

DR THULJA TRIKAMJEE (Orcid ID : 0000-0002-5951-6736)

MR WISDOM BASERA (Orcid ID : 0000-0002-4006-1858)

DR JON GENUNEIT (Orcid ID : 0000-0001-5764-1528)

DR CLAUDIA L GRAY (Orcid ID : 0000-0002-7765-1501)

PROFESSOR MICHAEL ELIAD LEVIN (Orcid ID : 0000-0003-2439-7981)

Article type : Original

Associations between Environmental Dust Composition and Atopic Dermatitis in Urban and Rural Settings

Running title: Environmental dust and AD in urban and rural settings

Thulja Trikamjee, MD

Wisdom Basera MPH

Maresa Botha, MD

Heidi E. Facey-Thomas, Dip. Nursing

Ben Gaunt, MD

Jon Genuneit MD, PhD

Claudia L. Gray, MD, PhD

Sabelo Hadebe

Carol Hlela

Frank Kirstein

Nonhlanhla Lunjani

Avumile Mankahla

Jordache Ramjith

Michael Levin, MD, PhD

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/PAI.13476

This article is protected by copyright. All rights reserved

Affiliations

Thulja Trikamjee, MD

Allergy and Immunology Unit, University of Cape Town Lung Institute, Cape Town,
South Africa

Wisdom Basera MPH

School of Public Health and Family Medicine, Faculty of Health Sciences, University
of Cape Town, Cape Town, South Africa

Burden of Disease Research Unit, South African Medical Research Council, South
Africa

Maresa Botha, MD,

Division of Paediatric Allergy, Department of Paediatrics, Faculty of Health Sciences,
University of Cape Town, South Africa

Heidi E. Facey-Thomas, Dip. Nursing

Division of Paediatric Allergy, Department of Paediatrics, Faculty of Health Sciences,
University of Cape Town, South Africa

Ben Gaunt, MD

Zithulele Hospital, Eastern Cape Department of Health, South Africa

School of Public Health and Family Medicine, Faculty of Health Sciences, University
of Cape Town, Cape Town, South Africa

Jon Genuneit MD, PhD

Institute of Epidemiology and Medical Biometry, Ulm University, Ulm, Germany

Pediatric Epidemiology, Department of Pediatrics, Medical Faculty, Leipzig

University, Leipzig, Germany

Claudia L. Gray, MD, PhD

Division of Paediatric Allergy, Department of Paediatrics, Faculty of Health Sciences,
University of Cape Town, South Africa

Sabelo Hadebe, PhD

Division of Immunology, Department of Pathology, Faculty of Health Sciences,
University of Cape Town, South Africa

Carol Hlela

Department of Dermatology, Faculty of Health Sciences, University of Cape Town,
Cape Town, South Africa

Frank Kirstein, PhD

Division of Immunology, Department of Pathology, Faculty of Health Sciences,
University of Cape Town, South Africa

Nonhlanhla Lunjani

Swiss Institute of Allergy and Asthma Research (SIAF), University of Zurich, Davos,
Switzerland.

Department of Dermatology, Faculty of Health Sciences, University of Cape Town,
Cape Town, South Africa.

APC Microbiome Ireland, University College Cork, Cork, Ireland.

Avumile Mankahla

Division of Dermatology, Department of Medicine and Pharmacology, Walter Sisulu
University, Mthatha, South Africa

Jordache Ramjith, MSc

Department for Health Evidence, Biostatistics Research Group, Radboud Institute for
Health Sciences, Radboud University Medical Center, Nijmegen, Netherlands

Michael Levin

Division of Paediatric Allergy, Department of Paediatrics, Faculty of Health Sciences,
University of Cape Town, South Africa

inVIVO Planetary Health, Group of Worldwide Universities Network (WUN)

Corresponding author

Prof Michael Levin

Division of Paediatric Allergy

Department of Paediatrics

Faculty of Health Sciences

University of Cape Town, South Africa

Email: michael.levin@uct.ac.za

Word count: 2876

Tables: 3

Figures: 0

Conflict of interest

The authors have no conflict of interest to declare.

Funding

This work is supported by a research grant from the Medical Research Council of South Africa and an Allergy Society of South Africa / MSD research award. FK is funded by NRF Competitive Program for Unrated Researchers (CSUR). SH is supported by NRF Thuthuka Grant (117721), MRC Self-initiated grant and Robert Bosch Stiftung Fellowship. NL was supported by an EAACI Research Fellowship Award, South African Medical Research Council (SAMRC), Max and Lillie Sonnenberg University of Cape Town Scholarship, and CK-CARE. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Abstract

Background:

Environmental exposures are involved in the pathogenesis of the allergic phenotype, and in determining which individual triggers a person becomes sensitized to. Atopic

dermatitis (AD) may modulate these effects through increased penetration through the skin modifying the immune system and AD may be triggered or intensified by environmental exposures. These exposures and immune-modulating factors may differ in urban and rural environments.

Objectives:

To compare house dust composition in urban and rural settings and correlate them with AD outcomes.

Methods:

Dust samples were collected from the beds of 156 children aged 6 months to 3 years. 42% of participants had atopic dermatitis. Samples were analysed for bacterial endotoxin, fungal (β -1,3-glucan) levels, and house dust mite, cockroach, dog, cat, mouse and peanut allergen. Exposures were compared in urban and rural environments, and in participants with and without AD.

Results:

Endotoxin but not fungal β -glucan exposure is higher in the environment of healthy controls than children with AD in both urban and rural settings. House dust mite allergen exposure is high in urban and rural settings with *Dermatophagoides* detected in 100% of samples. Cat and dog allergen exposure mirrors pet ownership patterns which differ slightly between groups and environments. Mouse allergen exposure is higher in urban homes.

Conclusion:

Environmental endotoxin may be protective against AD in both urban and rural settings. There are marked differences in allergen exposure in urban and rural settings, but these are unlikely to be important protective or risk factors.

Key words:

Aeroallergen, atopic dermatitis, eczema, endotoxin, environment, house dust,
urbanization

Abbreviations:

AD – Atopic dermatitis
Ara h – *Arachis hypogea*
AR – Allergic rhinitis
AS – Allergic sensitization
Bla g – *Blatella germanica*
Blo t – *Blomia tropicalis*
Can f – *Canis familiaris*
Der p – *Dermatophagoides pteronyssinus*
EU - Endotoxin units
Fel d – *Felis domesticus*
IQR – Interquartile range
LAL assay - limulus amebocyte lysate assay
Mus m – *Mus musculus*
OR – Odds ratio
PBS-T - Phosphate buffered saline 0.05% Tween 20
RAD – Rural atopic dermatitis
RC – Rural control
RT – Room temperature
SA – South Africa
SAFFA study – South African Food Allergy study
SPT – Skin prick test
UAD – Urban atopic dermatitis
UC – Urban control

Main text:

Introduction

Environmental exposures are known to influence the pathogenesis of the allergic phenotype, as well as in determining which individual triggers a person becomes sensitized to¹⁻⁵. The exponential rise in allergic diseases has been attributed to a number of factors, including increased urbanization with loss of protective rural effects and exposure to a westernized lifestyle⁶. Exposure to a diversity of bacteria and fungi or their products in a rural environment, and with direct exposure to farm animals (“farming effect”) has been considered beneficial⁷. The rural environment is protective against development of asthma⁸ and in urban settings, the risk declines as house dust composition becomes more similar to a farm household⁹.

Low microbial diversity and richness of house dust in the homes of children is associated with an increased risk of developing asthma^{8,10}. Farm homes have richer and more diverse microbial contents than urban homes¹⁰. Endotoxin exposure in the environmental dust of Amish children has been shown to be protective against allergies by stimulating the innate immune system¹¹.

The spectrum, timing, load, and route of allergen exposure have also been suggested to play a role in the development and severity of allergic disease and the pattern of sensitization with the skin barrier representing an important route of entry for allergens^{12,13}. Atopic dermatitis (AD) is a common childhood disorder and the first manifestation in the allergic march. The condition is increasing in frequency, but is less prevalent in rural than urban South Africa (SA)^{6,14}. Epicutaneous exposure to allergens is considered a risk factor in the development of AD, with the skin barrier impairment further increasing entry of allergens and affecting its severity. Food allergen sensitization via food in environmental dust can occur via the non-oral route in patients with skin barrier defects, putting them at higher risk of food allergy^{12,13,15,16}.

These environmental exposures and immune-modulating factors may be different in urban and rural environments. House dust mite (HDM) is the most important allergen in many parts of the world, including South Africa (SA)^{17,18}. The European house dust mite (*Dermatophagoides pteronyssinus*) and the American house dust

mite (*Dermatophagoides farinae*) are two immunogenically closely related species, with high sensitization rates found in many parts of SA (40-46%)¹⁷⁻¹⁹. Sensitization to the allergenically distinct tropical mite *Blomia tropicalis* has been described along the coastal regions of SA with a striking association with allergic rhinitis and asthma²⁰.

The SAFFA study demonstrated that the rural environment is protective against AD, asthma and rhinitis, food and aero-allergen sensitization, and confirmed food allergy^{6,14,21}. We analyzed environmental exposures of urban and rural children with and without AD from South Africa for markers of bacterial and fungal exposure as well as aeroallergens and the peanut allergen Ara h1.

Methods

Dust Sampling, Extraction and Analysis

Subjects

Urban control subjects (n=37) were recruited as a sub-study from non-allergic, non-food-sensitized subjects participating in the South African Food Allergy (SAFFA) study at randomly selected creches in the Cape Town metropole. As creches are rarely found in rural districts, rural controls (n=53) were recruited from children of eligible age from communities surrounding 10 district health clinics in the coastal Mqanduli district of the Eastern Cape. Patients with AD from urban settings were recruited from the paediatric dermatology department of the Red Cross War Memorial Children's Hospital in Cape Town (n=36) and rural patients with atopic dermatitis from the dermatology department of the Nelson Mandela Academic Hospital in Mthatha in the Eastern Cape highlands (n=30).

AD was diagnosed based on diagnostic criteria proposed by the American Academy of Dermatology including essential features of pruritus and eczematous rash with typical morphology and age-specific patterns, and a chronic or relapsing history²².

Collection and preparation of dust samples

Dust samples were collected in a standardized manner. The child's sleeping area was identified. Pillows, blankets and duvets were removed from the bed, but sheets were left *in situ*. Using sterile gloves, a nylon filter was inserted into Duststream collector (Indoor biotechnologies, Charlottesville, VA) and attached to the vacuum of a Dyson vacuum cleaner. The child's sleeping area of 1 x 0.5 meters was vacuumed methodically side to side and then perpendicularly until the whole area had been covered for a minimum of 2 minutes, to a maximum of 4 minutes. The filter was removed from the collector, covered with parafilm, labelled, placed into a ziplock bag and frozen at -20 degrees Celsius within 1 hour of collection.

Dust was allowed to defrost for at least 2 hours prior to use. Dust was sieved in a 300 micron device under sterile conditions, divided into 50mg aliquots and stored for further analysis at -80°C.

Analytical methods

Allergen Extraction

Dust samples were resuspended and extracted with 1ml phosphate buffered saline 0.05% Tween 20 (PBS-T) using a vortex mixer and then laboratory rocker at room temperature (RT) for 2 hours. Samples were centrifuged and supernatant was collected for analysis.

Allergen-specific ELISA

ELISAs were performed to Blo t5, Der p1, Mus m1, Fel d1, Can f1, Bla g1 and Ara h1 (Indoor Biotechnology, UK). Microtiter plates (NUNC Maxisorp) were coated with 100µl capture antibody and incubated overnight at 4°C. Plates were blocked for unspecific binding with 1% BSA, PBS-T. 100µl allergen standard was added in duplicates in 1 in 2, 1 in 10, 1 in 30 and 1 in 90 dilutions and incubated for 1 hour at RT. 100µl diluted detection antibody was added and plates were incubated for 1 hour at RT. 100µl/well diluted Streptavidin-Peroxidase (Sigma S5512) was added to Der p1, Mus m1, Blo t5, Fel d1, Ara h1) or 100µl/well goat anti-rabbit IgG (Jackson Laboratories) added to Can f1 and Bla g1. Plates were developed by adding 100µl/well TMB-substrate. The reaction was stopped by adding 100µl/well 1M H₂SO₄

and the absorbance was read at 450nm (SpectraMax microplate reader, Molecular Devices, USA). Concentrations were calculated from the standard curve using SoftMax Pro Microplate Data Analysis Software (Molecular Devices, USA). Concentrations in 1 ml (with 50mg of dust) were converted into ug allergen per g of dust by dividing by 50.

Endotoxin Test

Endotoxin in dust samples was quantified using LAL Chromogenic endpoint Assay (Hycult Biotech, Netherlands). Samples and reagents were brought to RT before use. Standard (50EU/ml) was diluted 1:1 with endotoxin free water (EFW) to make 25 EU/mL in an endotoxin free tube. For duplicate standard curve, 16 wells of a 96 well pryoplate (Pro Med Diagnostics) were filled with 50uL of EFW and the first dilution of 10EU/mL was achieved by pipetting 33uL of 25EU/mL, serial dilutions of 1:1.5 were performed. Samples (50uL/well) and respective controls were added. 50uL of reconstituted LAL reagent was added to all samples including standards and controls and incubated at RT. Plates were read at 405nm using a Biologic Microstation plate reader. Where samples were high/low (above/below the quantifiable limits), we used either more dilute or concentrated dilutions respectively. EU concentrations were calculated from the standard curve using appropriate dilutions. Samples that were undetectable below the level of 0.04EU/mL were further analyzed as 0.

Fungal (1→3)-β-D-glucan test

An endpoint assay procedure was performed using Glucatell kit (Cape Cod Inc. MA). 50μL of standards (40pg/mL - 0pg/mL) and unknown solutions diluted in endotoxin free water were placed in a 96 well plate (Cape Cod Inc. MA). 50μL of Glucatell reagent was added followed by incubation for 30 min at 37°C on a heating block. The reaction was stopped with 50μL of sodium nitrite. 50μL ammonium sulfamate and 50μL of n-(1-Naphthyl)ethylenediamine dihydrochloride was added for color development and the plate was read (SpectraMax microplate reader, Molecular Devices, USA) at optical density of 540nm. Concentrations were calculated from the standard curve using SoftMax Pro Microplate Data Analysis Software (Molecular

Devices, USA). Concentrations in 1 ml (with 50mg of dust) were converted into ug antigen per g of dust by dividing by 50.

Statistical Analysis

Data were entered into a Microsoft Access database and analyzed by using Stata (version 14.2; StataCorp, Texas). The Mann-Whitney test was used to investigate differences between numeric variables, all of which were non-normally distributed. The Fisher exact test was used to assess associations between pairs of categorical variables, and the z test using normal approximation to the binomial distribution was used to investigate differences in proportions. We performed a logistic regression for each of the binarized environmental exposures with study site, AD status and their interaction as covariates in the models. We estimated the odds ratios (ORs) (and 95% CI and p-values) of AD status for urban and rural participants for each outcome. The p-value for the interaction term was used to identify significant differences between the rural and urban AD status ORs for each outcome. A p-value of less than 0.05 was considered statistically significant.

Results

In total, 156 dust samples were collected, of which 83 (53.2%) were from rural and 73 (46.8%) from urban homes. Sixty-six (42.3%) patients had clinically confirmed AD, and ninety (57.7%) were recruited as controls. Among the participants with confirmed AD, thirty (45.5%) were from rural, and thirty-six (55.5%) from urban settings. The control AD cohorts comprised of fifty-three (58.9%) rural, and thirty-seven (41.1%) urban participants.

Endotoxin

House dust endotoxin prevalence and levels were highest in rural control homesteads (97.6%, median level 7759.3EU/mL), followed by urban control homes (97.2%, median 3174.3EU/mL), urban AD (77.4%, median 3106.4EU/mL), then rural AD (75.9%, median 2236.0EU/mL) (table 1, table 2, figure 1). In the rural environment both the prevalence and concentrations of endotoxin were lower in AD than controls (75.9% vs 97.6%; $p=0.01$ and median 2236.0EU/mL vs 7759.0EU/mL;

p<0.001). In the urban environment both the prevalence and concentrations of endotoxin were lower in AD than controls with the prevalence but not concentration differences achieving significance (77.4% vs 97.2%; p=0.02 and median 3106.4EU/mL vs 3174.3EU/mL; p=0.83). Logistic regression confirmed significantly lower prevalence of detectable endotoxin in AD than controls in both urban and rural environments (table 3).

Fungal β -glucan

Fungal exposure patterns did not show any significant differences between the rural and urban environments, or cohorts (table1, table 2, figure 1).

House dust mite

House dust mite was the most prevalent allergen, with Der p1 present in 100% of dust samples (table 1). Concentrations of Der p1 were significantly greater in the rural setting compared to the urban setting (median 4.73ug/g vs 1.02ug/g; p<0.001). Within the urban cohort, concentrations were lower in those with AD than the urban controls (table 2, figure 1).

Blo t5, the major allergen of *Blomia Tropicalis*, was detectable only at very low levels, but more frequently in urban (35.6%), than rural (7.2%) settings and lower prevalence in urban homes of subjects with AD (22.2%) than without (48.6%) (table 1, table 3). Levels were higher in the rural coastal Mqanduli control cohort than the rural highland Mthatha AD cohort (table 2, table 3).

Cat

Both prevalence (28.9% vs. 4.1%) and concentration (4.73ug/g vs. 0.08ug/g) of Fel d1 was higher in rural than urban settings (table 1, table 2). In the rural setting, Fel d1 was more frequently detected in the control (37.7%) homesteads than the Mthatha AD (13.3%) cohort but the difference in concentration was not significant (median 0.52ug/g vs 0.94ug/g; p<0.25). Logistic regression confirmed significantly lower prevalence of detectable Fel d1 in AD than controls in the rural environment (table 3).

Dog

Can f1 was found more frequently in rural (25.3%) than urban (11.0%) households. There were no differences between subjects with (16.7%) and without (20.0%) AD. The levels of allergen did not differ between different sites or cohorts (table 1, table 2, table 3).

Mouse

The presence and concentration of Mus m1 was highest in urban controls and lowest in rural controls (94.6% vs 26.4% and median 1.21ug/g vs 0.02ug/g; both $p < 0.001$). The intermediate prevalence of detectable allergen was similar in rural and urban homes of patients with AD, with a slightly higher allergen concentration in urban than rural homes of participants with AD. This resulted in a markedly lower prevalence and concentration of allergen in urban settings (83.6% vs 45.8%; median 0.36ug/g vs 0.03ug/g; both $p < 0.001$; table 1, table 3), predominantly driven by the high prevalence and concentration seen in urban control homes.

Rural homesteads showed a significantly higher prevalence of detectable mouse antigen and higher concentration in AD vs control homes (table 1, 2 and 3), however in urban settings a higher prevalence and concentration was seen in control homes (table 1, 2, 3).

Cockroach

Bla g1 was present in between 3.8 to 8.3% of homes. There were no urban vs rural differences or difference between participants with and without AD (table 1, table 2).

Peanut

Ara h1, was not detected in the homes of our study populations in both rural and urban environments with and without AD.

Discussion

The prevalence of allergic disease has is higher in high income, industrialized countries than lower- and middle-income countries, and higher in urban than rural and farming environments. The latter has been confirmed in the middle-income country South Africa in the SAFFA cohort ^{6,14,21}. Here, exposure to farm animals in rural children was associated with the reduced rates of allergy⁶. In rural Mqanduli of the SAFFA study regions, pets and livestock range freely and homesteads are widely open to the external environment. Electricity is rarely used and there is no access to purified tap water piped directly to the home, with water being obtained from rivers, rainfall or communal taps situated some distance away. Most homesteads are traditional circular mud or brick huts with a floor made from dried cow dung, a potent source of endotoxin. This stands in stark contrast to the urban environment in which farm animal exposure is rare and both electricity and purified piped water is almost universally available⁶.

Environmental endotoxin predominantly originates from the cell walls of non-pathogenic Gram-negative bacteria and is typically found in higher levels in farming environments and in rural settings²³. In this study healthy controls in both environments have markedly higher endotoxin exposure than participants with AD. This may reflect a direct protective effect of endotoxin or may reflect an increased quantity and quality of microbial exposure. Fungal β -glucan on the other hand, is equally present in urban and rural settings, and in homes of children with/without AD.

We show that rural homesteads have different allergen levels besides endotoxin compared to urban homes. In our study, *Dermatophagoides* mite allergen Der p1 was found at higher concentrations in rural settings. Lower exposure levels to HDM in the houses of children with AD in the urban environment may reflect a true effect, may be caused by slight differences in the season of collection of dust from AD and control houses or may reflect reverse causation due to enhanced cleaning practices in the bedrooms of such children, prompted by their skin disease.

Cat and dog allergen exposure is high in the rural environment. The difference in cat allergen exposure in rural children with and without AD probably reflects their slightly different recruiting environments with extremely high rates of pet ownership in the deeply rural cohort. Mouse allergen is more often detectable at higher levels in urban homes than rural homesteads, particularly homes from control children, which may again be due to cleanliness. This is strikingly reversed in the rural cohort, with higher levels in the AD Mthatha cohort than in the control children from deeply rural homesteads, possibly due to the high prevalence of cats in the deeply rural setting⁶. However, the presence of cats is unlikely to confound the interaction between rural/urban settings and mouse allergen levels because the relation between cat presence and mouse allergen is likely stable across rural and urban study sites. Although we did not test for confounding of the rural-urban difference, i.e. the interaction term in our models, due to the limited sample size, this strong interaction is unlikely to be fully due to residual confounding by other variables.

Peanut is easily transferable in the environment through hands or saliva, and has become an important route of peanut sensitization¹². Considering peanut consumption in these groups was 95.7% in urban, and 47% in rural children²¹, the absence of detectable peanut allergen in the environment may reflect methodological limitations of assessing dust for Ara h1 alone, as compared to prior studies which have used a polyclonal ELISA against whole peanut protein^{12,13}.

Limitations

The relatively small sample size limits our study to only detect effects with a large magnitude. The larger SAFFA study ascertained demographics and lifestyle characteristics including parental smoking, siblings, and presence or absence of other allergic diseases. Several of the known determinants of endotoxin, glucan and allergen levels differ between rural and urban families in our study⁶. While this is likely to drive the differing levels we detected, the determinant-allergen level association is unlikely to differ between rural and urban settings and thus allergen level determinants are unlikely to confound the interaction between study site and allergen level on the association with AD. Due to this and the limited sample size we

do not present adjusted models and our results may therefore be residually confounded.

Moreover, due to there being almost no children with moderate to severe AD in deeply rural Mqanduli where the control population were recruited, we recruited participants with AD from the closest referral center in Mthatha. There is no significant difference in latitude between the urban and rural sites, but seasonal variations in temperature and humidity, were not accounted for, with samples collected at different times of the year in each cohort. There are slightly different climates in these regions²⁴. Urban Cape Town is situated directly on the coast with a Mediterranean-style climate with wet winters and dry summers (Koppen Geiger climate Csb²⁴). Mthatha is situated 1170km North-East of Cape Town, and 80 km inland with a subtropical highland climate with uniform rainfall (Cfb²⁴). Mqanduli is situated a further 50 to 90 km South-East, against the coast with a humid subtropical climate (Cfa²⁴) with predominantly summer rainfall. These climatic differences may influence mites and fungi but are unlikely to have significant effects on pet ownership. In addition, Mqanduli is exclusively “rural” with all subjects living in traditional homesteads rather than the population in Mthatha situated on a main highway with mixed urban, peri-urban and traditional homes.

Conclusion

It seems likely that in this setting, environmental endotoxin associated with exposure to farm animals is associated with protection against AD development, whereas patterns of fungal and allergen exposure are unlikely to be marked protective or risk factors.

Acknowledgements: n/a

Key messages:

The rural environment is protective against allergy.

Endotoxin is more frequently detectable in rural and urban environments in the houses of control participants than those with AD and is detectable at far higher concentrations in the rural control environment.

Fungal β -glucan is equally present in urban and rural settings, and in homes of children with/without AD.

House dust mite allergen Der p1 is detectable in 100% of homes with higher concentrations in rural settings and does not correlate with AD development. The tropical mite allergen, blo t5 is commonly detectable and more frequently detectable in urban than rural settings.

Pet allergens are more prevalent and at higher levels in rural than urban environments. Mouse allergen is more prevalent in urban homes and detectable at higher levels. Cockroach allergen is equally present in urban and rural areas, and in homes of children with/without AD.

Impact statement:

Endotoxin exposure in rural settings is associated with protection against development of atopic dermatitis. Patterns of fungal and allergen exposure are unlikely to be marked protective or risk factors.

References:

1. Prescott SL, Larcombe D-L, Logan AC, et al. The skin microbiome: Impact of modern environments on skin ecology, barrier integrity, and systemic immune programming. *World Allergy Organ J.* 2017;10(1):29. doi:10.1186/s40413-017-0160-5
2. Ege MJ. The Hygiene Hypothesis in the Age of the Microbiome. *Ann Am*

- 539 *Thorac Soc.* 2017;14(Supplement_5):S348-S353.
540 doi:10.1513/AnnalsATS.201702-139AW
- 541 3. Strachan DP. Hay fever, hygiene, and household size. *BMJ*.
542 1989;299(6710):1259-1260. <https://www.ncbi.nlm.nih.gov/pubmed/2513902>.
- 543 4. Huang YJ, Marsland BJ, Bunyavanich S, et al. The microbiome in allergic
544 disease: Current understanding and future opportunities-2017 PRACTALL
545 document of the American Academy of Allergy, Asthma & Immunology and
546 the European Academy of Allergy and Clinical Immunology. *J Allergy Clin*
547 *Immunol.* 2017;139(4):1099-1110. doi:10.1016/j.jaci.2017.02.007
- 548 5. Lunjani N, Satitsuksanoa P, Lukasik Z, Sokolowska M, Eiwegger T, O'Mahony L.
549 Recent developments and highlights in mechanisms of allergic diseases:
550 Microbiome. *Allergy Eur J Allergy Clin Immunol.* 2018;73(12):2314-2327.
551 doi:10.1111/all.13634
- 552 6. Levin ME, Botha M, Basera W, et al. Environmental factors associated with
553 allergy in urban and rural children from the South African Food Allergy
554 (SAFFA) cohort. *J Allergy Clin Immunol.* 2020;145(1):415-426.
555 doi:10.1016/j.jaci.2019.07.048
- 556 7. Lampi J, Koskela H, Hartikainen AL, et al. Farm environment during infancy
557 and lung function at the age of 31: A prospective birth cohort study in Finland.
558 *BMJ Open.* 2015;5(7):e007350. doi:10.1136/bmjopen-2014-007350
- 559 8. Birzele LT, Depner M, Ege MJ, et al. Environmental and mucosal microbiota
560 and their role in childhood asthma. *Allergy Eur J Allergy Clin Immunol.*
561 2017;72(1):109-119. doi:10.1111/all.13002
- 562 9. Kirjavainen P V., Karvonen AM, Adams RI, et al. Farm-like indoor microbiota in
563 non-farm homes protects children from asthma development. *Nat Med.*
564 2019;25(7):1089-1095. doi:10.1038/s41591-019-0469-4
- 565 10. Karvonen AM, Kirjavainen P V., Täubel M, et al. Indoor bacterial microbiota
566 and development of asthma by 10.5 years of age. *J Allergy Clin Immunol.*
567 2019;144(5):1402-1410. doi:10.1016/j.jaci.2019.07.035

- 568 11. Stein MM, Hrusch CL, Gozdz J, et al. Innate Immunity and Asthma Risk in
569 Amish and Hutterite Farm Children. *N Engl J Med*. 2016;375(5):411-421.
570 doi:10.1056/NEJMoa1508749
- 571 12. Brough HA, Simpson A, Makinson K, et al. Peanut allergy: Effect of
572 environmental peanut exposure in children with filaggrin loss-of-function
573 mutations. *J Allergy Clin Immunol*. 2014;134(4):867–875.e1.
574 doi:10.1016/j.jaci.2014.08.011
- 575 13. Brough HA, Liu AH, Sicherer S, et al. Atopic dermatitis increases the effect of
576 exposure to peanut antigen in dust on peanut sensitization and likely peanut
577 allergy. *J Allergy Clin Immunol*. 2015;135(1). doi:10.1016/j.jaci.2014.10.007
- 578 14. Botha M, Basera W, Facey-Thomas HE, et al. Rural and urban food allergy
579 prevalence from the South African Food Allergy (SAFFA) study. *J Allergy Clin*
580 *Immunol*. 2019;143(2):662-668. doi:10.1016/j.jaci.2018.07.023
- 581 15. Gray CL, Levin ME, Zar HJ, et al. Food allergy in South African children with
582 atopic dermatitis. *Pediatr Allergy Immunol*. 2014;25:572-579.
583 doi:10.1111/pai.12270
- 584 16. Gray CL, Levin ME, du Toit G. Ethnic differences in peanut allergy patterns in
585 South African children with atopic dermatitis. *Pediatr Allergy Immunol*.
586 2015;26(8):721-730. doi:10.1111/pai.12459
- 587 17. Potter PC, Davis G, Manjra A, Luyt D. House dust mite allergy in Southern
588 Africa - Historical perspective and current status. *Clin Exp Allergy*.
589 1996;26(2):132-137. doi:10.1111/j.1365-2222.1996.tb00071.x
- 590 18. Potter PC. Common indoor and outdoor aero-allergens in South Africa. *Contin*
591 *Med Educ*. 2010;28(9):426-432.
- 592 19. Sinclair W, Coetzee L, Joubert G. House-dust mite species in Bloemfontein,
593 South Africa. *South African Med J*. 2010;100(3):164-167.
594 doi:10.7196/samj.3864
- 595 20. Jeevarathnum AC, van Niekerk A, Green RJ, Becker P, Masekela R. Prevalence
596 of *Blomia Tropicalis* allergy in two regions of South Africa. *South African Med*

- 597 J. 2015;105(7):567-569. doi:10.7196/SAMJnew.7786
- 598 21. Botha M, Basera W, Facey-Thomas HE, et al. Nutrition and allergic diseases in
599 urban and rural communities from the South African Food Allergy (SAFFA)
600 cohort. *Pediatr Allergy Immunol*. 2019;30(5):511-521. doi:10.1111/pai.13058
- 601 22. Eichenfield LF, Tom WL, Chamlin SL, et al. Guidelines of care for the
602 management of atopic dermatitis: Section 1. Diagnosis and assessment of
603 atopic dermatitis Work Group. *J Am Acad Dermatol*. 2014.
604 doi:10.1016/j.jaad.2013.10.010
- 605 23. Gereda JE, Leung DYM, Liu AH. Levels of environmental endotoxin and
606 prevalence of atopic disease. *J Am Med Assoc*. 2000;284(13):1652-1653.
607 doi:10.1001/jama.284.13.1652
- 608 24. Conradie D. StepSA Climate Indicators Koppen Geiger climate classification.
609 http://stepsa.org/climate_koppen_geiger.html. Accessed October 1, 2020.

Table 1: Prevalence of detectable endotoxin, beta glucan and allergens in dust from urban and rural AD and rural and urban controls

Characteristic	Atopic dermatitis (AD)	Controls (C)	Total Urban (U)	Total Rural (R)	Urban AD (UAD)	Rural AD (RAD)	Urban controls (UC)	Rural controls (RC)	AD vs C	U vs R	AD:U vs R	C:U vs R	U:AD vs C	R:AD vs C
Endotoxin														
absence	14/60 (23.3)	2/78 (2.6)	8/67 (11.9)	8/71 (11.3)	7/31 (22.6)	7/29 (24.1)	1/36 (2.8)	1/42 (2.4)	<0.001	0.90	0.89	1.00	0.02	0.01
presence	46/60 (76.7)	76/78 (97.4)	59/67 (88.1)	63/71 (88.7)	24/31 (77.4)	22/29 (75.9)	35/36 (97.2)	41/42 (97.6)						
Beta Glucan														
absence	19/49 (38.8)	26/88 (29.6)	20/57 (35.1)	25/80 (31.3)	8/21 (38.1)	11/28 (39.3)	12/36 (33.3)	14/52 (26.9)	0.27	0.64	0.93	0.52	0.72	0.26
presence	30/49 (61.2)	62/88 (70.4)	37/57 (64.9)	55/80 (68.7)	13/21 (61.9)	17/28 (60.7)	24/36 (66.7)	38/52 (73.1)						
Der p1														
absence	0/100 (0)	0/89 (0)	0/72 (0)	0/83 (0)	0/36 (0)	0/30 (0)	0/36 (0)	0/53 (0)						
presence	66/66 (100)	89/89 (100)	72/72 (100)	83/83 (100)	36/36 (100)	30/30 (100)	36/36 (100)	53/53 (100)	-	-	-	-	-	-
Blo t5														
absence	56/66 (84.9)	68/90 (75.6)	47/73 (64.4)	77/83 (92.8)	28/36 (77.8)	28/30 (93.3)	19/37 (51.4)	49/53 (92.5)	0.16	<0.001	0.10	<0.001	0.02	1.00
presence	10/66 (15.1)	22/90 (24.4)	26/73 (35.6)	6/83 (7.2)	8/36 (22.2)	2/30 (6.7)	18/37 (48.6)	4/53 (7.5)						
Fel d1														
absence	61/66 (92.4)	68/90 (75.6)	70/73 (95.9)	59/83 (71.1)	35/36 (97.2)	26/30 (86.7)	35/37 (94.6)	33/53 (62.3)	0.01	<0.001	0.17	<0.001	1.00	0.02
presence	5/66 (7.6)	22/90 (24.4)	3/73 (4.1)	24/83 (28.9)	1/36 (2.8)	4/30 (13.3)	2/37 (5.4)	20/53 (37.7)						
Can f1														
absence	55/66 (83.3)	72/90 (80.0)	65/73 (89.0)	62/83 (74.7)	30/36 (83.3)	25/30 (83.3)	35/37 (94.6)	37/53 (69.8)	0.60	0.02	1.00	0.003	0.15	0.20
presence	11/66 (16.7)	18/90 (20.0)	8/73 (11.0)	21/83 (25.3)	6/36 (16.7)	5/30 (16.7)	2/37 (5.4)	16/53 (30.2)						

Mus m1	absence	16/66 (24.2)	41/90	12/73 (16.4)	45/83 (54.2)	10/36 (27.8)	6/30 (20.0)	2/37 (5.4)	39/53 (73.6)	0.01	<0.001	0.46	<0.001	0.01	<0.001
	presence	50/66 (75.8)	(45.6) 49/90 (54.4)	61/73 (83.6)	38/83 (45.8)	26/36 (72.2)	24/30 (80.0)	35/37 (94.6)	14/53 (26.4)						
Bla g1	absence	61/66 (92.4)	86/90	68/73 (93.2)	79/83 (95.2)	33/36 (91.7)	28/30 (93.3)	35/37 (94.6)	51/53 (96.2)	0.50	0.74	1.00	1.00	0.67	0.62
	presence	5/66 (7.6)	(95.6) 4/90 (4.4)	5/73 (6.8)	4/83 (4.8)	3/36 (8.3)	2/30 (6.7)	2/37 (5.4)	2/53 (3.8)						
Ara h1	absence	66/66 (100)	90/90 (100)	73/73 (100)	83/83 (100)	36/36 (100)	30/30 (100)	37/37 (100)	53/53 (100)	-	-	-	-	-	-
	presence	0/66 (0)	0/90 (0)	0/73 (0)	0/83 (0)	0/36 (0)	0/30 (0)	0/37 (0)	0/53 (0)						

p-values derived by Fishers exact or Chi-square tests

Allergen levels reported as proportions, n/N (%)

Table 2: Median levels of endotoxin, beta glucan and allergens in dust from urban and rural AD and urban and rural controls

Allergen	Atopic dermatitis (AD)	Controls (C)	Total Urban (U)	Total rural (R)	Urban AD (UAD)	Rural AD (RAD)	Urban controls (UC)	Rural controls (RC)	AD vs C	U vs R	AD U vs R	Controls U vs R	Urban AD vs C	Rural AD vs C
Endotoxin (EU)	2667.5 (2136.6-8318.9)	6032.6 (2674.8-17516.6)	3150.0 (2514.8-10054.5)	392.6 (218.4-1751.7)	3106.4 (2413.0-9875.0)	2236.0 (1603.0-2980.3)	3174.3 (2514.8-12149.1)	7759.3 (2912.4-20652.8)	0.01	0.99	0.04	0.14	0.83	<0.001
Beta Glucan (ug/g)	2.16 (10.9-2.94)	1.39 (0.50-3.72)	1.38 (0.72-2.77)	1.49 (0.68-3.75)	1.38 (1.09-2.45)	2.37 (1.08-2.99.6)	1.44 (0.61-3.63)	1.39 (0.49-3.75)	0.33	0.59	0.41	0.85	0.63	0.38
Der p1 (ug/g)	1.54 (0.59-5.28)	3.34 (1.22-9.56)	1.02 (0.29-2.98)	4.73 (2.69-9.56)	0.81 (0.24-1.29)	4.60 (2.75-8.00)	1.50 (0.46-8.77)	5.00 (2.69-9.95)	0.01	<0.001	<0.01	0.01	0.03	0.85

Blo t5 (ug/g)	0.14 (0.02-0.24)	0.23 (0.02-0.27)	0.14 (0.12-0.27)	0.24 (0.22-0.50)	0.04 (0.01-0.31)	0.21 (0.21-0.21)	0.22 (0.18-0.27)	0.37 (0.24-0.50)	0.42	0.11	0.60	0.06	0.87	0.06
Fel d1 (ug/g)	0.77 (0.22-1.11)	0.44 (0.11-0.79)	0.08 (0.04-0.22)	0.55 (0.20-0.95)	0.04 (0.04-0.04)	0.94 (0.49-1.15)	0.15 (0.08-0.22)	0.52 (0.14-0.84)	0.62	0.04	0.16	0.21	0.22	0.25
Can f1 (ug/g)	0.42 (0.14-0.77)	0.19 (0.14-0.22)	0.31 (0.12-0.85)	0.19 (0.17-0.29)	0.31 (0.14-0.77)	0.45 (0.16-0.75)	0.55 (0.05-1.05)	0.19 (0.15-0.21)	0.28	0.59	0.86	1.00	1.00	0.41
Mus m1 (ug/g)	0.21 (0.10-0.36)	0.13 (0.03-1.94)	0.36 (0.13-1.66)	0.03 (0.02-0.17)	0.27 (0.19-0.37)	0.12 (0.02-0.24)	1.21 (0.10-2.50)	0.02 (0.01-0.02)	0.54	<0.001	0.01	<0.001	0.07	0.004
Bla g1 (ug/g)	0.06 (0.06-0.56)	0.09 (0.07-0.18)	0.10 (0.06-0.25)	0.07 (0.05-0.33)	0.06 (0.06-0.80)	0.31 (0.05-0.56)	0.18 (0.10-0.25)	0.07 (0.05-0.09)	0.81	0.33	0.56	0.12	0.56	0.44
Ara h1 (ug/g)	0 (0.0-0.0)	0 (0.0-0.0)	0 (0.0-0.0)	0 (0.0-0.0)	0 (0.0-0.0)	0 (0.0-0.0)	0 (0.0-0.0)	0 (0.0-0.0)	-	-	-	-	-	-

p-values derived by Mann Whitney U test

Allergen levels reported as median (IQR)

Figure 1: Median levels of endotoxin, beta glucan and Der p1 in dust from urban and rural AD and urban and rural controls

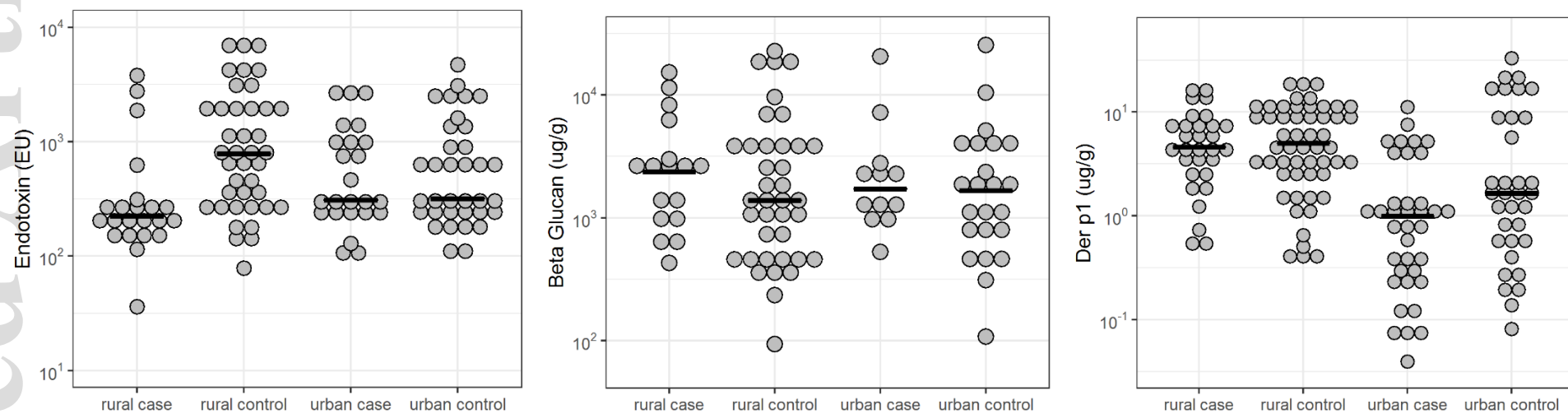


Table 3: Logistic regression for each environmental exposure with study site, AD status and their interaction as covariates in the model.

	Urban	Rural	
	AD vs Control	AD vs Control	Interaction p-value
Endotoxin	0.10 (0.01 – 0.60) p=0.035	0.08 (0.00 – 0.47) p=0.020	0.875
B-Glucan	0.81 (0.26 - 2.54) p=0.716)	0.57 (0.21 – 1.52) p=0.258	0.639
Der p1	NA	NA	NA
Blo t5	0.30 (0.10 – 0.81) p=0.021	0.88 (0.12 – 4.78) p=0.882	0.304
Bla g1	1.59 (0.25-12.66) p=0.623)	1.82 (0.21 – 15.86) p=0.559	0.923
Can f1	3.50 (0.74 – 25.12) p=0.142	0.46 (0.14 – 1.35) p=0.179	0.049
Fel d1	0.50 (0.02 – 5.45) p=0.579	0.25 (0.07 – 0.77) p=0.024	0.625
Mus m1	0.15 (0.02 – 0.62) p=0.020	11.14 (3.98 – 35.55) p<0.001	<0.001
Ara h1	NA	NA	NA