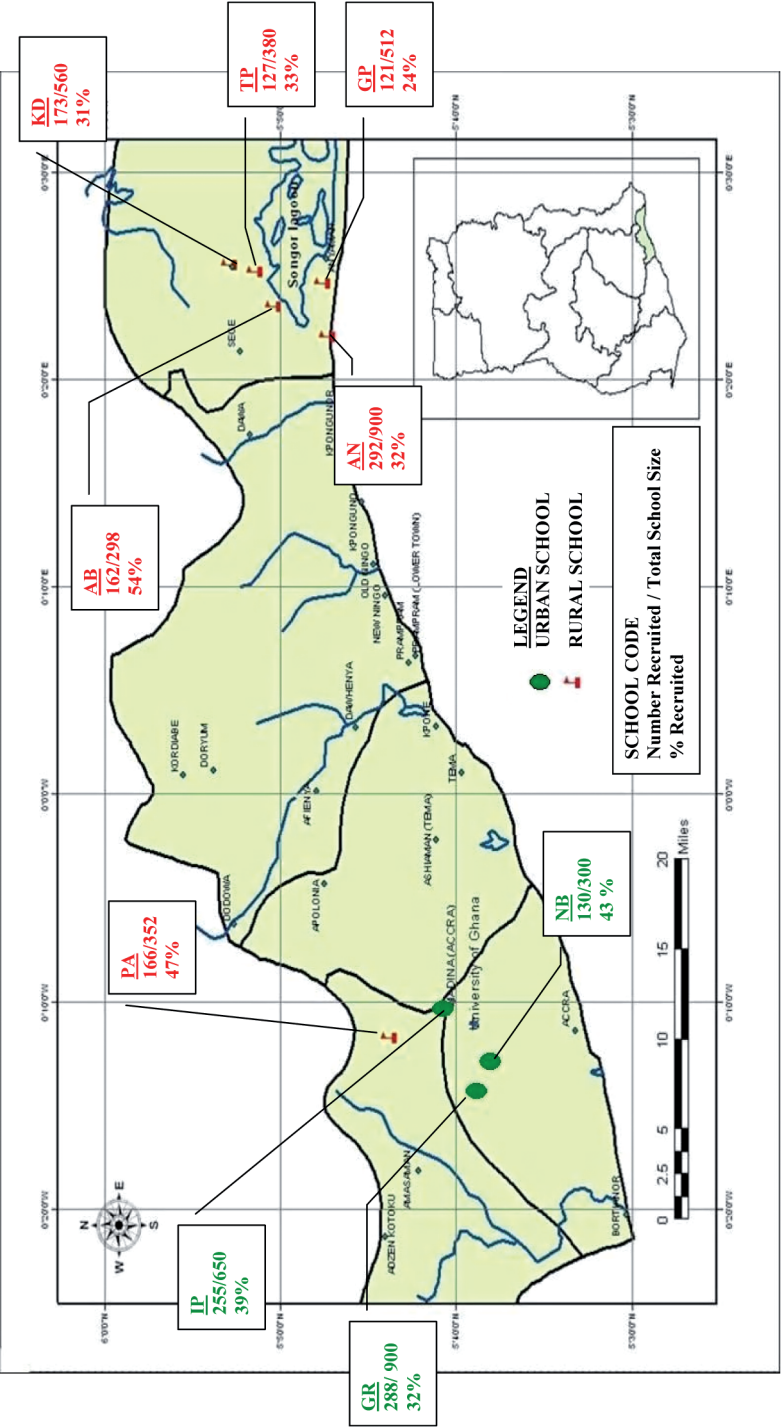


Figure E1. Map of the Greater Accra region of Ghana and study sites
The map shows the study sites and numbers of enrolled participants at each site.

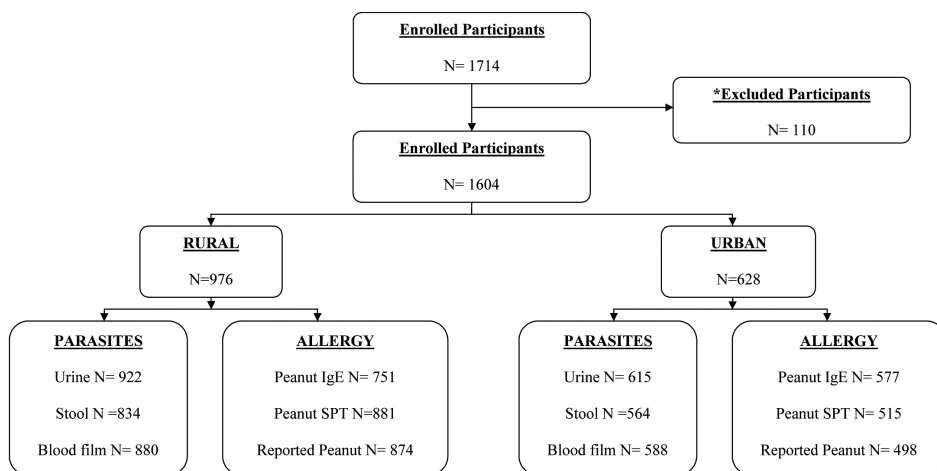


Figure E2: Flowchart of study participants

Flowchart of children recruited from the rural and urban study areas. The number of subjects with complete data for allergy parameters was 1004. The number of subjects with complete data for all parameters was 877.

* Excluded participants: 59 enrolled subjects were unavailable for data collection and 51 were outside of the age-range.

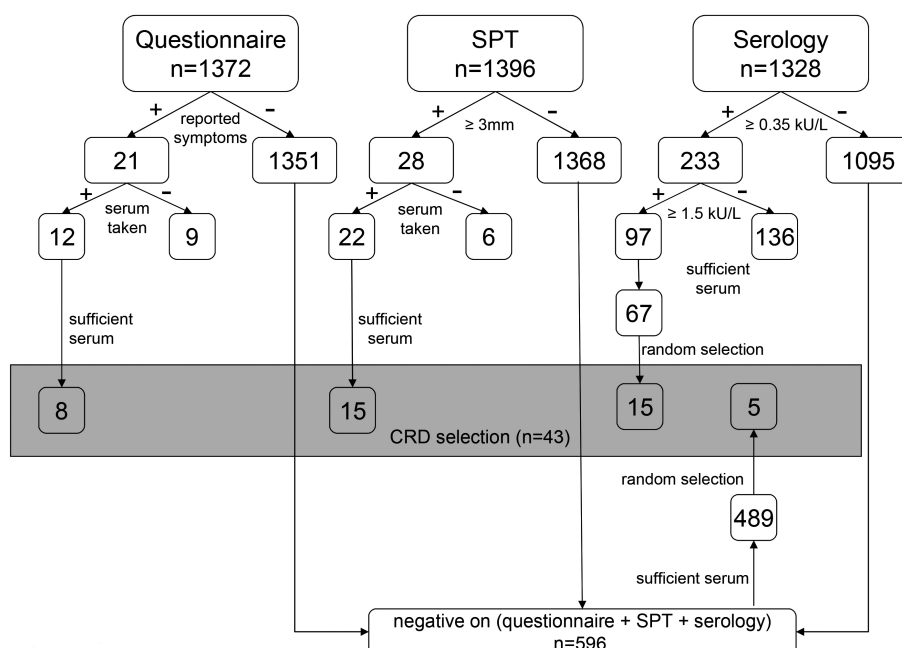


Figure E3. Flowchart of selection for component-resolved diagnostics

Flowchart of children selected for the component-resolved diagnostics component of the study.

Table E1. Characteristics of subset selected for component-resolved diagnostics

ID	Area	Age	Sex	Reported Peanut Reactions	Peanut SPT	Peanut SPT Wheal Size (mm)
AB011	R	7	F	ND	-	0.0
AB041	R	9	M	-	-	0.0
AB051	R	10	F	+	-	0.0
AN052	R	8	F	-	-	0.0
AN068	R	9	M	+	-	0.0
AN084	R	12	F	-	-	0.0
AN102	R	9	F	+	-	0.0
AN115	R	7	M	-	-	0.0
AN132	R	10	M	-	+	3.5
AN142	R	10	M	-	-	0.0
AN143	R	10	F	-	+	3.5
AN190	R	10	M	+	-	0.0
AN218	R	12	M	-	-	0.0
AN220	R	10	F	-	-	0.0
AN228	R	9	M	-	+	4.5
AN248	R	12	M	-	+	5.0
GP001	R	7	M	+	-	0.0
GP031	R	12	F	+	-	0.0
GP074	R	10	M	-	-	0.0
GP094	R	8	M	-	-	0.0
GR063	U	7	M	-	-	1.8
GR098	U	9	M	ND	+	5.0
GR101	U	10	M	-	-	0.0
GR104	U	10	F	-	-	0.0
GR145	U	16	M	-	+	4.0
GR211	U	13	F	-	+	4.0
GR280	U	13	M	-	+	4.5
IP161	U	12	F	-	-	0.0
IP183	U	14	F	ND	+	6.5
IP211	U	15	M	-	+	3.0
IP241	U	6	M	-	-	0.0
IP245	U	13	F	+	-	0.0
KD023	R	10	M	-	-	0.0
KD065	R	11	F	-	-	0.0
KD068	R	10	M	-	-	0.0
KD110	R	12	F	-	-	0.0
KD126	R	12	M	-	-	0.0
KD138	R	12	M	-	+	3.0
NB049	U	7	F	ND	+	3.5
NB071	U	13	M	-	+	3.5
PA058	R	12	M	-	+	3.5
TP095	R	10	M	+	-	0.0
TP116	R	12	F	-	+	3.0

R, rural; **U**, Urban, **M**, Male; **F**, Female; **+**, Positive; **-**, Negative; **SP**, Included in the serum pool for the inhibition assays; **SEA**, *S. haematobium* soluble egg antigen **BHR**, Included in BHR assays shown; **ND**, Not done.

ID	Specific IgE (kU/L)							In Serum Pool?	Individual	BHR Assay?
	Peanut	CCD	Ara h 1	Ara h 2	Ara h 3	Ara h 9	Phl p 12		Inhibition by SEA (%)	
AB011	24.90	25.90	0.30	0.44	0.48	0.46	0.43	SP	ND	ND
AB041	0.01	0.03	0.03	0.05	0.03	0.09	0.06	ND	ND	ND
AB051	40.20	1.83	0.12	0.18	0.18	72.80	0.29	ND	3.6	BHR
AN052	12.71	10.80	0.15	0.20	0.35	0.26	0.29	SP	ND	ND
AN068	0.00	0.02	0.01	0.02	0.01	0.01	0.03	ND	ND	ND
AN084	12.49	12.50	0.20	0.33	0.26	0.30	0.27	SP	ND	ND
AN102	2.45	2.50	0.11	0.11	0.10	0.25	0.18	ND	ND	ND
AN115	11.17	9.82	0.23	0.39	0.48	0.40	0.44	SP	ND	ND
AN132	0.46	0.44	0.04	0.10	0.06	0.21	0.10	ND	ND	ND
AN142	10.58	17.10	0.14	0.26	0.21	1.80	0.38	SP	ND	ND
AN143	34.59	38.40	0.48	0.80	0.59	0.67	0.58	SP	ND	ND
AN190	0.00	0.02	0.00	0.02	0.01	0.03	0.02	ND	ND	ND
AN218	18.11	25.50	0.19	0.22	0.22	0.33	0.32	SP	ND	ND
AN220	13.82	15.70	0.18	0.35	0.26	0.46	1.08	SP	ND	ND
AN228	2.18	1.79	0.08	0.10	0.07	0.20	0.15	ND	ND	ND
AN248	2.17	2.48	0.11	0.27	0.11	0.27	0.28	ND	ND	ND
GP001	0.03	0.05	0.03	0.06	0.03	0.09	0.09	ND	ND	ND
GP031	10.89	9.59	0.07	0.12	0.12	0.10	0.10	SP	ND	ND
GP074	19.59	16.40	0.27	0.34	0.24	0.34	0.58	SP	ND	ND
GP094	0.04	0.09	0.05	0.06	0.05	0.26	0.09	ND	ND	ND
GR063	13.62	0.77	0.60	1.26	0.64	ND	0.66	ND	ND	ND
GR098	4.51	1.02	0.08	0.09	0.07	0.10	0.13	ND	ND	ND
GR101	5.51	5.02	0.05	0.10	0.07	0.06	0.10	SP	ND	ND
GR104	0.00	0.02	0.01	0.02	0.01	0.00	0.03	ND	ND	ND
GR145	30.02	27.50	0.46	0.69	0.61	11.5	0.53	SP	ND	ND
GR211	21.00	4.89	0.05	0.10	0.06	77.4	0.13	ND	9.2	BHR
GR280	0.99	1.23	0.06	0.08	0.07	0.13	0.08	ND	ND	ND
IP161	1.58	1.27	0.08	0.10	0.07	0.11	0.16	ND	ND	ND
IP183	9.28	8.53	0.31	0.58	0.67	0.61	0.47	SP	ND	ND
IP211	1.20	1.36	0.07	0.12	0.11	0.11	0.12	ND	ND	ND
IP241	0.01	0.47	0.01	0.02	0.01	0.01	0.03	ND	ND	ND
IP245	0.94	0.97	0.14	0.19	0.18	0.18	0.33	ND	ND	ND
KD023	7.81	4.56	0.38	0.44	1.13	0.46	0.71	ND	ND	ND
KD065	29.92	29.70	0.82	1.19	1.01	0.79	1.08	SP	ND	BHR
KD068	20.60	20.40	0.09	0.14	0.11	0.26	0.22	SP	ND	ND
KD110	0.00	0.02	0.01	0.02	0.01	0.00	0.03	ND	ND	ND
KD126	64.28	78.30	0.84	1.22	1.01	0.72	1.04	SP	ND	BHR
KD138	13.14	11.80	0.32	1.13	0.38	0.71	0.42	SP	ND	ND
NB049	2.24	0.10	0.08	0.11	0.07	4.8	0.16	ND	ND	ND
NB071	0.52	0.68	0.09	0.10	0.08	0.15	0.19	ND	ND	ND
PA058	3.72	0.13	0.03	0.04	0.04	9.43	0.09	ND	ND	ND
TP095	0.01	0.03	0.01	0.02	0.02	0.06	0.04	ND	ND	ND
TP116	4.67	2.86	0.06	0.09	0.07	0.06	0.08	ND	83.1	ND

Table E2. Characteristics of subjects reporting adverse reactions to peanut

FACTOR	N (%)
Sex (N=21)	
Male	8 (38.1)
Females	13 (61.9)
Age (N=21)	
<11 years or less	13 (61.9)
>more than 11 years	8 (38.1)
Parasitic Infections	
Any intestinal helminth § positive (N=18)	2 (11.1)
<i>S.haematobium</i> positive (N=18)	1 (5.6)
<i>Plasmodium</i> species* (N=14)	3 (21.4)
Symptoms (N=21)	
Itching, tingling or swelling in the mouth, lips or throat	7 (33.3)
Difficulty swallowing	2 (9.5)
A rash, nettle sting-like rash or itchy skin	0 (0.0)
Diarrhoea or vomiting (other than food poisoning)	14 (66.7)
Runny or stuffy nose	3 (14.3)
Red, sore or running eyes	3 (14.3)
Breathlessness	2 (9.5)
Stiffness in your joints	4 (19.1)
Fainting or dizziness	2 (9.5)
Headaches	7 (33.3)
Reaction Time following Ingestion (N=21)	
Minutes	4 (19.1)
Hours	12 (57.1)
Days	2 (9.5)
Missing Information	3 (14.3)
How Long did Symptoms Last? (N=21)	
Minutes	2 (9.5)
Hours	7 (33.3)
Days	9 (42.9)
Missing Information	3 (14.3)

§ Any intestinal helminth= *Ascaris lumbricoides*, hookworm (*Ancylostoma duodenale* or *Necator americanus*), *Trichuris trichiura* or *Schistosoma mansoni*.

**Plasmodium* species = *Plasmodium falciparum* or *Plasmodium malariae* (the 2 malaria parasite species detected in our population).

Table E3 Characteristics of subjects reporting adverse reactions with peanut component-resolved diagnostics information

ID	Area	Age	Sex	Reported Peanut Reactions			Peanut		Specific IgE (kU/L)							
				Reaction Time	Symptoms		SPT	Wheal Size (mm)	Peanut SPT	CCD	Ara h 1	Ara h 2	Ara h 3	Ara h 9	Phlp12	
AB051	R	10	F	Hours	H		-	0.0	40.20	1.83	0.12	0.18	0.18	72.80	0.29	
AN068	R	9	M	ND	D		-	0.0	0.00	0.02	0.01	0.02	0.01	0.01	0.03	
AN102	R	9	F	Hours	I, F, H		-	0.0	2.45	2.50	0.11	0.11	0.10	0.25	0.18	
AN114	R	10	F	Hours	D		-	0.0	ND	ND	ND	ND	ND	ND	ND	
AN190	R	10	M	Hours	I, S, D, J, H		-	0.0	0.00	0.02	0.00	0.02	0.01	0.03	0.02	
AN193	R	9	M	Hours	D		-	0.0	ND	ND	ND	ND	ND	ND	ND	
AN224	R	6	F	Minutes	D		+	5.0	ND	ND	ND	ND	ND	ND	ND	
AN227	R	7	M	Days	D		-	0.0	ND	ND	ND	ND	ND	ND	ND	
AN242	R	9	F	Days	D		-	0.0	ND	ND	ND	ND	ND	ND	ND	
GP001	R	7	M	Hours	D		-	0.0	0.03	0.05	0.03	0.06	0.03	0.09	0.09	
GP031	R	12	F	Hours	D		-	0.0	10.9	9.59	0.07	0.12	0.12	0.1	0.10	
GR189	U	11	M	Hours	I, N,		-	0.0	0.00	ND	ND	ND	ND	ND	ND	
IP239	U	15	F	Minutes	I		ND	ND	ND	ND	ND	ND	ND	ND	ND	
IP245	U	13	F	Hours	I, N, E, B, J, H		-	0.0	0.94	0.97	0.14	0.19	0.18	0.18	0.33	
KD041	R	10	F	Hours	E, S, B, F, H		-	0.0	ND	ND	ND	ND	ND	ND	ND	
KD132	R	11	F	ND	ND		-	0.0	ND	ND	ND	ND	ND	ND	ND	
KD167	R	12	M	Minutes	D		-	ND	ND	ND	ND	ND	ND	ND	ND	
PA095	R	14	F	Minutes	D		-	0.0	0.07	ND	ND	ND	ND	ND	ND	
TP043	R	8	F	Hours	D, F, H		-	0.0	0.26	ND	ND	ND	ND	ND	ND	
TP095	R	10	M	ND	I, D		-	0.0	0.01	0.03	0.01	0.02	0.02	0.06	0.04	
TP128	R	11	F	Hours	I, S, D, N, E, J, H		-	0.0	0.02	ND	ND	ND	ND	ND	ND	

R, rural; **U**, Urban, **M**, Male; **F**, Female; **+**, Positive; **-**, Negative; **ND**, Not done/ No information provided

Reported Symptoms

I, Itching, tingling or swelling in the mouth, lips or throat **S**, Difficulty swallowing
D, Diarrhoea or vomiting (other than food poisoning) **N**, Runny or stuffy nose
B, Breathlessness **J**, Stiffness in your joints

R, A rash, nettle sting-like rash or itchy skin
E, Red, sore or running eyes
F, Fainting or dizziness **H**, Headaches

Table E4. Peanut consumption, preparation methods and associations with peanut sensitization (IgE and SPT) and reported adverse reactions to peanut

Factors	Peanut-specific IgE (≥ 0.35 kU/L vs. <0.35 kU/L)			Peanut SPT Positive (+ vs -)			Reported Adverse Reactions to Peanut (Yes vs. No)		
	Adjusted OR (95%CI)	Wald's Test P-value		Adjusted OR (95% CI)	Wald's Test P-value		Adjusted OR (95% CI)	Wald's Test P-value	
Peanut Consumption Frequency	Daily (yes vs. no)	1.06 (0.73 – 1.53)	0.76	0.36 (0.09 – 1.40)	0.14		1.06 (0.41 – 2.70)	0.91	
	Weekly (yes vs. no)	0.95 (0.68 – 1.32)	0.76	2.81 (0.94 – 8.40)	0.07		0.53 (0.22 – 1.30)	0.17	
	Monthly (yes vs. no)	0.91 (0.55 – 1.52)	0.73	0.79 (0.17 – 3.78)	0.77		0.43 (0.06 – 3.32)	0.42	
	Never (yes vs. no)	1.07 (0.41 – 2.83)	0.88	----	---		5.40 (1.47 – 19.80)	0.01	
Peanut Preparation Methods	Raw (yes vs. no)	0.74 (0.09 – 5.88)	0.78	5.57 (0.56 – 55.57)	0.14		17.14 (2.93 – 100.45)	0.002	
	Boiling ONLY (yes vs. no)	1.18 (0.55 – 2.54)	0.68	1.14 (0.13 – 9.80)	0.91		----	----	
	Frying ONLY (yes vs. no)	0.26 (0.03 – 2.05)	0.20	17.54 (1.57 – 196.33)	0.02		2.67 (0.34 – 21.29)	0.35	
	Roasting ONLY (yes vs. no)	0.90 (0.57 – 1.41)	0.64	2.02 (0.48 – 8.45)	0.34		1.04 (0.37 – 2.82)	0.93	
	Peanut oil (yes vs. no)	0.88 (0.27 – 2.85)	0.83	----	----		----	----	

Peanut-specific IgE models were adjusted for age, sex, area and *S. haematobium* infection.

Peanut SPT models were adjusted for age, sex, area and peanut-specific IgE.

Reported peanut reaction models adjusted for age, sex and area.

Values in boldface indicate significance.

---- Regression model does not generate estimates.

