Nkurunungi, Gyaviira; Lubyayi, Lawrence; Versteeg, Serge A; Sanya, Richard E; Nassuuna, Jacent; Kabagenyi, Joyce; Kabuubi, Prossy N; Tumusiime, Josephine; Zziwa, Christopher; Kizindo, Robert; +8 more... Niwagaba, Emmanuel; Nanyunja, Carol; Nampijja, Margaret; Mpairwe, Harriet; Yazdani-bakhsh, Maria; van Ree, Ronald; Webb, Emily L; Elliott, Alison M; (2019) Do helminth infections underpin urban-rural differences in risk factors for allergy-related outcomes? Clinical and experimental allergy, 49 (5). pp. 663-676. ISSN 0954-7894 DOI: https://doi.org/10.1111/cea.13335

Downloaded from: http://researchonline.lshtm.ac.uk/id/eprint/4651231/

DOI: https://doi.org/10.1111/cea.13335

Usage Guidelines:

Please refer to usage guidelines at https://researchonline.lshtm.ac.uk/policies.html or alternatively contact researchonline@lshtm.ac.uk.

Available under license: http://creativecommons.org/licenses/by/2.5/
Do helminth infections underpin urban-rural differences in risk factors for allergy-related outcomes?

Gyaviira Nkurunungi1,2 | Lawrence Lubyayi1,3 | Serge A. Versteeg4 |
Richard E. Sanya1,5 | Jacent Nassuuna1,2 | Joyce Kabagenyi1,2 | Prossy N. Kabuubi1,2 |
Josephine Tumusiime1,2 | Christopher Zziwa1,2 | Robert Kizindo1,2 |
Emmanuel Niwagaba1,2 | Carol Nanyunja1,2 | Margaret Nampijja1,2 |
Harriet Mpairwe1,2 | Maria Yazdanbakhsh6 | Ronald van Ree4 | Emily L. Webb7 |
Alison M. Elliott1,2

1Immunomodulation and Vaccines Programme, (MRC/UVRI and LSHTM) Uganda Research Unit, Medical Research Council/Uganda Virus Research Institute, Entebbe, Uganda
2Department of Clinical Research, London School of Hygiene and Tropical Medicine, London, UK
3Department of Epidemiology and Biostatistics, School of Public Health, University of the Witwatersrand, Johannesburg, South Africa
4Departments of Experimental Immunology and of Otorhinolaryngology, Amsterdam University Medical Centers, Amsterdam, The Netherlands
5College of Health Sciences, Makerere University, Kampala, Uganda
6Department of Parasitology, Leiden University Medical Center, Leiden, The Netherlands
7MRC Tropical Epidemiology Group, Department of Infectious Disease Epidemiology, London School of Hygiene and Tropical Medicine, London, UK

Correspondence
Gyaviira Nkurunungi, MRC/UVRI and LSHTM Uganda Research Unit, Entebbe, Uganda & London School of Hygiene and Tropical Medicine, London, UK.
Email: gyaviira.nkurunungi@mrc-uvri.org

Funding information
The LaVIISWA study and the urban survey were funded by the Wellcome Trust, grant

Summary

Background: It is proposed that helminth exposure protects against allergy-related disease, by mechanisms that include disconnecting risk factors (such as atopy) from effector responses.

Objective: We aimed to assess how helminth exposure influences rural-urban differences in risk factors for allergy-related outcomes in tropical low- and middle-income countries.

Methods: In cross-sectional surveys in Ugandan rural Schistosoma mansoni (Sm)-endemic islands, and in nearby mainland urban communities with lower helminth exposure, we assessed risk factors for atopy (allergen-specific skin prick test [SPT] reactivity and IgE [asIgE] sensitization) and clinical allergy-related outcomes (wheeze, urticaria, rhinitis and visible flexural dermatitis), and effect modification by Sm exposure.

Results: Dermatitis and SPT reactivity were more prevalent among urban participants, urticaria and asIgE sensitization among rural participants. Pairwise associations between clinical outcomes, and between atopy and clinical outcomes, were stronger in the urban survey. In the rural survey, SPT positivity was inversely associated with bathing in lakewater, Schistosoma-specific IgG4 and Sm infection. In the urban survey, SPT positivity was positively associated with age, non-Ugandan maternal tribe, being born in a city/town, BCG scar and light Sm infection. Setting (rural vs urban) was an effect modifier for risk factors including Sm- and Schistosoma-specific IgG4. In both surveys, the dominant risk factors for asIgE sensitization were Schistosoma-specific antibody levels and helminth infections. Handwashing and recent malaria treatment reduced odds of asIgE sensitization among rural but not urban participants. Risk factors for clinical outcomes also differed by setting. Despite suggestive...
Advances in health and hygiene practices have transformed high-income countries into “cleaner” environments, with reduced infection exposure. Consequently, homeostatic immunomodulatory effects of exposure to microbes and parasites that co-evolved with mammalian species (the “old friends hypothesis”) have been lost. The surge in allergy-related diseases alongside other chronic inflammatory diseases in high-income countries over recent decades has been partly attributed to this phenomenon. Although other environmental exposures may contribute, substantial support for the “old friends hypothesis” comes from studies in high-income countries, which show that traditional farming and related microbial exposures are associated with protection against allergy-related diseases. Additional evidence suggests a parallel relationship between ongoing urbanization and increasing allergy-related disease prevalence in tropical low- and middle-income countries (LMICs).

Akin to farming environments in high-income countries, rural LMIC settings are relatively protected against allergy-related diseases. Animal models and in vitro experiments in human samples have identified helminths as potent inhibitors of allergic reactions, leading to the hypothesis that they are partly responsible for the low overall prevalence of allergy-related diseases in tropical LMICs and the observed rural-urban disparities in allergy-related disease prevalence in the same settings. Helminths may dissociate risk factors, such as atopy, from allergy-related disease: work in Ugandan children showing that hookworm infection dissociates allergen-specific IgE from the effector phase of the allergic response is strongly suggestive. However, little comparative analysis of risk factors for allergy in rural vs urban LMIC settings has been conducted. Exploration of these factors in LMICs, where an epidemiological transition is ongoing, provides an unprecedented opportunity to better understand interactions between the environment and the allergic pathway and allergy-related disease outcomes.

Using data generated from two surveys in Uganda, one in rural helminth-endemic Lake Victoria island fishing villages and another in nearby mainland urban communities with lower helminth exposure, we investigated socio-demographic, behavioural, clinical and immunological characteristics as risk factors for allergy-related outcomes and assessed whether helminth infections contribute to rural-urban differences in these risk factors.

Conclusions and clinical relevance: Risk factors for allergy-related outcomes differ between rural and urban communities in Uganda but helminth exposure is unlikely to be the sole mechanism of the observed effect modification between the two settings. Other environmental exposures may contribute significantly.

KEYWORDS
allergy, effect modification, helminths, risk factors, Uganda, urban-rural

2 | METHODS

2.1 | Study settings and procedures

Rural participants were residents of 26 helminth-endemic fishing villages of Koome islands, Mukono district, Uganda (population 18 778 in 2014). Urban participants were residents of Entebbe Municipality, a lower helminth exposure area situated on the northern shores of Lake Victoria, 40 km southwest of the Ugandan capital, Kampala, and 35 km from Koome. The municipality had approximately 69 430 inhabitants in 2014 distributed across 24 sub-wards, the smallest administrative units.

The “rural survey” was part of the Lake Victoria Island Intervention Study on Worms and Allergy-related diseases (LaVIISWA; ISRCTN47196031), a cluster-randomized trial of standard vs intensive anthelminthic intervention, described elsewhere. A baseline household survey preceded the trial intervention; helminth-allergy associations at baseline have been reported. A household-based
Allergy outcomes survey (the “rural survey”) was conducted between September 2015 and August 2016, following 3 years of anthelmintic intervention: there was no difference in the prevalence of allergy outcomes between the two trial arms.26 Sampling for the survey involved random selection of 70 households from each village using a Stata program. All household members (1 year and older) of selected households were then invited to participate. Permission for household participation was granted by the household head.

The urban survey of allergy-related outcomes (September 2016–September 2017) was designed intentionally to collect data from Entebbe municipality for comparison with the helminth-endemic rural survey. Before the start of the survey, each sub-ward was mapped onto satellite imagery of the municipality. A random point generation function of ArcGIS software (version 10.4.1, Environmental Systems Research Institute, Redlands, CA) was then used to generate random starting points within each sub-ward. The number of starting points selected was proportional to the population size of the sub-ward. Coordinates of the random starting points generated were loaded onto geographic information system (GIS) devices (eTrex®, Garmin™ Ltd, Olathe, KS). These devices were then used in the field to identify the selected random points, from which the nearest four houses were surveyed.

There was no randomization to intensive or standard anthelmintic treatment in the urban survey; however, all other procedures were designed to be identical in both the urban and the rural survey.

Following written informed consent and assent, questionnaires were completed for each participant, capturing socio-demographic, clinical and behavioural characteristics as well as asthma, eczema and allergy symptoms. The latter employed questions based on the International Study on Allergy and Asthma in Children (ISAAC) questionnaire. Blood, stool and mid-stream urine were collected. Blood samples were used for haemo-parasitology, HIV serology and storage of plasma and cells for immunoassays. One stool sample per participant was examined for intestinal helminth infections using the Kato-Katz method27 (two slides, read by different technologists). The remaining sample was stored and later investigated for Schistosoma mansoni (Sm), Strongyloides stercoralis and hookworm (Necator americanus) infections using multiplex real-time PCR.28,29 Urine was assessed for Sm circulating cathodic antigen (CCA, Rapid Medical Diagnostics, Pretoria, South Africa). Schistosoma egg [SEA] and adult worm [SWA] antigen-specific immunoglobulin (Ig)E, IgG4 and IgG levels were assessed in plasma using in-house ELISAs (Data S1).

Ethics committees of Uganda Virus Research Institute (refs: GC/127/12/05/03 and GC/127/16/02/547) and London School of Hygiene and Tropical Medicine, (refs: 6187 and 10709) and the Uganda National Council for Science and Technology (ref: HS1183 and HS2036) approved both surveys.

2.2 Allergy-related outcomes

Outcomes were skin prick test (SPT) reactivity to allergens common in our setting,30 allergen-specific IgE (asIgE) sensitization, self-reported recent (previous 12 months) wheeze, recent rhinitis, recent urticarial rash and visible flexural dermatitis.

Skin prick test reactivity (wheal ≥3 mm diameter after 15 minutes in the presence of saline [negative] and histamine [positive] controls) to dust mites (Dermatophagoides mix, Blomia tropicalis) and German cockroach (Blattella germanica) (ALK-Abelló; supplied by Laboratory Specialities [Pty] Ltd., Randburg, South Africa) was

![Study flowchart](FIGURE 1)
assessed using standard procedures.\(^{31}\) SPT reactivity was defined primarily as a positive response to any of the three allergens. SPT reactivity was also analysed as a positive vs negative response to individual allergens.

Whole allergen (Dermatophagoides pteronyssinus, peanut [A. hypogaea] and B. germanica) extract-specific plasma IgE (asIgE) was measured by ImmunoCAP® (ThermoFisher Scientific, Uppsala, Sweden) in a sample of 780 and 345 rural and urban survey participants, respectively, randomly selected from those with sufficient volume of stored plasma. Allergen-specific IgE sensitization was defined as a positive ImmunoCAP response (IgE concentration $\geq 0.35$ kU/L) to any of the three allergens and as a positive vs negative ImmunoCAP response for individual allergens. ImmunoCAP IgE outcomes were also analysed as continuous variables.

Wheeze is considered a good proxy for asthma in epidemiological studies\(^{32}\) and was assessed separately in two age groups ($\geq 5$ years and $<5$ years) using an interviewer-administered ISAAC questionnaire. The principal age group of interest was $\geq 5$ years because wheeze cannot be assumed to represent asthma in children below 5 years.\(^{33}\)

Data on recent rhinitis (runny/blocked nose or sneezing accompanied by watery and itchy eyes, in the absence of cold or "flu") and urticarial rash (pruritic rash with weals, known as "ebilogologo" in the local language [Luganda]) were obtained by questionnaire. Visible flexural dermatitis was assessed (by staff trained on Williams’ online manual\(^{34}\)) as an erythematos rash with surface change in and around skin creases.\(^{35,36}\)

### 2.3 Statistical methods

Data analysis was conducted using Stata 13.1 (College Station, TX). The following were assessed as potential risk factors for allergy-related outcomes: socio-demographic characteristics (age, sex, presence of older/younger siblings, maternal tribe, paternal tribe, location of birth and occupation), behavioural characteristics (frequency of lake contact, type of bathing water, handwashing behaviour, footwear outside the house, smoking and alcohol use), clinical characteristics (helminth infections, exposure to anthelmintic treatment in utero, anthelmintic treatment in last 12 months, parental history of allergies, BCG scar, immunisation history, malaria treatment in last 12 months, malaria infection and HIV infection) and immunological characteristics (plasma SEA- and SWA-specific IgE, IgG4 and IgG levels). Additionally, allergy-related outcomes were independently assessed as risk factors for each other.

Stata "svy" commands were used to allow for clustering of participants within villages and for the non-self-weighting design of the rural survey\(^{24}\) and for clustering by sub-ward in the urban survey.

Logistic regression was used to compare the prevalence of outcomes and other characteristics between the rural and urban survey and to assess associations between each pair of allergy-related outcomes in both surveys. Population attributable fractions (PAFs) for pairs of allergy-related outcomes were calculated. Interaction tests were done to assess whether these associations differed by setting. Unadjusted and adjusted odds ratios (OR) for associations between exposures and allergy-related outcomes were estimated using univariable and multivariable logistic regression. Additionally, linear regression was used in secondary analyses of ImmunoCAP IgE outcomes as continuous variables. Age, sex (a priori) and factors showing evidence of crude association with an outcome ($P < 0.05$) were considered in multivariable analyses for that outcome. We hypothesized that helminth infections might be key mediating factors on the causal pathway between urban/rural residence and allergy-related outcomes; hence, helminths (and Sm-specific antibody responses and other "helminth-related" factors such as frequency of lake contact and occupation) were not included in multivariable analyses for other risk factors. The potential mediating role of helminths was then investigated separately by assessing whether associations between non-helminth-related risk factors and allergy-related outcomes changed substantially when adjusted for Sm infections and Schistosoma-specific antibody levels. These analyses were initially conducted separately for each survey. Subsequently, we merged data from the two surveys and tested for interaction between the rural and urban survey, to assess whether risk factors for allergy outcomes differed by setting. Here, we also assessed the potential role of helminths in urban-rural interactions by comparing interaction $P$ values before and after adjusting for Sm infection. A 5% significance level was used for all analyses.

### 3 RESULTS

#### 3.1 Participants’ characteristics

Flowcharts of the surveys are shown in Figure 1. Of 1820 households randomly selected for the rural survey (70 from each of the 26 villages), 1419 (78%) took part. There were 3566 individuals inhabiting the 1419 participating households; 3323 (93.2%) were interviewed and 3346 (93.8%) had data on at least one allergy-related outcome. Of 420 households randomly selected for the urban survey, 416 (99%) took part. There were 1747 individuals inhabiting the 416 households; 1339 (77%) were interviewed and 1523 (87%) had data on at least one allergy-related outcome.

Participant characteristics differed between the two study settings (Table 1). Significantly, rural, compared to urban participants, were more likely to be infected with helminths (including Sm), malaria and HIV, had higher median levels of Schistosoma-specific antibodies and were more likely to report anthelmintic or malaria treatment in the previous 12 months. Dermatitis and SPT reactivity were more prevalent among urban participants, while aslgE sensitization and urticaria were more common among rural participants (Table 1 and Figure 2A). The prevalence of wheeze and rhinitis was similar between the two communities.

#### 3.2 Associations between allergy-related outcomes

Crude associations between allergy-related outcomes are shown in Table 2. Individuals who were ImmunoCAP aslgE sensitized were
more likely to have a positive SPT response in both surveys; the PAF for SPT reactivity associated with asIgE sensitization was 86.1% and 80.9% for the urban and rural survey, respectively. Atopy measures (asIgE, SPT) were generally more strongly associated with other allergy-related conditions in the urban compared to rural survey; asIgE-rhinitis (interaction $P = 0.081$), asIgE-urticaria (interaction $P = 0.056$), SPT-rhinitis (interaction $P = 0.019$) and SPT-urticaria (interaction $P = 0.005$) associations approached statistical significance. Another major difference was that urticaria was associated with wheeze, rhinitis and SPT reactivity in the urban survey, but not with any allergy-related outcome in the rural survey.

We hypothesized that helminth infection, particularly Sm infection, might mediate this effect modification between the urban and rural setting (Figure 2B). However, the comparison of crude associations (reported above) with associations adjusted for current Sm infection (generally, or categorized by infection intensity) and Schistosoma-specific antibody concentrations did not show clear differences in the test statistics (Table S1); hence, any mediating role of current Sm infection, including effects on interactions between the rural and urban survey, was not evident.

### 3.3 Factors associated with skin prick test reactivity

Table 3 and Table S2 show factors associated with SPT reactivity to any of *Dermatophagoides* mix, *B* tropicalis or *B* germanica. In the urban survey, increasing age, non-Ugandan maternal tribe, being born in a city (compared to town or village) and having a BCG scar were positively associated with SPT reactivity. Additionally, light Sm infection (KK) and Sm infection (PCR) were positively associated with SPT reactivity in the urban survey, in sharp contrast to observations in the rural survey, where current Sm infection (KK, PCR and CCA) was associated with reduced odds of SPT reactivity. This rural-urban difference was statistically significant (interaction $P$ values = 0.002 and 0.015 for Sm-PCR and Sm-KK intensity, respectively). Other factors inversely associated with SPT reactivity in the rural survey were related to helminth infections and included bathing in lakewater and SWA-specific IgG4.

In addition to the Sm-SPT association, tests for interaction showed that associations between several other risk factors and SPT reactivity differed by survey setting. Being male ($P = 0.015$), maternal history of allergies ($P = 0.013$), SWA-specific IgG4 ($P = 0.011$) and hand washing ($P = 0.001$) were positively associated with SPT in the urban survey but inversely associated with the same outcome in the rural survey. The inverse association between SPT and being born in a village (compared to town or city) was stronger in the urban compared to rural survey ($P = 0.041$).

Associations with SPT reactivity to individual allergens are summarized in Table S3, and paint a similar picture.

Comparison of models with and without additional adjustment for current Sm infection (generally, or categorized by infection intensity) and Schistosoma-specific antibodies did not suggest any mediating role of Sm infection in associations between non-helminth-related risk factors and SPT reactivity, or in interactions between the rural and urban survey (Table S4A).

### 3.4 Factors associated with allergen-specific IgE sensitization

Table 4 and Table S5 show factors associated with ImmunoCAP IgE sensitization to any of *D* pteronyssinus, *A* hypogaea or *B* germanica extracts. In the urban survey, the presence of younger siblings and SWA-specific IgG were associated with asIgE sensitization. Rural participants who washed hands after toilet use, slept under a mosquito net and/or had recently been treated for malaria were less likely to be asIgE sensitized. Engaging in agricultural/fishing/lake-related activities or being unemployed, Sm infection (KK) and intensity, and elevated SWA-specific IgE increased the odds of asIgE sensitization.

The presence of younger siblings (interaction $P = 0.008$) and hand washing (interaction $P = 0.003$) were associated with reduced odds of asIgE sensitization in the rural but not the urban survey (Table 4). Adjusting for Sm infection in multivariable analysis models did not suggest a mediating role for Sm in these rural-urban differences (Table S4B).

Table S6 summarizes factors associated with ImmunoCAP asIgE sensitization to individual allergens: Schistosoma-specific antibody levels and helminth infections were the predominant risk factors in both surveys. Hygiene practices (washing and bathing) reduced the odds of sensitization in the rural but not urban survey.

### 3.5 Factors associated with clinical allergy-related outcomes

Factors associated with self-reported recent wheeze, urticarial rash and rhinitis are shown in Table S7. Risk factors for visible flexural dermatitis could not be assessed because it was rare in both settings. In the urban survey, the presence of older siblings, handwashing before eating, SWA-specific IgG and SEA-specific IgG were inversely associated with wheezing. In the rural survey, female sex and presence of any nematode infection were inversely associated with wheezing, while increasing age, SWA-specific IgG, SEA-specific IgG and paternal history of allergies increased the odds of wheezing. Non-Ugandan paternal tribe (interaction $P < 0.001$) increased the odds of wheezing in the urban but not rural survey, while SWA-specific IgG ($P < 0.001$) and SEA-specific IgG ($P = 0.001$) were positively associated with wheezing in the rural but not the urban survey.

Urban individuals who received any anthelminthic treatment in the previous 12 months were more likely to report urticarial rash. In the rural survey, increasing age, maternal history of allergies, SEA-specific IgE and recent malaria treatment were associated with urticaria. The association between SEA-specific IgE and urticaria was positive in the rural but not urban survey (interaction $P = 0.022$). No other significant interactions were observed.

Maternal and paternal history of allergies, and HIV infection were associated with rhinitis in the urban survey. The following were risk factors for rhinitis in the rural survey: increasing age, presence of...
**TABLE 1** Characteristics of study participants

| Characteristics                        | Urban survey n/N (%) | Rural survey n/N (%) | P value*  |
|----------------------------------------|----------------------|----------------------|-----------|
| **Socio-demographic**                  |                      |                      |           |
| Age in (y), median (IQR)               | 20 (8, 31)           | 24 (8, 34)           | 0.329**   |
| Male sex                               | 688/1610 (42.7)      | 1738/3350 (49.5)     | 0.002     |
| **Place of birth**                     |                      |                      |           |
| City                                   | 53/513 (10.3)        | 61/2406 (2.9)        |           |
| Town                                   | 138/513 (26.9)       | 254/2406 (10.4)      |           |
| Village                                | 322/513 (62.7)       | 2091/2406 (86.7)     | <0.001    |
| **Maternal tribe, larger region grouping** |                   |                      |           |
| Central Uganda                         | 605/1331 (45.5)      | 1197/3304 (36.5)     |           |
| Other, Ugandan                         | 607/1334 (46.8)      | 1556/3317 (47.5)     |           |
| Non-Ugandan, African                   | 119/1331 (8.9)       | 519/3304 (15.4)      | 0.020     |
| **Paternal tribe, larger region grouping** |                   |                      |           |
| Central Uganda                         | 593/1334 (44.5)      | 1343/3317 (39.8)     |           |
| Other, Ugandan                         | 624/1334 (46.8)      | 1556/3317 (47.5)     |           |
| Non-Ugandan, African                   | 117/1334 (8.7)       | 418/3317 (12.7)      |           |
| Maternal history of allergies (general)| 93/1187 (7.8)        | 366/2930 (12.7)      | <0.001    |
| Paternal history of allergies (general)| 30/1117 (2.6)        | 171/2796 (5.7)       | 0.005     |
| Maternal history of asthma             | 27/1266 (2.1)        | 93/2931 (3.4)        | 0.167     |
| Paternal history of asthma             | 27/1218 (2.2)        | 62/2796 (2.3)        | 0.950     |
| Maternal history of eczema             | 35/1229 (2.8)        | 131/2931 (4.5)       | 0.206     |
| Paternal history of eczema             | 15/1159 (1.3)        | 96/2795 (2.9)        | 0.028     |
| **Occupation, grouped by type**        |                      |                      |           |
| Student or child (not at school)       | 662/1339 (49.5)      | 1166/3323 (36.7)     |           |
| Unemployed or housewife                | 292/1338 (21.8)      | 301/3323 (8.7)       |           |
| Agricultural, fishing or lake related  | 60/1338 (4.5)        | 1389/3323 (38.8)     |           |
| Professional or service providers      | 324/1338 (24.2)      | 467/3323 (15.6)      | <0.001    |
| (Shops, saloons, bars, restaurants, entertainment) | | | |
| **Helminth infections**                |                      |                      |           |
| S mansoni (KK)                         | 86/1197 (7.2)        | 846/2751 (31.8)      | <0.001    |
| S mansoni intensity (KK)               |                      |                      |           |
| Uninfected                             | 1111/1397 (82.8)     | 1905/2751 (68.2)     |           |
| Low                                    | 41/1197 (3.4)        | 425/2751 (15.7)      |           |
| Moderate                               | 31/1197 (2.6)        | 231/2751 (9.1)       |           |
| Heavy                                  | 14/1197 (1.1)        | 190/2751 (7.1)       | <0.001    |
| S mansoni (urine CCA)                  | 581/1318 (44.1)      | 2445/2879 (85.6)     | <0.001    |
| S mansoni (PCR)                        | 204/1191 (17.1)      | 1338/2747 (50.0)     | <0.001    |
| A lumbricoides (KK)                    | 0/1197 (0.0)         | 14/2751 (0.4)        |           |
| Trichuris trichiura (KK)               | 21/1196 (1.8)        | 245/2751 (7.8)       | <0.001    |
| N americanus (PCR)                     | 56/1191 (4.7)        | 259/2747 (8.4)       | 0.016     |
| S stercoralis (PCR)                    | 29/1191 (2.4)        | 190/2747 (6.2)       | <0.001    |
| **Schistosoma-specific antibody levels** |                   |                      |           |
| SEA-specific IgE (μg/mL), median (IQR)| 2.7 (2.6, 2.8)       | 4.6 (4.3, 4.8)       | <0.001**  |
| SWA-specific IgE (μg/mL), median (IQR)| 2.2 (2.1, 2.4)       | 4.9 (4.6, 5.1)       | <0.001**  |
| SEA-specific IgG4 (μg/mL), median (IQR)| 30.8 (27.8, 37.3)    | 278.6 (228.7, 322.4) | <0.001**  |
| SWA-specific IgG4 (μg/mL), median (IQR)| 42.7 (40.5, 44.1)    | 108.6 (98.3, 124.7)  | <0.001**  |
TABLE 1  (Continued)

| Characteristics                                      | Urban survey n/N (%)a | Rural survey n/N (%)a | P value* |
|-------------------------------------------------------|-----------------------|-----------------------|----------|
| SEA-specific IgG (μg/mL), median (IQR)                | 777.9 (744.6, 806.1)  | 1975.4 (1848.0, 2096.4) | <0.001** |
| SWA-specific IgG (μg/mL), median (IQR)                | 795.4 (771.2, 828.6)  | 1497.2 (1429.4, 1561.5) | <0.001** |

Allergy-related outcomes

Skin prick test reactivity

|                          | Urban survey n/N (%) | Rural survey n/N (%) | P value* |
|--------------------------|----------------------|----------------------|----------|
| Any                      | 302/1317 (22.9)      | 576/3037 (19.1)      | 0.054    |
| Dermatophagoides mix     | 228/1317 (17.3)      | 326/3037 (10.5)      | <0.001   |
| B tropicalis             | 184/1317 (13.9)      | 229/3036 (7.9)       | <0.001   |
| B germanica              | 186/1320 (14.1)      | 350/3035 (11.8)      | 0.137    |

Allergen-specific IgE (≥0.35 kU/L, ImmunoCAP)

|                          | Urban survey n/N (%) | Rural survey n/N (%) | P value* |
|--------------------------|----------------------|----------------------|----------|
| Any                      | 148/345 (42.9)       | 437/780 (55.1)       | 0.007    |
| D pteronyssinus          | 104/345 (30.1)       | 264/780 (33.2)       | 0.421    |
| B germanica              | 118/345 (34.2)       | 393/780 (49.8)       | <0.001   |
| A hypogaea               | 41/345 (11.8)        | 114/780 (14.9)       | 0.266    |

Total IgE (kU/L), median (IQR)

|                          | Urban survey n/N (%) | Rural survey n/N (%) | P value* |
|--------------------------|----------------------|----------------------|----------|
| Wheeze in last 12 mo, age<5 y | 3/229 (1.3)         | 9/547 (1.4)          | 0.972    |
| Wheeze in last 12 mo, age ≥ 5 y | 24/1107 (2.2)     | 87/2776 (3.2)        | 0.190    |
| Visible flexural dermatitis | 22/1435 (1.5)      | 5/3111 (0.1)         | <0.001   |
| Rhinitis in last 12 mo    | 45/1336 (3.4)       | 104/3323 (3.2)       | 0.806    |
| Urticarial rash in last 12 mo | 53/1336 (3.9)    | 334/3322 (9.9)       | <0.001   |

Other

|                          | Urban survey n/N (%) | Rural survey n/N (%) | P value* |
|--------------------------|----------------------|----------------------|----------|
| Any worm treatment in the last 12 mo | 795/1296 (61.3)   | 2938/3307 (87.7)    | <0.001   |
| Malaria treatment in the last 12 mos | 506/1336 (37.8)  | 1993/3323 (60.8)    | <0.001   |
| P falciparum positivity by blood smear | 3/1347 (0.2)     | 102/2923 (3.7)      | <0.001   |
| HIV infection             | 66/1339 (4.9)       | 402/2399 (17.3)      | <0.001   |

CCA: circulating cathodic antigen; IQR: interquartile range; KK: Kato-Katz; PCR: polymerase chain reaction; SEA: Schistosoma egg antigen; SWA: Schistosoma adult worm antigen.

*Percentages adjusted for survey design. Percentages that are significantly higher in one setting compared to the other (P ≤ 0.05) are highlighted in bold. Adjusting for age and sex differences had no significant impact on these differences.

*P values obtained from survey design-based logistic regression.

**P values obtained from survey design-based linear regression.

FIGURE 2  Urban-rural differences in risk factors for allergy-related outcomes in Uganda: a role for helminths? A, summary of principal findings regarding prevalence of allergy-related outcomes in urban Uganda and in rural Ugandan fishing communities. B, Risk factors for allergy-related outcomes differed between urban and rural settings. Our data suggest that helminth exposure is unlikely to be the only factor involved in this effect modification. Additional hypothesized effect modifiers are indicated.
older siblings, being born in a city (compared to town or village) and bathing in lakewater. The positive association between HIV and rhinitis was stronger in the urban compared to the rural survey (interaction $P = 0.028$). No other significant interactions were observed.

We did not find any evidence to suggest that current Sm infection influenced associations between non-helminth-related risk factors and clinical allergy-related outcomes, and interactions between the rural and urban survey (Table S4, C-E).

| TABLE 2 | Crude associations between allergy-related outcomes |
|----------|---------------------------------------------------|
|          | SPT | Wheeze | Rhinitis | Urticaria |
| asIgE    |     |        |         |           |
| Urban    | OR (95% CI) | 21.4 (10.2, 44.6) | 5.5 (0.4, 68.6) | 3.7 (1.2, 11.9) | 3.7 (0.8, 16.2) |
|          | $P$ value | <0.001 | 0.171 | 0.028 | 0.075 |
|          | PAF (95% CI) | 86.1% (81.4, 88.3) | 65.5% (−120, 78.8) | 53.1% (12.1, 66.6) | 53.1% (−18.2, 68.2) |
| Rural    | OR (95% CI) | 10.3 (5.3, 19.8) | 3.9 (1.3, 11.5) | 1.1 (0.5, 2.6) | 0.9 (0.6, 1.3) |
|          | $P$ value | <0.001 | 0.015 | 0.793 | 0.651 |
|          | PAF (95% CI) | 80.9% (72.7, 85.1) | 62.2% (19.3, 76.5) | 5.7% (−57.9, 35.6) | −4.7% (−35.8, 12.4) |
|          | Interaction $P$ value | 0.127 | 0.792 | 0.081 | 0.056 |
| SPT      |     |        |         |           |
| Urban    | OR (95% CI) | 2.2 (0.6, 8.1) | 6.5 (3.4, 12.5) | 2.2 (1.6, 2.8) |
|          | $P$ value | 0.211 | <0.001 | <0.001 |
|          | PAF (95% CI) | 23.4% (−28.6, 37.6) | 54.2% (45.2, 58.9) | 20.8% (14.4, 24.6) |
| Rural    | OR (95% CI) | 3.0 (1.8, 5.1) | 2.6 (1.7, 3.9) | 1.2 (0.9, 1.6) |
|          | $P$ value | <0.001 | <0.001 | 0.243 |
|          | PAF (95% CI) | 29.2% (17.6, 31.9) | 23.4% (15.5, 27.9) | 3.6% (−2.4, 20.9) |
|          | Interaction $P$ value | 0.647 | 0.019 | 0.005 |
| Wheeze   |     |        |         |           |
| Urban    | OR (95% CI) | 7.4 (1.7, 33.2) | 4.9 (1.1, 21.7) |
|          | $P$ value | 0.011 | 0.035 |
| Rural    | OR (95% CI) | 11.9 (5.7, 24.9) | 1.4 (0.6, 3.3) |
|          | $P$ value | <0.001 | 0.403 |
|          | Interaction $P$ value | 0.557 | 0.127 |
| Rhinitis |     |        |         |           |
| Urban    | OR (95% CI) | 9.6 (5.6, 16.4) |
|          | $P$ value | <0.001 |
| Rural    | OR (95% CI) | 0.7 (0.3, 1.6) |
|          | $P$ value | 0.429 |
|          | Interaction $P$ value | <0.001 |

asIgE: ImmunoCAP IgE sensitization to any of D pteronyssinus, A hypogaea, or B germanica on ImmunoCAP; SPT: skin prick test reactivity to any of Dermatophagoides mix, B tropicalis or B germanica.

Odds ratios (ORs), $P$ values and population attributable fractions (PAFs) were obtained from survey design-adjusted analyses. Visible flexural dermatitis was not assessed because it was rare. Significant associations are highlighted in bold. Interaction $P$ values are shown to denote whether tests for interaction showed statistical evidence for urban-rural differences in associations between allergy-related outcomes, or not.

4 | DISCUSSION

We show risk factors for allergy-related outcomes in proximate Ugandan rural and urban settings. The rural setting was characterized by a significantly higher prevalence of Sm and nematode infections compared to the urban setting. The prevalence of SPT reactivity and visible flexural dermatitis was lower, and that of asIgE sensitization and urticaria higher, in the rural compared to urban setting. Risk factors for these outcomes differed by setting. We investigated the hypothesis that rural-urban differences in risk factors for allergy were attributable to differences in current Sm infection between the two settings.
### TABLE 3  Factors associated with SPT reactivity to any of *Dermatophagoides* mix, *B* tropicalis or *B* germanica

| Factor                              | Urban N (%)a | aOR (95% CI)bc | P   | Rural N (%)a | aOR (95% CI)d | P   | Interaction P |
|-------------------------------------|--------------|----------------|-----|--------------|----------------|-----|---------------|
| **Age**                             |              |                |     |              |                |     |               |
|                                     | 1.02 (1.00, 1.03) | 0.035          |     | 1.02 (1.00, 1.03) | 0.015         |     | 0.384         |
| **Sex**                             |              |                |     |              |                |     |               |
| Male                                | 132 (26)     | 1              |     | 285 (18)     | 1              |     |               |
| Female                              | 170 (21)     | 0.71 (0.49, 1.02) | 0.061 | 291 (20)     | 1.09 (0.79, 1.52) | 0.558 | **0.015**     |
| **Older siblings (Yes/No)**         |              |                |     |              |                |     |               |
| No                                  | 73 (22)      | 1              |     | 113 (24)     | 1              |     |               |
| Yes                                 | 194 (23)     | 1.58 (0.90, 2.76) | 0.103 | 341 (22)     | 0.76 (0.56, 1.03) | 0.076 | 0.133         |
| **Occupation**                      |              |                |     |              |                |     |               |
| Student or child (not at school)    | 111 (20)     | 1              |     | 136 (13)     | 1              |     |               |
| Unemployed or housewife             | 63 (24)      | 1.21 (0.70, 2.08) |     | 61 (22)      | 0.79 (0.34, 1.85) |     |               |
| Agricultural, fishing or lake related | 11 (20)     | 0.74 (0.29, 1.87) |     | 273 (22)     | 0.83 (0.39, 1.72) |     |               |
| Professional or service providers    | 82 (28)      | 1.26 (0.77, 2.06) | 0.709 | 103 (25)     | 0.93 (0.54, 1.62) | 0.932 | 0.473         |
| **Maternal tribe**                  |              |                |     |              |                |     |               |
| Central Uganda                      | 127 (25)     | 1              |     | 212 (20)     | 1              |     |               |
| Other, Ugandan                      | 113 (21)     | 0.82 (0.52, 1.30) |     | 272 (19)     | 0.86 (0.59, 1.27) |     |               |
| Non-Ugandan, African                | 26 (25)      | **1.77 (1.17, 2.70)** | 0.015 | 86 (18)      | 0.76 (0.44, 1.32) | 0.613 | 0.127         |
| **Maternal history of allergies**   |              |                |     |              |                |     |               |
| No                                  | 192 (21)     | 1              |     | 433 (20)     | 1              |     |               |
| Yes                                 | 34 (31)      | 1.68 (0.89, 3.18) | 0.107 | 71 (15)      | 0.90 (0.58, 1.41) | 0.644 | **0.013**     |
| **Location of birth**               |              |                |     |              |                |     |               |
| City                                | 16 (37)      | 1              |     | 12 (21)      | 1              |     |               |
| Town                                | 34 (28)      | 0.56 (0.30, 1.02) |     | 57 (24)      | 0.75 (0.37, 1.52) |     |               |
| Village                             | 60 (21)      | **0.34 (0.18, 0.61)** | 0.004 | 397 (21)     | 0.61 (0.29, 1.28) | 0.419 | **0.041**     |
| **BCG scar**                        |              |                |     |              |                |     |               |
| No                                  | 67 (19)      | 1              |     | 228 (19)     | 1              |     |               |
| Yes                                 | 234 (24)     | **2.22 (1.24, 3.97)** | 0.010 | 345 (19)     | 1.31 (0.96, 1.79) | 0.083 | 0.601         |
| **Lake contact**                    |              |                |     |              |                |     |               |
| Never                               | 72 (18)      | 1              |     |               |                |     |               |
| Rarely                              | 140 (27)     | 0.92 (0.50, 1.67) |     | 22 (33)      | 1              |     |               |
| Once a month                        | 29 (24)      | 0.78 (0.39, 1.61) |     |               |                |     |               |
| Once a week                         | 26 (23)      | 1.04 (0.42, 2.57) | 0.896 | 47 (24)      | 1.04 (0.64, 1.68) |     |               |
| Daily/almost daily                  | 385 (22)     | 0.89 (0.54, 1.48) | 0.499 |               |                |     |               |
| **Bathe in water from lake?**       |              |                |     |              |                |     |               |
| No                                  | 249 (23)     | 1              |     | 25 (36)      | 1              |     |               |
| Yes                                 | 18 (18)      | 0.75 (0.27, 2.06) | 0.558 | 429 (22)     | **0.41 (0.24, 0.71)** | 0.002 | 0.172         |
| **Hand washing after toilet**       |              |                |     |              |                |     |               |
| No                                  | 19 (12)      | 1              |     | 151 (23)     | 1              |     |               |
| Yes                                 | 248 (25)     | 4.67 (0.88, 24.8) | 0.068 | 303 (22)     | 0.78 (0.59, 1.02) | 0.068 | **0.001**     |
| SWA-specific IgG4ε                  | 1.04 (0.86, 1.24) | 0.691          |     | 0.77 (0.63, 0.94) | 0.013         | **0.011**     |
| SEA-specific IgEδ                   | 1.32 (0.90, 1.91) | 0.135          |     | 0.58 (0.29, 1.16) | 0.119         | 0.109         |
| **Sm infection (KK)**               |              |                |     |              |                |     |               |
| Uninfected                          | 221 (22)     | 1              |     | 376 (21)     | 1              |     |               |
| Infected                            | 20 (26)      | 1.47 (0.76, 2.83) | 0.239 | 127 (16)     | **0.68 (0.47, 0.97)** | 0.038 | 0.332         |
| **Sm infection intensity (KK)**     |              |                |     |              |                |     |               |
| Uninfected                          | 221 (22)     | 1              |     | 376 (21)     | 1              |     |               |

(Continues)
from several studies,\textsuperscript{37-39} SPT reactivity was less prevalent in the
in both settings and hence explore the role of
considerable exposure to light
The urban survey was done in the unusual context of a setting with
CCA positivity). However, this enabled us to adjust for
were approximately matched). Another potential limitation was the
risk factors, as both surveys were conducted by the same research
to look for patterns of association
large number of statistical tests, increasing likelihood of chance find-
seasonal effects were approximately matched). Another potential limitation was the
number of statistical tests, increasing likelihood of chance find-
required to account for observed urban-rural differences in allergy
risk factors, as both surveys were conducted by the same research
team, and covered approximately 1 year (so any seasonal effects
were approximately matched). Another potential limitation was the
large number of statistical tests, increasing likelihood of chance find-
ings. However, we were cautious to look for patterns of association
rather than interpreting individual results equally.
In keeping with the “old friends” hypothesis\textsuperscript{1} and observations
from several studies,\textsuperscript{37-39} SPT reactivity was less prevalent in the
helminth-endemic rural setting and was inversely associated with
helminth infections in the same setting. The only exception was \textit{Trichuris trichiura} infection, which was weakly positively associated with
\textit{Dermatophagoides} SPT (Table S3). This lone observation was also manifest in the same communities in a baseline household survey
3 years earlier,\textsuperscript{24} although no other helminth species were associated
with SPT then. The current observations beg further investigation
into the impact of anthelminthic treatment on SPT-helminth associa-
tions in a helminth-endemic setting. In mice, allergic airway inflam-
mation is increased during acute Sm infection but reduces drastically
with progression to chronic infection.\textsuperscript{40} In our urban setting, light Sm
infection was positively associated with SPT reactivity while moderate and heavy infections were inversely associated with the same
outcome (Table 3). “Helminth-related” behavioural characteristics were also inversely associated with SPT reactivity in the rural survey.
It is plausible that in these fishing communities, frequent lake con-
tact, bathing in lakewater and handwashing, for example, increase
the risk for Sm infection through contact with infected snails. Indeed, these characteristics were strongly associated with Sm infection
(\(P < 0.001\)). However, the same characteristics were also inversely associated with asIgE sensitization in the rural survey but not in the
urban survey.
As discussed earlier, Pinot de Moira and colleagues’ study in a
Ugandan village found that hookworm infection abrogated the

\begin{table}[h]
\centering
\begin{tabular}{|l|c|c|c|c|c|c|}
\hline
\textbf{Factor} & \multicolumn{2}{c|}{\textbf{Urban}} & \multicolumn{2}{c|}{\textbf{Rural}} & \textbf{Interaction} \\
 & N (%)\textsuperscript{a} & aOR (95% CI)\textsuperscript{b} & P & N (%)\textsuperscript{a} & aOR (95% CI)\textsuperscript{b} & P \\
\hline
Light & 15 (38) & 2.39 (1.24, 4.64) & & 65 (16) & 0.66 (0.43, 1.01) & \\
Moderate & 3 (12) & 0.76 (0.22, 2.61) & & 40 (18) & 0.83 (0.52, 1.34) & \\
Heavy & 2 (14) & 0.55 (0.05, 6.81) & 0.055 & 22 (12) & 0.49 (0.22, 1.14) & 0.053 \textbf{0.015} \\
\hline
\textbf{Sm infection (PCR)} & & & & & & \\
Uninfected & 188 (21) & 1 & & 289 (22) & 1 & \\
Infected & 48 (25) & 1.57 (1.01, 2.43) & 0.044 & 214 (17) & 0.66 (0.49, 0.89) & \textbf{0.010} \textbf{0.002} \\
\hline
\textbf{Sm infection (CCA)} & & & & & & \\
Negative & 163 (24) & 1 & & 114 (27) & 1 & \\
Positive & 115 (22) & 1.19 (0.69, 2.06) & 0.517 & 414 (18) & 0.56 (0.37, 0.83) & \textbf{0.006} \textbf{0.184} \\
\hline
\textbf{Malaria treatment, last 12 mo} & & & & & & \\
No & 163 (24) & 1 & & 234 (21) & 1 & \\
Yes & 100 (22) & 0.86 (0.52, 1.42) & 0.536 & 323 (18) & 1.08 (0.85, 1.38) & 0.502 \textbf{0.730} \\
\hline
\textbf{HIV} & & & & & & \\
Negative & 272 (22) & 1 & & 380 (19) & 1 & \\
Positive & 19 (32) & 1.82 (0.56, 5.93) & 0.302 & 98 (25) & 1.17 (0.74, 1.85) & 0.495 \textbf{0.440} \\
\hline
\end{tabular}
\caption{(Continued)}
\end{table}

Associations shown in this table are from adjusted analyses. Full table with crude associations is shown in supplementary Table S1. This table shows only factors that were associated with SPT reactivity (before and/or after adjustment) in either the urban or the rural survey. All other factors that were assessed are listed in the statistical methods section. Significant associations are highlighted in bold. Interaction \(P\) values are shown to establish whether associations between potential risk factors and SPT reactivity differed, or not, given the setting.

\textsuperscript{a}Number (percentage in parenthesis) of SPT reactive individuals in each category.
\textsuperscript{b}Odds ratios (ORs) and 95\% confidence intervals (CI) were adjusted for survey design.
\textsuperscript{c}ORs were adjusted for HIV infection status, maternal history of allergies, recent malaria treatment, presence/absence of older siblings, age and sex.
\textsuperscript{d}Log10 (concentration+1) transformation applied before analysis.
predicted association between *Dermatophagoides*‐specific IgE and basophil histamine release. We postulated that the rural setting might interfere with the link between atopic sensitization (asIgE, SPT) and clinical outcomes (reported wheeze and rhinitis) through high helminth exposure. Indeed, we found that associations between asIgE or SPT sensitization and clinical outcomes were weak among participants from the rural compared to the urban setting. However, statistical analyses did not suggest that this

| TABLE 4 | Factors associated with IgE sensitization (ImmunoCAP IgE > 0.35 kU/L) to any of *D pteronyssinus*, *A hypogaea* or *B germanica* |
|------------------|------------------|------------------|------------------|
| Factor | Urban | Rural | Interaction | |
| | N (%) | aOR (95% CI) | P | N (%) | aOR (95% CI) | P | |
| Age | | | | 0.99 (0.98, 1.01) | 0.547 | 1.01 (0.98, 1.03) | 0.589 | 0.728 |
| Sex | | | | 241 (64) | 1 | 196 (49) | 0.69 (0.42, 1.14) | 0.140 | 0.407 |
| Male | | | | 0.77 (0.51, 1.15) | 0.200 | 196 (49) | 0.69 (0.42, 1.14) | 0.140 | 0.407 |
| Female | | | | 101 (41) | 1 | 27 (33) | 1 | | |
| Younger siblings (Yes/No) | | | | 106 (46) | 2.07 (1.07, 4.01) | 0.030 | 313 (56) | 0.76 (0.53, 1.09) | 0.129 | 0.008 |
| No | | | | 313 (56) | 1.87 (1.04, 3.37) | 0.008 | 0.008 |
| Yes | | | | 313 (56) | 1.87 (1.04, 3.37) | 0.008 | 0.008 |
| Occupation | | | | 1.01 (0.98, 1.03) | 0.589 | 0.728 | 0.547 | 0.200 |
| Student or child (not at school) | | | | 0.77 (0.51, 1.15) | 0.200 | 196 (49) | 0.69 (0.42, 1.14) | 0.140 | 0.407 |
| Unemployed or housewife | | | | 0.70 (0.31, 1.60) | 0.200 | 196 (49) | 0.69 (0.42, 1.14) | 0.140 | 0.407 |
| Agricultural, fishing or lake related | | | | 0.56 (0.13, 2.46) | 0.200 | 196 (49) | 0.69 (0.42, 1.14) | 0.140 | 0.407 |
| Professional or service providers | | | | 0.61 (0.26, 1.43) | 0.200 | 196 (49) | 0.69 (0.42, 1.14) | 0.140 | 0.407 |
| Lake contact | | | | 251 (61) | 1.87 (1.04, 3.37) | 0.008 | 0.008 |
| Never | | | | 251 (61) | 1.87 (1.04, 3.37) | 0.008 | 0.008 |
| Rarely | | | | 251 (61) | 1.87 (1.04, 3.37) | 0.008 | 0.008 |
| Once a month | | | | 251 (61) | 1.87 (1.04, 3.37) | 0.008 | 0.008 |
| Once a week | | | | 251 (61) | 1.87 (1.04, 3.37) | 0.008 | 0.008 |
| Daily/Almost daily | | | | 251 (61) | 1.87 (1.04, 3.37) | 0.008 | 0.008 |
| Bathe in water from lake? | | | | 251 (61) | 1.87 (1.04, 3.37) | 0.008 | 0.008 |
| No | | | | 251 (61) | 1.87 (1.04, 3.37) | 0.008 | 0.008 |
| Yes | | | | 251 (61) | 1.87 (1.04, 3.37) | 0.008 | 0.008 |
| Hand washing after toilet | | | | 361 (57) | 0.42 (0.15, 1.11) | 0.078 | 0.065 | 0.174 |
| No | | | | 361 (57) | 0.42 (0.15, 1.11) | 0.078 | 0.065 | 0.174 |
| Yes | | | | 361 (57) | 0.42 (0.15, 1.11) | 0.078 | 0.065 | 0.174 |
| S. mansoni infection (KK) | | | | 158 (49) | 1.43 (0.88, 2.30) | 0.138 | 0.796 | 0.177 |
| Uninfected | | | | 158 (49) | 1.43 (0.88, 2.30) | 0.138 | 0.796 | 0.177 |
| Infected | | | | 158 (49) | 1.43 (0.88, 2.30) | 0.138 | 0.796 | 0.177 |
| Any nematode infection | | | | 158 (49) | 1.43 (0.88, 2.30) | 0.138 | 0.796 | 0.177 |
| No | | | | 352 (44) | 1.43 (0.88, 2.30) | 0.138 | 0.796 | 0.177 |
| Yes | | | | 352 (44) | 1.43 (0.88, 2.30) | 0.138 | 0.796 | 0.177 |

(Continues)
differed between rural and urban communities in this tropical and SEA. Our observations that recent anthelminthic treatment (urban survey) difference was mediated by current eLog10 (concentration cAll ORs were adjusted for hand washing after toilet use, mosquito net use, malaria treatment, age and sex. bOdds ratios (ORs) and 95% confidence intervals (CI) adjusted for survey design. aNumber (percentage in parenthesis) of IgE sensitized individuals in each category.

TABLE 4 (Continued)

| Factor                      | Urban N (%) | aOR (95% CI) | P    | Rural N (%) | aOR (95% CI) | P    | Interaction P |
|-----------------------------|-------------|--------------|------|-------------|--------------|------|---------------|
| Slept under mosquito net last night? |             |              |      |             |              |      |               |
| No                          | 35 (45)     | 1            |      | 203 (62)    | 1            |      |               |
| Yes                         | 97 (42)     | 0.93 (0.52, 1.66) | 0.958 | 172 (52)    | 0.63 (0.41, 0.97) | 0.037 | 0.316         |
| Malaria treatment, last 12 mo |             |              |      |             |              |      |               |
| No                          | 83 (45)     | 1            |      | 202 (63)    | 1            |      |               |
| Yes                         | 48 (39)     | 0.78 (0.46, 1.35) | 0.365 | 221 (51)    | 0.52 (0.34, 0.81) | 0.005 | 0.185         |

Associations shown in this table are from adjusted analyses. Full table with crude associations is shown in supplementary Table S1. This table shows only factors that were associated with IgE sensitization (before and/or after adjustment) in either the urban or the rural survey. All other factors that were assessed are listed in the statistical methods section. Significant associations are highlighted in bold. Interaction P values are shown to denote whether tests for interaction showed statistical evidence for urban-rural differences in associations with IgE sensitization, or not.

aOR: adjusted odds ratios; KK: Kato-Katz; SWA: Schistosoma adult worm antigen; SEA: Schistosoma egg antigen.

*Number (percentage in parenthesis) of IgE sensitized individuals in each category.

Odds ratios (ORs) and 95% confidence intervals (CI) adjusted for survey design.

All ORs were adjusted for hand washing after toilet use, mosquito net use, malaria treatment, age and sex.

All ORs were adjusted for age and sex.

Log10 (concentration+1) transformation applied before analysis.

Infection with any of Ascaris lumbricoides, Trichuris trichiura (assessed by KK), Necator americanus, Strongyloides stercoralis (assessed by PCR).

In conclusion, we show that risk factors for allergy-related outcomes differ between rural and urban communities in this tropical setting. However, our analyses did not confirm a role for current helmint (Sm) infection as the primary mechanism of the observed effect modification between the two settings, despite indicative trends. Differences in other environmental exposures may contribute significantly.

ACKNOWLEDGEMENTS

We thank Entebbe municipality and Koome sub-county community members for participating in the urban survey and the rural (LaVIISWA) study, respectively. These findings are presented on behalf of the following members of the LaVIISWA and urban survey research teams: project leaders, physicians, postdoctoral scientists: Richard Sanya, Margaret Nampijja, Harriet Mpairwe, Geraldine O’Hara; laboratory staff and collaborators: Gyaviira Nkurunungi, Joyce Kabagenyi, Jacent Nassuuna, Irene Namuya, Prossy Kabuubi, Emmanuel Niwagaba, Moses Kabunga, Gloria Oduru, Grace Kabami, John Vianney Tushabe, Elson Abayo, Eric Ssebagala, Fred Muwonge, Dennison Kizito, Stephen Cose, Serge Versteeg, Ronald van Ree, Linda Wammes, Jaco Verweij, Maria Yazdanbakhsh; statisticians and data managers: Emily Webb, Remy Hoek Spaan, Lawrence Muhangi, Lawrence Lubayi, Helen Akurut, Fatuma Nalukenge, Justin Okello, Sebastian Owilla, Wilber Ssembajjwe, Jacob Ochola, Jonathan Levin, Stephen Nash; clinical officers: Carol Nanyunja, Milly Namutebi, Christopher Zziwa; nurses: Esther Nakazibwe, Josephine Tumusiime, Caroline Ninsiima, Susan Amongi, Grace Kamukama, Susan Iwala, Florence Akello, Asherwin Ritah, Rehema Nampijja, Gloria Zalwango; internal monitor: Mirriam Akello; field workers: Robert Kizindo, Moses Sewankambo, Denis Nsibuga, Samuel Kiwanuka, Saadh Nsibuga Mwagalanyi, Samuelson Nambaale; social sciences: Edward Tumwesige; boatman: David Abiriga; driver: Richard Walusimbi; HIV counselling and testing: Cynthia Kabonesa; Vector Control Programme staff: James Kaweesa, Edridah Tukahebwa; administrative management: Moses Kizza; principal investigator: Alison Elliott.
CONFLICT OF INTEREST
The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS
AME conceived the LaVIISWA study and the urban survey. GN, AME, MY and RV-R designed the laboratory studies. GN, JK, JN and SV performed the laboratory experiments. AME, RES, MN, PNK, JT, CZ, RK, EN, HM and CN led and participated in field and clinic procedures. GN analysed the results with significant input from LL, ELW, HM, MY and AME. 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.

REFERENCES
1. Rook GA. 99th Dahlem conference on infection, inflammation and chronic inflammatory disorders: Darwinian medicine and the ‘hygiene’ or ‘old friends’ hypothesis. Clin Exp Immunol. 2010;160(1):70–79.
2. Okada H, Kuhn C, Feillet H, Bach JF. The ‘hygiene hypothesis’ for autoimmunity and allergic diseases: an update. Clin Exp Immunol. 2010;160(1):1–9.
3. Takizawa H. Impact of air pollution on allergic diseases. Korean J Intern Med. 2011;26(3):262–273.
4. Braun-Fahrlander C, Riedler J, Herz U, et al. Environmental exposure to endotoxin and its relation to asthma in school-age children. N Engl J Med. 2002;347(12):869–877.
5. Ege MJ, Frei R, Bieli C, et al. Not all farming environments protect against the development of asthma and wheeze in children. J Allergy Clin Immunol. 2007;119(5):1140–1147.
6. Ege MJ, Mayer M, Normand AC, et al. Exposure to environmental microorganisms and childhood asthma. N Engl J Med. 2011;364(8):701–709.
7. Ege MJ, Strachan DP, Cookson WO, et al. Gene-environment interaction for childhood asthma and exposure to farming in Central Europe. J Allergy Clin Immunol. 2011;127(1):138–144, 144 e131–134.
8. Stein MM, Hrusch CL, Gozdz J, et al. Innate immunity and asthma risk in Amish and Hutterite farm children. N Engl J Med. 2016;375(5):411–421.
9. Holbreich M, Genuneit J, Weber J, Braun-Fahrlander C, Waser M, von Mutius E. Amish children living in northern Indiana have a very low prevalence of allergic sensitization. J Allergy Clin Immunol. 2012;129(6):1671–1673.
10. von Mutius E. The microbial environment and its influence on asthma prevention in early life. J Allergy Clin Immunol. 2016;137(3):680–689.
11. Bousquet J, Bousquet PJ, Godard P, Daures JP. The public health implications of asthma. Bull World Health Organ. 2005;83(7):548–554.
12. Pawankar R, Canonica G, Holgate S, Lockey R. WAO White book on Allergy Update 2013. Milwaukee, WI: WAO; 2013.
13. Addo-Yobo EO, Woodcock A, Allotey A, Baffoe-Bonnie B, Strachan D, Custovic A. Exercise-induced bronchospasm and atopy in Ghana: two surveys ten years apart. PLoS Med. 2007;4(2):e70.
14. Keeley DJ,Neill P, Gallivan S. Comparison of the prevalence of reversible airways obstruction in rural and urban Zimbabwean children. Thorax. 1991;46(8):549–553.
15. Ng'ang'a LW, Odhiambo JA, Mungai MW, et al. Prevalence of exercise induced bronchospasm in Kenyan school children: an urban-rural comparison. Thorax. 1998;53(11):919–926.
16. Nicolau N, Siddique N, Custovic A. Allergic disease in urban and rural populations: increasing prevalence with increasing urbanization. Allergy. 2005;60(11):1357–1360.
17. Van Niekerk CH, Weinberg EG, Shore SC, Heese HV, Van Schalkwyk J. Prevalence of asthma: a comparative study of urban and rural Xhosa children. Clin Allergy. 1979;9(4):319–314.
18. Navarro S, Pickering DA, Ferreira IB, et al. Hookworm recombinant protein promotes regulatory T cell responses that suppress experimental asthma. Sci Transl Med. 2016;8(362):362ra143.
19. Qiu S, Fan X, Yang Y, et al. Schistosoma japonicum infection down-regulates house dust mite-induced allergic airway inflammation in mice. PLoS One. 2017;12(6):e0179565.
20. Wilson MS, Taylor MD, Balic A, Finney CA, Lamb JR, Maizels RM. Suppression of allergic airway inflammation by helminth-induced regulatory T cells. J Exp Med. 2005;202(9):1199–1212.
21. Schram ME, Tedja AM, Spijker R, Bos JD, Williams HC, Spuls PI. Is there a rural/urban gradient in the prevalence of eczema? A systematic review Br J Dermatol. 2010;162(5):964–973.
22. Pinot de Moira A, Fitzsimmons CM, Jones FM, et al. Suppression of basophil histamine release and other IgE-dependent responses in childhood Schistosoma mansoni/hookworm coinfection. J Infect Dis. 2014;210(8):1198–1206.
23. Uganda Bureau of Statistics. The National Population and Housing Census 2014 – Main Report. Uganda: Kampala; 2016.
24. Webb EL, Nampijja M, Kaweesa J, et al. Helminths are positively associated with atopy and wheeze in Ugandan fishing communities: results from a cross-sectional survey. Allergy. 2016;71(8):1156–1169.
25. Nampijja M, Webb EL, Kaweesa J, et al. The Lake Victoria island intervention study on worms and allergy-related diseases (LaVIISWA): study protocol for a randomised controlled trial. Trials. 2015;16(1):187.
26. Sanyu RE, Nkurunungi G, Hoek Spaans R, et al. The impact of intensive versus standard anthelmintic treatment on allergy-related outcomes, Helminth infection intensity, and Helminth-related morbidity in Lake Victoria Fishing Communities, Uganda: results from the LaVIISWA cluster-randomized trial. Clin Infect Dis. 2018 ciy761. https://doi.org/10.1093/cid/ciy761.
27. Katz N, Chaves A, Pellegrino J. A simple device for quantitative stool thick-smear technique in Schistosomiasis mansoni. Rev Inst Med Trop Sao Paulo. 1972;14(6):397–400.
28. Verweij JJ, Brienien EA, Ziem J, Yelifari L, Polderman AM, Van Lieshout P. Simultaneous detection and quantification of Ancylostoma duodenale, Necator americanus, and Oesophagostomum bifurcum in fecal samples using multiplex real-time PCR. Am J Trop Med Hyg. 2007;77(4):685–690.
29. Verweij JJ, Canales M, Palman K, et al. Molecular diagnosis of Strongyloides stercoralis in faecal samples using real-time PCR. Trans R Soc Trop Med Hyg. 2009;103(4):342–346.
30. Mpaiwe H, Muhangi L, Ndubazza J, et al. Skin prick test reactivity to common allergens among women in Entebbe, Uganda. Trans R Soc Trop Med Hyg. 2008;102(4):367–373.
31. Heinzerling L, Mari A, Bergmann KC, et al. The skin prick test - European standards. Clin Transl Allergy. 2013;3(1):3.
32. Pekkanen J, Pearce N. Defining asthma in epidemiological studies. Eur Respir J. 1999;14(4):951–957.
33. Deblay J, Stanoevic S, Filbrun AG, Subbarao P. Bronchodilator responsiveness in wheezy infants and toddlers is not associated with asthma risk factors. Pediatr Pulmonol. 2012;47(5):421–428.
34. Williams HC. So how do i define atopic eczema? A Practical manual for researchers wishing to define atopic eczema. http://www.nottingham.ac.uk/~mzzfaq/dermatology/eczema/contents.html. Accessed February 6, 2018.
35. Williams HC. Clinical practice. Atopic dermatitis. N Engl J Med. 2005;352(22):2314–2324.
36. Williams HC, Forsdyke H, Boodoo G, Hay RJ, Burney PG. A protocol for recording the sign of flexural dermatitis in children. Br J Dermatol. 1995;133(6):941-949.

37. Araujo Mi, Lopes AA, Medeiros M, et al. Inverse association between skin response to aeroallergens and Schistosoma mansoni infection. Int Arch Allergy Immunol. 2000;123(2):145-148.

38. Supali T, Djuardi Y, Wibowo H, van Ree R, Yazdanbakhsh M, Sartono E. Relationship between different species of helminths and atopy: a study in a population living in helminth-endemic area in Sulawesi, Indonesia. Int Arch Allergy Immunol. 2010;153(4):388-394.

39. Medeiros M Jr, Almeida MC, Figueiredo JP, et al. Low frequency of positive skin tests in asthmatic patients infected with Schistosoma mansoni exposed to high levels of mite allergens. Pediatr Allergy Immunol. 2004;15(2):142-147.

40. Smits HH, Hammad H, van Nimwegen M, et al. Protective effect of Schistosoma mansoni infection on allergic airway inflammation depends on the intensity and chronicity of infection. J Allergy Clin Immunol. 2007;120(4):932-940.

41. Hamid F, Amoah AS, van Ree R, Yazdanbakhsh M. Helminth-induced IgE and protection against allergic disorders. Curr Top Microbiol Immunol. 2015;388:91-108.

42. Igetei JE, El-Faham M, Liddell S, Schramm G, Doenhoff MJ. Antigenic cross-reactivity between Schistosoma mansoni and pollen allergens from the birch tree (Betula verrucosa) and Timothy grass (Phleum pratense): involvement of shared glycan epitopes and implications for the hygiene hypothesis. Int J Parasitol. 2018;48:345-357.

43. Tyagi N, Farnell EJ, Fitzsimmons CM, et al. Comparisons of allergenic and metazoan parasite proteins: allergy the price of immunity. PLoS Comput Biol. 2015;11(10):e1004546.

44. Santiago Hda C, Ribeiro-Gomes FL, Bennuru S, Nutman TB. Helminth infection alters IgE responses to allergens structurally related to parasite proteins. J Immunol. 2015;194(1):93-100.

45. Santiago HC, Bennuru S, Boyd A, Eberhard M, Nutman TB. Structural and immunologic cross-reactivity among filarial and mite tropomyosin: implications for the hygiene hypothesis. J Allergy Clin Immunol. 2011;127(2):479-486.

46. Santiago HC, LeeVan E, Bennuru S, et al. Molecular mimicry between cockroach and helminth glutathione S-transferases promotes cross-reactivity and cross-sensitization. J Allergy Clin Immunol. 2012;130(1):248-256. e249.

47. Nkurunungi G, Kabagenyi J, Nampijja M, et al. Schistosoma mansoni-specific immune responses and allergy in Uganda. Parasite Immunol. 2018;40(1):e12506.

48. Bakiri AH, Mingomataj EC. Parasites induced skin allergy: a strategic manipulation of the host immunity. J Clin Med Res. 2010;2(6):247-255.

49. Nahshoni A, Baum S, Barzilai A, Schwartz E. Chronic Urticaria in returning travellers: the role of anthelmintic treatment. Dermatology. 2016;232(4):468-471.

SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Nkurunungi G, Lubayai L, Versteeg SA, et al. Do helminth infections underpin urban-rural differences in risk factors for allergy-related outcomes. Clin Exp Allergy. 2019;00:1–14.
https://doi.org/10.1111/cea.13335