**Schistosoma mansoni**-specific immune responses and allergy in Uganda

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**Summary**
Low allergy-related disease (ARD) prevalence in low-income countries may be partly attributed to helminth infections. In the *Schistosoma mansoni* (*Sm*)-endemic Lake Victoria islands (Uganda), we recently observed positive helminth-allergy associations, despite low ARD prevalence. To understand how *Sm*-induced cytokine and antibody profiles might influence allergic response profiles in this population, we assessed *Schistosoma* worm (SWA)- and egg antigen (SEA)-specific Th1 (IFN-γ), Th2 (IL-5, IL-13) and regulatory (IL-10) cytokine profiles (*n* = 407), and total (*n* = 471), SWA-, SEA- and allergen (house dust mite [HDM] and cockroach)-specific (as)IgE and IgG4 profiles (*n* = 2117) by ELISA. Wheeze was inversely associated with SWA-specific IFN-γ (*P* < .001) and IL-10 (*P* = .058), and SEA-specific IL-5 (*P* = .004). Conversely, having a detectable asIgE response was positively associated with SWA-specific IL-5 (*P* = .006) and IL-10 (*P* < .001). Total, SWA-, SEA- and allergen-specific IgE and IgG4 responses were higher among *Sm* Kato-Katz positive (SmKK+) and skin prick test (SPT)+ individuals compared to SmKK- and SPT- individuals. However, total and asIgG4/IgE ratios were lower among SPT+ and wheezing individuals. We conclude that, in this population, helminth-induced antibody and cytokine responses may underlie individual positive helminth-atopy associations, while the overall IgG4-IgE balance may contribute to the low overall prevalence of clinical allergies in such settings.

**KEYWORDS**
allergy, cytokine, ELISA, immunoglobulin, *Schistosoma* spp

**1 | INTRODUCTION**

Helminths have a small range of antigens that are strikingly homologous to common allergens.1 These antigens induce immunoglobulin (Ig) E-mediated effector responses important for protection against helminth infection.2,3 To survive in the host, helminths modulate this atopic pathway, and this may have a bystander protective effect against allergy-related disease (ARD).4 While several animal and human studies provide compelling evidence of this protection,5,6 others suggest that in some circumstances helminths may actually promote enhanced responses to allergens.7,8

Mechanisms underlying helminth-allergy associations in low-income countries (LICs) are not fully understood. Hypothesized pathways that underpin these associations are shown in Figure 1. Helminth-induced cytokine and antibody profiles may influence allergic responses and consequently epidemiological trends pertaining to ARDs.5,9 Both helminth- and allergen-specific immune responses are characterized by elevated Th2-type responses (interleukin [IL]-4,
IL-5 and IL-13). Helminths, unlike allergens, further induce strong immunoregulation epitomized by IL-10 production. Typically, these cytokines influence the profile of antibodies involved in helminth infection and allergy. Helminth-induced IL-10 may drive immunoglobulin class switching to IgG4, which, akin to the Th2 cytokine-induced polyclonally stimulated IgE, may inhibit development of allergen-specific effector responses, leading to inverse helminth-allergy associations. Conversely, helminth-induced protein-specific IgE may promote strong, cross-reactive helminth- and allergen-specific responses, resulting in positive helminth-allergy associations.

Emerging epidemiological data on helminth-allergy associations in Uganda reflect the complex interaction between helminths and allergens: while observational analyses in a birth cohort suggested a protective effect of childhood and maternal helminths against childhood eczema that was reversed by maternal anthelminthic treatment, we recently reported positive helminth-allergy associations, with wheeze in the previous 12 months and atopy. Wheeze is widely used as a surrogate for asthma in epidemiological studies and was assessed for all ages using the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire. Such symptom questionnaires have been identified as the best way to estimate asthma prevalence in epidemiological studies. The ISAAC questionnaire was used to ask participants (or their caregivers) if they had ever wheezed and if so, if they had wheezed in the last 12 months. Details on aetiology were not collected. Atopy was defined as (i) SPT reactivity to any of Dermatophagoides mix, Blomia tropicalis or German cockroach (Blattella germanica) [ALK-Abelló; supplied by Laboratory Specialities Ltd., South Africa], and (ii) detectable IgE response (>312 ng/mL by ELISA) to Dermatophagoides pteronyssinus [hereinafter “house dust mite (HDM)’] and/or German cockroach whole allergen extracts (Greer Labs, USA).

Ethics committees of Uganda Virus Research Institute, London School of Hygiene and Tropical Medicine and Uganda National Council for Science and Technology approved the study.

2.2 | Laboratory methods

Two slides from one stool sample per individual were independently examined by different technicians for Sm eggs using the Kato-Katz method.

We assessed IFN-γ (Th1-type), IL-5, IL-13 (Th2-type) and IL-10 (regulatory) levels by ELISA using supernatants from six-day whole blood cultures stimulated with Schistosoma worm (SWA) and egg antigens (SEA), as previously described. Briefly, heparinized blood was diluted with RPMI 1640 medium (Life technologies, UK) supplemented with penicillin, streptomycin, glutamine and Hepes buffer (all from Life technologies, UK), plated in 96-well culture plates and stimulated (at 37°C, 5% CO2) with 10 μg/mL SWA or SEA (provided by Professor Mike Doenhoff, University of Nottingham) or mitogen (phytohaemagglutinin, PHA, Sigma, UK), or left unstimulated. Supernatants were harvested on day six and stored at −80°C until analysis. Cytokine
levels in supernatants were measured by ELISA (Becton Dickinson, USA). The net response to each stimulus was calculated by subtracting the concentration in the unstimulated control well. Response values that were below the dynamic range of the assay and those that were negative after subtraction of the response in the unstimulated well were assigned a value of zero.

HDM and cockroach extract-specific IgE and IgG4 were measured in plasma using an in-house ELISA described previously. Briefly, MICROLON® 96-well plates (Greiner bio-one, UK) were coated over-night at 4°C with 5 μg/mL HDM or cockroach allergens and twofold dilutions of human IgE (Calbiochem, Beeston, UK) or IgG4 (Sigma-Aldrich) standards. Plates were blocked at room temperature (RT) with 1% skimmed milk and incubated overnight at 4°C with plasma samples diluted 1/20 (IgE assay) or 1/40 (IgG4 assay) with 10% foetal bovine serum in PBS-Tween 20. Specific IgE or IgG4 was detected using biotinylated monoclonal mouse anti-human IgE or IgG4 (BD Pharmingen™) and a streptavidin-horseradish peroxidase conjugate (Mast Group Ltd, Bootle, UK). O-phenylenediamine (Sigma-Aldrich) was used as a substrate, and the reaction stopped with 2M sulphuric acid. Optical density values were measured at 490nm (reference wavelength 630nm) on a 96-well plate ELISA reader. IgE or IgG4 concentrations (ng/mL) were interpolated from standard curves using a five-parameter curve fit using Gen5 data collection and analysis software (BioTek Instruments Inc, Vermont, Winooski, USA). Total, SWA- and SEA-specific IgE and IgG4 ELISAs were performed using similar in-house procedures (detailed in this article’s supporting information).

![Image](422x739 to 461x759)

**TABLE 1** Characteristics of participants

| Characteristic                  | Survey population (N = 2316), n/N (%) | Cytokine responses\(^a\) (N = 407) | Allergen-, SWA- and SEA-specific IgE and IgG4\(^b\) (N = 2117) | Total IgE and IgG4\(^c\) (N=471) |
|---------------------------------|--------------------------------------|-----------------------------------|---------------------------------------------------------------|----------------------------------|
| Age in years, median (IQR)     | 24 (8, 32)                           | 9 (6, 16)                         | 25 (10, 33)                                                   | 19.5 (3, 31.25)                  |
| Male sex                       | 1268/2316 (54.7)                     | 168/407 (41.3)                    | 1152/2117 (54.4)                                             | 225/471 (47.7)                   |
| PZQ in last 12 mo              | 382/2255 (16.9)                      | 48/393 (12.2)                     | 368/2062 (17.8)                                              | 15/459 (15.5)                    |
| Helminth infections            |                                      |                                   |                                                               |                                  |
| S. mansoni (KK)                | 1041/1996 (51.4)                     | 204/373 (54.7)                    | 1008/1882 (53.6)                                             | 184/428 (42.9)                   |
| S. mansoni (urine CCA)         | 661/917 (72.0)                       | 94/128 (73.4)                     | 634/875 (72.5)                                               | 101/152 (66.5)                   |
| S. mansoni intensity (KK)      |                                      |                                   |                                                               |                                  |
| Uninfected                     | 995/1996 (48.6)                      | 169/373 (45.3)                    | 874/1882 (46.4)                                              | 244/428 (57.0)                   |
| Low                            | 429/1996 (21.0)                      | 77/373 (20.6)                     | 411/1882 (21.8)                                              | 70/428 (16.4)                    |
| Moderate                       | 288/1996 (13.7)                      | 56/373 (15.0)                     | 279/1882 (14.8)                                              | 51/428 (11.9)                    |
| Heavy                          | 324/1996 (16.6)                      | 71/373 (19.0)                     | 318/1882 (16.9)                                              | 63/428 (14.7)                    |
| Any nematode infection\(^d\)  | 788/2004 (39.3)                      | 129/373 (34.6)                    | 738/1889 (39.1)                                              | 87/428 (20.3)                    |
| Allergy-related outcomes       |                                      |                                   |                                                               |                                  |
| Wheeze in last 12 mo           | 107/2301 (4.7)                       | 14/404 (3.5)                      | 106/2103 (5.04)                                              | 58/468 (12.4)                    |
| Atopy (SPT)                    |                                      |                                   |                                                               |                                  |
| Any                            | 404/1976 (19.1)                      | 78/372 (20.9)                     | 403/1961 (20.6)                                              | 135/448 (30.1)                   |
| Dermatophagoides               | 190/1978 (9.0)                       | 33/372 (8.9)                      | 189/1963 (9.6)                                               | 61/448 (13.6)                    |
| Blomia                         | 205/1976 (9.6)                       | 31/372 (8.3)                      | 204/1961 (10.4)                                              | 67/447 (14.9)                    |
| Cockroach                      | 272/1977 (13.2)                      | 61/372 (16.4)                     | 272/1962 (13.9)                                              | 90/448 (20.1)                    |
| Atopy (detectable asIgE)       |                                      |                                   |                                                               |                                  |
| Any                            | 1685/2117 (79.6)                     | 320/403 (79.4)                    | 1685/2117 (79.6)                                              | 358/471 (76.0)                   |
| Dermatophagoides               | 1534/2115 (72.5)                     | 278/403 (68.9)                    | 1534/2115 (72.5)                                              | 326/471 (69.2)                   |
| Cockroach                      | 886/2117 (41.9)                      | 183/403 (45.4)                    | 886/2117 (41.9)                                              | 186/471 (39.5)                   |

PZQ, Praziquantel treatment; KK, Kato-Katz; CCA, circulating cathodic antigen; SPT, skin prick test; SWA, Schistosoma worm antigen; SEA, Schistosoma egg antigen; asIgE: allergen-specific IgE.

\(^a\) Assessed using samples from 1- to 17-year-olds, to allow comparison with related cellular immunology studies in an urban birth cohort (data not shown here).

\(^b\) Assessed in all survey participants that had sufficient plasma sample stored.

\(^c\) Samples randomly selected from individuals with antigen-specific antibody data.

\(^d\) Infection with any of Ascaris lumbricoides, Trichuris trichiura (assessed by KK), Necator americanus, Strongyloides stercoralis (assessed by PCR) and Mansonella perstans (assessed by modified Knott's method).
2.3 | Statistical methods

Our hypothesized mode of action of *S. mansoni*-induced cytokines and antibodies on allergy-related outcomes is illustrated in Figure 1. Using STATA 13.1 (College Station, Texas, USA), we performed cross-sectional analyses to assess whether Sm Kato-Katz positivity and allergy-related outcomes were associated with antibody and cytokine levels, using the “svy” command to allow for the non-self-weighting cluster survey design. Raw cytokine and antibody responses were skewed, so $\log_{10}$ (concentration+1)-transformed antibody and cytokine data were used in our regression models; we back-transformed the results to obtain geometric mean ratios and 95% confidence intervals. Crude and age- and sex-adjusted analyses were performed.

Associations between antibody responses were estimated using Spearman’s correlation coefficient ($r_s$). We used a 5% significance level for all analyses. $P$ values quoted in the main text are from adjusted analyses.

3 | RESULTS

Questionnaire data were obtained from 2316 participants. Their characteristics and those of participants for whom cytokine and antibody responses were assessed are shown in Table 1. Participants for whom cytokine ($n = 407$) and total antibody levels ($n = 471$) were assessed were a subset of participants who had allergen-, SWA- and SEA-specific antibody results ($n = 2117$). Cytokine responses were assessed using samples from 1- to 17-year-olds, to allow comparison with related cellular immunology studies in an urban birth cohort (data not shown). Allergen-, SWA- and SEA-specific responses were assessed in all survey participants that had sufficient plasma sample stored.

3.1 | *S. mansoni*-specific cytokines and allergy-related outcomes

Individuals who tested positive for Sm by Kato-Katz (SmKK+) had higher geometric mean concentrations of SWA-specific type 2 and regulatory cytokines compared to SmKK- individuals (Table 2), but this was statistically significant only for IL-5 ($P = .058$; Table 2), with SEA-specific IL-5/IFN-γ ratios ($P = .003$) and cockroach-specific IgE ($P = .003$) and cockroach-specific IgG4 ($P = .001$) were all inversely associated with wheeze.

4 | DISCUSSION

In this highly Sm-endemic setting, associations between wheeze and Sm-specific cytokines and antibodies, when significant, were inverse. However, SPT reactivity and detectable aslgE were positively associated with the same Sm-specific responses.

In this population, Sm exposure is almost universal, and infection much higher than indicated by Kato-Katz: urine assessment for Sm circulating cathodic antigen (CCA) indicated a prevalence of over 70%, compared to 51.4% prevalence by Kato-Katz. Therefore, Kato-Katz negativity in many study participants was indicative of lighter (rather than absent) infection. This explains why, although SWA-specific Th2-type and regulatory cytokine responses were generally higher among SmKK+ individuals, only SWA-specific IL-5 reached significant levels, and why SEA-specific responses were similar between SmKK+ and SmKK- individuals. Further support for these observations comes from supplementary analysis (Table S4A), which shows that cytokine responses were similar between SmKK-CCA+ and SmKK+CCA± individuals.
| Antigen | Cytokine | Geometric mean Geometric meana SmKK− n = 169 | SmKK+ n = 204 | Geometric mean Unadjusted Geometric mean Adjusted for age and sex GMR (95% CI)b P value GMR (95% CI)b P value |
|---------|---------|--------------------------------|----------------|--------------------------------|----------------|--------------------------------|----------------|
| SWA     | IFN-γ  | 1.16 1.13 | 1.06 (0.86, 1.30) .542 | 1.05 (0.87, 1.28) .531 |
|         | IL-5    | 14.92 49.47 | 1.43 (1.13, 1.81) .005 | 1.32 (1.02, 1.71) .034 |
|         | IL-13   | 7.01 17.56 | 1.20 (0.94, 1.54) .132 | 1.15 (0.88, 1.48) .282 |
|         | IL-10   | 3.99 11.58 | 1.21 (0.97, 1.51) .084 | 1.16 (0.91, 1.48) .207 |
| SEA     | IFN-γ  | 0.73 0.56 | 0.97 (0.81, 1.17) .760 | 0.98 (0.82, 1.18) .884 |
|         | IL-5    | 5.02 3.11 | 0.84 (0.59, 1.19) .320 | 0.84 (0.58, 1.19) .319 |
|         | IL-13   | 2.25 1.95 | 0.86 (0.71, 1.05) .127 | 0.88 (0.73, 1.06) .190 |
|         | IL-10   | 3.19 4.42 | 0.93 (0.78, 1.13) .486 | 0.93 (0.76, 1.13) .477 |
| SWA     | IFN-γ  | 1.27 0.23 | 0.60 (0.45, 0.80) .001 | 0.57 (0.44, 0.76) <.001 |
|         | IL-5    | 29.12 27.59 | 1.29 (0.72, 2.33) .373 | 1.14 (0.63, 2.08) .657 |
|         | IL-13   | 11.75 9.78 | 1.26 (0.65, 2.45) .465 | 1.17 (0.58, 2.36) .635 |
|         | IL-10   | 7.91 2.03 | 0.69 (0.47, 1.01) .059 | 0.66 (0.43, 1.02) .058 |
| SEA     | IFN-γ  | 0.66 0.33 | 0.83 (0.66, 1.04) .101 | 0.83 (0.65, 1.05) .121 |
|         | IL-5    | 4.37 1.20 | 0.52 (0.33, 0.83) .007 | 0.51 (0.33, 0.79) .004 |
|         | IL-13   | 2.25 0.96 | 0.75 (0.44, 1.25) .256 | 0.76 (0.44, 1.33) .327 |
|         | IL-10   | 4.34 0.59 | 0.70 (0.35, 1.38) .295 | 0.71 (0.37, 1.36) .291 |
| SPT− n = 294 | SPT+ c n = 78 | | | | |
| SWA     | IFN-γ  | 1.03 1.73 | 1.15 (0.96, 1.36) .115 | 1.13 (0.94, 1.34) .178 |
|         | IL-5    | 29.23 39.73 | 1.15 (0.86, 1.52) .330 | 1.02 (0.75, 1.38) .897 |
|         | IL-13   | 13.54 10.32 | 0.99 (0.72, 1.36) .961 | 0.92 (0.67, 1.26) .596 |
|         | IL-10   | 6.79 12.00 | 1.25 (1.00, 1.55) .048 | 1.21 (0.95, 1.54) .126 |
| SEA     | IFN-γ  | 0.55 0.99 | 1.11 (0.82, 1.51) .493 | 1.13 (0.83, 1.52) .423 |
|         | IL-5    | 4.54 3.68 | 1.00 (0.70, 1.45) .965 | 0.98 (0.67, 1.43) .935 |
|         | IL-13   | 2.81 1.09 | 0.84 (0.56, 1.25) .376 | 0.85 (0.58, 1.25) .413 |
|         | IL-10   | 4.12 3.09 | 0.94 (0.76, 1.16) .560 | 0.96 (0.75, 1.22) .730 |
| Undetectable asIgE n = 83 | Detectable asIgE d n = 320 | | | | |
| SWA     | IFN-γ  | 1.73 1.11 | 0.87 (0.62, 1.24) .444 | 0.86 (0.60, 1.23) .396 |
|         | IL-5    | 16.44 34.25 | 1.43 (1.16, 1.75) .001 | 1.32 (1.09, 1.61) .006 |
|         | IL-13   | 9.75 12.66 | 1.09 (0.77, 1.55) .599 | 1.04 (0.74, 1.47) .806 |
|         | IL-10   | 3.76 9.26 | 1.34 (1.18, 1.51) <.001 | 1.30 (1.16, 1.46) <.001 |
| SEA     | IFN-γ  | 1.12 0.56 | 0.79 (0.56, 1.13) .190 | 0.79 (0.57, 1.11) .176 |
|         | IL-5    | 3.78 4.17 | 1.13 (0.81, 1.58) .459 | 1.12 (0.82, 1.54) .450 |
|         | IL-13   | 2.21 2.21 | 0.88 (0.65, 1.22) .449 | 0.90 (0.67, 1.21) .484 |
|         | IL-10   | 3.49 4.17 | 1.05 (0.92, 1.21) .424 | 1.06 (0.93, 1.21) .382 |

SmKK−, Kato-Katz negative result (S. mansoni), single stool sample; SmKK+, Kato-Katz positivity for S. mansoni, single stool sample; SPT, skin prick test; SWA, Schistosoma worm antigen; SEA, Schistosoma egg antigen; asIgE, allergen-specific IgE; GMR, geometric mean ratio; 95% CI, 95% confidence interval. P values ≤.05 are highlighted in bold.

All cytokine concentrations in pg/mL.

Geometric mean ratios and 95% confidence intervals adjusted for the survey design.

SPT reactivity to any one of Dermatophagoides mix, Blomia tropicalis or Blattella germanica.

Detectable IgE to either Dermatophagoides pteronyssinus or Blattella germanica.
All statistically significant associations between atopy and Sm-specific cytokine responses were positive. Associations with whole blood cytokine responses are best interpreted taking into account total cell counts, but these data were unavailable. However, atopy-antibody associations were also positive. Besides, these results mirror our previous epidemiological observations in this population, where Sm infection was positively associated with *Dermatophagoides*-specific IgE, and atopy-wheeze associations were stronger in the presence of Sm infection.21

### TABLE 3  Associations between antibody (IgE and IgG4) levels and Kato-Katz positivity (S. mansoni), SPT reactivity and reported wheeze

| Antigen          | Antibody/antibody ratio | Geometric mean* | SmK+ | aGMR (95% CI)bc | P value |
|------------------|-------------------------|-----------------|------|-----------------|---------|
| SWA              | IgE                     | 1080            | 2433 | 1.54 (1.28, 1.84) | <.001   |
|                  | IgG4                    | 4031            | 27 355 | 3.71 (3.14, 4.37) | <.001   |
| SEA              | IgE                     | 1412            | 1833 | 1.32 (1.15, 1.52) | <.001   |
|                  | IgG4                    | 18 962          | 241 763 | 5.51 (4.55, 6.67) | <.001   |
| House dust mite  | IgE                     | 0.782           | 10.678 | 1.25 (1.07, 1.45) | .006    |
|                  | IgG4                    | 0.001           | 0.192 | 1.79 (1.51, 2.13) | <.001   |
|                  | IgG4/IgE ratio          | 0.002           | 0.033 | 1.18 (0.58, 2.41) | .629    |
| Cockroach        | IgE                     | 18.8            | 19.2 | 1.00 (0.82, 1.22) | .989    |
|                  | IgG4                    | 0.002           | 0.292 | 1.50 (1.34, 1.68) | <.001   |
|                  | IgG4/IgE ratio          | 0.001           | 0.027 | 1.32 (0.94, 1.85) | .110    |
|                  | Total IgE               | 969             | 3073 | 1.37 (1.22, 1.54) | <.001   |
|                  | Total IgG4              | 51 453          | 233 745 | 1.94 (1.49, 2.52) | <.001   |
|                  | Total IgG4/total IgE    | 52.16           | 75.24 | 1.36 (1.11, 1.67) | .005    |
|                  | Total IgE/cockroach IgE | 3.79            | 12.60 | 1.32 (1.06, 1.66) | .014    |
|                  | Total IgE/dust mite IgE | 0.562           | 1.301 | 1.13 (1.02, 1.25) | .016    |
|                  | SWA                     |                |      |                  |         |
|                  | IgE                     | 1704            | 1894 | 1.12 (0.94, 1.32) | .173    |
|                  | IgG4                    | 12 860          | 14 155 | 1.04 (0.85, 1.28) | .675    |
|                  | SEA                     |                |      |                  |         |
|                  | IgE                     | 1611            | 1876 | 1.12 (0.97, 1.29) | .092    |
|                  | IgG4                    | 84 831          | 101 778 | 1.08 (0.92, 1.27) | .319    |
|                  | Dust mite               |                |      |                  |         |
|                  | IgE                     | 2.6             | 42.2 | 1.59 (1.35, 1.89) | <.001   |
|                  | IgG4                    | 0.022           | 0.061 | 1.06 (0.87, 1.29) | .498    |
|                  | IgG4/IgE ratio          | 0.009           | 0.001 | 0.56 (0.36, 0.85) | .010    |
|                  | Cockroach               |                |      |                  |         |
|                  | IgE                     | 18.9            | 39.1 | 1.25 (1.08, 1.46) | .004    |
|                  | IgG4                    | 0.054           | 0.686 | 1.15 (0.95, 1.39) | .129    |
|                  | IgG4/IgE ratio          | 0.003           | 0.017 | 0.69 (0.47, 1.02) | .064    |
|                  | Total IgE               | 1462            | 2787 | 1.22 (1.05, 1.42) | .011    |
|                  | Total IgG4              | 90 643          | 126 688 | 0.84 (0.65, 1.07) | .163    |
|                  | Total IgG4/total IgE    | 60.41           | 45.80 | 0.75 (0.58, 0.95) | .022    |
|                  | Total IgE/cockroach IgE | 6.07            | 9.22 | 1.01 (0.82, 1.25) | .894    |
|                  | Total IgE/dust mite IgE | 0.849           | 0.868 | 0.93 (0.84, 1.03) | .140    |
|                  | Dust mite               |                |      |                  |         |
|                  | IgE                     | 1667            | 2409 | 1.26 (1.00, 1.57) | .043    |
|                  | IgG4                    | 13 088          | 12 565 | 1.04 (0.79, 1.36) | .744    |
|                  | SEA                     |                |      |                  |         |
|                  | IgE                     | 1623            | 1887 | 1.23 (0.99, 1.53) | .055    |
|                  | IgG4                    | 85 471          | 102 026 | 1.20 (0.85, 1.68) | .271    |
|                  | Dust mite               |                |      |                  |         |
|                  | IgE                     | 2.5             | 242.9 | 2.10 (1.57, 2.81) | <.001   |
|                  | IgG4                    | 0.020           | 0.245 | 1.41 (0.99, 1.99) | .052    |
|                  | IgG4/IgE ratio          | 0.009           | 0.001 | 0.49 (0.23, 1.04) | .064    |

(Continues)
Our results were unexpected in view of earlier findings from Gabon, which showed an inverse association between dust mite SPT and SWA-specific IL-10 (albeit we used whole blood cultures, compared to peripheral blood mononuclear cells in the Gabon study). However, although IL-10 is chiefly immunomodulatory, it may also enhance IgE production in already IgE-switched B cells; these may be abundant in individuals from this helminth-endemic setting. SWA- and SEA-specific IgE were weakly positively associated with HDM SPT reactivity, perhaps unsurprisingly, as helminth antigens may induce cross-reactive helminth- and allergen-specific IgE effector responses. Total serum IgE, elevated during helminth infection mainly due to increased synthesis of polyclonal IgE, has been proposed to inhibit allergic responses. However, contrasting evidence links high serum IgE levels to increased expression of IgE receptors on human basophils, and we show positive associations between total IgE and SPT reactivity to both cockroach and dust mite. In keeping with the original hypothesis, associations between wheeze and cytokine and antibody responses, when significant, were inverse. Furthermore, total and allergen-specific IgG4/IgE ratios were mostly inversely associated with atopy, implying that the regulatory role of IgG4 against allergy might best be assessed relative to IgE. Also, lower total/asIgE ratios among HDM SPT+ individuals are consistent with the perception that high total/asIgE ratios may be protective against allergic responses, because nonspecific polyclonal IgE may compete with asIgE to saturate IgE receptors.

One limitation of assessing helminth-allergy associations and underlying mechanisms in this population is the almost universal exposure to helminths, and lack of data on duration of infection. We also report a large number of statistical tests, so some apparently "significant" findings could have occurred by chance. As we anticipated that some of our measures might be correlated, we did not formally adjust for multiplicity, instead we focussed on patterns of association and consistency of results, and on biological plausibility with reference to other...
findings. Another potential limitation is that wheeze was relatively rare in the study population, and hence, some of our comparison groups (such as the age group 1-17 years) had a low prevalence. Besides, reported wheeze could easily be misclassified in this population due to lack of a direct translation of "wheeze" in the native languages.21

Nonetheless, our results generally agree with our epidemiological observations in the same population,21 where we found a very low prevalence of clinical allergies, despite positive helminth-atopy associations.

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DISCLOSURES

None.

AUTHOR CONTRIBUTIONS

AME conceived the main study. AME, RES and MN led the field and clinic teams. AME, GN and JK participated in the design of laboratory studies. GN, JK, BW and JN performed the experiments. GN and ELW analysed the results. GN wrote the manuscript, with all authors contributing to the interpretation of the results, and revision and approval of the final manuscript. GN is the guarantor of the article.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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APPENDIX

LaVIISWA trial team
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