Urbanisation and childhood asthma in a developing region of Latin-America: a cross-sectional analysis in northeastern Ecuador

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Declaration

I, Alejandro Rodriguez, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Signature

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Date: 13/09/2018

Abstract

Asthma is becoming increasingly frequent in children in urban and rural areas of low and middle-income countries (LMICs), with a high prevalence in urban centres. The urbanisation process has been suggested as a possible explanation for this temporal increase and urban-rural differences in asthma prevalence. This thesis aims to explore the influence of urbanisation on asthma prevalence in a developing region of Latin-America in which the rural-urban transition is occurring rapidly.

A systematic review evaluated how epidemiological studies have assessed the associations between asthma and urbanisation in LMICs. To understand better the relationship between urbanisation and wheeze/asthma in transitional areas, four cross-sectional studies were conducted in northwest Ecuador. In the first of these, the level of urbanicity at census ward level was quantified for children living in diverse urban and rural localities and urbanicity level was associated with asthma. The second and third study explored how internal migration, an important element of the urbanisation process, is associated with the prevalence of current wheeze in urban and rural populations, respectively. The fourth analysis identified lifestyle domains in urban and rural populations based on socioeconomic and behavioural characteristics and explored how these characteristics could explain the prevalence of wheeze in a transitional region.

This thesis provide evidence that even small-scale increases in levels of urbanicity are associated with a higher prevalence of wheeze/asthma. Analysis of internal migration showed that in urban areas, rural to urban migration was associated with an increase in the prevalence of wheeze while in rural areas the absence of the child's mother at home, through temporary or permanent migration, was associated with an increase in prevalence of wheeze, rhinitis and eczema. Finally, an analysis of lifestyle factors showed that living in substandard housing and a high level of sedentarism were associated with a greater prevalence of wheeze.

Preface

This thesis is written in the form of five research articles in various stages of publication. At the time of submission, three of the articles have been published and two have been submitted and are under revision. In the present thesis, each article represents a chapter, apart from the two articles discussing the effects of internal migration on asthma in urban and rural populations that comprise a single chapter. Research paper cover sheets giving the publication details are provided at the beginning of each chapter. Additionally, a short summary before each chapter is provided to maintain coherence of the thesis and ensure it is presented as one body of work. Although I have used distinct but complementary approaches in the course of writing this thesis to evaluate the effects of urbanisation on asthma, repetition in in places may occur particularly in the descriptions of study areas and populations. Nevertheless, the thesis does not exceed the word limit of 100,000. I, Alejandro Rodriguez, am the first author on all papers.

Two different projects conducted in the same tropical region of Ecuador provided the data used in this thesis. These projects have focused on identifying risk factors for asthma and other allergic diseases in populations living in diverse urban and rural environments. The information obtained through these projects was particularly well suited to study the effects of urbanisation on asthma because individual-level, household-level and census ward-level data were available for most of the study participants. I have played a key role in the collection of data for both studies and designed instruments that were used to collect data on factors related to urbanisation at individual, household, and community-levels in both.

This thesis is organized in six chapters: the first chapter is a general introduction to the studies describing objectives, populations and study areas; the second chapter is a systematic review of the scientific literature on the relationship between asthma and urbanisation; the third chapter discusses the construction of a multidimensional measure of urbanicity for studies of wheezing illness and asthma; the fourth chapter discusses the effects of internal migration on asthma; the fifth chapter discusses the changes in lifestyle and their relationship to asthma and the sixth chapter presents conclusions and recommendations for the study of urbanisation in asthma research.

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List of Acronyms and Abbreviations

AllDis	Allergic Diseases
BMI	Body mass index
CATPCA	Categorical Principle Component Analyses
DCs	Developing Countries
ECUAVIDA	Estudio eCUAtoriano del impacto de infecciones sobre Vacunas, Inmunidad y el Desarrollo de enfermedades Alergicas
FEPIS	Ecuadorian Foundation for Health Research
GIS	Geographical Information System
GPS	Geographical Position System
HICs	High Income Countries
INEC	National Institute of Statistics and Census
ISAAC	International Study of Asthma and Allergies in Childhood
LA	Latin America
LMICs	Low and Middle-Income Countries
LSHTM	London School of Hygiene and Tropical Medicine
MA	Multilevel Analyses
MCA	Multiple Correspondence Analyses
OR	Odds ratio
PRISMA	Preferred Reporting Items for Systematic reviews and Meta-Analyses
PROSPERO	International Prospective Register of Systematic Reviews
RAD	Related Allergic Diseases
STROBE	Strengthening the Reporting of Observational Studies in epidemiology
SCAALA	Social Change in Asthma and Atopy in Latin America
SES	Socioeconomic Status
WHO	World Health Organization

Chapter 1. Introduction

Background

General characteristics of Asthma

Over recent decades, asthma has emerged as a major challenge for health systems around the world affecting individuals of all ages.(1) While the global prevalence of asthma is difficult to determine because of lack of up-to-date information and gaps in data, the most recent global estimates suggest that 334 million people worldwide have asthma.(2) Among those who have asthma, children are most affected and asthma is now the most common chronic disease of childhood.(2) Although there are wide variations in the prevalence of asthma symptoms among countries, the International Study of Asthma and Allergies in Childhood (ISAAC) estimated that around 14% of the world's children experience asthma symptoms.(3) The global ranking of disability-adjusted life years among children placed asthma among the top 20 chronic conditions affecting school and working performance productivity.(2) Although asthma has no cure, symptoms can be controlled by treatment ranging from the identification and avoidance of the asthma triggers to the use of medications to relieve and control symptoms. However, significant direct and indirect costs are associated with this condition through emergency visits, physician visits, diagnostic tests, among other social costs.(4)

Asthma is widely recognized as a complex and heterogeneous disorder characterized often by chronic inflammation of the airways leading to the obstruction of airflow through the lungs.(5) Although asthma symptoms vary between and within patients in severity and frequency, wheezing is the most common symptom used to identify this disorder.(2) Other important clinical symptoms include chest tightness, breathlessness and cough. While important advances have been made in asthma research, we still have an incomplete understanding of the causes associated with the development of asthma. A complex combination of environmental exposures, changes in lifestyles and host genetics has been suggested as possible causes for asthma.(1) Substances or irritants that can start asthma symptoms or make them worse are considered as asthma triggers. Among the most common triggers are airborne substances such as pollen, dust mite or cockroaches, mold, pet dander; respiratory infections;

exercise; air pollutants; allergic reactions to certain foods; strong emotions and stress, certain medications, among others.(1)

Multiple asthma subgroups or phenotypes have been identified based on demographic, clinical or pathophysiological characteristics. Among the characteristics of asthma used for classification are frequency of symptoms, lung function, age of onset, type of the cells involved in inflammation, biomarkers for allergy (e.g. IgE and Th2 cytokines), and medication use.(1) The most widely used phenotype is that based on the presence of atopy. Atopic asthma (also called allergic or extrinsic asthma) is considered to be triggered by environmental antigens, such as dust, pollens, animal dander, and foods, that induce specific IgE responses and IgE-mediated inflammation. Patients with this condition usually have a positive family history of atopy, and asthmatic attacks are often preceded by allergic rhinitis, urticaria, or eczema.(6) In contrast, non-atopic asthma (also called intrinsic asthma) occurs when asthma symptoms are not caused by or at least not associated with measurable allergen-specific IgE or allergen skin test positivity.(6) It is estimated that 50% of childhood asthma and 30% all adult asthmatic patients could be non-atopic asthma.(6) Another important classification of asthma is that based on the severity of this condition (measured by the frequency of symptoms and lung function parameters) in which asthma has been defined as intermittent, mild persistent, moderate persistent and severe persistent.(7)

For research purposes, there is no a standard operational definition for asthma so different studies have used different definitions such as doctor diagnosis, the presence of clinical symptoms, bronchial hyperresponsiveness, pulmonary function tests, or a combination.(8) Additionally, a number of factors influence the clinical diagnosis of asthma within populations such as differences in awareness of asthma, medical training and experience as well as cultural and social factors.(2)

Asthma trends

Geographical and temporal trends in asthma and other related Allergic Diseases (AllDis) have been observed worldwide.(9) Among such studies, ISAAC was set up to generate estimates of asthma prevalence in different populations using standardized methodology. Four patterns were identified from these studies which were not explained by prevailing knowledge of asthma causation: 1) a progressive increase in the prevalence of asthma and other related AllDis over the previous 40 or so years.(9); 2) this increase was accompanied by variations in the prevalence between regions with different levels of development (10) showing trends of higher prevalence in high Income Countries (HICs), especially in speaking English countries, compared with Low and Middle Income Countries (LMICs); 3) that the prevalence of asthma in HICs appeared to have reached a plateau, while prevalence in LMICs continued to increase(11); and 4) differences in asthma prevalence between urban and rural populations in diverse geographic regions with a tendency for lower prevalence in rural populations.(12). This urban-rural difference has been attributed to diverse factors. In the case of rural areas, exposures related to a more traditional rural lifestyle such as farming have been suggested to provide protection against the development of asthma and other AllDis.(12) On the other hand, numerous exposures related to the acquisition of an urban lifestyle have been identified as potential risk factors for asthma.(13) However, recent evidence from studies conducted in the same locations but at different times (8-10 years apart), showed that asthma and other related AllDis are increasing in urban and even in rural populations in LMICs, reducing the prevalence gap between urban and rural areas.(14,15)

Urbanisation and asthma in LMICs

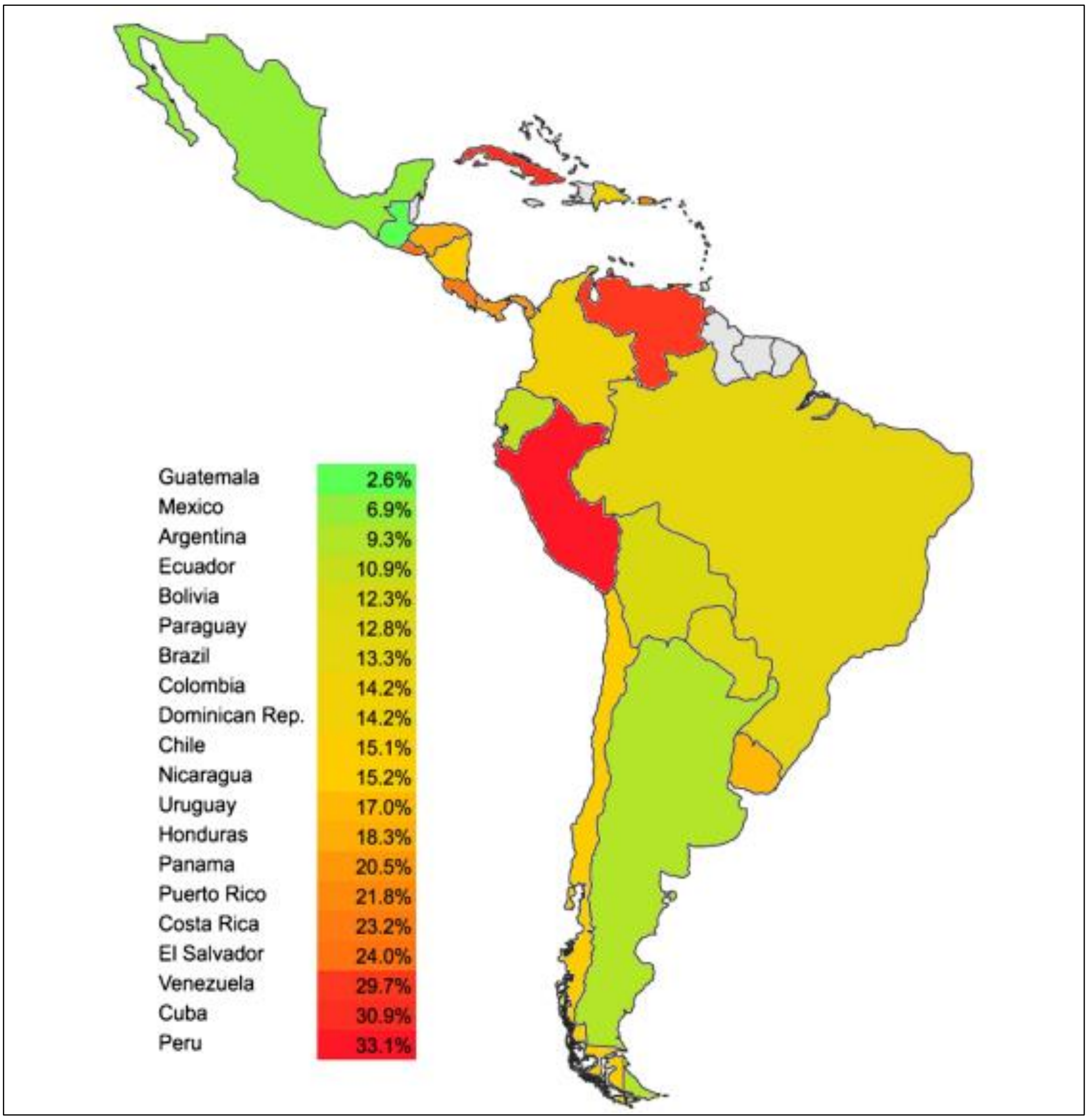
Epidemiological studies conducted in LMICs have suggested that changes from traditional/rural to modern/urban societies, especially the acquisition of a modern lifestyle, may be in part responsible for the geographical and temporal trends in asthma prevalence.(16) This hypothesis has been based on the fact that asthma prevalence is frequently higher in urban than rural areas, indicating that urban residence is a potential risk factor for asthma.(12) Further, evidence from studies in rural areas of LMICs have shown that the prevalence of asthma and other AllDis increase with increasing levels of urbanisation.(17) The increase in asthma prevalence in rural areas has been also associated with the extension of urbanisation and modernisation processes into rural populations (i.e. urban sprawl).(18) Epidemiological studies have identified that numerous environmental and social changes arise from the urbanisation process that are in themselves potential risk factors.(12,13) These factors may include changes in socioeconomic well-being, changes in diet, sedentarism, migration, reduction in the frequency of infections, reduction in family size, increasing vaccine coverage, use of antibiotics, increases in environmental pollution, household exposure to allergens. However, the nature of these associations remains poorly understood.(13)

Urbanisation also feeds rural-urban migration which is associated with changes in lifestyle and new environmental exposures. Most of what we know about the effects of migration on asthma comes from studies using international migrant populations, especially those who migrate from LMICs (presumed as being of low asthma risk) to HICs (considered to be higher risk).(19) These studies have shown that dimensions of the migration process such as place of origin, age of migration and time since migration, are important determinants of risk of asthma and other related AllDis.(20) Internal migration is an important part of the urbanisation process in LMICs and presents almost the same characteristics as international migration. Migrants from rural to urban areas may face similar social, psychological, economic and environmental changes as those who migrate to other countries. However, few studies have assessed the effects of internal migration on asthma.(21)

Asthma in Latin America

Although most countries in Latin America (LA) share similar cultural backgrounds, important geographical, political and economic differences characterize this region. Reflecting this diversity, the burden of asthma in children is highly variable across LA countries.(22) Only during the last 10 years has it been possible to get comparable and reliable information on the prevalence and severity of asthma in children in LA.(11) The first estimation of the prevalence of asthma or asthma symptoms in the region was provided by the ISAAC studies.(18) that showed that children living in cities in several LA countries reported similar or higher rates of asthma symptoms compared to those reported from HICs known to have a high prevalence of asthma.(23) Recent estimates have shown that the prevalence of childhood asthma ranges widely within LA from 2.6% in Guatemala to 33.1% in Peru (Figures 1).(23)

Figure 1. Asthma Prevalence in Latin America



Thorax 2015;70(9):898–905.

Although atopy has been associated consistently with asthma symptoms,(24) non-atopic wheeze/asthma seems to be the most common form of childhood wheeze/asthma in LA.(25) Cross-sectional studies conducted in Ecuador and Brazil have estimated that 2.4% (25) and 24.5% (26) of wheeze cases are attributable to atopy, respectively, and are considerably lower than the 40.7% average reported from developed countries.(27) While the regional variation in asthma prevalence is likely multifactorial, the process of urbanisation, migration, and the adoption of a modern lifestyle may play an important role within this region. Evidence from studies conducted in transitional populations in Ecuador (traditional to modern urban) have shown that the increase in asthma prevalence is associated with the level of urbanisation.(17) Other studies have shown that the acquisition of a "Western diet" or consumption of fast food is associated with higher asthma risk in Mexico and Brazil.(28,29) At the same time, air pollution and community violence are associated with asthma morbidity in urban LA.(18) However, asthma has been associated also with urban poverty.(30,31)

Study problem and objectives

Gap in understanding

Although it has been hypothesized that the urbanisation process could be in part responsible for the temporal increases in asthma prevalence in LMICs, very little is known of how and through which mechanisms this process may affect the increase in asthma prevalence. Worldwide, the population is becoming progressively more urban and it has been estimated that by 2030, approximately sixty per cent of the world's population will be urban, with most global population growth occurring in poorer regions of LMICs, especially in smaller cities.(32) According to this trend, one would predict that asthma prevalence will grow to epidemic levels in LMICs over the coming decades. The causes of asthma in LA are likely to be associated with urbanisation, migration, and the adoption of a modern lifestyle. It is important to understand how these processes and their exposures may increase or decrease the asthma risk to identify potential public health interventions. This knowledge could be used to alleviate the growing burden of asthma-related disability, particularly among the urban poor of LA, where asthma is highly prevalent and an important cause of morbidity.

Aim of the study

The present study aimed to evaluate the influence of urbanisation on asthma prevalence through different approaches and using diverse indicators based on contextual, household and individual variables, in order to identify urban living and urban conditions associated with childhood asthma in transitional populations in northwest Ecuador. To achieve this aim, the study first evaluated the approaches used by published epidemiological studies to study the relationship between urbanisation and asthma in LMICs. Taking into consideration, the different approaches used in asthma research, the study then used a multidimensional approach to measure the process of urbanisation generating a urbanicity scale to explore the gradual effect of urbanisation on asthma in transitional populations. Next, the study explored the effects of internal migration on asthma prevalence within urban and rural populations using several relevant indicators for migratory status. Finally, the study evaluated changes in lifestyle in urban and rural populations based on social, behavioural and household characteristics of the population and explored how these changes could explain the differences in asthma prevalence in transitional areas of Ecuador.

Methods

Context of the study

The present research is nested within two larger projects conducted in the province of Esmeraldas, Ecuador. These projects have been studying risk factors for asthma and other related AllDis in populations living in urban and rural areas.(33,34) The first project, ECUAVIDA (Impact of early life exposures to geohelminth infections on the development of vaccine immunity, allergic sensitization, and allergic inflammatory diseases in children living in tropical Ecuador), is an ongoing birth cohort with follow up to 8 years of age, designed to investigate the impact of pre- and postnatal exposures to soil-transmitted helminth parasites on the development of asthma and other related AllDis.(34) The study recruited 2,404 newborns between November 2006 and December 2009.

Detailed information has been collected for each child around the time of the birth, at 7 and 13 months and at 2, 3, 5, and 8 years. Data collection at 8 years finished in December 2017.The second project, SCAALA (Social Changes, Asthma and Allergy in Latin America), includes two cross-sectional studies conducted between 2005 and 2010. SCAALA aimed to study changes in the prevalence and risk factors for asthma and allergy in populations that migrated from rural to urban areas and examine how such changes may relate to changes in the risk of asthma and other related AllDis.(33)

Table1. Information collected by SCAALA and ECUAVIDA projects.

Indicator	Variables	SCAALA	ECUAVIDA
Demographic	Sex, age, ethnicity (child)	X	X
	Marital status (parents)		X
Socioeconomic	Household income	X	X
	Parental education and occupation	X	X
	Domestic goods and services	X	X
Household Characteristics	Building materials	X	X
	Area of residence	X	X
	Crowding	X	X
	Geographical Coordinates		X
Child Characteristics And exposures	Pet/farming/animal exposures	X	X
	Environmental tobacco smoke	X	X
	Use of medications/antibiotics		X
	Vaccination history		X
	Food frequency questionnaire	X	X
	Child health history		X
	Parents health history		X
	Child birth history		X
	Intestinal helminth infections	X	X
	Sedentarism and exercise	X	X
ISSAC Allergy Questions	Symptoms of wheezing	X	X
	Symptoms of eczema	X	X
	Symptoms of rhinitis	X	X
Clinical evaluations	Stool samples for parasites	X	X
	Blood samples for DNA	X	X
	Indicators of vaccine responses		X
	Allergen skin prick test	X	X
	Anthropometry	X	X
	Psychosocial evaluations		X
	Exercise-induced bronchospasm	X	
History of Migration	Place of origin and residence	X	
	Age and time of migration	X	
	Distance of migration	X	
Urbanisation	Urban Infrastructure	X	X
	Connectivity		X
	Population size and density		X
	Community violence		X
	Census ward data		X

Both studies collected extensive data including: demographic and lifestyle factors; psychosocial and dietary factors (ECUAVIDA only); childhood morbidity and clinical outcomes, clinical evaluations, stool samples for parasites; blood samples for measurement of vaccine responses (ECUAVIDA only); migration status; atopy measured by skin prick testing and IgE in sub-samples; anthropometric measurements for assessment of nutritional status; exercise testing for assessment of exercise-induced bronchospasm (SCAALA only); and dust sampling for measurement of household endotoxin and allergen levels.(Table 1).

Because of the widely available information of these studies, and the geographic and socioeconomic characteristics of their populations (transitional populations which are undergoing a rapid transformation from a traditional rural lifestyle to a more urban way of living), the ECUAVIDA and SCAALA studies were particularly well suited to study the effects of urbanisation on asthma.

Study design

Five scientific papers based on diverse approaches were prepared to explore the effects of urbanisation on asthma prevalence:

- Paper 1 comprises a systematic review assessing the relationship between asthma and urbanisation in studies conducted in LMICs.
- Paper 2 comprises a cross-sectional analysis generating an urbanicity scale in diverse urban and rural wards (within 3 Districts in Esmeraldas province) to evaluate the gradual effect of urbanisation on asthma prevalence.
- Paper 3 comprises a cross-sectional analysis exploring the effects of internal migration on the prevalence of asthma in an urban area.
- Paper 4 comprises a cross-sectional analysis exploring the effects of internal migration on the prevalence of asthma in a rural area.
- Paper 5 comprises a cross-sectional analysis evaluating changes in lifestyle in urban and rural populations to explore how new lifestyles could explain the differences in asthma prevalence in transitional areas.

Study site

The present research was based in the northern coastal province of Esmeraldas, Ecuador. This province comprises seven districts and covers an area of 15,256 km2, with a population of 534,092 inhabitants.(Figure 2) The district of Esmeraldas is the most important district in northwest Ecuador because it is home to the provincial capital, City of Esmeraldas, and the national oil refinery. The main economic activities in the province are oil industry, tourism, timber extraction, African palm oil, and other agricultural activities. Based on the last national census (2010), Esmeraldas province was considered to be one of the poorest regions of Ecuador, with a poverty rate by unsatisfied basic needs of 78.3% and an illiteracy rate of 9.5%. Basic services are deficient with 69% of the population lacking a household sewage system and only 36% of the households having access to piped water.(35)

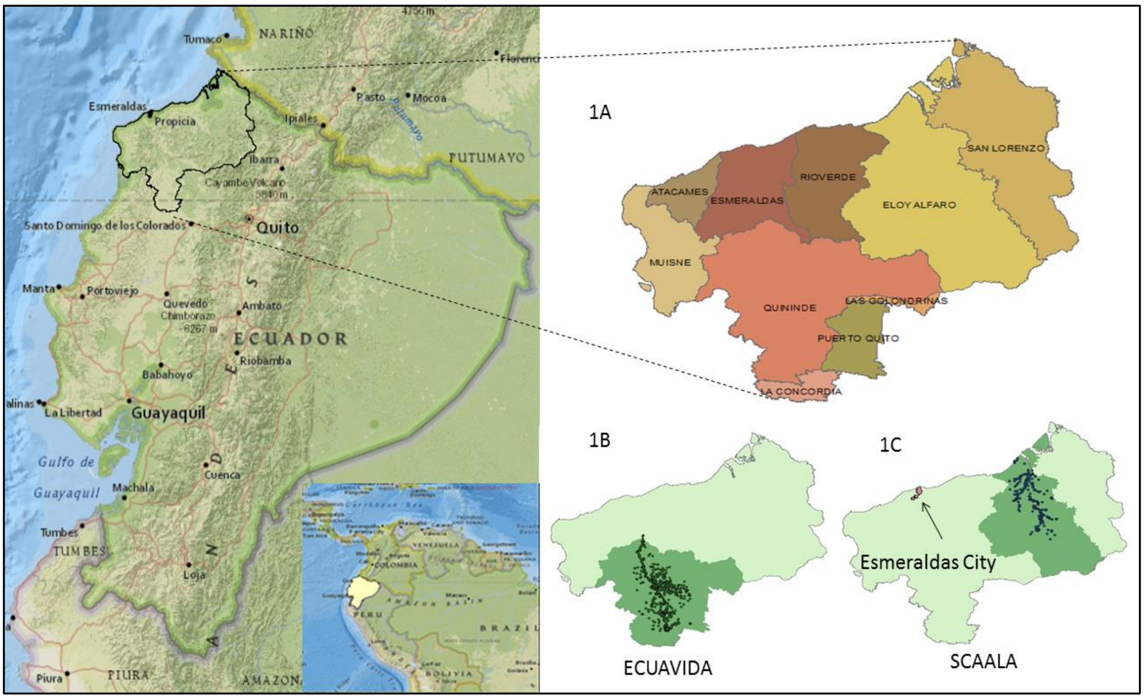


Figure 2. Study area in the province of Esmeraldas, Ecuador. Figure shows map of Ecuador: 1A- map of Esmeraldas and their districts, 1B- ECUAVIDA study area, 1C- SCAALA study area. Dots represent geographical distribution of the population households.

Study Population

Each paper used a different population from the ECUAVIDA and SCAALA studies with the exception of Paper 5 that used the populations from Papers 3 and 4. Study populations describing sample size, age and locations are shown in Table 2.

- Paper 2 evaluated 1,843 children of 5-7 years old living in diverse urban and rural areas of the district of Quinindé, La Concordia, Puerto Quito and Las Golondrinas. This population was used to evaluate urban conditions at the census ward level. Several characteristics were used to develop a multidimensional quantitative measure of urbanicity to investigate the associations between urban conditions and asthma prevalence. This study population belongs to ECUAVIDA cohort and at time of analysis most of the children were over 6 years of age.
- Paper 3 evaluated 2,510 schoolchildren, aged 7 to 16 years, living in the City of Esmeraldas. This population was used to evaluate the effects of internal migration on asthma prevalence in children living in urban neighbourhoods. In this population, the schools were selected from barrios or neighbourhoods in which there was a predominance of Afro-Ecuadorian migrants from two northern rural Districts, Eloy Alfaro and San Lorenzo. This study population belonged to the SCAALA study.
- Paper 4 evaluated 4,295 schoolchildren, aged 7 to 16 years, living in 59 rural communities of the Districts of Eloy Alfaro and San Lorenzo, in the north of the Esmeraldas province. This study population was used to evaluate the effects of internal migration on asthma prevalence in children living in rural environments. This study population belonged to the SCAALA study.
- Paper 5 evaluated 6,805 schoolchildren living in the City of Esmeraldas and 59 rural communities located in the District of Eloy Alfaro and San Lorenzo. This population was used to evaluate changes in lifestyles based on behavioural and socioeconomic characteristics of the children and their households to understand how changes in lifestyles might explain differences in asthma prevalence. This study population belonged to the SCAALA study

Data collection

Although the present research used data from the ECUAVIDA and SCAALA projects, especially that information related with the health status of the children, additional data related with household's characteristics of the children in the district of Quinindé was collected by the student (AR). Geographical coordinates and social and economic features of the households and the neighbourhoods where the children lived were collected using a questionnaire between August 2015 to July 2016.(Annex section) Demographic and socioeconomic data at the census ward level was obtained through the National Institute of Statistics and Census of Ecuador (INEC).

Table 2. Study populations by scientific papers

Paper	Population	Age (years)	Study Area	Area	Project
Paper 1	--	All years	LMICs	Urban Rural	--
Paper 2	1843	5-7	Quinindé	Urban Rural	ECUAVIDA
Paper 3	2510	7-16	City of Esmeraldas	Urban	SCAALA
Paper 4	4295	7-16	Eloy Alfaro San Lorenzo	Rural	SCAALA
Paper 5	6805	7-16	City of Esmeraldas Eloy Alfaro San Lorenzo	Urban Rural	SCAALA

Asthma Definition

Asthma symptoms were collected using a questionnaire based on ISAAC phase II.(36) The questionnaire was modified and adapted to the local conditions and translated into Spanish and extensively pre-tested in the relevant populations before use. A parent or guardian of each child was interviewed by a trained physician from the ECUAVIDA and SCAALA study teams. This research used several definitions of asthma, especially in the systematic review paper. However, most analyses were focused mainly on current wheeze defined as ***the presence of wheeze in the last 12 months***. This definition has been validated in different populations and is widely used in epidemiologic studies, especially those among populations with limited access to health care.(36)

Measures of Urbanisation.

Various definitions and criteria from different disciplines and studies were taken into account to select indicators susceptible to evaluating the multi-dimensional character of urbanisation.(32,37,38) According to our conceptual model, four dimensions were evaluated using different indicators at the census ward, household and individual level. 1) Demographic - representing the phenomenon of population concentration within restricted spaces (variables - population size and density). 2) Socioeconomic - representing changes in living conditions related to the rural-urban transition (non-agricultural activities, secondary education, commercial activities, housing constructed with cement, access to mobile phones, internet, computers and satellite tv, etc). 3) Built Environment - physical characteristics of the urban environment characterized by access to basic services, public and private institutions, and urban infrastructure (street paving, sewage access, electricity, and educational institutions and health institutions). 4) Geographical - representing the spatial distribution of the settlement where the child lives (geopolitical division, proximity to urban centres and access to highways). Table 3 describes a list of indicators by dimension and unit of analysis that were evaluated in the different papers conducted in this research.

Plan of analysis

The analysis was designed to address seven principal study questions: 1) Which methods are used by asthma studies to assess the effects of urbanisation in LMICs? 2) Is there a rural-urban gradient for the prevalence of asthma in LMICs and if so, to what extent? 3) How can we measure the level of urbanisation in transitional populations? 4) Is the level of urbanisation associated with asthma prevalence? 5)

Table 3. Urban indicators by dimension and unit of analyses

Indicators	Infrastructure	Socioeconomic	Geographical	Demographic
1. Population density (per/km^2)				C
2. Size of the population				C
3. Urban influence			C	C
4. Political division			C	C
5. Connectivity			H	
6. Tap water (access/coverage)	C	H		
7. Electricity (access/coverage)	C	H		
8. Sewerage (access/coverage)	C	H		
9. Telephone (access/coverage)	C	H		
10. Garbage collection (coverage)	C	H		
11. Presence of health institutions	C			
12. Presence of private institutions	C	C		
13. Number of commercial locals	C	C-I		
14. Presence of food retail provision	C	C-I		
15. Presence of recreation facilities	C			
16. Presence of public Transport	C			
17. Housing materials	C-H	H		
18. Housing: combustible for cooking		H		
19. Presence of pavement streets	C-H			
20. Presence of sidewalks	C-H			
21. Electrical appliances		H-I		
22. Access to cable or satellite tv	C-H	H-I		
23. Access to internet	C-H	H-I		
24. Employment		C-H		
25. Income		C-H		
26. Educational attainment		C-H		
27. Illiteracy		C		
28. Presence of educational institutions	C	C		
29. Food consumption patterns		I		
30. Physical Exercise		I		
31. Time watching tv		I		
32. Agricultural activities		H-I		
33. Raising livestock		H		
34. Migration history		H-I		
35. Family structure		H-I		
36. Level of violence		H		

***Level of analyses: C=Census ward, H= Household and I= Individual**. Some variables are included in more than one group of indicators because they represent different conceptual constructs

What urban characteristics are associated with the increase in asthma prevalence? 6) Are place of birth, age of migration and time since migration associated with asthma risk in children living in urban and rural areas? 7) What changes in lifestyles related to urbanisation increase the risk of asthma if so, are they potentially modifiable through intervention programmes? The statistical analysis for each question is detailed in each paper.

Ethical considerations

Ethical approval was granted for the ECUAVIDA cohort study by London School of Hygiene and Tropical Medicine (LSHTM- ref 8819) and the Ministry of Health in Ecuador (Subsecretaria General de Salud SSG-10-000285). The study protocol was approved by the Ethics Committees of the Hospital Pedro Vicente Maldonado, Universidad San Francisco de Quito, Pontificia Universidad Catolica del Ecuador, and Universidad Internacional del Ecuador and is registered as an observational study (ISRCTN 41239086). Ethical approval was granted for the SCAALA study by the ethics committees of the Hospital Pedro Vicente Maldonado and Pontificia Universidad Catolica del Ecuador and approval was obtained from the Ministry of Health in Ecuador.

Study Funding

The ECUAVIDA and SCAALA studies were funded by the Welcome Trust (grant 088862/Z/09/Z and 072405/Z/03/Z). Additionally, my research was also supported by the Ecuadorian Institute of Educational Credit through a 4-year scholarship that covers living expenses, research training, aeroplane tickets and some field work costs in UK and Ecuador.

Candidate's involvement

Since I trained as a sociologist in 2005 at the Catholic University in Quito, Ecuador, I have developed a particular interest in the processes of modernization and urbanisation in LMICs and their effects on human health particularly their effects on the development of asthma and other non-communicable diseases. I have been involved in a research programme supported by a Wellcome Trust Centres of Excellence in Latin America Award - SCAALA, run by Professor Mauricio Barreto in Salvador, Brazil and Dr Philip Cooper in Quininde, Ecuador. I have conducted several research studies within the SCAALA programme, especially studies evaluating the effects of modernization process on asthma and other AllDis in rural communities. Supported by the SCAALA programme, I did a Masters in degree in Epidemiology at the Instituto de Saude Coletiva, Universidad Federal de Bahia that was jointly supervised by Professors Mauricio Barreto and Philip Cooper.

Since 2012, I have been working in the ECUAVIDA cohort that is supported through a Wellcome Trust Senior Fellowship to Professor Philip Cooper. In Ecuador, the ECUAVIDA and SCAALA studies have been running by the Ecuadorian Foundation for Health Research (FEPIS). Currently, I am part of the research team and I am in charge of the social component of the two studies, working closely with Professor Philip J Cooper who is my co-supervisor in this PhD course. The present research is part of this social component and I have been responsible for the design, data collection, data analysis and manuscript preparation of all papers presented in this research.

Structure of the Thesis

Considering the objectives and the different methods used to evaluate the effects of urbanisation on asthma, the present research has been organized into five chapters:

Chapter 1 comprises a manuscript version of the study protocol. This chapter includes a general introduction of the current state of asthma in LMICs, objectives and context of the study, study locations and populations, methods, ethical approval, study funding and the structure of the thesis.

Chapter 2 describe a systematic review paper evaluating how epidemiological studies have assessed the relationship between asthma and urbanisation in LMICs and explored urban/rural differences in asthma prevalence. This chapter reports that prevalence is greater in urban than rural populations in LMICs, but the mechanisms by which urbanisation affects asthma are not clear, explained probably by the methods used to measure urbanisation.

Chapter 3 includes one paper approaching the relationship between the process of urbanisation and asthma using a multi-dimensional, quantitative measure of urbanicity. This chapter explores how increases in urbanicity levels are associated with a higher prevalence of asthma and show that the use of a multi-dimensional urbanicity indicator can identify better associations between wheeze/asthma and the process of urbanisation compared to widely-used urban vs. rural dichotomies.

Chapter 4 includes two papers evaluating the effects of internal migration on the prevalence of asthma in urban and rural areas. This chapter report how different dimensions of the migration process as place of birth, place of residence, age of migration and time since migration influence in the occurrence of asthma in children living in urban and rural environments.

Chapter 5 includes one manuscript exploring how changes in lifestyles between urban and rural areas could explain differences in asthma prevalence. The approach adopted in this study was to define lifestyle as a set of attributes representing groups of linked risk factors rather than the traditional approach of investigating the independent effects of individual risk factors. This chapter shows that the use of lifestyle as a set of attributes provides an alternative methodology for the evaluation of variations in wheeze prevalence in populations with different levels of development.

Chapter 6 includes a general discussion of the main findings in this research. It stresses the use of contextual and ecological analyses assessing the effects of urbanisation on asthma. It also highlights the relevance of this research in public health, epidemiology and social sciences. This chapter concludes with a short summary of the main results found in the five papers.

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Chapter 2.

Research Paper 1: Urbanisation and asthma in low and middle-income countries: a systematic review of the urban-rural differences in asthma prevalence

Overview Chapter 2

Over the last two decades, the suggestion that the process of urbanisation is associated with the increase of asthma prevalence in LMICs has become widely accepted. However, although a number of epidemiological studies conducted in urban and rural locations have provided important evidence to support this relationship, these studies are diverse in approaches, definitions and methodologies. Bearing in mind all these issues, our research starts with an overview of the scientific literature evaluating how epidemiological studies have assessed the relationship between asthma and urbanisation in LMICs. This part of the research discusses the most common epidemiological approaches used to evaluate the effects of urbanisation on asthma: studies comparing the prevalence of asthma between rural and urban areas; studies comparing the prevalence of asthma between cities, towns or communities within a country or across countries; and studies examining variations in the prevalence of asthma within cities. It then explains the advantages and limitations of these three approaches, focusing on the methodological issues inherent to each type of study. This chapter also discusses the urban-rural gap in asthma prevalence in LMICs and evaluates the magnitude of this difference using several asthma definitions. It concludes with a discussion on the need to use new approaches that include different dimensions of the urbanisation processes to a better understanding of the relationships between urbanisation and asthma.

Research paper Cover Sheet

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RESEARCH PAPER COVER SHEET

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SECTION A – Student Details

Student	**Alejandro Rodriguez**
Principal Supervisor	**Dr. Elizabeth Brickley**
Thesis Title	**Urbanisation and childhood asthma in a developing region of Latin-America: A Cross-sectional analysis in northeastern Ecuador**

If the Research Paper has previously been published please complete Section B, if not please move to Section C

SECTION B – Paper already published

Where was the work published?			
When was the work published?			
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion			
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Where is the work intended to be published?	**Thorax**
Please list the paper's authors in the intended authorship order:	**Alejandro Rodriguez, Laura C Rodrigues, Rebecca Normansell, Mauricio L Barreto, Elizabeth Brickley, Philip J Cooper.**
Stage of publication	**Submitted and accepted**

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	**Study design, data collection, data analysis, draft manuscript, and manuscript review.**

Student Signature: [redacted] **Date:** **06/08/2018**

Supervisor Signature: **Date:** 08/08/2018

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Abstract

Background: Urbanisation has been associated with increases in asthma prevalence in low and middle-income countries (LMICs). However, little is known of the mechanisms by which urbanisation and asthma are associated, perhaps explained by the methodological approaches used to assess the urbanisation-asthma relationship.

Objective: This review evaluated how epidemiological studies have assessed the relationship between asthma and urbanisation in LMICs, and explored urban/rural differences in asthma prevalence.

Methods: Asthma studies comparing urban/rural areas, comparing cities, and examining intra-urban variation were assessed for eligibility. Included publications were evaluated for methodological quality and Pooled Odds Ratios (OR) were calculated to indicate the risk of asthma in urban over rural areas.

Results: Sixty-six articles were included in our analysis. Fifty-nine compared asthma prevalence between urban and rural areas, five compared asthma prevalence between cities, and two examined intra-urban variation in asthma prevalence. Urban residence was associated with a higher prevalence of asthma, regardless of asthma definition: current-wheeze OR:1.43 (95%CI:1.21-1.70), doctor diagnosis OR:1.89 (95%CI:1.47-2.41), wheeze-ever OR:1.44 (95%CI:1.15-1.81), self-reported asthma OR:1.59 (95%CI:1.22-2.06), asthma questionnaire OR:1.57 (95%CI:1.07-2.29), and exercise challenge OR:1.96 (95%CI:1.32-2.91).

Conclusions: Most evidence for the relationship between urbanisation and asthma in LMICs comes from studies comparing urban and rural areas. These studies tend to show a greater prevalence of asthma in urban compared to rural populations. However, these studies have been unable to identify which specific characteristics of the urbanisation process may be responsible. An approach to understand how different dimensions of urbanisation, using contextual household and individual indicators, is needed for a better understanding of how urbanisation affects asthma.

Key words: urbanisation, asthma, urban and rural areas, LMICs

Introduction

The prevalence of asthma and Related Allergic Disorders (RAD) has been increasing over the past 4 decades.(1) However, recent evidence indicates that the prevalence may have reached a plateau in high-income countries (HICs) with a high prevalence, but continues to increase in lower prevalence LMICs, particularly among urban populations.(2,3) The factors that underlie such temporal and geographic trends in asthma prevalence are poorly understood, but are likely to reflect a complex interplay of biologic, environmental and social factors.(4)

It has been hypothesized that the urbanisation process could be in part responsible for the temporal and geographic variations of asthma prevalence in both HICs and LMICs.(5–7) This hypothesis has received support mainly by three observations. First, studies on wheezing or asthma in different regions of the world have regularly shown a lower asthma prevalence in rural settlements compared to cities.(5,8,9) Second, the low asthma prevalence in rural areas has been explained by possible protection provided by traditional rural exposures such as farming.(6,7) However, recent studies have shown that allergic disorders could be increasing in rural areas, reducing the urban-rural gap in asthma prevalence.(10–12) Third, exposures relating to environmental and lifestyle changes that originate from the urbanisation process have been identified as risk factors for asthma including changes in diet, sedentarism, reductions in childhood infections, smaller families, use of antibiotics, environmental pollution, and migration. (6,13)

Epidemiological studies have provided invaluable information about the relationship between urbanisation and asthma through use of diverse methods and indicators of urbanisation. However, studies evaluating the effects of urbanisation on asthma are complex and face several conceptual and methodological limitations. Firstly, there is no standard definition of urbanisation. Urbanisation is a highly complex process that affects all levels of human activity and no single definition can fully describe the multidimensional nature of this process.(14) Secondly, there is no universally accepted definition of what constitutes an urban area. Different countries use different definitions for urban areas mainly based on demographic, political or economic characteristics of their populations.(15) Thirdly, there is no agreed definition of asthma for research purposes, so different studies use different definitions such as doctor diagnosis, presence of clinical symptoms and bronchial hyper-responsiveness.(16,17)

In LMICs the specific features and mechanisms by which urbanisation affects asthma are not clear. Part of this problem may lie in the methods used by asthma studies to assess the effects of urbanisation on asthma. The aim of this systematic review is to provide a general overview of how epidemiological studies have assessed the relationship between asthma prevalence and urbanisation in LMICs.

In this review, we addressed the following research objectives:

- To examine the methods used to evaluate the effects of urbanisation on asthma.
- To examine rural /urban differences in asthma prevalence

Methods

We performed a systematic review of the scientific literature to identify studies that have assessed the relationship between asthma and urbanisation in LMICs following PRISMA guidelines.(18) The protocol can be accessed on PROSPERO (registration CRD 42017064470).

Inclusion and exclusion criteria

Population and context: Subjects of all ages living in urban or rural areas of LMICs. We excluded populations living in HICs.

Study designs: Cross-sectional, cohort and ecological studies. We excluded case-control studies, intervention, experimental and genetic studies. Studies that lacked essential data for calculating ORs were also excluded.

Exposure: Urban areas or urban environments defined by demographic, socioeconomic, administrative or other indicators associated to the urbanisation process. We excluded studies evaluating the effects of air pollution on asthma.

Outcomes: Studies using different asthma definitions such as wheeze/asthma in the last 12 months, clinical symptoms, doctor's diagnosis, questionnaire data and pulmonary function tests.

Search strategy

A literature search was done in PubMed, ScienceDirect and Scielo databases in February 2017 (Figure 1). To include all available evidence, past reviews, letters to the editor and publications discussing the relationship between urbanisation and asthma were also evaluated. Further, no restrictions were imposed regarding sample size, age, sex and publication date. Articles in English, Spanish and Portuguese were included in the search.

Figure 1. Major search terms in free text for search strategy in PubMed, ScienceDirect and Scielo.

((asthma) OR (asthma prevalence) OR (wheeze) OR (allergic diseases) OR (allergy)) AND ((urbanization) OR (urbanisation) OR (urban area) OR (rural area) OR (urban rural difference) OR (rural urban comparison) OR (urban rural environment) OR (urban rural population) OR (metropolitan) OR (city) OR (inner-city) OR (municipal) OR (urban neighbourhood) OR

Paper selection and retrieval process

Publications were grouped by three methods: a) studies comparing the prevalence of asthma between rural and urban areas; b) studies comparing the prevalence of asthma between cities, towns or communities within a country or across countries; and c) studies examining variations in the prevalence of asthma within cities.(19) Titles and abstracts of the articles identified with the initial search were screened by AR. Full-text papers were retrieved and classified based on the previously mentioned categories. Retrieved texts were evaluated by two reviewers (AR and PC) and a final decision upon their inclusion or exclusion was made based on the criteria previously outlined. In case of any doubts and uncertainties, a third author was consulted (LR). Review papers were included to provide a general overview of the topic and as a reference source only and did not provide primary data. A flow chart of the selection process is shown in Figure 2.

Data extraction

A working database was designed using SPSS-20 including relevant characteristics of the publications: authors(s) name, title, publication year, country, region, study design, study approach, area description, age range, sample size, indicators of urbanisation, urban area definition, asthma definition, urban-rural asthma prevalence, unadjusted OR, and P value for the urban-rural difference. For studies using more than one category for urban or rural settings (e.g. urban and peri-urban, or rural and peri-rural), those categories were grouped into either urban or rural area as appropriate.

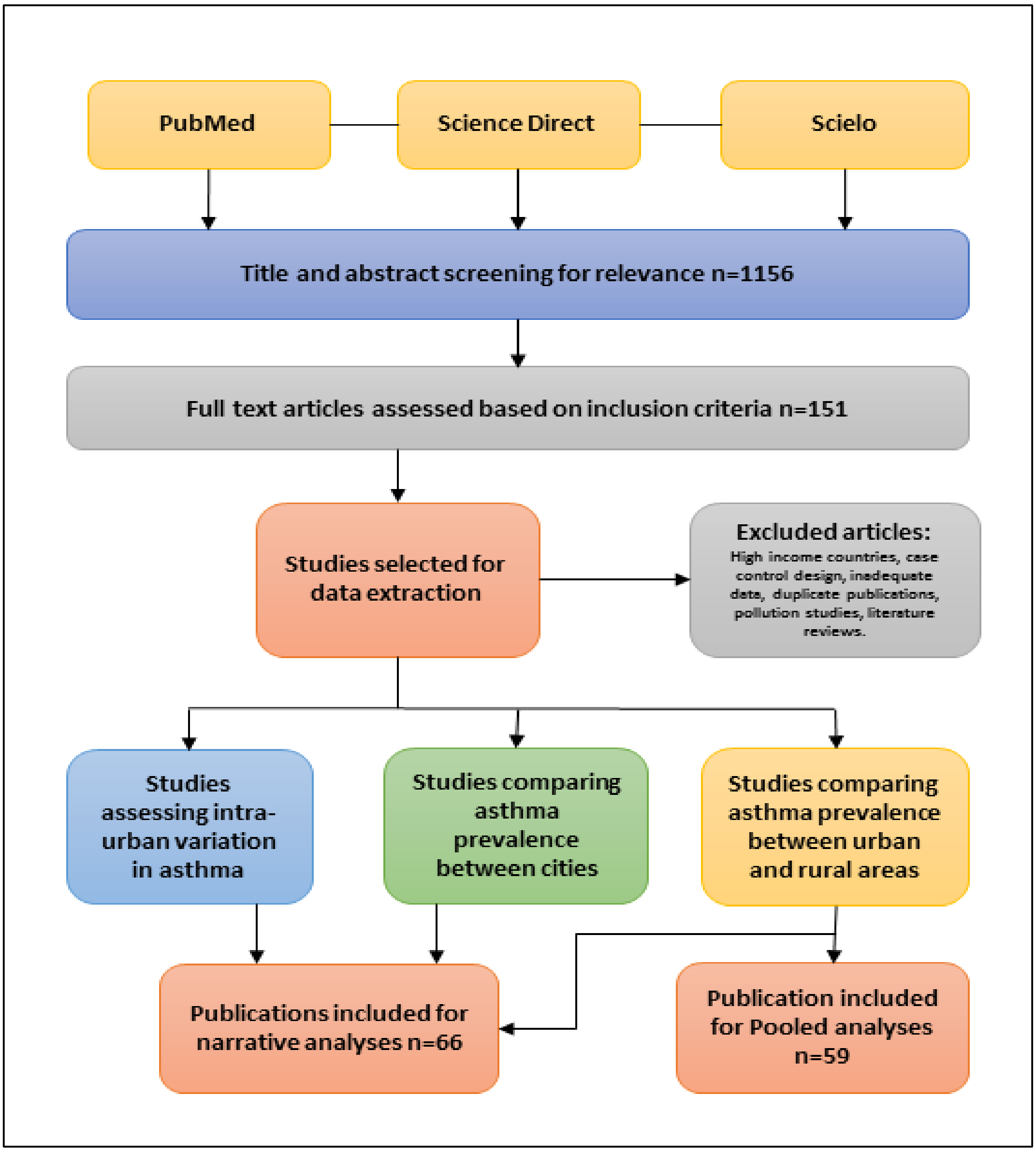
Study quality assessment

Study quality was assessed using STROBE (Strengthening the Reporting of Observational Studies in epidemiology) guidelines,(20) and "Critical Appraisal of Health Research Literature: Prevalence or Incidence of a Health Problem".(21) Seven criteria were considered (setting description, population description, sample method, sample size, urban definition, asthma definition, and adequate response rate) to classify study quality as high, medium or low. High-quality studies were those providing complete information for these criteria while medium quality studies lacked information for one criterion. Studies lacking information on more than one criterion were considered to be of low quality.

Statistical Analysis

A descriptive analysis was done based on the relevant characteristics of included publications. For studies comparing urban and rural areas, forest plots and unadjusted ORs were used to explore the association between asthma prevalence and area of residence. Because of the large degree of heterogeneity, studies were analysed by asthma definition. A single descriptive pooled OR (and 95%CI) was estimated for each definition using a random-effects model as a synthesis of available information. Results of individual studies were entered into the Cochrane Collaboration Review Manager 5 and analysed using Metaview 5. The I2 test was used to evaluate heterogeneity between studies.

Figure 2. Flow chart of publication selection process.

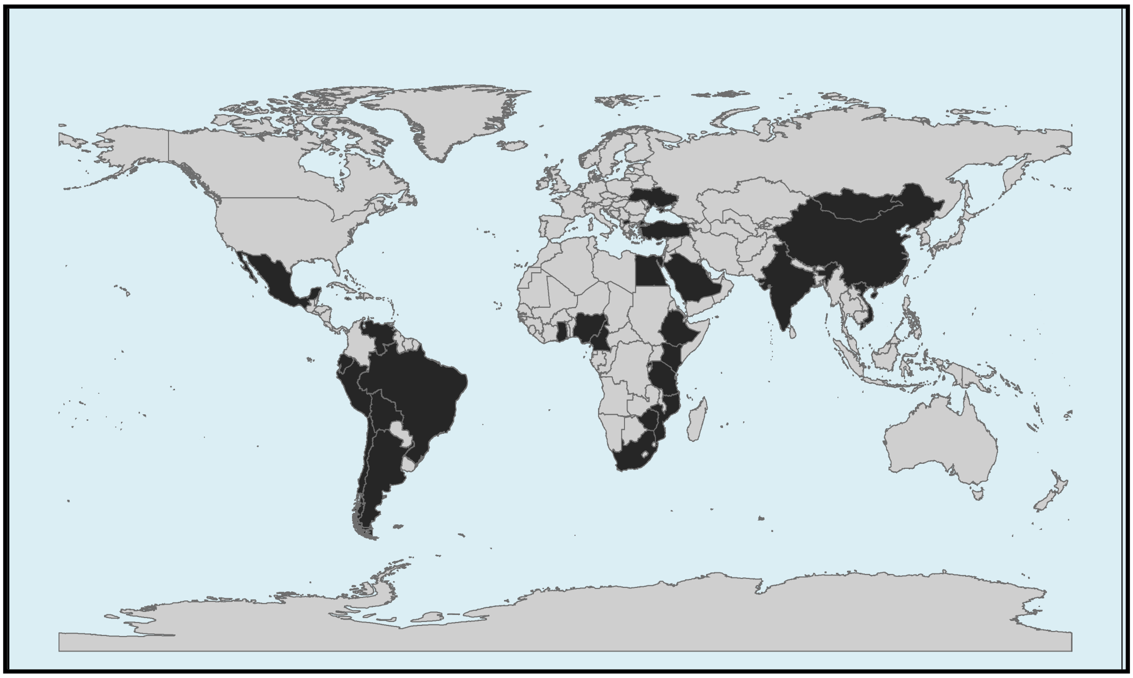


Results

Literature Search

From 1156 titles and abstracts identified for eligibility in the 3 databases, 151 articles were selected for a full text review. Sixty-six articles met our inclusion criteria after full-text review. We found two manuscripts with information for two locations in the same publication,(22,23) and 3 publications comparing asthma studies in the same location but at two different times.(10–12) These articles were included in our analysis considering each location (survey) as an independent study. We identified eleven asthma studies that used several categories to define urban and rural - these were re-categorized into a dichotomous urban versus rural classification for inclusion (Supplementary Table 1). (10,24–33) Although we did not consider review articles for data extraction, eleven review articles addressing the relationship between urbanisation and asthma in LMICs were identified in the literature search.(5,7,8,34–42) Eighty-five articles were excluded because they were conducted in high-income countries, case-control studies, studies that lacked data to estimate ORs, and duplicate publications.(Figure 2)

Figure 3. Map of countries in which studies on asthma and urbanisation have been done (countries in black)



Narrative Analysis

Sixty-six articles published between 1979 and 2017 met the inclusion criteria.(Table1) Fifty-nine publications compared asthma prevalence between urban and rural areas, five compared asthma prevalence between cities or rural settlements of the same country or among countries, and two studied intra-urban variations in asthma prevalence. Latin America (LA), Africa and Asia presented a similar number of publications (n=21, n=22, n=22, respectively).(Figure 3) Current wheeze was the most used asthma definition (42 publications). Forty-nine publications studied age groups ≤18 years including studies of children (0-12 years), adolescents (12-18 years), or both (0-18years).

Table 1. Characteristics of publications included in the systematic review

Variables and Categories	n (%)
Study approach:	
Comparing urban vs. rural areas	59 (89%)
Comparing cities or settlements	5 (8%)
Comparing intra-urban variation	2 (3%)
Region:	
Asia	22 (33%)
Africa	22 (33%)
Latin America	21 (32%)
Easter Europe	1 (2%)
Study design:	
Cross-sectional	58 (88%)
Ecologic	7 (10%)
Cohort	1 (2%)
Methodology:	
ISAAC**	34 (52%)
Other	32 (48%)
***Asthma Definition:**	
Wheezing ever	19 (16%)
Current wheeze	42 (35%)
Doctor diagnosis	24 (20%)
Exercise challenge test	10 (8%)
Self-report asthma	15 (12%)
Questionnaire diagnosis	11 (9%)
Age category (years):	
Children (0-12)	15 (23%)
Adolescent (12-18)	13 (18%)
Children and Adolescent (0-18)	21 (34%)
Adult (>18)	9 (14%)
All ages	7 (11%)
Year of the publication:	
Before 1990	2 (3%)
1990 – 1999	6 (9%)
2000 – 2009	23 (35%)
2010 – 2017	35 (53%)

*Some studies used 2 or 3 asthma definition, so percentages were calculated using the total number of definitions as denominator. **ISAAC – International Study of asthma and Allergies in Childhood

Asthma studies comparing rural and urban areas

We found fifty-nine cross-sectional studies conducted in thirty-two different countries of Africa,(10,23,26,27,30,31,33,43–56) Asia,(11,12,28,29,32,57–71) LA,(22,24,25,72–81) and Eastern Europe.(82) Figure 4 shows differences in asthma prevalence between urban and rural areas of these countries. Asthma prevalence was generally higher in urban areas. However, proportions of studies showing greater prevalence in urban compared to rural areas varied by asthma definition (Figure 5): current wheeze 69% (27/39 of which findings were statistically significant in 20 studies), wheezing ever 79% (15/19 of which 11 were significant), doctor diagnosis 90% (17/20 of which 11 were significant), exercise challenge test 90% (9/10 studies of which 6 were significant) self-reported asthma 80% (12/15 of which 8 were significant), and questionnaire diagnosis 92% (10/11 of which 5 were significant). Complete data are shown in Supplementary Table 2.

Pooled unadjusted ORs and forest plots for urban vs. rural comparisons of asthma prevalence by asthma definition are shown in Figures 6-8. Pooled ORs were: current wheeze, OR:1.43 (95%CI 1.21-1.70); doctor diagnosis, OR:1.89 (95%CI 1.47-2.41); wheeze ever, OR:1.44 (95%CI 1.15-1.81); self-reported asthma, OR:1.59 (95%CI 1.22-2.06); questionnaire-defined asthma, OR:1.57 (95%CI 1.07-2.29); and exercise-induced asthma OR:1.96 (95%CI1.32-2.91). A high statistical heterogeneity was found ($I^2 > 60$) for all definitions.

Asthma studies comparing cities

Publications in this group used ecological designs to compare different urban characteristics between locations in the same country or across countries to infer effects of the urban environment on asthma.(Table 2) Five studies were included in this group. The first evaluated associations between asthma prevalence and eleven health and socioeconomic indicators in 20 Brazilian cities and showed that indicators related to urban poverty and inequality were associated with a greater asthma prevalence.(83) The second evaluated 59 rural communities in Ecuador and correlated community asthma prevalence with different indices constructed to represent the process of urbanisation in the communities.(84) The study showed that greater levels of urbanisation, particularly with respect to lifestyle and socioeconomic indices, were positively associated with asthma prevalence. The third compared the prevalence of asthma between 31 urban centres across LA using several socioeconomic and environmental indicators.(85) This study found that social inequalities between cities could be a central determinant of the geographic variation in asthma prevalence within LA. A fourth study conducted in Brazil used 266 municipalities with more than 100,000 inhabitants as the unit of analysis.(86)

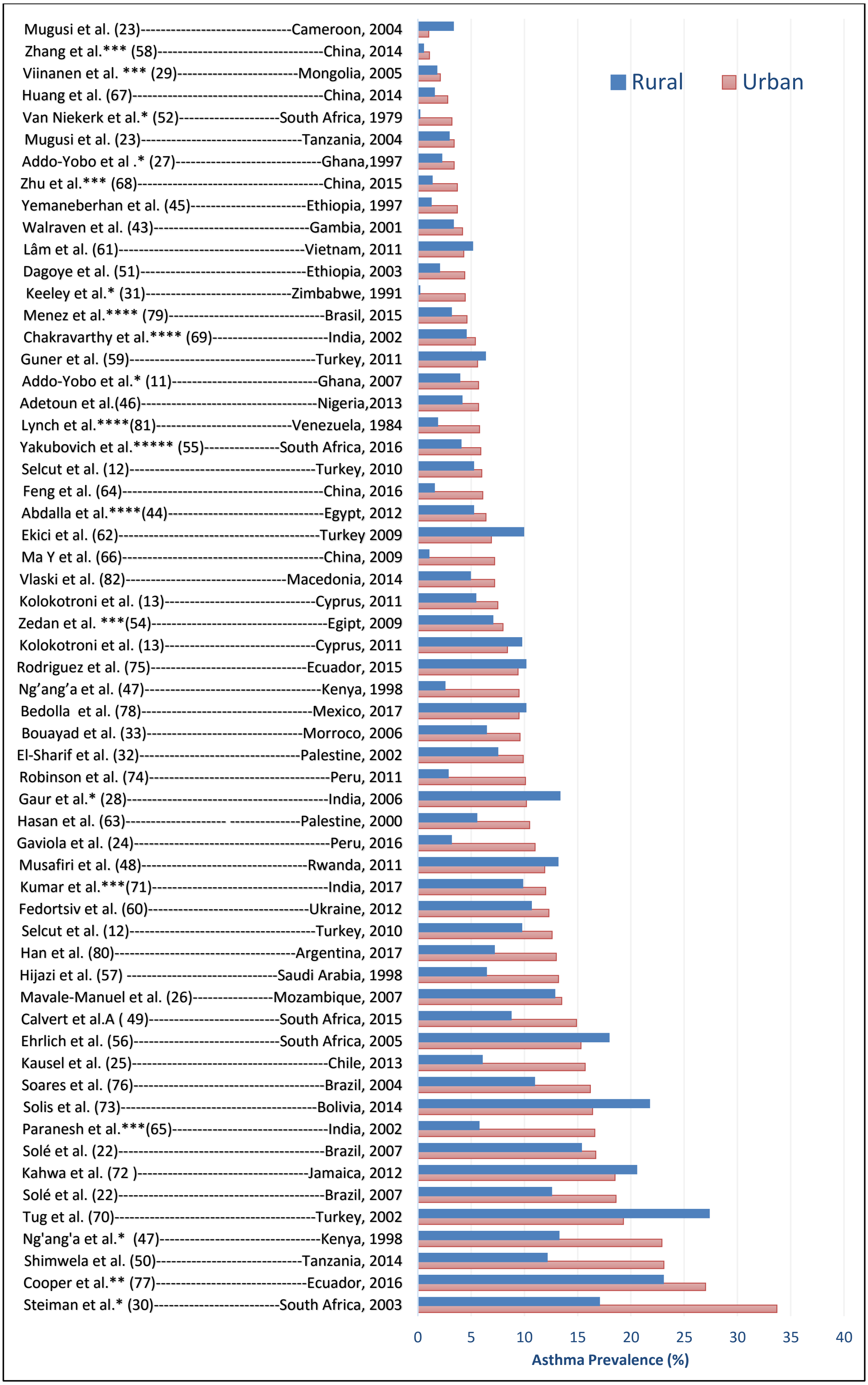

Mugusi et al. (23)-----------------------------Cameroon, 2004
Zhang et al.*** (58)--------------------------------China, 2014
Viinanen et al. *** (29)-----------------------Mongolia, 2005
Huang et al. (67)-------------------------------------China, 2014
Van Niekerk et al.* (52)-------------------South Africa, 1979
Mugusi et al. (23)-----------------------------Tanzania, 2004
Addo-Yobo et al .* (27)--------------------------Ghana,1997
Zhu et al.*** (68)---------------------------------China, 2015
Yemaneberhan et al. (45)---------------------Ethiopia, 1997
Walraven et al. (43)----------------------------Gambia, 2001
Lâm et al. (61)-----------------------------------Vietnam, 2011
Dagoye et al. (51)-----------------------------Ethiopia, 2003
Keeley et al.* (31)---------------------------Zimbabwe, 1991
Menez et al.**** (79)-------------------------------Brasil, 2015
Chakravarthy et al.**** (69)---------------------India, 2002
Guner et al. (59)------------------------------------Turkey, 2011
Addo-Yobo et al.* (11)---------------------------Ghana, 2007
Adetoun et al.(46)---------------------------------Nigeria,2013
Lynch et al.****(81)-------------------------Venezuela, 1984
Yakubovich et al.***** (55)--------------South Africa, 2016
Selcut et al. (12)-----------------------------------Turkey, 2010
Feng et al. (64)--------------------------------------China, 2016
Abdalla et al.****(44)------------------------------Egypt, 2012
Ekici et al. (62)-------------------------------------Turkey 2009
Ma Y et al. (66)-------------------------------------China, 2009
Vlaski et al. (82)------------------------------Macedonia, 2014
Kolokotroni et al. (13)----------------------------Cyprus, 2011
Zedan et al. ***(54)-----------------------------------Egipt, 2009
Kolokotroni et al. (13)----------------------------Cyprus, 2011
Rodriguez et al. (75)----------------------------Ecuador, 2015
Ng'ang'a et al. (47)--------------------------------Kenya, 1998
Bedolla et al. (78)---------------------------------Mexico, 2017
Bouayad et al. (33)-----------------------------Morroco, 2006
El-Sharif et al. (32)---------------------------Palestine, 2002
Robinson et al. (74)----------------------------------Peru, 2011
Gaur et al.* (28)--------------------------------------India, 2006
Hasan et al. (63)-------------------- -------------Palestine, 2000
Gaviola et al. (24)------------------------------------Peru, 2016
Musafiri et al. (48)-------------------------------Rwanda, 2011
Kumar et al.***(71)----------------------------------India, 2017
Fedortsiv et al. (60)------------------------------Ukraine, 2012
Selcut et al. (12)-----------------------------------Turkey, 2010
Han et al. (80)----------------------------------Argentina, 2017
Hijazi et al. (57) -----------------------------Saudi Arabia, 1998
Mavale-Manuel et al. (26)---------------Mozambique, 2007
Calvert et al.A (49)------------------------South Africa, 2015
Ehrlich et al. (56)---------------------------South Africa, 2005
Kausel et al. (25)--------------------------------------Chile, 2013
Soares et al. (76)-------------------------------------Brazil, 2004
Solis et al. (73)--------------------------------------Bolivia, 2014
Paranesh et al.***(65)------------------------------India, 2002
Solé et al. (22)--Brazil, 2007
Kahwa et al. (72)--------------------------------Jamaica, 2012
Solé et al. (22)--Brazil, 2007
Tug et al. (70)--Turkey, 2002
Ng'ang'a et al.* (47)-----------------------------Kenya, 1998
Shimwela et al. (50)----------------------------Tanzania, 2014
Cooper et al.** (77)-----------------------------Ecuador, 2016
Steiman et al.* (30)-----------------------South Africa, 2003
Rural
Urban
0
5
10
15
20
25
30
35
40
Asthma Prevalence (%)

Figure 4. Urban-rural gradient in asthma prevalence in low and middle-income countries. Asthma definition: (*) exercise challenge test, (**) wheeze ever, (***) asthma questionnaire, (****) Doctor diagnosis. All other studies were defined using current wheeze.

This study correlated indicators of socioeconomic factors and violence with the rate of hospital admissions for asthma. The study found a direct correlation between indicators of violence and rates of admission due to asthma, and an inverse correlation with indicators of development. The final study evaluated the effect of urbanisation on hospital admissions and death rates from asthma in 5,505 municipalities in Brazil using time series analysis in which urbanisation was defined as the proportion of people living in urban areas by municipality.(87) The study showed that urban population growth by municipality was associated with a rise in hospital admissions and death rates from asthma in children and young adults.(87)

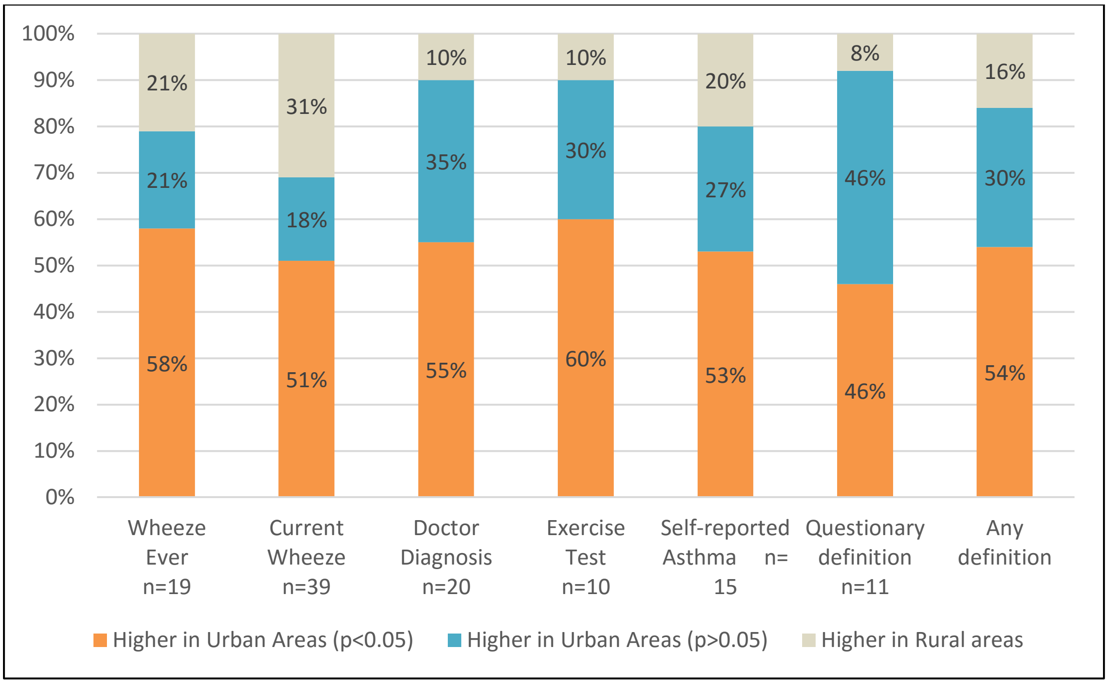
Asthma studies examining intra-urban variations within cities

We found two studies describing the spatial distribution of asthma and their relationships with social and health determinants in two Brazilian cities.(88,89) Both publications were ecological studies using census wards as the unit of analysis evaluating how living in a particular spatial setting within a city might be associated with asthma. The first study found that areas of Salvador whose population had lower levels of education and income, had a higher risk of hospitalization for respiratory diseases, particularly for asthma and pneumonia.(88) The second study conducted in Belo Horizonte found that hospital admissions for asthma were higher in areas of greater social vulnerability, suggesting that social and environmental factors may be determinants of variation in asthma prevalence.(89)

Table 2. Publications comparing asthma prevalence among cities and publication comparing variation in asthma within cities.

Publication	Methodology	Asthma definition	Urban indicators	Findings
Author: Cunha **Year: 2007** **Reference: 83**	-Approach: Comparing cities -Region/country: Brazil -Population: 6-7 & 13-14 years -Unit of analyses: Cities -Sample: 20	Current Wheezing	-**Socioeconomic indicators:** Illiteracy rate, Poverty rate, Income, Water supply, Sanitation, GINI, Human Development Index HDI. -**Health indicators:** Infant Mortality, Mortality for external Causes and Hospitals beds.	Asthma prevalence increased with poorer sanitation and with higher infant mortality, GINI index and external mortality. Poverty and inequality seems to be related with asthma prevalence in urban areas of Brazil.
Author: Rodriguez **Year: 2011** **Reference: 84**	-Approach: Comparing cities -Region/Country: Ecuador -Population: 5-15 years -Unit of analyses: Rural Communities -Sample: 59	Current Wheezing	-**Infrastructure Index:** Administrative grade, spatial organisation, transport access, electrical grid, pipe water system, telephone system, health centre, educational institutions. -**Socioeconomic Index:** Parent's Education, household income, material goods, access to urban services, housing materials, motors vehicle. -**Lifestyles Index:** Junk food consumption, physical exercise, tv viewing, farm activities, pets in house, migration and parasite infections.	Lifestyle and socioeconomic indicators had stronger overall effects on asthma prevalence than infrastructure indicators. Higher asthma prevalence was present in communities with a higher socioeconomic level and a more urbanised lifestyle.
Author: Fattore **Year: 2014** **Reference: 85**	-Approach: Comparing cities -Region/Country: Latin America -Population: 6-7 years -Unit of analyses: Cities -Sample: 31	Current Wheezing	-**Socioeconomic indicators:** Gini Index and Human Development Index HDI. -**Environmental variables:** Water supply, sanitation, crowding -**Health indicators:** Infant Mortality and Homicide mortality rate	Income inequality, lack of adequate sanitation, less crowding households, greater reduction in the infant mortality rates and high homicide rates were determinants of asthma symptoms in Latin American urban children.
Author: Tabalipa **Year: 2015** **Reference: 86**	-Approach: Comparing cities -Region/country: Brazil -Population: 6-7 & 13-14 years -Unit of analyses: Municipalities -Sample: 266	Hospital rate admissions (doctor diagnosis)	-**Index of Youth Vulnerability to Violence:** injury from external causes, incidences of homicides, traffic accidents, education, involvement in crime, poverty and unemployment.	Direct correlation between indicators of violence and rates of admission due to asthma, and an inverse correlation with indicators of development.
Author: Ponte **Year: 2016** **Reference: 87**	-Approach: Comparing cities -Region/country: Brazil -Population: 5-24 & 25-39 years -Unit of analyses: Municipality -Sample: 5505	Hospital rate admissions (doctor diagnosis)	-**Socioeconomic Indicators:** Per capita income, proportion of the population living in an urban area -**Health Indicators:** Number of physicians, number of hospital beds, rate of hospital admission from influenza, access to inhaled corticosteroid for asthma.	An increase in urban population by municipality was associated with lower odds for reduced hospital admissions and death rates from asthma in children and young adults
Author: Antunes **Year: 2014** **Reference: 88**	-Approach: Intra-urban variation -Region/country: Bahía, Brazil -Population: All population -Unit of analyses: Census Wards -Sample: 93	Hospital rate admissions (doctor diagnosis)	-**Socioeconomic Indicators:** Income, education, household crowding, presence of slums, GINI Index, sanitation, garbage collection.	Areas of Salvador whose population had lower levels of education and income had higher risk of hospitalization for respiratory diseases, particularly for asthma and pneumonia.
Author: Dias **Year: 2016** **Reference: 89**	-Approach: Intra-urban variation -Region/country: Belo Horizonte, Brazil -Population: 0-14 -Unit of analyses: Census Wards -Sample:	Hospital rate admissions (doctor diagnosis)	-**Health Vulnerability Index:** Inadequate water supply, sanitary sewage and inadequate garbage collection, housing, illiterate population, per capita income, race and ethnicity.	Hospital admissions for asthma were higher in areas of greater social vulnerability, suggesting that social and environmental factors may be determinants of variation in asthma prevalence in urban areas.

Figure 5. Proportions of studies showing greater prevalence of asthma in urban compared to rural areas by asthma definition.



Study quality

Information on study quality is provided in Supplementary Table 3. There was considerable variation in methodological quality between studies. Of the 66 studies included in this systematic review, 26 were considered of low methodological quality. Although most studies used schools as the unit of analysis (comparing urban and rural schoolchildren), the methods by which schools were selected were variable and generally not random but based on convenience samples (n=16). Twenty-three studies provided no information on response rates. Most studies used population size and administrative criteria to define urban and rural areas, comparing populations living in cities with those in rural towns or cities versus communities or villages. However, fifteen studies did not provide general information about the settings in which they were done (n=12). For studies comparing urban and rural areas, sample sizes ranged between 405 and 60,000 subjects. In the case of ecological studies sample size ranged between 20 and 5,505 units of analysis.

Figure 6. Forest Plot and unadjusted Odd Ratios for studies using current wheeze to define asthma comparing populations living in urban versus rural areas.

Study or Subgroup	Urban Events	Urban Total	Rural Events	Rural Total	Odds Ratio M-H, Random, 95% CI
Mugusi, Cameroon-2004	23	2265	68	2063	0.30 [0.19, 0.48]
Tug, Turkey-2002	476	2469	306	1122	0.64 [0.54, 0.75]
Ekici, Turkey-2009	1066	15554	1013	10289	0.67 [0.62, 0.74]
Solis, Bolivia-2014	281	1727	133	613	0.70 [0.56, 0.88]
Ehrlich, South Africa-2005	1311	8567	941	5259	0.83 [0.76, 0.91]
Lam, Vietnam-2011	91	2115	187	3667	0.84 [0.65, 1.08]
Kolokotroni, Cyprus-2011	118	1410	71	732	0.85 [0.62, 1.16]
Kahwa, Jamaica-2012	169	912	227	1105	0.88 [0.70, 1.10]
Guner, Turkey-2011	20	354	16	253	0.89 [0.45, 1.75]
Musafiri, Rwanda-2011	124	1036	104	788	0.89 [0.68, 1.18]
Rodriguez, Ecuador-2015	231	2510	421	4295	0.93 [0.79, 1.10]
Bedolla-Barajas, Mexico-2017	77	814	19	189	0.93 [0.55, 1.59]
Mavale-Manuel, Mozambique-2007	519	3853	149	1160	1.06 [0.87, 1.28]
Solé, Brazil_Rio Grande-2007	512	3066	468	3057	1.11 [0.97, 1.27]
Mugusi, Tanzania- 2004	36	1063	42	1443	1.17 [0.74, 1.84]
Selcuk, Turkey-2010	228	3792	84	1620	1.17 [0.90, 1.51]
Fedortsiv, Ukraine-2012	329	2665	234	2206	1.19 [0.99, 1.42]
Walraven, Gambia-2001	87	2062	104	3161	1.29 [0.97, 1.73]
Selcuk, Turkey-1994	547	4345	135	1390	1.34 [1.10, 1.63]
El-Sharif, Palestine-2002	155	1565	136	1817	1.36 [1.07, 1.73]
Kolokotroni, Cyprus-2000	270	3600	70	1296	1.42 [1.08, 1.86]
Adetoun Mustafa, Nigeria-2013	66	1153	10	244	1.42 [0.72, 2.80]
Vlaski, Macedonia-2014	355	4930	28	577	1.52 [1.03, 2.26]
Soares, Brazil-2004	541	3340	36	330	1.58 [1.10, 2.26]
Solé, Brazil_Pernambuco-2007	497	2674	44	352	1.60 [1.15, 2.22]
Bouayad, Morroco-2006	438	4422	80	1243	1.60 [1.25, 2.05]
Huang, China-2014	231	8248	113	7515	1.89 [1.50, 2.37]
Han, Argentina-2017	198	1525	12	168	1.94 [1.06, 3.56]
Dagoye, Ethiopia-2003	188	4285	58	2570	1.99 [1.47, 2.68]
Hasan, Palestine-2000	63	599	16	295	2.05 [1.16, 3.61]
Shimwela, Tanzania-2014	141	610	75	619	2.18 [1.61, 2.96]
Hijazi, Saudi Arabia-1998	135	1020	27	424	2.24 [1.46, 3.45]
Kausel, Chile-2013	512	3264	6	100	2.91 [1.27, 6.69]
Yemaneberhan, Ethiopia-1997	364	9844	36	3032	3.20 [2.26, 4.51]
Gaviola, Peru-2016	165	1501	45	1452	3.86 [2.75, 5.41]
Robinson, Peru-2011	73	725	20	716	3.90 [2.35, 6.46]
Odhiambo, Kenya-1998	54	568	15	604	4.13 [2.30, 7.40]
Feng, China-2016	423	7164	89	6087	4.23 [3.36, 5.33]
Ma Y, China-2009	254	3531	35	3546	7.78 [5.44, 11.11]
Total (95% CI)		**125147**		**77399**	**1.43 [1.21, 1.70]**
Total events	11368		5673		

Heterogeneity: Tau² = 0.27; Chi² = 751.80, df = 38 (P < 0.00001); I² = 95%
Test for overall effect: Z = 4.09 (P < 0.0001)

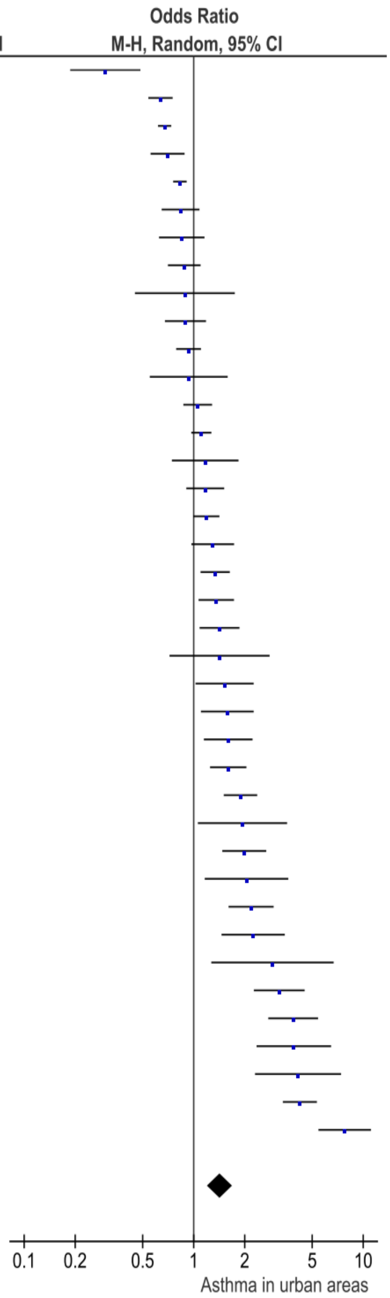


Figure 7. Forest Plots and unadjusted Odd Ratios for studies using wheezing ever and doctor diagnosis to define asthma comparing populations living in urban versus rural areas.

Study or Subgroup	Urban Events	Urban Total	Rural Events	Rural Total	Odds Ratio M-H, Random, 95% CI
Wheeze ever					
Mugusi, Cameroon-2004	53	2265	77	2063	0.62 [0.43, 0.88]
Solis, Bolivia-2014	470	1727	214	613	0.70 [0.57, 0.85]
Tug, Turkey-2002	636	2469	332	1122	0.83 [0.71, 0.97]
Bedolla-Barajas, Mexico-2017	191	814	47	189	0.93 [0.64, 1.34]
Mavale-Manuel, Mozambique-2007	946	3853	260	1160	1.13 [0.96, 1.32]
Solé, Brazil_Rio Grande-2007	1290	3066	1143	3057	1.22 [1.10, 1.35]
Cooper, Ecuador-2016	394	1458	141	611	1.23 [0.99, 1.54]
El-Sharif, Palestine-2002	302	1565	283	1817	1.30 [1.08, 1.55]
Bouayad, Morroco-2006	713	4422	160	1243	1.30 [1.08, 1.56]
Solé, Brazil_Pernambuco-2007	872	2674	92	352	1.37 [1.06, 1.76]
Chakravarthy, India-2002	114	584	39	271	1.44 [0.97, 2.14]
Hasan, Palestine-2000	98	599	35	295	1.45 [0.96, 2.20]
Lam, Vietnam-2011	118	2115	143	3667	1.46 [1.13, 1.87]
Mugusi, Tanzania- 2004	59	1063	50	1443	1.64 [1.11, 2.41]
Soares, Brazil-2004	1236	3340	78	330	1.90 [1.46, 2.47]
Huang, China-2014	544	8248	240	7515	2.14 [1.83, 2.50]
Hijazi, Saudi Arabia-1998	182	1020	31	424	2.75 [1.85, 4.10]
Feng, China-2016	1012	7164	248	6087	3.87 [3.36, 4.47]
Odhiambo, Kenya-1998	76	568	19	604	4.76 [2.84, 7.97]
Subtotal (95% CI)		**49014**		**32863**	**1.44 [1.15, 1.81]**
Total events	9306		3632		
Heterogeneity: Tau² = 0.23; Chi² = 396.75, df = 18 (P < 0.00001); I² = 95%					
Test for overall effect: Z = 3.17 (P = 0.002)					
Doctor diagnosis					
Adetoun Mustafa, Nigeria-2013	5	1153	7	244	0.15 [0.05, 0.47]
Han, Argentina-2017	64	1525	8	168	0.88 [0.41, 1.86]
Lam, Vietnam-2011	83	2115	139	3667	1.04 [0.79, 1.37]
Kahwa, Jamaica-2012	167	912	170	1105	1.23 [0.98, 1.56]
Abdallah, Egypt-2012	56	876	9	172	1.24 [0.60, 2.55]
Chakravarthy, India-2002	32	584	12	271	1.25 [0.63, 2.47]
Musafiri, Rwanda-2011	76	1036	45	788	1.31 [0.89, 1.91]
Ehrlich, South Africa-2005	360	8567	163	5259	1.37 [1.14, 1.66]
El-Sharif, Palestine-2002	179	1565	154	1817	1.39 [1.11, 1.75]
Bedolla-Barajas, Mexico-2017	69	814	11	189	1.50 [0.78, 2.89]
Menezes, Brazil-2015	2301	50015	316	10187	1.51 [1.34, 1.70]
Guner, Turkey-2011	37	354	18	253	1.52 [0.85, 2.74]
Fedortsiv, Ukraine-2012	55	2665	28	2206	1.64 [1.04, 2.59]
Feng, China-2016	475	7164	198	6087	2.11 [1.78, 2.50]
Lynch, Venezuela-1984	25	431	6	336	3.39 [1.37, 8.35]
Gaviola, Peru-2016	95	1501	19	1452	5.10 [3.10, 8.39]
Walraven, Gambia-2001	74	2062	22	3161	5.31 [3.29, 8.58]
Ma Y, China-2009	238	3531	39	3546	6.50 [4.62, 9.15]
Robinson, Peru-2011	94	725	16	716	6.52 [3.79, 11.19]
Odhiambo, Kenya-1998	24	568	1	604	26.60 [3.59, 197.31]
Subtotal (95% CI)		**88163**		**42228**	**1.89 [1.47, 2.41]**
Total events	4509		1381		
Heterogeneity: Tau² = 0.24; Chi² = 195.83, df = 19 (P < 0.00001); I² = 90%					
Test for overall effect: Z = 5.06 (P < 0.00001)					

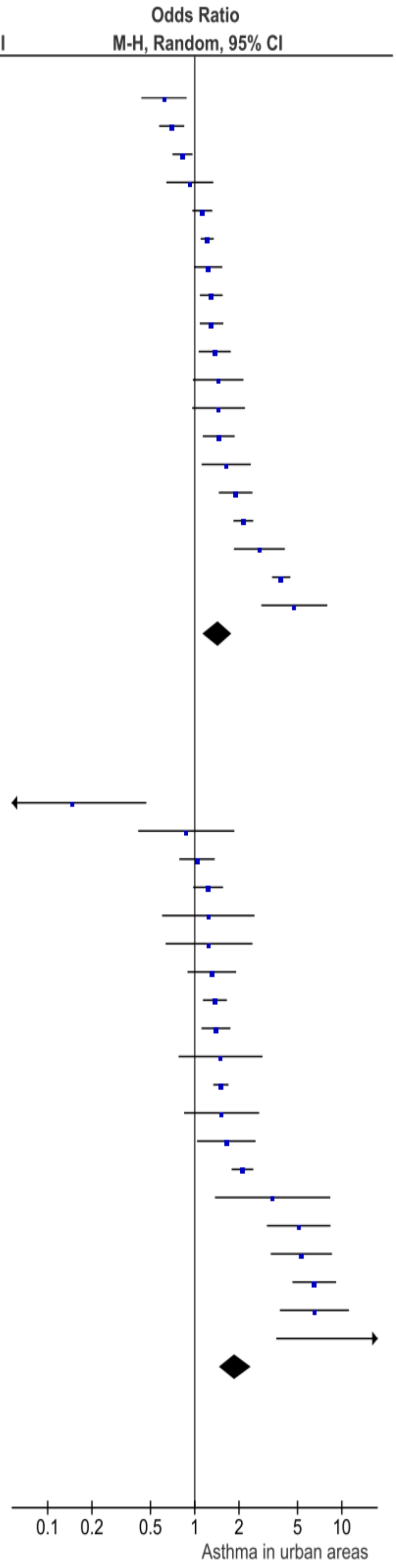
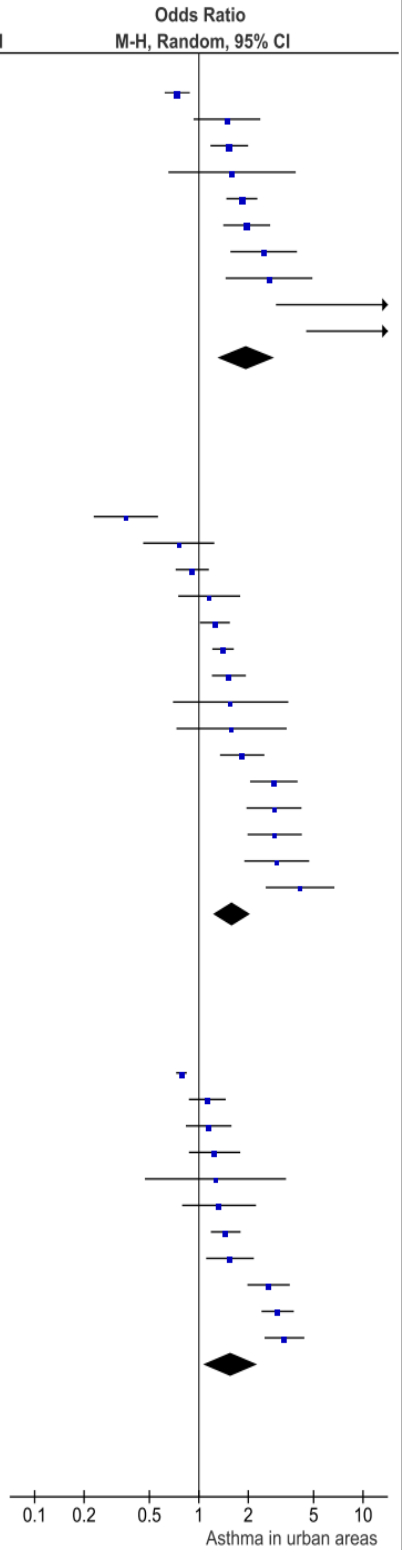


Figure 8. Forest Plots and unadjusted Odd Ratios for studies using exercise challenge test, self-reported asthma and asthma questionnaire to define asthma comparing populations living in urban versus rural areas.

Study or Subgroup	Urban Events	Urban Total	Rural Events	Rural Total	Odds Ratio M-H, Random, 95% CI
Exercise test					
Gaur, India-2006	444	4348	207	1552	0.74 [0.62, 0.88]
Addo-Yobo, Ghana-2007	69	1210	25	638	1.48 [0.93, 2.37]
Solis, Bolivia-2014	323	1727	80	613	1.53 [1.18, 2.00]
Addo-Yobo, Ghana-1997	28	819	6	276	1.59 [0.65, 3.89]
Calvert, South Africa-2010	246	1651	146	1671	1.83 [1.47, 2.27]
Ng'ang'a, Kenya-1998	131	573	63	479	1.96 [1.41, 2.72]
Steiman, South Africa-2003	65	193	36	212	2.48 [1.56, 3.96]
Shimwela, Tanzania-2014	38	610	15	619	2.68 [1.46, 4.92]
Van Niekerk, South Africa-1979	22	694	1	671	21.93 [2.95, 163.19]
Keeley, Zimbabwe-1991	62	1368	1	687	32.57 [4.51, 235.37]
Subtotal (95% CI)		**13193**		**7418**	**1.96 [1.32, 2.91]**
Total events	1428		580		
Heterogeneity: Tau² = 0.30; Chi² = 88.93, df = 9 (P < 0.00001); I² = 90%					
Test for overall effect: Z = 3.33 (P = 0.0009)					
Self-reported asthma					
Mugusi, Cameroon-2004	27	2265	67	2063	0.36 [0.23, 0.56]
Kausel, Chile-2013	518	3264	20	100	0.75 [0.46, 1.24]
Kolokotroni, Cyprus-2011	241	1410	135	732	0.91 [0.72, 1.15]
Mugusi, Tanzania- 2004	39	1063	46	1443	1.16 [0.75, 1.79]
Kolokotroni, Cyprus-2000	428	3600	126	1296	1.25 [1.02, 1.55]
Solé, Brazil_Rio Grande-2007	457	3066	339	3057	1.40 [1.21, 1.63]
Yakubovich, South Africa-2016	177	2989	119	3001	1.52 [1.20, 1.93]
Hasan, Palestine-2000	25	599	8	295	1.56 [0.70, 3.51]
Vlaski, Macedonia-2014	94	4930	7	577	1.58 [0.73, 3.43]
Soares, Brazil-2004	825	3340	50	330	1.84 [1.35, 2.51]
Yemaneberhan, Ethiopia-1997	354	9844	39	3032	2.86 [2.05, 3.99]
Solé, Brazil_Pernambuco-2007	564	2674	30	352	2.87 [1.95, 4.22]
Shimwela, Tanzania-2014	104	610	41	619	2.90 [1.98, 4.24]
Hijazi, Saudi Arabia-1998	149	1020	23	424	2.98 [1.89, 4.70]
Robinson, Peru-2011	84	725	22	716	4.13 [2.55, 6.69]
Subtotal (95% CI)		**41399**		**18037**	**1.59 [1.22, 2.06]**
Total events	4086		1072		
Heterogeneity: Tau² = 0.22; Chi² = 133.28, df = 14 (P < 0.00001); I² = 89%					
Test for overall effect: Z = 3.46 (P = 0.0005)					
Asthme questionnaire					
Ekici, Turkey-2009	1773	15554	1451	10289	0.78 [0.73, 0.84]
Selcuk, Turkey-1994	220	3792	84	1620	1.13 [0.87, 1.46]
Zedan, Egipt-2009	154	1935	55	785	1.15 [0.83, 1.58]
Musafiri, Rwanda-2011	89	746	53	541	1.25 [0.87, 1.79]
Viinanen, Mongolia-2005	8	385	8	484	1.26 [0.47, 3.40]
Guner, Turkey-2011	45	354	25	253	1.33 [0.79, 2.23]
Selcuk, Turkey-2010	526	4345	120	1390	1.46 [1.18, 1.80]
Zhang, China-2014	88	11792	57	11792	1.55 [1.11, 2.16]
Kumar, India-2017	200	1036	65	788	2.66 [1.98, 3.58]
Zhu, China-2015	497	13513	90	7209	3.02 [2.41, 3.79]
Paranesh, India-2002	925	5570	56	990	3.32 [2.51, 4.39]
Subtotal (95% CI)		**59022**		**36141**	**1.57 [1.07, 2.29]**
Total events	4525		2064		
Heterogeneity: Tau² = 0.38; Chi² = 266.27, df = 10 (P < 0.00001); I² = 96%					
Test for overall effect: Z = 2.31 (P = 0.02)					



Discussion

In this systematic review, we assessed how epidemiological studies conducted in LMICs have addressed the relationship between urbanisation and asthma. We compared also the reported prevalence of asthma in the urban and rural settings studied. Our analyses showed that almost all publications addressing the relationship between asthma and urbanisation come from studies comparing asthma prevalence between urban and rural populations. Few studies from LMICs have used more complex approaches to assess the relationship between urbanisation and asthma. This review provides evidence for an urban-rural gradient in asthma prevalence in LMICs, showing that the risk of asthma is higher in urban compared to rural areas, findings that were consistent irrespective of the asthma definition used. However, any interpretation of these data needs to be cautious because of the high level of heterogeneity between studies.

The study of urbanisation in asthma research has used different methodological approaches to measure the effects of urban areas and urban environments on asthma occurrence, of which the most widely used is comparison urban and rural populations. Although this approach has been useful to identify differences between environmental and social factors that could explain the urban-rural gradient in asthma prevalence,(6,90) they have limited usefulness understanding the multidimensional nature of urbanisation. Issues such as diverse dimensions of urban environments, differences in lifestyle between populations, distinct levels of urbanisation between urban centres and changes over time, cannot be properly addressed using this approach. For example, in our review, 13 studies reported a similar or a higher prevalence of asthma in rural compared to urban areas. It is likely that differences in lifestyle between urban and rural population may be responsible for these findings. Indeed, a non-systematic review of urban-rural comparisons of asthma prevalence showed only minimal differences, particularly where socioeconomic and environmental factors were comparable between urban and rural populations.(40) Thus, rural and urban populations that share similar living conditions and socioeconomic factors are likely to have comparable asthma risks. Such a situation is commonly found in HICs where rural and urban populations have similar lifestyles and standards of living, but also in LMICs where many urban (and peri-urban) localities may have similar living conditions to more rural settings, and in the case of urban slums living conditions may be worse than many rural settings.(91) This is important because of the frequent misconception in asthma studies that urban populations in LMICs live in cleaner and healthier environment.(92)

A second common approach has been to compare asthma prevalence or asthma hospitalization rates by different urban characteristics of cities, municipalities or communities - such as infrastructure, socioeconomic indicators, level of violence, urban services, health indicators, among others - to identify features of the urbanisation process that could be related to asthma prevalence. In studies comparing cities, a higher prevalence of asthma was observed in those cities with poor sanitation, high infant mortality, social inequalities and elevated levels of violence. Overall, these studies indicate that social deprivation in cities could contribute to asthma risk. In agreement with this, cross-sectional studies from the United States and LA have observed associations between asthma risk and poverty and lack of basic services in urban areas.(40,93,94) In the Ecuadorian study comparing rural communities, indices representing different domains of the urbanisation process as socioeconomic, lifestyle, urban infrastructure, and a summary urbanisation derived from representative variables of each of these, were associated with asthma prevalence. While significant heterogeneity was observed in the level of urbanisation between rural communities, the community prevalence of asthma increased with greater levels of urbanisation, especially with indices representing lifestyle and socioeconomic factors. These findings mirror those of other studies done in LMICs.(24,25,29,32) For example, a cross-sectional study from Mongolia compared the prevalence of asthma and RAD in localities with different levels of urbanisation: city, urban town and villages. The study showed an increasing prevalence of allergic diseases with greater level of urbanisation.(29) Comparisons of urban settlements within the same country offer advantages over inter-country studies: 1) the definition of urban is likely to be the same; and 2) lifestyle characteristics and a rural-urban comparison of these can be more reliably done within a country than between countries where other factors such as climate and culture are likely to be different. Such an approach may be more valid for generating aetiological hypotheses to explain why differences in asthma prevalence exist between urban areas. Similarly, comparisons between rural localities allow the study of urbanisation processes and *urban sprawl* in transitional societies where changes in lifestyle and environmental factors occur more rapidly. A weakness of studies using cities or settlements as the unit of analysis is the assumption that aggregate behaviours or characteristics at the city level are equally important for all residents. This ecologic fallacy requires a cautious interpretation of findings from such studies.(95)

Intra-urban studies evaluate how living in a particular area of a city may be associated with asthma outcomes. Such studies tend to use spatial groupings of individuals, commonly represented by neighbourhoods or census wards, to assess the effect of place of residence within an urban area on community or individual health.(19) These studies often require spatial and socio-economic information in these localities at individual and contextual levels, commonly provided by censuses and other publicly available data sources. For asthma research, this approach would be appropriate for addressing questions related to identifying the characteristics of areas within cities that may be associated with asthma. However, few such studies have been done in LMICs.

Limitations of this review

Studies evaluating specific characteristics of the urban environment, such as air pollution,(96) were not included. In the case of air pollution, there is a large literature and this topic may be better dealt with separately. We considered only studies done in LMICs because these countries share historical and developmental processes determining the evolution of the urban environment that are distinct from those that have occurred in HICs.(97) Case-Control studies were excluded from our analyses because all such studies were nested within cross-sectional studies already included in our search. Other ecological studies, especially those related to the International Study of Asthma and Allergies in Childhood (ISAAC), were not included here because they use populations from both LMICs and HICs.(98–100) Finally, because of the large degree of heterogeneity between studies (different study setting, population age, asthma definitions, urban-rural definitions) and variable methodological quality, pooled ORs estimated by asthma definition, need to be interpreted with caution.

Conclusions

This systematic review analysed the effects from the published literature of urbanisation on the prevalence of asthma in LMICs. Published epidemiological studies addressing this issue have mostly used one of 3 methodological approaches; comparisons of asthma prevalence between urban and rural areas, comparisons of cities within and between countries, and comparisons of areas within cities. Similarly, published studies have used a variety of definitions to define asthma. However, despite such heterogeneity in asthma definitions a number of consistent patterns emerged in this systematic review: 1) irrespective of the asthma definitions used, the prevalence of asthma was greater in urban than rural areas in most but not all studies; 2) indicators of social deprivation, inequality and or poverty within or between cities were associated with the prevalence of asthma or hospitalization rates for asthma; and 3) even at the rural level, indicators of urbanization, particularly lifestyle and socioeconomic factors, were associated with asthma prevalence. Overall, these findings provide evidence that urban residence and urbanisation are important determinants of asthma prevalence but do not permit us to identify which aspects of the urbanization process are most important as determinants of risk. There is a clear need for studies to address the multifactorial dimensions of the urbanisation process to identify specific urban factors or conditions that may be associated with asthma. Urbanisation is not a process confined to urban centres but is present in rural areas especially among population undergoing the transition from a traditional to a more urban and modern way of life. The study of transitional populations may provide the clearest clues as to how urbanisation affects asthma risk and urbanisation needs to be studied as a multidimensional process that affects all spheres of human activity.

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Chapter 3.

Research Paper 2: Measuring Urbanicity as a Risk Factor for Childhood Asthma in Transitional Areas of Ecuador: A Cross-sectional Analyses

Overview Chapter 3

As noted in the previous chapter, most of what we know about the relationship between urbanisation and asthma in LMICs is derived from studies comparing urban and rural areas. However, although these studies have been useful in defining the burden of asthma in urban and non-urban areas, issues such as diverse dimensions of urban environments, distinct levels of urbanisation between localities and urban environment and living conditions, cannot be properly addressed using this approach. To overcome these issues, the present chapter introduces the use of multi-dimensional approach to evaluate the effects of urbanisation on asthma in transitional populations. This novel approach is based on a composite measure of urbanicity based on different urban indicators and using geographical and census ward information. This section describes the methodology to build an urbanicity scale and stresses the usefulness of this measure in asthma research, especially compared to other commonly used measures of urbanicity such as political division and the rural-urban dichotomy. The chapter concludes with a discussion on the usefulness of this approach to provide novel insights into how urbanisation may affect asthma prevalence and especially how a population does not have to live in a city to experience many of the processes associated with urbanisation.

Research Paper Cover Sheet

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SECTION A – Student Details

Student	**Alejandro Rodriguez**
Principal Supervisor	**Dr. Elizabeth Brickley**
Thesis Title	**Urbanisation and childhood asthma in a developing region of Latin-America: A Cross-sectional analysis in northeastern Ecuador**

If the Research Paper has previously been published please complete Section B, if not please move to Section C

SECTION B – Paper already published

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SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	**Study design, data collection, data analysis, draft manuscript, and manuscript review.**

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Supervisor Signature: **Date:** 08/08/2018

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Abstract

Background: Although the process of urbanisation has been consistently associated with the increase in asthma prevalence in low- and middle-income countries, the nature of this association remains poorly understood.

Objective: This study explored the relationship between the process of urbanisation and asthma using a multi-dimensional, quantitative measure of urbanicity.

Methods: A cross-sectional analysis was conducted in 1843 children living in areas with diverse levels of urbanisation in the district of Quinindé, Ecuador. Categorical Principal Components Analysis was used to generate an urbanicity score conformed by 18 indicators representing demographic, socioeconomic, built environment and geographical dimensions of the process of urbanisation. Urban indicators were measured at census ward level using data from the last national census. Random effect logistic regression models were used to identify associations between urbanicity score, urban indicators and wheezing illness or asthma.

Results: The prevalence of wheeze ever, current wheeze and doctor diagnosis of asthma were 33.3%, 13% and 6.9% respectively. The urbanicity score ranged 0 to 10. Positive associations were observed between the urbanicity score and wheeze ever (OR=1.03) and doctor diagnosis (OR=1.05). For each point of increase in urbanicity score, the prevalence of wheeze ever and doctor diagnosis of asthma increased by 3% and 5%, respectively. Variables related with socioeconomic and geographical factors of the urbanisation process were associated with greater wheeze/asthma outcomes.

Conclusions: In transitional areas, even small-scale increases in urbanicity levels are associated with a higher prevalence of asthma. We show here that the use of a multi-dimensional urbanicity indicator can identify better associations between wheeze/asthma and the process of urbanisation compared to widely-used urban vs. rural dichotomies.

Key words: urbanisation, asthma, urban and rural areas, LMICs

Introduction

Studies conducted in low- and middle-income countries (LMIC) have consistently associated the process of urbanisation with temporal and geographical trends of increasing asthma prevalence.(1,2) These studies have shown that asthma prevalence is frequently higher in urban compared to rural settings, indicating urban residence to be a potential risk factor.(2,3) However, it is not yet clear how urban residence increases asthma susceptibility or the mechanisms by which the urbanisation process affects asthma prevalence in LMICs.

Numerous environmental, social and behavioural changes related to the process of urbanisation have been associated with differences in asthma prevalence between urban and rural populations.(4) Changes in diet, sedentarism, reduction in the frequency of infections, reduction in family size, use of antibiotics, increases in environmental pollution, migration, among other factors, have been identified as possible risk factors for asthma.(5–7) However, the nature of these associations remains poorly understood. Mechanisms to explain rural-urban gradients in in asthma prevalence in LMICs have been provided by the hygiene hypothesis in which childhood exposures to infectious diseases and a wide diversity of micro-organisms in the environment associated with traditional rural lifestyles are hypothesized to provide protection against asthma.(3) For example, helminth infections, endemic among many populations living in rural regions of LMICs and which have allergy-modulating effects, have been proposed as an explanation for the lower prevalence of asthma and allergies in rural populations.(8) However, the findings of studies investigating the effects of childhood infections and microbial diversity on asthma prevalence in LMICs have been far from conclusive(9) and there is a need to identify other factors associated with the process of urbanisation to explain urban-rural differences in asthma prevalence?

The process of urbanisation has been generally defined by the proportion of the population living in cities or urban areas.(10) However, the idea that urbanisation mostly affects populations living in cities is too simplistic a view of the urban process that inevitably reduces the concept to a phenomenon of population density. In broad terms, urbanisation can be defined as the gradual process of becoming urban and includes certainly a high concentration of people in relatively small areas, but also population growth by migration and natural increase, improvements in built infrastructure and changes in social and economic activities.(11) Although such a definition covers the multidimensional nature of the process, it also introduces a longitudinal perspective that is difficult to evaluate in cross-sectional studies. The use of the concept of "urbanicity" becomes relevant in this context to overcome this problem, concept which refers to the presence of conditions that are more common in urban areas than in non-urban areas at a given time.(12)

Our knowledge of the relationship between urbanisation and asthma in LMICs is derived from studies comparing urban and rural populations.(13) Although these studies have provided valuable information about the burden of the disease and differences in risk factors between urban and rural populations, they do not consider the multifactorial dimensions of the urbanisation process and cannot identify specific ways and conditions of living that may affect the prevalence of asthma, especially in populations within which levels of urbanisation are highly variable. The aim of this study was to develop a multidimensional quantitative measure of urbanicity to investigate the associations between urban conditions and asthma prevalence in a District of coastal Ecuador with a highly variable level of urbanisation.

In this analysis, we address the following objectives:

1. To develop a urbanicity scale using a set of indicators that represent the main dimensions of the urbanisation process.
2. To explore the associations between urbanicity scale, urban indicators and different definitions of asthma.
3. To compare the performance of the urbanicity scale with the urban-rural dichotomy and other commonly used definitions of urbanicity.

Methods

Context of the study

We used data from a birth cohort (the ECUAVIDA cohort) that followed 2404 newborns of mothers living within the District of Quininde, Esmeraldas Province, to 8 years of age. The primary objective of the cohort was to study effects of infectious diseases in early childhood on the development of asthma and allergies.(14) This cohort is using a multidisciplinary approach to understand better the causes of asthma and data that have been collected on children include information on demographic, lifestyle, psychosocial, and dietary factors, etc., in addition to clinical measurements. Data from this cohort are particularly well suited to study the effects of urbanisation on asthma because individual-level, household-level and census ward-level data are available for all study participants.

Study Area and Population

The study was conducted in the Districts of Quinindé and Puerto Quito, located in northwest Ecuador, a transitional area where the population is undergoing a rapid transformation from a traditional rural lifestyle to a more urban way of living. Although the recruitment of the cohort was defined by residence within the District of Quinindé, the District of Puerto Quito was included in the present analysis because of migration of cohort families to this District during follow-up. With an extension of 5019 km^2, and approximately 200,000 inhabitants, the study area is divided into 9 sub-districts and 330 census wards, of which 38 represent settlements of different population sizes as district capitals, towns and communities.(Figure 1) The settlements of La Concordia, Quinindé and Puerto Quito are the only 3 census wards considered as urban areas by the Ecuadorian census,(15) which classifies urban areas based mainly on administrative criteria rather than population size or coverage of urban services. Because of this, other settlements with significant populations in the study area (i.e. 5000 to 15,000 inhabitants) are not categorized as urban areas. The main economic activities in this region are focused on cattle and agriculture, especially cultivation of African palm oil and tropical fruits. Provision of basic services and other facilities are present in larger settlements where coverage, however, is deficient. (Table 1) The educational level is low (26% of urban and 17% of rural adults have completed secondary education) and main ethnic groups are Mestizo (52%) and Afro-Ecuadorian (35%).(15)

Study design and Sample

A cross-sectional analysis, nested within the birth cohort, to explore the effects of the process urbanisation on asthma prevalence. Asthma was measured at 5 years. Urban conditions were measured at the level of census wards where cohort children live, using data from the last national census in 2010.

Data Collection

Asthma symptoms were collected using a questionnaire that included the core asthma questions of the International Study of Asthma and Allergies in Childhood (ISAAC).(16,17) The questionnaire was administered to the child's mother by a trained physician (MV and MEC). Three different definitions were used for asthma: wheeze ever, current wheeze (wheeze in the last 12 months) and doctor diagnosis of asthma. Geographical coordinates of the child's household allowed each child to be referenced to a specific census ward. Geographical information on the study area and urban indicators by census ward were obtained through National Institute of Statistical and Census of Ecuador (INEC) where data on geographic, demographic, economic and social characteristics of household within each census ward are available.(15)

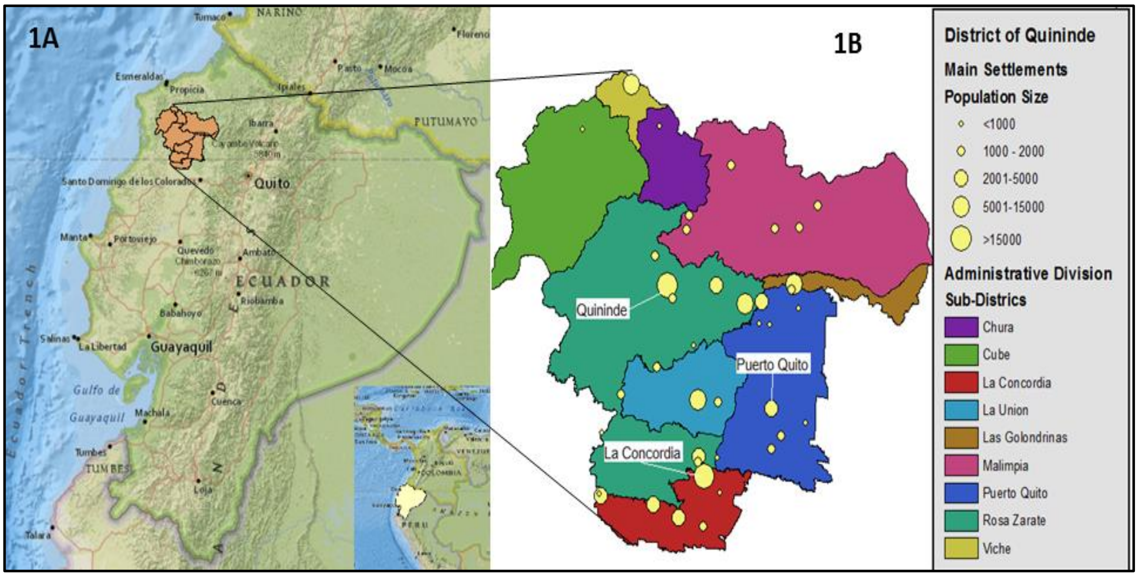

Figure 1. Study area by political division. Figure shows: 1A - map of Ecuador and location of study area. 1B - Geo-political divisions of the study area by parish and main population settlements.

Measures of Urban Conditions

Based on different dimensions of the urbanisation process and data available for analysis, 18 indicators were selected for inclusion in our urbanicity scale. These variables were classified into 4 groups representing some of the main dimensions of the urbanisation process: 1) Demographic - representing the phenomenon of population concentration within restricted spaces (variables - population size and density). 2) Socioeconomic - representing changes in living conditions related to the rural-urban transition (non-agricultural activities, secondary education, commercial activities, housing constructed with cement, access to mobile phones, internet, computers and satellite tv). 3) Built Environment - physical characteristics of the urban environment characterized by access to basic services, public and private institutions, and urban infrastructure (street paving, sewage access, electricity, and educational institutions and health institutions). 4) Geographical - representing spatial distribution of the settlement where the child lives (geo-political division, proximity to urban centres and access to highways). Urban indicators and definitions for each dimension group are shown in Table 1.

Spatial Analysis

A geographical information system was used to allocate observations and urban characteristics (educational, health institutions and roads) to respective census wards. Additionally, buffering analyses was used to build concentric circles of Euclidian distances around the main cities to calculate distances between households and core urban centres to evaluate urban sprawl. Maps representing the urbanicity scale and other characteristics of the study area were built using ArcGIS 10.2.2

Statistical Analyses

Categorical Principal Components Analysis (CATPCA) was used to generate a composite measure of urbanicity using demographic, socioeconomic, built environment and geographical indicators. CATPCA is a multivariate technique that summarises a large number of correlated variables in a reduced number of components or independent variables.(18) In contrast to similar techniques that are restricted to numeric variables, CATPCA integrates quantitative and qualitative variables in the analysis. This technique simultaneously concedes metric properties to the qualitative variables assigning numerical values to each category of nominal or ordinal variables through optimal scaling. (19). In CATPCA analysis, the first component explains the highest proportion of observed variance while the second component accounts for most of the variance not explained by component 1, and so on. The original variables are associated with each component through component loadings that show contributions to a given component. Values of correlation range -1 to +1, with a larger absolute value indicating a stronger contribution of a variable to that component. Each component produces a Z score for each observation (in our case by each census ward) that summarises the contribution of all variables to each component.(18) Two components were retained in our model and the score provided by the first one was used as a measure of urbanicity. The urbanicity score was interpreted such that higher values of component scores by census wards indicated a higher level of urbanicity. Subsequently, the urbanicity index was categorized in several groups representing diverse levels of urbanisation to assess its performance against other measures of urbanicity such as urban-rural classifications and geo-political division.

Bivariate analyses using random effects logistic regression models were used to identify associations between asthma definitions, urbanicity score and urban indicators, allowing for two-level data structure (i.e. at individual and census ward levels). Odds ratios (ORs) were estimated for each association. Variables with $P<0.05$ were considered statistically significant. Statistical analyses were done using SPSS V.24

Results

We evaluated 1843 children living in 157 census wards of 330 present within the two study districts. The study population represented 77% of the 2,404 newborns recruited into the ECUAVIDA birth cohort. Most of children not included in the study had either migrated outside the study area (15%) or were lost to follow-up (8%). The prevalence of wheeze ever, current wheeze and doctor diagnosis were 33.3%, 13% and 6.9%, respectively. Table 1 shows descriptive measures for 18 indicator variables representing demographic, socioeconomic, built environment and geographic dimensions for the 157 census wards included in this analysis and

Table 1. Summary of the urban indicators by dimensions and study area.

Dimensions	Indicators	Definitions	Categories	Quinindé district N= 330 wards		Study population N=157 wards	
				Mean (SD)*	Min – Max**	Mean (SD)*	Min - Max**
Demographic	Population Size	Number of people in each census ward		580 (2360)	36 - 29356	839 (3384)	96 - 29356
	Population density	Number of people per kilometre squared		332 (924)	1.5 - 4899	413 (1047)	1.6 - 4899
Socio-economics	Non-agriculture activities	Percentage of population working in non-agricultural activities		28.9 (19.1)	0 – 98.2	32.8 (20.4)	4.4 – 98.2
	Secondary Education	Percentage of population with secondary education.		12.5 (7.4)	0 – 43	14.5 (7.8)	2 - 43
	Commercial activities	Percentage of population working in commercial activities		9 (9.5)	0 – 51.7	10.6 (18.9)	0 – 51.7
	Concrete housing	Percentage of households with iron roof and concrete walls and floors.		30 (18)	0 – 77.3	33.1 (19.8)	0 – 77.3
	Mobile phone access	Percentage of households with mobile phones		70.7 (12.9)	19 – 97.2	72.7 (10.7)	33.3 - 97.2
	Internet access	Percentage of households with internet		1.9 (2.5)	0 – 13.3	2.3 (2.8)	0 – 13.3
	Computer access	Percentage of households with computers.		3.9 (4.6)	0 – 27.9	4.4 (5.1)	0 – 27.9
	Satellite TV access	Percentage of households with access to satellite TV.		3.3 (7.4)	0 – 66.2	4.3 (9.4)	0 – 66.2
Built Environment	Pavement street	Percentage of households with access to paved streets.		8.4 (15.7)	0 – 77.5	13.2 (19.8)	0 – 77.5
	Sewage system	Percentage of households with access to a sewage system.		1.7 (7.2)	0 - 74	1.6 (5.5)	0 – 51.3
	Electricity	Percentage of households with access to the electricity grid.		78.4 (22.6)	0 - 100	82.8 (19.3)	2.2 - 100
	Educational Institutions	Number of primary and secondary educational institutions		1.4 (2)	0 - 21	1.5 (2.6)	0 - 21
	Health Institutions	Type of health institution by census locality	Categories	Frequency	Percent %	Frequency	Percent %
			None	287	87	144	91.7
			Health centre	23	7	11	7
			Hospital	2	0.6	2	1.3

Continuation Table 1

Dimensions	Indicators	Definitions		Quinindé district N= 330 wards		Study population N=157 wards	
				Frequency	Percent %	Frequency	Percent %
Geographic	Geo-political division	Census wards classified by political/administrative division.	Countryside	292	88.5	134	85.4
			Community	30	9.1	18	11.5
			Town	5	1.5	3	1.9
			City	3	0.9	2	1.3
	Proximity to urban centres	Classify census wards by distance to main urban settlements within the district.	Distant from the city	271	82.1	116	73.9
			Close to the city	55	16.7	38	24.2
			City	4	1.2	3	1.9
	Close access to a highway	Classify wards based on distance to a National highway	No	221	67	92	58.6
			Yes	109	33	65	41.4

for all 330 census wards in the two districts. The urban characteristics of the 157 census wards included in this analysis were similar to those of the entire districts. There was considerable variation in indicator variables between census wards.

Table 2. Component loadings by infrastructure, socioeconomic and lifestyle indices. Component loadings were calculated using Categorical Principal Components Analysis

Component Loadings	**Dimensions**	
	1	**2**
Population Size	0.782	-0.476
Population density	0.794	-0.384
Non-agriculture activities	0.654	-0.172
Secondary of Education	0.803	0.25
Commercial activity	0.907	-0.161
Concrete housing	0.498	0.517
Mobile phone access	0.282	0.599
Internet access	0.644	0.231
Computer access	0.723	0.371
Satellite TV access	0.777	0.045
Pavement street	0.513	0.491
Sewage system	0.637	-0.244
Electricity	0.453	0.511
Educational Institutions	0.647	-0.499
Health Institutions	0.587	-0.358
Geo-political Division	0.74	-0.379
Proximity to Urban Centres	0.519	0.273
Close access to highway	0.404	0.493
Variance explained	**42.3%**	**15.1%**

CATPCA results are provided in Table 2. All 18 indicators were included in the model and all had positive loadings for the first component. Fourteen had loadings >0.5, 3 had loadings 0.4-0.5, and 1 (mobile phone access) had a low component loading <0.3). The total variance explained by the first component was 42.3%. The proportion of variance explained by each variable and the quantifications for each category for all variables are shown in Supplementary Figure SF1 and Supplementary Table ST1. To allow an easier interpretation of the urbanicity score with a scale of 0-10 (0 being the least and 10 the most urban), 1 was added to each of the z values for the first component.(Figure 2A)

The location of children's households within their respective census ward is shown in Figure 2B. Forty percent of the study population resided within census wards with urbanicity scores of >7.5, 23% resided in wards with scores of 2.5-7.5, and 37% resided in wards with an urbanicity score of <2.5. Comparisons between the urbanicity measure and the urban-rural dichotomy and political division are shown in figure 3. The urbanicity score was categorized into 7 groups representing an ascendant level of urbanisation based on census ward scores (Level 0=score<1; level1=1-1.9; level 2=2-2.9; level 3=3-3.9; level 4=4-4.9; level 5=5-5.9; level 6=≥6). Children living in wards level 2 (OR=1.72, CI:1.06-2.79, p=0.027), level 3 (OR=2.12, CI:1.25-3.56, p=0.005); level 4 (OR=1.45, CI:1.1-1.93, p=0.009), and level 6 (OR=1.57, CI:1.19-2.08, p=0.002), presented a higher prevalence of wheeze ever than those living in wards level cero. Likewise, children living in wards level 3 (OR 2.87 CI:1.77-4.66, p=<0.001) and level 6 (OR 1.58 CI:1.01-2.47, p=0.048) showed a higher prevalence of doctor diagnosis of asthma compared to children living in wards level cero.

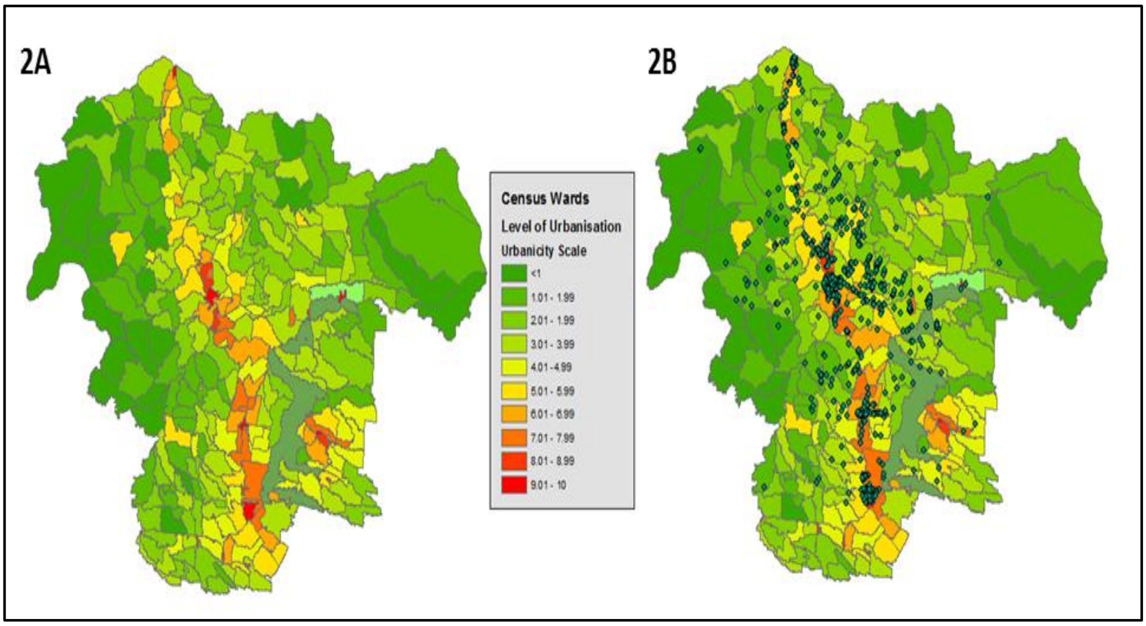

Figure 2. Study area by level of urbanicity. Figure shows 2A - district of Quinindé showing urbanicity score by census ward; 2B - district of Quinindé showing the geographic location of study households (green dots) within census wards.

Associations among urbanicity score, urban indicators, and asthma

The results of bivariate analyses between urbanicity scores, urban indicators and asthma definitions are shown in Table 3. Positive and significant associations were observed between the urbanicity score and wheeze ever (OR 1.029, p=0.049) and doctor diagnosis (OR 1.05, p=0.003). Thus, for a one-unit increase in urbanicity score, the risk of wheeze ever and doctor diagnosis increased by 2.9% and 5%, respectively. Associations between wheeze ever and urban indicators were observed for 3 of the 18 indicators: concrete house (OR 1.012, p=0.008), sewage system (OR 1.012, p=0.005) and close access to highway (1.41, p=0.021). Thus, for an increase in 10% of housing being of cement and proportion of households having access to a sewage system, the risk of wheeze increased by 12%. Similarly, children living in wards with close access to highways had 41% greater risk of having a history of wheeze compared to children living in wards without access to a highway. Two urban indicators were associated with current wheeze: adult secondary education levels (OR 1.015, p=0.04) and home access to a computer (OR 1.025, p=0.014). Twelve indicators were associated with doctor diagnosis of asthma: population size (OR 1.000014, p=0.003), adult rates of secondary education (OR 1.018, p=0.006), commercial activities (OR 1.011, p=0.007), mobile phone access (OR 1.024, p=0.034), home internet access (OR 1.046, p=0.019), home computer access (OR 1.032, p=0.001), home satellite TV access (OR 1.008, p=0.001), household sewage systems (OR 1.016, p=0.02), presence of educational (OR 1.021, p=0.007) and health institutions (hospital vs. none, OR 1.45, p=0.027), geo-political division (City vs. countryside, OR 1.46, p=0.034) and proximity to an urban centre (OR 0.62, p=0.006). Thus, for each increase in 10,000 inhabitants, the odds of doctor diagnosis of asthma increased by 14%; for each increase in 10% in rate of secondary education, commercial activities, mobile phone access, internet access, computer access, satellite TV access and sewage system, the odds of doctor diagnosis of asthma increased by in 18%, 11%, 24%, 46%, 32%, 8% and 16%, respectively. Children living in wards with hospitals had a 45% chance of a doctor diagnosis of asthma than those living in wards without any health institution, while children living in wards distant from urban centres has 38% less risk of doctor diagnosis of asthma compared with children living in cities.

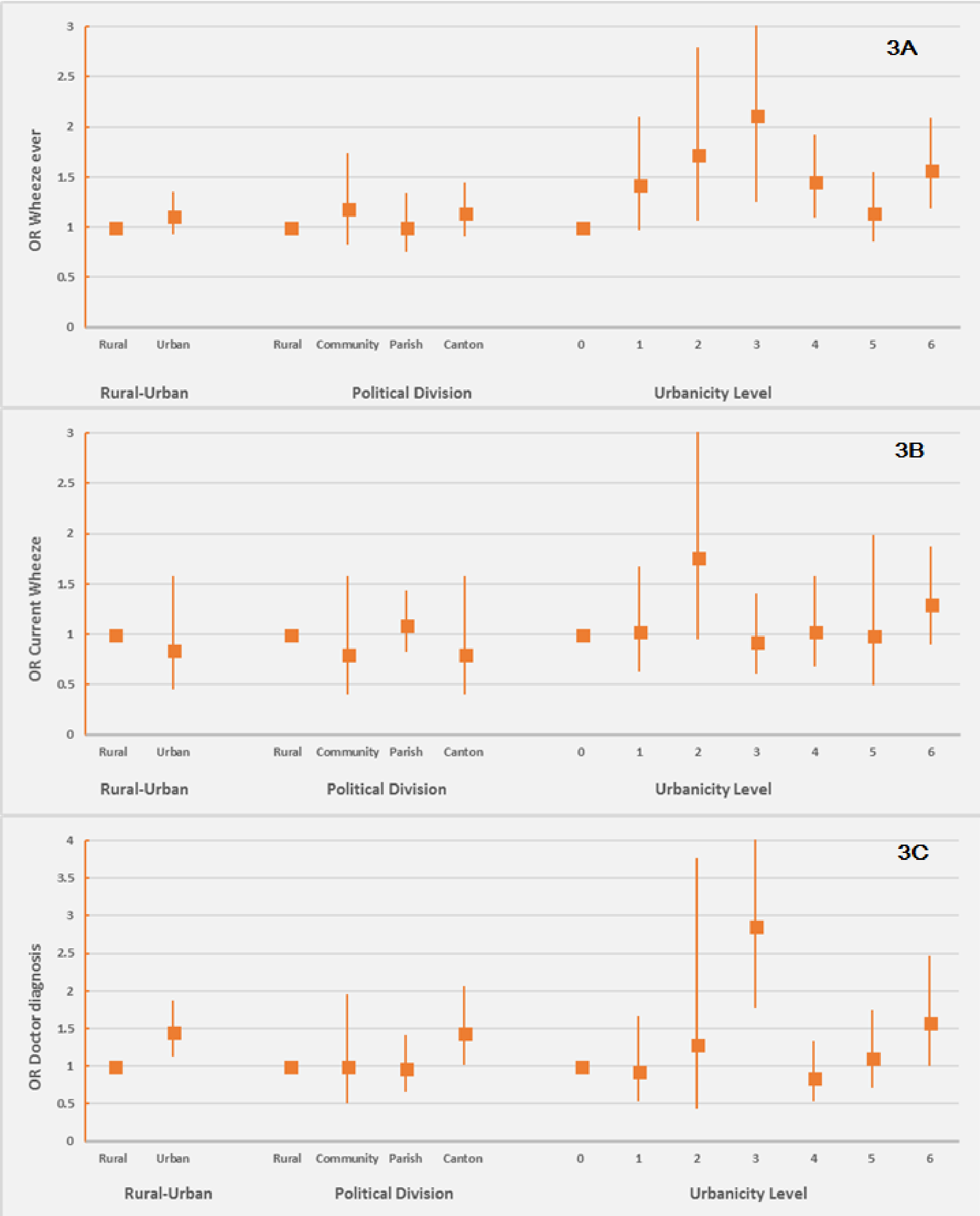

Figure 3. Comparison of the urbanicity measure with the urban-rural dichotomy and geo-political division. Figure shows 3A- Wheeze ever, 3B- Current wheeze and 3C – Doctor diagnosis of asthma.

Discussion

In the present study, we generated an urbanicity scale to understand better how the process of urbanisation may be associated with the prevalence of childhood asthma in transitional areas of an MIC. Clearly, a population does not have to live in a city to experience many of the factors associated with the process of urbanisation. Urban characteristics are present in small settlements and rural communities due to a gradual process of change occurring in rural towns or through the influence of cities on the rural areas that surround them. The degree of presence of these urban characteristics or urban conditions in a given time and locality is called Urbanicity level. Our data showed a wide variation in levels of urbanicity across census wards within the two Districts included in our analysis with evidence that the prevalence of childhood asthma was associated with increasing levels of urbanicity. Further, our data showed which indicators within the urbanicity index included in the analysis were more relevant as determinants of the prevalence of asthma symptoms at 5 years of age. Our analysis illustrates the benefits of using a multidimensional measure of urbanicity to study the epidemiology of asthma, allowing us to understand better which components may be most relevant, compared to more traditional urban-rural dichotomies.

Our findings are consistent with previous studies observing a higher risk of asthma in urban environments. Such studies have tended to use simple dichotomies of urbanicity in which populations living in large cities have been compared with those residing in rural towns or communities.(13) Several asthma studies have used more than two categories to represent different levels of urbanicity.(20–25) For example, studies conducted in Chile, Mozambique and Palestine compared asthma prevalence between urban, sub-urban, semi-rural and rural populations,(21,22,26) observing a lower prevalence of asthma in rural settlements that increased with increasing urbanization. Others studies have evaluated the effects of urbanisation on asthma by comparing populations living in different cities with diverse levels of urban development.(20,27,28) For example, a study conducted in Peru compared the prevalence of asthma between four geographically distinct sites with varying levels of urbanisation (urban vs. semi-urban vs. rural) and observed a greater prevalence with increasing levels of urbanisation.(20) Thus, in

Table 3. Bivariate logistic regression between urbanicity scale, urban conditions and asthma.

	Wheeze ever			Current wheeze			Doctor diagnosis		
Indicators	OR	95% CI	p	OR	95% CI	p	OR	95% CI	p
Urbanicity Score (0-10)	**1.029**	**(1.01-1.06)**	**0.049**	1.016	(0.98-1.06)	0.415	**1.05**	**(1.02-1.09)**	**0.003**
Population Size (by 10,000 inh)	1.04	(0.96-1.13)	0.356	0.96	(0.79-1.16)	0.660	**1.15**	**(1.04-1.27)**	**0.006**
Population density (by 1000 inh/km^2	1.02	(0.96-1.08)	0.533	0.98	(0.91-1.07)	0.702	1.08	(0.99-1.17)	0.082
Non-Agriculture activities	1.004	(1-1.01)	0.078	1.001	(0.99-1.01)	0.804	1.005	(0.99-1.01)	0.116
Secondary Education	1.010	(0.99-1.02)	0.09	**1.015**	**(1.0-1.03)**	**0.04**	**1.018**	**(1.01-1.03)**	**0.006**
Commercial activities	1.006	(0.99-1.01)	0.057	1.005	(0.99-1.018)	0.221	**1.011**	**(1.0-1.02)**	**0.007**
Concrete housing*	**1.012**	**(1.0-1.02)**	**0.008**	1.01	(0.99-1.02)	0.1	1.015	(0.99-1.03)	0.054
Mobile phone access	1.009	(0.99-1.02)	0.154	1.014	(0.99-1.03)	0.133	**1.024**	**(1.0-1.05)**	**0.034**
Internet access	1.021	(0.99-1.05)	0.153	1.016	(0.97-1.06)	0.429	**1.046**	**(1.01-1.08)**	**0.019**
Computer access	1.015	(0.99-1.03)	0.064	**1.025**	**(1.01-1.04)**	**0.014**	**1.032**	**(1.01-1.05)**	**0.001**
Satellite TV access	1.004	(0.99-1.01)	0.109	1.006	(0.99-1.01)	0.055	**1.008**	**(1.0-1.01)**	**0.001**
Pavement street	1.006	(0.99-1.01)	0.126	1.006	(0.99-1.01)	0.224	1.008	(0.99-1.02)	0.185
Sewage system	**1.012**	(1.0-1.02)	**0.005**	1.003	(0.99-1.01)	0.614	**1.016**	**(1.0-1.03)**	**0.02**
Electricity	1.001	(0.99-1.01)	0.843	1.013	(0.99-1.03)	0.22	1.020	(0.99-1.05)	0.137
Educational Institutions	1.005	(0.99-1.02)	0.443	0.99	(0.96-1.02)	0.576	**1.021**	**(1.06-1.04)**	**0.007**
Health Institutions									
Health centre vs None	1.13	(0.81-1.54)	0.467	1.12	(0.83-1.51)	0.462	0.98	(0.65-1.48)	0.922
Hospital vs None	1.16	(0.91-1.46)	0.218	0.87	(0.46-1.62)	0.656	**1.45**	**(1.04-2.03)**	**0.027**

Continuation Table 2.

	Wheeze ever			**Current wheeze**			**Doctor diagnosis**		
Indicators	OR	95% CI	p	OR	95% CI	p	OR	95% CI	p
Geo-political division									
Community vs Countryside	1.19	(0.79-1.76)	0.395	0.81	(0.41-1.58)	0.53	1.01	(0.5-2.02)	0.977
Town vs Countryside	1.008	(0.75-1.34)	0.955	1.08	(0.82-1.41)	0.562	0.98	(0.66-1.45)	0.921
City vs Countryside	1.17	(0.91-1.48)	0.214	0.81	(0.41-1.55)	0.526	**1.46**	**(1.03-2.07)**	**0.034**
Proximity to urban centres									
Distant from the city vs City	0.822	(0.61-1.1)	0.187	0.84	(0.56-1.26)	0.394	**0.62**	**(0.43-0.87)**	**0.006**
Close to the city vs City	1.305	(0.97-1.74)	0.076	1.43	(0.97-2.1)	0.067	0.88	(0.54-1.49)	0.591
Close access to a highway									
Yes vs No	**1.41**	**(1.05-1.88)**	**0.021**	1.22	(0.82-1.8)	0.333	1.19	(0.71-1.98)	0.505

contrast, to previous studies, we used a continuous measure of urbanicity to identify a wide variation in urbanicity levels within a geographically localized area in Ecuador, and were able to show that higher levels of urbanicity were associated with a greater risk of asthma. These findings extend previous observations from the same geographic region in Ecuador in which we showed that the community prevalence of asthma in rural settlements increased with greater levels of urbanisation (or urbanicity) at an ecologic level. The urban indicators most strongly associated with this were related to changes in socioeconomic and lifestyle factors.(29) However, not all 'urban' environments in LMICs increase asthma risk, at least when defined using a simple urban-rural dichotomy: some studies observed a lower prevalence of asthma in urban areas or were unable to detect a difference in prevalence between urban and rural populations.(30,31)

There is no generally accepted definition for asthma in epidemiological studies. Most studies have used a single definition, generally recent wheeze or a doctor diagnosis, which may measure different phenotypes and severity of asthma. Doctor diagnosis of asthma in poor settings in LMICs will also be affected by access to health care. In the present study we used 3 definitions for asthma - wheeze ever, current wheeze and doctor diagnosis - allowing us to explore better the associations between urbanicity indicators and a wider range of disease phenotypes represented by these definitions. Although several associations were identified, the presence and magnitude of these associations varied by phenotype. For example, doctor diagnosis of asthma was associated with 14 of 18 urban indicators, much greater than for wheeze ever (3 indicators) and recent wheeze (2 indicators). Differences in access to health care between urban and rural populations could be explain the greater number of associations with doctor diagnosis. Populations living in cities or other urban settlements have greater access to health care compared to rural populations because of the larger number of doctors and health institutions present.(32) Wheeze ever or current wheeze may be better definitions for asthma in populations with highly variable access to doctors. However, asthma defined as current wheeze, may be less useful for contextual indicators of urbanicity in cross-sectional analyses because of the restricted time the symptom would have been present (i.e. within the previous 12 months). Urban lifestyles and conditions may require years or even decades to affect human health outcomes.(33)

Some urban indicators appeared to be consistently associated with asthma, irrespective of the definition used. Positive trends between indicators for the socioeconomic dimension of urbanism such as rates of adult secondary education, commercial activities, cement housing, and home computer and satellite TV access were observed for more than one asthma definition. Indicators such as home computer and satellite TV access could be related to sedentarism within households.

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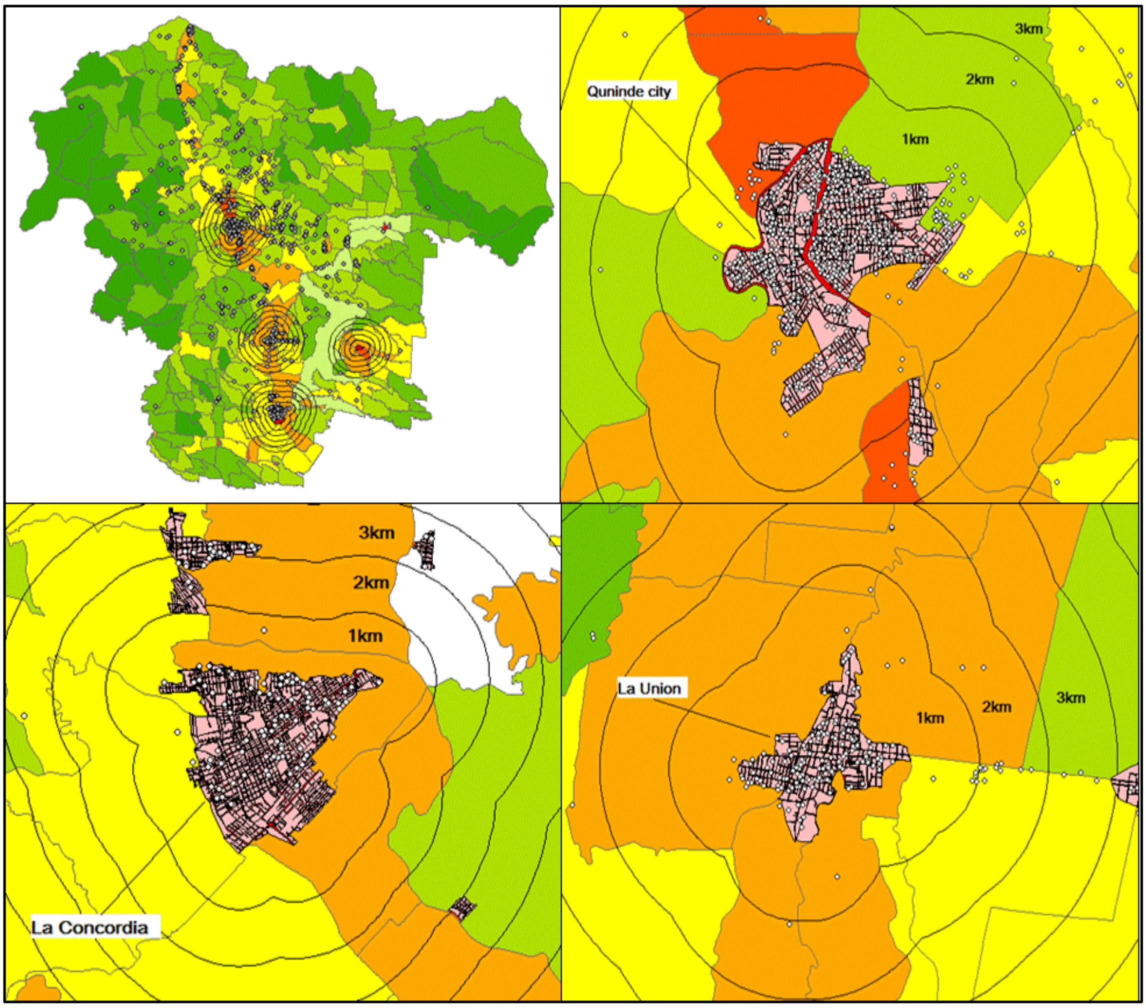

Figure 4. Evaluation of urban sprawl using buffer analyses. Figure shows the cities of Quininde, La Concordia and La Union bounded by concentring rings representing the influence of the urban areas on the rural surroundings

Low rates of physical activity and time spent indoors have been associated with asthma.(34,35) The association between the proportion of cement-built houses within a census ward and asthma, might be explained by higher levels of humidity and mold exposure. In transitional areas, such houses are generally built without regulatory controls and often suffer from poor ventilation and high humidity. Several studies have observed associations among previous respiratory infections, humidity within the house, and asthma.(36) Geographic indicators of the process of urbanisation may also be determinants of asthma prevalence, especially in rural populations. Children residing in census wards close to cities and with easy access to the highway had a higher prevalence of asthma compared to children living in distant areas. Such associations could be explained by "urban sprawl" that extends urban influences from cities to the rural areas surrounding them such that rural populations living close to urban centres can experience the similar social and environmental exposures to those living within the urban environment.(32) In this study, we evaluated the effect of urban sprawl by building buffers around the main cities of the study area (Quinindé, La Concordia and La Union). Households located within 3 km of these cities were included within cities and children living within these households had 44% greater risk of wheeze ever compared to those living outside these areas. (Figure 4)

Several epidemiological studies have used scale-based approaches to measure the effects of urbanicity on the risk of other non-communicable diseases.(37,38) Of these, most have used community-level data to develop urbanicity scales although the urban indicators used tended to vary depending on study context, level of urbanisation, unit of analysis and availability of information. Some of these studies have applied methodologies with predefined scale algorithms to rank each indicator which when added were used to quantify level of urbanicity.(39,40) Other studies have used more complex statistical procedures such as data reduction techniques in order to generate indexes or scales of urbanicity.(29,30) The scale in the present analysis used relevant urban indicators from publicly available data (from a national census at ward level) to derive an urbanicity index by principal components analysis. We included a wide range of urban indicators that included sociological, geographic and demographic factors allowing us to detect associations with asthma prevalence and urbanicity, when analysed either as a continuous or categorical variable. As a categorical variable, our urbanicity measure identified differences in asthma prevalence (measured as wheeze ever) between 7 differences levels of urbanicity, differences that were not detected by standard urban-rural geo-political approaches. (Figure 3)

The present analysis is subject to a number of limitations. The cross-sectional design does not allow us to determine the direction of the effects between urbanicity and asthma risk. Further, we have not considered potential effects of migration between areas of different urbanicity, something that may be frequent in this study population. This analysis did not include urban indicators related to the lifestyle of the population because such data is not available from the census. Further, while the data is derived from the 2010 census, data on asthma outcomes were collected between 2013 and 2015. Future studies will plan to analyse the effects on asthma prevalence of urbanicity indicators that are being collected at the level of each household.

Urbanisation is a process that takes place over time and which involves a range of processes determined by cultural, historical, economic, and other dimensions that are difficult to define and measure. Inclusion of all such factors in any analysis is challenging. However, to date most studies exploring the effects of urbanization on asthma have used simple urban-rural dichotomies, that can only identify the totality of multiple and often counter-balancing effects acting on asthma. Such an approach does not allow us to consider the multifactorial dimensions of the urbanisation process and cannot identify specific factors or conditions associated with asthma risk. .(11) We need to start thinking about more complex chains of causation in urban studies and asthma. An important issue for studies of the effects of urbanisation and asthma is a lack of an adequate conceptual model for how social, psychological, and biological determinants within urbanization processes interact to affect asthma risk. A better understanding of how such processes operate is likely to lead to a better understanding of asthma causation and potential strategies to the primary prevention of this important debilitating disease.

Conclusion

This study has used a multi-dimensional urbanicity approach to evaluate the effects of urbanisation on asthma prevalence. Our data provide evidence that even small-scale increases in urbanicity levels in rural populations may be associated with a higher prevalence of asthma. The present study also shows that the use of a multicomponent scale for measuring urbanicity can identify more clearly the relationship between the process of urbanisation and asthma and, challenges the prevailing use of urban-rural dichotomies in epidemiological studies of asthma. We believe that this multifactorial approach in asthma studies will provide novel insights into how urbanisation may affect asthma prevalence.

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Chapter 4.

Research Paper 3: Rural to urban migration is associated with increased prevalence of childhood wheeze in a Latin-American city

Overview Chapter 4

As discussed in the previous chapter, urbanisation is a complex process that comprises an array of dimensions which have different effects on asthma prevalence. However, although some of the most relevant dimensions of the urbanisation process were considered in our study, the dimension of migration was excluded due to its complexity. The process of internal migration is a key dimension of urbanisation and it has been largely overlooked by asthma studies in LMICs. The migration process, especially from rural to urban areas, involves exposures to new environmental and social factors as pollution, housing conditions, diet and accessibility to medical services, factors that have been associated with asthma. The present chapter presents two papers, one conducted in an urban population and other conducted in a rural population, discussing the effects of internal migration on asthma prevalence. This part of the study explores how distinctive characteristics of the migrant population such as area of origin and residence, age of migration and time since migration could be possible determinants of asthma prevalence. This section also discusses the social and economic conditions of the migrant populations and their possible implications on asthma prevalence. The chapter concludes with a discussion of how social phenomenon associated with internal migration such as the feminization of migration and circular migration could be in part responsible for the temporal and geographical trends in asthma prevalence in LMICs.

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Student	**Alejandro Rodriguez**
Principal Supervisor	**Dr. Elizabeth Brickley**
Thesis Title	**Urbanisation and childhood asthma in a developing region of Latin-America: A Cross-sectional analysis in northeastern Ecuador**

If the Research Paper has previously been published please complete Section B, if not please move to Section C

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Where was the work published?	**Open Respiratory Research**		
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Rural to urban migration is associated with increased prevalence of childhood wheeze in a Latin-American city

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ABSTRACT

Introduction The urbanisation process has been associated with increases in asthma prevalence in urban and rural areas of low-income and middle-income countries (LMICs). However, although rural to urban migration and migration between cities are considered important determinants of this process, few studies have evaluated the effects of internal migration on asthma in urban populations of LMICs. The present study evaluated the effects of internal migration on the prevalence of wheeze in an urban area of Latin America.

Methods We did a cross-sectional analysis of 2510 schoolchildren living in the city of Esmeraldas, Ecuador. Logistic regression was used to analyse associations between childhood wheeze and different aspects of migration among schoolchildren.

Results 31% of schoolchildren were migrants. Rural to urban migrants had a higher prevalence of wheeze, (adj. OR=2.01,95% CI1.30 to 3.01, p=0.001) compared with non-migrants. Age of migration and time since migration were associated with wheeze only for rural to urban migrants but not for urban to urban migrants. Children who had migrated after 3 years of age had a greater risk of wheeze (OR 2.51, 95% CI 1.56 to 3.97, p=0.001) than non-migrants while migrants with less than 5 years living in the new residence had a higher prevalence of wheeze than non-migrants (<3 years: OR=2.34, 95% CI 1.26 to 4.33, p<0.007 and 3–5 years: OR=3.03, 95% CI 1.49 to 6.15, p<0.002).

Conclusions Our study provides evidence that rural to urban migration is associated with an increase in the prevalence of wheeze among schoolchildren living in a Latin-American city. Age of migration and time since migration were important determinants of wheeze only among migrants from rural areas. A better understanding of the social and environmental effects of internal migration could improve our understanding of the causes of the increase in asthma and differences in prevalence between urban and rural populations.

Key messages

- Internal migration process could be related with the increase and differences in asthma prevalence between urban and rural areas of Latin-America.
- Few studies have evaluated the effect of internal migration on asthma in an urban area of low-income and middle-income countries.
- Our findings show how some migrant categories based on temporal and spatial characteristics area associated with asthma/wheeze prevalence.

INTRODUCTION

Over the past 40 years or so, there has been a progressive increase in the prevalence of asthma and other allergic diseases particularly in high-income countries (HICs) and in urban areas of HICs and low-income and middle-income countries (LMICs).[1] However, in recent years the prevalence of allergic disorders may have reached a plateau in some HICs but continues to increase in LMICs.[2] The reasons for these variations remain unexplained but are likely to be caused by a complex interplay of biological, environmental and social factors.[3 4]

Urbanisation is a social process that has been causally implicated in trends of increasing asthma prevalence in LMICs,[3–6] and the lower prevalence in rural compared with urban populations.[3 7] Rural to urban differences in asthma prevalence have been attributed to the protective effects of environmental exposures such as farming that are typical of a rural way of life.[8] However, recent studies have shown that allergic disorders may be increasing in rural areas thus reducing the prevalence gap between urban and rural settings.[9 10] Furthermore, an array of environmental and social changes stemming from the urbanisation process have been identified as potential risk factors for asthma in urban and rural areas.[4 8]

Rural to urban migration and migration between cities are important components of the urbanisation process in LMICs and are related to environmental, socioeconomic and behavioural changes.[11] However, few epidemiological studies have investigated the influence of internal migration on asthma and other allergic diseases in

LMICs.[12 13] Most studies of the effects of migration on allergic diseases have investigated populations migrating from LMICs (presumed low risk for allergic diseases) to HICs (presumed high risk).[14 15] These studies have shown that being born in an area of low risk provides protection against asthma,[16 17] but this protection may decline with the length of residence in the new environment.[18] Others studies have shown that age of migration and time since migration are associated with the risk of asthma and other allergic diseases,[14] often leading to a higher risk of atopy and allergic diseases among migrants than the local population.

Although internal migration can be defined as the movement of an individual between two geographical locations (rural to urban or urban to urban) of the same country or region,[19] the social, economic and environmental conditions of migrant populations transform this simple movement into a more complex process with different effects on health.[20] Temporal, spatial and social characteristics of the population produce different groups of migrants each with specific features with potentially differing effects on asthma risk. Further, the consequences of migration are relevant to individual migrants and to their families and communities both in place of origin and destination.[21] A better understanding how these factors, relating to the migration process may alter risk of asthma and other allergic diseases, may contribute to our comprehension of the causes of temporal increases in asthma prevalence and the differences in prevalence between urban and rural populations of Latin America (LA).[22 23] The aim of the present study was to explore the effects of internal migration on the prevalence of wheeze in schoolchildren living in a coastal city in Ecuador.

METHODS

Study population

The study was conducted in the city of Esmeraldas, the provincial capital of the tropical coastal province of Esmeraldas in north-western Ecuador, located 140 km south of the Colombian border. With approximately 190 000 inhabitants, Esmeraldas is the principal northern port and is home to the country's largest oil refinery. The main economic and industrial activities of the population are based on oil processing and export, commerce, agriculture (especially tropical fruit and palm oil), timber, fishing and tourism. Based on the last national census of 2010, the coverage of basic services in the city is deficient: 28% of the households have no access to running water, 22% lack a sewage system and 5% have no access to electricity. The educational level of the population is low compared with the national average: 6% of the population is illiterate while only 18% has higher education.[24]

Study design and sample

A cross-sectional study was conducted in schoolchildren aged 5–16 years to evaluate risk factors for allergy and asthma and history of migration.[12] A convenience sample of 10 schools was selected from nine barrios or neighbourhoods in which there was a predominance of Afro-Ecuadorian migrants from the rural districts of the province. . All children attending the schools at the time of the survey were eligible for inclusion. The response rate was 90.8% (of the annually updated school lists). Data collection was done between November 2007 and January 2010.

Data collection

Detailed information on risk factors and symptoms of wheeze was collected using a questionnaire based on the International Study of Asthma and Allergies in Childhood (ISAAC) phase II.[25] The questionnaire, translated into Spanish and adapted to local conditions, was administered to the parent or guardian of each child by trained field workers. Wheeze was defined as a positive response to the question 'Has your child had wheeze in the chest in the last 12 months'. The questionnaire was also used to collect information on migration history of the children and their parents.

Migrant categories

Migration data were analysed based on the recommendations of the United Nations Secretariat to measure internal migration.[19] Detailed information about place of birth (community/city, parish, province and country) and temporal characteristics of migratory movements were collected for each child. Migration was defined as a change of residence from one civil division to another in which community was the minor division. *Migration status* was measured based on the place of birth of the children by the question '*Where was the child born?*' Children who had been born in the city of Esmeraldas were treated as non-migrants and all others as migrants. Migrants were classified into several categories based on spatial and temporal characteristics of their migratory movements: (1) *Direction of migration* classified migrants by direction of migration between place of birth and current place of residence (rural to urban or urban to urban), (2) *Locality of migration* classified migrants by the population size at the place of birth. (Categories explained in table 1), (3). *Age of migration* classified migrants by age when they left their place of birth (≤3 years and >3 years), and (4) *Time since migration* classified migrants by time spent in the city of Esmeraldas (<3 years, 3–5 years and >5 years). Migrant status of parents was also included. Categories and definitions are provided in table 1. Parents were treated as non-migrants if they were born in the city of Esmeraldas and as migrants if they were born elsewhere. Due to the proximity of the city of Esmeraldas to the international border with Colombia (140 km), the variable 'Colombian children' was included also representing children

Table 1 History of migration, demographic variables and socioeconomic variables

Dimensions/ indicators	Definitions	Categories	n	Total population	Migrant population
Migrant status	Classified children by difference between place of birth and place of residence	NMa	1694	68.7%	
		Migrant	772	31.3%	100%
Direction of migration	Classified migrants by migration movement between place of birth and place of residence based on the political division of Ecuador	NM	1694	68.7%	
		Rural to urban	286	11.6%	37%
		Urban to urban	486	19.7%	63%
Locality of migration	Classified migrants by size of population of place of birth: *communities* (rural village), *small city* (urban towns), *medium city* (provincial capitals) and *large city* (migrants from Quito and Guayaquil, the two largest cities in the country)	NM	1694	68.7%	
		Community	286	11.6%	37%
		Small city	170	6.9%	22%
		Medium city	124	5%	16%
		Large city	192	7.8%	25%
Age at migration	Classified migrants considering the age when children left their place of birth (years)	NM	1694	68.7%	
		≤3	291	11.8%	38%
		>3	481	19.5%	62%
Time since migration	Classified migrants by time spent in current locality (years)	NM	1694	68.7%	
		<3	275	11.2%	36%
		3–5	178	7.2%	23%
		>5	319	12.9%	41%
Migration status of parents	Parents were treated as non-migrants if they were born in the city of Esmeraldas and as migrants if they were born elsewhere	NM	575	24%	
		One migrant parent	877	36%	
		Two migrant parents	981	40%	
Colombian children	First-generartion or secondgeneration migrants from Colombia	No	2332	92.9%	
		Yes	103	4.1%	
Age of the children	Age of the children (years)	≤9	1294	51.6%	
		>10	1216	48.4%	
Sex	Sex of the child	Male	1321	52.7%	
		Female	1189	47.4%	
Farm environment	Classified houses of the children based on farm characteristics (eg, presence of animals and agricultural activities)[26]	No	2141	85.3%	
		Yes*	369	14.7%	
Type of house	Classified houses of the children based on construction materials, presence of urban services and electrical appliances[26]	Urban†	2190	87.3%	
		Transitional‡	320	12.7%	
Consumption of junk food	Classified children by fizzy drink consumption	Barely	738	33.7%	
		Sometimes/week	1132	51.6%	
		Daily	323	14.7%	

Continued

Table 1 Continued

Dimensions/indicators	Definitions	Categories	n	Total population	Migrant population
Parents living in the child's house	Classified children based on the presence of parents at home	Both	1138	45%	
		One	889	36%	
		None	483	19%	

*Houses characterised by farm activities and peridomestic animal breeding.
†Urban house: residences with connection to running water, concrete building materials for walls, use of a flushing toilet, ownership of a set of appliances and two to three urban services.
‡Transitional: residences with an incomplete set of electric appliances, latrine bathroom and use of mixed materials in house construction.
a NM: no migrant

with a history of migration from Colombia as first-generation or second-generation international migrants (ie, children born in Colombia or born to migrant Colombian parents). Additional data collected for each child included age, sex, parents living in the child's home (both, one, none), farm environment (households characterised by farming activities and presence of peridomestic animals), type of house (*urban house*: residences with connection to running water, concrete building materials for walls, presence of a flushing toilet, ownership of a set of electric appliances and two to three urban services; *transitional house*: residences with an incomplete set of electric appliances, latrine for bathroom, use of mixed materials for house construction) and consumption of junk food (consumption of fizzy drinks). Variables to represent farm environment and type of housing were created using multiple correspondence analysis (MCA) and methodology is explained elsewhere.[26] The variables used in MCA to define 'farm environment' were parental agricultural activities, contact with animals in farms and animal breeding in or around the household. Variables to define 'type of house' were basic services, disposal of faeces, electrical appliances, household construction materials and type of cooking fuel (table 1).

Statistical analysis

Logistic regression was used to explore associations between migrant categories and wheeze in the last 12 months. Each migrant category was adjusted for age, sex, farm environment, type of house and consumption of junk food. Associations with $p<0.05$ were considered statistically significant. All analyses were done using SPSS V.23.

RESULTS

We evaluated a total of 2510 schoolchildren in the city of Esmeraldas of whom 44 were excluded due to lack of information on child's birthplace. The prevalence of wheeze in the last 12 months was 9.4%.

History of migration

Almost a third (31.3%) of schoolchildren were migrants (table 1). Among migrants, 63% had a history of urban to urban migration and 37% of rural to urban migration, while 25% came from large cities (Quito or Guayaquil), 16% from medium cities (provincial capitals), 22% from small cities (city towns) and 37% from rural communities. Age at migration was most frequently reported after 3 years of age (62%) and most migrants (41%) had lived more than 5 years in the city of Esmeraldas. Of the total study population, 4% were first-generation or second-generation international migrants from Colombia, and history of parental migration was reported for 76%.

Table 2 Demographic and socioeconomic variables by direction of migration

		Direction of migration of children						
		Non-migrant		Rural to urban		Urban to urban		χ^2
Variables	Categories	n	%	n	%	n	%	p Value
Migration status of parents	NM	466	28.4%	20	7.2%	75	15.9%	<0.001
	One migrant parent	600	36.6%	83	30.1%	182	38.5%	
	Two migrant parents	574	35.0%	173	62.7%	216	45.7%	
Colombian children	No	1598	97.4%	270	97.8%	420	88.4%	<0.001
	Yes	42	2.6%	6	2.2%	55	11.6%	
Age of children (years)	≤9	901	53.2%	121	42.3%	246	50.6%	0.003
	>10	793	46.8%	165	57.7%	240	49.4%	
Sex of children	Male	930	54.9%	130	45.5%	239	49.2%	0.003
	Female	764	45.1%	156	54.5%	247	50.8%	
Farm environment	Non-farm	1464	86.4%	236	82.5%	403	82.9%	0.059
	Farm	230	13.6%	50	17.5%	83	17.1%	
Type of house	Basic urban	1491	88.0%	242	84.6%	416	85.6%	0.148
	Transitional	203	12.0%	44	15.4%	70	14.4%	
Consumption of junk food	Barely	525	35.3%	73	31.7%	129	29.9%	0.041
	Sometimes/week	733	49.3%	130	56.5%	243	56.4%	
	Daily	230	15.5%	27	11.7%	59	13.7%	
Parents living in child's house	Both	876	51.7%	70	24.5%	176	36.2%	<0.001
	One	580	34.2%	103	36.0%	183	37.7%	
	None	238	14.0%	113	39.5%	127	26.1%	

Sociodemographic characteristics of the migrant population

A higher proportion of migrants than non-migrants was female (45.1 vs 52.6%) while rural to urban migrants tended to be older than the other groups (table 2). Farm environment was more common in migrants (17.3%) than non-migrants (13.6%). Only 25% of the rural to urban migrant children were living with both parents compared with 52% for non-migrant children. Daily consumption of junk food was greater in non-migrants.

Associations between history of migration and wheeze

Positive associations were observed with direction of migration and locality of migration in unadjusted analyses (table 3). Rural to urban migrant children (or migrants from communities) had greater odds of wheeze than non-migrants (OR 1.66, 95% CI 1.15 to 2.41, p=0.007). Children with a history of migration from Colombia had a higher prevalence of wheeze (OR 1.83, 95% CI 1.05 to 3.18, p=0.032) compared with Ecuadorian children. Multivariable analyses were adjusted for sex, age, farm environment, type of housing and consumption of junk food. Adjusted analyses showed a higher risk of wheeze compared with non-migrant children for the following: (1) Direction and locality of migration—children who migrated from rural (or communities) to urban areas (OR 2.01, 95% CI 1.30 to 3.01, p=0.001) had two times more wheeze than non-migrant children; (2) History of migration of the parents—children for whom both parents had a history of migration had a greater risk of wheeze than children whose parents had not (OR 1.52, 95% CI 1.01 to 2.29, p=0.046) and (3) Colombian children had greater risk of wheeze (OR 2.15, 95% CI 1.22 to 3.78, p=0.008) than Ecuadorian children.

Table 4 shows the adjusted associations between wheeze and age at migration and time since migration, stratified by direction of migration. Positive and statistically significant associations were observed for rural to urban migrants but not for urban to urban migrants. Among rural to urban migrants, those who had migrated after 3 years of age had a greater risk of wheeze (OR 2.5, 95% CI 1.56 to 3.67, p<0.001) than non-migrant children and those who had spent less than 3 years and between 3–5 years in the new area of residence had a higher prevalence of wheeze than non-migrant children (OR 2.34, 95% CI 1.26 to 4.33, p<0.007 and OR 3.03, 95% CI 1.49 to 6.15, p=0.002, respectively).

DISCUSSION

In the present analysis, we have explored how internal migration affects the prevalence of recent wheeze among schoolchildren living in a city in a coastal tropical area of LA. Clearly, the study of migration is complex because of the spatial and temporal dimensions of migratory movements and also due to different social backgrounds within migrant populations. These characteristics produce

Table 3 ORs and 95% CIs for associations between wheeze in the last 12 months and history of migration adjusted for age, sex and socioeconomic variables

		Wheeze	Univariable			Multivariable		
Dimensions	**Categories**	**Prevalence**	**OR**	**(95% CI)**	**p**	**OR***	**(95% CI)**	**p**
Migration status	Non-migrant	9.1%	1			1		
	Migrant	10.4%	1.15	(0.86 to 1.53)	0.342	1.25	(0.91 to 1.71)	0.175
Direction of migration	Non-migrant	9.1%	1			1		
	Rural to urban	14.3%	1.66	(1.15 to 2.41)	0.007	2.01	(1.30 to 3.01)	0.001
	Urban to urban	8%	0.87	(0.60 to 1.25)	0.443	0.91	(0.61 to 1.63)	0.660
Locality of migration	Non-migrant	9.1%	1			1		
	Communities	14.3%	1.66	(1.15 to 2.41)	0.007	2.01	(1.30 to 3.01)	0.001
	Small city	5.3%	0.56	(0.28 to 1.11)	0.095	0.59	(0.29 to 1.20)	0.145
	Medium city	12.1%	1.37	(0.78 to 2.40)	0.278	1.68	(0.92 to 3.07)	0.089
	Large city	7.8%	0.84	(0.48 to 1.46)	0.540	0.80	(0.42 to 1.52)	0.486
Age at migration	Non-migrant	9.1%	1			1		
	≤3 vs NM	10.3%	1.14	(0.75 to 1.73)	0.530	1.20	(0.76 to 1.89)	0.437
	>3 vs NM	10.4%	1.15	(0.82 to 1.61)	0.410	1.28	(0.86 to 1.87)	0.204
Time since migration (years)	Non-migrant	9.1%	1			1		
	<3	12.4%	1.40	(0.94 to 2.08)	0.095	1.36	(0.87 to 2.13)	0.185
	3–5	9%	0.98	(0.57 to 1.68)	0.943	1.12	(0.63 to 1.98)	0.705
	>5	9.4%	1.03	(0.68 to 1.55)	0.885	1.23	(0.77 to 1.94)	0.388
History of migration of the parents	Non-migrant	7.8%	1			1		
	One migrant parent	9.5%	1.23	(0.84 to 1.80)	0.282	1.39	(0.92 to 2.11)	0.123
	Two migrant parents	10.3%	1.35	(0.94 to 1.95)	0.108	1.52	(1.01 to 2.29)	0.046
Colombian children	No	9.1%	1			1		
	Yes	15.5%	1.83	(1.05 to 3.18)	0.032	2.15	(1.22 to 3.78)	0.008

*OR adjusted by sex, age, farm environment, quality of the house and consumption of junk food.

several types of migrants each with specific population features.[20 21] Additionally, migration is a multistage process with effects not only at the individual level, but also at family and community levels.[21] Considering this, we have taken a multidimensional approach to analyse migration, using categories based on temporary and spatial characteristics of the migratory movements of children and their parents. Our data provide evidence that internal migration, specifically rural to urban migration, was associated with a higher risk of wheeze. Further, international history of migration of the children, specifically migrants from Colombia, was associated with a greater prevalence of wheeze: Colombian children had a twofold greater risk of wheeze compared with Ecuadorian children. Our data extend our previous observations on the effects of migration on allergic diseases in Afro-Ecuadorian

Table 4 ORs and 95% CIs for associations between wheeze in the last 12 months and age at migration and time since migration, stratified by direction of migration. ORs adjusted for age, sex and socioeconomic variables

		Rural to urban			Urban to urban		
Dimensions	**Categories**	**OR***	**(95% CI)**	**p**	**OR***	**(95% CI)**	**p**
Age at migration (years)	Non-migrant	1			1		
	≤3 vs NM	1.05	(0.44 to 2.49)	0.909	1.30	(0.76 to 2.19)	0.319
	>3 vs NM	2.51	(1.56 to 3.97)	<0.001	0.67	(0.38 to 1.18)	0.164
Time since migration (years)	Non-migrant	1			1		
	<3	2.34	(1.26 to 4.33)	0.007	0.94	(0.51 to 1.73)	0.840
	3–5	3.03	(1.49 to 6.15)	0.002	0.41	(0.15 to 1.13)	0.086
	>5	1.15	(0.54 to 2.46)	0.714	1.28	(0.74 to 2.20)	0.374

*OR adjusted by sex, age, farm environment, quality of the house and consumption junk food.

schoolchildren living in rural communities in Esmeraldas Province in Ecuador[13] in which we have shown that migration before 1 year of age and international migration (from nearby areas of the border between Ecuador and Colombia) to a rural community were associated with a higher prevalence of recent wheeze and rhinitis in transitional rural communities. We also observed that the absence of the mother at home, due to temporary or permanent migration, was associated with an increase in the occurrence of wheeze, rhinitis and eczema in rural areas.[13]

Although internal migrants account for nearly four times as many individuals as international migrants,[21] associations between migration status and asthma and other allergic diseases have generally been investigated in populations migrating between countries, mostly by comparing those that have migrated from LMICs to HICs. Two publications have reviewed studies of differences in prevalence of asthma and other allergic diseases between international migrants, the host population and the population of origin. The first, by Rottem and colleagues who reviewed available literature published before 2003,[14] concluded from 14 published studies that international migrants from LMICs to HICs tended to develop more allergies and asthma compared with their populations of origin in a time-dependent fashion, had a greater risk if migration occurred before 2 years of age and were more prone to allergies than the host populations. The second (a systematic review by Cabieses and colleagues),[15] evaluated 54 studies of which 41 were published in the last 10 years. The authors concluded that the prevalence of asthma but not 'allergies' was lower in migrants compared with the host population and that the prevalence of asthma tended to converge with that of the host population over time. Further, the study also concluded that asthma prevalence was generally higher in the second-generation compared with the first-generation of migrants. Although the overall conclusions of these reviews of published studies were consistent with the premise that migrants from LMICs suffer less asthma symptoms than host populations for a period following migration, not all studies supported such a conclusion. A recent analysis of data from the ISAAC phase III studies that included study centres from both LMICs and HICs indicated that being born outside the country of residence was associated with a lower prevalence of asthma, rhinoconjunctivitis and eczema but only for migrants to affluent countries.[27] The results for non-affluent countries showed a higher prevalence of eczema symptoms in migrants and no associations for asthma and rhinoconjunctivitis.[27]

Studies conducted in Asia and LA have used history of rural residence to evaluate the effects of rural/farm environment on allergic diseases in urban populations.[28 29] Although these studies did not focus on the study of migration as a risk factor, they provided a good starting point to evaluate the effects of internal migration on asthma in LMICs. The first study, conducted in

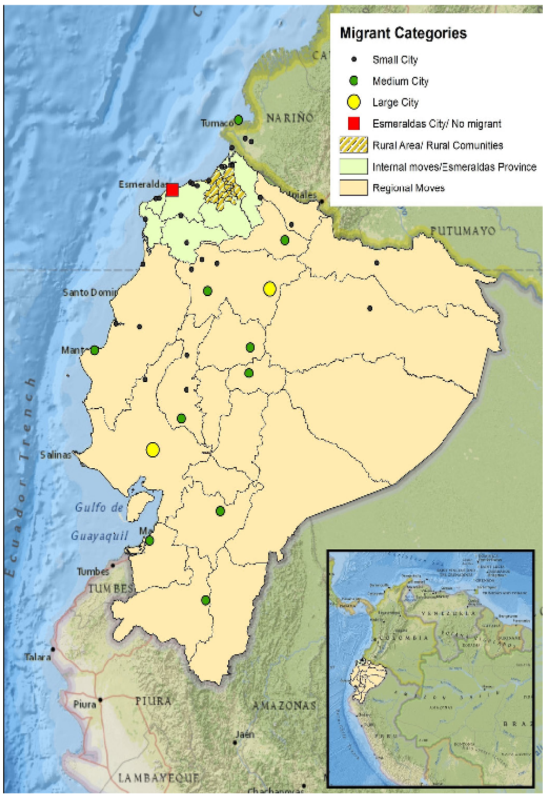

Figure 1 Study site. Map of Ecuador showing migrant categories by location. Red square represents Esmeraldas city (study area), green area represents internal migrations in the Esmeraldas Province and yellow area represents internal moves (migrants of other provinces of Ecuador). Yellow, green and black circles represent large, medium and small cities, respectively.

Mongolia, showed that subjects aged 10–60 years who relocated from a small rural village into a town, were more likely to develop asthma than subjects who lived in a town from birth, although this trend was not statistically significant.[28] Another study conducted in an urban area of Argentina showed that adolescents aged 13–14 years with a history of rural residence had the same prevalence of wheeze compared with those who had always lived in the urban area.[29] Our study focused on the effects of internal migration on wheeze prevalence in an urban population of an LMIC, where migrants formed a diverse group including migrants from rural communities, migrants from other urban settings, and those crossing voluntarily or being displaced by civil conflict across the international border with Colombia (figure 1). In this setting, we found that rural to urban migration is an important determinant of a higher risk of wheeze in an urban population. Further, family history of migration was associated with an increase in wheeze prevalence that was especially marked among Colombian children. However, in contrast to previous international comparisons, our data showed that migrants from populations considered to be at low risk for allergic diseases (rural communities) actually had a slightly higher prevalence of wheeze than the host population in the urban area (14.3% vs 9.1%), challenging the assumption that rural residence protects against allergic diseases.[4] At the same time, the prevalence of asthma in rural migrants was slightly higher (14.3% vs 10.1%) than that of the population of origin (rural communities located north of the city of Esmeraldas),[26] indicating an increase in risk related to the migration process itself. Another important finding was the effect of time since migration and age of migration in rural migrants: the risk of wheeze increased with greater time since arriving in the city up to 5 years, after which the risk disappeared (table 4). Our data also indicate that age of migration was associated an increased risk of wheeze among children who migrated after 3 years of age.

In an international context, the apparently lower prevalence of asthma and allergic diseases in migrants could be explained by the '*healthy migrant effect*' in which recent migrants, including those migrating from LMICs to HICs, are on average healthier than the native population.[30] International migrants are not a random sample of their country of origin but a highly selected group who are able or motivated to deal with the stress, cost and organisation that such a process entails. Individuals or families who migrate are in a relatively advantageous position, whether financial or social. However, migrant health may deteriorate with increasing length of residence in the new country.[29] In the case of rural to urban migration, evidence for a healthy migrant effect is limited.[31] In our study, socioeconomic variables of the migrant population were not statistically different than those of the non-migrant population. However, the tough social conditions that new rural migrants face in LMIC cities could explain partly the higher prevalence of wheeze/asthma in rural migrants. It is well known that rural migrants move to the cities in search of work or to improve their quality of life. Most rural migrants settle at the periphery of growing cities in areas that lack basic services and infrastructure. Such newly established neighbourhoods are characterised by low quality of life, poor housing and poverty.[32] Several studies conducted in urban centres of LA have shown an increased risk of wheeze/asthma to be associated with factors indicative of poverty, dirt and infections.[33 34] Thus, the adverse urban environment in which new rural migrants find themselves could increase asthma risk. Further, psychosocial stressors arising from the adaptation process in the new environment and family dissolutions consequent to migration could contribute to an increase in wheeze/asthma in migrant populations.[35–37] A high proportion of migrants in LMICs are women who provide the primary economic support for their families working in urban areas in unskilled service jobs.[38] In our study, 75% of the children with history of rural migration lived in families with one parent or without parents (table 2). As we have seen previously, the absence of parents at home (especially the mother) is an important determinant in the increase of wheeze in children of migrant parents.[13] Another factor that could explain the higher prevalence of wheeze in rural to urban migrants could be migration in search of medical attention for asthma (reverse causality), emphasising the importance of the migration process in the spatial and temporal distribution of asthma between urban and rural areas.

The study of migration using various categories provides a better understanding of the possible factors and mechanisms affecting the development of asthma in urban areas of LMICs. For example, in addition to 'direction of migration' which classified migrants according to rural or urban birthplaces, we used 'locality of migration' to describe more precisely the possible social and physical environment of the previous residence of the children. In our study, for example, migrants from medium cities (capital cities of Ecuadorian provinces) had 68% more wheeze than non-migrants. We also identified an important migration flow from Colombia, especially from Nariño Department, a region that borders the province of Esmeraldas and which is located less than 150 km from the city of Esmeraldas (figure 1). Although this is a group of international migrants, they provided a useful comparison group because of proximity and a social and ecological environment similar to that found in Esmeraldas Province. Children born in Colombia or born of Colombian parents had a greater prevalence of wheeze compared with Ecuadorian children. Many of these migrants are involuntary migrants or refugees fleeing guerrilla and paramilitary violence in Colombia and the higher prevalence of wheeze in this population might be explained by psychosocial stressors related to displacement rather than changes in lifestyles.[39–41] However, our study is subject to several limitations. First, the cross-sectional design does not permit assumptions of the direction of causality. Second, misreporting of birthplace related to recall bias and misclassification is possible, especially for boundary changes in the geographical units of study. However, the use of place of birth to define migration status is more precise than previous studies that have either not defined migration status or used other variables (eg, use of ethnic surnames as a surrogate marker for migrant status[15]). Further, specific information about the history of migration of the parents was limited. Third, not all wheezing is asthma, although this definition is probably more useful in rural populations where access to healthcare is limited and where alternative definitions such as doctor diagnosis may be subject to significant misclassification. Fourth, because selection of schools resulted in a sample of predominantly Afro-Ecuadorian schoolchildren (to represent the original source population of migrants from rural districts in the north of the province), our findings cannot necessarily be generalised to populations of differing ethnic compositions within the province. However, we believe that our findings provide novel insights into how social and demographic factors may affect asthma burden in LMICs.

Clearly, internal migration is a major contributor to the urbanisation process in LMICs and has a direct effect on the prevalence of asthma in urban and rural populations.[42] It is possible that the differences in asthma prevalence between urban and rural areas or between LA countries could be in part explained by different rates of urbanisation and migration within the region. As for international migrants, internal migrants also face important economic, social and environmental changes, especially those associated with changes in diet, physical activity, housing, family composition and air pollution, all factors related to asthma risk.[43] Special consideration must be given to rural migrants living in poor conditions in their new urban environments, generally slums and informal settlements. Populations living in such environments are exposed to a number of factors that could increase the risk of asthma or exacerbate existing disease such as inadequate housing, increased risk of respiratory infections through overcrowding and high levels of violence.[29] Finally, migration results in exposure to a new set of pollutants and allergens, and to new socioeconomic and cultural factors resulting in important changes to the social and family environment in which the new arrivals find themselves and which may contribute to asthma risk. A better understanding of the effects of migration on asthma will allow us to identify potential public health interventions that could be tested with the aim of alleviating the growing burden of asthma disability, particularly among the urban poor of LA.

CONCLUSION

We evaluated the effects of different migrant categories on the prevalence of wheeze in an urban area of LA. Our study provides evidence that rural to urban migration is a risk factor for wheezing in urban schoolchildren. Age of migration and time since migration were associated with an increased risk of wheeze only for rural to urban migrants but not for urban to urban migrants. Temporal, spatial and socioeconomic dimensions of the migration process may have different effects on the prevalence of wheeze/asthma and other allergic diseases. Further studies in different populations living in rural and urban areas of LMICs, that are subject to migration processes, are required in which detailed information is collected at individual, household and community levels.

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Research Paper 4: Migration and allergic diseases in a rural area of a developing country

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Migration and allergic diseases in a rural area of a developing country

To the Editor:

Studies in developing countries (DCs) have frequently reported a lower prevalence of allergic diseases (ADs) in rural areas compared with urban settings, and this has been attributed to the protective effects of environmental exposures such as rural lifestyle.[1] Recent evidence from studies conducted in Africa and Asia showed that ADs are increasing in urban and even in rural settings, reducing the urban-rural prevalence gap.[2,3] It has been hypothesized that temporal increases in the prevalence of ADs might be associated with urbanization processes, especially with the change from rural to more modern urban lifestyles.[1]

Migration is an important component of the urbanization process and involves socioeconomic, environmental, and lifestyle changes in rural and urban populations. However, the effects of migration on ADs in urban and rural settings of DCs have not been explored.[4] The impact of migration on ADs has been largely investigated by comparing populations that have migrated from DCs (presumed low risk for ADs) to developed countries (presumed high risk).[5] These studies have shown that being born in a country of low risk provides protection against asthma, but this protection may decline with the length of residence in the new environment.[5] Others studies have shown that age of migration and time since migration are associated with the risk of asthma and other ADs, often leading to a higher risk of atopy and allergy among migrants than among the local population.[6]

The Social Changes, Asthma and Allergy in Latin America (SCAALA) study has been investigating the effects of migration on the prevalence of ADs in schoolchildren living in rural and urban areas.[4] We studied 4295 rural and 2510 urban children aged 5 to 16 years attending a convenience sample of schools in Esmeraldas province, Ecuador. Data on potential risk factors, migration (direction and distance of migration, age at migration, and time since migration), and wheeze, rhinitis, and eczema symptoms within the previous 12 months were collected using an investigator-administered questionnaire that included the core allergy questions of the International Study of Asthma and Allergies in Childhood (ISAAC phase II).[4] Atopy was measured by skin prick testing to 7 aeroallergens.

Results from the rural area showed that children who migrated during the first year of life had a greater risk of wheeze and rhinitis than did nonmigrant children and that children with a history of international migration (children from rural areas of Colombia) had a higher prevalence of rhinitis than did nonmigrant children (Table I). The study also evaluated the effects of maternal migration on allergic outcomes in children using the variables maternal history of migration and children living with one or no parent. These analyses suggested that children whose mothers had a history of migration had a greater risk of eczema compared with children whose mother did not, and children who did not live with any parent had more wheeze than did children living with both parents (Table I). The magnitude of the latter association was greater for all allergic symptoms among children of migrant mothers (Table II). No associations were observed for atopy (at least 1 positive allergen skin test result).

The present study is unique in investigating migrants within a rural area of a DC, where migrants come from urban and rural settings. In this setting, age at migration and international migration were important factors associated with a higher risk of ADs in rural populations. A novel observation was the effect of migrant status of the mother on the prevalence of ADs: children of migrant mothers not living with either parent had a 2-fold greater risk of all 3 ADs compared with children living with both parents. These data raise a question: *Could it be that social effects of migration, such as absence of parents at home, are important determinants of the increase in ADs in rural populations of DCs*? To answer this question, we need to consider some demographic patterns in these regions. It is well known that people in rural villages move to urban areas, temporally or permanently, in search of work to improve their quality of life. A high proportion of these rural migrants are single women who provide economic support for their families. Most of these women leave their children in the community of origin to be cared for by relatives. Some of these immigrants are able to settle in the city while others return to their rural communities.[7] In the SCAALA rural population, 31% of the children and 23% of the mothers had a history of migration and 15% of the children lived with no parent.

If the absence of parents at home (especially the mother) is an important determinant of the increase in ADs in DCs, then 2 migration trends that have occurred over recent decades might help us understand temporal trends in ADs. In the past, most economic migrants were young men, but now "feminization of migration" is a growing trend worldwide because of a greater demand for female labor.[8] Second, "circular migration" is a common phenomenon in regions that are undergoing high levels of urbanization, and it refers to repeated migrations between rural and urban areas due to improvements in transport and modern forms of communication.[9]

Migration affects not only the individuals who migrate but also their family. Migration impacts on roles, support structures, and responsibilities of family members, resulting in changes in social and psychological factors. In the case of maternal migration, children who remain in their community may experience heightened levels of stress and depression because of separation from their primary carer. Psychological mechanisms have been proposed to explain how emotional factors, in the context of family, might affect the development of allergic diseases.[10] For this reason, we propose that the absence of the parents at home, through temporary or permanent migration, may contribute to the increase in ADs in rural and urban populations of DCs.

Finally, further analyses in different populations living in rural and urban areas evaluating the effects of migration on ADs are required. A better understanding of the social, psychological, and environmental effects of migration on ADs in DCs is required.

TABLE I. ORs and 95% CIs for associations between migration variables and allergic symptoms adjusted for sex, age, and socioeconomic status

			OR (95% CI)		
Variable	**Category**	**N**	**Wheeze**	**Rhinitis**	**Eczema**
Direction of migration	NM	2964	1	1	1
	Rural to rural	555	1.13 (0.84-1.52)	1.02 (0.7-1.49)	1.23 (0.82-1.83)
	Urban to rural	776	0.97 (0.74-1.27)	1.18 (0.86-1.61)	1.16 (0.81-1.66)
Distance of migration	NM	2964	1	1	1
	National	1263	0.99 (0.79-1.25)	1.04 (0.79-1.38)	1.21 (0.90-1.64)
	International	68	1.71 (0.88-3.32)	2.39 (1.16-4.92)*	0.64 (0.16-2.66)
Age at migration (y)	NM	2964	1	1	1
	<1	269	1.47 (1.02-2.12)*	1.59 (1.03-2.46)*	1.25 (0.73-2.14)
	1-5	560	0.96 (0.71-1.31)	1.18 (0.83-1.69)	1.17 (0.78-1.75)
	>5	502	0.88 (0.62-1.24)	0.76 (0.48-1.19)	1.16 (0.75-1.79)
Time since migration (y)	NM	2964	1	1	1
	<3 vs NM	383	0.98 (0.68-1.4)	0.94 (0.6-1.49)	0.96 (0.57-1.61)
	3-5 vs NM	197	0.56 (0.31-1.02)	0.9 (0.48-1.69)	1.53 (0.86-2.7)
	>5 vs NM	751	1.21 (0.94-1.58)	1.26 (0.92-1.73)	1.21 (0.85-1.73)
Maternal history of migration	No	3314	1	1	1
	Yes	981	1.22 (0.96-1.53)	1.24 (0.93-1.65)	1.88 (1.39-2.53)*
Parents living in the child's house	Both	2490	1	1	1
	One	1146	1.07 (0.84-1.36)	1.16 (0.87-1.54)	1.21 (0.88-1.67)
	None	659	1.57 (1.2-2.05)*	1.29 (0.92-1.81)	1.27 (0.86-1.86)

NM, No migrant; *OR*, odds ratio.

Outcomes were defined as recent wheeze—reported wheezing during the previous 12 months; recent eczema—having a reported itchy rash with a flexural distribution in the previous 12 months; and recent rhinitis—nasal stuffiness or sneezing without a cold accompanied by itchy eyes in the previous 12 months.

*$P < .05$.

TABLE II. ORs and 95% CIs for associations between allergic symptoms and parents living in the child's home (live with parents) stratified by maternal history of migration*

		Maternal history of migration					
		No			Yes		
Allergic symptom	**Live with parents**	**OR***	**95% CI**	***P* value**	**OR***	**95% CI**	***P* value**
Wheeze	One vs both	1	0.76-1.34	.976	1.2	0.77-1.87	.429
	None vs both	1.44	1.06-1.95	.02	2.17	1.25-3.77	.006
Rhinitis	One vs both	1.03	0.73-1.46	.858	1.46	0.85-2.52	.171
	None vs both	1.1	0.74-1.64	.627	2.07	1.05-4.08	.036
Eczema	One vs both	0.96	0.63-1.46	.857	1.63	0.95-2.77	.074
	None vs both	1.03	0.64-1.65	.916	2.12	1.07-4.17	.031

OR, Odds ratio.

*ORs adjusted for sex, age, and socioeconomic status.

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Persistent nodal histoplasmosis in nuclear factor kappa B essential modulator deficiency: Report of a case and review of infection in primary immunodeficiencies

To the Editor:

Histoplasma capsulatum var. *capsulatum* is an endemic dimorphic intracellular fungus that typically causes asymptomatic and self-limited infections in immunocompetent individuals.[1] Inversely, immunocompromised patients, particularly patients with the acquired immunodeficiency syndrome and other cell-mediated immune defects, are at increased risk for disseminated histoplasmosis.[1]

Here, we report the first case of persistent nodal histoplasmosis in a patient deficient in nuclear factor kappa B (NF-κB) essential modulator (NEMO). NEMO is required for activation of NF-κB, a key transcription factor for both innate and adaptive immunity. Multiple cell surface receptors converge on the induction of NF-κB activation, including CD40, the Toll-like receptors, TNF-α receptor, IL-1 receptor, and the receptor for ectoderm formation, ectodysplasin.[2]

Amorphic mutations in the X-linked gene for NEMO (*IKBKG*) are lethal prenatally in males, but cause *incontinentia pigmenti* in females.[3] Lyonization allows cells with normal NEMO expression to survive; however, those cells with completely absent expression die with inflammatory consequences.[3] Hypomorphic NEMO mutations resulting in reduced activation of NF-κB permit male survival and cause a spectrum of conditions, including ectodermal dysplasia, invasive pneumococcal diseases, and mycobacterial disease.[3,4]

Reduced natural killer cell function, variable hypogammaglobulinemia, and impaired function of the receptors of innate immunity predispose to viral, pyogenic bacterial, and mycobacterial infections.[5] NEMO mutations can also impair CD40 signaling in monocytes, leading to diminished IL-12 secretion and therefore impaired IFN-γ production and increased susceptibility to mycobacterial infections, suggesting that activation of NF-κB by NEMO is essential for the destruction of intracellular pathogens.[4]

A 52-year-old man with NEMO deficiency presented to our institution with 1 year of mildly worsening dyspnea and intermittent night sweats. A mutation had been identified previously in exon 1b of *IKBKG* (c.1-16C>G), resulting in abnormally spliced transcripts.[6] He had a history of *Haemophilus influenzae* pericarditis in childhood and disseminated *Mycobacterium avium* predominantly involving the thigh and perineum in his 30s, which was treated successfully with antimycobacterials and IFN-γ. His nontuberculous mycobacterial infection had been well controlled for over 10 years on ethambutol, azithromycin, and moxifloxacin, but IFN-γ had been stopped.

Computed tomography (CT) of the chest showed a new right superior perihilar mass (2.2 cm × 1.6 cm) with necrosis and areas of peribronchial thickening (Fig 1, *A*). Repeat chest CT 3 months later showed interval increase (2.5 cm × 1.8 cm) of the perihilar mass with development of tree-in-bud opacities and larger mediastinal lymph nodes.

Histopathology of a paratrachal lymph node biopsy showed chronic inflammatory changes with granulomatous inflammation and rare small yeast forms (Fig 1, *B*). Fungal culture from the lymph node biopsy grew *H capsulatum*. Urine was also positive for *Histoplasma* antigen. Bronchoalveolar lavage, blood, and urine fungal cultures were all negative. Posaconazole 300 mg daily was initiated. At 3 months, he was clinically improved and CT showed improvement in lung opacities and right hilar lymphadenopathy.

A search of the National Institutes of Health patient databases for unpublished cases of histoplasmosis in patients with known primary immunodeficiencies (PIDs) was conducted. All patients had provided informed consent on approved protocols of the National Institute of Allergy and Infectious Diseases at the National Institutes of Health. We identified 4 unpublished cases of histoplasmosis: disseminated histoplasmosis in GATA2 deficiency (patient 1), CD40L deficiency (patient 2), and idiopathic CD4 lymphocytopenia (ICL; patient 4) and lymphadenitis in CD40L deficiency (patient 3). Patients' clinical descriptions are provided in this article's Online Repository at www.jacionline.org and are summarized in Tables E1 and E3 in this article's Online Repository at www.jacionline.org.

A systematic search of all published cases of histoplasmosis in patients with PID was conducted through August 2015. In PubMed, PubMed Central, Scopus, Embase, and Web of Science, search terms were histoplasma, histoplasmosis, disseminated, and immunodeficiency. All publications involving secondary or acquired immune deficiencies were excluded. For specific PIDs that can be defined clinically or molecularly, the following descriptive terms were used: X-linked hyper-IgM = CD40L deficiency; hyper-IgM syndrome = clinical phenotype; STAT3 deficiency = autosomal-dominant hyper-IgE; hyper-IgE syndrome = clinical phenotype.

We identified 47 patients with histoplasmosis and a PID defined molecularly or clinically. All references are summarized in Tables E1 and E3. We classified infections as disseminated on the basis of original classification in the publication, the presence of *H capsulatum* in 2 or more tissues, or the suspected involvement of multiple tissues and a positive urine or serum *Histoplasma* antigen (see Table E1).

Disseminated histoplasmosis was classified in 32 of 47 patients (68%) with PID identified in our review. Two deaths occurred because of progression of disseminated histoplasmosis. All 8 documented recurrences of histoplasmosis occurred in the context of disseminated infection. Disseminated histoplasmosis had variable clinical manifestations, most commonly with systemic symptoms (82%), pulmonary symptoms (74%), splenomegaly (56%), and lymphadenopathy (56%) (see Table E2 in this article's Online Repository at www.jacionline.org). Notably, out of 10 cases of histoplasmosis in molecularly or clinically diagnosed autosomal-dominant hyper-IgE syndrome (STAT3 deficiency), 8 patients presented with gastrointestinal complaints, including 3

Chapter 5.

Research Paper 5: Lifestyle domains as determinants of wheeze prevalence in urban and rural schoolchildren in Ecuador: cross sectional analysis

Overview Chapter 5

The process of urbanisation has a profound impact on the lifestyle of populations not only in urban but also in rural areas. Changes in dietary patterns, health behaviours, work activities, economic status, housing materials, among many others, are all associated with the urbanisation process. In asthma research, changes in lifestyles, especially the acquisition of a modern way of life, have been associated with the increase in asthma and differences in asthma prevalence between rural and urban populations. However, it has been extremely difficult for epidemiologists interested in the effects of lifestyle factors on the prevalence of asthma to disentangle independent effects of individual risk factors that together constitute lifestyle. The current chapter introduces a new approach to define lifestyle as a set of attributes representing groups of linked risk factors rather than the traditional approach of investigating independent effects of individual risk factors. This chapter describes the methodology to identify lifestyle domains based on a combination of social, behavioural and housing characteristics of the population. It uses multiple correspondence analysis to recognise groups of associated personal characteristics. The chapter also discusses how urban processes such as internal migration and urban sprawl are constantly modifying the lifestyle in urban and rural areas of LMICs. The chapter concludes discussing how the effects of lifestyle may be better understood through a combination of effects or a set of attributes allowing us to identify common features that may help explain differences in asthma prevalence between populations.

Research Paper Cover Sheet

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SECTION A – Student Details

Student	**Alejandro Rodriguez**
Principal Supervisor	**Dr. Elizabeth Brickley**
Thesis Title	**Urbanisation and childhood asthma in a developing region of Latin-America: A Cross-sectional analysis in northeastern Ecuador**

If the Research Paper has previously been published please complete Section B, if not please move to Section C

SECTION B – Paper already published

Where was the work published?	**Environmental Health**		
When was the work published?	**4 February 2015**		
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion	**N/A**		
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SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	**Study design, data collection, data analysis, draft manuscript, and manuscript review.**

Student Signature: [redacted] **Date:** **06/08/2018**

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HYPOTHESIS

Open Access

Lifestyle domains as determinants of wheeze prevalence in urban and rural schoolchildren in Ecuador: cross sectional analysis

Alejandro Rodriguez[1,2,3*], Maritza G Vaca[1], Martha E Chico[1], Laura C Rodrigues[3], Mauricio L Barreto[4] and Philip J Cooper[1,2,5]

Abstract

Background: The acquisition of a modern lifestyle may explain variations in asthma prevalence between urban and rural areas in developing countries. However, the effects of lifestyle on asthma have been investigated as individual factors with little consideration given to the effects of lifestyle as a set of attributes. The aim of the present study was to identify modern lifestyle domains and assess how these domains might explain wheeze prevalence in urban and rural areas.

Methods: We analysed data from cross-sectional studies of urban and rural schoolchildren in Esmeraldas Province, Ecuador. Variables were grouped as indicators of socioeconomic factors, sedentarism, agricultural activities and household characteristics to represent the main lifestyle features of the study population. We used multiple correspondence analyses to identify common lifestyle domains and cluster analysis to allocate children to each domain. We evaluated associations between domains and recent wheeze by logistic regression.

Results: We identified 2–3 lifestyle domains for each variable group. Although wheeze prevalence was similar in urban (9.4%) and rural (10.3%) schoolchildren, lifestyle domains presented clear associations with wheeze prevalence. Domains relating to home infrastructure (termed transitional, rudimentary, and basic urban) had the strongest overall effect on wheeze prevalence in both urban (rudimentary vs. basic urban, OR = 2.38, 95% CI 1.12-5.05, p = 0.024) and rural areas (transitional vs. basic urban, OR = 2.02, 95% CI 1.1-3.73, p = 0.024; rudimentary vs. basic urban, OR = 1.88, 95% CI 1.02-3.47, p = 0.043). A high level of sedentarism was associated with wheeze in the rural areas only (OR = 1.64, 95% CI 1.23-2.18, p = 0.001).

Conclusions: We identified lifestyle domains associated with wheeze prevalence, particularly living in substandard housing and a high level of sedentarism. Such factors could be modified through programmes of improved housing and education. The use of lifestyle domains provides an alternative methodology for the evaluation of variations in wheeze prevalence in populations with different levels of development.

Keywords: Lifestyle domains, Wheeze, Schoolchildren, Urban, Rural, Tropics, Latin America

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Introduction

Secular trends of increasing prevalence of childhood asthma have been documented worldwide [1]. This observation, in parallel with differences in asthma prevalence between developed and developing countries and between urban and rural areas, has led to the suggestion that alterations in environmental and lifestyle factors are the most plausible explanation for such trends [2]. There is evidence also that asthma prevalence may have reached a plateau in developed countries in contrast with many developing regions where the prevalence of asthma continues to increase [3].

Asthma prevalence varies considerably between countries and may be lower in rural populations [3,4]. The lower prevalence in rural populations could be explained by farming exposures associated with a traditional lifestyle that may confer protection against allergic diseases [5]. The prevalence of asthma appears to be increasing in urban and rural populations in developing countries [6,7], and increasing urbanization and the acquisition of modern lifestyle, coupled perhaps with environmental triggers for non-atopic symptoms and or the persistence of exposures that attenuate atopy [8,9], has been the most cited factors to explain these epidemiological trends [10,11].

The most common lifestyle factors associated with the increase in asthma related to the process of urbanization are dietary changes, economic development, sedentary habits, changes in housing and a decline in farming activities, among others [12]. Commonly, the association between lifestyle factors and asthma has been examined in terms of the independent effects of individual risk factors, and such analyses can explain asthma prevalence only partially. The effects of lifestyle may be better understood through a combination of effects or a set of attributes allowing us to identify common features that may help explain differences in asthma prevalence between populations, and understand how aggregations of behaviours and personal characteristics shared by groups of individuals may alter disease risk [13].

In Latin America, asthma or wheeze prevalence has been associated with changes in lifestyles in urban and rural settings [11]. Nevertheless, there are no studies evaluating how differences in lifestyle affect the prevalence of asthma in both urban and rural areas. The present study identified common lifestyle domains in urban and rural populations of schoolchildren based on social and behavioural characteristics of the children and the households in which they live, and explore how these patterns could explain the prevalence of wheeze in a tropical region of Ecuador.

Methods

Study area and population

The study was conducted in urban and rural areas of a tropical region of Esmeraldas Province in northwest Ecuador. The population is predominantly Afro-Ecuadorian, although mestizo and indigenous populations are also present in this area. The rural study population comprised small communities in the Districts of San Lorenzo and Eloy Alfaro, located in the north of the province. Economic activities of these communities are focused on subsistence agriculture, hunting, fishing, logging, and more recently African palm oil cultivation. Commerce and the provision of services are available in the larger communities. The educational level of the population is generally very low with a high rate of adult illiteracy. Housing materials are a mixture of traditional and modern materials - wood and bamboo for walls and corrugated iron for roofing with the use of cement blocks for walls becoming increasingly common. Drinking water comes directly from rivers although some communities have untreated piped water sourced from streams. There is no sewage system in any of the rural communities and disposal of faeces is generally by household or community pit latrines or in the open. A number of communities are connected to the national electrical grid but few have access to telephone services or mobile networks. Transportation for most communities is by river, although small roads have been built to connect some of these communities with the provincial capital [14].

The urban study population was in Esmeraldas city, especially in neighbourhoods of Afro-Ecuadorian migrants coming from the districts of San Lorenzo and Eloy Alfaro (rural study population). Esmeraldas city is the provincial capital located ~100 km to the south of the rural study area. It is a medium sized coastal city with approximately 189,504 inhabitants, is the principal port in northwest Ecuador, the terminal for trans-Andean oil pipeline, and location of the country's largest refinery. More than half of oil refined in Ecuador is refined in Esmeraldas, and most of the country's oil exports are shipped from its port. Industrial facilities such as thermoelectric power plant (run on fuel oil processed by the refinery), a wood processing plant and the refinery are located in an area in the south of the city, being an important source of pollution for the city. The main economic and industrial activities are based on chemical and oil exports, commerce, agriculture (especially tropical fruit and palm oil), timber, fishing, and tourism. Basic services are of limited availability: 28% of the households have no potable water, 23% of the households lack a sewage system and 17% of the houses in city are constructed with traditional materials (wood and or bamboo). Extreme poverty is present in 21% of the population and educational level is low with just 10% of the population having 10 or more years of education [15].

Study design

Cross-sectional studies were done in schoolchildren aged 5–16 years, in a convenience sample of 59 rural communities and 9 urban schools to evaluate lifestyle domains. The rural and urban schools were chosen as convenience samples to provide schools in which the children were predominantly Afro-Ecuadorian. The rural study was done in two rural Districts (Eloy Alfaro and San Lorenzo) in which small rural communities were selected each with a single school and <150 pupils on the school roll. In the urban area in the City of Esmeraldas, schools were selected from barrios or neighbourhoods in which there was a predominance of Afro-Ecuadorian migrants from the two northern rural Districts where the rural study was conducted. All children attending the schools at the time of the survey were eligible for inclusion. The response rate was 91.3% of children in rural schools and 90.8% of those attending urban schools (of the annually updated school lists). Selection of rural and urban Afro-Ecuadorian schoolchildren of similar ancestry should have minimized differences in lifestyle related to social and cultural differences between populations. Data collection for the rural population was done between March 2005 and May 2007 and for the urban population between November 2007 and January 2010. The original study was designed to investigate risk factors for allergy and asthma in Afro-Ecuadorian children associated with rural–urban migration and is described in detail elsewhere [16].

Data collection

A parent or guardian of each child was interviewed by trained field workers using a questionnaire modified from the International Study of Asthma and Allergies in Childhood (ISAAC) phase II study [17] and adapted to the local conditions and translated into Spanish. We used recent wheeze as a proxy for asthma (defined as the presence of wheeze in the last 12 months), a definition that has been validated in different populations and that is widely used in epidemiologic studies [17,18], and is especially useful in populations with limited access to health care [9]. Indicators to evaluate lifestyle were selected and classified into 4 variable groups (Table 1). 1) Socioeconomic status (SES) of the household, representing the socioeconomic position of the household in relation to the study population. We included variables for monthly household income and parental education and employment. 2) Characteristics of the child's home, representing the type of housing in which the child lived including presence of basic services (electricity and running water), disposal of faeces (type of bathroom), electrical appliances, household construction materials and type of cooking fuel. 3) Sedentary characteristics of the child, representing the level of sedentarism including variables such as nutritional status (overweight vs. normal weight), consumption of fast foods (fizzy drinks and hamburgers), hours of daily TV viewing and frequency of vigorous physical exercise. Nutritional status was defined by z scores of body mass index (weight[kg]/height[m]^2) to classify children as overweight (Z scores greater than or equal to the 85th centile) or of normal weight [19]. 4) Agricultural activities of the household, representing the child's potential farming exposures including parental agricultural activities, contact with animals in farms and animal breeding in or around the household home. A fifth group of variables (Other asthma risk factors) representing other important risk factors for asthma including sex, age, maternal history of asthma, maternal smoking during pregnancy, environmental tobacco smoke exposure in the child's household and household overcrowding were included also in the analyses (Table 1).

Statistical analysis

Multiple correspondence analyses (MCA) is a technique that allows researchers to use a multivariate approach to lifestyle studies. MCA [20] is a descriptive, exploratory technique designed to analyse the pattern of relationships of several categorical dependent variables in order to produce a graphic illustration of the original information in a low dimensional space. MCA summarizes a set of categorical variables into a small number of orthogonal variables named "dimensions" that are related to the original variables through a pair of descriptive measures, discrimination and inertia. Discrimination measures show the correlation (−1 to 1) of each variable with a dimension, and inertia measures the proportion of variance of the original variables explained by each dimension. Dimensions produce a set of coordinate values (Z scores) that summarise subjects and categories permitting the associations between subjects and between categories to be displayed. Dimensions form the axes of the graphical representation of the associations between categories or subjects that can be visualized as clusters or patterns. A high proximity between categories represents a high level of association.

To identify lifestyle domains, we developed four models using MCA, one for each variable group (but not for the fifth group – Other asthma risk factors). We retained two dimensions in each model, evaluating measures of discrimination and inertia for each dimension. Graphical displays of the variable categories and subjects (children) were constructed using two dimensions for each variable group (i.e. by building a series of two-dimensional graphs). Graphs were evaluated to identify visually prominent lifestyle domains based on associations between variable categories. Having identified lifestyle patterns, we used non-hierarchical cluster analysis to allocate individual subjects to domains using Z scores for each subject [21]. Scatter plots were constructed to see how cluster analysis assigned children to lifestyle domains and were compared

Table 1 Characteristics of the study population by variable groups and area of residence

Groups	Variables	Categories	Rural		Urban	
			n	%	n	%
Socioeconomic Status of the household	Father's [A] education	<6 years	2512	58.5%	513	20.4%
		6-11 years	1395	32.5%	1268	50.5%
		>11 years	388	9.0%	729	29.0%
	Mother's [A] education	<6 years	2444	56.9%	502	20.0%
		6-11 years	1498	34.9%	1296	51.6%
		>11 years	353	8.2%	712	28.4%
	Father's employment	Farm worker	3356	78.1%	433	17.3%
		Employee	459	10.7%	1223	48.7%
		Trader	323	7.5%	507	20.2%
		Professional	157	3.7%	347	13.8%
	Mother's employment	House wife	2880	67.1%	1254	50.0%
		Farm worker	610	14.2%	21	0.8%
		Employee	450	10.5%	735	29.3%
		Trader	195	4.5%	313	12.5%
		Professional	160	3.7%	187	7.5%
	Household income [B]	≤$170	3523	82.0%	1077	42.9%
		$171-$340	627	14.6%	913	36.4%
		>$340	145	3.4%	520	20.7%
Characteristics of the child's home	Basic Services	0-1 Services	4044	94.2%	210	8.4%
		2-3 Services	251	5.8%	2300	91.6%
	Source of drinking water	River/well	3052	71.1%	14	0.6%
		Piped	977	22.7%	185	7.4%
		Potable	266	6.2%	2311	92.1%
	House construction materials	Wood/Bamboo	2892	67.3%	710	28.3%
		Concrete/others	725	16.9%	638	25.4%
		Concrete	678	15.8%	1162	46.3%
	Bathroom	Field	1553	36.2%	97	3.9%
		Latrine	2742	63.8%	671	26.7%
		Toilet	0	0%	1742	69.4%
	Electrical appliances	0-2 appliances	2465	57.4%	417	16.6%
		3 appliances	1030	24.0%	943	37.6%
		4 appliances	800	18.6%	1150	45.8%
	Cooking fuel	Only gas	3074	71.6%	2355	93.8%
		Gas/wood/charcoal	1221	28.4%	155	6.2%
Sedentary characteristics of the child	Nutritional Status[C]	Normal weight	3786	88.1%	2069	82.4%
		Overweight	509	11.9%	441	17.6%
	Fizzy drink consumption	Sometimes	938	21.9%	843	33.7%
		1-4 times/ week	2476	57.8%	1293	51.7%
		>4 times/week	871	20.3%	364	14.6%
	Hamburger consumption	Never	3211	74.9%	968	38.8%
		Sometimes	718	16.7%	881	35.3%
		1 in a month	359	8.4%	649	26%

Table 1 Characteristics of the study population by variable groups and area of residence *(Continued)*

	Vigorous exercise	Daily	3197	74.7%	1862	74.2%
		1-3 times/week	970	22.7%	603	24%
		Sometimes	57	1.3%	17	0.7%
		Barely	55	1.3%	27	1.1%
	TV viewing	<1 hours daily	1136	26.4%	242	9.6%
		1-3 hours daily	2459	57.3%	1670	66.5%
		> = 4 hours daily	700	16.3%	598	23.8%
Groups	Variables	Categories	Rural		Urban	
			n	%	n	%
Agriculture activities of the household	Farm activities	No	851	19.8%	2071	82.5%
		Yes	3444	80.2%	439	17.5%
	Contact with animals in farms	No	2981	69.4%	2294	91.4%
		Yes	1314	30.6%	216	8.6%
	Pigs breeding around home	No	2203	51.4%	2104	83.8%
		Yes	2086	48.6%	406	16.2%
	Chicken breeding around home	No	644	15.0%	954	38.0%
		Yes	3648	85.0%	1554	62.0%
	Other farm animals around home	No	2523	58.8%	1789	71.3%
		Yes	1768	41.2%	719	28.7%
Others asthma risk factors	Sex	Male	2206	51.4%	1321	52.7%
		Female	2089	48.6%	1187	47.3%
	Age	<11 years	2224	51.8%	1689	67.3%
		≥11 years	2071	48.2%	821	32.7%
	Maternal asthma	No	3223	79.1%	1919	81.4%
		Yes	854	20.9%	439	18.6%
	Matrenal smoking during pregnancy	No	3720	88.2%	2300	94.2%
		Yes	499	11.6%	142	5.8%
	Environmental tobacco smoke[D]	No	2297	53.7%	1600	63.8%
		Yes	1981	46.3%	909	36.2%
	Household overcrowding[E]	≤3	2670	62.2%	1578	62.9%
		>3	1625	37.8%	932	37.1%

[A]<6 years = Incomplete primary or ilitare; 6-11 years = complete primery and incomplete secundary; >11 = complete secundary or higher. [B]Household income based on the basic family wage in 2007 (US$170). [C]Defined by z scores of body mass index (weight[kg]/height[m]^2) to classify children as overweight (Z scores greater than or equal to the 85th centile [16]) or of normal weight. [D]Exposure to a smoker living in the child's household. [E]Persons per sleeping room.

visually with those for the four variable categories that were built for each of the 4 MCA models. Similarly, cross-tabulation frequencies between domains and original variables were done to assess the distributions of each variable within each domain.

Unadjusted and multivariable logistic regression was used to evaluate the associations between lifestyle domains and wheeze prevalence. For multivariate logistic regression analyses we constructed models for rural and urban areas using the 4 lifestyle domains and other asthma risk factors (fifth variable group). The final models were selected using back-wards step-wise regression and were those that explained the most variation in wheeze prevalence, those with the smallest mean square error, and the highest value of adjusted R^2. Distributions of variable categories by area of residence were evaluated using the Chi-squared test. Population-attributable fractions (PAFs) were calculated for each pattern [(proportion of exposed cases) × (OR −1)/OR], using adjusted ORs. Statistical significance was inferred by $p < 0.05$. All the analyses were done with SPSS version 18 (IBM SPSS, New York, USA).

The ethics committee of the Hospital Pedro Vicente Maldonado, Ecuador, approved the study protocol. Written informed consent was obtained from a parent and signed minor assent from the child.

Results

We evaluated a total of 6,805 children of whom 4,295 lived in the rural area and 2,510 in the urban area. The prevalence of wheeze was similar in rural and urban schoolchildren (10.3% vs. 9.4%, respectively) with an overall prevalence of 9.7%. Characteristics of the study populations by variable groups and area are shown in Table 1. The frequencies of almost all variables were significantly different between urban and rural areas ($p < 0.05$), except sex and overcrowding.

Graphic presentations of the two dimensions obtained by MCA for each of the 4 variable groups are shown in Figures 1A-D. Discrimination measures and the proportion of variance accounted for by each dimension are provided in Additional file 1: Table S1. The model for socioeconomic status (SES) of the household identified three domains (Figure1A): a) Low SES - associated with a rural setting and represented by farm workers, low income (<=$170 or lower than the basic family wage in 2007) and low parental educational level (<6 years or incomplete primary education); b) Medium SES - associated with an urban setting represented by trade and non-professional employment, 6–11 years of education (or incomplete secondary education) and monthly income of $171-$340; c) High SES - represented by professional occupations, higher parental educational level (>11 years or complete secondary or higher level education) and income of > $340 (or more than 2 basic family wages). The model for characteristics of the child's residence identified three domains (Figure 1B): a) Transitional - represented by presence of 3 electric appliances, gas for cooking, latrine bathroom and use of concrete and wood in house construction; b) Rudimentary - associated with rural areas and characterized by 1 or fewer basic services, less than 2 electric appliances, defaecation in the open, use of river or well water for drinking and use of wood or bamboo in house construction; c) Basic urban - associated with urban residence and represented by a household connection to potable water, concrete building materials for walls, use of a flushing toilet, ownership of a set of appliances and 2 or 3 basic services. The model for Sedentary characteristics of the child identified three domains (Figure 1C): a) High sedentarism – represented by consuming fizzy drinks > 4 times by week, watching television ≥ 4 hours daily and consumption of hamburgers sometimes; b) Medium sedentarism represented by vigorous physical activity 1–3 times per week, watching television 1–3 hours daily, consumption of fizzy drinks 1–4 times weekly, being overweight and consuming hamburgers once month; c) Low sedentarism - associated with rural settings and characterized by never consuming fizzy drinks and hamburgers, watching television <1 hour daily, normal weight and daily vigorous physical exercise. The model for agricultural activities of the household (Figure 1D) identified two domains: a) Farming - associated with rural settings and characterized by farm activities and peri-domestic animal breeding; b) Non-farming - associated with urban settings and represented by no farm activities, no animal husbandry around the household and no contact with farm animals. Cluster analysis was used to allocate the children to domains for each of the 4 variable groups. Scatter plots derived from these cluster analyses show the distributions of children by lifestyle domains in which dots represent individual children and dot colour represents lifestyle domains for each of the 4 variable groups (Figures 1E-H). Frequencies of original variables stratified by each domain for each model are shown in Additional file 1: Tables S2-S5 to illustrate the relative distributions of variable categories between domains. Low SES status (64.1%), rudimentary residence (51.6%), low sedentarism (67.9%) and farming environment (61.5%) were more common among rural school children (Table 2), while medium SES status (63.9%), basic urban type of residence (87.3%), low sedentarism (48.2%) and non-farming environment (85.3%) were more common among urban children (Table 2).

There was evidence of differences in the prevalence of recent wheeze by lifestyle patterns for the four lifestyle variable groupings, and estimates of effect from univariate and multivariate analyses are shown in Table 3. Univariate analyses showed: 1) socioeconomic status of the household - a higher risk of wheeze in children of low versus high socioeconomic status in the urban but not the rural sample (interaction $p = 0.026$); 2) Characteristics of the child's home - living in a basic urban house compared to a transitional or rudimentary house provided protection against wheeze in both urban and rural children; 3) sedentarism of the child – a high compared to low level of sedentarism was associated with an increased risk of wheeze in rural but not urban children (interaction $p = 0.027$); 4) agricultural activities of the household – farming compared to non-farming exposures were associated with wheeze in the urban but not rural samples (interaction $P = 0.032$).

Multivariate analyses (Table 3) showed that in the rural area, children living in transitional and rudimentary houses had 2.02 and 1.88-fold greater risk of wheeze, respectively, compared to children living in basic urban houses, while children with a high level of sedentarism had 1.64-fold greater risk of wheeze than those with a low level of sedentarism and this risk increased depending to SES of the household: Low SES 1.39, Medium SES 1.80 and High SES 3.26 (Table 4). In the urban area, children living in rudimentary houses had 2.38-fold greater risk of wheeze compared with those living in basic urban houses.

The findings for univariate and multivariate associations between recent wheeze and other asthma risk factors are shown in Table 3. In multivariate analyses, children aged

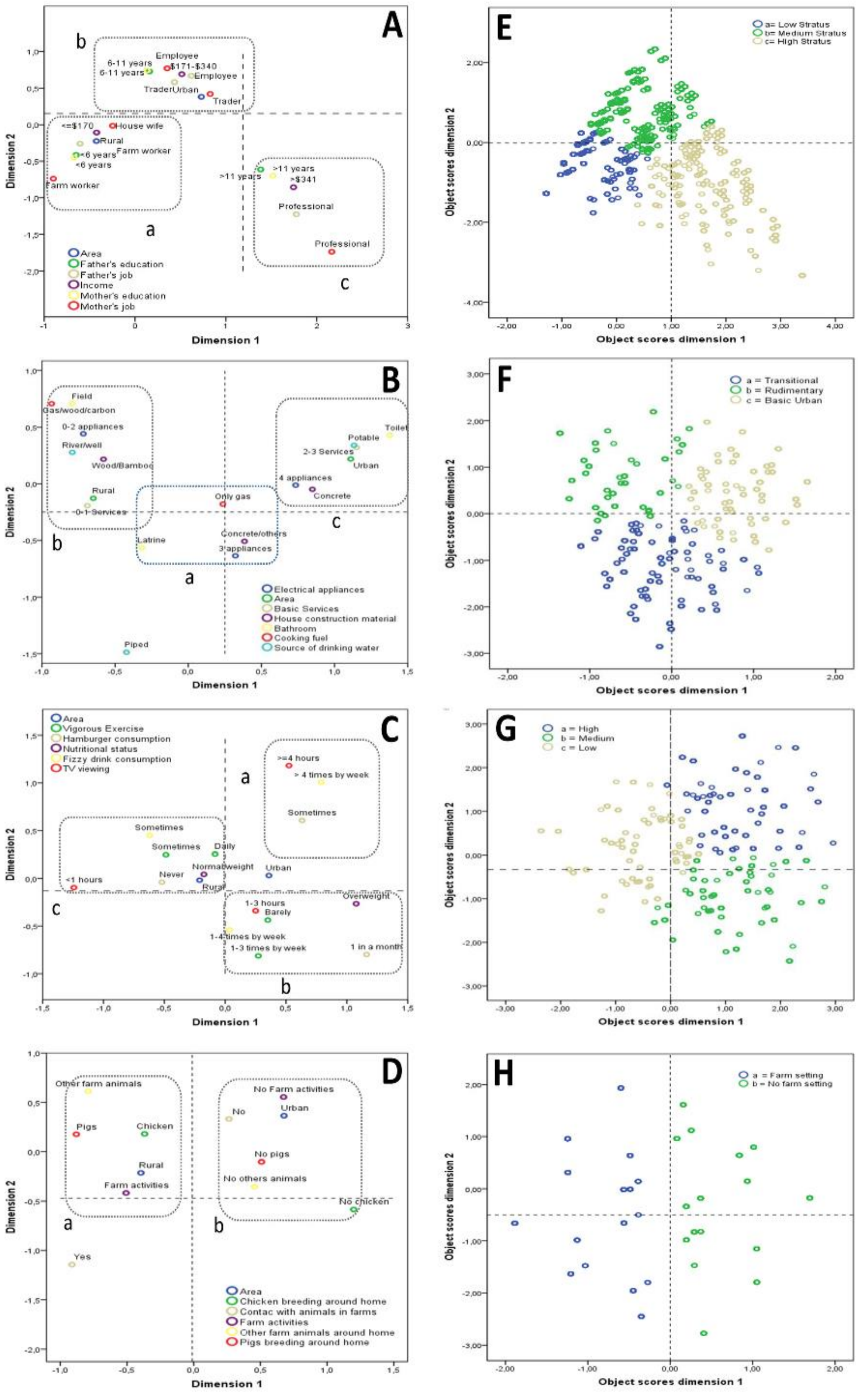

Figure 1 (See legend on next page.)

(See figure on previous page.)
Figure 1 Multiple Correspondence Analyses by: A) Socioeconimc status of the household, **B)** Characteristics of the chikld's home, **C)** Sedentary characteristics of the child, **D)** Agricultural activities of the household. Colour boxes around groupings of categories represent lifestyle domains. Axes represent z scoress by dimension. Cluster Analyses using objects scores of each MCA: **E)** Socioeconimc status of the household, **F)** Characteristics of the child's home, **G)** Sedentary characteristics of the child, **H)** Agriculture activities of the household. Colour circles represent the number of children in each lifestyle domains.

less than 11 years (versus ≥11 years) and children of mothers with a history of asthma had 1.81 and 3.03-fold greater risk of wheeze, respectively. In the urban area, children with a maternal history of asthma and of mothers who smoked during pregnancy had a 3.48 and 1.86-fold greater risk of wheeze, respectively.

We estimated the population fractions of wheeze attributable to each of the lifestyle domains (Table 5). In rural areas, transitional or rudimentary (versus basic urban) housing explained 22-24% of wheeze and high and medium versus low sedentarism accounted for 6% and 3% of wheeze, respectively. In contrast, lifestyle domains seemed to have poor explanatory power among urban schoolchildren. We stratified also the four lifestyle domains by sex and age groupings (<11 years versus ≥11 years) (see Additional file 1: Tables S6 and S7): in the rural area, associations between high sedentarism and wheeze were stronger in males and older children, while in urban areas, associations between housing characteristics and wheeze were stronger in females and in younger children.

Discussion

Lifestyle represents a complex constellation of highly associated factors involving social, behavioural and economic activities in which humans are engaged and environmental factors to which they are exposed. Lifestyle is in a constant state of change as human societies evolve, particularly under the influences of globalisation and increasing urbanization of humans worldwide – even the remotest of human populations have been strongly influenced by these processes. It has been extremely difficult for epidemiologists interested in the effects of lifestyle factors on the prevalence of wheeze and asthma to disentangle independent effects of individual risk factors that together constitute lifestyle. The approach adopted in the present analysis has been to define lifestyle as a set of attributes representing groups of linked risk factors rather than the traditional approach of investigating independent effects of individual risk factors [13]. In the present study, we used multiple correspondence analyses to identify key lifestyle domains and then cluster analysis to allocate individuals to specific lifestyle domains to explore how these patterns relate to the risk of wheeze in a large sample of school children living in urban and rural areas of tropical Ecuador. Our data provide evidence that factors associated with sub-standard and poor housing (i.e. lack of basic services including electricity, traditional construction materials and cooking fuel) may be an important determinant of the risk of wheeze overall while among generally highly physically active rural schoolchildren with generally high levels of activity, those with a high levels of sedentarism (low physical activity, overweight, and consumption of junk foods) are at greatest risk. Our data provide further insights into how groups of risk

Table 2 Distributions of children in lifestyle domains and prevalence of wheeze by study area

Groups	Lifestyle Domains	Rural		Wheeze	Urban		Wheeze
		n	%	%	n	%	%
Socioeconomic Status of the household	Low	2751	64.1%	10.1%	231	9.2%	14.8%
	Medium	1255	29.2%	10.4%	1603	63.9%	8.7%
	High	289	6.7%	11.4%	676	26.9%	9.1%
Characteristics of the child's home	Transitional	1860	43.3%	10.8%	257	10.2%	13.9%
	Rudimentary	2217	51.6%	10.3%	63	2.5%	15.8%
	Basic Urban	218	5.1%	5.7%	2190	87.3%	8.7%
Sedentarism of the child	High	584	13.6%	14.4%	533	21.2%	9.6%
	Medium	793	18.5%	10.7%	766	30.5%	8.9%
	Low	2918	67.9%	9.4%	1211	48.2%	9.6%
Agricultural activities of the household	Farming	2642	61.5%	10.1%	369	14.7%	12.4%
	Non-farming	1653	38.5%	10.7%	2141	85.3%	9.4%

Table 3 Associations between recent wheeze and lifestyle domains or other asthma risk factors

Groups	Lifestyle domains/ Categories	Rural						Urban						Interact. [A]
		Univariate			Multivariate			Univariate			Multivariate			
		OR	95% CI	P value	OR	95% CI	P value	OR	95% CI	P value	OR	95% CI	P value	P value
Socioeconomic status of the household	Low vs High	0.88	0.59-1.30	0.518				1.74	1.10-2.74	0.016	1.61	0.99-2.63	0.054	0.026
	Medium vs High	0.90	0.60-1.37	0.628				0.96	0.70-1.32	0.796	0.88	0.62-1.23	0.451	0.819
Characteristics of the child's home	Transitional vs Basic urban	2.01	1.10-3.66	0.023	2.02	1.1-3.73	0.024	1.70	1.15-2.50	0.007	1.36	0.90-2.05	0.143	0.645
	Rudimentary vs Basic urban	1.89	1.04-3.44	0.037	1.88	1.02-3.47	0.043	1.97	0.95-4.08	0.067	2.38	1.12-5.05	0.024	0.932
Sedentarism of the child	High vs Low	1.63	1.24-2.12	<0.001	1.64	1.23-2.18	0.001	0.99	0.70-1.41	0.957				0.027
	Medium vs Low	1.16	0.89-1.50	0.269	1.22	0.93-1.60	0.154	0.91	0.66-1.25	0.568				0.253
Agricultural activities of the household	Farming vs. non-farming	0.93	0.76-1.15	0.513				1.45	1.02-2.06	0.036				0.032
Other asthma risk factors														
Sex	Male vs Female	0.93	0.76-1.14	0.480				0.92	0.70-1.20	0.526				0.926
Age (years)	<11 vs ≥11	1.72	1.40-2.12	<0.001	1.81	1.46-2.24	<0.001	1.27	0.94-1.72	0.115	1.39	1.0-1.92	0.051	0.108
Maternal sthma	Yes vs No	3.04	2.46-3.76	<0.001	3.03	2.44-3.75	<0.001	3.47	2.58-4.66	<0.001	3.48	2.58-4.71	<0.001	0.473
Matrenal smoking during pregnancy	Yes vs No	1.04	0.76-1.41	0.826				1.82	1.12-2.96	0.016	1.86	1.12-3.08	0.016	0.054
Environmental tobacco smoke[B]	Yes vs No	1.01	0.82-1.23	0.945				1.07	0.81-1.42	0.631				0.726
Household overcrowding[C]	>3 vs ≤ 3	1.19	0.98-1.46	0.086				0.99	0.75-1.32	0.959				0.297

Shown are univariate and multivariate analyses with estimated odds ratios (OR) and 95% confidence intervals (95% CI).
[A]Interaction p value between urban and rural in univariate analyses. [B]Exposure to a smoker living in the child's household. [C]Persons per sleeping room.

Table 4 Associations between recent wheeze and sedentarism domains stratified for each level of socioeconomic domains among rural schoolchildren

Socioeconomic status domains	Domains of Sedentarism	Rural population		
		OR	95% CI	p
Low SES	High vs Low	1.39	0.95-2.04	0.095
	Medium vs Low	1.48	1.07-2.04	0.017
Medium SES	High vs Low	1.80	1.15-2.81	0.011
	Medium vs Low	0.77	0.45-1.32	0.341
High SES	High vs Low	3.26	1.24-8.55	0.016
	Medium vs Low	1.27	0.47-3.43	0.634

Shown are multivariate analyses with estimated odds ratios (OR) and 95% confidence intervals (95% CI).
ORs also adjusted for age, characteristics of the child's home, and agricultural activities.

factors relating to lifestyle patterns may affect the risk of wheeze in a population undergoing the rapid transition from a traditional to a more modern and urban way of life.

Previous epidemiological studies have reported associations between prevalence of wheeze/asthma and lifestyle factors related to urbanization [5,10,12] although causal risk factors and mechanisms have not been clearly identified. These associations are often inconsistent between studies of urban and rural populations and between regions at different levels of development. For example, studies conducting in Zimbabwe and Ghana observed a higher prevalence of wheeze/asthma among urban children of higher SES [22,23], while studies of children in urban areas of Latin America have reported a higher prevalence among the poor [11,24]. In industrialized countries, associations between low SES and childhood asthma severity has been observed in different international studies [25-27]. Risk factors such as sedentary habits, an increase in the consumption of 'junk' food, and obesity have been identified as possible explanations for the increasing asthma prevalence in both developing and developed countries [28,29], nevertheless some studies found no association between overweight/obesity and asthma trends [30]. Traditional lifestyles, especially those related to farming, have been postulated to provide protection against allergic diseases and atopy [5], and explain, at least in part, the lower prevalence in rural areas [4]. However, not all farming environments protect against childhood wheeze/asthma [31,32]. It has been hypothesized that changes in housing construction creating a more artificial living environment might predispose to growth of indoor allergens affecting the occurrence of asthma in developed countries [33]. Housing with excessive moisture and dampness, inadequate or poorly maintained heating and ventilation systems, crowding, pest infestations, deteriorated carpeting, and structural defects may increase exposure to asthma triggers [34].

Our data showed that characteristics of the child's household were associated with the prevalence of wheeze in both urban and rural areas. Children living in rudimentary and transitional households had a higher risk. In urban areas, there were trends of increased prevalence of wheeze in poor households and those with agricultural exposures. Our findings are consistent with a previous study done in City of Esmeraldas 20 years ago, which showed an association between low socioeconomic status and asthma prevalence, especially for those socioeconomic factors relating to housing materials [35]. Other studies from urban centres in Latin America have also shown associations between wheeze/asthma and risk factors indicative of poverty, dirt and infections [11,24,32]. In rural areas of our study, wheeze was more prevalent in children with a high level of sedentarism. Similarly, data from elsewhere have shown that an association between diet (and 'junk' food consumption) and asthma prevalence [28,36].

In a previous ecologic study of the same rural communities analysed here, we showed that factors associated with greater socioeconomic level and changes towards a more urban lifestyle were associated with the community prevalence of childhood asthma [37]. The same ecologic analysis showed an association between community SES and wheeze prevalence. Our data here indicate that SES, in

Table 5 Population fractions (PAF%) of recent wheeze attributable to each lifestyle domain

Groups	Lifestyle Domain	Rural			Urban		
		Exposed cases	OR^B	PAF %	Exposed cases	OR^B	PAF %
Socioeconomic Status of the household	Low vs. High	0.64	0.88	<0	0.09	1.74	4%
	Medium vs. High	0.29	0.90	<0	0.64	0.96	<0
Characteristics of the child's home	Transitional vs. Basic urban	0.43	2.01	22%	0.10	1.70	4%
	Rudimentary vs. Basic urban	0.52	1.89	24%	0.03	1.97	2%
Sedentarism of the child	High vs. Low	0.14	1.63	5%	0.21	0.99	<0
	Medium vs. Low	0.19	1.16	3%	0.31	0.91	<0
Agricultural activities of the household	Farming vs Non-farming	0.62	0.93	<0	0.15	1.45	5%

PAF% - populationa attribuatble fraction estimated using the proportion of exposed cases for each lifestyle pattern // OR^B = univariate odds ratios.

fact, may be acting as a confounder at the individual level. In the rural area, SES was strongly associated with the level of consumption and acculturation: a household needs an electrical connection to watch TV and TV viewing influences the level of consumption, while traditional exercise patterns will be modified by living close to a school and not having to fetch water because of a household water connection. In fact, our data showed that children allocated to the high SES and high sedentarism domains in rural areas had 3.26 greater risk of wheeze compared to children of high SES and low sedentarism.

Treating lifestyle as a set of attributes or domains allows us to understand better how urbanization processes may affect the prevalence of wheeze in rural and urban areas of a developing country. In transitional societies, it is common to find rural lifestyles co-existing in urban areas, particularly at the poor peripheries of cities that lack many basic services and where rural migrants settle and gradually become acculturated through the gradual adoption of modern habits and behaviours although remaining socially marginalized. This phenomenon could be observed in our urban study population by the proportion of households that retained agricultural activities (14.7% were classified as having farming attributes) and that lived in transitional and rudimentary households (10.8% & 10.3%, respectively). In urban areas, maintaining a traditional way of life is commonly associated with poverty and an increased risk for a number of diseases [38]. In the present study, there was evidence in the urban area for an effect of poverty on wheeze prevalence for domains within 3 of the 4 variable groups (e.g. low socioeconomic level, transitional housing, and farming exposures). This evidence is supported by the fact that severe asthma, which is less susceptible to misclassification of asthma, is more common among the poor, a pattern observed in epidemiological studies done in different geographical regions [25,26]. On the other hand, urban influences in rural areas have led to the adoption of 'modern' behaviours, patterns of consumption, diet, and housing – seen in the rural area of the present study by the proportions of children with sedentary lifestyles (18.5% with medium and 13.6% with high sedentarism) and the proportion of children living in transitional homes (43.3%). Some components of the urbanization process have been clearly identified as detrimental for human health, especially asthma, such as consumption of junk food and the acquisition of sedentary habits [28,36]. Nevertheless, other components of the urbanization process have been related to a reduction in the burden of disease through benefits gained by the provision of basic services and improvements in housing [39]. Our data indicate that a child living in better quality housing (termed basic urban) had a lower risk of wheeze than those living in rudimentary and transitional households.

Different lifestyle attributes may have distinct effects on the risk of wheeze in urban and rural populations. The level of development of each attribute and the speed by which they change in an relentless (and unstoppable) process of increasing urbanization could explain at least partly the increasing prevalence of wheeze/asthma that has been documented in some developing countries [3], and the declining disparity in prevalence between urban and rural areas [6,7]. Furthermore, lifestyle factors probably operate at several levels on the risk of asthma such as individual, family, and community levels (i.e. contextual) – future studies addressing this question should collect data at all these levels and use a multilevel approach to understand better the differences in the observed prevalence of wheeze/asthma prevalence by geography and wealth [40].

The methodological limitations of our study include its cross-sectional design and the potential recall bias using questionnaire data. Furthermore, we restricted the study to mainly Afro-Ecuadorian children - until the findings are replicated in other populations, we cannot conclude their generalizability. Another limitation is that not all wheezing is asthma. Nonetheless, this definition, based on a symptom questionnaire, has been shown to distinguish between asthmatics and non asthmatics and has advantages in terms of cost, convenience, and the resulting optimization of sample sizes and response rates [17,18]. Also, this definition is more practical in rural areas where access to health care is limited and use of doctor diagnosis may be subject to significant bias because of differential access to health care between populations. There was time period of up to 5 years between the start of data collection in the rural study population and completion of data collection in the urban area. It is possible that lifestyle exposures might start to change within such period in the two populations but we do not believe that such changes would be marked over such a relatively short period and that significant changes occur over decades rather than years. Finally, lifestyle involves a range of influences determined by cultural, economic and environmental factors, among others, that are hard to define and measure; including all such factors in an analysis is difficult. We approached this problem by selecting urban and rural populations that share a common history, culture and geography, and choosing common indicators based on asthma literature, sociological approach [37] and information available in our databases. Despite the fact that we collected detailed information related with lifestyle, other data that are likely to be important (eg, environmental sampling for air pollution, seasonal or geographical data) were not available. Smoking was not considered as a modern lifestyle indicator because smoking of tobacco (using 'cachimbas') in the rural area is a widespread and traditional practice, in contrast to the urban area where modern cigarettes are smoked. We did not collect data to distinguish smoking of traditional versus modern cigarette. In fact, the rural population had a higher proportion of mothers who smoked during pregnancy and household exposure to environmental tobacco smoke compared with the urban population (Table 1).

In conclusion, we have used a novel analytic approach to explore the effects of lifestyle domains or patterns on the prevalence of recent wheeze in schoolchildren living in rural and urban areas of Ecuador. Our data provide evidence that lifestyle domains relating to poverty (e.g. low socioeconomic status and living in transitional housing) are associated with a greater risk of wheeze in urban areas, and domains related to greater sedentarism and living in substandard housing are associated with a greater prevalence of wheeze in rural areas. These data indicate that different components of lifestyle may have distinct effects on the risk of wheeze between (i.e. rural vs. urban) and within populations at different stages of urbanization.

Additional file

Additional file 1: Table S1. Discrimination measures (dimensions) and proportion of variance explained for each dimension by variable groups. **Table S2.** Socioeconomics status of the household: variables stratified by domains. **Table S3.** Characteristics of the child's home: variables stratified by domains. **Table S4.** Sedentary characteristics of the child: variables stratified by domains. **Table S5.** Agricultural activities of the household: variables stratified by domains. **Table S6.** Estimates odds ratios and 95% confidence intervals of recent wheeze for lifestyle domains controled by age group in urban and rural areas. **Table S7.** Estimates odds ratios and 95% confidence intervals of recent wheeze for lifestyle domains controled by sex in urban and rural areas.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
Study design: AR, PJC, MEC, LCR, MLB. Data collection; AR, MGV, Data analysis: AR. Draft manuscript: AR, PJC. Manuscript review: AR, PJC, LCR, MLB. All authors read and approved the final manuscript.

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Chapter 6. Conclusions

The study of the effects of urbanisation on health is complex, not only because of the multidimensional nature of this process but also because of the methodological and conceptual limitations inherent in epidemiological and urban studies. In the case of asthma research, the study of the relationships between urbanisation and asthma is no exception. While numerous of epidemiological studies have provided a large body of evidence for the relationship between urbanisation and asthma, these studies have differed in their conceptual approaches, methodologies, and definitions. Additionally, due to different historical, socio-economic and political backgrounds of societies, diverse levels of urbanisation are present in different countries and regions, making comparisons between studies difficult. Taking this into consideration, our research has taken a multidimensional approach to study the effects of urbanisation on asthma, conducting several analyses in different populations of Ecuador. Our studies provide novel evidence about how the process of urbanisation could be in part responsible for the temporal and geographical trends in asthma prevalence in LMICs, and identify specific urban characteristics associated with asthma prevalence. The five scientific papers presented in this research evaluated methodological issues related to asthma studies assessing urbanisation, identified urban living and urban conditions in transitional populations associated with asthma prevalence, measured the different levels of urbanisation and their relationship with asthma, identified migrant characteristics in urban and rural populations related to asthma prevalence, and identified lifestyle domains associated with the occurrence of asthma.

Asthma studies and urbanisation

Over the last decades, the number of asthma studies has increased dramatically, making the amount of information generated almost overwhelming due to the multifactorial nature of this condition. To help make sense of this information, more and more review articles that pool the results of multiple asthma studies are needed to simplify the information available. Among this information, studies assessing the association between urbanisation and asthma have become more frequent both in HICs and LMICs. However, although some articles have documented and discussed the possible effects of the urbanisation process on asthma,(1–3) the asthma-urbanisation association has not been evaluated using a systematic review or a meta-analysis. In the present thesis, we provide the first systematic review and meta-analysis assessing the relationship between asthma and urbanisation in LMICs. While difference approaches, definitions and methodologies were used by asthma studies to evaluate the possible effects of urbanisation, we have reduced these studies into three principal groups: a) studies comparing the prevalence of asthma between rural and urban areas; b) studies comparing the prevalence of asthma between cities, towns or communities within a country or across countries; and c) studies examining variations in the prevalence of asthma within cities. These three categories were based on epidemiological and urban research methodologies addressing the effects of urban areas on health.(4) Our analysis combined all this information through a systematic review and meta-analysis to generate a general overview of how epidemiological studies assess the relationship between urbanisation and asthma in LMICs.

The systematic review showed that studies comparing the prevalence of asthma between rural and urban areas is the predominant approach by asthma studies. Our analysis suggests that the lack of knowledge on the mechanism and specific urban factors associated with asthma occurrence could be related to limitations that characterize the urban-rural approach. The systematic review also described the methodological and conceptual issues that epidemiological studies face assessing the effects of urbanisation on asthma, especially those related to the lack of a clear definitions of urban areas and operational definitions of asthma. However, although meta-analysis is a powerful approach to summarizing and comparing results from empirical literature, several conditions needs to be fulfilled for such a meta-analysis to be sound.(5) These conditions are: 1) Well-defined objectives, including precise definitions of clinical variables and outcomes; 2) An appropriate and well-documented study identification and selection strategy; 3) Evaluation of bias in the identification and selection of studies; 4) Description and evaluation of heterogeneity; 5) Justification of data analytic techniques; 6) Use of sensitivity analysis. While our systematic review and meta-analysis considered all these conditions, the broad nature of our aims encompassed a substantial variety of settings, populations, methodologies and asthma definitions yielding a high level of statistical heterogeneity (*I^2 test<90)* in the meta-analysis. However, despite significant heterogeneity among studies included in the meta-analysis, we presented pooled ORs and forest plots by asthma definition and age because they provide useful general statistics (subgroup analysis), but we do emphasize that these results should be interpreted with caution.

A subgroup analysis comparing forest plots and meta-analyses based on different sets of studies (such as asthma definition, age, income per-capita and region) were conducted in our review. However, although these comparisons are an important method to identify possible causes of heterogeneity,(6) subgroup analysis present some limitations: 1) information about specific characteristics or variables that can be used for comparison may not be available in published studies; 2) Individual patient data, rather than published summary statistics, have greater power to carry out informative subgroup analyses; 3) frequently, the findings of these subgroup analyses fail to be confirmed by later research, and 4) the underlying risk of occurrence of asthma is related to the level of exposure, that is to say, populations living in a more urban environment are more likely to develop asthma, 5) confounding factors could exist between the variable used to compare groups and the outcome.(6) Attempting to utilise a meta-analysis to produce more than a simple overall effect estimate is tempting, but needs to be treated cautiously, for the reasons detailed above. Trying to explore possible reasons for heterogeneity is likely to lead to over-interpretation of the results. Moreover apparent, even statistically significant, heterogeneity may always be due to chance and searching for its causes would then be misleading.

Alternative approaches to comparing the strength of associations of urbanisation with different measures of asthma are narrative analysis (as done in Chapter 5) and meta-regression analyses.(7) The advantages of the latter are that continuous (and categorical) variables can be simultaneously modelled but this approach is not considered adequate where numbers of studies with information on the relevant variables are small. In the case of urbanisation, meta-regression could be used to model effects of urbanisation on asthma prevalence, taking into account the effects of variables such as age, per capita income, human development index, infant mortality rate, etc. However, such data were not available for most of the studies included in the meta-analysis, making meta-regression inappropriate in this case.(8)

Urban dimensions and urban indicators in asthma studies

The starting point of this research project was the observation that many rural communities in the north of Ecuador presented a number of urban characteristics which varied substantially between communities. Aware of the possible relationship between the urbanisation process and the increase in asthma prevalence, we have done previously an ecological analysis of the associations between the level of urbanisation - measured through infrastructure, socioeconomic and lifestyle indexes - and the prevalence of asthma in 59 rural communities of the north of Ecuador.(9) The study showed that the prevalence of asthma increases with increasing levels of urbanisation, and factors associated with greater socioeconomic level and changes towards a more urban lifestyle were particularly relevant.(9) However, although this study was potentially useful in identifying urban features that could be related to the increase of asthma prevalence in transitional communities, the ecological approach presented limited usefulness in determining how these urban characteristics may be associated with individual asthma risk.(4) Other important limitations of the previous ecologic approach were the risk of an ecological fallacy and the lack of data on relevant urban indicators. To overcome these limitations, we set out with a broader approach which included a set of studies addressing the urbanisation-asthma relationship through different perspectives (ecological, contextual and multilevel analysis) and using different urban indicators at individual, household, community and census ward level. The papers presented in chapter three and five are part of this broader approach.

Traditionally, asthma research has focused on individual level risk factors to explain the differences in asthma prevalence between urban and rural population, giving less attention to contextual or ecological factors. This trend is evident in our systematic review where only 11% of studies assessing the relationship between urbanisation and asthma used information at the ecological or contextual level. A reason for the limited use of ecological-level data in asthma research could be the fear of falling prey to the ecological fallacy. The ecological fallacy is the incorrect inference that a relationship observed at an aggregate level will be observed at the same direction and magnitude at the individual level.(10) However, it is important to distinguish between the improper use of aggregate data as a proxy for individual data (the ecological fallacy) and the analysis of the effects of urban indicators (an ecological perspective).(11) This distinction is highlighted by the fact that urbanisation is mainly represented by ecological variables, due to the ecological nature of this process. The process of urbanisation affects different levels of human activity such that urban indicators can be operationalised at different levels at individual and household levels.

In epidemiological research, the effects of urbanisation on health have been commonly analysed at the contextual and the individual level. The contextual level refers to the effects of urbanisation measured through ecological variables or group variables, often based on census data (contextual effects). These variables commonly represent geographical locations (neighbourhoods, census wards, cities) or specific feature of an urban area or an urban population (e.g. presence of urban services, percentage of population engaged in agricultural activities, population density, etc). At the individual level, epidemiological research uses personal characteristics related to the urbanisation process that can be measured at the individual level such as access to urban services, quality of housing, lifestyle, level of sedentarism, etc. However, It is important to understand that contextual variables sometimes have an analogue at the individual-level (e.g., mean neighbourhood income and individual-level income), both variables could be measuring different urban constructs.(11) For example, the construct of community smoking prevalence is distinct from individual consumption of tobacco, and both are important to asthma. The presence of an electricity grid, measured as physical infrastructure, is distinct from the access to electricity service by the population. The main idea behind this reasoning is that contextual variables may provide information that is not captured by its individual-level analogue. Moreover, urban constructs defined at a contextual level may be important in explaining variability at an individual level, and constructs defined at an individual level may be important in explaining variability at a contextual level.(11)

We conducted a review of various definitions of urban, urbanisation and urbanicity, derived from different disciplines to identify the most relevant dimensions and indicators of the urbanisation process in transitional populations.(12–15) This review helped to establish demographic, social, economic, geographical and spatial dimensions of urbanisation. Once the dimensions were established, we identified a set of variables to represent each dimension. For example, in the demographic dimension were considered indicators such as population concentration, population density, population growth and dynamics. The spatial dimension, for example, was represented by urban environment – concentration and expansion, land use/land cover, housing type materials and construction etc. However, although a number of indicators were identified for each dimension in the literature review, the operationalization issues with urban variables and the lack of existing information in the national and regional census reduced significantly the number of indicators in the selection phase.

In our research, we started conducting an ***ecological analysis*** where both independent and dependent variables were measured at group level. A set of indicators based on infrastructure, socioeconomic and lifestyle characteristics of the communities were associated with the community asthma prevalence using correlations and linear regressions. This ecological analysis helped us to generate hypotheses about specific characteristics and dimensions of the urbanisation process that might affect the occurrence of asthma. Next, we conducted a ***contextual analysis*** where independent variables where measured at census ward level but the dependent variable was measured at individual level (Chapter three). Eighteen indicators representing demographic, socioeconomic, built environment-infrastructure and geographical dimensions of the process of urbanisation were associated with individual occurrence of asthma using random effects logistic regression models. Contextual analyses attributed to each individual, a variable that represents whether or not one lives in a census ward with a specific urban characteristic. A ***second contextual analysis*** (Chapter five) was conducted to understand how lifestyles domains affect the prevalence of asthma. Seventeen indicators related to the process of urbanisation were included in this analysis. Four group-level variables were identified using multiple correspondence analysis and cluster analysis: socioeconomic status of the household, characteristics of the children home, sedentarism of the child, agricultural activities of the household. Characteristics of these four group variables were ascribed to the individual and using logistic regression were associated with individual risk of asthma. In the future, a ***multilevel analysis*** using data from the chapter 3 is planned. This study will integrate contextual and individual variables in the same analysis to understand how urban indictors measured at different levels interplay among them and affect the risk of asthma. A table summarizing dimensions, indicators and study analyses is provided above.

Our studies identified a number of urban indicators associated with asthma, especially those related to changes in lifestyle. Greater access to computer or satellite TV, in the case of children living in Quinindé district (Chapter three), or high levels of sedentarism, in the case of children living in the rural communities of Eloy Alfaro (Chapter five), were particularly important. These factors represent the acquisition of a modern lifestyle in transitional populations and suggest the possible mechanisms associated to the increase of asthma prevalence in LMICs. The urban way of life is easily adopted in transitional or rural populations through the presence of new technology, especially communications technologies. These technologies introduce and shape new lifestyles in these populations. For example, in the case of a family, the access of a satellite TV could modify the dietary patterns through the consumptions of new foods advertised or seen on TV or simply reduce the physical activity because of the time watching TV or working at home on a computer. There is evidence for an association between the consumption of fast food or lack of physical activity with asthma prevalence.(16–20) Another important factor associated with asthma prevalence in our research was the quality of the house where the children lived.

		Study approach and unit of analysis			
Dimensions	**Indicators**	**Ecological Analysis[1]**	**Contextual Analysis[2] (Chapter 3)**	**Contextual Analysis[3] (Chapter 5)**	**Multilevel analysis[4] (future)**
Demographic	Population Size		*		*
	Population density		*		*
Infra-Structure Or Built environment	Pavement street		*		*
	Sewage system		**		*
	Piped water system	*	*		*
	Electricity	**	*		*
	Educational Institutions	*	*		*
	Health Institutions	**	*		*
	Transport access	**			*
	Telephone system	*			*
	Presence of Pharmacies	**			*
	Number of Shops	**			*
Socio-economic	**Non-agriculture activities**	**	**	*	*
	Type of Employment			*	*
	Secondary Education	*	**	*	*
	Commercial activities	*	**		*
	Concrete housing/ housing materials	**	**	**	*
	Mobile phone access		*		*
	Internet access		*		*
	Computer access		**		*
	Satellite TV access		**		*
	Gas for cooking	**		**	*
	Household income	**		*	*
	Access to electricity	**		**	*
	Access to basic services			**	*
	Motor vehicles	**			*
	Material goods	**		*	*
	Uncrowded household	*		*	*
Lifestyle	**Consumption of hamburgers**	**		**	*
	Consumption of fizzy drinks	**		**	*
	No physical exercise	**		**	*
	Nutritional status			**	*
	Television in house	*	*		*
	TV viewing	**		**	*
	Cat in house	**			*
	Dog in house	*			*
	Migration	*			*
	Parasite infection rate	*			*
	Contact with animals in farms			*	*
Geographic	Geo-political division	*	*		*
	Proximity to urban centres		**		*
	Close access to a highway		**		*
	Spatial organisation (community)	*			*

*Indicators included in the analysis. ** Significant associations between urban indicators and asthma (current wheezing).
[1] Ecological study: Urbanisation is associated with prevalence of childhood asthma in diverse, small rural communities in Ecuador.
[2]Contextual study (Chapter 3): Measuring Urbanicity as a Risk Factor for Childhood Asthma in Transitional Areas of Ecuador: A Cross-sectional Analyses. [3]Contextual study (Chapter 5): Lifestyle domains as determinants of wheeze prevalence in urban and rural schoolchildren in Ecuador: cross sectional analysis. [4] Future analysis: Multilevel analysis using data from the chapter 3 integrating contextual and individual variables in the same analysis.

In the study evaluating lifestyle domains, asthma risk was related to poor housing, both in urban and rural areas. In the study measuring the level of urbanicity, this association was related to the proportion of houses constructed with cement. In both cases, the use of new housing materials and housing design may be important determinants of asthma prevalence in transitional populations.(21,22) As we discussed in previous chapters, these houses are frequently made with bricks and cement without plastering and most lack windows in bedrooms or adequate ventilation. This type of house is characterized by high levels of humidity and mold in the presence of overcrowding, providing environments with a likely increase risk of respiratory infections and exposures to respiratory irritants, both of which are important risk factors for asthma, especially for non-atopic asthma(23,24) which is the most common phenotype in LA.

Based on the above table, several considerations need to be taken into account. First, some urban dimensions cannot be evaluated at the individual level. In our model, the demographic dimension is represented by population size and density. These two variables do not have an analogue indicator at individual level. Second, some urban variables can represent different constructs and dimensions. For example, the variable "housing materials" could characterize the infrastructure dimension (measured as a built environment indicator - the proportion of houses built with concrete) or the socioeconomic dimension (measured as a socioeconomic characteristic of the household). Third, contextual variables of urbanisation mostly depend on availability of census data. Four, urban indicators related to housing materials seems to be an important determinant of asthma, regardless of level of analysis or approach. The three studies presented associations between asthma prevalence and housing materials indicators. Although important issues were identified in these analyses, we must be aware that the study samples and locations of the studies in the chapter 3 and 5 belong to different populations in different regions within the same Province in Ecuador, so the level of exposure to urban environment or urban characteristics could vary considerably among individuals in the two studies.

Finally, since the acquisition of a modern lifestyle has been the most common explanation for the increase in asthma and differences in asthma prevalence between LMICs, efforts to define and measure a "modern lifestyle" need to be done through epidemiological studies. Our study in chapter five, offers a novel approach to identify and measure 'modern lifestyle' based on a group of variables that represent socioeconomic factors, sedentarism, agricultural activities and household characteristics of the study population. We believe that this approach is superior to analysis of individual risk factors, particularly because individual lifestyle indicators have failed to account fully for the rise in and regional differences in asthma prevalence. However, we are aware of the limitations of this approach, especially those related with the uncertainty of exposure allocation for individual subjects. It is clear that, different methods of clustering usually give very different results. This occurs because of the different criterion for merging clusters (including cases). Additionally, when an observation is allocated to a certain group the characteristic of the group is ascribed to the individual with a fixed effect so all the individuals in the group will have the same effect. Alternative approaches can be used to classify and analyse these types of data such as discriminant analysis or principal components analysis.

Internal Migration and asthma

The study of the effects of internal migration on asthma is poorly understood. The different types of migrations and the psychological and socioeconomic conditions of migrants before and after moving,(25) make difficult the evaluation the specific effects of the migration process on asthma.

In epidemiological literature, studies evaluating the effects of the internal migration on asthma are rare. Most that we know about the relationship between migration and asthma comes from studies evaluating international immigrants, especially those who move from LMICs to HICs. This absence of studies assessing internal migration seem surprising especially because internal migration is considered as one of the most important components of the urbanisation process which has been frequently associated with the increase and differences in asthma prevalence. The major reason for the small number of studies evaluating the association between internal migration and asthma is attributed to the absence of individual formal records for this type of migration, contrary to the international migration where each individual is registered in the country of origin as well as in the country of destination. However, although other sources of information and methodologies are available to collect this information, asthma studies seem to shy away from this type of analysis. The current thesis presents first study evaluating the effects of internal migration on asthma in a LMICs, both in rural and urban areas. Our results showed that, in urban areas, rural-to-urban migrants had 2-fold greater risk of asthma compared with the host population (non-migrants), and this association increase depending of the age of the children and time since migration. However, urban-to-urban migration was not associated with differences in asthma prevalence. In the rural area, although an association was observed between age of migration and wheeze, the most relevant finding was the relationship between the history of migration of the mothers and asthma prevalence in their children. Children with mothers with history of migration had 2-fold greater risk of asthma. Urban-to-rural migration was not associated with asthma prevalence in rural areas.

Numerous epidemiological studies have shown evidence that migration from countries of low asthma prevalence(LMICs) to those of high asthma prevalence (HICs) provide certain protection to immigrants to develop asthma compared to the host population.(26–28) A systematic review assessing the association between international migration and asthma showed that, the prevalence of asthma and allergic diseases in immigrants is generally lower than the host population and this immigrant health advantage diminishes over time substantially.(29) Also, second generation of immigrants presents a higher asthma prevalence than the first generation, and with length of residence in the host country the prevalence of asthma and allergies increases steadily.(29) Contrary to the idea of international migration studies, that immigrants from a low asthma prevalence area moving to a high asthma prevalence area had a less risk of present asthma, our result showed that rural to urban migration is associated with a higher risk of asthma. This apparently paradox could have several explanations: 1) The low prevalence of asthma in immigrants living in HICs could be caused by the "healthy migration effect": healthy people are more likely to migrate and the host states may prefer healthier people. Additionally, the sick or unhealthy immigrants could return to the home state (especially adult population), which may possibly cause the statistical bias of immigrants' health status.(30) 2) Differences in socioeconomic characteristics between international and internal migrants could be producing differences in asthma prevalence. Rural urban migration is still triggering by economic factors. Access to social services and labour opportunities in the rural areas are still considerable worse than in urban areas. Disadvantaged economic and social conditions of rural areas have been responsible for the rapid process of urbanisation in Latin America. Additionally, civil violence has also promoted this type of flow, as we saw in the rural migration study where a number of immigrants from Colombia were found. In the other hand, international migrants are not a random sample of their country of origin, they are a highly selected group who are able or motivated to deal with the stress, cost and organisation that such a process entails. Individuals or families who migrate are in a relatively advantageous position, whether financial or social, compared with the native population.(31) 3) The high prevalence of asthma in rural urban migrants could be specifically caused by factors related to the settlement and adaptation stages. Factors as psychosocial stressors or family dissolutions consequent to migration could contribute to an increase in wheeze/asthma in migrant populations.(32) 4) Additionally, we believe that a considerable number of children migrating from rural to urban areas are children that did not live with their parents, so the cause of their migration is family reunification. Considered this, the prevalence of asthma in migrant population could be high due to the over represented cases related to absence of parents at home and, as we saw in the rural study, children with mothers with history of migration presented a higher risk of asthma.

The effects of internal migration on asthma are complex and difficult to disentangle. Urban migrants form a hugely diverse group that comprises internal migrants from rural and urban areas, cross border migrants, internally displaced persons and urban refugees. Because of this, internal migration has become more complex to quantify. It now involves a multiplicity of places of origin and destination and also a change of sociodemographic characteristics of migrants. Additionally, migration impacts not only the individual but also family and community. At the level of the family, migration has effects on roles, support structures, and responsibilities of family members resulting in changes in social and psychological factors that might be related to the occurrence of allergic diseases. At the level of the community, migrants and other mobile population reflect the health characteristics of their place and environment of origin and carry several of these characteristics with them when they move affecting the burden of diseases in the place of origin and destination. A better understanding of the social and environmental effects of internal migration on asthma is necessary - further analyses in different populations living in rural and urban areas of LMICs that are subject to migration are required in which detailed information is collected at individual, household and community levels, and by place of origin and place of destination.

Relevance of the Study

This study is of general interest to public health researchers, epidemiologists and social scientists. For public health, our data offer specific urban factors associated with the increase of asthma prevalence that could be modified through public health programmes such as improved housing and education for healthy lifestyles. Our findings are likely to be of general relevance to epidemiologists interested in the potential role of urbanisation and migration process in explaining asthma prevalence in LMICs. This study also offers new approaches to assess the effects of urbanisation on asthma, thus increasing the methodological armamentarium in epidemiological studies of asthma. For social scientist, this study shows the relevance of social processes on population health and the importance of integrating new concepts and methodologies widely used in other scientific fields. In conclusion, this information is relevant to identify urban factors associated with an increased risk of asthma in transitional populations, to improve the understanding of the causes of the increase in asthma prevalence in LMICs and to identify potentially modifiable factors to inform the design of prevention programmes to reduce the risk of asthma in urban and rural populations of LA.

Future Studies

Although the current research thesis has produced five studies in order to respond the scientific questions posed at the beginning of the project, the information obtained by these studies has generated several new questions that needs to be addressed with new studies. Additionally, several methodological and practical limitations presented in our studies need to be corrected with new methodologies, designs and statistical analysis. Among the most important limitations we can cite: 1) Most of my analysis relied on cross-sectional design even when the original design of one of them was a cohort. This is a crucial point because cross-sectional analysis are limited in their ability to identify causal effects of urbanisation and cannot deal appropriately with problems of migration and stability of the population. 2) In the national census database, information related to lifestyle factors at the census ward level is scarce. 3) The unit of analysis was defined using a geographical criteria (census ward), this could cause misclassification in some urban indicators, unless the boundaries of census ward coincide exactly with this boundaries dimensions. 4) My studies are focused in transitional communities and small cities. It is quite possible that a new theoretical frame and a new set of variables will be necessary to evaluate the effect of urbanisation on asthma in middle size and big cities. 5) Conceptual issues with the definition of urban area and urbanisation. 6) Cities are characterized by multiple factors that in many ways make each city unique. The complexity of cities and of city living may mean that urban characteristics that are important in one city may not be important in others, limiting the generalizations that can be drawn about how urban living influences asthma.

Considering all these points, my futures studies will be focus in three main groups:

a) Asthma studies using new analysis and study designs. Two studies are already underway. The first one is a multilevel analysis to evaluate independent effects of the associations between asthma prevalence and urbanisation using variables at census ward, household and individual levels. Data of the ECUVIDA study is being used for this analysis. Due to the number of urban indicators in the three levels is considerable, urbanicity indices will be built by census ward level (as in chapter three) to reduce the number of variables to be included in the multilevel models. The second study will evaluate the history of migration on the occurrence of childhood asthma. This study will use retrospective data of the children participating in ECUAVIDA cohort based on the annual periodic visit conducted over 8 years. This study will evaluate how migration and mobility within a city are associated with asthma prevalence. The study not only will include information on the history of migration of the children but also housing and individual health information.

b) Studies evaluating the intra-urban variability in asthma prevalence (neighbourhood studies). At the moment, only one study has been considered for this group. As we saw in the chapter two, studies evaluating the intra-urban variability in asthma prevalence are conducted exclusively in cities, using as a unit of analysis the neighbourhoods or urban census wards. Due to the availability of geographical, socioeconomic and health information, I will conduct this kind of study in the city of Quininde, Ecuador. Data will be obtained from the National Institute of statistic and census and ECUAVIDA cohort. This study will help to understand how urban issues in cities such as violence, urban poverty, gentrification, socio-spatial segregation, urban slums, and other factors could be associated with the higher prevalence of asthma in urban settings. We hope that future studies of this kind can be carried out in bigger cities of Ecuador as Quito or Guayaquil.

c) **Theoretical studies.** New concepts and theories are needed to understand the relationship between urban living, urban conditions and asthma. Definitions as urban areas, urbanisation, urbanicity and lifestyle must be more precise. Particularly, I am interested to define the concept of modern lifestyle in asthma research, so I will elaborate a theoretical paper related with this topic.

Final Conclusions

The aetiology of asthma is increasingly recognized as a complex interplay of factors operating at several levels, including the individual, the family and the community. As a part of this growing complexity, the process of urbanisation seems to play an important role at each level. Additionally, internal migration may be a key determinant in the increase in asthma prevalence in LMICs, not only because most of population growth in urban areas is attributable to population movements, but also because migration, as a social process, involves a variety of environmental, social, economic changes that are associated with asthma. However, it has been difficult to disentangle the effects of the urbanisation and internal migration on asthma prevalence, especially identifying the mechanisms and specific factors associated with asthma. The present research used different methodologies to identify several issues in asthma studies but also identified urban living and urban conditions associated with the prevalence of asthma in transitional populations, which are summarized in the next points:

- Most that we know about the relationship between urbanisation on asthma come from studies comparing urban and rural populations, an approach which does not allow us to identify specific urban characteristics associated with asthma.
- In LMICs, urban residence is a potential risk factor for asthma.
- In urban areas of LMICs, urban indicators related to poverty and social deprivation were associated with asthma prevalence.
- In a transitional area, even small-scale increases in urbanicity levels were associated with a higher prevalence of asthma.
- In a transitional area, contextual indicators of urbanisation related to changes in lifestyle and socioeconomic factors were associated with the prevalence of asthma.
- The process of internal migration was associated with the prevalence of asthma in urban and rural populations.
- In an urban area, rural to urban migration was associated with a higher prevalence of asthma, and this association varied according the age and time since migration.
- In a rural area, a higher prevalence of asthma in children was associated with the history of migration of the mother.

- Lifestyle domains related to poor housing conditions were associated with asthma in urban and rural areas.
- Lifestyle domains related with sedentarism was associated with asthma prevalence in a rural area.

All these findings provide evidence that urbanisation and internal migration are important determinants of asthma prevalence in LMICs. Additionally, the use of a multidimensional approach to study urbanisation allow us to identify specific urban living and urban conditions associated with asthma occurrence and help us to understand better the possible mechanisms to explain the temporal and geographical trends in asthma prevalence. Finally, the historical view of asthma being a disease of HICs no longer holds - most people affected by this condition are now in LMICs - and asthma prevalence is estimated to be increasing fastest in LMICs. It is possible that this increase may be associated with the urbanisation and migration processes occurring within these regions.

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Appendices

Appendix I. SCAALA Study Protocol

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Study protocol

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Risk factors for asthma and allergy associated with urban migration: background and methodology of a cross-sectional study in Afro-Ecuadorian school children in Northeastern Ecuador (Esmeraldas-SCAALA Study)

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Abstract

Background: Asthma and allergic diseases are becoming increasingly frequent in children in urban centres of Latin America although the prevalence of allergic disease is still low in rural areas. Understanding better why the prevalence of asthma is greater in urban migrant populations and the role of risk factors such as life style and environmental exposures, may be key to understand what is behind this trend.

Methods/design: The Esmeraldas-SCAALA (Social Changes, Asthma and Allergy in Latin America) study consists of cross-sectional and nested case-control studies of school children in rural and urban areas of Esmeraldas Province in Ecuador. The cross-sectional study will investigate risk factors for atopy and allergic disease in rural and migrant urban Afro-Ecuadorian school children and the nested case-control study will examine environmental, biologic and social risk factors for asthma among asthma cases and non-asthmatic controls from the cross-sectional study. Data will be collected through standardised questionnaires, skin prick testing to relevant aeroallergen extracts, stool examinations for parasites, blood sampling (for measurement of IgE, interleukins and other immunological parameters), anthropometric measurements for assessment of nutritional status, exercise testing for assessment of exercise-induced bronchospasm and dust sampling for measurement of household endotoxin and allergen levels.

Discussion: The information will be used to identify the factors associated with an increased risk of asthma and allergies in migrant and urbanizing populations, to improve the understanding of the causes of the increase in asthma prevalence and to identify potentially modifiable factors to inform the design of prevention programmes to reduce the risk of allergy in urban populations in Latin America.

Background

Large increases in the prevalence of asthma and allergic diseases have been reported in industrialized countries during the last twenty to thirty years [1,2], although there is evidence that the observed increase in asthma has reached a plateau in some industrialized countries [3,4]. There is strong evidence for differences in the prevalence of allergic diseases between urban and rural areas in Europe and in non-industrialized countries, with higher prevalences of allergic diseases reported in urban areas [5-7].

Allergic diseases are caused by a complex interaction between host genetics and environmental exposures. Temporal trends in allergy prevalence in developed countries [4], and the differences in allergy risk between urban and rural populations of the same ethnicity in developing countries [8], indicate that changes in environmental exposure rather than genetic factors are the most likely explanation for these epidemiological observations.

Rural residence is consistently identified as the strongest protective factor against asthma in epidemiological studies [7,9] and is likely to be associated with environmental exposures such as life style, diet, and hygiene [3,4,10]. Numerous epidemiological studies have provided evidence that migration from countries of low asthma prevalence (non-industrialized countries) to those of high asthma prevalence (industrialized countries) is associated with an increased risk of allergic disease [11-14], but the environmental factors that determine this have not been identified. There are few published studies of allergic diseases among migrant populations within non-industrialized countries [8] that migrate from low-risk rural areas to high-risk urban areas.

Changes in exposures to many different environmental and life style factors are likely to occur in populations that migrate from rural to urban areas, and studies of migrants to urban areas and comparisons of environmental exposures with the original rural and with more established urban populations should allow the effects of environmental exposures in early life (e.g. causing immune programming) on allergy risk to be distinguished from the effects of current exposures. Clarification of the dynamics behind the causation of allergy and asthma in urban migrant populations will further improve our understanding of what is behind the increase of frequency of these diseases with urbanization and westernization. The identification of modifiable risk factors could lead to new public health initiatives to reduce the burden of allergic disease among urbanizing populations.

Latin American countries are undergoing a rapid process of population change that includes urbanization, migration, economic development and adoption of a "modern" lifestyle. Among the burgeoning urban populations of Ecuador and other Latin American countries, asthma and allergic diseases are perceived to be an increasingly important public health problem of children [15], although there is limited data quantifying the magnitude of the problem and the associated risk factors. The International Study of Asthma and Allergies in Childhood (ISAAC) compared the prevalence of allergic disease in school-age children using a standardized questionnaire and found that urban centres in Latin America have among the highest rates of allergic symptoms worldwide [16]. School children living in rural areas of Ecuador appear to have very low risks of allergic disease [17]. Current trends of continuing rural-urban migration, coupled with the large expected increases in the urban population of Ecuador over the next 10 years, make it likely that allergic diseases, including asthma, will become a significant public health problem in the future.

The study described here is part of the Programme "Social Change, Asthma and Allergy in Latin America" (SCAALA), a research programme being conducted in Ecuador and Brazil (in the North Eastern city of Salvador), funded by The Wellcome Trust as part of the programme of Major Awards to Centres of Excellence in Latin America. The SCAALA collaboration aims to clarify the social and biological mechanisms that mediate the effect of population and lifestyle changes on the frequency of atopic diseases. This paper deals with the methodological aspects of the study being conducted in Ecuador.

The study in Ecuador aims to study changes in the prevalence and risk factors for asthma and allergy in populations that migrate from rural to urban areas and examine how such changes may relate to changes in the risk of atopic disease. The study will measure the frequency of symptoms of asthma, allergic rhinitis and atopic dermatitis in school children in urban and rural areas, and will collect detailed information on life style factors and environmental exposures that may affect the frequency of atopic diseases in the urban and rural environments. The study will investigate also changes in key immunologic factors (i.e. cytokines) and environmental exposures (i.e. exposure to aeroallergens in the household) that may be associated with altered asthma risk in the rural and urban study groups. On the other hand, the SCAALA study in Brazil aims to investigate the associations between the prevalence and incidence of allergic diseases, environmental exposures, particularly hygiene-related exposures, and immunological parameters in a cohort of children in the city of Salvador in the State of Bahia, and has been described elsewhere [18].

Methods/design

Ecuador is among the poorest of South American countries, with an estimated per capita GDP of US$4,300 in 2005 and industrialization that is far less advanced compared to richer countries in the region, such as Chile (GDP per capita, US$11,300 and Argentina (GDP per capita, US$13,100) [19]. In Ecuador, the process of industrial development accelerated from the 1970s as a consequence of revenues available from oil exploitation in the Eastern forest region. Industrial development has been associated with a high rate of population growth in the cities, estimated to be 2.5% annually in 2000 [20]. There have been large increases in the urban proportion of Ecuador's population that rose from 42% to 65% during the period 1975 to 2000 and is projected to reach 76% by 2015 [20]. The new urban centres are home to 50% of the country's poor [20].

Study site

The study is based in Ecuador's northern coastal province, Esmeraldas Province. The Province covers an area of 15,237 km^2 and has a population of approximately 429,000. The main economic activities are oil industry, tourism, timber extraction, and African palm oil. The principal city in the Province is Esmeraldas, with a population of 250,566 that is home to approximately 80% of the Ecuador's Afro-Ecuadorian population [21]. Esmeraldas Province is one of the poorest regions of Ecuador, with a per capita income that is less than half the national average. An estimated 70% of the economically active population in the city of Esmeraldas is unemployed or underemployed, and 60% have no access to basic services such as electricity, drinking water and sanitation [21]. Over the past 30 years there have been large migrations of Afro-Ecuadorians from rural areas of the Province to the city of Esmeraldas, largely as a consequence of displacement by African palm oil plantations and by migrants from other Provinces. The comparison of environmental exposures between Afro-Ecuadorians that continue to live in a rural environment with those (of the same genetic 'stock') that have migrated from the same rural environment to live in an urban environment provides an important opportunity to investigate how changes in environmental and life style factors that follow urban migration may contribute to changes in allergy risk.

Study population

The study will be conducted among school children attending rural schools in Afro-Ecuadorian communities in the District of Eloy Alfaro, in a tropical coastal area of Esmeraldas Province, and in marginal Afro-Ecuadorian 'barrios' in an urban centre, where migrants from the rural area (called norteños) congregate.

Study design

The study forms two parts: (i) a cross-sectional study of environmental factors associated with atopy and allergic symptoms in school-age children in the rural District of Eloy Alfaro in Esmeraldas Province and in 'barrios' of the city of Esmeraldas where rural migrants congregate; and (ii) a nested case-control study of environmental risk factors for asthma using asthma cases identified from the cross-sectional study in urban and rural school children and a random sample of non-asthmatic controls.

1. Cross-sectional study

A total of 4,000 school children, aged 7 to 15 years, living in the District of Eloy Alfaro and 2,500 children of the same age range, living in the city of Esmeraldas, will be assessed to estimate the frequency of atopy and allergic diseases, including asthma, rhinitis and eczema, and identify and compare risk factors associated with these outcomes in urban and rural study populations. School children in urban areas will be defined by place of birth, period of residence in urban area and at what age, and whether their parents are migrants (i.e. born in a rural area) or not. The cross-sectional study will examine the relationship between atopy and allergic symptoms in urban and rural school children and the environmental factors that modify this relationship.

2. Nested case-control study

Asthma cases (200 cases in each of the urban and rural areas) and non-asthmatic controls (800 controls in each of the rural and urban areas), respectively, will be identified from the results of an allergy symptom questionnaire performed in the cross-sectional study by the questions – "Have you had wheezing or whistling in the chest in the last 12 months? " and "Have you ever had wheezing or whistling in the chest at any time in the past?" Non-asthmatic controls will be randomly selected from all children in each of the urban and rural areas that respond negatively to the second question. Nested case-control studies in the urban and rural areas will identify risk factors associated with symptoms of recent wheeze using both quantitative and qualitative epidemiological methods, investigations of immunological function and measurements of allergens and endotoxin in the environment.

Sample size and study power

Data are available for the prevalence of recent wheeze in urban and rural study areas, from pilot surveys of 245 and 536 school children, respectively. These surveys estimate the prevalence of recent wheeze to be greater in the urban area (urban 13.4% versus rural 7.3%). The prevalence of atopy was similar in urban and rural school children (urban 11.3% vs. rural 13.5%). A sample of 4,000 children in the rural area and 2,500 children in the urban area will provide approximately 820 cases of atopy for the cross-sectional study, and 292 and 335 cases of asthma for the case-control study in the rural and urban areas, respectively. Two hundred asthma cases from each area will be recruited into case-control studies (allowing a drop-out rate of ~ 30%). The study will have a case-control ratio of 1:4 (400 cases vs. 1,600 controls). With a study power of 80% and $P < 0.05$, the nested case-control studies combined (i.e. urban and rural) will be able to detect significant effects on asthma of common exposures (prevalences of 40–60% – e.g. geohelminth infections and household pets) with OR<0.7 and rare exposures (10%, e.g. family history of allergic disease) with OR<0.5. Likewise, the individual case-control studies (i.e. urban vs. rural) with the same power and level of significance will be able to detect an effect of OR<0.6 for common exposures (40–60% prevalence), and OR<0.4 for rare exposures (10%).

Main exposures and definitions

Asthma, rhinitis and eczema will be defined according to the core allergy symptom questions of the International Study of Allergy and Asthma in Childhood (ISAAC) [22]. Atopy will be defined as a positive skin prick test to any of the panel of aeroallergens tested. Allergy will be defined by the presence of a history of appropriate symptoms of a clinical allergic condition (asthma, rhinitis or eczema) in the presence of atopy [23]. Information on important risk factors and environmental exposures that affect the risk of atopy and asthma will be collected as listed in Table 1.

Data collection and instruments

1. Questionnaires

A. *Cross-sectional study*: The questionnaire has been modified from the ISAAC Phase II study questionnaire [24], and has been translated into Spanish and extensively field tested [17,25]). The questionnaire includes the core allergy symptoms questions of ISAAC and will collect information on maternal and paternal educational level and occupation; house construction; family possession of material goods; family income; cooking materials; access to water, electricity, and sanitation; household crowding; exposures to pets and animals; exposures to farming, including consumption of unpasteurized milk; dietary questionnaire; physical activity and time spent watching television; number of siblings and birth order; breast feeding; day care attendance; family history of allergic disease; exposure to smoking; detailed history of residence in urban and rural areas of father and mother; and detailed history of residence in urban and rural areas of child. The questionnaire will be administered to the parent or guardian of each child by trained field workers in the presence of the child.

B. *Case-control study*: A questionnaire for a more detailed history of asthma, rhinitis and eczema symptoms, based on the ISAAC phase II supplementary questionnaires [24], will be used. The questionnaire is designed to distinguish between asthma and other common respiratory disorders. The questionnaire includes also questions of the management of asthma [24] and of risk factors for asthma. The case-control study will include qualitative epidemiological techniques to assess knowledge, attitudes, and practice about asthma (KAP questionnaire). KAP questionnaires will be administered to the parents of asthma cases and non-asthmatic controls and also to health care personnel in health centres in urban and rural areas. Deep interviews with key informants in urban barrios and rural communities, focus groups and other qualitative epidemiological techniques will be used to evaluate relevant lifestyle factors, and how different patterns between urban and rural areas may contribute to risk (e.g. factors that are protective in rural areas, such as rearing of livestock, may be risk factors in urban areas as recent migrants 'ruralise' their urban environment).

2. Exercise test

The presence of exercise-induced bronchospasm (EIB) will be assessed in all children in the case-control as described previously [25,26]. Briefly, peak expiratory flow rate (PEFR) is measured before and after 6 minutes of vigorous exercise and a fall of 15% in PEFR is considered to indicate EIB.

2. Nutritional assessment

Finger prick blood samples will be collected from all subjects in the cross-sectional study to estimate hemoglobin level using standard procedures. Anthropometric measurements will be performed using standardised methodology and will include weight (kg), height (cm) and triceps skin fold thickness (mm). *z*-scores for weight-for-age, weight-for-height and height-for-age will be calculated using the EPINUT program (Epi Info 6.0; CDC. Atlanta, GA, USA).

3. Examination of stool samples for parasites

Single stool samples will be collected from all children and examined using the modified Kato-Katz and formol-ether concentration methods [27]. Intestinal helminth parasite burdens (Kato-Katz) will be quantified as eggs per gram of faeces. Infections with protozoan pathogens (formol-ether concentration) will be graded as positive or negative. All children with intestinal helminth infections will be offered appropriate treatments with albendazole and children with trophozoites of *Entamoeba histolytica* (with ingested red cells) and *Giardia intestinalis* will be offered appropriate doses of tinidazole.

4. Examination of dust samples

Dust samples will be collected from the homes of the children in the case-control study using a ~ 1200 W vacuum cleaner, weighed and stored as described previously [18].

Table 1: Variables to be collected in the cross-sectional and nested case-control studies

	Cross-sectional	Case-control
Allergy questions		
Core asthma symptom questions (ISAAC phase II)	X	X
Core rhinitis symptom questions (ISAAC phase II)	X	X
Core eczema symptom questions (ISAAC phase II)	X	X
Supplementary asthma questions (ISAAC phase II)		X
Asthma management questions (ISAAC phase II)		X
Rhinitis management questions (ISAAC phase II)		X
Eczema management questions (ISAAC phase II)		X
Knowledge attitude practice questions (KAP)		X
Demographic and socio-economic		
Sex	X	X
Age	X	X
Race	X	X
On socio-economic level:		
parental schooling	X	
parental occupation	X	X
domestic goods	X	X
monthly income	X	X
Environmental factors		
Sanitation and water supply	X	X
Does the house have electricity?	X	X
Pets		
Has the child ever had a cat or dog living in the house?	X	
Does the child have a cat or dog living in the house currently?	X	X
Presence of animals outside household	X	X
Indoor smoking	X	X
Exposure to a farming environment	X	X
Housing (e.g. construction, number of rooms)	X	X
Presence of allergens in dust (mites and cockroach)		X
Presence of endotoxin in the house		X
Cooking materials	X	X
Maternal and family related factors		
Maternal smoking during pregnancy	X	
Asthma and other allergic diseases in the family	X	
Breast-feeding duration	X	
Vaccination (BCG, DTP, measles)	X	

Table 1: Variables to be collected in the cross-sectional and nested case-control studies *(Continued)*

Whether attended day care (and for what period)	X	
Number of people who live in each household	X	X
Number of siblings or children in the household	X	X
Birth order	X	X
Diet		
Food frequency questionnaire	X	X
Consumption of unpasteurized milk	X	
Migration		
Place of birth of child	X	
Place of birth of father	X	
Place of birth of mother	X	
Was the father brought up in an urban or rural area?	X	
Was the mother brought up in an urban or rural area?	X	
Period of time and places living outside place of birth during first 5 years of life	X	
Details of relatives living in the city of Esmeraldas	X	
Exercise/sedentarism		
Number of hours of television watched daily	X	X
Frequency of vigorous exercise	X	X
Nutritional assessment		
Hemoglobin	X	X
Height	X	X
Weight	X	X
Mid-upper arm circumference	X	
Triceps skin fold thickness	X	
Specific Infections		
Intestinal helminth and protozoal infections (stool examinations)	X	X
A. lumbricoides IgE		X
IgG for Toxoplasma. Gondii, Helicobacter pylori and viral infections		X
Exercise test for exercise-induced bronchospasm		X
Atopy		
Skin prick test	X	X
Total and specific IgE titres to house dust mite and American cockroach		X
Immunoregulatory cytokines		
IL-10, IL-13, IFN-γ		X

Surveys of mites in dust samples and of cockroaches in houses in the rural area show a predominance (>90%) of both *Dermatophagoides pteronyssinus* mite and the American cockroach, *Periplaneta americana*. Dust samples will be analyzed for endotoxin and fungal β-glucans (Limulus lysate assay, BioWhittaker, MD, USA) and allergens from *D. pteronyssinus* (Der p 1) and *P. americana* (Per a 1) (Indoor Biotechnologies, VA, USA).

5. Examination of blood samples

Blood samples (7 mL) will be collected by venipuncture from participants in the case-control study and will be used for obtaining serum for the measurement of: polyclonal IgE [28]; IgE antibodies specific for *D. pteronyssinus*, American cockroach, and *Ascaris lumbricoides* using the Pharmacia CAP system (Phadia AB, Uppsala, Sweden); IgG antibodies specific for hepatitis A virus, *Helicobacter pylori*, herpes simplex virus, herpes zoster virus, Epstein-Barr virus, and *Toxoplasma gondii* using commercially available assays; and for whole blood cultures stimulated with mitogen and relevant allergens for the measurement of the regulatory cytokines, IFN-γ, IL-13, and IL-10, as described elsewhere [18]. A whole blood pellet sample will be stored at -40°C and shipped frozen to Imperial College, London, UK, for genotyping of single nucleotide polymorphisms using the Sequenom system (Sequenom Inc, San Diego, CA, USA), as described previously [24].

6. Allergen skin prick testing

All children in the cross-sectional study will be tested for immediate hypersensitivity responses to relevant aeroallergens as described previously [25,28]. A positive test will be taken as a wheal with a mean diameter of at least 3 mm greater than the saline control 15 minutes after pricking the allergen into the right forearm using an ALK Lancet (ALK-Abello, Horsholm, Denmark). The following allergens from Greer Laboratories Inc (Lenoir, NC, USA) will be tested: *D. pteronyssinus*/*D. farinae* mix, American cockroach, *Alternaria tenuis*, cat, dog, '9 Southern grass mix', and 'New stock fungi mix' .

Overview of statistical plan of analysis

The analysis will be designed to address five principal study questions: 1) What is the frequency of atopy and asthma in school children from the rural and urban study areas? 2) What environmental exposures are associated with atopy and asthma and how do these interact with area of residence (rural vs. urban) to affect the relationship between atopy and wheeze symptoms? 3) Do environmental exposures associated with wheeze differ between migrant and established urban populations and, if so, are they potentially modifiable through intervention programmes? 4) Are place of birth (rural vs. urban) and/or period of residence in rural and urban areas associated with risk of allergy? 5) How do complex inter-relationships between factors at different levels (e.g. immunologic factors and social factors) interact to affect the risk of asthma in urban and rural populations? Statistical analysis will be conducted according to a conceptual framework that defines a proposed causal pathway and the complex analytic approach to be used has been described in detail in a companion paper [18]. Methods (epidemiological and laboratory measurements) have been standardized between the Salvador-SCAALA [18] and this study (Esmeraldas-SCAALA), a procedure that will permit comparisons between the two studies, and may allow the identification of common risk/protective factors as well as those that are peculiar to the different study sites.

Ethical considerations

Ethical approval for the study has been obtained from the Hospital Pedro Vicente Maldonado, Provincia de Pichincha, Ecuador. Written informed consent to participate in the study will be obtained from the parent of each child and signed minor assent will be obtained from each child. The parent or guardian of each child will be provided with a copy of all laboratory results and if, appropriate, treatment recommendations will be made by a trained clinician that will review each case.

Discussion

Atopic asthma and other allergic diseases are becoming increasingly important public health problems in Latin American cities and there is little published information on the causes of this disease epidemic. The SCAALA (Social Change, Asthma and Allergy in Latin America) research initiative includes two epidemiological studies being conducted in urbanizing populations (Esmeraldas Province, Ecuador and the city of Salvador in Brazil, respectively) that are investigating the environmental causes of allergy in urban Latin America and the biological and social mechanisms that underlie these epidemiological trends. While the SCAALA-Salvador aims to investigate the associations between the prevalence of asthma and other allergic diseases (rhinitis, atopic eczema) and potential risk factors that includes living conditions and early life and current exposures to infections [18], the SCAALA-Esmeraldas study aims to study frequency of atopy and allergic diseases and exposure to potential risk factor in rural populations and in migrants from rural to urban areas and examine how these may explain the risk of atopic diseases in migrants from rural to urban areas. Both studies will investigate how the association between environment factors, allergic diseases and markers of atopy (i.e. skin-prick test and total and specific serum IgE levels) may be mediated by interleukins from antigen-stimulated leukocytes.

The Esmeraldas-SCAALA study in Ecuador includes cross-sectional and nested case-control studies conducted in rural and urban contexts. The studies are investigating the impact of urban migration on asthma risk and the environmental exposures that are associated with an increased risk of asthma in populations that migrate from rural to urban areas. The study will focus on a single ethnic cultural group, Afro-Ecuadorians, that traditionally has lived in the remote rural North Eastern region of Esmeraldas Province, but that has migrated in significant numbers over the past 30 years to cities such as the provincial capital, Esmeraldas. The study of a single and easily identifiable group that presumably shares the same genetic 'stock' and that has migrated locally (within the same Province) should allow important environmental risk factors to be identified more easily and should not suffer from the biases that limit the interpretation of studies that have investigated populations that have migrated between countries. Specifically, the study, by investigating a migrant population, will distinguish between the effects of early life exposures (inducing immune programming) and current exposures in determining allergy risk.

The knowledge generated from this study will help to define the size of the public health problem of allergy in Ecuador and may identify possible environmental exposures that could be considered for primary prevention public health strategies. The study in Brazil is a prospective study investigating the effects of early life exposures to environmental factors, and the potential effects of these on the immune system and the risk of allergy and has been described in detail in a separate paper [18].

The causes of the allergy epidemic in Latin America are assumed to be multifactorial and an important strength of the SCAALA studies is the use of similar causal frameworks and the sharing of methodology and expertise in a wide range of scientific disciplines (e.g. epidemiology, immunology, microbiology, biostatistics and social sciences). The two studies are complementary and are likely to yield important information on the underlying causes of the allergy epidemic in urban Latin America.

In summary, the aim of the proposed programme is to investigate the biological and social mechanisms that underlie the epidemic of allergic diseases in urban Latin America. Urbanization and the health problems associated with this phenomenon probably represent the single most important challenge for health researchers working in developing countries in the 21st century, and allergic diseases are likely to emerge as the most prevalent of chronic diseases of childhood in Latin America during this century. Latin America urbanization has its roots on the intense process of displacement of the poor rural population that move to the urban centres looking for work and other improvements in their life conditions. It is expected that the knowledge generated from the SCAALA studies will help identify public health interventions that may ameliorate the adverse effects of urbanization on the prevalence and severity of asthma and allergic diseases.

Abbreviations

ISAAC: International Study of Asthma and Allergies in Childhood

SCAALA: Social Change, Asthma and Allergy in Latin America

Competing interests

The author(s) declare that they have no competing interests.

Authors' contributions

PJC and LCR had the original idea for the study. PJC designed the study and drafted the manuscript. MC, MV were involved in study design and co-ordination. NAN and LPC were responsible for the immunological methods. BG was responsible for the statistical analysis plan. LR, AR, AC, RS and MB were involved in study design. All authors helped draft the manuscript, and read and approved the final version of the manuscript.

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Appendix II: ECUAVIDA Study Protocol

Description of study:

The study is based on an established birth cohort, the Ecuador Life (ECUAVIDA) study, set up to investigate the impact of early life infectious and microbial exposures, especially STH infections, on protective immune responses to childhood vaccines and the development of atopy and allergic diseases in the rural tropics. The study is being conducted in the rural District of Quinindé in Esmeraldas Province in a tropical region of Ecuador. The cohort of 2,404 newborns was recruited at the time of birth between 2005 and 2009 with periodic follow-up to 5 years of age for determination of study outcomes. Primary study exposures were maternal STH infections and childhood STH infections during the first two years of life. Primary study outcomes are the development of protective immunity to childhood vaccines and the development of atopy, eczema, and asthma during childhood. Follow-up for measurement of exposures and determination of study outcomes were done around the time of birth, and at 7 and 13 months, and at 2, 3, and 5 years of age. Sampling at these time point included blood (for buffy coats, plasma, and whole blood cultures) and stool samples (for detection of enteric parasites and intestinal microbiota). Evaluations included questionnaires, physical examination, environmental sampling (i.e. mattress dust), and allergen skin prick testing (from 2 years of age). Follow-ups at 13 months, 2, 3, and 5 years of age have been greater than 90%. We now wish to continue follow-up of the cohort at 9 years of age. At the start of the study we expanded the range of exposures being measured to allow a more complete evaluation of early childhood infections and exposures to microbes. We now wish to expand the exposures being measured at 9 years of age to include an evaluation of the effects of environmental and lifestyle changes associated with the process of urbanization, and expand the outcomes to allow a more thorough evaluation of other chronic non-communicable diseases that are of growing importance in the study population, namely hypertension and type-2 diabetes.

General objective

Our research addresses how STH parasites, infections, and microbes in the environment affect clinically relevant inflammation through the development of atopy and asthma; how such exposures might affect the development of protective antibody responses to common childhood vaccines; and how such exposures and increasing urbanization may contribute to the growing epidemic of not just allergic but other chronic non-communicable diseases such as hypertension and type-2 diabetes.

Specific objectives

Objective 1. To determine the effect of maternal and childhood STH infections on the development of atopy and asthma at 9 years of age.

Objective 2. To determine the effect of other infections and microbial exposures in early life on the risk of atopy and asthma at 9 years.

Objective 3. To determine the effects of early childhood infections, specifically STH parasites, on the protective antibody response to common childhood vaccines and to determine if host genetic factors might contribute to poor vaccine responsiveness.

Objective 4: To determine the effects of infections, including STH parasites, and microbial exposures in early childhood on the development of systemic inflammation and the childhood trajectory of markers of chronic non-communicable diseases (i.e. type-2 diabetes and hypertension).

Objective 5: To determine the effects of urbanization on the childhood trajectory of markers of chronic non-communicable diseases (i.e. type-2 diabetes and hypertension).

Study design and methods

Design

The study is a birth cohort (the ECUAVIDA cohort) of 2,404 children designed to investigate the effects of pre- and postnatal exposures to soil-transmitted helminth (STH) parasites on the development of atopy and allergic diseases in childhood. The cohort was recruited around the time of birth in Hospital "Padre Alberto Buffoni," Quininde, Esmeraldas Province. The cohort has been followed-up since birth with periodic evaluations to 5 years of age for measurement of study exposures and outcomes (defined below). Study children children are now aged 6-9 years. We now wish to evaluate the cohort at 9 years of age.

Study population

The study population included eligible newborns delivered at the Hospital "Padre Alberto Buffoni" (HPAB) between November 2006 and December 2009. HPAB is the only hospital serving the rural District of Quinindé, Esmeraldas Province, Ecuador. During recruitment, members of the study team visited daily the maternity and vaccination departments of HPAB. Inclusion criteria at recruitment were: 1) healthy baby less than 14 days old; 2) at least one stool sample collected from the mother in the third trimester of pregnancy or around the time of delivery of the child; 3) the family has lived in the District of Quinindé for the last 2 years and does not plan to move out of the District over the following 3 years; 4) the home was accessible; and 5) the family has no ethical or religious reasons that might interfere with their participation. Mothers were initially interviewed for eligibility and if interested and eligible, a home visit was scheduled where informed written consent was obtained and a standardized questionnaire administered. The mothers of 2,404 eligible newborns agreed to participate in the study. The District of Quinindé is primarily dependent economically on agriculture. Within the District are 3 towns with populations greater than 10,000 inhabitants, Quinindé, La Union and La Concordia. About 70% of the cohort lives in rapidly expanding urban and peri-urban neighbourhoods of these 3 towns and the remainder in rural recintos (or settlements).

Inclusion and exclusion criteria at 9 years of age:

Criteria for inclusion into the present study:

- Inclusion:
  a) Recruited into birth cohort according to inclusion criteria outlined above
  b) 9 years of age
  c) Able to locate the child's household
  d) Mother, father, or representative signs informed written consent, and child signs minor assent that ensures that the child accepts to be involved in the study.

- Exclusion:
  a) Not Recruited into birth cohort
  b) Less than or greater than 9 years of age
  c) Unable to locate child's household
  d) The mother father, or representative do not sign informed written consent or child does not accept to be involved in the study.

Study exposures and outcomes:

Primary study exposures were maternal soil-transmitted helminth (STH) infections and childhood STH infections during the first two years of life. Primary study outcomes are the development of protective immunity to childhood vaccines and the development of atopy, eczema, and asthma during childhood. Follow-up for measurement of exposures and determination of study outcomes were done around the time of birth, and at 7 and 13 months, and at 2, 3, and 5 years of age. Sampling at these time points included blood (plasma and whole blood cultures), stool samples (for detection of enteric parasites and intestinal microbiota), and nasopharyngeal and hypopharyngeal swabs (for measurement of respiratory infections). Evaluations included questionnaires, physical examination, environmental sampling (i.e. mattress dust), and allergen skin prick testing (at 2, 3, and 5 years of age).

Exposures to be measured and or analysed at 9 years of age:

Primary:

1. Soil-transmitted helminth (STH) infections:
   a) Maternal STH infections – a positive stool sample for any STH parasite during the third trimester of pregnancy or around the time of birth of the child
   b) Early childhood infections – a positive stool sample for any STH parasite collected during the first 2 years of life

Secondary:

1. Chronic systemic amd local infections:
    a) Chronic infections/carrier states: presence of specific IgG in plasma at 3 years of age to Helicobacter pylori, Toxopasma gondii, Hepatitis A, Epstein-Barr virus, and Herpes simplex (Alcantara-Neves et al, 2012).
    b) Documented respiratory tract infections and diarrhoea during the first 3 years of life
2. Household microbiota:
    a) Levels of endotoxin in mattress dust sample collected at 13 months of age.
    b) Drinking water: contamination of drinking water sample with coliforms to be measured at 9 years.
3. Urbanization: An urbanizationindex will be defined. Definition will include data on community infrastructure, household environment and socioeconomic factors, lifestyle (e.g. diet and physical activity), and migration history. See References (Rodriguez et al, 2011; Rodriguez et al, 2015) for further details
4. Host genetics: Gene polymorphisms associated with poor vaccine responses to common childhood vaccines (see outcome at 13 months below).

Tertiary:

Intestinal and hypopharyngeal microbiota: exploratory analyses will be done to explore the effects of early childhood microbiota on the development of allergic sensitization and asthma. Faecal microbiota will be used as a surrogate for intestinal microbiota and hypopharyngeal microbiota as a surrogate for lower airways microbiota.

<u>Outcomes to be analysed at 13 months of age:</u>

a) Protective antibody levels in a plasma sample collected at 13 months of age to measure soecific IgG or IgA antibodies to Diptheria, tetanus toxoid, Pertussis, Haemophilus influenzae type B, types 1 and 3 polioviruses types, measles, rubella, and rotavirus. Antibody levels will be defined as protective (Yes vs. No, using previously defined standard antibody thresholds to define protection) or by antibody titer.

<u>Outcomes at 9 years of age:</u>

Primary:

a) Asthma/wheeze – defined as parentally-reported wheeze in the previous 12 months.
b) Atopy – defined as any positive skin prick test to 7 aeroallergens and 3 food allergens at 9 years

c) Eczema – defined using the UK refinement of the Hanifin and Rajka diagnostic criteria (Williams and Flohr, 1996)

Secondary:

a) Blood pressure: systolic and diastolic blood pressure at rest
b) Blood glucose and glycosylated haemoglobin: defined by capillary glucose/Hba1c on fasting capillary blood samples

Power calculation

The sample size is fixed at 2,404 neonates that were recruited between 2005 and 2009 from children born in the Hospital Padre Alberto Buffoni, the public hospital of the District of Quininde in Esmeraldas Province, Ecuador. The primary study exposure is maternal geohelminth infections and infant infections during the first 2 years of life. The prevalence of these exposures was 45.9% for maternal geohelminths and 28.6% for STH infections in children during the first 2 years of life. Follow-up at 9 years is expected to be greater than 85% or ~2,050 children. Power estimates for study outcomes are provided in Table 2 below (source: Cooper PJ et al. BMC Infectious Diseases 2011; 11; 184) using an estimated prevalence of primary exposures of 50% for maternal geohelminth infections and 30% for childhood infections to 2 years of age but with estimated follow-ups of 77% and 72% for 3 and 5 years, respectively, compared to actual follow-ups of 90% and ~85%, respectively. We we will have >80% power at P<0.05 for all primary study outcomes.

Table 2 Power for analysis of primary exposure-outcome associations

Primary exposures (geohelminth infections)	Outcome	Sample size available	Analysis sample size	Exposure prevalence	Expected prevalence of outcomes (exposed vs. unexposed groups)	A	Power
Maternal	Vaccine antibodies @ 7 mths						
	Hib	2,300	1,000	50%	65% vs. 75%	0.01	80%
	Rotavirus	2,300	1,000	50%	40% vs. 60%	0.01	> 99%
Maternal	Vaccine antibodies @ 2 yrs						
	TT	1,955	1,000	50%	85% vs. 95%	0.01	> 99%
	HBV	1,955	1,750	50%	80% vs. 85%	0.05	80%
	OPV type 3	1,955	500	50%	80% vs. 95%	0.01	> 99%
Infant	Vaccine antibodies @ 5 yrs						
	TT	1,725	1,000	35%	60% vs. 80%	0.01	99%
	HBV	1,725	1,500	35%	70% vs. 80%	0.01	95%
	OPV type 3	1,725	1,000	35%	50% vs. 70%	0.01	> 99%
Maternal	SPT @ 3 years	1,840	1,840	50%	15% vs. 25%	0.01	99%
Infant	SPT @ 3 years	1,840	1,840	35%	15% vs. 25%	0.01	> 99%
Maternal	Eczema @ 3 years	1,840	1,840	50%	25% vs. 35%	0.01	99%
Maternal	Asthma @ 5 years	1,725	1,725	50%	17% vs. 23% #	0.05	86%
Infant	Asthma @ 5 years	1,725	1,725	35%	17% vs. 23% #	0.05	83%
Infant	Asthma @ 5 years	1,725	1,207	20%	25% vs. 15% #	0.01	93%

Random samples of the study population will be selected for analysis of the protective levels of vaccine antibodies. Based on data from the cohort we estimate that 20% of children will have allergen skin test reactivity to any allergen at 5 years, that 30% of children will have at least one documented episode of eczema by 3 years of age and that 20% of children will have asthma at 5 years. Based on data from the cohort we estimate that ~50% of mothers are infected with any geohelminth parasite and ~30% of children will have at least one documented geohelminth infection during the first 2 years of life.

#-expected effects of geohelminth infections on asthma prevalence using data from cross-sectional analyses in Ecuador [58] and Brazil [59].

Statistical analysis

Primary analyses will analyse the associations between maternal and early childhood STH infections (i.e. first 2 years of life) and the development of primary study outcomes (atopy, wheeze, eczema, and protective antibody responses to standard childhood vaccines). These analyses will be done using bivariate logistic regression analyses followed by multivariate logistic regression adjusting for confounding variables. Secondary analyses will explore exposure-outcome associations for secondary exposures and outcomes using logistic regression for binary outcomes and linear regression for continuous outcomes. Results will be expressed as odds ratios and 95% confidence intervals for binary outcomes and as fold changes (of geometric means) for continuous outcomes (ie titers of protective antibodies to vaccines). Where necessary statistical corrections will be made for multiple comparisons. Analysis of multiple correlated variables to measure urbanization will use appropriate data reduction techniques such as principal components analysis and multiple correspondence analysis. All analyses will be done using Stata 11.

Study procedures

Children in the cohort are presently aged 5 to 9 years. Routine procedures will be done at a single time point when the child is aged 9 years. At the same time data on arterial blood pressure and fasting glucose and glycosylated haemoglobin will be collected from the child's mother and father. All these procedures will be done by trained and highly experienced study physicians (Dr Maritza Vaca and or Dr Martha Chico) at our cohort outpatient facility in the Hospital Padre Alberto Buffoni (HPAB), the District hospital in the District of Quininde where the study is based. Whenever the child changes household, new information will be collected for that household.

<u>Routine in cohort children</u>

1. Questionnaire: a questionnaire will be administered in Spanish to the child's mother or guardian by one of the study physicians (Dr Martha Chico or Dr Maritza Vaca). The questionnaire will include data for the child on: demographics, socioeconomic factors, location of the child's household, number of siblings and birth order, household crowding, changes of address, type of bathroom, schooling, carers, household smokers, pets and animals in and around the household, household pests, diet, vaccination history, illnesses and hospitalizations, and symptoms of wheeze/rhinitis/eczema, and family history of chronic diseases. Data on any current illnesses will be collected also.
2. Home questionnaire: a questionnaire will be administered in Spanish to the child's mother or guardian by one of the study workers while visiting the child's household to collect information on location and characteristics of the household, access to services such as potable water and sewage,

construction, electrical goods, and migration history of the child. At the time of the home visit a 50 mL sample of drinking water will be collected for microbiological testing.

3. Clinical examination and anthropometry: The child will undergo a complete physical examination with particular emphasis on the presence of flexural dermatitis. Weight, height, head circumference, abdominal circumference, mid-upper arm circumference, and bioimpedance (Bodystat 1500) will be measured.
4. Nasopharyngeal and hypopharyngeal swabs: a nasopharyngeal and hypopharyngeal swab will be collected.
5. Allergen skin prick testing; Allergen skin prick testing will be done to measure atopy. Skin prick testing will be done to house dust mite, American cockroach, mixed fungi, grass pollen, mosquito, dog, cat, peanut, milk, and egg extracts and positive histamine and negative saline controls using commercial extracts from Greer laboratories. Allergen extracts are placed on the forearm and then pricked using bloodless 2 mm Lancets. Wheal sizes are measured after 15 minutes and a positive reaction is a mean wheal size of 3 mm of greater than the saline control.
6. Forced expiratory nitric oxide (FeNO): FeNO will measure inflammation in the airways. FeNO will be measured in exhaled breath at a standardized flow rate using a handheld portable device (NOBreath, Bedfont).
7. Spirometry: Pulmonary function and reversibility will be measured by standard spirometry before and after inhalation of 200 mcg of salbutamol. Forced spirometry (i.e. expiratory) for FEV1 and FVC is done before and 15 minutes after inhalation of 200 mcg of salbutamol using a spacer device. Three reproducible FEV1 readings using a portable Microloop spirometer (Micro Direct, UK) are recorded.
8. Nasal wash: The presence of inflammatory cells in the upper airway (e.g. eosinophils) will be measured using a nasal wash. A small volume of aerosolized saline (2 ml) is sprayed into each nasal fossa and the liquid collected into a 5 mL tube using a glass funnel.
9. Physical activity: Physical activity will be measured using a portable accelerometer (Actigraph). The device is placed on the hip with a belt and recordings done continuously for seven days.
10. Arterial pressure: arterial pressure while seated will be measured using an electronic device (Omron).
11. Stool samples: one to two stool samples will be collected and examined for the presence of enteric parasites.
12. Blood sample: A 5 mL blood sample will be collected from the forearm into a Vaccutainer tube containing sodium heparin as anticoagulant.
13. Fasting glucose and glycosylated haemoglobin: fasting glucose will be measured in venous blood using commercial devices (fasting glucose - Accucheck Performa; glycosylated haemoglobin – iChroma, Korea).
14. Urine sample: a urine sample will be collected for dipstick measurement of standard urinary markers of disease and microscopy.

Procedures for child's mother and father:

1. Arterial pressure: arterial pressure while seated will be measured using an electronic device (Omron).
2. Fasting glucose and glycosylated haemoglobin: fasting glucose will be measured in capillary finger prick blood using commercial devices (fasting glucose - Accucheck Performa; glycosylated haemoglobin – iChroma, Korea).

Collection and storage of data

All data will be collected directly into 7-inch tablets programmed with ODK software and downloaded directly to the ODK website. Paper forms will be used for providing results to parents and guardians. Data will be stored on a password protected central computer. The central computer is kept at a locked facility at the FEPIS research centre and is backed continuously through a secure remote server (Crash Plan). Consent forms, lists of children's names with their study numbers and other identifiable data (contact details with detailed map of home location) will be stored in a locked filling cabinet in research centre, with access limited to the project team. Laboratory data will be recorded in paper notebooks in locked drawers in the study laboratory using the subjects identification number and the notebooks will transcribed to the central computer periodically. Completed notebooks are kept in a locked filing cabinet at the FEPIS research centre in Quininde. All data collected, stored and shared will comply with GCP standards.

Clinical samples (stools, plasma, swabs, etc.) will be stored at -20C or -80C in a secure facility at Laboratorio de FEPIS, Pontificia Universidad Catolica del Ecuador with 24/7-back-up power.

Justification of the study

The study will address the effects of childhood infections and microbial exposures in early childhood on the development of immune regulation, atopy and allergic diseases in later childhood. The study will address also the role of maternal helminth infections and host genetic factors on protective immunity to infant vaccines, and how infectious and microbial exposures in early childhood may contribute to the development of childhood markers for other chronic non-communicable diseases such as type-2 diabetes and hypertension.

<u>Allergy</u>: Allergic diseases such as asthma, rhinitis and eczema, are the major chronic diseases of childhood in industrialized countries [Eder et al, 2006]. The prevalence of these diseases has increased over recent decades [Eder et al, 2006], particularly in urban populations living in low and middle-income countries (LMICs) [Asher et al, 2006; Cooper et al, 2009]. Studies of the impact of early childhood exposures to infections and microbes on the development of atopy and

allergic diseases could identify novel mechanisms amenable to primary prevention programmes in childhood.

Infant vaccines: Infectious diseases are the leading cause of child mortality in LMICs, and a large proportion of these deaths are preventable. Immune responses to infant vaccines tends to be much poorer in populations in LMICs compared to those in high-income countries and it has been suggested that exposures to infections such as helminths may contribute to this effect [Cooper et al, 2000; Labeaud et al, 2009]. Improvements in vaccination strategies and vaccine immunogenicity among children in poor populations of LMICs, that bear a dispropratelionate burden burden of infectious diseases, is likely to have a significant beneficial impact on morbidity and mortality in such populations.

Chronic non-communicable diseases (NCDs): Type-2 diabetes and cardiovascular diseases such as hypertension, are currently the major causes of morbidity and mortality in Ecuador [MSP, 2010]. These diseases, that primarily affect adults after the 4th decade of life, are considered to have their origins in early life and may arise from complex interactions between genetic and environmental factors that determine the regulation of inflammation in early childhood. Lifestyle and environmental factors are also considered to be major determinants of allergic diseases and NCDs, and the increases in morbidity and mortality attributed to these disaeses in LMICs are likely to be associated with changes in such exposures in the context of urbanization. A better understanding of how systemic infammation originating in early childhood, and changes in lifestyle associated with urbanization, contribute to the development of NCDs could identify novel strategies for their prevention.

A failure of immune regulation contributes to the growing burden of inflammatory diseases: Chronic inflammatory diseases of childhood such as asthma are a growing problem in low and middle-income countries (LMIC) [Masoli et al, 2004]. The causes of the asthma epidemic in urban populations of LMIC are poorly understood. The currently favoured explanation, the 'hygiene' hypothesis - originally proposed to explain the sibling effect on hay fever prevalence [Strachan, 1989] and later modified [Martinez and Holt, 1999; Rook, 2013] - explains these epidemiologic trends in terms of a failure of immune regulation in childhood caused by a decline in the variety and intensity of microbial and infectious exposures. It has been suggested that a decline particularly of infections with which we have co-evolved may be particularly relevant [Rook, 2013]. Such infections are characterized by chronicity or latency and can be maintained within the host through regulation of inflammatory responses. Helminth infections are the classic examples of such infections because of their ubiquity in rural populations living in conditions of poor hygiene, their tendency to cause chronic infections, and their ability to induce tightly regulated inflammatory responses in the host directed against the parasite but with spill-over effects on homeostatic regulatory mechanisms [Maizels et al, 2014; Figueiredo et al, 2010; Reina et al, 2010]. Other chronic parasitic and non-parasitic infections (e.g. protozoa) may contribute to this effect. Because these largely orofecally-transmitted infections have declined in urban and urbanizing populations with access to clean water and sanitation [Barreto et al, 2008], the human immune system must now rely for its education on other microbial signals in the environment [Rook, 2013]. The replacement of natural with artificial environments has reduced considerably these compensatory exposures over a relatively short period in LMIC settings [Rook, 2013]. Although evidence from experimental animal models supports a key role of early life microbial exposures in the induction of immune regulation and the prevention of inflammatory states [Belkaid and Hand, 2014], there are limited data from human studies particularly from non-affluent settings.

STH parasites are potent modulators of the host immune response: Helminth infections are the most potent known inducers of Th2 immune responses in humans [Mahanty et al, 1993; Cooper et al, 2000], the arm of the immune response that is critical for protective immunity against these parasites [Zaph et al, 2014]. A characteristic feature of chronic helminth infections is a blunting or tolerization of the allergic inflammatory response, called the modified Th2 response [Maizels et al, 2014; Reina et al, 2010], that allows parasites to survive in the host but also the host in the presence of parasites. The regulation of inflammatory responses during chronic infections not only affects Th2 but also other inflammatory pathways (e.g. Th1 and Th17) [Maizels et al, 2014]. The regulation of allergic inflammation during chronic infections in children has been associated with a reduced risk of allergy [Wammes et al, 2014; Cooper, 2009], particularly a suppression of immediate hypersensitivity effector responses such as those measured by skin prick test reactivity (SPT) to aeroallergens [Fear et al, 2011]. The effects of STH parasites against allergic diseases are less consistent [Cooper, 2009; Leonardi-Bee et al, 2006]. There are few data on the effects of STH infections in the regulation of non-allergic inflammatory responses.

Effects of helminth infections and host genetic factors impaired immunogenicity of infant vaccines: Studies in children and adults have shown that infection with STH parasites can suppress immunogenicity and/or protective immune response to both parenteral [Elias et al, 2001; Labeaud et al, 2009] and oral [Cooper et al, 2000] vaccines. Because children living in areas that are endemic for STH parasites, acquire infections in the second year of life, an affect of STH infections on infant vaccine responses would have to be mediated through maternal helminth infections. There is evidence that maternal helminth infection modifies the fetal immune response [Labeaud et al, 2009], an effect that persists in childhood that may interfere with the protective immunity associated with BCG vaccine [Malhotra et al, 1999]. Similarly, maternal infection with *Ascaris* is associated with sensitization to antigens of *A. lumbricoides* at birth [Guadalupe, et al, 2009]. Thus, maternal helminth infections could contribute to poor vaccine responses during infancy by modifying the neonatal and infant immune response. Alternatively, impaired infant vaccine responses could be mediated by nutritional effects or host genetic factors, particularly interactions between environmental exposures in infancy and underlying host genetic susceptibility.

A regional asthma collaboration in Latin America: Major contributions to our understanding of the role of the hygiene hypothesis in the Latin American context have been provided by regional research collaboration, SCAALA (Social Changes Asthma and Allergies in Latin America), with study centres in Brazil [Barreto et al, 2006] and Esmeraldas Province, Ecuador [Cooper et al, 2006]. The SCAALA studies have emphasized a role of childhood infections including STH parasites in attenuating atopy (Rodrigues et al, 2008; Alcantara-Neves et al, 2012; Moncayo et al 2013; Endara et al, 2014] but not necessarily asthma which is primarily non-atopic [Barreto et al, 2010; Cooper et al, 2014] and that the underlying immunological mechanisms do not necessarily conform to the classic Th1/Th2/Treg paradigm Figueiredo et al, 2013]. Data from our birth cohort in Ecuador showed that maternal STH infections were associated with a reduced prevalence of SPT to house dust mite (HDM) in children at 3 years of age (Cooper et al, manuscript submitted) indicating that important effects may occur much earlier in life than previously thought. Wheezing illness among schoolchildren in rural Esmeraldas was mild and was strongly associated with IgE sensitization to Ascaris but not house dust mite [Moncayo et al, 2013; Endara et al, 2014]. Mite-specific IgE emerged as an important risk factor for wheeze only in urban schoolchildren [Endara et al, 2014] while most severe asthma disease presenting to the hospital in the City of Esmeraldas was explained largely by IgE monosensitization to HDM (Ardura et al, manuscript submitted). Thus, we have observed that STH parasites attenuate allergic sensitization to HDM, and clinically relevant asthma only emerges in the urban environment in the context of specific sensitization to HDM, particularly at high titer (Ardura et al, manuscript submitted). An explanation for the mildness of disease in rural Esmeraldas is the atopy-attenuating effects of STH parasites but these parasites explain only part of the effect [Moncayo et al, 2013; Endara et al, 2014].

The diversity of infectious and microbial exposures is important: STH parasites are not alone in their atopy-attenuating effects, and similar observations have been made for other chronic bacterial (e.g. *Helicobacter pylori*), viral (e.g. Epstein-Barr virus) and parasitic (e.g. *Toxoplasma gondii*) infections [Alcantara-Neves et al, 2012]. However, it appears likely that a variety of other microbial exposures may contribute to the regulation of allergic responses. Traditionally, these exposures would have come from living in a rural environment (e.g. contact with soil and vegetable matter within [e.g. dirt floors, wood/bamboo walls, etc.] and outside the household, and exposure to the faeces of household members and domestic pets and farm animals) [Rook, 2013].

How might these exposures mediate their effect? The primary interaction between infections/microbes and the host is through the innate immune response. Signalling of the innate immune responses may induce inflammation if such signals are perceived to be dangerous. Little is known on how the human immune response is educated in early childhood to respond appropriately to such danger signals, but regulation of the inflammatory response is likely to be critical. In the relative absence of exposures to infections/microbes, humans may not learn to regulate adequately responses to inflammatory stimuli and be vulnerable to the clinical consequences of poorly controlled inflammation [Rook, 2013; McDade, 2012].

The transition from a pre-to post-hygiene living environment: An important contributory factor to the failure of immune regulation is the transition from the pre- to post-hygiene environment in which the diversity of microbial exposures decline rapidly. This is represented in the urban environment by the ubiquity of cement and asphalt, metals and plastics, soap and detergents, clothes and shoes, etc., that have replaced contact with bugs, soil and vegetable matter in the natural environment. A shift in pre- to post-hygiene lifestyle and environment may be critical factors for an increased risk of atopy and mite sensitization in particular, allergic airways disease, and poorly controlled non-allergic systemic inflammation, that are consequent to a failure to regulate the inflammatory response.

Age and intensity of exposures: The education of the human regulatory response likely occurs in early life. Several environmental exposures have been shown to protect against allergy such as STH parasites [Wammes et al, 2014; Cooper, 2009; Feary et al, 2011], farming [von Mutius and Vercelli, 2010; Wlasiuk and Vercelli, 2012] and household pets [Lodge et al, 2012]. The farming and pet effects are strongest when exposures occur *in utero* and during the first few years of life [Wlasiuk and Vercelli, 2012; Lodge et al, 2012; Cooper et al, 2003]. Our cohort data indicate a protective effect of maternal STH infections against mite atopy (Cooper et al, manuscript submitted). Previous prospective and cross-sectional studies have shown that the level of protection against SPT increase with parasite burden [Rodrigues et al, 2008; Ege et al, 2011]. The protective effects of farming and pets may be mediated through microbial exposures [Ege et al, 2011]. A study of the protective effect of farming exposures against the development of allergy showed that the protective effect against asthma was associated with a greater diversity of microbes in household dust [Ege et al, 2011]. Early life and high intensity microbial exposures may reduce also systemic inflammation – individuals in the Philippines who spent childhood in households with a high level of contamination with animal faeces had lower levels of plasma CRP as adults [McDade, 2012].

Poor hygiene, infections, immune regulation, and the epidemic of chronic non-communicable chronic diseases: Inflammation, and particularly a failure to regulate inflammation, associated with decline in microbial and infectious diseases of childhood, may also contribute to the emerging epidemic other chronic non-communicable diseases or NCDs [Rook, 2013; McDade, 2013;Prescott, 2014] - here we refer to type-2 diabetes, hypertension, and cardiovascular diseases, that are now the major causes of death in Ecuadorian adults (MSP, 2010), under the umbrella term NCDs. Underlying systemic inflammation starting in early life (Barouki et al, 2012, McDade, 2012, Prescott, 2013) is considered to be an important contributory factor to the pathaetiology of NCDs (McDade, 2012; Prescott, 2013), and a failure to regulate such inflammation from early childhood may be key to the current epidemic of NCDs among adult populations in LMIC. Previous studies have demonstrated that the blood glucose and blood pressure track over the life course such that elevated blood glucose and blood pressure in childhood is related to risk of type-2 diabetes and hypertension, respectively, in adulthood (Nguyen et al, 2010; Chiolero et al, 2013).

Urbanization may contribute to the development of asthma and other NCDs: We have previously shown in rural communities in the province of Esmeraldas that the community prevalence of asthma is related to increasing urbanization, particularly socioeconomic status and adoption of a more urban lifestyle (Rodriguez et al, 2011). More recently, we have shown that specific lifestyle characteristics are associated with an increased risk of asthma, and explain up to 30% of the variation in asthma prevalence among children living in rural Esmeraldas Province (Rodriguez et al, 2015). Increasing urbanization, with greater exposure to relevant lifestyle and other factors occuring within this process, are likely to be improve our understanding of the mechanisms underlying temporal trends of increasing prevalence of NCDs in LMICs.

The ECUAVIDA cohort: The ECUAVIDA cohort is a birth cohort based in the rural District in Quininde in Esmeraldas Province within a population exposed to a high burden of infectious diseases, a high intensity of microbial exposures, and that is undergoing the transition from a pre- to post-hygiene environment and from a traditional to a more urban way of living. The ECUAVIDA cohort, which to our knowledge is the only birth cohort study being conducted in a rural region of Latin America, provides us with the unique opportunity to address the study questions outlined in this protocol.

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Ethical aspect of the study

All children and their mothers are part of an on-going birth cohort study in which informed written consent has been obtained at the start of the study and at 5 years of age. Appointments for 9 years will be scheduled with study mothers or guardians for them to come to the cohort outpatient facility at the Hospital Padre Alberto Buffoni in Quininde. At the time of the scheduled appointment, the objectives of the study and study procedures will be explained to the mother or guardian. The mother will be informed that there are general principles to be applied in all participating in the study: (1) participation in this study is completely voluntary, and (2) if the mother and child wishes they may withdraw at any time during the study without prejudice. If agreeing to participate, an informed written consent form will be read out to the parent or guardian to be signed by them, and a minor assent form to be signed by the child. Children aged 9 years are vulnerable. At the time of obtaining consent, the study procedures will be described to the child, in a simple manner for him/her to understand. If the child is happy with proceeding, then the child will be asked to sign a minor's assent form. The informed consent form will include a summary of the importance of the study, its objectives, and the procedures and when they will be carried out, the risks and benefits of participating in the study, and the options and rights they have as participants. Any questions that the child's mother or guardian has, will be addressed by the study investigator to ensure that all the points included in the informed consent and assent forms are fully understood. All parents or guardians signing the informed consent form will be given the informed consent and minor assent form to take home to discuss with other members of the family before consent if required. All parents or guardians signing the informed consent form will be given a copy of the form to take with them in lieu of an information sheet.

Risks

Potential risks include:

1. Blood sampling: An experienced nurse or laboratory technician will obtain a 5 mL sample of venous blood from each participant at 9 years of age. During the procedure of venous venipuncture, the child may suffer slight pain or discomfort, or a small haematoma may appear afterwards, and very rarely the child may faint. The same sample will be used to measure blood glucose and glycosylated haemoglobin which carries no additional risk or discomfort to the child.
2. Nasal wash: May cause slight discomfort and itching during the procedure. The nasal wash requires the child's collaboration. Saline is aerosolized into the nasal fossa and volumes (2.5 ml aerosolized into each nasal fossa) are too small to represent a significant risk (i.e. through accidental inhalation).
3. Spirometry and bronchodilator response: Children need to collaborate in order to carry out spirometry correctly. They will be asked to blow into a spirometer and, after reproducible tests are obtained, will be given an inhaled bronchodilator (β2- agonists, salbutamol) that will open the airways if they have any residual bronchoconstriction. This drug is standard treatment for asthma and episodes of wheezing. This drug may cause side effects such as trembling or shakiness of arms or legs, faster heart rate, headaches, muscle cramps or palpitations, and very rarely metabolic problems, vasodilatation of the extremities, feelings of muscle tension, heart problems, hyperactivity, or hypersensitivity reactions. Spirometry will be repeated after administration of salbutamol. Spirometry presents no risks to the child.
4. FeNO measurement: Children need to collaborate to carry out this procedure. The child id asked to blow into a handheld machine at a constant flow rate. There is no risk or associated discomfort.
5. Questionnaire: Some of the questions may be slightly discomforting for the parents or guardian to answer, as we may require details about the characteristics of the house in which the child is living and socioeconomic information.
6. Skin prick testing: may cause local discomfort and itching when there is a positive reaction. Itching subsides within 30-45 minutes.
7. Arterial blood pressure: the cuff may cause discomfort through pressure when it is being inflated.

8. Nasopharyngeal and hypopharyngeal swabs: the nasopharyngeal swab may cause local discomfort and the hypopharyngeal swab may cause the child to gag if applied incorrectly.

Benefits

We have been following up the cohort children since birth through regular follow-up evaluations and through evaluation of thc hildren when they are ill at a dedicated paediatric outpatient facility at HPAB. Children when ill have been treated with essential medicines or offered specialist care within within the Ministry of Health where necessary. We will continue to do this.

The parents or guardians of the children will receive the results of the tests we do on stool, blood, and faecal samples. The results will be explained to the parents/guardians by a study physician and where necessary appropriate treatment will be provided such as antiparasite treatments for parasites and iron/vitamins for anaemia. The results of other tests (e.g. allergen skin testing and pulmonary function) will be explained to the parents. Where necessary further evaluation will be offered by referral within the Ministry of Public Health. Each participant will be followed closely by the study team while they remain in the project.

Potential benefits to society of the study

There are few studies examining the effects of infections and other environmental exposures in early life on the development of allergic and other chronic diseases to which underlying inflammation is considered to contribute to pathogenesis (e.g. type-2 diabetes and hypertension). Asthma has emerged as a major cause of morbidity in Latin America over recent decades and diabetes and hypertension are now the major causes of death in Ecuadorian adults.

The antecedents of allergy and other chronic non-communicable diseases are considered to occur in early life and, in the case of allergy, protective environmental exposures are likely to have stronger effects when exposures occur early. Our birth cohort, set in a rural District of Latin America, is to our knowledge the only such study in progress and is likely to provide important data on the potential role of early life microbial exposures and infections, the effects of such exposures on the regulation of inflammation, and how such exposures may affect the risk of development of asthma and other NCDs in childhood and later in life. Our research has the potential to identify modifiable infectious and other environmental exposures responsible for the education of immune regulation, and potential mechanisms through which they operate, informing the design of novel primary prevention strategies for asthma in LMICs.

Data Confidentiality

Questionnaire data and data for all procedures apart from laboratory evaluations will be collected directly from the child's parent or guardian by the study team on to an android tablet. Tablets will be downloaded daily onto a central computer at the FEPIS research centre in Quininde and the files then eliminated from the tablet memory. The central computer is password-protected and kept at a locked facility at the FEPIS research centre and is backed continuously through a secure remote server (CrashPlan). Consent forms, lists of children's names with their study numbers and other identifiable data (contact details with detailed map of home location) are/will be stored in a locked filling cabinet in the research centre in Quininde, with access limited to the project team. Clinical laboratory data will be recorded in paper notebooks in locked drawers in the study laboratory using the subjects identification number and the notebooks will transcribed to the central computer periodically. Completed notebooks are kept in a locked filing cabinet at the FEPIS research centre in Quininde.

Laboratory samples will be labelled with the participant's unique identifier number at the collection or extraction point.

Data or samples that are shared with collaborators will be identifiable only by their unique identifier number and collaborators will not have access to any identifying information.

Study collaborators

The study is being conducted by the Laboratorio de Investigaciones FEPIS (Quininde, Esmeraldas Province, Ecuador) [whose legal representative is PJC] in collaboration with the Centro de Investigacion en Enfermedades Infecciosas, Pontificia Universidad Catolica del Ecuador (Quito, Ecuador) where PJC holds an honorary appointment as Visiting Professor. Many of the analyses will be done at the FEPIS laboratory at PUCE, Quito, but where analysis in Ecuador is not feasible, analyses will be done in collaboration with several international collaborators who will receive anonymised clinical samples from the cohort: 1) extracted DNA or buffy coats will be shipped to the Wellcome Trust Sanger Institute for genomewide analysis in collaboration with Professor Adrian Hill, Dr Manjindher Sandhu, and Dr Alex Mentzer as part of the VaccGene consortium comprising academic partners in Asia (International Centre for Diarrhoeal Disease Research, Dhaka, Bangladesh), Africa (Uganda Virus Research Institute, Entebbe, Uganda; University of Witwatersrand, South Africa; Centre National de Recherche, Burkina Faso), and Latin America (PUCE) that is investigating potential genetic determinants of poor vaccine responses observed in infants living in poor populations worldwide. Analysis will use the Illumina 2.5M chip array (based on bead array technology this chip is able to identify 2.5 million SNP loci). The data will be registered in European Genome-Phenome archive in accordance with archiving policy of the Wellcome Trust and scientific journals; 2) plasma samples for biomarkers of inflammation will be analysed at the Max Planck Institute for Infection Biology, Munich, Germany (Professor Stefan

Kaufmann) using a panel of 9 pro-inflammatory cytokines and chemokines including IL-1α, IL-1β, IL-6, IL-8, IFN-γ, GM-CSF, MCAF, TNF-α, IL-10: 3) plasma samples for total and allergen-specific IgE will be measured in plasma samples at the Asthma and Allergy Center of the University of Virginia, Charlottesville, Virginia, USA (Professor Tom Platts-Mills); 4) hypopharyngeal swabs for microbiota will be analysed using the illumina MiSeq sequencing platform at Imperial College, London, UK (Professors Bill Cookosn and Miriam Moffatt); 5) faecal samples for intestinal microbiota will be analysed using the illumina MiSeq sequencing platform at the Asthma and Allergy Center of the University of Virginia, Charlottesville, Virginia, USA (Professor Tom Platts-Mills), the Michael Smith Laboratories, University of Vancouver, Canada (Professor Brett Finlay), and Laboratory of Intestinal Immunology, Ecole Polytechnique Federale de Lausanne, Switzerland (Professor Nicola Harris); and 6) nasopharyngeal swabs for respiratory viral infections will be analyzed at the Johns Hopkins Bloomberg School of Public Health, Baltimore, USA (Dr Ruth Karron).

Study timetable

Year	2015			2016				2017				2018				2019			
Quarter	1	2	3	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Data collection and basic laboratory analyses	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔						
Stool PCR for parasites		✔	✔	✔	✔	✔	✔		✔	✔	✔		✔	✔	✔		✔		
IgE measurements (Univ. Virginia)	✔			✔				✔				✔				✔			
Inflammatory biomarkers																	✔	✔	✔
Serology for systemic infections @ 3 yrs						✔	✔												
Genetic analyses (Sanger Institute)	✔	✔																	
Data analysis			✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔
Report writing			✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔

Appendix III. Households characteristics questionnaire

NÚMERO: ____________________ FECHA ____/_______/________

CUESTIONARIO: ESTILO DE VIDA URBANO Y ENFERMEDADES ALERGICAS

A. DATOS GENERALES

1. Nombre del niño/a							
Coordenadas de la vivienda	**2. Latitud**	N	**3. Longitud**	W	**4. Elevación**		metros

B. DATOS DE LA VIVIENDA

5. Cantón:

- ☐ Quinindé
- ☐ La Concordia
- ☐ Otros..................................

6. Parroquia:

- ☐ Rosa Zárate
- ☐ Cube
- ☐ Chura
- ☐ Malimpia
- ☐ Viche
- ☐ La Unión
- ☐ La Concordia
- ☐ Las Golondrinas
- ☐ Otra......................................

7. Nombre del barrio o recinto

Barrio:...
Recinto:...

8. La vivienda se encuentra en un:

- ☐ Barrio Urbano
- ☐ Barrio Peri-urbano
- ☐ Recinto
- ☐ En las afueras del recinto
- ☐ Completamente alejada del recinto

9. A la vivienda se accede por vía o calle:

- ☐ Pavimentada, adoquinada o concreto
- ☐ Lastrada, empedrada o tierra
- ☐ Río

10. Tipo de vivienda donde vive el niño/a

- ☐ Casa
- ☐ Departamento en casa o edificio
- ☐ Cuarto en casa de inquilinato
- ☐ Casa en finca

11. El material del techo de la casa es:

- ☐ A. Losa/cemento
- ☐ B. Zinc
- ☐ C. Eternit, Eurolit (asbesto)
- ☐ D. Madera/caña/otros

Si el techo de la vivienda está compuesto por más de un material especificar.

12. ¿Cuál es el material del piso de la vivienda

- ☐ A. Madera
- ☐ B. Caña
- ☐ C. Cemento, ladrillo.
- ☐ D. Cerámica, baldosa
- ☐ E. Tierra

Si tiene más de 2 tipos de materiales especificar

13. El material de las paredes de la vivienda es:

- ☐ A. Cemento, bloque, ladrillo (enlucido)
- ☐ B. Cemento, bloque, ladrillo (sin enlucir)
- ☐ C. Madera
- ☐ D. Caña

Si tiene más de 2 tipos de materiales especificar

14 ¿Cuántos cuartos en total (incluyendo la cocina, sala, comedor y habitaciones) posee la vivienda?

☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8 ☐9 ☐10 ☐>10

15. ¿De estos cuantos son solo para dormir?

☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐>=7

16. ¿Cuántas personas viven en la vivienda?

☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8 ☐9 ☐10 ☐11 ☐12

17. El servicio higiénico de la vivienda es:

- ☐ Conectado a red pública de alcantarillado
- ☐ Conectado a pozo séptico
- ☐ Letrina (conectado a pozo ciego)
- ☐ Con descarga directa al río, estero, etc.
- ☐ Campo

NÚMERO: ____________________ FECHA ___/_______/________

18. La cocina de la vivienda es:

- ☐ Un cuarto exclusivo solo para cocinar
- ☐ Un cuarto utilizado también para dormir
- ☐ Cocina/sala/comedor
- ☐ Otras...

19. ¿Con qué frecuencia en la casa se utiliza leña o carbón para cocinar?

- ☐ Nunca
- ☐ Rara vez
- ☐ Una vez al mes
- ☐ Una vez a la semana
- ☐ Dos a tres veces por semana

20. El agua que recibe la vivienda proviene de:

- ☐ A. Red pública de agua potable
- ☐ B. Agua entubada no tratada
- ☐ C. Pozo
- ☐ D. Río, vertiente, acequia
- ☐ E. Tanquero

Si existe varias fuentes especifique

21. El agua que toman los miembros de la vivienda:

- ☐ A. La beben tal como llega al hogar
- ☐ B. La hierven
- ☐ C. Le ponen cloro
- ☐ D. La filtran
- ☐ E. Compran agua en botellones

Si existe varias opciones especifique

22. ¿Las personas en esta vivienda se bañan en:

- ☐ Un cuarto de baño o ducha
- ☐ En el patio con tanque de recolección de agua
- ☐ En el río o estero

23. Equipamiento del hogar :

	SI	NO
A. Refrigeradora	☐	☐
B. Licuadora	☐	☐
C. Televisión	☐	☐
D. Ventilador	☐	☐
E. Micro Ondas	☐	☐
F. Equipo de Sonido	☐	☐
G. DVD	☐	☐
H. Computadora	☐	☐
I. Lavadora	☐	☐

24. La vivienda poseen:

	SI	NO
A. Internet	☐	☐
B. Teléfono fijo	☐	☐
C. TV cable/pagada	☐	☐

25. La vivienda presenta problemas como:

	SI	NO
A. Goteras en el techo	☐	☐
B. Fuga de agua en tuberías	☐	☐
C. Excesiva humedad	☐	☐
D. Presencia de plagas	☐	☐
E. Otros.................................	☐	☐

26. ¿Cómo eliminan la basura en la vivienda?

- ☐ Por carro recolector
- ☐ La arrojan a un botadero, quebrada o río
- ☐ La queman
- ☐ La entierran

27. ¿En la vivienda crían?

	SI	NO
A. Perros	☐	☐
B. Gatos	☐	☐
C. Chanchos	☐	☐
D. Vacas/caballos	☐	☐
E. Gallinas	☐	☐
F. Otros	☐	☐

28. La vivienda que ocupa este hogar es:

- ☐ Propia
- ☐ Arrendada
- ☐ Prestada
- ☐ Compartida (Vive con los padres o familiares)

29. ¿En el último año se han realizado cambios o mejoras en la vivienda?

☐ SI ☐ NO

30. Especifique que tipo de cambios

	SI	NO
A. Mejora en las paredes	☐	☐
B. Mejora en los pisos	☐	☐
C. Mejoras en el techo	☐	☐
D. Mejoras en los baños	☐	☐
E. Mejora en la fachada	☐	☐
F. Cambió de vivienda	☐	☐

31. ¿En el último año se han realizado cambios o mejoras en el barrio o la comunidad?

☐ SI ☐ NO

32. Especifique que tipo de cambios

	SI	NO
A. Vías	☐	☐
B. Servicios básicos	☐	☐
C. Canchas y parques	☐	☐
D. Otros...........................	☐	☐

33. Cuantas horas al día mira la televisión el niño?

..............horas

34. Con qué frecuencia el niño realiza ejercicios?

☐ A diario ☐ 2-3veces por semana

☐ A veces ☐ Rara vez

NÚMERO: ____________________ FECHA ____/_______/________

C. CARACTERÍSTICAS SOCIOECONOMICAS DEL HOGAR

35. ¿Cuál es la relación del niño con la señora de la casa?

☐ Madre ☐ Madrastra
☐ Abuela ☐ Ausente
☐ Tía ☐ Hermana
☐ Otro……………………………………

36. Nivel de instrucción de la señora de la casa:

☐ Analfabeta ☐ No Sabe
☐ Primaria Incompleta ☐ Primaria Completa
☐ Secundaria Incompleta ☐ Secundaria Completa
☐ Superior Incompleta ☐ Superior Completa
☐ Cuarto nivel

37. ¿Cuál es la relación del señor de la casa con el niño/a?

☐ Padre ☐ Padrastro
☐ Abuelo ☐ Ausente
☐ Tío ☐ Hermano
☐ Otro……………………………………………………………

38. ¿Nivel de instrucción del señor de la casa?

☐ Analfabeta ☐ No Sabe
☐ Primaria Incompleta ☐ Primaria Completa
☐ Secundaria Incompleta ☐ Secundaria Completa
☐ Superior Incompleta ☐ Superior Completa
☐ Cuarto nivel

39. ¿Cuál es el ingreso mensual del hogar?

$……………………………………………

40. En comparación con el año anterior, la situación económica del hogar es:

☐ Mejor ☐ Igual ☐ Peor

Ocupación del señor de la casa		Ocupación de la señora de la casa	
41. Trabajo	42.Ocupación	43. Trabajo	44.Ocupación
☐ Agricultor (su propia tierra)	☐ **Actividades agrícolas** Actividades relacionadas con el campo	☐ Agricultor (su propia tierra)	☐ **Actividades agrícolas**
☐ Jornalero (para terceros)		☐ Jornalero (para terceros)	
☐ Cría de animales		☐ Cría de animales	
☐ Albañil	☐ **Empleado no profesional** Aquellas personas que se dedican a tareas básicas o con poca capacitación o instrucción informal para realizar dicho trabajo.	☐ Quehaceres domésticos	☐ **Empleado no profesional**
☐ Chofer (no dueño automotor)		☐ Empleada doméstica	
☐ Obrero de fábrica (operarios)		☐ Obrera de fábrica (operarios)	
☐ Guardia de seguridad/portero		☐ Vendedora	
☐ Cocinero		☐ Lavandera	
☐ Empleado		☐ Empleada	
☐ Oficial en taller		☐ Ayudante en taller	
☐ Cargador		☐ Cocinera	
☐ Tienda	☐ **Comerciante** Aquella persona que es propietaria de un negocio propio, profesional o no Profesional.	☐ Tienda/abastos/panadería	☐**Comerciante**
☐ Local Comercial		☐ Local Comercial	
☐ Salón		☐ Salón/restaurante	
☐ Puesto de comida		☐ Puesto de comida	
☐ Panadería		☐ Gabinete de belleza	
☐ Taller (todo tipo de taller)		☐ Costurera	
☐ Chofer (dueño del vehículo)		☐ Taller	
		☐ Chofer (dueño del vehículo)	
☐ Profesor	☐ **Profesional** Aquellas personas con educación superior y que ejercen su profesión	☐ Profesora	☐ **Profesional**
☐ Empleado Público (e)		☐ Empleada Pública (e)	
☐ Ingeniero		☐ Ingeniera	
☐ Abogados		☐ Abogados	
☐ Administradores (contadores)		☐ Administradores/secretaria	
☐ Policías/militares/bomberos		☐ Enfermera	
☐ Otros (especificar)		☐ Otros (especificar)	
☐ Desempleado		☐ Desempleada	

NÚMERO: ____________________ FECHA ___/_______/________

D. MOVILIDAD Y MIGRACIÓN

45. La madre del niño se crió en:
- ☐ Campo
- ☐ Ciudad
- ☐ No sabe

46. ¿Dónde se crió la madre del niño/a?

....

Provincia/Cantón/Parroquia/Comunidad/Barrio

47. Actualmente la madre del niño vive en una ciudad diferente a la que vive el niño/a

☐ NO ☐ SI

Indique la dirección donde la madre vive y el tiempo

48. Dirección...

49. Tiempo ....meses

50. El padre del niño se crió en:
- ☐ Campo
- ☐ Ciudad
- ☐ No sabe

51.. ¿Dónde se crió el padre del niño/a?

....

Provincia/Cantón/Parroquia/Comunidad/Barrio

52. Actualmente la madre del niño vive en una ciudad diferente a la que vive el niño/a

☐ NO ☐ SI

Indique la dirección donde el padre vive y el tiempo

53. Dirección:...

54. Tiempo...meses

55. ¿El niño/a ha vivido en esta casa toda su vida?

☐ NO ☐ SI

56. ¿Cuántas veces el niño/a se ha cambiado de casa?

☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ >4

Si el niño no ha vivido en esta casa toda su vida, a continuación detalle los lugares donde el niño/a ha estado:

57. Dirección:..	58. Edad del niño: 0 - ....meses	59. Tiempo en meses	60. Lugar ☐ Campo ☐ Recinto ☐ Ciudad	61. ¿Criaba animales en la casa? ☐ SI ☐ NO
62. ¿Cuál fue el material del piso de la vivienda? ☐ A. Tierra ☐ B. Caña/Madera ☐ C. Cemento, ladrillo. ☐ D. Cerámica	63. ¿Cuál fue el material de las paredes de la vivienda? ☐ A. Bloque, ladrillo (enlucido) ☐ B. Bloque, ladrillo (sin enlucir) ☐ C. Madera/Caña ☐ D. Mixta	64. La casa tenía: A. Agua Potable SI ☐ NO ☐ B. Alcantarillado SI ☐ NO ☐ C. Calle asfaltada SI ☐ NO ☐ D. TV cable SI ☐ NO ☐	65. Cuántas personas vivían en la casa? .... 66. Cuántas habitaciones poseía la casa?	67. Calidad de la vivienda en comparación a la actual ☐ Peor ☐ Igual ☐ Mejor
68. Dirección:..	69. Edad del niño: 0 - ....meses	70. Tiempo en meses	71. Lugar ☐ Campo ☐ Recinto ☐ Ciudad	72. ¿Criaba animales en la casa? ☐ SI ☐ NO
73. ¿Cuál fue el material del piso de la vivienda? ☐ A. Tierra ☐ B. Caña/Madera ☐ C. Cemento, ladrillo. ☐ D. Cerámica	74. ¿Cuál fue el material de las paredes de la vivienda? ☐ A. Bloque, ladrillo (enlucido) ☐ B. Bloque, ladrillo (sin enlucir) ☐ C. Madera/Caña ☐ D. Mixta	75. La casa tenía: A. Agua Potable SI ☐ NO ☐ B. Alcantarillado SI ☐ NO ☐ C. Calle asfaltada SI ☐ NO ☐ D. TV cable SI ☐ NO ☐	76. Cuántas personas vivían en la casa? .... 77. Cuántas habitaciones poseía la casa?	78. Calidad de la vivienda en comparación a la actual ☐ Peor ☐ Igual ☐ Mejor

NÚMERO: ____________________ FECHA ____/________/_________

79. Dirección:...	80. Edad del niño: 0 - ....meses	81. Tiempo en meses	82. Lugar ☐ Campo ☐ Recinto ☐ Ciudad	83. ¿Criaba animales en la casa? ☐ SI ☐ NO
84. ¿Cuál fue el material del piso de la vivienda? ☐ A. Tierra ☐ B. Caña/Madera ☐ C. Cemento, ladrillo. ☐ D. Cerámica	85. ¿Cuál fue el material de las paredes de la vivienda? ☐ A. Bloque, ladrillo (enlucido) ☐ B. Bloque, ladrillo (sin enlucir) ☐ C. Madera/Caña ☐ D. Mixta	86. La casa tenía: A. Agua Potable SI ☐ NO ☐ B. Alcantarillado SI ☐ NO ☐ C. Calle asfaltada SI ☐ NO ☐ D. TV cable SI ☐ NO ☐	87. Cuántas personas vivían en la casa? .... 88. Cuántas habitaciones poseía la casa?	89. Calidad de la vivienda en comparación a la actual ☐ Peor ☐ Igual ☐ Mejor
90. Dirección:...	91. Edad del niño: 0 - ....meses	92. Tiempo en meses	93. Lugar ☐ Campo ☐ Recinto ☐ Ciudad	94. ¿Criaba animales en la casa? ☐ SI ☐ NO
95. ¿Cuál fue el material del piso de la vivienda? ☐ A. Tierra ☐ B. Caña/Madera ☐ C. Cemento, ladrillo. ☐ D. Cerámica	96. ¿Cuál fue el material de las paredes de la vivienda? ☐ A. Bloque, ladrillo (enlucido) ☐ B. Bloque, ladrillo (sin enlucir) ☐ C. Madera/Caña ☐ D. Mixta	97. La casa tenía: A. Agua Potable SI ☐ NO ☐ B. Alcantarillado SI ☐ NO ☐ C. Calle asfaltada SI ☐ NO ☐ D. TV cable SI ☐ NO ☐	98. Cuántas personas vivían en la casa? .... 99. Cuántas habitaciones poseía la casa?	100. Calidad de la vivienda en comparación a la actual ☐ Peor ☐ Igual ☐ Mejor
101. Dirección:...	102. Edad del niño: 0 - ....meses	103. Tiempo en meses.......................	104. Lugar ☐ Campo ☐ Recinto ☐ Ciudad	105. ¿Criaba animales en la casa? ☐ SI ☐ NO
106. ¿Cuál fue el material del piso de la vivienda? ☐ A. Tierra ☐ B. Caña/Madera ☐ C. Cemento, ladrillo. ☐ D. Cerámica	107. ¿Cuál fue el material de las paredes de la vivienda? ☐ A. Bloque, ladrillo (enlucido) ☐ B. Bloque, ladrillo (sin enlucir) ☐ C. Madera/Caña ☐ D. Mixta	108. La casa tenía: A. Agua Potable SI ☐ NO ☐ B. Alcantarillado SI ☐ NO ☐ C. Calle asfaltada SI ☐ NO ☐ D. TV cable SI ☐ NO ☐	109. Cuántas personas vivían en la casa? .... 110. Cuántas abitaciones poseía la casa?	111. Calidad de la vivienda en comparación a la actual ☐ Peor ☐ Igual ☐ Mejor
112. Dirección:...	113. Edad del niño: 0 - ....meses	114. Tiempo en meses....................	115. Lugar ☐ Campo ☐ Recinto ☐ Ciudad	116. ¿Criaba animales en la casa? ☐ SI ☐ NO
117. ¿Cuál fue el material del piso de la vivienda? ☐ A. Tierra ☐ B. Caña/Madera ☐ C. Cemento, ladrillo. ☐ D. Cerámica	118. ¿Cuál fue el material de las paredes de la vivienda? ☐ A. Bloque, ladrillo (enlucido) ☐ B. Bloque, ladrillo (sin enlucir) ☐ C. Madera/Caña ☐ D. Mixta	119. La casa tenía: A. Agua Potable SI ☐ NO ☐ B. Alcantarillado SI ☐ NO ☐ C. Calle asfaltada SI ☐ NO ☐ D. TV cable SI ☐ NO ☐	120. Cuántas personas vivían en la casa? .... 121. Cuántas habitaciones poseía la casa?	122. Calidad de la vivienda en comparación a la actual ☐ Peor ☐ Igual ☐ Mejor

Appendix IV. Ethics Approval and Permissions

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Observational / Interventions Research Ethics Committee

Professor Laura Rodrigues
Professor of Epidemiology of Infectious Diseases
Department of Infectious Disease Epidemiology (IDE)
Epidemiology and Population Health (EPH)
LSHTM

2 June 2015

Dear Laura

Study Title: Effects of STH infections in pregnant mothers and infants on atopy and immunity to vaccine in infants living in rural tropical Ecuador: the ECUAVIDA birth cohort

LSHTM Ethics Ref: 8819

Thank you for responding to the Observational Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Conditions of the favourable opinion

Approval is dependent on local ethical approval having been received, where relevant.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document Type	File Name	Date	Version
Protocol / Proposal	ECUAVIDAProtocol25-5-15PartA	25/05/2015	2
Covering Letter	LSHTMethicsResponse	27/05/2015	1

After ethical review

The Chief Investigator (CI) or delegate is responsible for informing the ethics committee of any subsequent changes to the application. These must be submitted to the Committee for review using an Amendment form. Amendments must not be initiated before receipt of written favourable opinion from the committee.

The CI or delegate is also required to notify the ethics committee of any protocol violations and/or Suspected Unexpected Serious Adverse Reactions (SUSARs) which occur during the project by submitting a Serious Adverse Event form.

At the end of the study, the CI or delegate must notify the committee using an End of Study form.

All aforementioned forms are available on the ethics online applications website and can only be submitted to the committee via the website at: http://leo.lshtm.ac.uk

Additional information is available at: www.lshtm.ac.uk/ethics

Yours sincerely,

[redacted]

Professor John DH Porter
Chair

ethics@lshtm.ac.uk
http://www.lshtm.ac.uk/ethics/

HOSPITAL PEDRO VICENTE MALDONADO

Pedro Vicente Maldonado 09 de diciembre del 2004

ACTA DEL COMITÉ DE ÉTICA DEL HOSPITAL PEDRO VICENTE MALDONADO

Luego de haber conocido y analizado la propuesta y el protocolo del proyecto de investigación "FACTORES DE RIESGO ASOCIADOS CON CAMBIOS EN ATOPÍA Y ASMA ENTRE NIÑOS QUE VIVEN EN ÁREAS URBANAS Y RURALES DEL ECUADOR", el comité de ética reunido el jueves 02 de diciembre del 2004, resolvió aceptar la ejecución del mismo ya que satisface todas las inquietudes planteadas desde el punto de vista ético.

Por el comité de ética

[redacted]

Dr. Augusto Maldonado G.
PRESIDENTE

[redacted]

CORPORACION COMUNITARIA DE SALUD
HOSPITAL PEDRO VICENTE MALDONADO

Comité de Bioética. Universidad San Francisco de Quito
El Comité de Revisión Institucional de la USFQ
The Institutional Review Board of the USFQ
Aprobación MSP, Oficio No. MSP-SDM-10-2013-1019-O, Mayo 9, 2013

Quito, 20 de Octubre de 2015

Señor
Dr. Philip Cooper
Investigador Principal
UNIVERSIDAD SAN FRANCISCO DE QUITO
Ciudad

De mi mejor consideración:

Por medio de la presente, el Comité de Bioética de la Universidad San Francisco de Quito se complace en informarle que su solicitud del estudio de investigación **"Impacto de las infecciones con geohelmintos en madres embarazadas e infantes sobre atopia e inmunidad a vacunas en infantes que viven en zonas rurales tropicales del Ecuador: el cohorte de nacimiento ECUAVIDA"**, ha sido aprobada el día de hoy como un estudio *full board,* debido a que la investigación va a tomar datos personales pero el investigador asegura que serán codificados para el análisis y presentación de los resultados y una vez concluido el estudio cualquier dato que pudiere identificar al participante será borrado.

El Comité de Bioética de la Universidad San Francisco de Quito aprueba el estudio ya que cumple con los siguientes parámetros:

- El proyecto de investigación muestra metas y/o objetivos de significancia científica con una justificación y referencias.
- El protocolo de investigación cuenta con los procedimientos para minimizar sus riesgos de sus participantes y/o los riesgos son razonables en relación a los beneficios anticipados del estudio.
- Los participantes del estudio tienen el derecho a retirarse del estudio y su participación su conseguida a través de un proceso de consentimiento informado
- El protocolo cuenta con provisiones para proteger la privacidad y confidencialidad de los participantes del estudio en sus procesos de recolección, manejo y almacenamiento de datos
- El protocolo detalla las responsabilidades del investigador

Además el investigador principal de este estudio ha dado contestación a todas las dudas y realizado todas las modificaciones que este Comité ha solicitado en varias revisiones. Los documentos que se aprueban y que sustentan este estudio es la versión # 2 de Octubre , 2015 que incluyen:

- Solicitud de revisión y aprobación de estudio de investigación, 20 páginas;
- Solicitud de aplicación al consentimiento informado por escrito, 6 páginas;
- Modelo Asentimiento del menor, 1 página;
- Cuestionario de Estilo de Vida Urbano y Alergias, 5 páginas;
- Instrumentos y protocolo del proyecto, 65 páginas

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2015-125E
20 de Octubre de 2015

Esta aprobación tiene una duración de **un año (365 días)** transcurrido el cual se deberá solicitar una extensión si fuere necesario. En toda correspondencia con el Comité de Bioética favor referirse al siguiente código de aprobación: **2015-125E.** El Comité estará dispuesto a lo largo de la implementación del estudio a responder cualquier inquietud que pudiere surgir tanto de los participantes como de los investigadores.

Favor tomar nota de los siguientes puntos relacionados con las responsabilidades del investigador para este Comité:

1. El Comité no se responsabiliza por los efectos de eventos adversos que pudieran ser consecuencia de su estudio, los cuales son de entera responsabilidad del investigador principal. Sin embargo, es requisito informar a este Comité sobre cualquier novedad, especialmente eventos adversos, dentro de las siguientes 24 horas, explicando las medidas se tomaron para enfrentar y/o manejar el mencionado evento adverso.

2. El Comité no se responsabiliza por los datos que hayan sido recolectados antes de la fecha de esta carta; los datos recolectados antes de la fecha de esta carta no podrán ser publicados o incluidos en los resultados.

3. El Comité de Bioética ha otorgado la presente aprobación en base a la información entregada por los solicitantes, quienes al presentarla asumen la veracidad, corrección y autoría de los documentos entregados.

4. De igual forma, los solicitantes de la aprobación son los responsables por la ejecución correcta y ética de la investigación, respetando los documentos y condiciones aprobadas por el Comité, así como la legislación vigente aplicable y los estándares nacionales e internacionales en la materia.

Deseándole los mejores éxitos en su investigación, se solicita a los investigadores que notifiquen al Comité la fecha de terminación del estudio.

Atentamente,

[redacted]

William F. Waters, PhD
Presidente Comité de Bioética USFQ
cc. Archivo general, Archivo protocolo

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Appendix V: Supplementary Files Paper 1

Supplementary Table 1. Re-classification of publications using more than one category to define urban or rural settings in asthma studies.

Publication	Asthma definition (AD)	Categories	New Categories	AD 1 (%)	AD 2 (%)	AD 3 (%)	Urban vs rural Prevalence (%)
Addo-Yobo et al. (11) Ghana, 2007	AD1. Exercise challenge test	Urban Rich	Urban	8.3			5.65 vs 3.9
		Urban Poor	Urban	3			
		Rural	Rural	3.9			
Addo-Yobo et al. (27) Ghana, 1997	AD1. Exercise challenge test	Urban Rich	Urban	4.2			3.42 vs 2.2
		Urban Poor	Urban	1.4			
		Rural	Rural	2.2			
Biuyad et al. (33) Morroco, 2006	AD1. Current wheeze AD2. Wheezing ever	Casablanca city	Urban	16.2	23.9		9.91 vs 6.44
		Marrakech city	Urban	4.6	10.3		16.13 vs 12.9
		Ben Slimane city	Urban	8.9	14.2		
		Boulmane rural area	Rural	6.4	12.9		
El-Sharif et al. (32) Palestine, 2002	AD1. Current wheeze AD2. Wheeze ever AD3. Doctor diagnosis	Refugee camps	Urban	12.6	22.1	15.6	9.9 vs 7.46
		Cities	Urban	7.2	16.5	7.3	19.3 vs 16.6
		Semi urban	Rural	6.3	12.9	5.6	11.45 vs 8.5
		Typical rural	Rural	10.2	18.1	10	
		Primitive	Rural	5.9	15.8	9.9	
Keeley rt al. (31) Zimbabwe, 1991	AD1. Exercise challenge test	Urban North	Urban	5.8			4.45 vs 0.15
		Urban South	Urban	3.1			
		Rural	Rural	0.01			

Continuation Supplementary Table 1

Publication	Asthma definition (AD)	Categories	New Categories	AD 1 (%)	AD 2 (%)	AD 3 (%)	Urban vs rural Prevalence (%)
Steiman et al. (30) South Africa, 2003	AD1. Exercise challenge test	Urban School	Urban	32.9			33.65 vs 17
		Recently urbanized	Urban	34.4			
		Rural school	Rural	17			
Viinamen et al. (29) Mongolia, 2005	AD1. Questionnaire diagnosis	City	Urban	2.1			2.1 vs 1.75
		Rural towns	Rural	2.4			
		Villages	Rural	1.1			
Gaur et al. (28) India, 2006	AD1. Exercise challenge test	Urban city	Urban	7.7			10.21 vs 13. 3
		Urban slum	Urban	11.9			
		Rural	Rural	13.3			
Mavale-Manuel et al (26) Mozambique, 2007	AD1. Current wheeze AD2. Wheeze ever	Urban	Urban	13.6	25.15		13.5 vs 12.8
		Suburban	Urban	13.5	24		24.58 vs 22.6
		Semirural	Rural	12.9	22.6		
Kausel et al.(25) Chile, 2013	AD1. Current wheeze AD2. Self-reported asthma	Urban	Urban	16	15.8		15.69 vs 6
		Semiurban	Urban	10	17.6		16 vs 20
		Rural	Rural	6	20		
Gaviola et al.(24) Peru, 2016	AD1. Current wheeze AD2. Doctor diagnosis	Urban_Lima	Urban	9.9	8.2		5.85 vs 1.85
		Urban_Puno	Urban	1.8	2.6		5.4 vs1.15
		Semiurban_Tumbes	Rural	2.5	1.7		
		Rural_Puno	Rural	1.2	0.6		

Supplementary Table 2. Differences in asthma prevalence between urban and rural populations by asthma definition.

	Wheeze ever		Current Wheeze		Doctor diagnosis		Exercise test		Self-reported		Questionnaire	
Author (reference)	**Urban vs Rural**	**diff**	**Urban vs Rural**	**diff**	**Urban vs Rural**	**diff**	**Urban vs Rural**	**diff**	**Urban vs Rural**	**diff**	**Urban vs Rural**	**diff**
Addo-Yobo et al.(11)							5.7 vs 3.9	**				
Selcut et al. (12)			6 vs 5.2	**							5.8 vs 5.2	**
Selcut et al. (12)			12.6 vs 9.7	***							12.1 vs 8.6	***
Kolokotroni et al. (13)			7.5 vs 5.4	***					11.9 vs 9.7	**		
Kolokotroni et al. (13)			8.4 vs 9.7	*					17.1 vs 18.4	*		
Kahwa et al. (72)			18.5 vs 20.5	*	18.3 vs 15.4	**						
Mavale-Manuel et al. (26)	24.6 vs 22.4	**	13.5 vs 12.8	**								
Hijazi et al. (57)	17.8 vs 7.3	***	13.2 vs 6.4	***					14.9 vs 5.4	***		
Walraven et al. (43)			4.2 vs 3.3	**	3.6 vs 0.7	***						
Solis et al. (73)	27.4 vs 34.9	*	16.4 vs 21.7	*			19.2 vs 13.1	**				
Solé et al. (22)	32.6 vs 23	***	18.6 vs 12.5	***					21.1 vs 8.5	***		
Solé et al. (22)	42.1 vs 37.4	***	16.7 vs 15.3	**					14.9 vs 11.1	***		
Steiman et al. (30)							33.7 vs 17	***				
Kausel et al. (25)			15.7 vs 6	***					16 vs 20	*		
Zhang et al. (58)											1.1 vs 0.5	***
Keeley et al. (31)							4.5 vs 0.2	***				
Robinson et al. (74)			10.1 vs 2.8	***	13 vs 2.2	***			11.6 vs 3.1	***		
Abdalla et al. (44)					6.4 vs 5.2	**						
Yemaneberhan et al. (45)			3.7 vs 1.2	***					3.6 vs 1.3	***		
Addo-Yobo et al . (27)							3.4 vs 2.2	**				
Vlaski et al. (82)			7.2 vs 4.9	***					1.9 vs 1.2	**		
El-Sharif et al. (32)	19.3 vs 15.6	***	9.9 vs 7.5	***	11.5 vs 8.5	***						

Continuation Supplementary Table 2

	Wheeze ever		Current Wheeze		Doctor diagnosis		Exercise test		Self-reported		Questionnaire	
Author (reference)	Urban vs Rural	diff	Urban vs Rural	diff	Urban vs Rural	diff	Urban vs Rural	diff	Urban vs Rural	diff	Urban vs Rural	diff
Adetoun et al.(46)			5.7 vs 4.1	**	0.4 vs 2.9	*						
Viinanen et al. (29)											2.1 vs 1.8	**
Gaur et al.A (28)							10.2 vs 13.3	*				
Ng'ang'a et al. (47)							22.9 vs 13.2	***				
Musafiri et al. (48)			11.9 vs 13.1	*	7.3 vs 5.7	**					19.3 vs 8.3	***
Calvert et al. (49)							14.9 vs 8.7	***				
Shimwela et al. (50)			23.1 vs 12.1	***			6.3 vs 2.4	***	17.1 vs 6.6	***		
Guner et al. (59)			5.6 vs 6.3	*	10.5 vs 7.1	**					12.7 vs 9.9	**
Fedortsiv et al. (60)			12.4 vs 10.6	**	2.6 vs 1.3	***						
Lâm et al. (61)	5.6 vs 3.9	***	4.3 vs 5.1	*	3.9 vs 3.8	**						
Dagoye et al. (51)			4.4 vs 2	***								
Ekici et al. (62)			6.9 vs 9.9	*							11.4 vs 14.1	*
Rodriguez et al. (75)			9.4 vs 10.1	*								
Van Niekerk et al.A (52)							3.2 vs 0.1	***				
Hasan et al. (63)	16.4 vs 12	**	10.5 vs 5.5	***					4.2 vs 2.8	**		
Odhiambo et al. (53)	13.4 vs 3.2	***	9.5 vs 2.5	***	4.2 vs 0.2	***						
Feng et al. (64)	14.6 vs 4.2	***	6.1 vs 1.5	***	6.9 vs 3.4	***						
Paranesh et al. (65)											16.6 vs 5.7	***
Soares et al. (76)	37 vs 23.6	***	16.2 vs 10.9	***					24.7 vs 15.2	***		
Ma Y et al. (66)			7.2 vs 1	***	6.3 vs 1.1	***						
Huang et al. (67)	6.6 vs 3.2	***	2.8 vs 1.5	***								
Cooper et al. (77)	27 vs 23	**										

Continuation Supplementary Table 2

	Wheeze ever		Current Wheeze		Doctor diagnosis		Exercise test		Self-reported		Questionnaire	
Author (reference)	Urban vs Rural	diff	Urban vs Rural	diff	Urban vs Rural	diff	Urban vs Rural	diff	Urban vs Rural	diff	Urban vs Rural	diff
Bedolla et al. (78)	23.5 vs 24.9	*	9.5 vs 10.1	*	8.5 vs 5.8	**						
Zhu et al. (68)											3.7 vs 1.3	***
Chakravarthy et al. (69)	19.6 vs 14.4	**			5.4 vs 4.5	**						
Menezes et al. (79)					4.6 vs 3.1	***						
Tug et al. (70)	25.8 vs 29.6	*	19.3 vs 27.3	*								
Zedan et al. (54)											8 vs 7	**
Mugusi et al. (23)	5.6 vs 2.9	***	3.4 vs 2.9	**					3.7 vs 3.2	**		
Mugusi et al. (23)	2.3 vs 3.7	*	1 vs 3.3	*					1.2 vs 3.3	*		
Gaviola et al. (24)			11 vs 3.1	***	6.3 vs 1.3	***						
Yakubovich et al.E (55)									5.9 vs 4	***		
Kumar et al. (71)											11.9 vs 9.8	**
Han et al. (80)			13 vs 7.1	***	4.3 vs 4.8	*						
Ehrlich et al. (56)			15.3vs17.9	*	4.2vs3.1	***						
Lynch et al. (81)					5.8 vs 1.8	***						
Bouayad et al. (33)	16.13 vs12.9	***	9.9 vs 6,4	***								

***Higher in urban area and statistically significant. ** Higher in urban area and no statistically significant, * Higher in rural area.

Supplementary Table 3. Methodological quality of studies included in systematic review

Author (Country-year of publications)	Area description	Population description	Sample method	Sample size	Urban definition	Response rate	Overall quality
Van Niekerk et al. (South Africa-1979)	+	+	-	+	+	unk	Low
Lynch et al. (Venezuela-1984)	+	+	-	-	+	unk	Low
Keeley et al. (Zimbabwe-1991)	+	+	-	+	+	unk	Low
Addo-Yobo et al. (Ghana*-1997)	+	+	-	+	+	unk	Low
Yemaneberhan et al. (Ethiopia-1997)	-	-	+	+	+	unk	Low
Hijazi et al. (Saudi Arabia-1998)	+	-	+	+	+	unk	Low
Ng'ang'a et al. (Kenya-1998)	+	+	+	+	+	+	High
Odhiambo et al. (Kenya-1998)	+	+	+	+	+	+	High
Hasan et al. (Palestine-2000)	+	+	+	+	+	+	High
Walraven et al. (Gambia-2001)	+	+	+	+	+	+	High
Chakravarthy et al. (India-2002)	+	-	-	+	-	+	Low
El-Sharif et al. (Palestine-2002)	+	+	+	+	-	+	Medium
Paranesh et al. (India-2002)	+	+	-	+	-	unk	Low
Tug et al. (Turkey-2002)	+	+	-	+	+	+	Medium
Dagoye et al. (Ethiopia-2003)	-	-	-	+	+	unk	Low
Steiman et al. (South Africa-2003)	+	+	-	-	+	+	Low
Mugusi et al. (Tanzania-2004)	+	+	+	+	+	unk	Medium
Mugusi et al. (Cameroon-2004)	+	+	+	+	+	unk	Medium
Maia et al. (Brazil-2004)	+	+	+	+	+	unk	Medium
Ehrlich et al. (South Africa-2005)	-	+	+	+	+	+	Medium

Continuation Supplementary Table 3

Author (Country-year of publications)	Area description	Population description	Sample method	Sample size	Urban definition	Response rate	Overall quality
Viinanen et al. (Mongolia-2005)	+	+	+	+	+	+	High
Bouayad et al. (Morroco-2005)	+	+	-	+	+	-	Low
Gaur et al. (India*-2006)	+	+	-	+	+	unk	Low
Addo-Yobo et al. (Ghana*-2007)	+	+	-	+	+	unk	Low
Cunha et al. (Brazil-2007)	-	+	+	+	+	na	Medium
Mavale-Manuel et al. (Mozambique-2007)	+	+	+	+	+	+	High
Solé et al. (Brazil^-2007)	+	+	+	+	+	unk	Medium
Solé et al. (Brazil^-2007)	+	+	+	+	+	unk	Medium
Ekici A et al. (Turkey-2009)	+	-	+	+	-	+	Low
Ma Y et al. (China-2009)	-	+	+	+	-	unk	Low
Zedan et al. (Egipt-2009)	-	-	+	+	+	+	Low
Calvert et al. (South Africa-2010)	+	+	+	+	+	unk	Medium
Selcuk et al. (Turkey*-2010)	+	+	+	+	+	+	High
Selcuk et al. (Turkey*-2010)	+	+	+	+	+	+	High
Guner et al. (Turkey-2011)	-	-	+	-	-	+	Low
Kolokotroni et al. (Cyprus*-2011)	-	-	-	+	+	unk	Low
Kolokotroni et al. (Cyprus*-2011)	-	-	-	+	+	unk	Low
Lam et al. (Vietnam-2011)	+	+	+	+	+	+	High
Musafiri et al. (Rwanda-2011)	+	+	+	+	+	+	High
Robinson et al. (Peru-2011)	+	+	-	+	+	+	Medium

Continuation Supplementary Table 3

Author (Country-year of publications)	Area description	Population description	Sample method	Sample size	Urban definition	Response rate	Overall quality
Rodriguez et al. (Ecuador-2011)	+	+	+	+	+	na	High
Abdallah et al. (Egypt-2012)	+	+	+	+	+	+	High
Fedortsiv et al. (Ukraine-2012)	-	-	+	+	-	+	Low
Kahwa et al. (Jamaica-2012)	+	+	+	+	+	+	High
Adetoun Mustapha et al. (Nigeria-2013)	+	+	+	+	+	+	High
Kausel et al. (Chile-2013)	+	-	-	+	+	unk	Low
Antunes et al (Brazil-2014)	-	-	+	+	+	na	Low
Fatore et al. (Latin America-2014)	+	+	+	+	+	na	High
Huang et al. (China-2014)	+	+	-	+	+	unk	Low
Shimwela et al. (Tanzania-2014)	+	+	+	+	+	+	High
Solis et al. (Bolivia-2014)	+	+	+	+	+	+	High
Vlaski et al. (Macedonia-2014)	+	+	+	+	+	+	High
Zhang et al. (China-2014)	-	+	+	+	+	+	Medium
Menezes et al. (Brasil-2015)	-	-	+	+	-	unk	Low
Rodriguez et al. (Ecuador-2015)	+	+	-	+	+	+	Medium
Cooper et al. (Ecuador-2016)	+	+	-	+	-	+	Low
Tabalipa et al. (Brazil-2015)	+	+	+	+	+	na	High
Feng, et al. (China-2015)	+	+	+	+	+	+	High
Zhu et al. (China-2015)	+	+	+	+	+	+	High
Yakubovich et al. (South Africa-2016)	+	+	+	+	+	unk	Medium

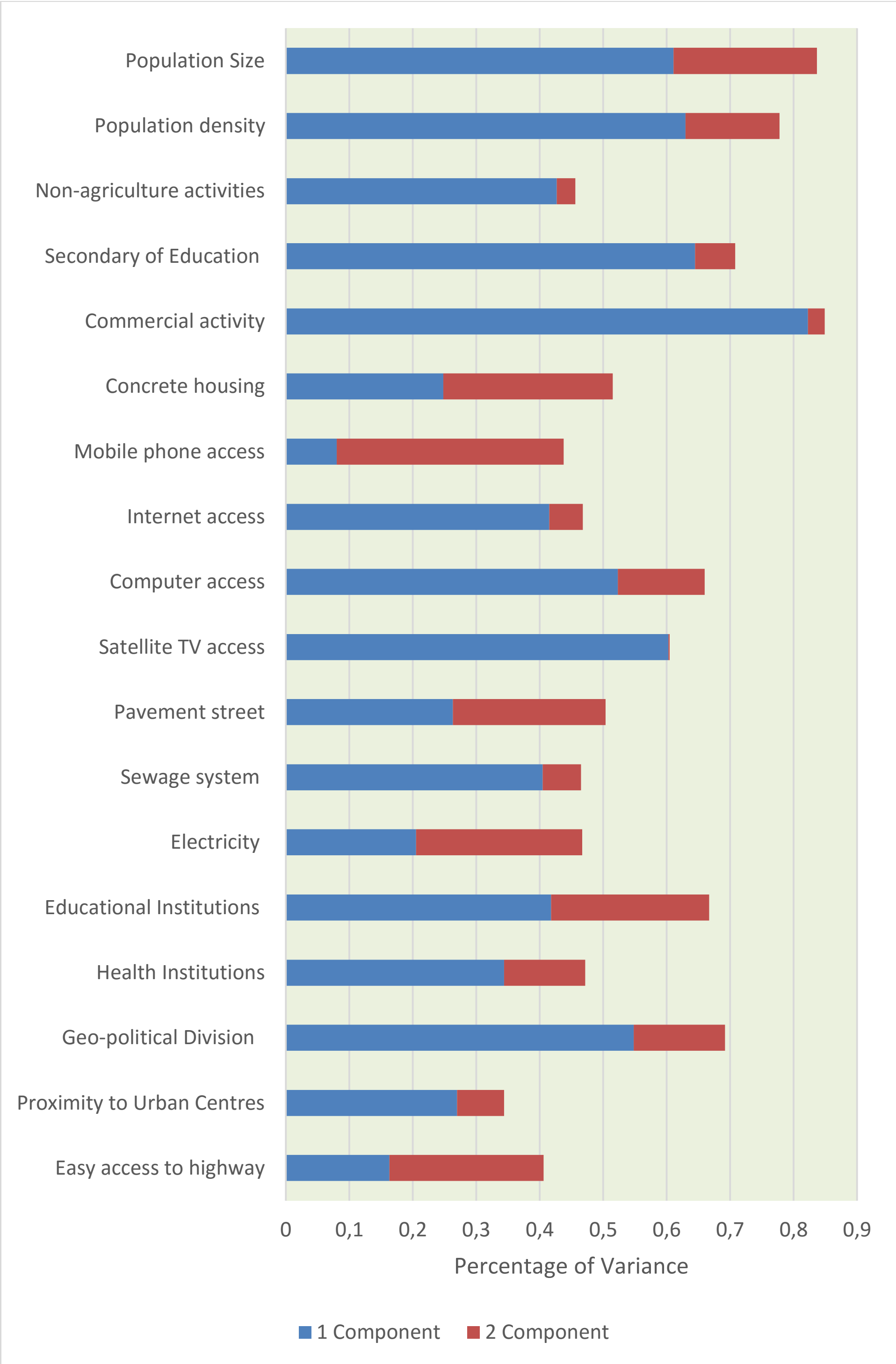
Continuation Supplementary Table 3

Author (Country-year of publications)	Area description	Population description	Sample method	Sample size	Urban definition	Response rate	Overall quality
Dias et al. (Brazil-2016)	+	+	+	+	+	na	High
Ponte et al. (Brazil-2016)	-	+	+	+	+	na	Medium
Gaviola et al. (Peru-2016)	+	+	+	+	+	+	High
Bedolla-Barajas et al. (Mexico-2017)	+	+	+	+	+	+	High
Han et al. (Argentina-2017)	+	+	-	+	+	+	Medium
Kumar et al. (India-2017)	-	+	+	+	-	+	Low

(na) not applicable; (unk) unknown; (+) adequate; (-) not adequate

Appendix VI: Supplementary Files Paper 2

Supplementary Figure SF1. Percentage of variance accounted by variables of each dimension.



Supplementary Table 1. Categories and Quantifications derived from Categorical Principle Component Analysis.

Population Size			4296.85	1	3.114	78.95	1	2.945
Category	**Freq**	**Value**	4583.00 - 4587.76	2	3.114	82.28	1	3.439
36 - 226	98	-0.315	4864.89 - 4899.78	2	9.148	85.07	1	3.439
227 - 461	189	-0.231	**Non-agricultural activities**			86.22 - 86.31	2	5.432
468 - 682	25	0.688	**Category**	**Freq**	**Value**	95.7	1	5.432
706 - 922	7	1.288	0	1	-0.953	98.15	1	5.432
1142	1	1.367	3.85 - 4.76	5	-0.718	**Adult secondary education**		
2597 - 2767	2	2.14	5.08 - 6.90	15	-0.718	**Category**	**Freq**	**Value**
3080 - 3186	2	2.971	6.94 - 8.55	10	-0.718	0	1	-1.197
3306	1	2.971	8.82 - 10.68	18	-0.718	0.81	1	-1.197
3634	1	4.297	10.74 - 12.62	21	-0.605	1.2	1	-1.197
4726	1	4.942	12.63 - 14.49	19	-0.524	1.89 - 2.31	7	-1.197
10169	1	6.015	14.58 - 16.39	19	-0.469	2.73 - 3.23	6	-1.17
29310 - 29356	2	9.225	16.51 - 18.33	19	-0.469	3.32 - 3.92	7	-0.991
Population density			18.75 - 20.18	14	-0.45	3.98 - 4.69	15	-0.899
Category	Freq	Value	20.54 - 21.98	12	-0.45	4.76 - 5.44	13	-0.723
1.49 - 9.02	36	-0.288	22.22 - 23.76	14	-0.351	5.47 - 6.15	17	-0.723
9.09 - 98.97	248	-0.288	24.36 - 25.68	9	-0.351	6.25 - 6.94	18	-0.669
107.34 - 175.27	7	0.152	26.09 - 27.59	9	-0.156	7.02 - 7.62	15	-0.661
209.28 - 282.03	2	0.453	28.00 - 29.55	14	-0.156	7.73 - 8.40	17	-0.599
474.79 - 558.14	2	0.453	30.00 - 31.51	15	-0.156	8.44 - 9.09	16	-0.593
884.51	1	0.475	32.14 - 33.62	6	-0.15	9.19 - 9.92	16	-0.261
956.23	1	0.475	34.01 - 35.36	10	0.166	10.00 - 10.67	8	-0.261
1346.62 - 1384.59	2	0.672	35.59 - 37.44	6	0.166	10.67 - 11.29	14	-0.261
1399.89	1	0.672	38.16 - 39.31	6	0.166	11.46 - 12.08	7	-0.261
1543.19	1	0.672	39.39 - 41.24	5	0.166	12.20 - 12.88	14	0.173
1600.85	1	0.672	41.28 - 42.86	12	0.262	12.90 - 13.61	16	0.173
1692.75	1	0.672	43.33 - 45.05	6	0.262	13.73 - 14.29	9	0.173
1831.26	1	0.672	45.28 - 46.81	9	0.262	14.49 - 15.10	12	0.173
1948.75 - 1984.76	2	1.304	47.68 - 48.59	6	0.466	15.24 - 15.85	7	0.173
2125.48	1	1.304	49.04 - 50.79	8	0.466	15.89 - 16.56	11	0.194
2289.62	1	1.304	52.48	1	0.466	16.67 - 17.31	9	0.297
2320.82 - 2325.26	2	1.304	52.83 - 54.55	6	0.466	17.53 - 17.91	6	0.537
2414.12 - 2465.53	4	2.275	54.90 - 56.52	4	0.466	18.13 - 18.83	7	0.537
2502.83	1	2.547	56.94 - 58.33	3	0.466	19.30 - 19.39	3	0.537
2696.57	1	2.547	59.19 - 59.70	2	1.396	19.59 - 20.26	7	0.537
2923.71	1	2.547	61.11 - 62.21	3	1.396	20.55 - 20.72	3	0.871
3053.99	1	2.547	62.86 - 63.21	4	1.396	21.13 - 21.58	8	0.871
3196.02	1	2.547	64.71	1	1.396	21.96 - 22.50	3	0.871
3260.98	1	2.547	66.85 - 67.46	2	2.945	22.65 - 22.96	4	0.871
3357.47	1	2.547	68.75 - 69.44	4	2.945	23.49 - 23.59	2	1.785
3541.13	1	2.547	71.82	1	2.945	24.20 - 24.66	4	1.785
3688.84	1	2.547	73.13	1	2.945	24.82 - 25.25	3	1.785
3773.54	1	2.547	74.6	1	2.945	25.7	1	1.785
4038.19	1	3.114	75.66 - 76.44	2	2.945	26.39 - 27.01	2	1.785

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27.08 - 27.74	5	1.785	30.51 - 30.85	2	1.881	65.38	1	1.097
27.84 - 28.00	3	1.785	31.62	1	1.881	66.67 - 66.94	3	1.097
28.72 - 28.99	3	1.785	33.89	1	3.44	68.83 - 69.81	3	1.097
30.29 - 30.58	2	1.785	34.58 - 34.68	2	3.44	72	1	1.097
31.03 - 31.33	2	1.785	37.86	1	3.44	72.50 - 74.19	3	1.097
32.39	1	1.83	38.24 - 38.90	3	3.44	76.67 - 77.30	2	1.097
33.5	1	2.315	39.09	1	3.44	**Mobile phone access**		
33.98	1	5.315	44.22	1	4.135	**Category**	**Freq**	**Value**
38.39	1	6.972	51.05	1	5.274	18.97	1	-2.49
43.04	1	6.972	51.71	1	10.878	30.36	1	-2.49
Commercial activity			**Cement housing**			33.33	2	-2.49
Category	**Freq**	**Value**	**Category**	**Freq**	**Value**	35.00 - 35.14	2	-2.49
0	23	-0.449	.00 - 1.08	24	-2.052	37.18 - 37.86	4	-2.226
.53 - .93	12	-0.449	1.56 - 3.16	14	-1.353	38.24	1	-1.971
1.10 - 1.87	25	-0.449	3.39 - 5.17	6	-1.199	39.76 - 40.00	3	-1.971
1.94 - 2.88	41	-0.449	5.88 - 6.90	4	-1.199	41.18	1	-1.971
2.90 - 3.75	26	-0.429	7.41 - 9.09	13	-1.01	42.35 - 42.86	3	-1.971
4.00 - 4.76	21	-0.429	9.33 - 10.94	6	-1.01	44.23	1	-1.655
4.84 - 5.71	24	-0.382	11.11 - 12.99	9	-1.01	47.23 - 47.73	4	-1.655
5.75 - 6.56	14	-0.382	13.21 - 15.00	18	-0.964	48.78	1	-1.655
6.73 - 7.58	12	-0.382	15.22 - 16.67	12	-0.41	50	1	-1.655
7.69 - 8.54	9	-0.252	17.24 - 18.82	9	-0.41	51.58	1	-1.655
8.65 - 9.46	9	-0.252	19.61 - 20.75	9	-0.312	52.07 - 53.33	6	-1.655
9.52 - 10.40	14	-0.015	21.05 - 22.77	12	-0.312	53.62 - 54.24	4	-1.655
10.57 - 11.36	9	0.104	23.44 - 24.64	7	-0.018	55.93 - 56.72	5	-0.703
11.59 - 12.06	3	0.104	25.00 - 26.83	14	-0.018	57.45 - 57.89	6	-0.703
12.50 - 12.88	9	0.104	27.06 - 28.85	6	-0.018	58.70 - 59.78	7	-0.703
13.33 - 13.91	8	0.104	28.89 - 30.77	10	-0.018	60.22 - 60.53	6	-0.689
14.34 - 14.96	5	0.104	30.95 - 32.56	13	0.2	61.18 - 62.07	7	-0.536
15.29 - 16.09	4	0.27	32.91 - 34.67	9	0.734	62.77 - 63.64	11	-0.536
16.18 - 17.07	7	0.442	34.78 - 36.36	12	0.734	64.00 - 64.94	11	-0.536
17.14 - 17.88	7	0.496	37.04 - 38.46	12	0.734	64.95 - 66.20	9	-0.536
18.06 - 18.78	4	0.496	39.53 - 40.54	5	0.734	66.28 - 67.44	11	-0.536
19.15 - 19.87	4	0.496	41.03 - 42.55	8	0.895	68.00 - 68.75	10	-0.536
20.75	1	0.496	42.94 - 44.44	9	0.895	68.89 - 70.00	13	-0.536
21.37 - 21.67	2	0.496	44.64 - 46.51	10	1.068	70.18 - 71.25	22	-0.536
22.22 - 22.58	3	0.496	46.81 - 48.50	11	1.068	71.43 - 72.46	11	0.314
23.20 - 23.49	3	0.836	48.68 - 50.50	14	1.068	72.73 - 73.91	17	0.314
24.53 - 24.64	2	0.836	50.65 - 52.50	9	1.068	74.00 - 75.18	16	0.314
24.71 - 25.30	4	0.836	52.63 - 54.26	5	1.068	75.32 - 76.47	13	0.472
25.76	1	0.836	54.72 - 56.45	8	1.068	76.54 - 77.78	19	0.646
26.81 - 27.14	4	0.836	56.79 - 57.14	3	1.097	78.02 - 79.09	15	0.646
27.54 - 28.30	2	0.836	59.09 - 60.27	5	1.097	79.25 - 80.36	15	0.646
28.57 - 28.87	3	0.836	60.53 - 62.35	7	1.097	80.41 - 81.61	16	0.874
29.46	1	1.881	62.90 - 64.17	4	1.097	82.00 - 82.98	10	0.874

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83.15 - 84.21	6	0.874	9.52	1	2.933	21.62	1	2.804
84.38 - 85.53	6	1.18	10.28 - 10.34	2	2.933	23.08	1	2.804
85.71 - 86.84	8	1.18	10.69	1	4.077	24.84	1	5.014
87.18 - 87.50	6	1.18	11.76	1	4.077	27.94	1	5.483
88.24 - 89.19	6	1.286	12.9	1	4.077	**Satellite TV access**		
89.58 - 90.70	3	1.286	13.04	1	4.077	**Category**	**Freq**	**Value**
90.91 - 91.67	3	1.286	13.33	1	4.077	0	165	-0.561
92.16 - 92.52	2	3.002	**Computer access**			.83 - 1.37	31	-0.263
93.33	1	3.002	**Category**	**Freq**	**Value**	1.45 - 2.13	27	-0.263
95.74	1	3.002	0	101	-0.887	2.15 - 2.86	14	0.373
96.97	1	3.002	.70 - .87	2	-0.663	3.03 - 3.61	18	0.373
97.22	1	3.002	.91 - 1.33	15	-0.659	3.65 - 4.35	7	0.487
Internet access			1.37 - 1.79	17	-0.457	4.38 - 5.08	12	0.487
Category	**Freq**	**Value**	1.89 - 2.20	16	-0.231	5.17 - 5.63	4	0.487
0	139	-0.686	2.30 - 2.70	18	-0.231	5.88 - 6.52	8	0.763
.66 - .73	3	-0.686	2.82 - 3.13	17	-0.231	6.82 - 7.14	3	0.763
.88 - 1.00	8	-0.686	3.23 - 3.64	17	-0.182	7.46 - 7.81	3	0.763
1.03 - 1.26	24	-0.273	3.65 - 4.00	11	0.091	8.41 - 8.77	4	0.763
1.27 - 1.49	12	-0.259	4.11 - 4.55	15	0.263	8.82 - 9.52	7	0.763
1.52 - 1.75	20	-0.092	4.60 - 4.92	5	0.263	9.68 - 10.14	5	1.679
1.79 - 2.00	7	-0.092	5.05 - 5.48	12	0.263	10.39 - 10.91	2	1.679
2.04 - 2.17	8	-0.092	5.62 - 5.83	5	0.263	12.00 - 12.43	4	1.679
2.27 - 2.50	14	-0.086	5.97 - 6.39	9	0.529	12.77	1	1.679
2.53 - 2.74	10	0.208	6.45 - 6.82	8	0.529	16.68	1	1.705
2.78 - 2.92	4	0.208	6.90 - 7.14	5	0.529	17.28	1	1.705
3.03 - 3.23	13	0.6	7.44 - 7.66	4	0.89	18.42 - 18.82	2	1.705
3.28 - 3.45	9	0.6	7.81 - 8.24	6	1.02	19.70 - 19.80	2	1.927
3.51 - 3.70	6	0.6	8.33 - 8.70	7	1.183	23.21	1	1.927
3.77 - 3.97	4	0.6	8.82 - 8.93	3	1.183	24.44	1	1.927
4.00 - 4.05	3	0.6	9.24 - 9.52	5	1.183	29.6	1	1.927
4.29 - 4.48	7	0.6	9.68 - 10.02	4	1.183	32.18	1	1.927
4.55	2	0.6	10.34 - 10.53	3	1.183	39.13	1	1.927
4.88 - 4.92	2	0.6	10.61 - 10.94	3	1.94	41.34	1	4.017
5.00 - 5.10	2	0.672	11.54 - 11.91	2	2.282	45.95	1	4.017
5.26 - 5.41	6	1.914	12.06	1	2.282	52.49	1	8.541
5.49 - 5.62	3	1.914	12.5	1	2.282	66.18	1	8.541
5.77	1	1.914	12.91 - 13.16	2	2.305	**Paved streets**		
6.00 - 6.19	4	2.138	13.54 - 13.64	2	2.305	**Category**	**Freq**	**Value**
6.25 - 6.38	3	2.138	14.57	1	2.432	.00 - 1.30	178	-0.734
7.14 - 7.23	2	2.138	15.15	1	2.432	1.37 - 2.70	30	-0.253
7.49 - 7.69	2	2.933	15.19 - 15.52	2	2.432	2.94 - 4.44	18	0.113
8.11	1	2.933	16	1	2.432	4.49 - 6.00	15	0.113
8.82	1	2.933	17.54 - 17.78	3	2.432	6.10 - 7.58	7	0.213
9	1	2.933	18.79	1	2.804	7.81 - 8.89	3	0.918
9.3	1	2.933	19.57	1	2.804	9.32 - 10.71	8	0.918

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11.76 - 12.06	3	1.168	9.09	1	1.264	77.27 - 79.25	9	-0.324
12.50 - 13.79	5	1.168	9.38	1	1.264	79.59 - 81.63	8	-0.324
14.00 - 14.81	3	1.168	12.5	1	1.264	81.82 - 83.72	13	-0.208
15.56 - 16.82	4	1.168	15.49	1	1.264	84.04 - 85.98	24	-0.208
17.59 - 18.06	3	1.168	22.8	1	5.878	86.25 - 88.28	34	-0.171
19.98 - 20.00	3	1.876	25.99	1	5.878	88.52 - 90.70	33	0.631
21.88 - 22.78	4	1.876	30.37	1	5.878	90.91 - 92.94	31	0.817
23.81 - 24.14	2	1.876	35.17	1	5.878	93.12 - 95.12	32	0.817
25.00 - 25.71	3	1.876	41.68	1	5.878	95.29 - 97.06	27	1.149
27.42 - 27.63	2	1.876	51.33	1	5.878	97.67 - 99.01	12	1.398
28.17	1	1.876	61.17	1	5.878	100	10	1.398
29.63 - 31.03	4	1.876	74.03	1	5.878	**Educational institutions**		
31.43	1	1.876	**Electricity**			**Category**	**Freq**	**Value**
32.91 - 34.21	2	1.876	**Category**	**Freq**	**Value**	0	83	-0.2
35.48	1	1.876	0	2	-2.48	1	145	-0.2
36.00 - 37.31	2	1.876	1.35 - 2.22	3	-2.48	2	61	-0.069
37.50 - 38.46	3	1.876	2.90 - 3.57	2	-2.33	3	22	0.037
39.53	1	1.899	6.67	1	-2.33	4	11	1.072
40.58	1	1.899	7.84 - 8.51	2	-2.33	5	3	1.072
42.06 - 43.33	5	1.899	15.05	1	-2.33	7	1	1.072
44.78	1	1.899	17.39 - 17.44	2	-2.3	8	1	5.607
45.61	1	1.899	22.22 - 22.89	3	-2.3	9	1	6.683
47.41 - 48.08	2	1.899	25	1	-2.3	20	1	7.697
48.65 - 48.98	3	1.899	25.33 - 27.50	2	-2.3	21	1	12.959
51.46 - 51.76	2	1.899	29.55	1	-2.25	**Health institutions**		
56.53	1	1.899	32.81 - 34.15	2	-2.25	**Category**	**Freq**	**Value**
58.59	1	1.899	34.62 - 35.80	2	-2.25	None	305	-0.277
60	1	1.899	36.67 - 38.46	3	-1.68	Health centre	23	3.109
65.22	1	1.899	38.96 - 40.91	5	-1.24	Hospital	2	6.494
66.67	1	1.899	42.06	1	-1.24	**Close access to highway**		
71.11	1	1.899	44.12	1	-1.24	**Category**	**Freq**	**Value**
71.79	1	1.899	45.68 - 47.83	3	-1.24	No	221	-0.702
76.60 - 77.50	2	2.839	49.00 - 50.00	2	-1.24	Yes	109	1.424
Sewage system			50.60 - 52.27	5	-1.24	**Geo-political division**		
Category	**Freq**	**Value**	53.03 - 53.33	4	-1.14	**Category**	**Freq**	**Value**
.00 - .66	238	-0.36	55.17 - 55.70	2	-1.14	Rural area	292	-0.324
.67 - 1.35	20	0.254	57.14 - 58.33	4	-1.14	Community	30	1.877
1.41 - 2.08	22	0.366	59.38 - 60.53	2	-0.83	Town	5	3.978
2.13 - 2.72	17	0.497	61.54 - 62.71	7	-0.83	City	3	6.179
2.86 - 3.45	9	0.497	65.33	1	-0.83	**Proximity to urban centres**		
3.61 - 3.95	6	0.497	66.28 - 67.44	6	-0.75	**Category**	**Freq**	**Value**
4.65	1	0.497	68.42 - 70.11	7	-0.32	Far from the city	271	-0.452
5.13 - 5.38	3	0.497	70.49 - 72.17	6	-0.32	Close to the city	55	1.914
5.98	1	1.264	73.44 - 74.51	4	-0.32	City	4	4.279
7.67	1	1.264	75.00 - 77.01	10	-0.32			

Appendix VII: Supplementary Files Paper 5

Supplementary Table S1. Discrimination measures (dimensions) and proportion of variance explained for each dimension by variable groups.

Variable groups	Variables	Dimensions 1	2	Mean
Socioeconomic Status of the household	Father's education	0.510	0.345	0.428
	Mother's education	0.562	0.389	0.476
	Father's job	0.555	0.300	0.428
	Mother's job	0.478	0.321	0.372
	Household income	0.478	0.086	0.333
	% of Variance [A]	50.59	30.82	40.71
Characteristics of the child's home	Basic Services	0.794	0.062	0.428
	Drinking water source	0.799	0.456	0.628
	House construction materials	0.402	0.078	0.240
	Bathroom	0.688	0.328	0.508
	Electrical appliances	0.403	0.199	0.301
	Cooking fuel	0.221	0.127	0.174
	% of Variance [A]	55.11	20.83	37.97
Sedentary characteristic of the child	BMI Z value	0.188	0.011	0.100
	Soda	0.214	0.400	0.307
	Hamburger	0.460	0.182	0.321
	Exercise	0.026	0.204	0.115
	TV viewing	0.403	0.338	0.371
	% of Variance [A]	25.84	22.70	24.27
Agriculture activities of the household	Farm activities	0.342	0.231	0.287
	Contact with animals in farms	0.241	0.380	0.311
	Pigs breeding around home	0.447	0.018	0.233
	Chicken breeding around home	0.442	0.105	0.273
	Other farm animals around home	0.361	0.216	0.288
	% of Variance [A]	36.64	19	27.82

[A] % variance represents the sum of inertias for each constituent variable belonging to each variable group.

Supplementary Table S2. Socioeconomic status of the household: variables stratified by domains.

Variables	Categories	Domains					
		Low SES		Medium SES		High SES	
		n	%	n	%	n	%
Father's education[A]	<6 years	2356	79.0%	601	21.0%	68	7.0%
	6-11 years	488	16.4%	1966	68.8%	209	21.7%
	>11 years	138	4.6%	291	10.2%	688	71.3%
Mother's education[A]	<6 years	2385	80.0%	498	17.4%	63	6.5%
	6-11 years	485	16.3%	2095	73.3%	214	22.2%
	>11 years	112	3.8%	265	9.3%	688	71.3%
Father's job	Farm worker	2662	89.3%	1006	35.2%	121	12.5%
	Employee	174	5.8%	1197	41.9%	311	32.2%
	Trader	117	3.9%	600	21.0%	113	11.7%
	Professional	29	1.0%	55	1.9%	420	43.5%
Mother's job	House wife	2171	72.8%	1595	55.8%	368	38.1%
	Farm worker	573	19.2%	50	1.7%	8	0.8%
	Employee	158	5.3%	894	31.3%	133	13.8%
	Trader	73	2.4%	310	10.8%	125	13.0%
	Professional	7	0.2%	9	0.3%	331	34.3%
Income[B]	<=$170	2734	91.7%	1608	56.3%	258	26.7%
	$171-$340	198	6.6%	1106	38.7%	236	24.5%
	>$341	50	1.7%	144	5.0%	471	48.8%

[A] <6 years (incomplete primary); 6-11 years (incomplete secondary; >11 years (complete secondary and higher). [I]ncome is stratified by number of basic wages based on on a basic family income (canasta familiar) of US$170 in 2007.

Supplementary Table S3. Characteristics of the child's home: variables stratified by domains.

Variables	Categories	Domains					
		Transitional		Rudimentary		Basic Urban	
		n	%	n	%	n	%
Basic Services	0-1 Services	2017	95.3%	2211	97%	26	1.1%
	2-3 Services	100	4.7%	69	3%	2382	98.9%
Source of drinking water	River/well	899	42.5%	2165	95%	2	0.1%
	Piped	1119	52.9%	28	1.2%	15	0.6%
	Potable	99	4.7%	87	3.8%	2391	99.3%
House construction materials	Wood/Bamboo	925	43.7%	2003	87.9%	674	28.0%
	Concrete/others	672	31.7%	124	5.4%	567	23.5%
	Concrete	520	24.6%	153	6.7%	1167	48.5%
Bathroom	Field	288	13.6%	1291	56.6%	71	2.9%
	Latrine	1757	83.0%	987	43.3%	669	27.8%
	Toilet	72	3.4%	2	0.1%	1668	69.3%
Electrical appliances	0-2 appliances	623	29.4%	1851	81.2%	408	16.9%
	3 appliances	889	42.0%	240	10.5%	844	35.0%
	4 appliances	605	28.6%	189	8.3%	1156	48%
Cooking fuel	Only gas	1872	88.4%	1270	55.7%	2287	95%
	Gas/wood/charcoal	245	11.6%	1010	44.3%	121	5%

Supplementary Table S4. Sedentary characteristics of the child: variables stratified by domains.

		Domains					
		High		Medium		Low	
Variables	Categories	n	%	n	%	n	%
BMI Z score	Normal weight	850	76.1%	1014	65.0%	3991	96.7%
	Overweight	267	23.9%	545	35.0%	138	3.3%
Fizzy Drinks	Sometimes	170	15.2%	155	10.0%	1456	35.4%
	1-4 times by week	261	23.4%	1274	82.0%	2234	54.3%
	> 4 times by week	686	61.4%	125	8.0%	424	10.3%
Hamburger	Never	260	23.3%	621	39.9%	3298	80.2%
	Sometimes	615	55.1%	233	15.0%	751	18.3%
	once a month	242	21.7%	703	45.2%	63	1,5%
Exercise	Daily	922	82.6%	623	40.0%	3514	85.4%
	1-3 times per week	168	15.1%	882	56.7%	523	12.7%
	Sometimes	16	1.4%	5	0.3%	53	1.3%
	Barely	10	0.9%	46	3.0%	26	0.6%
TV viewed (daily)	<1 hours	21	1.9%	58	3.7%	1299	31.5%
	1-3 hours	354	31.7%	1430	91.7%	2345	56.8%
	>=4 hours	742	66.4%	71	4.6%	485	11.7%

Supplementary Table S5. Agricultural activities of the household: variables stratified by domains

Variables	Categories	Domains			
		Farm environment		Non-farming environment	
		n	%	n	%
Farm activities	No	416	13.8%	2506	66.1%
	Yes	2595	86.2%	1288	33.9%
Contact with animals on farms	No	1738	57.7%	3537	93.2%
	Yes	1273	42.3%	257	6.8%
Pigs breeding around house	No	914	30.4%	3393	89.5%
	Yes	2095	69.6%	397	10.5%
Chicken breeding around house	No	59	2.0%	1539	40.6%
	Yes	2951	98.0%	2251	59.4%
Other farm animals around house	No	1051	34.9%	3261	86.1%
	Yes	1959	65.1%	528	13.9%