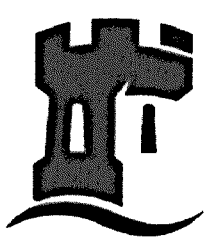

The University of Nottingham

EARLY LIFE DETERMINANTS OF WHEEZE AND ALLERGIC DISEASE: A LONGITUDINAL STUDY IN AN ETHIOPIAN BIRTH COHORT

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ABSTRACT

Background

The hypothesis that paracetamol may increase the risk of asthma and other allergic disease has gained consistent support from epidemiological studies, but evidence from longitudinal cohort studies, particularly those looking at the timing and dose of exposure are lacking. Epidemiological studies have also reported an inverse relation between gastro-intestinal infections including *Helicobacter pylori*, commensal bacteria and geohelminths and asthma and allergic disease, however, data from longitudinal birth cohort study are scarce. This thesis has therefore investigated the effects of paracetamol, *H. pylori* and other gastro-intestinal infections on the incidence and prevalence of allergic diseases and sensitization in a low-income birth cohort in which confounding by social advantage and other medical interventions is unlikely to play a role.

Methods

In 2005/6 a population based cohort of 1065 pregnant women from Butajira, Ethiopia was established, to whom 1006 live singleton babies were born, and these children have been followed-up from birth to age five. At ages one, three and five, the International Study of Asthma and Allergies in Children (ISAAC) questionnaires were administered to the mothers to obtain data on wheeze, eczema and rhinitis. Allergen skin tests to *Dermatophagoides pteronyssinus* and cockroach were performed at ages three and five. Data on child's use of paracetamol, and various early life putative risk factors, including levels of Der p 1 and Bla g 1 allergen in the child's bedding and symptoms of respiratory tract infections were also measured. Stool samples were collected at ages three and five for analysis of *H. pylori* antigen using a rapid test (Medimar immunocard),

as well as for geohelminths (at ages one, three and five) and selected commensal bacteria (at age three). Multivariate logistic regression was used to determine the independent effects of various markers of paracetamol use on the incidence of each outcome between age one and five, as well as on prevalence at age five. Similar analyses were also carried out to determine the independent effects of *H. pylori,* geohelminths and commensals on the incidence and prevalence of each outcome.

Results

Effects of paracetamol

Of the 1006 children in the cohort at birth, 863 children were successfully followed up at age five (94% of surviving mother-child dyads). Wheeze and eczema incidence between the ages of one and five were reported in 5.9% (40/676) and 5.8% (39/700) of children respectively, and rhinitis and sensitization incidence between ages three and five were found in 3.9% (31/798) and 2.0% (15/766) of children respectively. Paracetamol use in the first three years of life was common, with 18% reported use at age one but not three, 23% at age three but not one and 21% at both time points. Use in the first year of life was significantly associated with a dose-dependent increased risk of incident wheeze between ages one and three (fully adjusted ORs, 95% CI, 1.77; 0.96, 3.26 for 1-3 tablets and 6.78; 1.89, 24.39 for ≥ 4 tablets in past month versus never), but not eczema. The risk of incident wheeze, eczema, rhinitis and sensitization between ages three and five was increased in those exposed, significantly so for incident eczema ($p=0.02$) and borderline significant for rhinitis ($p=0.07$), with fully adjusted odds ratios (ORs), including for symptoms of respiratory tract infections, for persistent exposure (ages one and

three) versus never of 3.82 (95% CI 1.36, 10.73) and 3.10 (1.00, 9.57) respectively. Borderline significant trends were also seen between paracetamol dose in the first three years of life and incident eczema and rhinitis, with adjusted ORs for heavy reported use compared to low of 1.59 (0.44, 5.74; p trend=0.06) and 2.31 (0.72, 7.46; p trend=0.07) respectively, but not with incident wheeze (fully adjusted OR=3.64; 1.34, 9.90, p trend=0.11). Cross-sectional analysis at age five resulted in significant positive dose-response effects of lifetime use (use at ages one, three and five) in relation to the prevalence of all outcomes.

Effects of gastro-intestinal infection

H. pylori infection was found in 17% of the children at age three but not five, 21% at age five but not three years, and 25% at both ages. In the longitudinal analysis, *H. pylori* infection at age three was significantly associated with a decreased risk of incident eczema between ages three and five years (adjusted OR, 95% CI, 0.31; 0.10, 0.94, p=0.02), but the associations with incident wheeze, rhinitis and sensitization were not significant. In cross-sectional analysis at age three, *H. pylori* infection was associated with a borderline significant reduced risk of eczema (adjusted OR, 95% CI, 0.49; 0.24, 1.01, p=0.05) and *D. pteronyssinus* sensitization (adjusted OR, 95% CI, 0.42; 0.17, 1.08, p=0.07), and a significant inverse association between current exposure to *H. pylori*, and any sensitization at age five (adjusted OR, 95% CI, 0.26; 0.07, 0.92, p=0.02). However, no significant associations were seen for wheeze and rhinitis.

The prevalence and intensity of geohelminth infection (hookworm, *Ascaris*

lumbricoides and *Trichuris trichiura*) were found to be low in this cohort, with only 4% of children infected at age one, 9% at age three and only 0.2% at both ages. The risk of new onset wheeze between ages one and three was lower in those infected at age one (3.6%) than uninfected (7.8%), but infection was insufficiently prevalent to compute estimates of effect. Exposure to geohelminth infections in the first three years of life was not significantly associated with the incidence of reported outcomes or sensitization. However, *A. lumbricoides* infection was associated with a borderline increased risk of incident eczema between ages three and five (adjusted OR, 95% CI, 2.86; 1.04, 7.86, p=0.07). Children at age three were commonly colonized with enterococci 38% (207/544), lactobacilli 31% (169/544) and bifidobacteria 19% (103/544). However, none of these commensal bacteria were associated significantly with either incidence or prevalence of allergic outcomes.

Conclusions

This longitudinal study from a developing country birth cohort provides further support for an association between early life use of paracetamol and increased risk of wheeze and allergic disease, which is unlikely to be explained by aspirin avoidance, reverse causation or confounding by indication. Furthermore, among young children in this cohort, the study found novel evidence to support the hypothesis of a protective effect of *H. pylori* infection on the risk of allergic disease, but no evidence to support an etiological role for the microflora enterococci, lactobacilli or bifidobacteria. The power of the study to explore the role of geohelminth infection on wheeze and allergic disease was limited by few infected children, and therefore understanding on this particular relation has not been much further advanced.

PAPERS ARISING FROM THIS THESIS

The work in this thesis has been published in the following journals (Appendix I):

Papers directly related to the work presented in this thesis:

1. Amberbir A, Medhin G, Alem A, Britton J, Davey G, Venn A. The role of acetaminophen and geohelminth infection on the incidence of wheeze and eczema: a longitudinal birth-cohort study. Am J Respir Crit Care Med. 2011;183 (2):165-70.
2. Amberbir A, Medhin G, Erku W, Alem A, Rebecca S, Robinson K, Fogarty A, Britton J, Venn A, Davey G. Effects of *Helicobacter pylori*, geohelminth infection, and selected commensal bacteria on the risk of allergic disease and sensitization in 3-year-old Ethiopian children. Clin Exp Allergy. 2011(41):1422-1430.

Other papers arising from the cohort but not directly related to the thesis:

1. Belyhun Y, Amberbir A, Medhin G, Erko B, Hanlon C, Venn A, Britton J, Davey G. Prevalence and risk factors of wheeze and eczema in 1-year-old children: the Butajira birth cohort, Ethiopia. Clin Exp Allergy. 2010 (40):619-626.
2. Amberbir A, Medhin G, Hanlon C, Britton J, Venn A, Davey G. Frequent use of paracetamol and risk of allergic diseases among women in an Ethiopian population. PLoS ONE 2011; 6(7): e22551.

PRESENTATIONS/ABSTRACTS

1. Amberbir A, Medhin G, Alem A, Britton J, Davey G, Venn A. The role of paracetamol, geohelminths and other environmental exposures on the incidence of wheeze and eczema in an Ethiopian birth cohort. Am J Respir Crit Care Med 181; 2010: A2505 (American Thoracic Society (ATS) 2010) – oral presentation.
2. Amberbir A, Medhin G, Erku W, Alem A, Robinson K, Fogarty A, Britton J, Venn A, Davey G. Effects of Helicobacter pylori and intestinal microflora on the risk of allergic disease and sensitization in young children. Am J Respir Crit Care Med 181; 2010: A4143 (ATS 2010) – poster discussion.
3. Amberbir A, Medhin G, Alem A, Hanlon C, Britton J, Venn A, Davey G. Frequent use of paracetamol and risk of allergic diseases among women in an Ethiopian population. Eur Respir J, E5252 (European Respiratory Society (ERS) 2010) - E-communication session.
4. Amberbir A, Medhin G, Abegaz W, Hanlon C, Robinson K, Fogarty A, Britton J, Venn A, Davey G. Exposure to Helicobacter pylori infection in early childhood and the risk of allergic disease and atopic sensitization: a longitudinal birth cohort study. Am J Respir Crit Care Med, A28908 – Poster discussion (Accepted for the forthcoming ATS conference in May 2012).
5. Amberbir A, Medhin G, Hanlon C, Britton J, Davey G, Venn A. Early life exposure to acetaminophen and the prevalence and incidence of allergic disease and sensitization: 5 year follow-up of an Ethiopian birth cohort. Am J Respir Crit Care Med, A28671 – Poster presentation (Accepted for the forthcoming ATS conference in May 2012).

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Very special thanks to my family, my wife, Misrak Melese, my daughter, Saron Alemayehu, who were always my source of love and great inspiration. I have always thought I had a special family - I am greatly indebted to you.

STATEMENT OF AUTHOR'S ROLE

I played a significant role in designing the study and fieldwork for Butajira birth cohort from just before the three year follow-up in February 2008 until the five year follow-up in June 2011. During that time, I was responsible for everything from obtaining ethical approval for the study; organising and overseeing the data collection including providing training and day-to-day supervision of data collectors as they visited homes to collect questionnaire and skin test data, stool samples and dust samples; establishing a new rudimentary microbiology laboratory in Butajira (and learning the techniques required); preparing and importing the consumables needed for the fieldwork; organising dust samples analysis in Nottingham; designing databases for data entry; carrying out all data analyses; and writing all manuscripts relating to the research that I have led. The only follow-up which I was not responsible was data from the first year follow up of the cohort which was supervised by the Master's student before I joined the project.

I was also responsible for the dissemination of the findings to the local and international audiences. I have presented the findings at the American Thoracic Society conference (ATS) in May 2010 and at the European Respiratory Society meeting (ERS) in September 2010. I have also presented the work within Ethiopia and Africa at the Ethiopian Public Health Association annual conference (EPHA) in October 2010 and at the International Network for the Demographic Evaluation of Populations and Their Health in Developing Countries (INDEPTH Network) annual conference in September 2010, in Accra, Ghana.

DEDICATION

To my wife Misrak, my daughter Saron and to my kindest sister Tsige who accompanied me on this journey. Saron, I am sure you will understand when you see this thesis; the reason why I missed your birth while I was in the UK. I love you very much!

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LIST OF ABBREVIATIONS

Ag	Antigen
ALSPAC	Avon Longitudinal study of Parents and Children
ATS	American Thoracic Society
BCG	Bacillus Calmette-Guerin
BHR	Bronchial hyperresponsiveness
Bla g 1	Cockroach allergen type 1
BMI	Body mass index
BRHP	Butajira Rural Health Program
CagA	Cytotoxin-associated gene A
COPD	Chronic obstructive pulmonary disease
DALY	Disability-adjusted life year lost
Der p 1	Dust mite allergens type 1
DHS	Demographic and Health Survey
DPT/DTP	Diphtheria, Pertussis and Tetanus
DSS	Demographic Surveillance Site
ECHRS	European Community Respiratory Health Survey
EDHS	Ethiopian Demographic and Health Survey
ELISA	Enzyme-Linked Immunosorbent Assay
EOS	Outreach Strategy for Child Survival
ERS	European Respiratory Society
FeNO	Fractional exhaled nitric oxide
FEV_1	Forced expiratory volume in 1 second
FVC	Forced vital capacity
GP	General Practitioner
GSH	Glutathione
GSTP1	Glutathione S-transferase pi gene

HSDP	Health Sector Development Program
HSEP	Health Service Extension Program
IFN	Interferon
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IL	Interleukin
ISAAC	International Study of Asthma and Allergies in Childhood
MAS	German Multicentre Allergy cohort
NO_2	Nitrogen dioxide
NCDs	Non Communicable Diseases
NHANES	National Health and Nutrition Examination Survey
NSAIDs	Nonsteroidal anti-inflammatory drugs
OR	Odds ratio
PAR	Population-attributable risks
PCR	Polymerase chain reaction
PIAMA	Prevention and Incidence of Asthma and Mite Allergy
PM_{10}	Aerodynamic diameter of <10 μm
RCT	Randomized Clinical Trial
SO_2	Sulfur Dioxide
SPT	Skin prick test
TCRS	Tucson Children's Respiratory Study
TGF	Transforming growth factor
Th cells	T helper cells
Treg cells	Regulatory T-cells
95% CI	95% confidence interval

1 INTRODUCTION

1.1 ASTHMA AND ALLERGY

Asthma and allergic conditions are prevalent throughout the world, and have become one of the most common chronic disorders among children and adults.[1-3] At a conservative estimate, around 300 million people in the world have asthma and it accounts for 4 in every 1000 deaths worldwide.[4] It has been ranked 25th amongst the leading causes of death in terms of disability-adjusted (DALYs) life years lost, and the economic cost is significant.[4-6]

1.1.1 Definitions

Asthma is a chronic disorder of the airways often associated with variable airflow obstruction, and manifested by episodes of wheezing, cough, dyspnoea and chest tightness.[7] A disease condition characterised by the existence of hypersensitivity or sensitivity[a] reactions and initiated by specific immunological mechanisms (antibody- or cell-mediated) is termed allergic disease.[8] Asthma therefore can be allergic (resulting from immunological reactions, mostly immunoglobulin E mediated, but also non-IgE mediated) or non-allergic

[a] Objectively reproducible symptoms or signs initiated by exposure to a defined stimulus at a dose tolerated by normal persons.

asthma.[7, 8] Rhinitis is a hypersensitivity syndrome characterised by itching, sneezing, and nasal obstruction, and can also be accompanied by conjunctivitis which can be allergic (also known as hay fever) or non-allergic.[8] The term rhinitis will be used in this thesis. Eczema is a localized inflammation of the skin which may be atopic (dominated by IgE-antibody associated reaction) or non-atopic.[8] Atopy (also referred to as sensitization in the thesis) is a complex condition under genetic influence in which IgE-sensitised to allergens[b] occurring in the environment are produced.[8]

1.1.2 Measuring asthma in epidemiological studies

There is no gold standard definition for asthma owing to the complex nature of the disease including, multiple aetiological causes and variable symptom expression. Epidemiological studies have, either alone or in combination, used reported symptoms of asthma, doctor diagnosis of asthma or objective measurements such as bronchial hyperresponsiveness (BHR).[9-11]

Use of doctor's diagnosis of asthma as an epidemiological tool is unreliable because of wide variation in the diagnostic criteria.[10, 12] It is also influenced by availability of diagnostic facilities, and comparisons between populations, a focus of epidemiological research, are especially difficult.[9] An issue with the clinical

[b] An allergen is an antigen causing allergic disease.

labelling of asthma by a clinician is also that a diagnosis might have been given for transient symptoms that have since resolved.[10]

Despite a continuing debate about measuring asthma in epidemiological studies, [10] symptoms based questionnaires are widely used,[9, 13] and wheezing has been shown to be symptom that best predicts asthma (75% sensitivity and 87% specificity).[11] Luyt and colleagues found a reliability of 97% for 12-month prevalence of asthma and ever wheeze questionnaires responses, compared with the clinical diagnosis of asthma.[12] Even though most population based studies tend to use symptom questionnaires (e.g. wheezing or whistling in last 12 months) which are simple to use, cost effective and encourage a good response rate;[9, 13] bias associated with symptom recognition and recall is a problem.[9] Bronchial hyperresponsiveness (BHR),[9, 10] is often used as an adjunct to symptom-based questionnaires in epidemiological studies,[9, 10] because it has generally higher specificity (over 80%), although sensitivity is lower (less than 50%).[10]

1.1.3 Wheezing phenotypes in children

Several wheezing phenotypes in children have been described based on clinical and epidemiological observations.[14-16] The Tucson Children's Respiratory Study (TCRS) identified four wheezing phenotypes: no wheezing, transient early wheezing, late-onset wheezing, and persistent wheezing.[14] Interestingly, these wheezing phenotypes characteristically have distinct risk factors; maternal asthma, smoking, rhinitis, eczema, and atopy were seen to predict the persistent wheezing phenotype, and these children had significant lung function

impairments, whilst maternal smoking was seen to predict the transient phenotype and these children had normal lung function.[14] More recently, comparable wheeze phenotypes were identified from the Avon Longitudinal study of Parents and Children (ALSPAC),[15] and the Prevention and Incidence of Asthma and Mite Allergy (PIAMA)[16] birth cohorts using longitudinal latent class analysis. The PIAMA[16] study presented five wheezing phenotypes (never/infrequent wheeze, transient early wheeze, intermediate-onset wheeze, late-onset wheeze, and persistent wheeze) which were broadly similar to the findings from the ALSPAC cohort.[15] Characteristically the persistent phenotype was associated with asthma, atopy, lower lung function tests, and bronchial hyperresponsiveness (BHR)[15, 16] consistent with that identified by Martinez and colleagues.[14] Overall, it may be possible to infer that the transient wheezing phenotype has a favourable outcome (most of the children grow out of it),[14] whereas children with persistent wheezing phenotype may be destined to have a considerable deficit in lung function and airflow limitation, and progress to adult-onset asthma.[14-16]

1.1.4 Relation between atopy, asthma and allergic diseases

Various studies have independently identified atopy as a predictor of asthma in children and adults.[17-20] A prospective study recently reported a 6-fold increased risk of wheeze at the age of 12 years in those who were positive to house dust mite sensitization at 2 years.[20] A similar relation was reported from the ISAAC phase II multicountry study, however, it appeared in this study that the pooled association between atopy and asthma for the affluent countries was 2-fold higher than the non affluent countries, and that the strength of association increased with economic development.[18] Overall, Pearce and co-workers[19]

estimated, based on the available evidence, that the proportion of asthma cases attributable to atopy (defined as skin prick test positivity) in children is about 38%, with a similar attributable risk in adults.[19] Rhinitis, alone or with atopy, has also been shown to be related to asthma.[21, 22] The German Multicentre Allergy cohort (MAS) reported a nearly 4-fold increased risk of developing wheezing during adolescence in children with rhinitis in the first 5 years of life.[22] Shaaban *et al*[21] recently found evidence from longitudinal analyses of the ECRHS I and II study in adults that rhinitis predicts adult onset asthma with odds ratios ranging from 2.7 for non-allergic rhinitis to 3.5 for allergic rhinitis.[21] As with rhinitis and atopy, studies have also shown a link between childhood eczema and asthma.[23, 24] A meta analysis and systematic review which pooled data from the available birth cohort studies, reported that children with eczema in the first four years of life had an estimated 36% increased risk of developing asthma later in childhood.[23]

1.1.5 Geographical and temporal variation in the prevalence of asthma and allergic disease

1.1.5.1 Worldwide variation

The International Study of Asthma and Allergies in Childhood (ISAAC),[25, 26] conducted in children aged 6-7 and 13-14 years, and the European Community Respiratory Health Survey (ECHRS),[27] based on adults aged 20-40 years, were designed to assess the geographical and temporal variations in asthma and allergy.[4] The highest prevalence rates of current wheezing in childhood, as a measure of prevalence of current asthma, were reported in the UK (15.3%), New Zealand (15.1%), Australia (14.7%), Ireland (14.6%) and Canada

(14.1%), and the lowest prevalence estimates were found in Ethiopia (3.1%), Nepal (1.5%), Indonesia (1.1%) and Macau (0.7%), which gives an indication of the difference between developed and developing countries.[4] There was also found to be a 30-fold variation (range 1.4-39.7%) between countries in the prevalence of symptoms of rhinoconjunctivitis, with the lowest reported prevalence centres being similar to those seen for asthma symptoms.[28] There was also a substantial variation in the prevalence of reported eczema between centres, ranging from 2.0 to 15.6%.[28] The prevalence of atopy has also shown to vary greatly by country, with the highest being in China (45.3%) and the lowest found in rural Ghana (1.7%).[18] Marked differences in the prevalence of asthma and atopy were also documented in the ECRHS in adults, a higher prevalence being in English speaking countries than Eastern Europe and Mediterranean region.[27] Overall, there exists a wide geographical variation in the prevalence of asthma and allergic conditions worldwide, with substantial differences seen between developing and developed countries. This observation raises the possibility that a range of environmental factors including adoption of more affluent living styles, or other factors related to socio-economic disparity across the countries likely to be important.[4, 27, 28]

1.1.5.2 Temporal changes

A range of independent epidemiological studies, using the ISAAC and ECHRS allergy and environmental questionnaire, have demonstrated an increasing trend in asthma prevalence in both developed and developing countries over the last three decades.[28-33] Comparable data from the ISAAC Phase One and Phase Three studies in children aged 6-7 years between 1992-2003 showed the prevalence of asthma symptoms increased in 64% of the participated centres.[32]

Similarly, the prevalence of symptoms of rhinoconjunctivitis and eczema have shown an increase in 83% and 85% of the centres respectively.[32] The increase in all three symptoms were particularly evident in regions where the prevalence was previously low, including Asian-Pacific, India, Northern and Eastern Europe, and Africa.[32] This observation is particularly important as the increase was reported in low and middle income countries with a growing economy and industrialisation, and the role of environmental factors for the observed trend have been emphasised elsewhere.[30, 32]

More recently, however, there are studies reporting a stabilizing, or even a declining pattern of asthma and allergic conditions in developed countries.[34-38] Gupta and colleagues in the UK using data from primary care consultations and GP records, showed hospitalisation for eczema had stabilized whilst for rhinitis has fallen since 1995.[34] Another study in the British Isles also reported a similar decline in the prevalence of self reported wheezing, allergic rhinoconjunctivitis and eczema.[37] A multicenter study from the ECRHS between 1998 and 2003 has reported an increase in asthma diagnosis, and use of medication, but no increase in wheezing and other bronchial symptoms across the European countries. Most of the decreasing or stabilising trend in asthma and allergic conditions however is reported from developed countries, therefore the possibility that the observed changes have arisen from improved diagnostic facilities, use of effective medication and uptake of the service by the patients remain unclear.[39]

1.1.5.3 Asthma and allergic disease in Africa

Data on the prevalence of asthma and allergic diseases in most African countries come from the ISAAC multicountry studies. The ISAAC study divided the African study centres into English (South Africa, Nigeria, Kenya and Ethiopia) and French speaking (Algeria, Morocco and Tunisia), and higher prevalences were reported in the former.[32] The prevalences were generally less than those reported in developed countries, with only South Africa seeing rates approaching those found in the UK (12-month wheeze, rhinoconjunctivitis, and eczema prevalence of 20.3%, 20.7% and 13.3% respectively).[32] Studies have also reported a marked variation between urban and rural area within African countries; for example in Ghana[40], Kenya[41] and Zimbabwe,[42] a higher prevalence of asthma was seen in the urban than rural areas.

Comparison between the ISAAC Phase One and Three studies between 1992-2003 also found an increase in the prevalence of asthma, rhinoconjunctivitis and eczema symptoms in most African countries, however, the increase was more apparent in 13- to 14 years age group than in the younger aged 6 to 7 children.[32] A further insight into the trends of asthma prevalence in Africa has been reported in Ghanaian schoolchildren by Addo-Yobo and colleagues.[40] This study showed a 2.1% increase in the prevalence of exercise-induced bronchospasm between 1993 to 2003, and a doubling in prevalence of sensitization over the ten-year period.[40]

1.1.5.4 Asthma and allergic disease in Ethiopia

Prevalence data from Ethiopia, the country in which the research presented in this thesis is set, come from a variety of sources including the ISAAC multicentre study[32] and elsewhere from prevalence studies.[43-45] The 12-month prevalence of wheeze among 13- to 14 years old children in Addis Ababa was 9.1%, rhinoconjunctivitis 9.9%, and eczema 19.0%.[32] A study of adults in Jimma, southwest Ethiopia, over five years before the ISAAC Phase Three study,[32] using comparable measurements, reported a 12-month wheeze prevalence much lower than the ISAAC Phase Three study (3.7% in urban and 1.2% in rural areas), and dust mite sensitization was 4% in urban and 11.8% in rural.[43] Another study in Jimma of 1 to 5 year-old-children reported a higher prevalence of wheeze in urban (4.4%) than rural areas (2.0%);[44] the prevalence of eczema was also low, overall being 1.2% and higher in urban than rural areas.[46] Prevalence studies were also reported from Butajira, central Ethiopia, the same area in which the research in this thesis is based. Davey and colleagues[45] in a population sample of urban and rural adults and children found a high prevalence of wheeze of 10.5% which was higher in urban than rural areas.[45]

In summary, asthma prevalence appears to be increasing in African countries, at a time where many are experiencing an epidemiologic and economic transition.[47] Factors relating to this transisition may therefore have contributed to the observed variations and trends in asthma and allergic disease.

1.2 RISK FACTORS OF ASTHMA AND ALLERGIC DISEASE

1.2.1 Genetic factors

Many studies have identified the importance of gene-environment interactions in the causation of asthma and allergic diseases,[48-52] though most agreed that genetics by itself is unlikely to explain the worldwide increasing prevalence of asthma, and allergic disease.[53] A Danish birth cohort comprising 20,888 twin pairs revealed higher concordance for asthma in monozygotic than dizygotic twins, evidence supporting a role of genetics in asthma aetiology.[50] The epigenetics of asthma has been reviewed by Miller and colleagues,[48] and in the past decade, several gene-by-environment interactions have been identified. These include, interactions between gene variant and both active[54] and passive smoking[55], gene CD14 and farm exposure, [56] gene β_2-Adrenalgic receptor and obesity,[57] and gene IL-4 and infection with *H. pylori.*[58]

Overall, it has been suggested that a range of environmental factors, interacting possibly with genetics, might altogether explain the worldwide changes in the prevalence of asthma and allergic disease. The next sections describe the most common environmental factors identified in epidemiological studies which are linked to asthma and allergic diseases.

1.2.2 The hygiene hypothesis

In the mid 1970s Gerrard and colleagues examined serum IgE levels and the prevalence of certain atopic diseases in 819 individuals in 176 white Canadian

families living in central Saskatchewan and in 275 individuals in 58 Indian (called Metis) families living in northern Saskatchewan.[59] The prevalence of asthma, eczema and urticaria was greater in the white community than in the more rural Metis community who had increased exposure to geohelminths, bacterial and viral infection and diminished access to medical care. In contrast to this, the geometric mean total IgE was three-fold higher in the Metis community compared with the white, suggesting that atopic disease may be the price paid due to over-cleanliness.[59]

In the late 1980s, David Strachan showed independent inverse associations between hay fever and family size and presence of older sibling and eczema.[60] He suggested that declining family size, improvements in household facilities, and hygienic behaviour have reduced the opportunity for infection in smaller families,[60] a theory which then became colloquially known as the 'hygiene hypothesis. The hygiene theory proposes that lower exposure to infections at a younger age may be responsible for the increase in atopic diseases, and this theory has since gained support from a range of epidemiological studies.[61-67] Studies since have shown consistent findings with relation to older siblings.[65, 66] A review of 53 studies of the effects of having a higher number of siblings on the risk of allergic diseases, found that there was an inverse relation in all but 2 of the 11 studies of eczema, in all but 10 of the 31 studies of wheezing and asthma and in all but 2 of the 16 studies of sensitization.[67] However, evidence for an asthma outcome was found to be less consistent.[63, 68] Possible mechanisms for the infection and immune link have been given by Holt *et al*[69] based on the T-cells immune modulation theory. Holt *et al* proposed that T-cell memory is driven by immune stimulation of Th1 or Th2 cells, which primarily occurs

prenatally or at birth.[69, 70]

The hygiene hypothesis, however, has been argued in recent decade by Douwes and Pearce.[71, 72] They have proposed the importance of other factors in the aetiology of asthma and atopy particularly in view of a study by Ponsonby *et al,*[73] whom reported a decline in asthma in Australian schoolchildren, and a rise in eczema symptoms in recent decades. Douwes and Pearce[71, 72] explained that the immune modulation in the case of the hygiene theory, possibly works for atopic asthma more than non atopic asthma, and if so the hygiene hypothesis might not fully explain this trend,[73] and other factors need to be sought. The mechanism has also been challenged by Heaton and colleagues[74] who showed heterogeneity of an individual immune response, and that the Th1/Th2 pattern may not apply to everyone.[74]

1.2.2.1 Infection, immunization and daycare

Various cross-sectional and longitudinal studies have explored the hypothesis that the decrease in early childhood infection, through immunization, increases the prevalence of allergic diseases. Among the studies, the protective effect of childhood infection and immunization, particularly measles, was reported in a British national birth cohort,[75] Aberdeen school children, UK,[65] in a west Africa population (Guinea-Bissau),[76] and in Ethiopia.[43] However, others have shown no associations, for example between infection with measles, mumps, or rubella,[77] and BCG vaccination[78] and allergic disease. The larger international evidence on immunization and risk of allergy came from the ISAAC ecological analysis of immunization data for tuberculosis, diphtheria, tetanus toxoid (DTP) and

measles, and concluded that the increase in allergic disease was unlikely to be explained by variation in immunization.[79]

Another specification of the hygiene hypothesis relating to childhood infection is daycare attendance, though the evidence is inconsistent.[80-82] Daycare attendance has been positively associated with respiratory tract infections,[80] but no effect seen on allergic disease.[80-82]

In summary, the current evidence on childhood infection, daycare attendance and allergy, both in the first[63] and second decade,[83] provides little or weak support for the hygiene hypothesis.

1.2.2.2 Farm environment

Several studies have shown that children who have grown up on a farm are less likely to have allergic disease and sensitization than those not living in a farm environment,[84-87] and the evidence to date is intriguing.[88] Possible explanations include farm environment increasing exposure to infection, for example exposure to commensal bacteria, parasites and microbial products, or farm environment may be a 'proxy marker' for other risk factors such as larger family size, pets exposure, dietary habit, or the association could be due to other unknown factors.[61, 63, 68]

1.2.2.3 Allergen exposure

Several studies have reported that exposure to various sources of perennial and seasonal allergens increase the risk of asthma and allergic diseases.[43, 46, 89] This observation is important since increasing urbanisation and changes in domestic environment, including use of mattresses, favours exposure to allergens. In this regard, particularly exposure to house dust mite and the risk of asthma and allergic disease has received consistent support.[89] Despite support from epidemiological studies, however, allergen avoidance interventions are less successful to date.[90, 91] Several reasons were speculated for this disappointing outcomes from the trials, including a focus on mono-allergen avoidance intervention (mainly to house dust mite) and inability to reduce the exposure to a clinically relevant level.[90, 91] The former speculation was supported by Schayck *et al* meta-analysis whom reported that multifaceted intervention (intervention involving multiple environmental allergens) decreased the risk of asthma in children by 27% whilst the monofaceted intervention (an intervention aiming at reducing a single environmental allergen) shown to have no effect.[90]

1.2.3 Gastro-intestinal infections

1.2.3.1 Geohelminth exposure

The hypothesis that geohelminth infection, which tends to be more common in rural populations, may protect against asthma was first proposed in the 1970s,[92] but appears to have been generally disregarded after an epidemiological review in 1985 concluded that the data 'neither refute nor support the theory that parasite infection protects against asthma'.[92] However, in the past two decades,

a range of independent studies including cross-sectional,[45, 93-101] case control[102-104] and intervention studies[105-107] have shown a link with asthma, clinical allergy and atopy (Table 1.1).

Our group's previous work in adults in Jimma in south west Ethiopia showed a three-fold higher prevalence of asthma symptoms in urban than in rural areas,[43] and found a strong protective effect of hookworm infection (adjusted OR, 0.48, 95% CI; 0.24, 0.93).[104] More recent work in Butajira, southern Ethiopia, with a relatively low prevalence of geohelminth infection and intensity than Jimma, however, showed no significant protective effect of any geohelminth infection against wheeze or asthma.[45]

Despite this body of evidence, studies in children are limited. Inverse associations between geohelminth infection and allergy in children were reported from Brazilian,[93] Ecuadorian[95] and Vietnamese school children.[96] Rodrigues and colleagues in urban Brazil, an area with higher wheeze prevalence, showed early life exposure to *T. trichiura* (at age 2) protected from atopy later in life (at age 7).[93] The Ecuadorian study by Cooper *et al*[95] reported an inverse relation between current or chronic exposure to hookworm, *A. lumbricoides*, *T. Trichiura* and atopy.[95] Our group's work in Jimma, in children of aged 1 to 4, found higher prevalence of asthma symptoms in urban area (4.4%) than rural (2%), and *A. lumbricoides* appeared in the study to be protective, however the association for either hookworm or *T. trichiura* was non significant.[97]

Several meta analyses have reported pooled estimates of the available

observational studies.[108, 109] Among the meta-analyses conducted in the past six years, Leonardi-Bee and colleagues showed a 50% reduction in asthma risk with hookworm infection which was intensity related.[108] However, the study showed an increased risk of asthma with exposure to *A. lumbricoides*.[108] The other meta-analysis explored the link between atopy and geohelminth infection and found a decreased risk of atopy with current parasite infection (OR, 95% CI, 0.68; 0.60, 0.76).[109] However, it appeared in species specific analysis that the odds of atopy significantly reduced with exposure to *A. lumbricoides* and *T. trichiura*, but marginally with hookworm.[109] Plausible mechanisms for apparent protection of geohelminth infection includes production of immune regulatory cytokines IL-10[110, 111] and transforming growth factor-ß (TGF- ß).[111]

Intervention studies are limited to few studies. Cooper and colleagues[105] randomized controlled trial found no evidence of the effect of deworming and risk of atopy or clinical allergy.[105] The Gabonese intervention study in schoolchildren however showed a 2.5-fold increased risk of atopy following a deworming programme.[112] The first experimental hookworm trial was recently conducted in the UK in previously unexposed adults and found no difference between the experimental and placebo arm in respect to bronchial responsiveness, atopy status or use of asthma medication.[106]

In summary, the role of geohelminths in the development of asthma and allergic diseases, particularly with atopy is relatively consistent; and there is independent support for hookworm whilst the effects of *A. lumbricoides* and *T. trichiura* are conflicting. Results from the available intervention studies are disappointing, although most of them are eradication studies among previously

infected subjects. Most of the studies to date are cross-sectional or case control where reverse causation is difficult to exclude, and cohort studies in children particularly, prospective birth cohort studies are remarkably scarce.

Table 1.1 Studies showing the association between geohelminth infection, asthma and allergic disease

Authors	Study area	Study design	Age group	Outcome	Main findings (OR, 95% CI)
Rodrigues *et al* 2008[93]	Brazil	Cross-sectional	12-13 yr	Atopy	*Hookworm (0.89; 0.24, 3.38)* *A. lumbricoides (0.71; 0.39, 1.30)* *T. trichiura (0.29; 0.08, 0.98)*
Cooper *et al* 2004[94]	Ecuador	Cross-sectional	1-17 yr	Atopy	*Any helminth (0.65; 0.47, 0.91)*
Cooper *et al* 2003[113]	Ecuador	Cross-sectional	5-19 yr	Atopy	*Hookworm (0.39; 0.18, 0.85)* *A. lumbricoides (0.74; 0.60, 0.91)* *T. trichiura (0.82; 0.67, 1.01)*
Flohr *et al* 2006[96]	Vietnam	Cross-sectional	6 -18 yr	Sensitization (dust mite)	*Hookworm (0.61; 0.39, 0.96)* *A. lumbricoides (0.28; 0.10, 0.78)*
Dagoye *et al* 2003[97]	Jimma, Ethiopia	Cross-sectional	1-4 yr	Wheeze	*Hookworm (0.6; 0.2, 1.8)* *A. lumbricoides (0.5; 0.3, 0.9)* *T. trichiura (1.1; 0.6, 1.8)*
Davey *et al* 2004a[45]	Butajira, Ethiopia	Cross-sectional	5 or more	Wheeze	*Hookworm (0.92; 0.73, 1.14)* *A. lumbricoides (1.00; 0.80, 1.25)* *Any geohelminth (0.98; 0.83, 1.15)*
Davey *et al* 2004b[45]	Butajira, Ethiopia	Cross-sectional	5 or more	Dust mite sensitization Cockroach sensitization	*Hookworm (1.03; 0.83, 1.29)* *A. lumbricoides (1.19; 0.97, 1.47)* *Hookworm (0.79; 0.61, 1.03)* *A. lumbricoides (0.92; 0.72, 1.18)*
Wordemann *et al* 2008[100]	Cuba	Cross-sectional	4-14 yr	Rhinoconjunctivitis Eczema	*Hookworm (2.81; 1.23, 6.42)* *A. lumbricoides (0.23; 0.08, 0.68)*
Nyan *et al* 2001[98]	Gambia	Cross-sectional	15 or more	atopy	*Any helminth (0.30; 0.11, 0.80)*
Palmer *et al* 2002[99]	China	Cross-sectional	8-18 yr	Asthma	*A. lumbricoides (1.85; 1.37, 2.49)*
Scrivener *et al* 2001a[104]	Jimma, Ethiopia	Case control	14 or over	Wheeze	*Hookworm (0.48; 0.24, 0.93)* *A. lumbricoides (0.69; 0.38, 1.25)*

					T. trichiura (0.75; 0.45, 1.26)
Haileamlak *et al* 2005[103]	Jimma, Ethiopia	Case-control	1-5 yr	Eczema	*Hookworm (1.01; 0.45, 2.28)* *A. lumbricoides (1.31; 0.95, 1.81)* *T. trichiura (1.64; 1.18, 2.28)*
Schafer *et al* 2005[102]	Germany	Case-control	5-14 yr	Eczema Allergen specific IgE Abs	*Any helminth (Ascaris or Oxyuris) (0.45; 0.33, 0.60)* *Any helminth (Ascaris or Oxyuris) (0.74; 0.60, 0.92)*
Cooper *et al* 2006[105]	Ecuador	Randomized controlled trial	School age children (mean age 9.4 yr)	Atopy Wheeze	*Albendazole vs. no treatment group (0.97; 0.68,1.39)* *Albendazole vs. control group (1.07; 0.54,2.11)*
Feary *et al* 2010[106]	UK	Randomized placebo-controlled trial	18 or more	Change in $PD_{20}AMP$ (doubling dose DD)	*Mean difference DD hookworm vs. placebo group (-0.51; -1.79, -2.80)*

1.2.3.2 Helicobacter pylori infection

Helicobacter pylori is a Gram negative, urease positive, bacterium which is common and exclusively colonizes the stomach.[114] It is acquired early in childhood, with new infections usually occurring before the age of 10 years.[115] *H. pylori* can readily be cultured from vomitus, and occasionally from saliva or diarrhea stool.[116] It is now evident that the prevalence of *H. pylori* in developed countries has been rapidly declining over the last 4-5 decades. Bantavala *et al* studied 631 samples collected between 1969 and 1989 and showed that *H. pylori* prevalence deceased by 26% per decade.[117] Similarly, rapid declines in the $CagA^+$ strains of *H. pylori* between 1973 and 1974 were reported by Perez *et al*[118] and Kosunen *et al*[119] where markers of allergy increased by more than 3-fold with the increase mainly occurring in those *H. pylori* negative subjects.[119] A comparable decline in *H. pylori* infection was also reported in the UK, described as a 'birth cohort effect', with a reported 8-fold decrease in prevalence between 1930 and 1970.[120] This decrease in the prevalence of *H. pylori* in developed countries over the last decades, when markers of affluence became more apparent,[117] were paralleled by a dramatic increase in the prevalence of allergy and asthma (Table 1.2).[32]

As part of the hygiene hypothesis, therefore, recent interest has been focused on the protective role of *H. pylori* infections in the etiology of asthma and allergic disease, which has gained support from a range of epidemiological[58, 119, 121-133] and epigenetic studies.[58] Reduced risk of atopy and asthma has been seen in many studies[58, 118, 123-128, 130, 130, 134, 135] both in children,[121, 122, 133] and adults.[123, 125, 127, 128, 130] However, most of the studies to date are either cross-

sectional,[121-128] or case-control,[58, 119, 129-133] A cross-sectional study by Chen and colleagues[123] showed an inverse relation between *H. pylori* and reported asthma, current and ever rhinitis and sensitization, with greater effects seen in children with onset of *H. pylori* infection before the age of 15.[123] Herbarth and colleagues work in children, independent of potential confounders, also showed the protective role *H. pylori* infection on eczema (OR=0.37), but not with respiratory disease.[122]

Conflicting findings have been reported from case-control studies in adults,[58, 119, 129, 131-133] and reviewed by Blaser *et al.*[135] The review found consistent inverse associations from cross-sectional studies, but no assocations from case-control studies. The review,[135] as in the authors of the case-control studies,[124] highlighted that the lack of an association could be attributed to the power of the study.[136]

Plausible mechanisms include the development of cell-mediated immunity of T-helper 1 type, and higher IL-10 responses in children with *H. pylori,*[137] or stimulation of H. *pylori* associated Treg cells that suppress immune responses.[138]

In summary, most of the studies of *H. pylori* and allergic diseases to date are cross-sectional and case control and based in developed country adult populations, with only three studies conducted in children. Alternative explanations including reverse causation and bias due to antibiotic eradication therapy affecting *H. pylori* acquisition are therefore difficult to exclude.

Table 1.2 Epidemiological studies showing associations between *H. pylori*, asthma and allergic disease

Author	Study area	Study design	Age group	Exposure measure	Outcome	Main findings
Chen *et al.* 2008[121]	US	Cross-sectional	3-19 yr	IgG Abs	Asthma, rhinitis, wheeze and eczema	*OR for ever had asthma, 0.49 (0.30,0.80), current asthma, 0.41 (0.24,0.69), allergic rhinitis, 0.31 (0.17,0.57), wheeze, 0.73 (0.57,0.94), and eczema, 0.73 (0.57,0.94)*
Chen *et al.* 2007[123]	US	Cross-sectional	≥17 yr	IgG Abs, IgG CagA	Asthma, rhinitis, wheeze, eczema, and sensitization	*OR for asthma, 0.79 (0.63,0.99); allergic rhinitis, 0.77 (0.62,0.94), and significantly associated with sensitization*
Jarvis *et al.* 2004[124]	UK	Cross-sectional	20-44	IgG Abs	Asthma, rhinitis, IgE, and sensitization	*OR for wheeze, 1.21 (0.71, 1.72), rhinitis, 1.01 (0.70, 1.52), and any sensitization, 1.13 (0.81, 1.59). Associated significantly with sensitization to grass.*
Herbarth *et al.* 2007[122]	Germany	Cross-sectional	Mean age 6.3 yr	C-urea breath test	Eczema	*OR for eczema, 0.37 ($p<0.01$), bronchitis, 1.99 ($p<0.01$)*
McCune *et al.* 2003[125]	UK	Cross sectional	Adults	C-urea breath test	Asthma, eczema, and rhinitis	**A 30% reduction in asthma, eczema and rhinitis risk*
Fullerton *et al.* 2009[126]	UK	Cross-sectional	18-70 yr	IgG Abs	FEV_1, FVC, bronchial reactivity, sensitization, and IgE	*OR for wheeze, 0.94 (0.74,1.19), chronic bronchitis, 1.00 (0.75, 1.33), rhinitis, 1.00 (0.79,1.26), asthma, 1.09 (0.77,1.54) and atopy, 0.92 (0.74,1.15)*
Janson *et al.* 2007[127]	Sweden	Cross-sectional	42 yr	IgG Abs	Asthma, eczema, rhinitis, IgE	**H pylori infection protects from atopy (24.8% vs. 36.6%, $p<0.01$).*
Hertzen *et al.* 2006[128]	Finland and Russia	Cross-sectional	25-54 yr	IgG Abs	Sensitization	*H pylori infection alone explains 32% of the difference in the prevalence of atopy between Finland and Russia.*
Reibman *et al.* 2008[130]	New York	Case control	18-65 yr	IgG Abs	Total IgE, and Spirometry	*OR for asthma, 0.57 (0.36, 0.89)*

Tsang *et al.* 2000[129]	Hong Kong	Case control	41-56 yr	IgG Abs	Asthma, FEV_1, FVC	**No association*
Jun *et al.* 2005[131]	Japan	Case control	Adults	IgG, $CagA^+$	Asthma	**No association*
Bodner *et al.* 2000[132]	UK	Case control	39-45 yr	IgG Abs	Asthma, IgE, and sensitization	**No association*
Kosunen *et al.* 2002[119]	Finland	Longitudinal	15-54 yr	IgG Abs	Allergen specific IgE	*OR for allergen specific IgE Abs, 0.63 (0.17,2.40)*
Cam *et al.* 2009[139]	Turkey	Prospective	13-17 yr	C-urea breath test	Wheeze, rhinitis, eczema, and sensitization	**No association*
Seiskari *et al* 2007[133]	Russia and Finland	Case control	7-15 yr	IgG Abs	Total and specific IgE	*OR for IgE in Russia Karelia children, 0.33 (0.1, 0.9)*
Pessi *et al* 2004[58]	Finland	Case control	30 or over	IgG Abs	Sensitization and asthma	**Inverse association with sensitization*

*OR not reported in the study

1.2.3.3 Commensal bacteria

Commensal microflora that live in symbiosis with human hosts, contain various microbial antigens that may contribute to individual risk of allergy.[140] The development and composition of the commensal bacteria from birth are likely affected by feeding pattern,[141] hygienic condition, dietary difference, use of antibiotics and other environmental factors,[142] and may in part contribute to the worldwide differences in the prevalence of asthma and allergic diseases. The 'microflora hypothesis' first debated by Sepp and colleagues[143] about two decades ago, has since received support from cross-sectional,[88, 144, 145] case control,[141, 146, 147] and small[148-152] and large[153, 154] scale birth cohort studies (Table 1.3). The intestinal microflora species believed most important in induction of immune stimulation early in life, are enterococci, lactobacilli and bifidobacteria.[140, 143, 144, 150]

The most pioneering small scale study by Sepp colleagues[143] in a cohort of Estonian and Swedish children, identified faecal composition of intestinal flora in Estonian (less affluent, and lower prevalence of asthma) and Swedish (more affluent, and higher prevalence of asthma) one year infants.[143] The result showed high counts of lactobacilli, eubacteria and enterococci in Estonian children (sign of undisturbed flora) while more clostridia were found in the Swedish children, which is a sign of disturbed microbial balance.[143] A five year follow up study of 47 Swedish infants, by the same group, further reported that children who developed allergic symptoms and atopy were significantly less colonized by strains of lactobacilli and bifidobacteria in the first two months of life. Inverse relation between atopy and bifidobacteria colonization during infancy was also reported by Suzuki *et al*[155] and Kalliomaki *et al.*[151] The latter

prospective study demonstrated that the gut microflora may precede the development of atopic symptoms, though it was based on small sample size of 76 infants.[151] These findings over the year were replicated in other studies particularly in relation to atopic eczema,[141, 145, 147, 151] though associations with specific bacterial strains have been conflicting.[147, 151]

The largest epidemiological study so far is recently reported by Ege *et al*[88] presenting data from two large cross-sectional studies in children comparing living on farms with the reference group. Even though, the study analysed microbial and fungal species from the dust samples, the findings showed that children living on farms had a 24 to 51% reduced risk of asthma, and a 49 to 76% reduced risk of atopy, and that it was positively associated with detectable microorganisms in dust samples.[88] Even though the study was unable to identify specific microbes, it appeared that a diversity of bacterial and fungal genera were protective with odds ratios of 0.37 and 0.57 respectively.[88] A similar role of bacterial diversity in asthma aetiology more than a single bacterial strain has also been reported elsewhere in molecular based studies.[146, 148] The plausible mechanisms for down-regulation of the immune system includes the production of anti-inflammatory cytokines and regulatory T-cells (Treg cells),[156] though regulatory mechanisms might differ depending on the specific commensal stains.[157, 158]

Whilst studies have reported inverse association between commensal bacteria and risk of asthma and allergic disease, others have reported no associations.[154, 159-161] The Manchester Asthma and Allergy case-control study of children aged 3 to 5 years showed no significant difference in the composition of lactobacilli or

bifidobacteria between atopic wheezy and non atopic non wheezy children.[159] Similar findings were also reported from the Danish[154] and the European birth cohort studies.[161]

In summary, most studies exploring the 'microflora hypothesis' are cross-sectional, and are primarily based in developed countries. Such studies are susceptible to reverse causation, and differences in commensal composition between allergic and non-allergic subjects probably arise from antibiotic therapy or changes in dietary habit. Moreover, longitudinal studies, particularly from developing countries, are lacking.

Table 1.3 Association between selected commensal bacteria, asthma and allergic disease

Commensal bacteria	Study area	Study design	Age group	Exposure measure	Outcome	Main results (with vs. without outcome)
Enterococci spp						
Bjorksten *et al* 1999[144]	Estonia and Sweden	Cross-sectional	2 yr	Stool culture	Allergy/atopy	*Colonization and atopy at 2 years (85% vs. 89%), P>0.05*
Bjorksten *et al* 2001[150]	Estonia and Sweden	Cohort	Up to 2 yr	Stool culture	Eczema and/or atopy	*Colonization at 1 week and allergy at 2 years (67% vs. 96%), p=0.03*
Kalliomaki *et al* 2001[151]	Finland	Cohort	Up to 1 yr	Stool culture	Atopy	*Colonization at 3 weeks and atopy at 12 months (100% vs. 95.9%), p=0.36*
Lactobacilli spp						
Bjorksten *et al* 1999[144]	Estonia and Sweden	Cross-sectional	2 yr	Stool culture	Allergy/atopy	*Colonization and atopy at 2 years (44% vs. 60%), p<0.01*
Bjorksten *et al* 2001[150]	Estonia and Sweden	Cohort	Up to 2 yr	Stool culture	Eczema and/or atopy	*Colonization at 1 week and allergy at 2 years (39% vs. 8%), p=0.03*
Penders *et al* 2007a[154]	Netherland	Cohort	Up to 2 yr	PCR	Wheeze	*OR for wheeze at 2 years and colonization at 1 month; 1.22 (0.77, 1.93), p>0.05*
Penders *et al* 2007b[154]	Netherland	Cohort	Up to 2 yr	PCR	Eczema	*OR for eczema at 2 years and colonization at 1 month; 1.23 (0.91,1.65), p>0.05*
Penders *et al* 2007c[154]	Netherland	Cohort	Up to 2 yr	PCR	Atopy	*OR for atopy at 2 years and colonization at 1 month; 1.04 (0.71,1.53), p>0.05*
Kalliomaki *et al* 2001[151]	Finland	Cohort	Up to 1 yr	Stool culture	Atopy	*Colonization at 3 weeks and atopy at 12 months (90% vs. 89.8%), p=0.98*
Bifidobacteria spp						
Bjorksten *et al* 1999[144]	Estonia and Sweden	Cross-sectional	2 yr	Stool culture	Allergy/atopy	*Colonization and atopy at 2 years (59% vs. 71%), p<0.05*
Bjorksten *et al* 2001[150]	Estonia and Sweden	Cohort	Up to 2 yr	Stool culture	Eczema and/or atopy	*Colonization at 1 week and allergy at 2 years 17% vs. 50%, p=0.03*
Suzuki *et al* 2007[155]	Japan	Case control	Up to 6 months	PCR	Allergic symptoms/atopy	*Colonization and allergy at 6 months (70% vs. 12.5%), p<0.01*
Murray *et al* 2005a[159]	Manchester	Case control	Mean age 4.4 yr	PCR	Wheeze, and atopy	*Median colonization among sensitized wheezy and non sensitized non wheezy children*

						1.71% vs. 1.88%, p=0.70
Murray *et al* 2005b[159]	Manchester	Case control	Mean age 4.4 years	PCR	Wheeze, atopy and eczema	*Median colonization among sensitized wheezy plus eczema and non sensitized non wheezy and non eczema children (1.6% vs. 4.0%), p=0.05*
Penders *et al* 2007a[154]	Netherland	Cohort	Up to 2 yr	PCR	Wheeze	*OR for wheeze at 2 years and colonization at 1 month; 1.32 (0.85,2.06), p>0.05*
Penders *et al* 2007b[154]	Netherland	Cohort	Up to 2 yr	PCR	Eczema	*OR for eczema at 2 years and colonization at 1 month; 1.02 (0.77,1.35), p>0.05*
Penders *et al* 2007c[154]	Netherland	Cohort	Up to 2 yr	PCR	Atopy	*OR for atopy at 2 years and colonization at 1 month; 1.23 (0.85,1.77), p>0.05*

PCR – polymerase chain reaction

1.2.4 Other risk factors

1.2.4.1 Birth weight and body mass index

The association between birth weight,[162-165] a marker of maternal diet during pregnancy or other mechanisms related to lung growth, and the risk of asthma has been explored in various studies. Whilst several studies have shown low birth weight increased the risk of asthma and allergic disease,[162, 164, 165] others have shown no associations.[166] Studies have also looked at the role of body mass index (BMI),[165, 167-169] which may be a proxy for low physical inactivity, dietary habit, and other factors including hormonal imbalance. Higher body mass index has been reported to be positively associated with wheeze[170] including maternal obesity and risk off-spring asthma.[169] Despite heterogeneity among the literature, however, recent a meta-analysis suggested a 50% increased risk of incident asthma in obese and overweight adults.[168]

1.2.4.2 Diet and breastfeeding

Several studies have proposed that alteration to a more westernized diet may be fuelling the worldwide variation and increasing prevalence of asthma and allergic disease.[171-174] In this context, dietary hypotheses, particularly those relating to the role of antioxidant deficiency (vitamin C, vitamin E, carotinoids, flavonoids and selenium), have gained support from epidemiological studies and been reviewed in detail.[172-176] The beneficial effect of vitamin D on asthma morbidity has also gained recent interest,[177] which may operate through altering viral infections or increasing responsiveness to asthma therapy.[178] A meta-analysis including 37 observational studies (23 case control, 15 cross-sectional

and 2 cohort studies) reported an inverse association between vitamin A, and C status and measures of asthma, but evidence to support the role of vitamin E was found to be limited.[176]

The role of breast feeding in asthma and allergic disease aetiology has also been explored in a range of epidemiological studies, however to date the scientific evidence is controversial.[179, 180] A recent meta-analysis of the available observational studies in the past 10 years found no association between any or exclusive breast feeding and wheezing symptoms in younger children. The results of this meta-analysis were also supported by an earlier large scale randomized clinical trial of breast feeding promotion programme in children on asthma and atopy.[179]

In summary, despite an inverse association between dietary antioxidant and asthma, data from clinical trials to date, for example supplementation of vitamin C in asthma,[181] and the role of breast feeding[179] are disappointing.

1.2.4.3 Pollutants and exposure to tobacco smoke

Among the risk factor studies of asthma and allergic disease in the past two decades, exposure to road traffic pollution, and other pollutants of particulate matters have gained considerable attention, though the evidence is often conflicting.[182, 183] The available epidemiological studies of exposure to air pollution and risk of asthma were summarized by Anderson *et al*[182] and Weinmayr *et al.*[183] The first study included 21 multi-community studies of long-term exposure to NO_2,

SO_2 and particulate matter with aerodynamic diameter of <10 μm (PM_{10}).[182] The pooled estimate from this meta-analysis showed no evidence of an association between these exposures and the prevalence of asthma (wheeze symptom and asthma diagnosis).[182] The second meta-analysis however demonstrated exposure to PM_{10} increased the risk of asthma symptom, but the effect of NO_2 appeared less clear, though a high degree of heterogeneity was observed among the studies.[183] In general, the role of air pollution on asthma and allergic disease is uncertain and multicentre studies have reported weaker associations than individual studies. A number of factors including distance from traffic sources, level and dose of exposure and bias associated with the measurement of the exposure probably lead to these inconsistent findings.[182]

Exposure to tobacco smoke has been consistently shown to influence the risk of asthma, though the association with clinical allergy is inconsistient.[184, 185] A systematic review by Cook and Strachan reported consistent findings with odds ratios ranging from 1.2 to 2 for the effects of parental smoking (maternal, paternal or both) on the prevalence and incidence of respiratory disease in children, but the effects on allergy was generally less strong.[184] Most recently a review by Burke and colleagues[186] concurred with findings of the earlier reviews,[184, 185] and reported an adverse effect of pre- or postnatal passive smoke exposure on the incidence of wheeze and asthma. This review included 79 cohort studies and found exposure to postnatal maternal smoking was associated with a 70% increased risk of incident wheeze in children ≤2 years and a similar increased risk of exposure to prenatal maternal smoking on incident asthma in younger children.[186]

1.2.4.4 Paracetamol

Varner and colleagues were the first to speculate that the decreased use of aspirin in favour of paracetamol (also known as acetaminophen) in the early 1980s, due to the adverse effect of aspirin related Reye's syndrome in children with febrile illness,[187] may be fuelling the world wide increase in the prevalence of asthma and allergic disease. Since then paracetamol remains one of the most prescribed, over-the-counter pain reliever, and is the safe analgesic and antipyretic drug of choice in the world.[188]

There is now increasing epidemiological evidence from worldwide multicounty studies,[189-191] systematic reviews[192-195] and meta-analyses[193, 196] implicating paracetamol use in the etiology of asthma and other allergic disease. The evidence to date has been remarkably consistent,[187, 189, 190, 192, 193, 195, 197-206] with adverse effects reported in relation to paracetamol exposure *in utero*,[197-200] during infancy,[200, 201] in childhood[189, 190, 202] and during adult life.[203-206] The effect also extends to asthma severity amongst asthmatics.[201, 204-206] However, most of these studies were cross-sectional or case-control and reverse causation is difficult to exclude (Table 1.4).

The largest studies so far are the reports of the ISAAC multicounty study in children by Beasley *et al.*[189, 190] These two studies reported significant dose dependent associations between current and first year use of paracetamol and the risk of asthma, eczema and rhinoconjunctivitis in 6-7 years old children,[189] which persisted when the children reached adolescence.[190] According to the ISAAC multicentre study, the population-attributable risks (PAR) of severe

asthma symptoms in children due to paracetamol use in the first year of life range from 22% to 38%.[189]

Various other cross-sectional,[207-210] case control,[211] and one longitudinal study[212] also reported an adverse role of exposure to paracetamol on childhood asthma, and meta-analysis of the available observational studies by Etminan and colleagues[193] estimated a 63% increased risk of asthma among children using paracetamol. Our group's previous work in Butajira, Ethiopia showed a significant and dose-dependent increased risk of allergic symptoms among paracetamol users,[203] which accompanying qualitative work suggested was unlikely to be explained by aspirin avoidance.[213] Lowe *et al*[212] from Australia also found a similar positive association between child's exposure to paracetamol and the risk of asthma, however, it appeared that the associations were confounded by respiratory tract infections, and became non significant on adjustment.[212]

Shaheen SO *et al*, using the ALSPAC cohort in the UK, were the first to report positive associations between prenatal use of paracetamol during pregnancy and the risk of childhood asthma and allergic disease.[197] This study found that use of paracetamol in late pregnancy (20 to 32 weeks) was associated with an increased risk of wheezing in children at 30-40 months.[197] Similar findings were reported elsewhere from study in Spain,[214] a US birth cohort[215] and a Danish birth cohort[199], all linking exposure *in utero* with increased risks of off-spring asthma and allergic diseases. Overall, a pooled estimate from meta-analysis of the available observational studies for use of paracetamol during pregnancy and childhood wheeze showed an increased risk for use prenatally (adjusted OR, 95% CI, 1.21; 1.02, 1.44).[196]

Among the observational studies in adults are two United States-based studies.[204, 206] The study by Barr *et al*[206] prospectively examined paracetamol use on new onset asthma in women and found an increase in adult onset-asthma. The other study by McKeever and colleagues[204] showed paracetamol use to be associated with increased risk of asthma and COPD.

The Randomized controlled trial (RCT) is a gold standard design to establish a cause-effect relationship, however, is limited to one study of paracetamol due to ethical dilemmas. The only randomized clinical trial in asthmatic febrile children from the US showed increased outpatient visit in the paracetamol arm compared to the ibuprofen arm (adjusted OR, 95% CI, 1.79; 1.05, 2.94),[201] and weak positive association with rates of hospitalization. Whilst the trial was double-blinded, the lack of placebo control arm in this trial, limits its interpretation.

In *vivo* and *in vitro* studies support these epidemiological observations and provide plausible mechanisms, including depletion of pulmonary glutathione concentration,[216, 217] inhibition of basal INF-α and IL-6 protein secretion in the lung.[217]

In summary, studies reporting the role of paracetamol in the development or maintenance of asthma and allergic disease, particularly for childhood wheezing are more consistent. However, evidence on eczema and sensitization is limited to a few studies, and data from longitudinal studies are lacking. Moreover, most of the studies were based in developed countries and alternative explanations including aspirin avoidance is difficult to exclude.

Table 1.4 Studies showing link between paracetamol, asthma and allergic disease

Author	Study area	Study design	Age group	Outcome	Main results
Beasley *et al* 2011[190]	ISAAC multicountry study	Cross-sectional	13-14 yr	Asthma, rhinoconjunctivitis and eczema	*OR for current asthma for use of paracetamol (high dose) vs. never: 2.51 (2.33, 2.70), current wheeze (video): 2.35 (2.13, 2.60), rhinoconjunctivitis: 2.39 (2.24, 2.55), and eczema: 1.99 (1.82, 2.16).*
Beasley *et al* 2008[189]	ISAAC multicountry study	Cross-sectional	6-7 yr	Asthma, rhinoconjunctivitis and eczema	*OR for asthma at 6 to 7 yrs for use of paracetamol in the first years of life vs. never: 1.46 (1.36, 1.56), rhinoconjunctivitis: 1.48 (1.38, 1.60), and eczema: 1.35 (1.26, 1.45)* *Current use also associated with the outcomes at 6 to 7 years.*
Davey *et al* 2005[203]	Butajira, Ethiopia	Cross-sectional	≥5 yr	Wheeze, rhinitis, eczema, asthma, and sensitization	*OR for wheeze for use ≥3 tablets per month vs. never: 1.89 (1.51, 2.36), eczema: 1.90 (1.39, 2.61), rhinitis: 2.52 (1.99, 3.20), dust mite sensitization: 1.17 (0.92, 1.48), and cockroach sensitization: 1.40 (1.10, 1.79)*
Vlaski *et al* 2007[207]	Republic of Macedonia	Cross-sectional	13-14 yr	Wheeze, asthma, rhinitis, eczema	*OR for wheeze for use once per month vs. never: 2.04 (1.31, 3.20), asthma ever: 2.77 (1.06, 7.26), rhinitis: 2.25 (1.36, 3.70), current eczema: 0.93 (0.41, 2.10)*
Garcia-Marcos *et al* 2008[214]	Spain	Cross-sectional	3-5 yr	Wheeze	*OR for wheeze for use of paracetamol during pregnancy once per month vs. never: 1.71 (1.15, 2.53), for asthmatic mothers: 0.95 (0.15, 5.79), and for non-asthmatic mothers: 1.74 (1.15, 2.61)*
Cohet *et al* 2004[209]	New Zealand	Cross sectional	6-7 yr	Asthma, wheeze, eczema, and rhinitis	*OR for current wheeze at 6 to 7 yrs for use in the first year of life vs. never: 1.38 (1.04, 1.83), asthma ever: 1.72 (1.32, 2.23), current allergic conjunctivitis: 1.25 (0.92, 1.70), and eczema ever: 1.27 (1.02, 1.58)*
Barragan-meijuerio *et al* 2006[210]	Mexico	Cross - sectional	6-7 yr	Wheeze, rhinitis, eczema	*OR for wheeze for paracetamol use in past month vs. never: 3.30 (1.54, 7.18), rhinitis: 1.61 (1.33, 1.95), eczema: 1.82 (1.24, 2.66)*
Mckeever *et al* 2004[204]	US	Cross-sectional	20-80 yr	Asthma, COPD and lung function tests	*OR for asthma for daily use vs. never: 1.20 (1.12, 1.28), for COPD: 1.16 (1.09, 1.24, and for FEV_1*

					(ml): -54.0 (-90.3, -17.7).
Newson *et al* 2000[202]	ISAAC and ECRHS multi centre study	Ecological study	6-7, 13-14, and 20-44 yr	Reported asthma, wheeze, eczema, rhinoconjunctivitis	*Regression coefficient for wheeze for every gram increase in per capital paracetamol sales: +0.26 (0.16, 0.37), for rhinitis: +0.35 (0.21, 0.49), for Atopy: 0.20 (-0.02, 0.41), and BHR: -0.02 (-0.03, 0.00) for adults 20-44 years.*
Lesko *et al* 2002[201]	US	Randomized clinical trial	6-12 months	Asthma (outpatient visit and hospitalization)	*OR for outpatient visit for asthma for paracetamol arm vs. ibuprofen: 1.79 (1.05,2.94)*
Shaheen *et al* 2000[205]	UK	Case control	16-49 yr	Asthma, and rhinitis	*OR for asthma for daily use vs. never: 2.38 (1.22, 4.64), and rhinitis in those without asthma: 2.33 (1.09, 4.96).*
Shaheen *et al* 2008[191]	Multicentre study (European Network)	Case control	20-45 yr	Diagnosed and reported asthma	*OR for asthma for weekly use vs. less frequent use:* 2.87 (1.49-5.37).
Lowe *et al* 2010[212]	Australia	Longitudinal birth cohort	6-7 yr	Asthma, wheeze, rhinitis, and eczema	*OR for asthma for increasing total days of use of paracetamol further adjusted for respiratory tract infections at 5 to 7 yr: 1.08 (0.91, 1.29), incident asthma: 0.97 (0.76, 1.25), allergic rhinitis: 1.17 (0.96, 1.43), and eczema: 1.10 (0.93, 1.29)*
Kang *et al* 2009[218]	UK	Longitudinal (*in utero*)	0-6 yr	Diagnosed asthma	*OR for asthma:* 0.67 (0.53-1.10)
Shaheen *et al* 2002[197]	UK	longitudinal study (*in utero*)	18-40 months	Wheeze and eczema	*OR for wheeze for use of paracetamol at 20 to 32 weeks of pregnancy for daily/most days use vs. never: 2.10 (1.30, 3.42), eczema: 1.04 (0.65, 1.66), and for use at infancy for eczema: 1.22 (1.06, 1.41) and wheeze: 1.64 (1.13, 2.39)*
Perzanowski *et al* 2008[215]	US	longitudinal study (*in utero*)	5 yr	Wheeze	*Exposure to paracetamol during pregnancy (particularly during the third trimester) increased risk of wheeze at the age of 5 years. But no effect before this age.*
Rebordosa *et al*	Denmark	longitudinal study (*in*	18 months-7 yr	Diagnosed asthma and hospitalization	*RR for physician diagnosed asthma or bronchitis at 18 months for use of paracetamol at any time*

2008[199]		*utero*)		for asthma	*during pregnancy: 1.17 (1.13, 1.23), hospitalization due to asthma: 1.24 (1.11, 1.38) and physician diagnosed asthma at 7 yr: 1.15 (1.02, 1.29)*
Persky *et al* 2008[200]	US	longitudinal study (*in utero*)	1 yr	Respiratory symptoms, wheeze, and asthma	*OR for wheeze for use of paracetamol during middle to early pregnancy: 1.80 (1.10, 3.00), asthma: 2.00 (0.60, 7.20). However no significant effect with exposure early in pregnancy*
Shaheen *et al* 2005[219]	UK	longitudinal study (*in utero*)	18 months-7 yr	Wheeze, eczema, rhinitis, sensitization and serum IgE	*OR for asthma for use of paracetamol during late pregnancy for those who took most days/daily vs. never: 1.62 (0.86, 3.04), wheezing: 1.86 (0.98, 3.55), and total IgE (geometric mean ratio): 1.14 (1.03, 1.26).* *No association with eczema or rhinitis*
Barr *et al* 2003[206]	US	longitudinal study	30-35 yr (women)	Physician diagnosed asthma	*OR for asthma for use of paracetamol for more than 14 days per month vs. never: 1.63 (1.11, 2.39).*

1.3 SUMMARY

From the risk factors discussed above, it can be concluded that a range of environmental factors, interacting possibly with genetics, may explain the worldwide changes in the prevalence of asthma and allergic diseases. However, no specific harmful or protective putative exposure has been identified yet.

Among the risk factors explored in the past decade, the role of paracetamol in the development or maintenance of asthma and allergic disease is intriguing. However, in studies based in developed countries, the possibility that paracetamol and asthma are associated through reverse causation, in turn arising from medical advice to avoid aspirin use in people with asthma, is difficult to exclude. Moreover, prospective evidence has been limited to a handful of studies, and only two studies looked at personal consumption: one in children and the other in adult women, and both are based in developed countries. Studies have also tended to rely on recall of paracetamol use in the past, or not adjusted for confounding by early respiratory infections. Further work is therefore needed, particularly that in developing countries, where avoidance of aspirin due to asthma risk is unlikely,[203] and bias due to the availability of multiple formulations of paracetamol is less of a problem.

Recent interest has also been focused on the protective role of various gastro-intestinal organisms on asthma and allergic disease, including the stomach-colonizing bacterium *H. pylori*, geohelminths and commensal bacteria. Most of the *H. pylori* studies are cross-sectional or case control, in which alternative

explanations including reverse causation are a problem; and no prospective study has been reported in children. Moreover, the majority of the studies have been in adults, with only three studies conducted in children; furthermore as all are based in developed countries, bias arising from universal *H. pylori* eradication therapy, that this is not the case in developing countries, is difficult to exclude. The relation between geohelminth infection, particularly hookworm, and asthma and allergic disease has proven controversial. In tropical areas, like Ethiopia, the first exposure to geohelminth infections typically occurs at an early age, during a crucial period of immune development. However, to date, most studies have been based on older children or adult populations, and evidence from prospective birth cohort studies from developing countries is remarkably scarce. Finally, the role of commensal bacteria in the pathogenesis of asthma and allergic disease has been debated over the past 15 years, and remains unclear. As with the other gastro-intestinal exposures, studies exploring this hypothesis are mainly based in developed countries, in which the possibility that bias arising from antibiotic therapy and changes in the dietary habit is difficult to untangle. Moreover, the available prospective studies, with a few exceptions, are small scale studies, often include less than 100 subjects, and power is an issue. No longitudinal study from a developing country to date is available, therefore making a case for this study.

At present, the relationship between each of these risk factors and asthma and allergy remains uncertain. The association between geohelminth infection, commensal bacteria and *H. pylori*, and asthma and allergic disease is potentially very important, because it raises the possibility that products might have a therapeutic value. Furthermore, paracetamol remains one of the most prescribed over-the-counter pain reliever, and the safe analgesic and antipyretic

drug of choice in the world, and Africa; and further investigation of this enigma in asthma; if this is a true effect, could have potential preventive value.

This thesis has therefore explored these associations in a birth cohort of children followed to age five in Butajira, Ethiopia, where previously established bias associated with each exposure is less pronounced, and where asthma and allergic disease are continuing to emerge as clinical problems. The study aims to establish, for the first time prospectively in a longitudinal cohort, the influence of these risk factors on the incidence and prevalence of allergic diseases.

1.4 OBJECTIVES OF THIS THESIS

The aim of this thesis is to investigate the hypothesis that early infection with geohelminths, commensal bacteria, and *H. pylori* protects against asthma and other allergic disease, and paracetamol increases the risk of these conditions using an Ethiopian birth cohort. More specifically the objectives of this thesis are:

1. To determine the independent effect of paracetamol use in early life on the incidence and prevalence of wheeze, eczema, rhinitis, and skin sensitization in young children.

2. To determine the independent of effect of *H. pylori* infection on the incidence and prevalence of wheeze, eczema, rhinitis, and sensitization in young children.

3. To determine the independent effect of geohelminth infection, specifically hookworm, *A. lumbricoides*, and *T. trichiura* infection, on the incidence and prevalence of wheeze, eczema, rhinitis and sensitization in young children.

4. To determine the independent effects of selected commensal bacteria, namely enterococci, lactobacilli, and bifidobacteria, on the incidence and prevalence of wheeze, eczema, rhinitis and sensitization in young children.

2 METHODS

2.1 COUNTRY PROFILE: ETHIOPIA

2.1.1 Geography

Ethiopia is situated in the Horn of Africa between 3^0 and 15^0 north and 33^0 and 48^0 east. It is the tenth largest country in Africa with an area of 1.1 million km^2. Ethiopia is bordered to the north by Eritrea, Sudan and South Sudan to the west, Djibouti and Somalia to the east, and Kenya to the south. Ethiopia is an ecologically diverse country with a range of deserts, tropical forests and Afro-mountain. The principal climatic zones are tropical rainy, dry and warm temperate climates. It is administratively divided in to nine regional states and two city administrations, and the capital city is Addis Ababa[220] (Figure 2.1).

2.1.2 Demography and education

Ethiopia is the second most populous nation in Africa. According to the most recent census in 2007, it has a total population of 73.9 million, with annual growth rate of 2.6%.[220] Of these, 37.3 million (50.5%) are males, and the rest (36.6 million, 49.5%) females. Most of the population live in rural areas (84%), while the rest live in urban areas (16%). The population of under five children (0-4 years) is 10.8 million.[220] The dependency ratio is high, with 48.9% of the population age under 15 or over 65, and 51.9% in the working age group (15-64 years).[220] The largest proportion of the country's population are found in Oromia region (36.7%), followed by Amhara (23.3%) and Southern Nations, Nationalities and People's Region (SNNPR) (20.4%) (Figure 2.1).[220] The majority

of the population are Orthodox Christian by religion (43.5%), followed by Muslim/Islam (33.9%), Protestant (18.6%), traditional religion group followers (2.6%), and Catholic (0.8%).[220] Ethiopia is a multi-cultural country with more than 80 ethnic groups[220] and over hundred dialectics; Amharic being the official language. In regards to education, the Gross Enrolment Ratio (GER) at primary level (grade 1-8) is 85.8%, with a Net Enrolment Ratio of 73.9% and a male to female gender gap of 14.4%.[221] Although Ethiopia has shown improvement in the education sector, a significant share of the primary school age population has an unmet need of education.[221]

Figure 2.1 Regional map of Ethiopia

Source: United Nations Office for the Coordination of Humanitarian Affairs (UNOCHA). Available at; http://www.ocha-eth.org/Maps/downloadables/ETH.GENERAL.pdf

2.1.3 Economy

Ethiopia is among the least developed counties, with a per capita gross national income (GNI) in 2009 of US$330.[222] The main economy is based on agriculture which employs about 80% of the population and accounts for about 90% of the exports,[223] and 51% of the gross domestic product (GDP).[222] According to the crop production survey conducted by the Central Statistical Authority, the major crops produced from 2007 to 2009 were cereals (maize, wheat, sorghum, barley, finger millet, rice, oats and *teff*), and oil seeds (mainly rapeseed and groundnuts).[224] Ethiopia is a major producer of coffee, which is the main source of export economy for the country.[224]

2.1.4 Health

According to the Ethiopian Federal Ministry of Health report on the Health Sector Development Program (HSDP), the health service coverage in 2006/07 is 87%.[225] The number of hospitals in 2006/2007 is 143, while the number of health centres and health posts (the smallest primary health care unit) is 690 and 9,914 respectively.[225] The doctor-to-population ratio is 1:42,706 and nurse-to-population ratio 1:4,207.[225] The Health Service Extension Program (HSEP), an approach that brings health care to the village level, is the main actor of the health care delivery particularly at community level, with the health extension worker-to-population ratio being 1:4,369 (Table 2.1).[225] Safe water and sanitation supply are rudimentary in the country with only around 47% and 30% of the population estimated to have access to safe water and sanitation respectively (Table 2.1).[226]

Given the high population growth rate, and the poor health care delivery system, Ethiopia has poor health and health-related indicators. According to the preliminary report of the Ethiopian Demographic and Health Survey (EDHS) 2011, the total fertility rate (the number of children a woman would have in her child bearing age) three years preceding the survey is 4.8 per 1000 women aged 15-49.[227] As expected, the fertility rate is considerably higher in rural (5.5) than urban areas (2.6).[227] Regardless of type of contraceptive, overall 29% of currently married women are using any family planning methods.[227]

Amongst the main health problems in Ethiopia, HIV/AIDS created a significant burden in the health system with an estimated adult prevalence in 2010 of

2.4%, and around 1.2 million people living with the disease.[228] A trend analysis however showed that the urban epidemic appears to have levelled off whilst there is no change in the rural epidemic.[228] Tuberculosis (TB) and malaria are the other major health problems in the country with about 126,106 new cases of TB registered in 2006/2007,[225] and estimated malaria parasite prevalence in 2007 of 0.7%.[229]

Twenty four percent of the children aged between 12 to 23 months in 2011 are fully vaccinated whilst 15% have never received any vaccination.[227] Malnutrition is highly prevalent in Ethiopia with 44% of the children less than five years stunted, indicating chronic malnutrition, whereas 10% of them are wasted, a condition reflecting acute malunitrion.[227] The most important causes of childhood morbidity and mortality, among others, are acute respiratory tract infections (ARI), fever and diarrhoea with dehydration. The Ethiopian Demographic and Health Survey (EDHS) 2011 found that overall, 7% and 17% of children under five showed symptoms of ARI and fever in the two weeks preceding the survey, of whom 27% and 24% respectively sought treatment from the health facility.[227]

In relation to child mortality, the neonatal morality rate (the chance of dying within the first month of life) is 37 per 1,000 live births and the infant mortality rate (the chance of dying before the first birthday) 59 per 1,000 live births. The estimated child mortality (the probability of dying between the first and fifth birth day) is 31 deaths per 1,000 children surviving to 12 months of age, and the overall under-5 mortality rate (the probability of dying between the birth and fifth birthday) is 88 deaths per 1,000 live births (Table 2.1).[227] The maternal mortality rate in 2005 was 673/100,000 live births,[230] with the major causes of

death being bleeding, infection (sepsis), obstructed labour, and abortion. The proportion of mothers who attended antenatal care and delivered in an institution with a skilled birth attendant was 29% and 10%, respectively.[227]

Comprehensive data on Non Communicable Diseases (NCDs) are not available in Ethiopia. However, some studies have shown that the prevalence and incidence of chronic diseases and risk factors such as high blood pressure, physical inactivity, overweight, smoking, and alcohol drinking are increasing.[231-235] A recent population based survey among 25-64 years living in Addis Ababa showed that about 20% of males and 38% of females were overweight, and 31% and 17% of females and males respectively were reported to have low physical inactivity.[233] The burden of high blood pressure is also showing an increasing trend with almost a third of the population in Addis Ababa found to be hypertensive.[233] Furthermore from the same population it was documented that current smoking among men was 11%, alcohol intake was 10.4%, and khat (*Catha edulis Forsk*) chewing was 15.9%.[234] Table 2.1 summarises key maternal, child, and environmental and basic health indicators in Ethiopia.

Table 2.1 Key health and health related indicators in Ethiopia

Health indicators	
General health*	
Potential health service coverage	87%
Doctor/population ratio	1:42,706
Nurse/population ratio	1:4,207
Health extension worker/population ratio	1:4,369
Child health*	
Neonatal mortality rate	37
Infant mortality rate	59
Child mortality rate	31
Under-five mortality rate	88
All basic vaccinations (BCG, measles and 3 doses of DPT)	24.3%
Symptoms of acute respiratory tract infections (cough, and fast breathing)	7%
Fever (2 weeks preceding the survey)	17%
Diarrhoea (2 weeks preceding the survey)	13%
Maternal Health*	
Antenatal care coverage	34%
Institutional delivery	9.9%
Maternal mortality rate[¥]	673
Environmental health[c]	
Access to safe water (2004/05)	47%
Access to sanitation (2004/05)	30%

* Source:[225] Federal Ministry of health of Ethiopia, Health and Health related indicators, 2006/2007.

† Source:[227] EDHS 2011, and all rates expressed per 1,000 live birth, except for child mortality which is expressed per 1,000 children surviving to 12 months of age.

¥Source:[230] EDHS 2005, rates expressed per 100,000 live births.

[c]Source:[226] Federal Ministry of health of Ethiopia, Health and Health related indicators, 2004/2005.

2.2 BUTAJIRA RURAL HEALTH PROGRAM (BRHP)

2.2.1 The Butajira Rural Health Program and reasons for choice of Butajira

The shortage of data on vital and other health related events in low income settings led to the development of other strategies for obtaining this information. One such example in Ethiopia is the Demographic Surveillance Site (DSS) in Butajira, called the Butajira Rural Health Program. The BRHP is part of the International Network for the Demographic Evaluation of Populations and Their Health in Developing Countries (INDEPTH Network), which is a global network of members working in 19 countries in Africa, Asia and Oceania who conduct longitudinal health and demographic evaluation of populations in low and middle income settings (www.indepth-network.org). The BRHP was initiated in mid 1986 by performing a census of the population of ten randomly selected *kebeles* (the smallest administrative unit) using the probability proportionate to size technique in the districts of Meskan, Mareko and Silti.[236]

2.2.2 The study setting: geography

The Butajira DSS is located in the Meskan, Mareko and Silti Districts, Gurage Zone, in the Southern Nations, Nationalities and Peoples Regional state (SNNPR) of Ethiopia (Figure 2.2). According to the 2007 population and housing survey, the total population of the study area (Mareko, Meskan, Silti Wodeda and Butajira town) is 434, 036, with a female to male ratio of 1:1.02. The majority live in rural areas (84%), and the rest live in urban areas (16%).[220] The estimated size of the District is 797 km^2 and Butajira town covers approximately

9km^2. The district capital, Butajira, is located 135 km south of the capital city, Addis Ababa, and 50 km to the west of Zway town in the Rift valley. Historically the district was part of 'Gurageland' and acquired its name in 1954. The naming of the town originating from the presence of a *'shifta'* (bandit) in the area by the name of 'Buta': traders coming into the town called out 'Buta jira' to ask 'is Buta there?', and thus coined the name 'Butajira'.[236]

Butajira is located at an elevation of around 2,100 meters above sea level, ranging from 1,750 meters in the lowlands to 3,400 meters in the mountainous area (Figure 2.2). The climate varies from arid dry lowland areas to cool mountainous areas. The main rainy season is from June to September with rainfall ranging between 900 to 1,400 mm per annum.[236]

The district was divided administratively into approximately 86 units, mostly rural, but including the urban Butajira town.[237] Out of the ten initially sampled study units, nine were rural *kebeles* (also called Peasants' Associations), and one was an urban *kebele* (also called Urban Dwellers Association) (Figure 2.2). The nine rural *kebeles* (administrative units) are: 'Dobena', 'Bati', 'Hobe', and 'Mekakelegna Jare Demeka' from the lowland areas, 'Misrak Meskan', 'Wurib', 'Yeteker', 'Bido' and 'Dirama' from the highland areas, and 'Butajira 04' from the town (Figure 2.2). An initial census at the end of 1986 enumerated all the residents of the ten selected communities and formed the basis for an open cohort study (where members of the cohort enter by birth or in-migration, or exit by out-migration or death), which is still continuing. The initial census was used to obtain baseline population information and to establish a system of demographic surveillance with continuous registration of vital events (birth,

death, marriage, new household formation, out-migration, in-migration, and internal moves within the study units) at the household level. In addition, the study base aimed to generate information on morbidity, mortality and provide a sampling frame for other health-related research and interventions.[236] The demographic and other health related indicators of the DSS were very similar to the EDHS, and also to the national figures, reflecting the representativeness of the BRHP.[236, 238]

The Butajira area was chosen purposefully as being potentially representative of the Ethiopian population on the basis of being 130km from the capital Addis Ababa (but also near enough to Addis Ababa University where the study administration is based), combining a mixture of the highland and lowland areas typical of Ethiopia, and being home to a mixture of geographical, ethnic, cultural and religious groups.

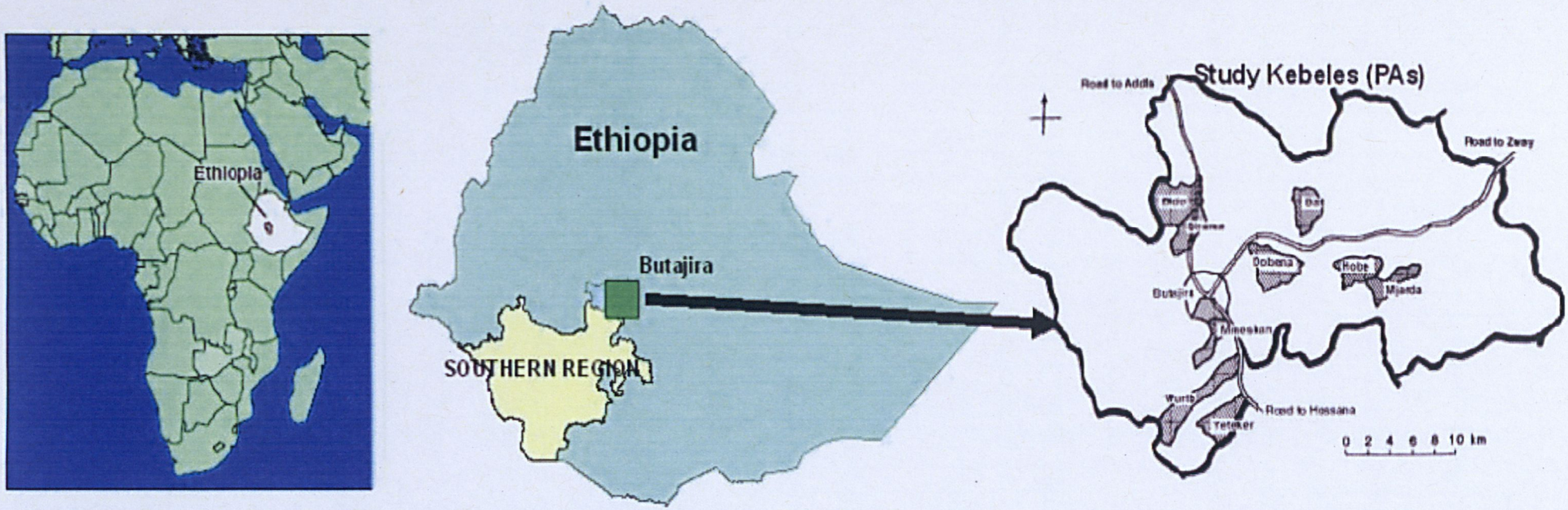

Figure 2.2 Map of Ethiopia showing the study area – Butajira and sampling units

Source (with some modification)[236]

2.2.3 The BRHP population: demography and health

The BRHP population is estimated to be 60,000, and contains more than 13,268 women of reproductive age.[236] Butajira, the district capital, has a total population of 33,393, with male to female ratio of 1:1.03.[220] Two thirds of the district population are followers of Islam religion followed by Orthodox and other Christian. Various ethnic groups such as the Meskan, Mareko, Silti and Sodo, live in the district. The predominant language, *'Guragigna'* belongs to the Ethio-semitic language group, and the working language is Amharic which is spoken by most of the population in the district.[236]

The livelihood of the majority of the residents is based on mixed farming. Khat (*Cata edulis Forsk*) and chilli-peppers are the main cash crops, while maize and "false banana" or Ensete (*Ensete ventricosun*) are the main staples.[236] The main occupations are related to farming in the rural area, and small-scale businesses in the town.[236] An all-weather road connects Butajira town north with Addis Ababa and south with Hosanna, another all-weather road connects it to Zway. The villages in the district, with few exceptions, are connected to the town by dry-weather roads.[236] The lowland areas are drought prone and have been affected by major drought in the past decade,[236] the most recent being in 2002/2003. The Butajira town has 24-hour electricity, and telephone and postal service, none of which are available in the rural areas.[236]

The study population is served by one government owned hospital, one private hospital, four health centres (more health centres are under construction), at

least one health post per study unit, and several privately owned drug stores and clinics. Childhood mortality indicators in the study area resemble the national indicators, with the perinatal mortality rate reported at 22 per 1,000 live births, the infant mortality rate 65/1,000 live births, the childhood mortality rate 31.5/1,000 live births, and the under-five mortality rate 40/1,000 live births.[238] The major causes of morbidity and mortality in the district are malaria, respiratory tract infection (pneumonia), tuberculosis, diarrhoea and gastroenteritis, acute febrile illness and other infective and parasitic diseases (*giardiasis*, and *amoebiasis*). In terms of adult mortality, the crude mortality rate (between 15-64 years) was 7.8 per 1,000 person years, of which 53% were attributed to communicable diseases and the rest to NCDs, showing the emergence of NCDs in the study area.[239] The cause of death data were derived from verbal autopsy questionnaires and therefore need careful interpretation as bias due to misclassification is possible.

The BRHP is now well established and is used for a variety of nested studies, involving many international collaborators from across the world.[237] One such nested study is the Butajira birth cohort, on which this thesis is based, and which is described in the next section.

2.3 BUTAJIRA BIRTH COHORT

2.3.1 Establishment of the Butajira Birth Cohort

In 2004, collaboration between the Institute of Psychiatry, King's College London, and the Department of Psychiatry, Addis Ababa University received funding from The Wellcome Trust to set up a birth cohort to investigate the hypothesis that common maternal mental health disorders in pregnancy and the post natal period are associated with infant health, growth, development and survival (grant number WT081504/Z/06/Z). Earlier collaboration over risk factors for asthma and allergic diseases between investigators at Addis Ababa University, Ethiopia and the University of Nottingham, United Kingdom, led to an extension of this birth cohort to include additional hypotheses relating to aetiology of asthma and allergic disease (funded by Asthma UK, grant number 07/036). The collaboration was started just after the birth of the children; however my involvement in the cohort started after the follow-up at age one, and this thesis therefore focuses on outcomes collected after this time (age three and five).

The methodology relating to the initiation of the cohort and data collection during pregnancy has been described elsewhere.[240, 241] In brief, after validation and qualitative work,[241] pregnant women living in the BRHP were recruited into this birth cohort (Figure 2.3). Eligible women were those aged between 15-49 years, in the third trimester of pregnancy during the study recruitment period (July 2005 to February 2006), living in the area covered by the BRHP, and able to speak the national language of Ethiopia, Amharic. These women were identified by the BRHP fieldworkers during a quarterly surveillance interviews.[240]

Of the 1234 eligible women, 134 were excluded because they delivered before interview, 26 were not identified before birth or not traced, and nine refused to participate, leaving 1065 women (86% of eligible) who were successfully recruited in the study (Figure 2.3). Non-participating women did not differ from participating women in respect to socio-demographic characteristics.[240] The project data collectors visited the pregnant women at home and an interview-led questionnaire was administered. Information collected during pregnancy, as part of the main study among others are: antenatal common mental health disorder (CMD), psychosocial exposures, physical illness and somatic symptoms.[240] Furthermore socio-demographic data including age of the mother, area of residence, ethnicity, religion, marital status, maternal occupation, education, household income, and housing characteristics were available during this follow-up.[240]

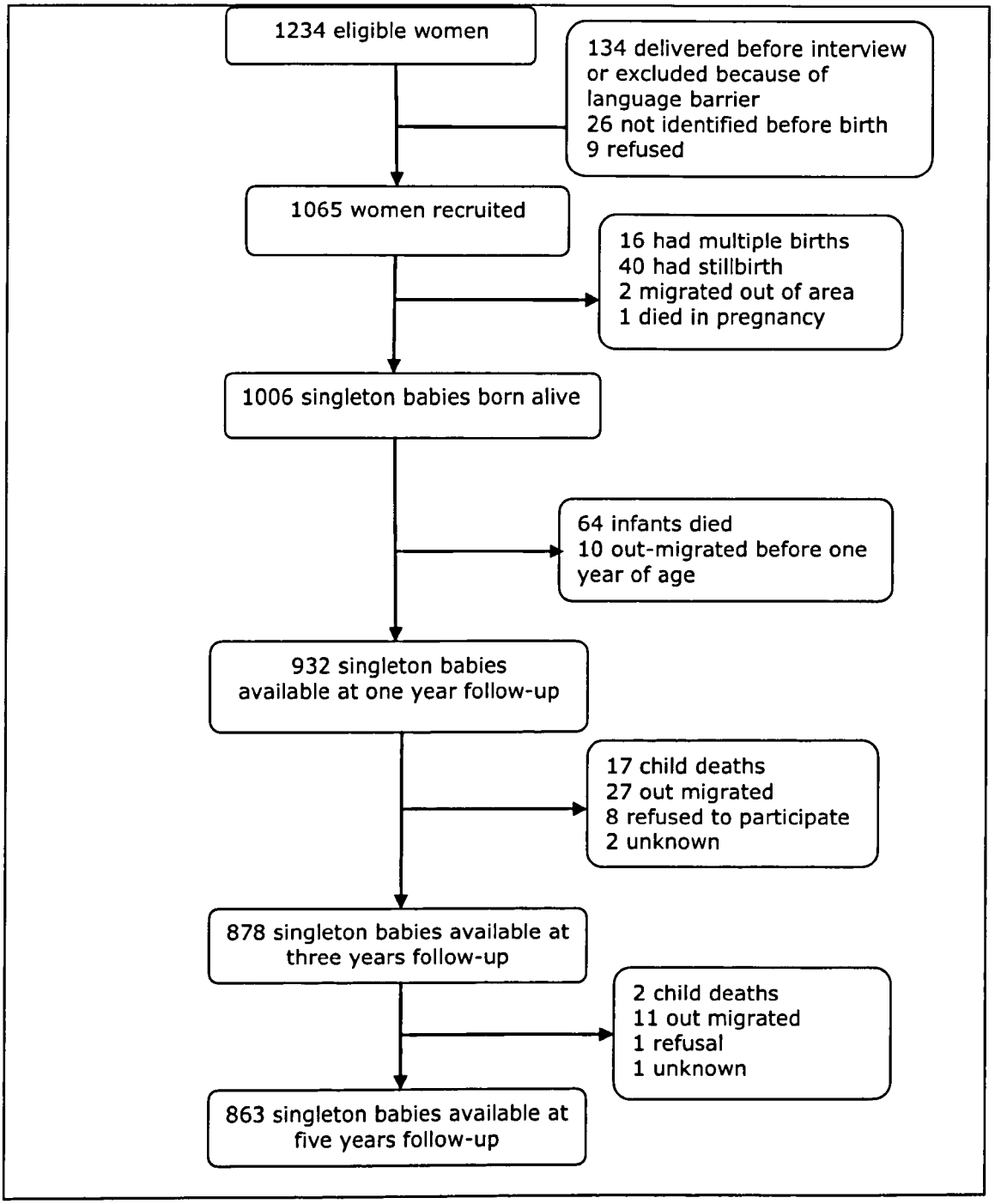

Figure 2.3 The Butajira Birth Cohort from recruitment up to year five

2.3.2 Characteristics of the women at recruitment

The age range of the women was 15 to 46 years (mean [SD] 26.9 [6.4] years), the great majority (85%) were in the age group 15-34 years, and the rest were older. Less than one quarter of mothers (20.6%) had formal education, the rest had no education (Table 2.2). Over 85% of the mothers were from rural areas, 76% of them lived in houses with thatched roofs, and more than 80% of them were housewives. The largest Ethnic group was Meskan (46%), and the majority of the women (78%) were Muslim (Table 2.2).

Table 2.2 Baseline characteristics at recruitment

Baseline characteristics	Number (%)
Area of residence	
Urban	152 (14.3)
Rural	913 (85.7)
Age of the mother	
15-24	410 (38.5)
25-34	495 (46.5)
35-46	160 (15.0)
Ethnic origin	
Meskan	485 (45.5)
Silti	257 (24.1)
Mareko	147 (13.8)
Others*	176 (16.5)
Religion	
Muslim	826 (77.6)
Christian	239 (22.4)
Education of the mother	
No formal education	846 (79.4)
Had formal education	219 (20.6)
Occupation of the mother	
House wife	901 (84.6)
Any other jobs†	164 (15.4)
Marital relation of the mother	
Monogamous marriage	855 (80.3)
Polygamous marriage	200 (18.8)
Single	10 (0.9)
Type of roof	
Thatched roof	806 (75.7)
Corrugated iron sheet	259 (24.3)

N=1065

*Sodo, Dobi, Oromo, Amhara and Wolene

†Farm related, profession related, trade related and daily laborer

2.3.3 Birth of babies in cohort

Of the 1065 women recruited into the study, two migrated out of the area before giving birth, one died during pregnancy, 40 had still births, 16 had multiple births and 1006 had live singleton babies[240] (Figure 2.3). These 1006 babies from the birth cohort subsequently followed-up. Birth weights were collected; mostly within 48-hours of birth on over 60% of cohort babies (measured in districts where a suitable health worker was available) (Table 2.3).

In addition, information on breastfeeding status (exclusive breastfeeding) and immunization for age (BCG and polio at birth, and DPT_1 vaccines) were available at two months (Table 2.3). These variables were ascertained by asking the mother whether the child was immunized for age (Yes/No), and if available, registration cards were checked, and BCG scar verified. Information on symptoms of respiratory tract infection (cough, fast breathing and fever) was also available. The mothers were asked whether the child had coughing (Yes/No), fast breathing (Yes/No) or fever (Yes/No) since birth.

The demographic and early life characteristics of the babies in the cohort are shown in Table 2.3. The sex ratio was 1:1.05. Most (84.0%) of the infants were exclusively breast fed till the age of two months, more than 90% of those weighed had birth weights of >2.5kg. Around 59% of the infants had received at least one vaccination by two months.

Table 2.3 Demographics and early life characteristics of the babies

Characteristics	Number (%)
Gender (N=1006)	
Male	516 (51.3)
Female	490 (48.7)
Birth weight (N=653)	
Low (<2.5kg)	48 (7.4)
Normal	605 (92.7)
Vaccination at 2 months[‡] (N=955)	
Yes	562 (58.9)
No	393 (41.2)
Exclusive breastfeeding status at 2 months (N=955)	
Yes	802 (84.0)
No	153 (16.0)

[‡] Any vaccination history at 2 months (BCG, Polio, and DPT_1)

2.3.4 Follow-up of cohort at age one

The first year follow-up of the birth cohort was conducted by a previous Master's student project between July 2006 and May 2007. The Butajira data collectors, who knew and visited the mothers bi-annually since the establishment of the birth cohort administered an interview-led questionnaire (within two weeks of the first birthday). Of the 1006 babies in the cohort, 64 (6.4%) had died and 10 (0.9%) migrated from the study area before their first birthday, leaving 932 children successfully followed-up at age one (Figure 2.3). The prevalence of and risk factors for wheeze and eczema at age one have been reported elsewhere.[242]

2.3.4.1 Wheeze and allergic disease measures at age one

The questionnaire included questions on wheeze, asthma and eczema based on the International Study of Asthma and Allergies in Children (ISAAC) core allergy and environmental questionnaire,[25] and used previously in Ethiopian

populations[43, 104, 243, 244] (Table 2.4). Data on wheeze and eczema were collected at year one, however questions on rhinitis were not asked, consistent with other studies in very young children as it is unlikely to represent an allergic phenotype.[197, 199] These outcomes were identified by a positive answer to the following questions, and the full questionnaire (English and Amharic translated) can be found in Appendix II to Appendix V.

Wheeze

- *'In the last 12 months/since birth has your child had wheezing or whistling in their chest?'*);

Asthma

- *'In the last 12 months/since birth has your child had asthma?'*, and *'has this been confirmed by a doctor?'* and;

Eczema

- *'In the last 12 months/since birth has your child ever had an itchy skin rash which has affected the skin creases, eg, front of the elbow, behind the knees, the front of the ankles, around the neck, or around the eyes?')*

Table 2.4 Summary of variables measured during pregnancy, at birth and at ages one, three and five

Variables	Follow up points					
	During pregnancy	At birth	At 2 months	At Year 1	At Year 3	At year 5
Reported allergy outcomes						
Wheeze symptom question				x	x	x
Eczema symptom question				x	x	x
Rhinitis symptom question					x	x
Reported/confirmed asthma				x	x	x
Socio-demographic characteristics						
Age/sex/area of residence	x	x				
Ethnicity/religion	x					
Maternal occupation/education	x					
Household income	x					
Environmental risk factors						
Sanitation and water supply	x			x	x	x
Roof/wall/floor type	x			x	x	x
Indoor smoking	x			x	x	x
Presence of animals	x			x	x	x
Insecticide use	x			x	x	x
Indoor/outdoor cooking	x			x	x	x
Family factors						
Family history of allergy				x	x	x
Household size				x	x	x
Child's place of sleep				x	x	x
Number of siblings				x	x	x
Birth order				x	x	x
Childhood characteristics						
Birth weight		x				
Breast feeding history		x	x	x	x	
Vaccination		x	x	x	x	
Use of paracetamol				x	x	x
Symptoms of respiratory tract infections (cough, fast breathing and fever)			x	x	x	x
Specific infections						
Geohelminths				x	x	x
Selected commensal bacteria					x	
Helicobacter pylori					x	x
Sensitization						
Skin prick test (*D. Pteronyssinus* and cockroach allergen)					x	x
Allergen						
Dust samples (Der p1 and Bla g1)					x	

2.3.4.2 Paracetamol exposures at age one

Questions related to paracetamol exposure were asked through mothers on behalf of the child. The questions on paracetamol exposure were adapted from the ISAAC Phase three environmental questionnaires,[189] and had been previously used in the same Butajira population.[203, 213] The mothers were asked whether the child had taken or been given paracetamol in the past 12 months (*"Has your child taken any paracetamol in the last year (e.g. Panadol, or Pamol)?"*), and a positive response was recorded as use of paracetamol in the past year. To assess a dose-response relationship, those who reported use in the past year was further asked to quantify the dose of paracetamol consumed in the past month (*"How many tablets of paracetamol has the child taken in the last month?")*

2.3.4.3 Geohelminth analysis at age one

Each child's faecal sample was analyzed for geohelminths at age one. Stool pots with scoops and 4ml diluted formaldehyde were used to collect stool samples for parasitological examination. Stool samples were transported to Aklilu Lemma Institute of Pathobiology at Addis Ababa University for geohelminth analysis. Samples were examined qualitatively using the modified formol-ether concentration method.[245] Using an applicator stick, approximately 1g of faeces was placed in a clean 15ml conical centrifuge tube containing 7ml of diluted formalin (10%). The sample was mixed and suspended. The resulting suspension was filtered through a sieve into a beaker and the filtrate was poured back into the same tube. The debris trapped on the sieve was discarded. After adding 3ml of ether to the solution, the contents were shaken and centrifuged at

300rpm for one minute. The supernatant was poured off, and the sediment was then examined under a microscope using 10x and 40x objective lenses (Appendix VI). The prevalence of and risk factors for geohelminth infection at age one has been previously reported.[246]

2.3.4.4 Measurement of potential confounders at age one

Information was collected on a range of potential confounders (Appendix II to Appendix V), and these are summarized in Table 2.4. This information was collected for adjusted analysis to be explored, and includes:

- Familial factors: Maternal and paternal history of asthma and allergy: parental history of allergy was measured by using three questions on asthma (*'In the last 12 months have you or your husband had asthma?'*), rhinitis (*'In the last 12 months have you or your husband had problems with sneezing or running nose when not have cold or flu, or problems with itchy watery eyes?'*) and eczema (*'In the last 12 months have you or your husband had an itchy skin condition affecting the skin creases (front of the elbow, behind the knees, the front of the ankles, around the neck, or around the eyes?'*));

- Childhood risk factors: Immunization, and vaccination for age, breastfeeding status and duration, birth order, and number of siblings, child's use of antibiotic, presence or absence of symptoms of respiratory tract infections – cough, fast breathing and fever;

- Household characteristics: roof, wall and floor type, household size, and child's sleeping place;

- Environmental risk factors: waste disposal sites, soap use, and source of water supply, type of toilet, indoor pollution including indoor cooking, indoor kerosene use, presence of animals, and insecticide use.

2.3.4.5 Demographics of the children at age one

Of the 932 singleton babies at age one, 33 had missing data on main outcomes and exposures, leaving 899 (89%) children available for analyses. Just over half were male (50.5%) and the majority (83.1%) were from rural areas. The majority of the households had thatched roofs (76.6%) and mud walls (99.6%) (Table 2.5). Most households (63.3%) had animals inside overnight, and cooking took place inside the main house in 80.9%. Fifty percent of the children slept on locally made grass matting, 42.5% on the floor and only 7.6% on a bed or platform. Most of the children (54.7%) had 1 to 3 older siblings, and 56.3% lived together with 4 to 6 members of the household (Table 2.5).

Table 2.5 Distribution of demographics and potential risk factors at age one

Risk factor	Overall N *(%)*
Child's Gender	
Male	454 (50.5)
Female	445 (49.5)
Place of residence	
Urban	117 (13.0)
Rural	782 (87.0)
Insecticide use	
Yes	748 (83.2)
No	151 (16.8)
Number of people in house	
1-3	115 (12.8)
4-6	506 (56.3)
7+	278 (30.9)
Older siblings	
0	138 (15.4)
1-3	492 (54.7)
4-10	269 (29.9)
Child's sleeping place*	
Bed/Platform	68 (7.6)
Floor	382 (42.5)
Grass matting	448 (49.9)
Cooking site	
Inside	727 (80.9)
Outside	172 (19.1)
Indoor kerosene use	
Yes	98 (10.9)
No	801 (89.1)
Animals living in the home*	
Yes	569 (63.4)
No	329 (36.6)

N=899
**One missing data (n=858)*

2.3.5 Follow-up of cohort at age three

2.3.5.1 Field preparation

The field preparation for the three year follow-up, the time when I got fully involved, was started from late 2007. This involved designing a questionnaire; setting-up a laboratory for the analysis of geohelminths and bacteriological samples in Butajira; recruitment, training, and preparation of manuals for data collectors and laboratory personnel; import of skin test allergen solution and skin-prick-test lancets, vacuum and dust filters, bacteriological agar media, and *H. pylori* stool Ag kits from the UK; and designing a database for data entry. The actual data collection was started in July 2008 through to June 2009.

2.3.5.2 Data collection process, training and pre testing

Data collectors were recruited from the local area. They were all women, and had completed high school education (grade 10 or 12); three were clinical nurses. Women data collectors were purposefully chosen to create rapport with the mothers in the cohort. Alongside the data collectors, two data entry clerks, also women, and high school completed with computer-related training, were employed. Four laboratory technicians, with two years laboratory training, and at least five years of work experience, were recruited to work part time from the Butajira health centre for the three year follow-up. Two supervisors were recruited whose role was organizing the field work and checking the consistency of the questionnaire. Data collection, on average, was undertaken every morning between 0700 and 1400.

The data collectors were given three days of intensive training on the aim, consent process, contents of the questionnaire, conducting the skin prick test, and stool and dust sample collection. Pre-test of the instruments was done in a population other than those included in the study, and feedback was given accordingly. The laboratory personnel were trained at Aklilu Lemma Institute of Pathobiology at Addis Ababa University for two weeks on the stool concentration technique for geohelminths, culture microbiology, and analysis of rapid stool Ag test for *H. pylori*. The trainers were from the Institution who had training and experience on stool microscopy and culture microbiology. Data collection and bench laboratory manuals were provided for daily reference (Appendix VI and Appendix VII).

Every questionnaire was checked, edited, marked and passed onto the data entry clerks who further inspect the variables on the hardcopy, and recorded into an Excel sheet using key identifier and the date of birth. Incomplete or missing data were returned to the field for re-collection and the database updated accordingly. Refresher training and feedback to the data collectors and data entry clerks were given every Friday by the PhD candidate to explain any confusion and queries arising from the field.

As for the questionnaire data, the stool and dust samples were checked for amount and consistency and the key identifiers recorded in an Excel sheet. The samples were then taken to the Butajira health centre laboratory for analysis. Reports were collected on a daily basis, checked against the original sample, and entered in the same database as the questionnaire data. About 10% of the positive and negative stool samples and microscopy slides were reserved and

transported, when possible, to the Aklilu Lemma Institute of Pathobiology at Addis Ababa University for quality assurance, and feedback was given based on the results.

At three year follow up, 17 (1.8%) children had died since the one year follow up, 27 (2.9%) had out-migrated either temporarily or permanently, eight (0.9%) refused to participate in the study and two (0.2%) were missing for unknown reasons, leaving 878 children who provided outcome and exposure data (Figure 2.3).

2.3.5.3 Wheeze, allergic disease and skin test measurements at age three

The questionnaire included questions, similar as in the one year follow up in section 2.3.4.1, on wheeze, asthma and eczema, and additionally questions on rhinitis were included (see section 2.3.4.1, and Appendix II and Appendix V). Besides the outcomes asked in the past year as in section 2.3.4.1; outcomes referring since birth (i.e. ever wheeze, asthma, eczema, and rhinitis) were measured at age three, as follow:

Wheeze

- *'Has your child ever had wheezing or whistling in their chest?'*

Asthma

- *'Has your child ever had asthma?'*

Eczema

- *'Has your child ever had an itchy skin rash which has affected the skin crease (e.g. front of the elbow, behind the knees, the front of the ankles, around the neck, or around the eyes?')*

Rhinitis

- *'Has your child had problems with sneezing or running nose in the past 12 months (when not affected by cold or flu), or problems with itchy watery eyes?'* and;
- *'Has your child ever had problems with sneezing or running nose (when not affected by cold or flu), or problems with itchy watery eyes?'*

In addition to the reported outcome measures, allergen skin sensitization to *D. pteronyssinus* and cockroach allergen (*Blatella germenica*) (Biodiagnostics, Upton-upon-Severn, UK) was measured on each child using skin-prick lancets. These two allergens were previously found to be common in an Ethiopia population.[104] Glycerol saline and histamine dihydrochloride were used as negative and positive controls, respectively. Skin sensitization was conducted at ages three and five, but not at year one. The field work instructions for skin prick testing is available in the Appendix VIII. In brief, after the data collectors explained the aim of skin testing to the mother and requested consent, an 8cm line was drawn long ways down the middle of the palmar side of the forearm, and crossed three times to create four sections (one for each allergen solution). A drop of each solution was then applied, and using a lancet, the upper dermis was lifted and released. After 15 minute, the areas that were raised and red were measured (two diameters at right angles to one another) and recorded. A

positive test were defined as an average of two perpendicular wheal diameters, one of which was the maximum measurable diameter, of at least 3mm greater than the saline control response.

Reliability testing was done among the 11 data collectors who performed the test in 44 children aged three and five years who were not included in the main study. The results showed that there was reasonably good interrater agreement[247] both for cockroach (Kappa=0.67, $p<0.01$) and *D. Pteronyssinus* (Kappa=0.63, $p<0.01$), and sensitization to any allergen (Kappa=0.75, $p<0.01$).

2.3.5.4 Paracetamol exposure at age three

The questions used for measuring paracetamol exposure at age three were identical to those used at age one (see section 2.3.4.2).

2.3.5.5 Geohelminth analysis at age three

The laboratory technique used for the analysis of geohelminth infections was also the same as year one (see section 2.3.4.3 and Appendix VI). However, at year three, in addition to qualitative identification, infection intensity was determined quantitatively using three slides per sample.

2.3.5.6 *Analysis of Helicobacter pylori Ag in stool samples at age three*

A rapid test (Medimar immunocard) was used to determine *Helicobacter pylori* antigen in the stool samples (Biohit, Unit 1 Barton Hill Way, UK) in a randomly chosen subsample at year three due to logistical reasons (N=616) (Table 2.4). The laboratory analysis was conducted in Butajira (Appendix VII). In brief, for a liquid stool, the extraction liquid bottle was opened and 6-7 drops of the stool sample were homogenized in the extraction liquid in the same bottle. For solid stool, a quantity of stool equivalent to 3 or 4 grains of rice was inserted into the extraction liquid and dissolved using a vortex mixer. After 5 minutes, 4 drops of the mixture were dropped on the sampling window and results were read within a maximum of 10 minutes. If only one transverse blue line (control) appeared in the test window, the test was regarded as *H. pylori* negative. While if a second, red transverse line appeared, the test was considered *H. pylori* positive. This method has been accepted for determining current infection status in young children.[248, 249]

2.3.5.7 *Analysis of selected intestinal microflora in stool samples at age three*

Bacteriological analysis for the intestinal microflora enterococci, lactobacilli, and bifidiobacterium was undertaken in a random sample of 544 children at age three (Table 2.4). As for the *H. pylori* stool sample analysis, this analysis was undertaken on the day of sample collection. In brief, a weighed sample of 1gm of faeces brought in Amis transport medium, within 6 hours of collection, was mixed into 10ml sterile buffered peptone water and serially diluted from 10^{-2} -

10^{-9} over Bunsen burner. 0.1ml (100µ) of the mixture from each of the last three dilutions (10^{-7}, 10^{-8}, 10^{-9}) was taken and spread onto freshly prepared media in duplicate on:

i) Bile Esculine agar (Lab M Limited, Topley House, UK), incubated at 37^{0}C for 24 hour. Colonies with dark brown surroundings were counted and reported. For presumptive identification of enterococci, 2-5 representative colonies from each plate were taken to separately sub-culture in Tryptone Soya Broth grown overnight at 37^{0}C. It was then streaked separately to Tryptone Soya Agar for purification and incubated for 24 hour at 37^{0}C to undergo the catalase test. Those with no reaction on the catalase test underwent gram staining, and any gram positive chained coccus under microscopy was reported as enterococci positive.

ii) To identify lactobacillus a separate selective agar media was used. Those spread from each dilution above were inoculated into de Man-Rogosa-Sharpe agar (MRS) (Oxoid LTD, Basingstoke, Hampshire UK) in duplicate and grown anaerobically in an aerobic jar at 37^{0}C for 72 hours. Colonies were counted for each Petri dish using a colony counter and recorded on a form prepared for this. Then up to 2-5 representative colonies were taken from the plate to separately sub-culture in MRS Broth or Brain Heart Infusion (BHI) incubated overnight at 37^{0}C. This was streaked separately into MRS agar for purification and anaerobic incubation at 37^{0}C for 24 hours to undergo catalase test. Those which showed no reaction to the catalase test were gram stained and examined microscopically. If gram positive rods with no spores were identified microscopically, they were presumptively reported as lactobacillus.

iii) Similarly, to identify bifidiobacterium, MRS agar was again used. Those spread from each dilution were inoculated into MRS agar in duplicate and grown anaerobically in an aerobic jar at 40^0C for 72 hours. Colonies were counted in each Petri dish and recorded. Two to five representative colonies were taken from the plate to separately sub-culture in MRS Broth or Brain Heart Infusion incubated overnight at 40^0C. This was streaked separately into MRS agar for purification and anaerobic incubation at 40^0C for 24 hours to undergo the catalase test. Those which were catalase test negative were gram stained and examined microscopically. Gram positive, Y or V shaped bifurcating rods, and rods without spores were presumptively identified as bifidiobacterium. This culture-dependent method for measurement of intestinal microflora has been used in a range of epidemiological studies.[143, 144, 150]

2.3.5.8 Measurement of potential confounders at age three

A range of potential confounders, similar with year one, including residential smoking and antibiotic use were collected at age three (see section 2.3.4.4), and questionnaires found in Appendix II and Appendix V (Table 2.4). In addition to confounders measured using risk factor questionnaire, a dust sample was collected at year three from each child's bedding by 2-3 minute suction through a portable Dyson vacuum cleaner (Dyson LTD, Malmesbury, UK), and DUSTREAM™ collector and filter (Indoor Biotechnologies LTD, The Old Brewery, UK) (Table 2.4). The methods for analysis for dust samples were described below.

2.3.5.9 Dust sample analysis

Samples were brought to the UK and assayed for Der p1 and Bla g1 allergen. Dust was filtered, weighed and extracted following the Indoor Biotechnologies procedure.[250] Dust was sieved through 100uM cell nylon filter to remove large debris and a sample between 95 to 105mg of fine dust was placed into a 2ml Eppendorf. 1ml per 50gm of phosphate buffered saline solution (PBS-T) was added, vortexed briefly and mixed end over end for 2 hours at room temperature (18°c), and centrifuged for 20 minutes and 1200g at 4°C. The supernatant was removed and stored at -20°C until analysed for allergen content. The Der p 1 and Bla g 1 dust assays were performed using a standardized monoclonal antibody-based Enzyme-Linked Immunosorbent Assay (ELISA) method, developed in the Division of Respiratory Medicine, University of Nottingham. The lowest dilution of the sample used was 1/10 (greater than 0.49ng/ml), and a sample found to have no allergen in the 1/10 sample was further analysed for a neat sample using the same ELISA technique.

2.3.6 Follow-up of cohort at age five

2.3.6.1 Field preparation

The five year follow-up was conducted from July 2010 to March 2011. As for the three year follow-up, it has involved field preparation including refresher training for data collectors and laboratory technicians; preparation of laboratory consumables; and import of vacuum and dust filters, *H. pylori* stool Ag kits, and skin test allergen solutions from the UK.

2.3.6.2 *Data collection process, training and pre testing*

The same data collectors who knew the cohort from pregnancy up until age three were also followed the cohort at age five. The data collection process, training, and pre testing were described in section 2.3.5.2.

At age five, 863 children (86% of the original cohort at birth, and 97% of those available at year three follow up) were followed-up (Figure 2.3). Two children died between ages three and five, 11 out migrated, one refused, and one left the cohort under unknown circumstances (Figure 2.3).

2.3.6.3 *Wheeze, allergic disease and skin test measurements at age five*

The same ISAAC questionnaire[25] used at age one and three were administered at age five (see sections 2.3.4.1 and 2.3.5.3, and Appendix II and Appendix V). The only exception was the time frame in which in addition to the outcomes measured in the past year (in the past 12 months at age five) and outcome ever (since birth), we have also measured wheeze, eczema, rhinitis, and asthma in the past two years (since three year follow-up) as presented below:

Wheeze

- *'Has your child had wheezing or whistling in their chest in the past two years?'*

Asthma

- *'Has your child had asthma in the past two years?'*

Eczema

- *'Has your child had an itchy skin rash which has affected the skin creases in the past two years (e.g. front of the elbow, behind the knees, the front of the ankles, around the neck, or around the eyes?')*

Rhinitis

- *'Has your child had problems with sneezing or running nose in the past two years (when not affected by cold or flu), or problems with itchy watery eyes?'*

Data on allergen skin sensitization to *D. pteronyssinus* and cockroach allergen were measured on each child similar with year three follow-up (section 2.3.5.3).

2.3.6.4 Exposure measures

As in the three year follow up, the main exposures collected at age five were child's use of paracetamol, exposure to geohelminth and *H. pylori* infections. However, data on commensal bacteria were not measured at year five consistent with previous suggestion for the timing of measurement for microflora.[140]

2.3.6.5 Paracetamol exposure at age five

Similar questions as ages one and three were used (see section 2.3.4.2). In addition, at year five, data on indications for use of paracetamol, availability *(Is paracetamol available close to where you live?)*, affordability *(Is paracetamol affordable to you?)*, and preference of use of analgesics including aspirin use *(Do you prefer to give aspirin or paracetamol for your child?)* were collected. The questions on indications for use included (*Can you name any symptoms for which you have given your child paracetamol*?), and the choices were: fever of any origin, malaria, common cold, wheezing illness, coughing, rhinitis, eczema, and any other indications. Identification of paracetamol from aspirin was also verified by showing strips of paracetamol and aspirin and asking the mother to differentiate.

2.3.6.6 Geohelminth analysis at age five

Geohelminth infections were analysed qualitatively as age one (section 2.3.4.3), and quantitatively as age three (see section 2.3.5.5 and Appendix VI) follow-up.

2.3.6.7 Analysis of Helicobacter pylori Ag in stool samples at age five

H. pylori infection was measured in all the children available at age five (N=863). The methods of analysis was by using the same rapid stool antigen test from a similar supplier as at age three, and identical procedure (see section 2.3.5.7 and Appendix VII).

2.3.6.8 Measurement of potential confounders at age five

Information on various potential confounders, similar to year one (see section 2.3.4.4) and year three follow up (see section 2.3.5.8 and Appendix II and Appendix V), was collected. However, data on dust allergen were not available at age five.

2.3.7 Data entry and management

Questionnaire, skin test and all laboratory data were double entered using EpiData version 3.1 (EpiData, Odense, Denmark) on a daily basis. On a weekly basis, the data were exported to Stata 11 (Statacorp, College Station, TX), and frequency tables generated to check data consistency. If data collection errors were found, they were corrected the same day. The datasets from the different time points (pregnancy, birth, two months, one, three and five years) were finally cleaned, coded, and merged ready for analysis using Stata 11. The final merged dataset comprised the 1006 live singleton children born into the cohort.

2.3.8 Statistical analyses

As the study utilized various statistical approaches, the specific statistical methods used are presented in the next sections. The first section describes the natural history of wheeze, eczema, rhinitis and sensitization between age one and five, followed by longitudinal analyses (chapter three, five and six), which includes computation of each incident outcome (incident wheeze, eczema, rhinitis and sensitization) and exposures in the first three years of life (paracetamol,

geohelminth infection, *H. pylori* and commensal bacteria), and finally the cross-sectional analyses (chapter four, five and six) including computation of the prevalent outcomes and these exposures. In both longitudinal and cross-sectional analyses, the modelling strategies for multivariate analysis and the statistical parameters utilized are described in detail for each.

2.3.8.1 Natural history of wheeze and allergic disease

The patterns of wheeze and eczema between age one, three and five, and rhinitis and sensitization between age three and five were explored by cross tabulation of the outcomes found at each follow up. The frequencies falling into each subgroup were then computed and presented graphically using a flow chart.

2.3.8.2 Longitudinal analyses

For analysis of new onset wheeze between age one and three (chapter three), those children without reported wheeze ever at age one (n=756) were selected for analysis and the outcome defined as reported wheeze ever at age three (Figure 3.1). Similarly, children without eczema ever at age one (n=780) were selected for analysis of incident eczema, defined as a positive response at age three (Figure 3.2). Asthma was very rarely reported in this birth cohort (1%), and therefore not pursued further. Data on rhinitis and sensitization were not available at age one but were included in the subsequent follow up as described below.

For longitudinal analysis between age three and five (chapter five and six), the primary outcome measures were incidence of new-onset wheeze, eczema, rhinitis and allergen sensitization between age three and five. To compute the incidence of wheeze, a wheeze-free cohort comprising all children with a negative response to the wheeze questions at ages one and three (wheeze in the past 12 months at years one and three, and wheeze ever at year three) was selected (n=698), and any children who reported wheeze at the five year follow-up were defined as having new onset wheeze (Figure 5.1). The incidence of eczema (computed from eczema free cohort at age one and three; n=723) and rhinitis (rhinitis free cohort at age three; n=798) were computed in a similar way, although the rhinitis-free cohort at age three was based on responses given at age three only (rhinitis ever and in the past 12 months) (Figure 5.2 and Figure 5.3). For sensitization, those children who were not sensitized at age three (n=789) (defined as an average of two perpendicular wheal diameters, one of which was the maximum measurable diameter, of at least 3mm greater than the saline control response, also described in the methods) were selected for analysis (skin tests not performed at age one), and new-onset sensitization defined as a positive result at age five (Figure 5.4). Since the prevalence of cockroach allergen at age five was low (only one), a separate analysis of each allergen was not possible; instead a combined variable 'any sensitization' was created to refer to sensitization to either *D. pteronyssinus* or cockroach allergen.

For analysis of child's use of paracetamol in the first year of life (chapter three), the two original variables (use in the past year and dose of exposure in the past month), were combined to create a new four level categorical variable: 'never', 'yes but not in the last month', '1-3 tablets in last month' and '≥4 tablets in last

month.' Due to small numbers, it was also necessary to merge the two highest paracetamol categories in the analysis of eczema as: 'never', 'yes but not in the last month', '≥1 tablet in last month.' To explore evidence of a dose-response trend across paracetamol categories, a new baseline category of 0 tablets in the past month was created by merging the first two paracetamol groups ('never' and 'yes but not in the last month'), and p for trend computed across the categories (0, 1-3, 4+ tablets in past month). This dose-response relationship could only be assessed in relation to wheeze as numbers for the two highest paracetamol categories for eczema analysis were too small for trend analysis.

Two exposure variables were computed for paracetamol exposure in the first three years of life (chapter five). Firstly a variable called 'early life paracetamol use' representing paracetamol use prior to the disease outcomes (i.e. first three years of life) was computed from responses to the questions on use in the past year (*'has your child taken any paracetamol in the last year')* asked at ages one and three: 'never exposed' (negative responses to the question at both ages one and three), 'exposed at year one not at year three', 'exposed at year three not at year one', and 'persistently exposed' (positive responses to the question at both age one and three). A second exposure variable 'early life paracetamol dose' was computed as a marker of dose during this time period, based on the assumption that those who reported use in the past month were the more 'regular users'. The categories were computed as 'low exposure' (negative response to use in the past month at age one and three), 'medium exposure' (positive response to use in past month at ages one or three), and 'heavy exposure' (positive response to use in past month at both time points).

Data on geohelminth infection (hookworm, *Ascaris lumbricoides* and *Trichuris trichiura*) were available at ages one and three. As the prevalence and intensity of individual geohelminth infections in the first year of life were low in the cohort,[242] this limited any species level analysis at the age 3 follow-up (chapter three).It was therefore necessary to create a combined variable 'any geohelminth infection' defined as positive infection with any of hookworm, *A. lumbricoides* or *T. Trichiura* at age one. For longitudinal analysis between age three and five (chapter five), a variable called 'early life geohelminth exposure to any geohelminth' representing exposure to any of hookworm, *A. lumbricoides* or *T. trichiura* prior to the disease outcomes (first three years of life) was computed from exposure status at year one and three: 'never exposed' (no infection at ages one and three), or 'exposed at any age up to year three' (exposure at age one, or three or both). Similar variables were computed for the individual species hookworm and *A. lumbricoides* but not *T. trichiura* as the prevalence was very low. As there were few children with reported incident outcomes infected persistently with geohelminths at ages one and three, or infected at year one but not at year three, it was not possible to use a more detailed exposure variable representing the different timing of infections.

An exposure variable representing infection with *H. pylori* at age three was computed for longitudinal analysis between age three and five. *H. pylori* status in the cohort was only available on a random subsample of 616 children at year three (described in chapter 2, section 2.3.5.6), and therefore the longitudinal analysis included this group of children.

The last exposure variables considered in the longitudinal analysis (between age

3 and 5) were colonization with the selected commensal bacteria, enterococci, lactobacilli and bifidobacteria at age three (binary exposure variable). Data on these commensals were available from a subsample of 544 children in the cohort at age three. The effects of each of the commensal bacteria (enterococci, lactobacilli, and bifidobacteria) on incident outcomes were separately analysed.

In each disease-free cohort the relation between early sensitization (sensitization at age three) and incident outcomes between ages three and five was assessed using univariate analysis with crude odds ratios and 95% confidence interval, along with the p value. The association between demographic characteristics, potential confounders including symptoms of respiratory tract infections and incident outcomes were also computed, and crude odds ratios and 95% confidence interval reported, and the analyses tables included, accordingly, in the results chapter. Since risk factor analysis was not the aim of this thesis, these potential confounders were considered in the adjusted analysis of the main exposures. The relation between child's use of paracetamol at age one and various potential confounders were also assessed using univariate analysis with crude odds ratios and 95% confidence interval, along with the p value.

Univariate analyses with crude odds ratios (OR) and 95% confidence intervals (CI) for each outcome in relation to child's use of paracetamol, geohelminth infection, *H. pylori* and commensal bacteria variables were conducted. Multivariate logistic regression was then used to determine the independent effects of these exposures on each incident outcome, controlling for the *a priori* confounders and adjusted ORs, and 95% CIs obtained. These *a priori* confounders were place of residence (urban/rural), child's gender and maternal education (as a marker of socioeconomic

status) recoded as formal or non formal. The impact of further controlling for any other potential confounders collected in the first year of life including early life respiratory tract infections (cough, fever and fast breathing) was also explored. These covariates were retained in the model if they have altered the odds ratios for the main exposure of interest by more than 10%. The significance of the association between exposure and outcome in the model was assessed using a likelihood ratio test for the association (LRT).

2.3.8.3 Cross-sectional analyses

For cross-sectional analysis at age three (chapter 4), the prevalence of each outcome (wheeze, eczema, rhinitis, and sensitization to *D. Pteronyssinus* and cockroach allergen) at age three was computed. As the prevalence of asthma was small (only two children reporting asthma), this outcome was not analyzed further. A variable 'any sensitization' was created representing sensitization to either dust mite or cockroach allergen. Similarly, for analysis at age five (chapter five and six), the outcomes were wheeze, eczema and rhinitis in the past 12 months as reported at age five, and sensitization to either *D. pteronyssinus* or cockroach allergen. As the prevalence of sensitization at age five was low, allergen-specific analyses were not possible.

For cross-sectional analysis of outcomes at age three (chapter 3), exposure to any geohelminth infection was defined as infection with any of hookworm, *A. lumbricoides* or *T. trichiura* at age three. The prevalence of *T. trichiura* was very low (<1%), and a separate analysis was therefore impossible, so *T. trichiura* was analysed under 'any geohelminths' infection (a combined variable referring

to infection with any of these parasites). Exposure to 'any microflora' was also computed as colonization with any of enterococci, lactobacilli, and bifidobacterium. Similarly, for analysis of outcomes at age five (chapter six), the prevalence of *T. trichiura* was low for species-specific analysis and merging was necessary with the other geohelminth infections. A variable called 'lifetime geohelminth exposure to hookworm, *A. lumbricoides* or any geohelminth' was created, reflecting infection status at ages one, three and five: 'never exposed' (no exposure at ages one, three and five), and 'exposed at any age up to year three' (representing exposure at age one, three or five or all). As in the longitudinal analysis above, the low prevalence of geohelminths meant a separate analysis by window of age was not possible.

The effects of *H. pylori* infection, for all available children at year five, were analysed by first creating a new exposure variable with categories representing different combinations of infection status at age three and five: 'never exposed' (never exposed at both time points), 'exposed at year three but not at year five', 'exposed at year five but not at year three', or 'persistently exposed' (exposed at both time points). For the sensitization outcome at year five, however, numbers of children in some exposure categories were low (for example n=0 for category 'exposed at year five not at year three') and it was therefore necessary to merge the exposed categories to create a single category 'exposed at any age up to year five'. Moreover, an additional separate analysis of the effects of *H. pylori* infection using only data collected at age five was conducted (infected vs not infected at age 5) to increase statistical power, since *H. pylori* data were available in all the children at year five.

Two markers of paracetamol exposure were computed (chapter five) for cross-sectional analysis of outcomes at age 5. Firstly a marker of lifetime paracetamol exposure was computed called 'lifetime paracetamol use' which had the following categories to reflect different timings of exposure over the child's lifetime: 'never exposed' (negative response to use in past year reported at ages one, three and five), 'past exposure only' (positive response to use in past year reported at ages one or three but negative response at age five)', 'current exposure with or without past exposure' (positive response to use in past year reported at age five), and 'persistently exposed' (positive response to use in past year reported at ages one, three and five). A second exposure variable called 'lifetime paracetamol dose' was computed to reflect total lifetime dose and was created using a composite score by adding each exposure at year one, three and five, and computed as: 'never exposed', 'light exposure', 'medium exposure', and 'heavy exposure.'

Outcomes were summarised overall and within each exposure category using percentage frequencies. The relation between reported prevalent symptoms and sensitization was assessed using binary logistic regression analysis to compute crude ORs and associated 95% confidence intervals. Indications for use of paracetamol, availability and preference data collected at year five were summarized using percentages, and presented graphically. Der p 1 and Bla g 1 allergens in the dust sample were categorized into tertiles as '0', '0.02-1.96 U/g' and '2.17-13.95 U/g' for Bla g 1 allergen, and '0', '0.54-500 µg/g' and '≥501 µg/g' for Der p 1 allergens. Median and interquartile range were used as a summary statistics as the data were not normally distributed. The associations between potential confounders (details of which were presented in sections

2.3.4.4 and 2.3.5.8 of chapter two), and the outcomes wheeze, eczema, rhinitis, and skin sensitization were determined and are presented in the results section as appropriate.

Univariate analysis using binary logistic regression was carried out to explore the association of each exposure variable on each outcome, and crude ORs presented. Multivariate analysis, yielding adjusted ORs and 95% CIs, was conducted to determine the independent effects of these exposures on each prevalent outcome adjusting for *a priori* confounders (area of residence, child's gender and maternal education) and symptoms of respiratory tract infections, and further adjusted for various potential confounders collected at year five. The modelling strategies were the same as in the longitudinal analysis described above.

2.3.9 The study power

The sample size of the birth cohort was originally determined to address objectives other than this thesis, [240, 241, 251, 252] however, retrospective power calculations show that for an outcome with 8% prevalence, our sample of children followed between one and five years provided approximately 80% power at the 5% significance level to detect an odds ratio of around 0.45 for gastro-intestinal exposure,[253] and 2.00 for use of paracetamol.[242]

2.3.10 Ethics and consent

Ethical approval was granted by the Institutional Review Board (IRB) of Addis Ababa University, the National Ethical Review Committee of the Ethiopian Science and Technology Ministry, and the University of Nottingham UK. Written, informed consent was obtained from all participants at each study visit. Local authorities and village representatives were formally informed about the study. The principles of health research ethics (consent, autonomy, and confidentiality) were maintained throughout the study period. All mothers and children with geohelminth infections were treated with anti-helminthics based on the national protocol. In keeping with the requirements of the Ethiopian ethics committee, medical costs incurred by each mother or child were reimbursed throughout the study period. Dissemination of the findings was conducted in the presence of representatives from different sectors in Butajira, and the region, and selected women who participated in the study. The ethics approval forms including Material Transfer Agreement (MTA) and correspondence from Ethiopia and the UK are shown in Appendix IX.

3 LONGITUDINAL ANALYSIS OF EARLY LIFE RISK FACTORS FOR INCIDENT WHEEZE AND ECZEMA BETWEEN AGE ONE AND THREE

3.1 INTRODUCTION

In May 2009, follow-up of the cohort at children's third birthday was complete, and longitudinal analysis of the outcomes collected at ages one and three could therefore be conducted. The aim of this analysis was to investigate the effects of child's use of paracetamol and exposure to geohelminth infection (hookworm, *A. lumbricoides* and *T. trichiura*) in the first year of life on the incidence of wheeze and eczema between age one and three. Outcome data on rhinitis and skin sensitization, and exposure to bacterial commensals and *H. pylori* were not available at year one and hence were not included in this analysis. Information on other early life risk factors including socio-demographic, environmental and lifestyle factors, which are not focus of the analysis, are treated as potential confounders and univariate associations between these and the outcomes are presented in the results.

In this chapter, first the results will be described, followed by a short summary of the findings, with the main discussion presented in chapter seven.

3.2 RESULTS

The description of the birth cohort between birth and age one, along with the demographics, was presented in chapter two, sections 2.3.3 and 2.3.4. The findings reported here are based on 899 children with complete outcome data at age one followed up to age three (Figure 2.3).

3.2.1 Natural history of wheeze between age one and three

Figure 3.1 shows natural history of wheeze between age one and three. Of the 96 children with reported wheeze ever at age one and who were successfully followed-up, 26 (27.1%) still reported wheeze ever at age three, while 70 (72.9%) did not (Figure 3.1). Of the 756 non wheezers at age one, 58 (7.7%) reported wheeze ever at age three and were defined as incident wheezers (Figure 3.1).

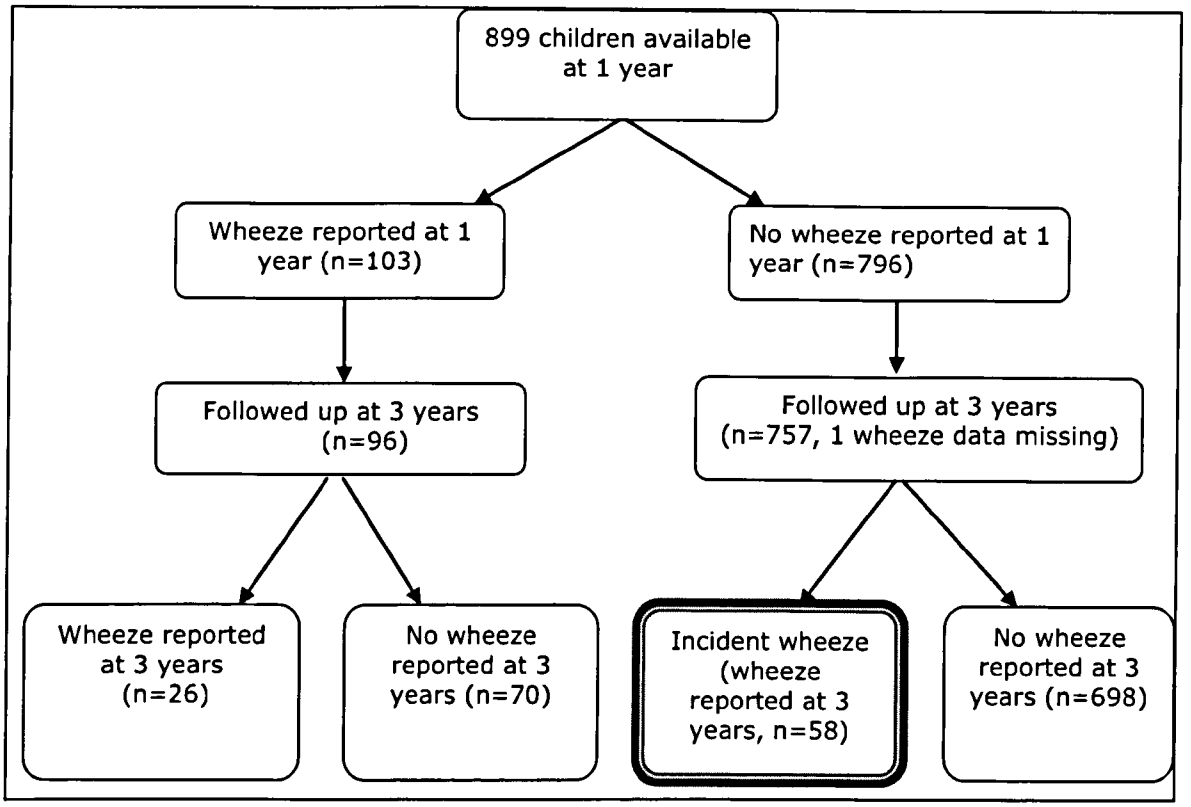

Figure 3.1 Flow chart showing reporting of wheeze between one and three years

3.2.2 Natural history of eczema between age one and three

As shown in Figure 3.2, of the 72 children with reported eczema ever at age one, 9 (12.5%) still reported eczema at age three, but not the other 63 (87.5%). Incident eczema was reported in 7.3% (57/780) of children without reported eczema at age one followed to age three (Figure 3.2).

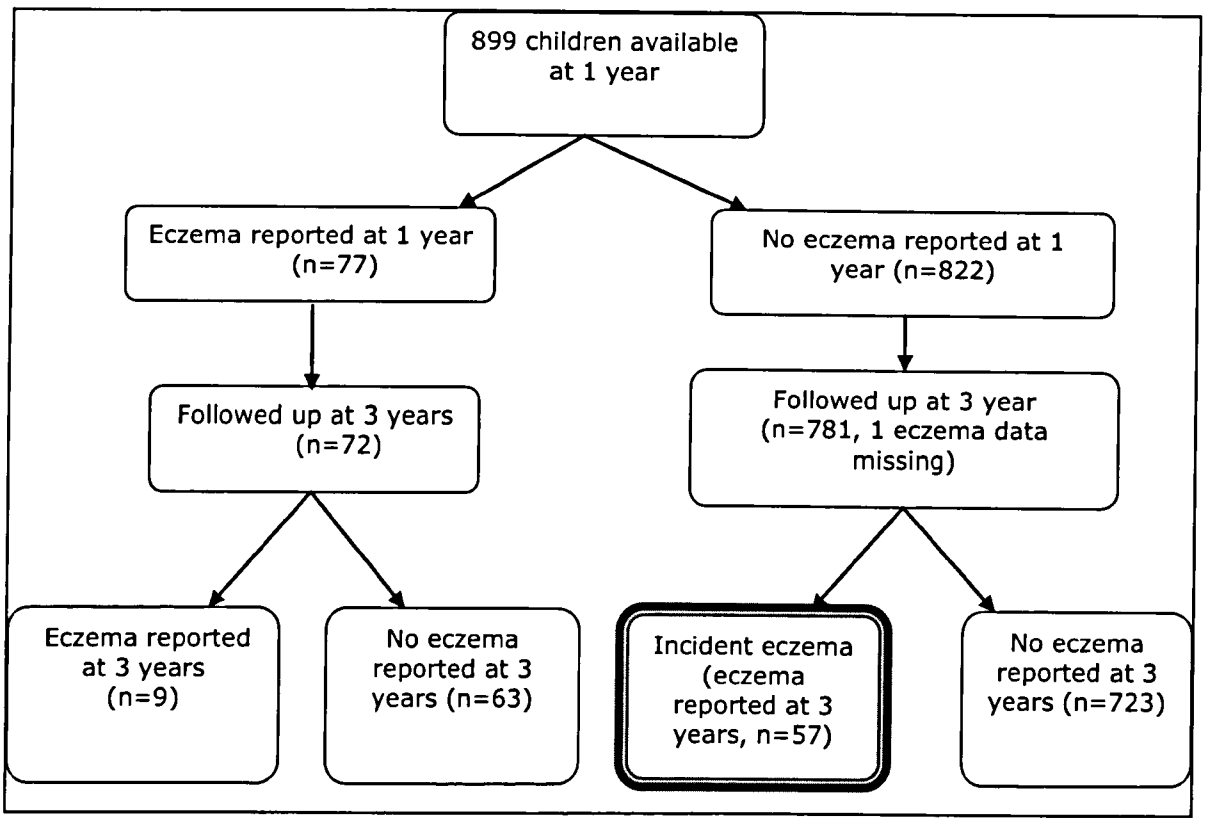

Figure 3.2 Flow chart showing reporting of eczema between one and three years

3.2.3 Distribution of potential confounders with incident wheeze and eczema

3.2.3.1 Demographic and life style risk factors

Table 3.1 and Table 3.2 show the distribution of demographic and potential confounding variables amongst the wheeze and eczema free children at age one on whom subsequent analyses were performed. The risk of incident wheeze was higher, although not significantly so, in urban children (crude OR, 95% CI, 1.50; 0.73, 3.08, p=0.27) and boys (crude OR, 95% CI, 1.35; 0.79, 2.32, p=0.28), and significantly greater in those of low birth weight (crude OR, 95% CI, 2.73; 1.06, 7.04, p=0.04) (Table 3.1). There was a borderline significant decreased

risk of new onset eczema in boys compared to girls (crude OR, 95% CI, 0.59; 0.34, 1.03, p=0.06). There was also a borderline significant increased risk of incident eczema with large households (crude OR, 95% CI, 1.93; 0.71, 5.20 for seven or more households compared with less than four household size, p for trend=0.06) and a significant increased risk with increasing number of older siblings (crude OR, 95% CI, 4.33; 1.28, 14.61 with four to ten number of older siblings versus zero, p trend=0.02) (Table 3.2). A number of other potential confounders collected during pregnancy, at birth and during infancy, including maternal age, maternal education, child's immunization and breastfeeding history, parental history of allergy, use of insecticide, child's sleeping place, indoor cooking, and roof types were not significantly associated with either incident wheeze or eczema (Table 3.1 and Table 3.2).

Table 3.1 Distribution of potential confounders in the first year of life in relation to incident wheeze between ages 1 and 3

Variables	Wheeze never at age one (N=756)			
	Overall N (%)	n (%)new wheeze*	Crude OR (95% CI)	P-value
Residence				
Urban	95 (12.6)	10 (10.5)	1.50(0.73,3.08)	0.27
Rural	661 (87.4)	48 (7.3)	1	
Maternal age				0.92[†]
15-24	293 (38.8)	21 (7.2)	0.88 (0.39,1.99)	0.71[‡]
25-34	351 (46.4)	28 (8.0)	0.99 (0.45,2.17)	
35-44	112 (14.8)	9 (8.0)	1	
Gender				
Male	378 (50)	33 (8.7)	1.35 (0.79,2.32)	0.28
Female	378 (50)	25 (6.6)	1	
Maternal education				
Formal	148 (19.6)	16(10.8)	1.63 (0.89,2.99)	0.11
No formal	608 (80.4)	42 (6.9)	1	
Exclusively breastfed[¥]				
Yes	633 (84.1)	51 (8.1)	1.41 (0.63,3.20)	0.40
No	120 (15.9)	7 (5.8)	1	
Vaccination at 2 months[¶]				
Yes	443 (58.8)	35 (7.9)	1.07 (0.62,1.85)	0.81
No	310 (41.2)	23 (7.4)	1	
Birth weight				
Low (< 2.5kg)	35 (7.0)	6 (17.1)	2.73 (1.06,7.04)	0.04
Normal	468 (93.0)	33 (7.1)	1	
Parental allergic history				
Yes	39 (5.2)	4(10.3)	1.40 (0.48,4.08)	0.54
No	714 (94.8)	54 (7.6)	1	
Insecticide use in home				
Yes	623 (82.4)	46 (7.4)	0.81 (0.42,1.57)	0.53
No	133 (17.6)	12 (9.0)	1	
Household size				0.58[†]
1-3	98 (13.0)	7 (7.1)	1	0.58[‡]
4-6	422 (55.8)	36 (8.5)	1.21 (0.52,2.81)	
7[+]	236 (31.2)	15 (6.4)	0.88 (0.35,2.24)	

Table 3.1 (continued)

Variables	Wheeze never at age one (N=756)			
	Overall N (%)	n (%)new wheeze*	Crude OR (95% CI)	P-value
No. of older siblings				0.52†
0	111 (14.7)	7 (6.3)	1	0.81‡
1-3	415 (54.9)	36 (8.7)	1.41 (0.61,3.26)	
4-10	230 (30.4)	15 (6.5)	1.04 (0.41,2.62)	
Child's sleeping place				0.87†
Bed/platform	59 (7.8)	5 (8.5)	1	
Floor	324 (42.9)	23 (7.1)	0.83 (0.30,2.26)	
Grass matting	372 (49.3)	30 (8.1)	0.95 (0.35,2.55)	
Indoor cooking				
Yes	609 (80.9)	46 (7.6)	0.90 (0.46,1.74)	0.75
No	144 (19.1)	12 (8.3)	1	
Indoor kerosene use				
Yes	88 (11.7)	6 (6.8)	0.86 (0.36,2.07)	0.74
No	665 (88.3)	52 (7.8)	1	
Type of roof				
Thatched	578 (76.8)	41 (7.1)	0.71 (0.39,1.28)	0.26
Corrugated iron	175 (23.2)	17 (9.7)	1	

†Likelihood ratio test

‡ p for trend

¥ Exclusive breast feeding status at two months

¶ Any vaccination history at two months

*Reported wheeze and eczema ever at year three follow-up.

Table 3.2 Distribution of potential confounders in the first year of life in relation to incident eczema between ages 1 and 3

Variables	Eczema never at age one (N=780)			
	Overall N (%)	n (%)new eczema*	Crude OR (95% CI)	P-value
Residence				
Urban	98 (12.6)	8 (8.2)	1.15 (0.53,2.50)	0.73
Rural	682 (87.4)	49 (7.2)	1	
Maternal age				0.32†
15-24	297 (38.1)	19 (6.4)	1.25 (0.49,3.22)	0.96‡
25-34	367 (47.1)	32 (8.7)	1.75 (0.71,4.30)	
35-44	116 (14.9)	6 (5.2)		
Gender				
Male	394 (50.5)	22 (5.6)	0.59 (0.34,1.03)	0.06
Female	386 (49.5)	35 (9.1)	1	
Maternal education				
Formal	155 (19.9)	12 (7.7)	1.08 (0.56,2.10)	0.82
Non formal	625 (80.1)	45 (7.2)	1	
Exclusively breastfed¥				
Yes	659 (84.7)	49 (7.4)	1.11 (0.51,2.42)	0.78
No	119 (15.3)	8 (6.7)	1	
Vaccination at 2 months¶				
Yes	463 (59.5)	34 (7.3)	1.01 (0.58,1.74)	0.98
No	315 (40.5)	23 (7.3)	1	
Birth weight				
Low (< 2.5kg)	39 (7.4)	1 (2.6)	0.32 (0.04,2.40)	0.27
Normal	487 (92.6)	37 (7.6)	1	
Parental allergic history				
Yes	42 (5.4)	3(7.1)	0.97 (0.29,3.25)	0.96
No	736 (94.6)	54 (7.3)	1	
Insecticide use in home				
Yes	648 (83.3)	50 (7.7)	1.47 (0.65,3.32)	0.36
No	130 (16.7)	7 (5.4)	1	
Household size				0.11†
1-3	89 (11.4)	5 (5.6)	1	0.06‡
4-6	446 (57.3)	27 (6.1)	1.08 (0.41,2.89)	
7+	243 (31.2)	25(10.3)	1.93 (0.71,5.20)	
No. of older siblings				0.01†
0	104 (13.4)	3 (2.9)	1	<0.01‡
1-3	437 (56.2)	27 (6.2)	2.22 (0.66,7.45)	
4-10	237 (30.5)	27(11.4)	4.33(1.28,14.61)	

Table 3.2 (continued)

Variables	Eczema never at age 1(N=780)			
	Overall N (%)	n (%)new eczema*	Crude OR (95% CI)	P-value
Child's sleeping place				0.89†
Bed/platform	59 (7.6)	5 (8.5)	1	
Floor	344 (44.3)	26 (7.6)	0.88 (0.32,2.40)	
Grass matting	374 (48.1)	26 (7.0)	0.81 (0.30,2.19)	
Indoor cooking				
Yes	628 (80.7)	46 (7.3)	1.00 (0.50,1.98)	0.99
No	150 (19.3)	11 (7.30	1	
Indoor kerosene use				
Yes	87 (11.2)	6 (6.9)	0.93 (0.39,2.23)	0.87
No	691 (88.8)	51 (7.4)	1	
Types of roof				
Thatched	595 (76.5)	42 (7.1)	0.85 (0.46,1.57)	0.61
Corrugated iron	183 (23.5)	15 (8.2)	1	

†Likelihood ratio test

* p for trend

¥ Exclusive breast feeding status at two months

¶ Any vaccination history at two months

*Reported wheeze and eczema ever at year three follow-up.

3.2.3.2 Symptoms of respiratory tract infections and the risk of incident wheeze and eczema

Information on symptoms of respiratory tract infections during infancy was available from the cohort. This information was collected to account for confounding by indication for the main exposure variable, paracetamol. Tables 3.3 and 3.4 below show infant symptoms of respiratory infections (cough, fast breathing and fever) by new-onset wheeze and eczema between age one and three. At two months, coughing symptoms were reported by 298 (39.6%), fast breathing by 180 (23.9%), and fever by 248 (32.9%) of the cohort babies. Similarly, cough was reported by 443 (58.6%), fast breathing by 251 (33.2%), and fever by 581 (76.9%) of the children at one year. Reported symptoms at age two months were not related to incident wheeze (Table 3.3) or incident

eczema (Table 3.4). All three symptoms in the first year of life were significantly positively associated with the incidence of wheeze between age one and three i.e. children with symptoms of respiratory tract infections were more likely to report new-onset wheezing than children without these symptoms (Table 3.3). However, all the three symptoms at year one were unrelated to incident eczema (Table 3.4).

Table 3.3 Prevalence of symptoms of respiratory infections in early life in those with and without incident wheeze between age one and three

Symptoms of respiratory infections n (%)	**Wheeze never at age one (N=756)**			
	All children	With incident wheeze	Without incident wheeze	p-value
Respiratory infection symptoms at two months				
Cough	298 (39.6)	22 (37.9)	276 (39.7)	0.79
Fast breathing	180 (23.9)	15 (25.9)	165 (23.7)	0.72
Fever	248 (32.9)	21 (36.2)	227 (32.7)	0.58
Respiratory infection symptoms at one year				
Cough	443 (58.6)	41 (70.7)	402 (57.6)	0.05
Fast breathing	251 (33.2)	27 (46.6)	224 (32.1)	0.03
Fever	581 (76.9)	51 (87.9)	530 (75.9)	0.04

Three missing data on symptoms of respiratory infection at age two months

Table 3.4 Prevalence of symptoms of respiratory infections in early life in those with and without incident eczema between age one and three

Symptoms of respiratory infections n (%)	Eczema never at age one (N=780)			
	All children	With incident eczema	Without incident eczema	p-value
Respiratory infection symptoms at two months				
Cough	318 (40.9)	23 (40.4)	295 (40.9)	0.93
Fast breathing	192 (24.7)	13 (22.8)	197 (24.8)	0.73
Fever	275 (35.4)	17 (29.8)	258 (35.8)	0.37
Respiratory infection symptoms at one year				
Cough	487 (62.4)	37 (64.9)	450 (62.2)	0.67
Fast breathing	297 (38.1)	24 (42.1)	273 (37.8)	0.52
Fever	618 (79.2)	48 (84.2)	570 (78.8)	0.34

Two missing data on symptoms of respiratory infection at age two months

3.2.4 Associations between paracetamol use and early life risk factors

The use of paracetamol, as an over-the-counter drug, may be confounded by demographic and lifestyle risk factors. Paracetamol use in the first year of life was commonly reported, with around a quarter reporting use in the past month. As shown in Table 3.5, paracetamol use was associated with symptoms of respiratory infections in the first year of life ($p<0.01$ for cough, fast breathing and fever), with rural residence (crude OR, 95% CI, 1.59; 0.99, 2.56, $p=0.06$), and with living in a thatched house (crude OR, 95% CI, 1.41; 0.98, 2.02, $p=0.06$). Indoor cooking was associated with increased exposure to paracetamol (crude OR, 95% CI, 1.52; 1.02, 2.26, $p=0.04$). Maternal use of paracetamol was associated with child's use (36.7% use amongst children of mothers who also used, compared to 18.3% in children of mothers who did not, $p<0.01$). Low birth weight children were more likely to have been exposed to paracetamol

than normal birth weight babies (p=0.09). Other factors explored were not seen to be associated with child's use of paracetamol (Table 3.5).

Table 3.5 Use of paracetamol in the first year of life by demographic, life style and early childhood factors

Variables	Use of paracetamol in the past month*			
	Overall N (%)	Yes n(%)	Crude OR (95% CI)	P-value
Residence				
Urban	117 (13.0)	24 (20.5)	0.63(0.39,1.01)	0.06
Rural	782 (87.0)	227 (29.0)	1	
Gender				
Male	454 (50.5)	128 (28.2)	1.03 (0.77,1.38)	0.85
Female	445 (49.5)	123 (27.6)	1	
Maternal education				
Formal	181(20.1)	43 (23.8)	0.76 (0.52,1.12)	0.16
No formal	718 (79.9)	208 (29.0)	1	
Birth weight				
Low (<2.5kg)	42 (7.0)	17 (40.5)	1.73 (0.91,3.29)	0.09
Normal	556 (93.0)	157 (28.2)	1	
Cough at 1 year				
Yes	344 (38.3)	183 (33.0)	2.00 (1.45,2.75)	<0.01
No	555 (61.7)	68 (19.8)	1	
Fast breathing at 1 year				
Yes	337 (37.5)	126 (37.4)	2.09 (1.55,2.81)	<0.01
No	562 (62.5)	125 (22.2)	1	
Fever at 1 year				
Yes	704 (78.3)	234 (33.2)	5.21 (3.09,8.78)	<0.01
No	195 (21.7)	17 (8.7)	1	
Parental allergic history				
Yes	57 (6.3)	18 (31.6)	1.21 (0.68,2.15)	0.53
No	842 (93.7)	233 (27.7)	1	
Maternal paracetamol at one year				
Yes	472 (52.5)	173 (36.7)	2.59 (1.90,3.53)	P<0.01
No	427 (47.5)	78 (18.3)	1	
Insecticide use in home				
Yes	748 (83.2)	216 (28.9)	1.35 (0.89,2.03)	0.16
No	151 (16.8)	35 (23.2)	1	
Household size				0.34†
1-3	115 (12.8)	27 (23.5)	1	0.86‡
4-6	506 (56.3)	150 (29.6)	1.37 (0.86,2.20)	
7+	278 (30.9)	74 (26.6)	1.18 (0.71,1.96)	

Table 3.5 (continued)

Variables	Use of paracetamol in the past month*			
	Overall N (%)	Yes n(%)	Crude OR (95% CI)	P-value
No. of older siblings				0.64†
0	138 (15.4)	34 (24.6)	1	0.47‡
1-3	492 (54.7)	140 (28.5)	1.22 (0.79,1.88)	
4-10	269 (29.9)	77 (28.6)	1.23 (0.77,1.96)	
Child's sleeping place				
Bed/platform	68 (7.6)	20 (29.4)	1	0.19
Floor	382 (42.5)	118 (30.9)	1.07 (0.61,1.89)	
Grass matting	448 (49.9)	113 (25.2)	0.81 (0.46,1.42)	
Site of cooking				
Inside	727 (80.9)	214 (29.4)	1.52 (1.02,2.26)	0.04
Outside	172 (19.1)	37 (21.5)	1	
Types of roof				
Thatched	689 (76.6)	203 (29.5)	1.41 (0.98,2.02)	0.06
Corrugated iron	210 (23.4)	48 (22.9)	1	

N=899

‡Likelihood ratio test

† p for trend

*Use of paracetamol in the past month created from never use, use but not in the past month, 1-3 tablets in the past month and ≥4 tablets in the past month

3.2.5 Determinants of incident wheeze and eczema

3.2.5.1 Exposure to geohelminth infections and risk of incident wheeze and eczema

As part of the primary hypothesis, the effects of geohelminths (hookworm, *A. lumbricoides,* and *T. trichiura*) in the first year of life were investigated. Any geohelminth infection (infection with either hookworm, *A. lumbricoides,* or *T. trichiura*) at age one was found in only 4% of children, with hookworm being the most common infection (2.3%), followed by *A. lumbricoides* (1.4%) and *T. trichiura* (0.4%). Single infection was found in 40 children (4.4%), and the rest 4 (0.4%) with double infection.

In univariate analysis, the risk of new onset wheeze was lower in those infected (3.6%) than uninfected (7.8%), but the 95% CI was wide and significance not reached (crude OR, 95% CI, 0.44; 0.06, 3.27) for any infection, including hookworm infection (crude OR, 95% CI, 0.80; 0.10, 6.16) (Table 3.6). As only one infected child had incident wheeze, no further adjusted analysis could be carried out. No children with geohelminth infection reported incident eczema, and therefore ORs could not be computed or further analysis performed (Table 3.6).

Table 3.6 OR for incident wheeze and eczema in relation to geohelminth exposures in the first years of life

Geohelminths	Overall N (%)	Incident wheeze (N=756) Yes n (%)	Crude OR (95%CI)	P-value
Any†	28 (3.7)	1 (3.6)	0.44 (0.06,3.27)	0.39
Hookworm	16 (2.1)	1 (6.3)	0.80 (0.10,6.16)	0.91
Ascaris	11 (1.5)	0 (0)	-	-
Geohelminths	**Overall N (%)**	**Incident eczema (N=780) Yes n (%)**	**Crude OR (95%CI)**	**P-value**
Any†	26 (3.3)	0 (0)	-	-
Hookworm	16 (2.1)	0 (0)	-	-
Ascaris	10 (1.3)	0 (0)	-	-

†Hookworm, *Ascaris lubmricoids* or *Tricuris Tricuria*

3.2.5.2 Exposure to paracetamol and risk of incident wheeze

Paracetamol use in the first year of life was commonly reported in 36% of the wheeze cohort, with around a quarter (24.8%) reporting use in the past month (Table 3.7). In the univariate analyses, use was significantly associated with an increased risk of incident wheeze (p=0.01), with increased risks seen amongst those taking >=4 tablets per month (crude OR, 95% CI, 7.34; 2.09, 25.72) and

those taking 1-3 tablets per month (crude OR 1.79; 95% CI 0.98, 3.26), compared with never users (Table 3.7). The association was found to be dose-dependent with a highly significant trend seen across the 'use in the past month' categories (0, 1-3 and 4+ tablets) (p trend <0.01; Table 3.7).

Table 3.7 Univariate associations between child's use of paracetamol in the first year of life and incident wheeze between age one and three

Paracetamol use in the first year of life	Incident wheeze (N=756)			
	Overall N (%)	N (%) new disease	Crude OR (95%CI)	P-value
Never	486 (64.3)	31 (6.4)	1	0.01‡
Yes but not in the past month	83 (11.0)	4 (4.8)	0.74 (0.26,2.16)	<0.01¶
1-3 tablets per month	175 (23.2)	19 (10.7)	1.79 (0.98,3.26)	
≥ 4 tablets per month	12 (1.6)	4 (33.3)	7.34 (2.09,25.72)	

‡Overall p-value (likelihood ratio test); ¶ P value for trend (computed for dose of paracetamol intake in the past month: 0, 1-3, and ≥ 4 tablets)

In multivariate analyses, exposure to paracetamol was still significantly associated with the incidence of wheeze ($p<0.01$) and the odds ratios were little changed with risks still increased in the 1-3 tablets/month group (adjusted OR, 95% CI, 1.88; 1.03, 3.44) and the ≥4 tablets/month group (adjusted OR 7.25; 2.02, 25.95) compared with the never users. A significant trend across the categories of dose in the past month (0, 1-3, >4 tablets) was also seen (p trend <0.01) (Table 3.8). Further control for other potential confounders collected in Table 3.1, including postnatal maternal paracetamol use (which was strongly related to child's use), did not materially alter the associations (Table 3.8). To explore the effects of confounding by indication, the model was further adjusted for symptoms of respiratory tract infections. Exposure to paracetamol was associated with symptoms of respiratory infections in the first year of life, and adjustment for these slightly reduced the strength of the wheeze association but overall significance remained (further adjusted OR, 95% CI, 6.78; 1.89, 24.39, for ≥4 tablets/month group versus never users; overall p=0.01; p trend <0.01) (Table 3.8).

Table 3.8 Multivariate analysis: child's use of paracetamol in the first year of life and incident wheeze between age one and three

Paracetamol use in the first year of life	Incident wheeze (N=756)			
	Adjusted OR* (95% CI)	P-value	Further adjusted OR† (95% CI)	P-value
Never	1	<0.01‡	1	0.01‡
Yes but not in the past month	0.73 (0.25,2.14)	<0.01¶	0.70 (0.24,2.04)	<0.01¶
1-3 tablets per month	1.88 (1.03,3.44)		1.77 (0.96,3.26)	
≥ 4 tablets per month	7.25 (2.02,25.95)		6.78 (1.89,24.39)	

*ORs adjusted for gender, urban/rural residence and maternal education

†ORs adjusted for child's gender, place of living and maternal education and additionally adjusted for symptoms of respiratory infections in the first year of life.

‡Overall p-value (likelihood ratio test)

¶ P value for trend (computed for dose of paracetamol intake in the past month: 0, 1-3, and ≥ 4 tablets)

3.2.5.3 Exposure to paracetamol and risk of incident eczema

Use of paracetamol in the first year of life was reported by 39% of children in the eczema cohort. The numbers of children in the eczema cohort who had taken ≥4 tablets/month were very small; this category was therefore merged with the 1-3 tablets/month, becoming ≥1 tablet/month, making dose-response analysis impossible (Table 3.9 and Table 3.10).

In univariate analysis, the odds ratios were increased but not to the level of significance (crude OR, 95% CI, 1.61; 0.90, 2.89, p=0.18 for those taking ≥1 tablet/month versus never users) (Table 3.9).

Table 3.9 Univariate analysis: child's use of paracetamol in the first year of life and incident eczema

Paracetamol use in the first year of life	Incident eczema (N=780)			
	Overall N (%)	n(%) new disease	Crude OR (95%CI)	P-value
Never	477 (61.3)	30 (6.3)	1	0.18‡
Yes but not in the past month	86 (11.1)	6 (7.0)	1.12 (0.45,2.77)	
≥ 1 tablet per month	215 (27.6)	21 (9.8)	1.61 (0.90,2.89)	

‡Overall p-value (likelihood ratio test)

Results of analyses adjusted for a *priori* confounders and additionally for respiratory infections are shown in Table 3.10, and are similar to the univariate results. The odds ratio for use of paracetamol in the past month was still increased (fully adjusted OR, 95% CI, 1.62; 0.89, 2.96) compared to never use, but no overall significant association was seen (p=0.30) (Table 3.10). Further control for potential confounders collected in Table 3.2 had little effect on the odds ratio.

Table 3.10 Multivariate analysis: child's use of paracetamol in the first year of life and incident eczema

Paracetamol use in the first year of life	Incident eczema (N=780)			
	Adjusted OR* (95% CI)	P-value	Further adjusted OR† (95% CI)	P-value
Never	1	0.25‡	1	0.30‡
Yes but not in the past month	1.17 (0.47,2.91)		1.14 (0.45,2.87)	
≥ 1 tablet per month	1.66 (0.92,2.98)		1.62 (0.89,2.96)	

*ORs adjusted for gender, urban/rural residence and maternal education

†ORs adjusted for child's gender, place of living and maternal education and additionally adjusted for symptoms of respiratory infections in the first year of life.

‡Overall p-value (likelihood ratio test)

3.3 SUMMARY

In summary, exposure to paracetamol in the first year of life, which was reported in over one third of infants, was significantly associated with an increased risk of incident wheeze between age one and three, in a dose-response manner. However, this positive dose-response association was not seen for incident eczema.

These effects were independent of various early life potential confounders including confounding by indication (paracetamol being taken for respiratory tract infections in early life by children who are also more susceptible to the subsequent development of allergic diseases).[254] After adjusting for the main symptoms of respiratory infections early in life, the wheeze association did reduce in magnitude slightly, but remained highly statistically significant.

The effects of geohelminth infection in the first year of life were explored to determine independent effects on incident wheeze and eczema. Whilst the study found no significant associations, the observed effects were in the expected direction. However, the analyses were limited by insufficient statistical power as a result of the low prevalence of geohelminth infection and small numbers with the outcomes amongst the infected children. The low prevalence of geohelminths in children in this population is probably linked to a mass de-worming strategy in under-five year olds in study area.[255] We anticipate that as the cohort ages, more children will become infected and we will be better able to assess these associations.

Clearly from these findings further follow-up of the birth cohort beyond the first year of life is important. The birth cohort will continued to be followed, and the next chapters explore cross-sectional and further longitudinal associations of geohelminth infections, other gastro-intestinal infections including *H. pylori*, commensal bacteria, and exposure to paracetamol on the development of allergic diseases and sensitization at ages three and five. The discussion of the findings including strengths and weaknesses and comparison with previous studies will be made in chapter seven.

4 CROSS-SECTIONAL ANALAYSIS OF GEOHELMINTHS INFECTION, *HELICOBACTER PYLORI*, COMMENSAL BACTERIA AND ALLERGIC DISEASES AT THE AGE OF THREE

4.1 INTRODUCTION

This chapter presents cross-sectional findings from the three year follow up of the Butajira birth cohort. During this follow-up period, the study investigated the hypothesis that gastro-intestinal infections (geohelminths, *H. pylori,* and commensal bacteria), which become less prevalent with increased urbanization and better hygiene, may play a protective role in the aetiology of asthma and allergy.[256] Data on *H. pylori* and commensal bacteria were not available at age one, however this further follow up allowed for this analysis to be explored. In addition to wheeze and eczema, rhinitis and sensitization outcomes were able to be investigated.

The relationship between each of these gastro-intestinal infections and allergy remains uncertain. This analysis therefore aimed to determine the independent effects of *H. pylori*, geohelminth infections and selected commensal bacteria on allergic diseases and sensitization in a birth cohort of three-year-old children. The next sections describe the results, followed by a brief discussion of the main findings.

4.2 RESULTS

The analyses reported here are based on cross sectional data from the 878 singleton children available at the three year follow up (Figure 2.3), and details of the statistical analyses were described in chapter two.

4.2.1 The birth cohort at age three

At year three, 876 (94.2%) mother-child pairs provided questionnaire information, and 866 (93%) successfully provided an adequate stool sample. Dust samples were collected from 871 (93.5%) child sleeping areas, and 783 of these were analysed for Der p 1 and Bla g 1 dust assays, the rest being inadequate for dust sample analysis. Information on skin sensitization was available on 864 (92.7%) children and bacteriological data on intestinal microflora, and *H. pylori* from a sub sample of 544 and 616 respectively. In fewer than 2% of the children the stool sample was not adequate for laboratory analysis. The demographic characteristics of the children were described previously in chapter two under section 2.3.3.

4.2.2 Prevalence of wheeze, eczema and rhinitis

Self-reported asthma was present in 0.2% (2/876) of children with diagnosis confirmed by a doctor. Wheeze was reported in 9.1% (80/876) of children, half of these reporting 1-3 episodes in the last 12 months. Eczema was reported in 6.3% (55/876) of children and rhinitis reported in 5% (44/876) (Table 4.1). Wheeze and eczema were reported slightly more frequently, and rhinitis slightly

less frequently in urban than rural children, but these differences were not statistically significant (Table 4.1).

Table 4.1 Prevalence of self reported wheeze and allergic symptoms by area of residence

Variables	Overall n (%)	Urban† Yes n(%)	Rural‡ Yes n (%)	Crude OR (95% CI)	p-value
Wheeze (N=876)	80 (9.1)	14 (12.6)	66 (8.6)	1.53 (0.83,2.83)	0.17
Eczema (N=876)	55 (6.3)	8 (7.2)	47 (6.1)	1.17 (0.54,2.58)	0.67
Rhinitis (N=876)	44 (5.0)	4 (3.6)	40 (5.2)	0.68 (0.24,1.93)	0.46

† n=111 and ‡ n=765

4.2.3 Prevalence of skin sensitization

Sensitization to dust mite and cockroach allergen was found in 5.6% (48/864) and 4.2% (36/864) of the children respectively, and sensitization to any allergen was found in 8.7% (75/864) of the children (Table 4.2). Almost identical proportions of urban and rural children were sensitized to cockroach and dust mite allergen, 5.6% versus 6.5% respectively (Table 4.2).

Table 4.2 Prevalence of skin sensitization by area of residence

Variables	Overall n (%)	Urban Yes n(%)	Rural Yes n (%)	Crude OR (95% CI)	p-value
Dust mite (N=864)	48(5.6)	7 (6.5)	41 (5.4)	1.22(0.53,2.80)	0.64
Cockroach (N=864)	36 (4.2)	6(5.6)	30 (4.0)	1.44 (0.58,3.55)	0.43
Any sensitization† (N=864)	75 (8.7)	12 (11.2)	63 (8.3)	1.39 (0.72,2.67)	0.32

¥ Sensitization to either dust mite or cockroach allergen

Number of urban = 107 and rural= 757

4.2.4 Associations between allergic symptoms and sensitization

Wheeze, eczema and rhinitis were not significantly associated with sensitization to dust mite, cockroach or any sensitization. As only few children who were sensitized to these allergens reported allergic symptoms, no further adjusted analysis could be carried out (Table 4.3).

Table 4.3 OR for wheeze, eczema and rhinitis in relation to skin sensitization

Allergic symptoms	Skin sensitization	Overall N (%)	Yes n (%)	Crude OR (95%CI)	P-value
Wheeze (N=876)	Dust mite	48(5.6)	2 (4.2)	0.41 (0.10,1.72)	0.45
	Cockroach	36 (4.2)	0 (0.0)	-	-
	Any sensitization[¥]	75 (8.7)	2 (2.7)	0.25 (0.06,1.03)	0.12
Eczema (N=876)	Dust mite	48(5.6)	1 (2.1)	0.30 (0.04,2.21)	0.23
	Cockroach	36 (4.2)	0 (0.0)	-	-
	Any sensitization	75 (8.7)	1 (1.3)	0.18 (0.03,1.35)	0.09
Rhinitis (N=876)	Dust mite	48(5.6)	0 (0.0)	-	-
	Cockroach	36 (4.2)	1 (2.8)	0.52 (0.07,3.89)	0.54
	Any sensitization	75 (8.7)	1 (1.3)	0.23 (0.03,1.72)	0.16

[¥] Sensitization to either dust mite or cockroach allergen

4.2.5 Distribution of potential confounders

4.2.5.1 Relation between potential confounders and wheeze, eczema and rhinitis

Allergic disease symptoms were not significantly related to gender or other socio-demographic characteristics (Table 4.4 to Table 4.6). Maternal allergic history was associated with an increased risk of wheeze (crude OR 2.16, 95% CI 0.97, 4.79, p=0.05), eczema (crude OR 2.33, 95% CI 0.94, 5.75, p=0.06) and rhinitis (crude OR 3.75, 95% CI 1.57, 8.92, p<0.01) although only reaching borderline significance for wheeze and eczema. Paternal allergic history was also significantly associated with an increased risk of wheeze (crude OR 2.09, 95% CI 1.01, 4.29, p=0.04) and eczema (crude OR 2.47, 95% CI 1.11, 5.49,

p=0.02), but no effect was seen for rhinitis. Use of paracetamol (crude OR 3.72, 95% CI 2.11, 6.55, use in past month vs. never, p<0.01) and antibiotics in the child (crude OR 2.23, 95% CI 1.39, 3.55, p<0.01) were both significantly associated with an increased risk of wheeze, but not eczema and rhinitis. There was a positive significant trend between siblingship and rhinitis (p trend=0.05), but not with wheeze and eczema.

The number of dust samples from children's bedding positive for Der p 1 allergens[c] was 128 (16.4%) and for Bla g 1 allergen was 216 (27.6%). The level of Der p 1 allergen (median [interquartile range (IQR)] = 782.5 µg/g [120.29-1355.23] was much higher than Bla g 1 allergen (median [IQR] = 2.3U/g [0.58-5.49]. Der p 1 and Bla g 1 allergen levels in bedding were not significantly related with each of allergic symptoms. The other environmental and life style variables collected were not significantly associated with the reported allergic symptoms (Table 4.4 to Table 4.6).

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[c] Above detection threshold of ELISA

Table 4.4 Distribution of potential confounders and associations with wheeze at age 3

Variables	Overall N (%)	n (%) wheeze	Crude OR (95% CI)	P-value
Child's gender				
Male	434 (49.4)	45 (10.2)	1.29 (0.81,2.05)	0.28
Female	442 (50.5)	35 (8.1)	1	
Maternal education				
Formal	172 (19.6)	22 (12.8)	1.63 (0.97,2.75)	0.06
Informal	704 (80.4)	58 (8.1)	1	
Maternal allergic history				
Yes	47 (5.4)	8 (17.0)	2.16 (0.97,4.79)	0.05
No	829 (94.6)	72 (8.7)	1	
Paternal allergic history				
Yes	61 (7.0)	10 (16.4)	2.09 (1.01,4.29)	0.04
No	815 (93.0)	70 (8.6)	1	
Breast feeding at 3yr				
Yes	93 (10.6)	4 (4.3)	0.42 (0.15,1.17)	0.09
No	783 (89.4)	76 (9.7)	1	
Reported duration of breast feeding				0.66
≤12 mo	45 (5.9)	5 (11.1)	1	0.42*
12-24 mo	443 (58.5)	38 (8.6)	0.75 (0.28,2.02)	
>24 mo	269 (35.5)	28 (10.4)	0.93 (0.34,2.55)	
Older siblings				0.35
0	132 (15.1)	10 (7.6)	1	0.71*
1-3	480 (54.8)	50 (10.4)	1.42 (0.70,2.88)	
4-10	264 (30.1)	20 (7.6)	1.00 (0.45,2.20)	
Paracetamol use				
Never	496 (56.6)	25 (5.0)	1	<0.01
Yes but not in the past month	204 (23.3)	26 (12.8)	2.75 (1.55,4.89)	
Yes in past month	176 (20.1)	29 (16.5)	3.72 (2.11,6.55)	
Antibiotic use				
Yes	250 (28.5)	36 (14.4)	2.23 (1.39,3.55)	<0.01
No	626 (71.5)	44 (7.0)	1	
Smoking in the residential house				
Yes	109 (12.4)	14 (12.8)	1.57 (0.85,2.90)	0.15
No	767 (87.6)	66 (8.6)	1	
Charcoal fuel use				
Yes	134 (15.3)	14 (10.5)	1.19 (0.65,2.20)	0.57
No	742 (84.7)	66 (8.9)	1	

Table 4.4 (continued)

Variables	Overall N (%)	n (%) wheeze	Crude OR (95% CI)	P-value
Insecticide use				
Yes	519 (59.3)	46 (8.9)	0.92 (0.58,1.47)	0.74
No	357 (40.8)	34 (9.5)	1	
Type of roof				
Thatched	661 (77.2)	58 (8.8)	0.84 (0.49,1.44)	0.52
Corrugated iron	195 (22.8)	20 (10.3)	1	
Der p 1 allergens(µg/g) (N=783)				0.17
0	655 (83.7)	58 (18.9)	1	0.93*
0.54-500	53 (6.8)	1 (1.9)	0.20 (0.03,1.46)	
≥501	75 (9.6)	8 (10.7)	1.23 (0.56,2.68)	
Bla g 1 allergens (U/g) (N=783)				0.36
0	567 (72.4)	51 (9.0)	1	0.28*
0.02-1.96	101 (12.9)	10 (10.0)	1.12 (0.55,2.30)	
≥2.17	115 (14.7)	6 (5.2)	0.56 (0.23,1.33)	

* p value for trend

Table 4.5 Distribution of potential confounders and associations with eczema at age 3

Variables	Overall N (%)	n (%) eczema	Crude OR (95% CI)	p-value
Child's gender				
Male	434 (49.4)	24 (5.4)	0.75 (0.43,1.29)	0.30
Female	442 (50.5)	31 (7.1)	1	
Maternal education				
Formal	172 (19.6)	10 (5.8)	0.90 (0.45,1.83)	0.78
Non formal	704 (80.4)	45 (6.4)	1	
Maternal allergic history				
Yes	47 (5.4)	6 (12.8)	2.33 (0.94,5.75)	0.06
No	829 (94.6)	49 (5.9)	1	
Paternal allergic history				
Yes	61 (7.0)	8 (13.1)	2.47 (1.11,5.49)	0.02
No	815 (93.0)	47 (5.8)	1	
Breast feeding at 3yr				
Yes	93 (10.6)	5 (5.4)	0.83 (0.32,2.15)	0.70
No	783 (89.4)	50 (6.4)	1	
Reported duration of breast feeding				0.33
≤12 mo	45 (5.9)	1 (2.2)	1	0.36*
12-24 mo	443 (58.5)	27 (6.1)	2.86 (0.38,21.62)	
>24 mo	269 (35.5)	21 (7.8)	3.73 (0.48,28.68)	
Older siblings				0.24
0	132 (15.1)	8 (6.1)	1	0.23*
1-3	480 (54.8)	25 (5.2)	0.85 (0.37,1.93)	
4-10	264 (30.1)	22 (8.3)	1.41 (0.61,3.26)	
Paracetamol use				
Never	496 (56.6)	27 (5.4)	1	0.35
Yes but not in the past month	204 (23.3)	13 (6.4)	1.18 (0.60,2.34)	
Yes in past month	176 (20.1)	15 (8.5)	1.62 (0.84,3.11)	
Antibiotic use				
Yes	250 (28.5)	17 (6.8)	1.13 (0.62,2.04)	0.69
No	626 (71.5)	38 (6.1)	1	
Smoking in the residential house				
Yes	109 (12.4)	7 (6.4)	1.03 (0.45,2.33)	0.95
No	767 (87.6)	48 (6.3)	1	
Charcoal fuel use				
Yes	134 (15.3)	10 (7.5)	1.25 (0.61,2.54)	0.54
No	742 (84.7)	45 (6.1)	1	

Table 4.5 (continued)

Variables	Overall N (%)	n (%) eczema	Crude OR (95% CI)	p-value
Insecticide use				
Yes	519 (59.3)	33 (6.4)	1.03 (0.59,1.81)	0.91
No	357 (40.8)	22 (6.2)	1	
Type of roof				
Thatched	661 (77.2)	40 (6.1)	0.90 (0.47,1.72)	0.75
Corrugated iron	195 (22.8)	13 (6.7)	1	
Der p 1 allergens(µg/g) (N=783)				0.72
0	655 (83.7)	43 (6.6)	1	0.82*
0.54-500	53 (6.8)	2 (3.8)	0.56 (0.13,2.37)	
≥501	75 (9.6)	5 (6.7)	1.01 (0.39,2.65)	
Bla g 1 allergens (U/g) (N=783)				0.96
0	567 (72.4)	36 (6.4)	1	
0.02-1.96	101 (12.9)	6 (6.0)	0.94 (0.39,2.30)	0.86*
≥2.17	115 (14.7)	8 (7.0)	1.10 (0.50,2.44)	

* p value for trend

Table 4.6 Distribution of potential confounders and associations with rhinitis at age 3

Variables	Overall N (%)	n (%) rhinitis	Crude OR (95% CI)	P-value
Child's gender				
Male	434 (49.4)	21 (4.8)	0.89 (0.49,1.64)	0.71
Female	442 (50.5)	23 (5.3)	1	
Maternal education				
Formal	172 (19.6)	9 (5.2)	1.06 (0.50,2.24)	0.89
Non formal	704 (80.4)	35 (5.0)	1	
Maternal allergic history				
Yes	47 (5.4)	7 (14.9)	3.75 (1.57,8.92)	<0.01
No	829 (94.6)	37 (4.5)	1	
Paternal allergic history				
Yes	61 (7.0)	3 (4.9)	0.98 (0.29,3.25)	0.97
No	815 (93.0)	41 (5.0)		
Breast feeding at 3yr				
Yes	93 (10.6)	5 (5.4)	1.08 (0.42,2.82)	0.87
No	783 (89.4)	39 (5.0)		
Reported duration of breast feeding				
≤12 mo	45 (5.9)	2 (4.4)	1	0.96
12-24 mo	443 (58.5)	23 (5.2)	1.18 (0.27,5.17)	0.84*
>24 mo	269 (35.5)	13 (4.8)	1.09 (0.24,5.02)	
Older siblings				0.08
0	132 (15.1)	5 (3.8)	1	0.05*
1-3	480 (54.8)	19 (4.0)	1.05 (0.38,2.86)	
4-10	264 (30.1)	20 (7.6)	2.08 (0.76,5.68)	
Paracetamol use				
Never	496 (56.6)	20 (4.0)	1	0.11
Yes but not in the past month	204 (23.3)	16 (7.8)	2.03 (1.03,3.99)	
Yes in past month	176 (20.1)	8 (4.6)	1.13 (0.49,2.62)	
Antibiotic use				
Yes	250 (28.5)	15 (6.0)	1.31 (0.70,2.50)	0.40
No	626 (71.5)	29 (4.6)	1	
Smoking in the residential house				
Yes	109 (12.4)	7 (6.4)	1.35 (0.59,3.12)	0.46
No	767 (87.6)	37 (4.8)	1	
Charcoal fuel use				
Yes	134 (15.3)	3 (2.2)	0.39 (0.12,1.28)	0.11
No	742 (84.7)	41 (5.5)	1	

Table 4.6 (continued)

Variables	Overall N (%)	n (%) rhinitis	Crude OR (95% CI)	P-value
Insecticide use				
Yes	519 (59.3)	28 (5.4)	1.22 (0.65,2.28)	0.54
No	357 (40.8)	16 (4.5)	1	
Type of roof				
Thatched	661 (77.2)	33 (5.0)	1.09 (0.51,2.31)	0.83
Corrugated iron	195 (22.8)	9 (4.6)	1	
Der p 1 allergens(µg/g) (N=783)				0.80
0	655 (83.7)	35 (5.4)	1	0.53*
0.54-500	53 (6.8)	2 (3.8)	0.69 (0.16,2.97)	
≥501	75 (9.6)	3 (4.0)	0.74 (0.22,2.46)	
Bla g 1 allergens (U/g) (N=783)				0.16
0	567 (72.4)	25 (4.4)	1	0.36*
0.02-1.96	101 (12.9)	9 (9.0)	2.14 (0.97,4.74)	
≥2.17	115 (14.7)	6 (5.2)	1.19 (0.48,2.98)	

* p value for trend

4.2.5.2 Relation between potential confounders and sensitization

A large number of familial, environmental and lifestyle exposures, including parental allergic history, breastfeeding, siblingship, smoking in the residential house, insecticide use, and type of housing, were unrelated to dust mite or cockroach sensitization, or sensitization to any allergens (Table 4.7 and Table 4.8). The risk of dust mite was decreased in those who used paracetamol (crude OR 0.14, 95% CI 0.03, 0.61, use in the past month versus never, $p<0.01$), but not with cockroach sensitization. Use of charcoal for cooking and heating increased the risk of cockroach sensitization (crude OR 2.29, 95% CI 1.07, 4.86, $p=0.03$), but was unrelated to dust mite sensitization. There was a significant positive trend of Der p 1 allergen level in child's bedding and cockroach sensitization (crude OR 2.26, 95% CI 0.82, 6.22 for ≥501 µg/g and, 2.66; 0.87,

8.10 for 0.54 to 500 μg/g compared to 0, p for trend= 0.05), but not with dust mite (Table 4.7 and Table 4.8).

Table 4.7 Distribution of potential confounders and associations with dust mite sensitization at age 3

Variables	Overall N (%)	n (%) Dust mite sensitization	Crude OR (95% CI)	P-value
Child's gender				
Male	439 (50.8)	25 (5.7)	1.06 (0.59,1.89)	0.86
Female	425 (49.2)	23 (5.4)	1	
Maternal education				
Formal	166 (19.2)	6 (3.6)	0.59 (0.24,1.40)	0.23
Non formal	698 (80.8)	42 (6.0)	1	
Maternal allergic history				
Yes	46 (5.3)	1 (2.2)	0.36 (0.05,2.70)	0.30
No	816 (94.7)	47 (5.8)	1	
Paternal allergic history				
Yes	60 (7.0)	2 (3.3)	0.57 (0.13,2.39)	0.43
No	802 (93.0)	46 (5.7)	1	
Breast feeding at 3yr				
Yes	93 (10.8)	6 (6.5)	1.19 (0.49,2.89)	0.70
No	769 (89.2)	42 (5.5)	1	
Reported duration of breast feeding				0.35
≤12 mo	44 (5.9)	2 (4.6)	1	0.33*
12-24 mo	434 (58.4)	29 (6.7)	1.50 (0.35,6.54)	
>24 mo	265 (35.7)	11 (4.2)	0.91 (0.19,4.26)	
Older siblings				0.07
0	125 (14.5)	6 (4.8)	1	0.21*
1-3	477 (55.3)	34 (7.1)	1.52 (0.62,3.71)	
4-10	260 (30.2)	8 (3.1)	0.63 (0.21,1.86)	
Paracetamol use				
Never	490 (56.8)	37 (7.6)	1	<0.01
Yes but not in the past month	201 (23.3)	9 (4.5)	0.57 (0.27,1.21)	
Yes in past month	171 (19.8)	2 (1.2)	0.14 (0.03,0.61)	
Antibiotic use				
Yes	243 (28.2)	9 (3.7)	0.57 (0.27,1.20)	0.32
No	619 (71.8)	39 (6.3)	1	
Smoking in the residential house				
Yes	108 (12.5)	3 (2.8)	0.45 (0.14,1.48)	0.18
No	754 (87.5)	45 (6.0)	1	

Table 4.7 (continued)

Variables	Overall N (%)	n (%) Dust mite sensitization	Crude OR (95% CI)	P-value
Charcoal fuel use				
Yes	129 (15.0)	10 (7.8)	1.54 (0.75,3.17)	0.24
No	733 (85.0)	38 (5.2)	1	
Insecticide use				
Yes	511 (59.3)	28 (5.5)	0.96 (0.53,1.73)	0.89
No	351 (40.7)	20 (5.7)	1	
Type of roof				
Thatched	656 (77.7)	35 (5.3)	0.91 (0.45,1.82)	0.78
Corrugated iron	188 (22.3)	11 (5.9)	1	
Der p 1 allergens(µg/g) (N=783)				0.61
0	645 (83.8)	34 (5.3)	1	0.41*
0.54-500	51 (6.6)	3 (5.9)	1.12 (0.33,3.79)	
≥501	74 (9.6)	2 (2.7)	0.50 (0.12,2.12)	
Bla g 1 allergens (U/g) (N=783)				0.99
0	556 (72.2)	28 (5.0)	1	0.92*
0.02-1.96	101 (13.1)	5 (5.0)	0.98 (0.37,2.61)	
≥2.17	113 (14.7)	6 (5.3)	1.06 (0.43,2.62)	

* p value for trend

Table 4.8 Distribution of potential confounders and associations with cockroach sensitization at age 3

Variables	Overall N (%)	n (%) Cockroach sensitization	Crude OR (95% CI)	P-value
Child's gender				
Male	439 (50.8)	16 (3.6)	0.77 (0.39,1.50)	0.44
Female	425 (49.2)	20 (4.7)	1	
Maternal education				
Formal	166 (19.2)	6 (3.6)	0.84(0.34,2.04)	0.69
Non formal	698 (80.8)	30 (4.3)	1	
Maternal allergic history				
Yes	46 (5.3)	2 (4.4)	1.05 (0.24,4.49)	0.95
No	816 (94.7)	34 (4.2)	1	
Paternal allergic history				
Yes	60 (7.0)	2 (3.3)	0.78 (0.18,3.32)	0.74
No	802 (93.0)	34 (4.2)	1	
Breast feeding at 3yr				
Yes	93 (10.8)	4 (4.3)	1.04 (0.36,3.00)	0.95
No	769 (89.2)	32 (4.2)	1	
Reported duration of breast feeding				0.66
≤12 mo	44 (5.9)	2 (4.6)	1	0.42*
12-24 mo	434 (58.4)	21 (4.8)	1.07 (0.24,4.72)	
>24 mo	265 (35.7)	9 (3.4)	0.74 (0.15,3.55)	
Older siblings				0.57
0	125 (14.5)	4 (3.2)	1	0.87*
1-3	477 (55.3)	23 (4.8)	1.53 (0.52,4.52)	
4-10	260 (30.2)	9 (3.5)	1.08 (0.33,3.59)	
Paracetamol use				
Never	490 (56.8)	24 (4.9)	1	0.20
Yes but not in the past month	201 (23.3)	9 (4.5)	0.91 (0.42,1.99)	
Yes in past month	171 (19.8)	3 (1.8)	0.35 (0.10,1.17)	
Antibiotic use				
Yes	243 (28.2)	6 (2.5)	0.50 (0.20,1.21)	0.12
No	619 (71.8)	30 (4.9)	1	
Smoking in the residential house				
Yes	108 (12.5)	2 (1.9)	0.40 (0.09,1.69)	0.20
No	754 (87.5)	34 (4.5)	1	

Table 4.8 (continued)

Variables	Overall N (%)	n (%) Cockroach sensitization	Crude OR (95% CI)	P-value
Charcoal fuel use				
Yes	129 (15.0)	10 (7.8)	2.29 (1.07,4.86)	0.03
No	733 (85.0)	26 (3.6)	1	
Insecticide use				
Yes	511 (59.3)	22 (4.3)	1.08 (0.55,2.15)	0.82
No	351 (40.7)	14 (4.0)	1	
Type of roof				
Thatched	656 (77.7)	28 (4.3)	1.15 (0.50,2.68)	0.74
Corrugated iron	188 (22.3)	7 (3.7)	1	
Der p 1 allergens(µg/g) (N=783)				0.08
0	645 (83.8)	20 (3.1)	1	0.05*
0.54-500	51 (6.6)	4 (7.8)	2.66 (0.87,8.10)	
≥501	74 (9.6)	5 (6.8)	2.26 (0.82,6.22)	
Bla g 1 allergens (U/g) (N=783)				0.80
0	556 (72.2)	20 (3.6)	1	0.86*
0.02-1.96	101 (13.1)	5 (5.0)	1.40 (0.51,3.81)	
≥2.17	113 (14.7)	4 (3.5)	0.98 (0.33,2.93)	

* p value for trend

4.2.6 Effects of geohelminths on wheeze, eczema and rhinitis

4.2.6.1 Prevalence of geohelminths

A total of 866 stool samples were examined and three species of geohelminths were identified with an overall prevalence of 8.5% in three year old children. Hookworm, *A. lumbricoides* and *T. trichiura* were detected in 4.9% (42/866), 4.3% (37/866) and 0.1% (1/866) of the children, respectively (Table 4.9). Infection intensity was low (median [interquartile range (IQR)] = 6 eggs per gram of faeces [3-10] for hookworm and (median [interquartile range (IQR)] =

12 eggs per gram of faeces [3-39] for *A. lumbricoides*, therefore this was not analysed further.

Hymenolepsis nana was the most frequently detected intestinal parasite with a prevalence of 13.6% (118/866). Enterobias vermicularis was found in four children (0.5%), and Taenia species found in one (0.1%). Strongyloides and Schistosoma infections were not found in the cohort. Among protozoa, Giardia lamblia, and Entamoeba species were found in 9.1% (79/866), and 7.5% (65/866) of children respectively.

Emphasis is now given to the findings relating to geohelminths as this was the *a priori* hypothesis, and was previously reported to be linked to asthma and allergy. However, these other parasites were looked at as potential confounders, though adjustment was found to make little change in the odds ratios reported below.

Wheeze

In this cross-sectional analysis, none of the geohelminth parasites were significantly associated with wheeze at age 3 (Table 4.9). A non-significant slight increased risk of wheeze was observed in relation to hookworm infection (adjusted OR, 95% CI, 1.11; 0.38, 3.21, p=0.53). However, a non-significant decreased risk of wheeze was seen with *A. lumbricoides* infection (adjusted OR, 95% CI, 0.28; 0.04, 2.09, p=0.22), but confidence intervals were wide and no associations were statistically significant (Table 4.9).

Table 4.9 OR for wheeze in relation to geohelminth infection at age 3

Geohelminth	Overall N (%)	Wheeze (N=876) Yes n (%)	Crude OR (95%CI)	Adjusted OR* (95% CI)	P-value
Hookworm					
Yes	42 (4.9)	4 (9.5)	1.03 (0.36,2.97)	1.11 (0.38,3.21)	0.85
No	824 (95.1)	76 (9.3)	1	1	
A. lumbricoides					
Yes	37 (4.3)	1 (2.7)	0.26 (0.04,1.94)	0.28 (0.04,2.09)	0.22
No	829 (95.7)	79 (9.6)	1	1	
Any geohelminths†					
Yes	75 (8.5)	5 (6.7)	0.69 (0.27,1.77)	0.74 (0.29,1.90)	0.53
No	791 (91.3)	75 (9.5)	1	1	

*OR adjusted for gender, urban rural residence and maternal education,

†Hookworm, *A. lumbricoides, or T. trichiura*

Eczema

Eczema was reduced in relation to hookworm (adjusted OR, 95% CI, 0.36; 0.05, 2.68, p=0.32), and *A. lumbricoides* (adjusted OR, 95% CI, 0.38; 0.05, 2.86, p=0.35) infection (Table 4.10). However, only few children with eczema were infected by either hookworm or *A. Lumbricoides* infection, and confidence intervals were wide, and none of these associations were significant (Table 4.10).

Table 4.10 OR for eczema in relation to geohelminth infections

Geohelminth	Overall	Eczema (N=876)			
		Yes	Crude	Adjusted	P-
	N (%)	n (%)	OR (95%CI)	OR* (95% CI)	value
Hookworm					
Yes	42 (4.9)	1 (2.4)	0.35 (0.05,2.57)	0.36 (0.05,2.68)	0.32
No	824 (95.1)	54 (6.6)	1	1	
A. lumbricoides					
Yes	37 (4.3)	1 (2.7)	0.40 (0.05,2.96)	0.38 (0.05,2.86)	0.35
No	829 (95.7)	54 (6.5)	1	1	
Any geohelminths†					
Yes	75 (8.5)	2 (2.7)	0.39 (0.09,1.62)	0.39 (0.09,1.63)	0.19
No	791 (91.3)	53 (6.7)	1	1	

*OR adjusted for gender, urban rural residence and maternal education,

†Hookworm, *A. lumbricoides, or T. trichiura*

Rhinitis

As in the wheeze and eczema associations, none of the geohelminths were significantly associated with rhinitis though the effect was in the expected direction. For hookworm the adjusted OR was 0.44 (95% CI 0.06, 3.30, p=0.43), and for *A. lumbricoides* infection the adjusted OR was 0.54 (95% CI 0.07, 4.02, p=0.54) but again the number of infected children with the outcome was very low (Table 4.11).

Table 4.11 OR for rhinitis in relation to geohelminth infection

Geohelminth	Overall N (%)	Rhinitis (N=876)			
		Yes n (%)	Crude OR (95%CI)	Adjusted OR* (95% CI)	P-value
Hookworm					
Yes	42 (4.9)	1 (2.4)	0.45 (0.06,3.37)	0.44 (0.06,3.30)	0.43
No	824 (95.1)	42 (5.1)	1	1	
A. lumbricoides					
Yes	37 (4.3)	1 (2.7)	0.52 (0.07,3.88)	0.54 (0.07,4.02)	0.54
No	829 (95.7)	42 (5.1)	1	1	
Any geohelminths†					
Yes	75 (8.5)	2 (2.7)	0.50 (0.12,2.09)	0.49 (0.12,2.09)	0.33
No	791 (91.3)	41 (5.2)	1	1	

*OR adjusted for gender, urban rural residence and maternal education,

†Hookworm, *A. lumbricoides, or T. trichiura*

4.2.7 Effects of geohelminths on skin sensitization

Dust mite sensitization

Hookworm infection was associated with a non-significant decreased risk (adjusted OR, 95% CI, 0.86; 0.20, 3.69, p=0.84), and *A. lumbricoides* with a non-significant increased risk of dust mite sensitization (adjusted OR, 95% CI, 1.48; 0.43, 5.04, p=0.53) (Table 4.12). However, as in the symptom outcomes, the number of infected children with the outcomes was low (Table 4.12).

Table 4.12 OR for dust mite sensitization in relation to geohelminth infections

Geohelminth	Overall N (%)	Dust mite sensitization (N=864)			
		Yes n (%)	Crude OR (95%CI)	Adjusted OR* (95% CI)	P-value
Hookworm					
Yes	42 (4.9)	2 (4.8)	0.88 (0.21,3.75)	0.86 (0.20,3.69)	0.84
No	824 (95.1)	44 (5.4)	1	1	
A. lumbricoides					
Yes	37 (4.3)	3 (8.1)	1.60 (0.47,5.41)	1.48 (0.43,5.04)	0.53
No	829 (95.7)	43 (5.2)	1	1	
Any geohelminths†					
Yes	75 (8.7)	5 (6.7)	1.24 (0.48,3.23)	1.18 (0.45,3.09)	0.74
No	791 (91.3)	41 (5.2)	1	1	

*OR adjusted for gender, urban rural residence and maternal education

†Hookworm, *A. lumbricoides, or T. trichiura*

Cockroach sensitization

The risks of cockroach sensitization was reduced in those with hookworm infection (adjusted OR, 95% CI, 0.58; 0.08, 4.34, p=0.59), and increased in those with *A. lumbricoides* infection (adjusted OR, 95% CI, 1.99; 0.57, 6.89, p=0.28) (Table 4.13). However, none of these associations were statistically significant.

Table 4.13 OR for cockroach sensitization in relation to geohelminth infections

Geohelminth	Overall N (%)	Cockroach sensitization (N=864)			
		Yes n (%)	Crude OR (95%CI)	Adjusted OR* (95% CI)	P-value
Hookworm					
Yes	42 (4.9)	1(2.4)	0.54 (0.07,4.07)	0.58 (0.08,4.34)	0.59
No	824 (95.1)	35 (4.3)	1	1	
A. lumbricoides					
Yes	37 (4.3)	3 (8.1)	2.11(0.62,7.21)	1.99 (0.57,6.89)	0.28
No	829 (95.7)	33 (4.0)	1	1	
Any geohelminths†					
Yes	75 (8.7)	4 (5.3)	1.33 (0.46,3.88)	1.34 (0.46,3.92)	0.59
No	791 (91.3)	32 (4.1)	1	1	

*OR adjusted for gender, urban rural residence and maternal education

†Hookworm, *A. lumbricoides, or T. trichiura*

Any sensitization

None of the geohelminth parasites were significantly associated with sensitization to any allergen (either dust mite or cockroach allergens), and again numbers of infected children with the outcome were too small (Table 4.14).

Table 4.14 OR for any sensitization in relation to geohelminth infection

Geohelminth	Overall N (%)	Any sensitization¥ (N=864)			
		Yes n (%)	Crude OR (95%CI)	Adjusted OR* (95% CI)	P-value
Hookworm					
Yes	42 (4.9)	3 (7.1)	0.82 (0.25,2.77)	0.83 (0.25,2.76)	0.76
No	824 (95.2)	70 (8.6)	1	1	
A. lumbricoides					
Yes	37 (4.3)	5 (13.5)	1.73 (0.65,4.59)	1.62 (0.61,4.32)	0.34
No	829 (95.7)	68 (8.3)	1	1	
Any geohelminths†					
Yes	75 (8.5)	8 (10.7)	1.29 (0.59,2.79)	1.25 (0.57,2.73)	0.58
No	803 (91.5)	67 (8.5)	1	1	

*OR adjusted for gender, urban rural residence and maternal education

†Hookworm, *A. lumbricoides, or T. trichiura*

¥ Sensitization to either dust mite or cockroach allergen

4.2.8 Effects of H. pylori and intestinal microflora on wheeze, eczema and rhinitis

The most common commensal bacteria detected were enterococci (38.1% - 207/544) followed by lactobacilli (31.1% - 169/544) and bifidobacteria (19.0% - 103/544). *H. pylori* was identified in 41.1% (253/616) of children (Table 4.15).

Wheeze

The presence of enterococci (adjusted OR, 95% CI, 1.27; 0.72, 2.24, p=0.40), lactobacilli (adjusted OR, 95% CI, 1.19; 0.66, 2.16, p=0.56), and bifidobacteria (adjusted OR, 95% CI, 1.24; 0.63, 2.47, p=0.53) in stool tended to be

associated with a non significant increased risk of wheeze (Table 4.15). Infection with *H. pylori* was associated with a non significant decreased risk of wheeze (adjusted OR 0.80, 95% CI 0.46 to 1.38, p=0.41). However, none of these associations were statistically significant (Table 4.15).

Table 4.15 OR for wheeze in relation to *H. pylori* and microflora

Infection	Overall N (%)	Wheeze			
		Yes n (%)	Crude OR (95%CI)	Adjusted OR* (95% CI)	P-value
Helicobacter pylori					
Yes	253 (41.1)	24 (9.6)	0.88 (0.51,1.50)	0.80 (0.46,1.38)	0.41
No	363 (58.9)	39 (10.7)	1	1	
Enterococci					
Yes	207 (38.1)	24 (11.6)	1.24 (0.71,2.17)	1.27 (0.72,2.24)	0.40
No	337 (61.9)	32 (9.6)	1	1	
Lactobacilli					
Yes	169 (31.1)	19 (11.2)	1.15 (0.64,2.07)	1.19 (0.66,2.16)	0.56
No	375 (68.9)	37 (9.9)	1	1	
Bifidobacteria					
Yes	103 (18.9)	12 (11.8)	1.20 (0.61,2.36)	1.24 (0.63,2.47)	0.53
No	441 (81.1)	44 (10.0)	1	1	
Any microflora†					
Yes	317 (58.3)	37 (11.7)	1.44 (0.81,2.58)	1.49 (0.83,2.69)	0.18
No	227 (41.3)	19 (8.4)	1	1	

*OR adjusted for gender, urban rural residence and maternal education

†Enterococci, lactobacilli and bifidobacteria

Eczema

None of the intestinal microflora detected were significantly associated with eczema (adjusted OR, 95% CI, 1.07; 0.53, 2.17, p=0.84 for enterococci, adjusted OR, 95% CI, 1.00; 0.48, 2.10, p=0.99 for lactobacilli, and adjusted OR, 95% CI, 1.19; 0.18, 1.50, p=0.23 for bifidobacteria) (Table 4.16).

However, infection with *H. pylori* was associated with a borderline significant decreased risk of eczema (adjusted OR 0.49, 95% CI; 0.24, 1.01, p=0.05) (Table 4.16), and the magnitude of these effects changed little following adjustment for other potential confounders shown in Table 4.5, and further mutual adjustment for geohelminth and microflora exposure.

Table 4.16 OR for eczema in relation to *H. pylori* and microflora

Infection	Overall	Eczema			
		Yes	Crude	Adjusted	P-
	N (%)	n (%)	OR (95%CI)	OR* (95% CI)	value
Helicobacter pylori					
Yes	253 (41.1)	11 (4.4)	0.51 (0.25,1.04)	0.49 (0.24,1.01)	0.05
No	363 (58.9)	30 (8.3)	1	1	
Enterococci					
Yes	207 (38.1)	14 (6.8)	1.08 (0.54,2.18)	1.07 (0.53,2.17)	0.84
No	337 (61.9)	21 (6.3)	1	1	
Lactobacilli					
Yes	169 (31.1)	11 (6.5)	1.01 (0.48,2.12)	1.00 (0.48,2.10)	0.99
No	375 (68.9)	24 (6.4)	1	1	
Bifidobacteria					
Yes	103 (18.9)	4 (3.9)	0.54 (0.19,1.56)	0.52 (0.18,1.50)	0.23
No	441 (81.1)	31 (7.1)	1	1	
Any microflora†					
Yes	317 (58.3)	22 (7.0)	1.23 (0.60,2.49)	1.20 (0.59,2.45)	0.61
No	227 (41.3)	13 (5.8)	1	1	

*OR adjusted for gender, urban rural residence and maternal education

†Enterococci, lactobacilli and bifidobacteria

Rhinitis

The presence of lactobacilli (adjusted OR, 95% CI, 1.14; 0.54, 2.41, p=0.74), bifidobacteria (adjusted OR, 95% CI, 1.49; 0.65, 3.43, p=0.35), and *H. pylori* (adjusted OR, 95% CI, 1.86; 0.92, 3.78, p=0.09) were associated with a non significant increased risk of rhinitis (Table 4.17). The relation between the commensal enterococci bacteria and rhinitis was also not significant (p=0.85), though the direction of effect was in the expected direction (Table 4.17).

Table 4.17 OR for rhinitis in relation to *H. pylori* and microflora

Infection	Overall N (%)	Rhinitis			
		Yes n (%)	Crude OR (95%CI)	Adjusted OR* (95% CI)	P-value
Helicobacter pylori					
Yes	253 (41.1)	18 (7.2)	1.79 (0.89,3.63)	1.86 (0.92,3.78)	0.09
No	363 (58.9)	15 (4.1)	1	1	
Enterococci					
Yes	207 (38.1)	12 (5.8)	0.92 (0.44,1.91)	0.93 (0.45,1.94)	0.85
No	337 (61.9)	21 (6.3)	1	1	
Lactobacilli					
Yes	169 (31.1)	11 (6.5)	1.11 (0.53,2.35)	1.14 (0.54,2.41)	0.74
No	375 (68.9)	22 (5.9)	1	1	
Bifidobacteria					
Yes	103 (18.9)	8 (7.8)	1.41 (0.62,3.23)	1.49 (0.65,3.43)	0.35
No	441 (81.1)	25 (5.7)	1	1	
Any microflora†					
Yes	317 (58.3)	22 (7.0)	1.46 (0.69,3.08)	1.51 (0.71,3.19)	0.28
No	227 (41.3)	11 (4.9)	1	1	

*OR adjusted for gender, urban rural residence and maternal education,

†Enterococci, lactobacilli and bifidobacteria

4.2.9 Effects of H. pylori and intestinal microflora on skin sensitization

Dust mite sensitization

The commensal bacteria enterococci was associated with a non significant increased risk of dust mite sensitization (adjusted OR, 95% CI, 1.03; 0.44, 2.43, p=0.94). Lactobacilli (adjusted OR, 95% CI, 0.76; 0.29, 1.96, p=0.57), and bifidobacteria (adjusted OR, 95% CI, 0.61; 0.18, 2.11, p=0.44) were associated with a non significant decreased risk of dust mite sensitization (Table 4.18).

The association between *H. pylori* and a reduced risk of dust mite sensitization, however, did reach borderline significance (adjusted OR, 95% CI, 0.42; 0.17, 1.08, P=0.07) (Table 4.18). Further adjustment for potential confounders presented in Table 4.7, including mutual adjustment for geohelminths and commensal bacteria made little change on the size of the risk estimate.

Table 4.18 OR for dust mite sensitization in relation to *H. pylori* and microflora

Infection	Overall N (%)	Dust mite sensitization			
		Yes n (%)	Crude OR (95%CI)	Adjusted OR* (95% CI)	P-value
Helicobacter pylori					
Yes	253 (41.1)	6 (2.4)	0.41 (0.16,1.05)	0.42 (0.17,1.08)	0.07
No	363 (58.9)	20 (5.6)	1	1	
Enterococci					
Yes	207 (38.1)	9 (4.4)	1.04 (0.44,2.46)	1.03 (0.44,2.43)	0.94
No	337 (61.9)	14 (4.2)	1	1	
Lactobacilli					
Yes	169 (31.1)	6 (3.4)	0.77 (0.30,2.00)	0.76 (0.29,1.96)	0.57
No	375 (68.9)	17 (4.6)	1	1	
Bifidobacteria					
Yes	103 (18.9)	3 (3.0)	0.64 (0.19,2.19)	0.61 (0.18,2.11)	0.44
No	441 (81.1)	20 (4.6)	1	1	
Any microflora†					
Yes	317 (58.3)	12 (3.8)	0.77 (0.33,1.78)	0.76 (0.33,1.75)	0.51
No	227 (41.3)	11 (4.9)	1	1	

*OR adjusted for gender, urban rural residence and maternal education

†Enterococci, lactobacilli and bifidobacteria

Cockroach sensitization

The association between intestinal microflora and cockroach sensitization was not significant; however the observed effects were in the expected direction.

Infection with *H. pylori* was also associated with a non significant decreased risk of cockroach sensitization (adjusted OR, 95% CI, 0.59; 0.22, 1.56, p=0.29) (Table 4.19).

Table 4.19 OR for cockroach sensitization in relation to *H. pylori* and microflora

Infection	Overall N (%)	Cockroach sensitization			
		Yes n (%)	Crude OR (95%CI)	Adjusted OR* (95% CI)	P-value
Helicobacter pylori					
Yes	253 (41.1)	6 (2.4)	0.60 (0.23,1.59)	0.59 (0.22,1.56)	0.29
No	363 (58.9)	14 (3.9)	1	1	
Enterococci					
Yes	207 (38.1)	6 (2.9)	0.74 (0.28,1.98)	0.72 (0.27,1.95)	0.52
No	337 (61.9)	13 (3.9)	1	1	
Lactobacilli					
Yes	169 (31.1)	8 (4.8)	1.64 (0.65,4.16)	1.65 (0.65,4.19)	0.29
No	375 (68.9)	11 (3.0)	1	1	
Bifidobacteria					
Yes	103 (18.9)	3 (3.0)	0.80 (0.23,2.81)	0.75 (0.21,2.64)	0.65
No	441 (81.1)	16 (3.7)	1	1	
Any microflora†					
Yes	317 (58.3)	10 (3.2)	0.79 (0.32,1.97)	0.76 (0.30,1.92)	0.57
No	227 (41.3)	9 (4.0)	1	1	

*OR adjusted for gender, urban rural residence and maternal education

†Enterococci, lactobacilli and bifidobacteria

Any sensitization

None of the commensal bacteria in the stool, nor infection with *H. pylori* were significantly associated with sensitization to any allergens (Table 4.20).

Table 4.20 OR for any sensitization in relation to *H. pylori* and microflora

Infection	Overall N (%)	Any sensitization¥ Yes n (%)	Crude OR (95%CI)	Adjusted OR* (95% CI)	P-value
Helicobacter pylori					
Yes	253 (41.1)	12 (4.8)	0.62 (0.31,1.24)	0.61 (0.30,1.23)	0.16
No	363 (58.9)	27 (7.5)	1	1	
Enterococci					
Yes	207 (38.1)	13 (6.3)	0.87 (0.43,1.75)	0.85 (0.42,1.72)	0.65
No	337 (61.9)	24 (7.2)	1	1	
Lactobacilli					
Yes	169 (31.1)	13 (7.8)	1.22 (0.60,2.45)	1.20 (0.59,2.42)	0.62
No	375 (68.9)	24 (6.5)	1	1	
Bifidobacteria					
Yes	103 (18.9)	6 (5.9)	0.83 (0.33,2.03)	0.78 (0.31,1.93)	0.59
No	441 (81.1)	31 (7.1)	1	1	
Any microflora†					
Yes	317 (58.3)	20 (6.4)	0.83 (0.43,1.63)	0.80 (0.41,1.58)	0.59
No	227 (41.3)	17 (7.6)	1	1	

*OR adjusted for gender, urban rural residence and maternal education

†Enterococci, lactobacilli and bifidobacteria

¥ Sensitization to either dust mite or cockroach allergen

4.3 SUMMARY

In this population-based study of young Ethiopian children, cross-sectional analyses at age three resulted in a borderline significant decreased risk of eczema symptoms and dust mite sensitization in relation to *H. pylori*, in which alternative explanations including medical interventions are unlikely to play a role. The study however found no evidence to support an etiological role of the commensal bacteria on allergic diseases. The power to assess effects of geohelminths was limited. Further follow up of the cohort beyond three years is important to clarify conflicting findings, and to investigate whether the

associations seen in these cross-sectional analyses could also be replicated in a longitudinal analysis.

The study also showed that the reported prevalence of wheeze, eczema and the levels of sensitization to domestic allergens in three year old children were low in both urban and rural areas, and unrelated to geohelminth exposure and commensal bacteria. Furthermore, reported outcome measures were unrelated to sensitization to dust mite and cockroach allergens at this age. This may be due to the low prevalence of reported symptoms and sensitization in the study.

The prevalence and intensity of geohelminths in this cohort of children was very low, and hookworm (4.8%) and *A. lumbricoides* (4.3%) were the predominant geohelminths identified. The low prevalence of geohelminths in the study is likely linked to a mass de-worming program,[257] and may account for the low statistical power for this analysis.

The cohort was followed further, and results of this continued follow up are described in the next chapters: chapter five focuses on further analyses (longitudinal and cross-sectional) of the effects of paracetamol on allergic symptoms and sensitization at the age of five years, and chapter six covers longitudinal and cross-sectional analyses of the roles of geohelminths, commensal bacteria, and *H. pylori* on allergic disease and sensitization at the same age.

5 EARLY LIFE EXPOSURE TO PARACETAMOL AND THE PREVALENCE AND INCIDENCE OF WHEEZE AND ALLERGIC DISEASE AT AGE 5

5.1 INTRODUCTION

In May 2011, follow-up of the cohort at age five was complete which allowed further exploration of the role of paracetamol in the aetiology of allergic disease to be carried out. So far this thesis has investigated the effects of paracetamol in the first year of life on the incidence of wheeze and eczema between ages one and three but the analysis was limited to two outcomes, wheeze and eczema. Data collected at this later time point allows analysis of two additional outcomes, rhinitis and allergic sensitization. In addition, as the children grow older, it may likely that the wheeze outcome will represent asthmatic phenotype, rather than a marker of respiratory tract infection. Furthermore, exploration of the relative importance of timing and dose of paracetamol exposure is possible at this follow-up by incorporating exposure responses given at age one and three. Furthermore, the availability, preference and indications for use of paracetamol could be explored for the first time at this follow-up.

This chapter will therefore present further prospective findings of the five year follow-up of the same children in which paracetamol use and outcomes have been prospectively measured at ages one, three and five. The primary aim is therefore to determine the longitudinal association between early life

paracetamol use (dose and timing) on the incidence of wheeze, eczema, rhinitis and skin sensitization between ages three and five. In addition, this chapter aims to determine the cross-sectional association between the use of paracetamol and these allergic outcomes using current and previous exposures at the age of five years (lifetime use of paracetamol), and to investigate indications for use of paracetamol. Analysis of five year follow-up of the other early life risk factors of interest, namely geohelminths, *H. pylori* infection, and commensal bacteria, will be reported in chapter six.

5.2 RESULTS

5.2.1 The birth cohort at age five

At age five, 863 (86% of the original cohort at year one, and 98% of those available at year three follow up) were followed-up (see section 2.3.6 and Figure 2.3). Reported symptom outcome data were available from 852 children; for the other 11 children, mothers were not around to provide the information. Skin tests were performed on 855 children (three of these children did not provide symptom questionnaire data but skin tests were able to be carried out in the presence of another guardian). Children who were lost to follow up (less than 2% between year three and five) were not different in terms of paracetamol exposure or the outcomes collected.

5.2.2 Natural history of the outcomes between ages one and five

Of the wheeze-free cohort up to three years of age (n=698), incident wheeze

was reported in 5.9% (40/676) of children between ages three and five (Figure 5.1). Of the 26 children who were persistent wheezers (wheezers at year one and three), 6 (23.1%) still reported wheeze ever at age five whereas 20 (76.9%) did not (Figure 5.1).

Among the eczema-free cohort up to year three (n=723), incident eczema was reported in 5.8% (39/700) (Figure 5.2). Of the eight children who reported eczema at ages one and three and followed up to year five, only 1 (12.5%) still reported eczema at age five, the remaining 7 (87.5%) did not (Figure 5.2).

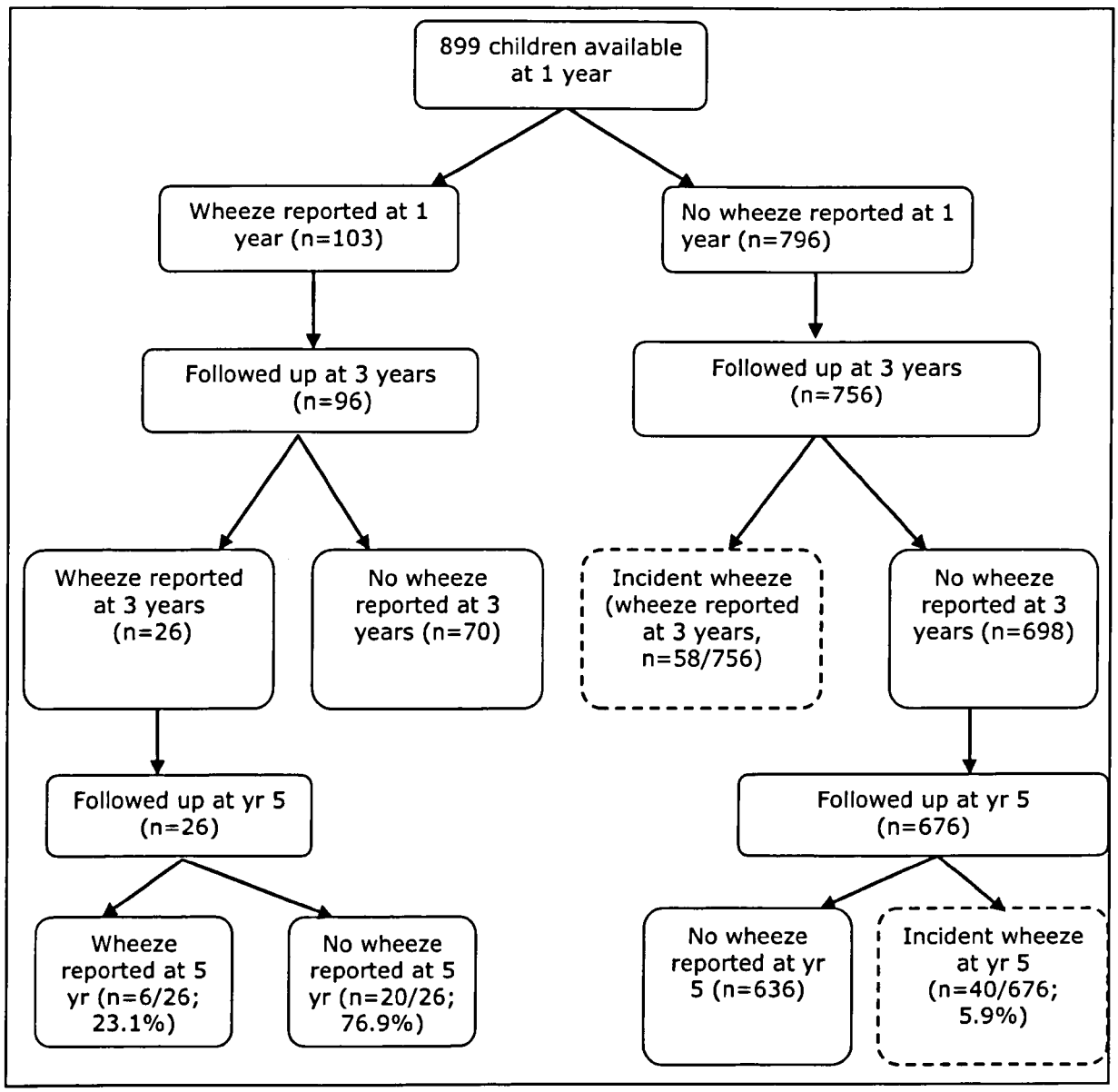

Figure 5.1 Wheeze reporting between one and five years

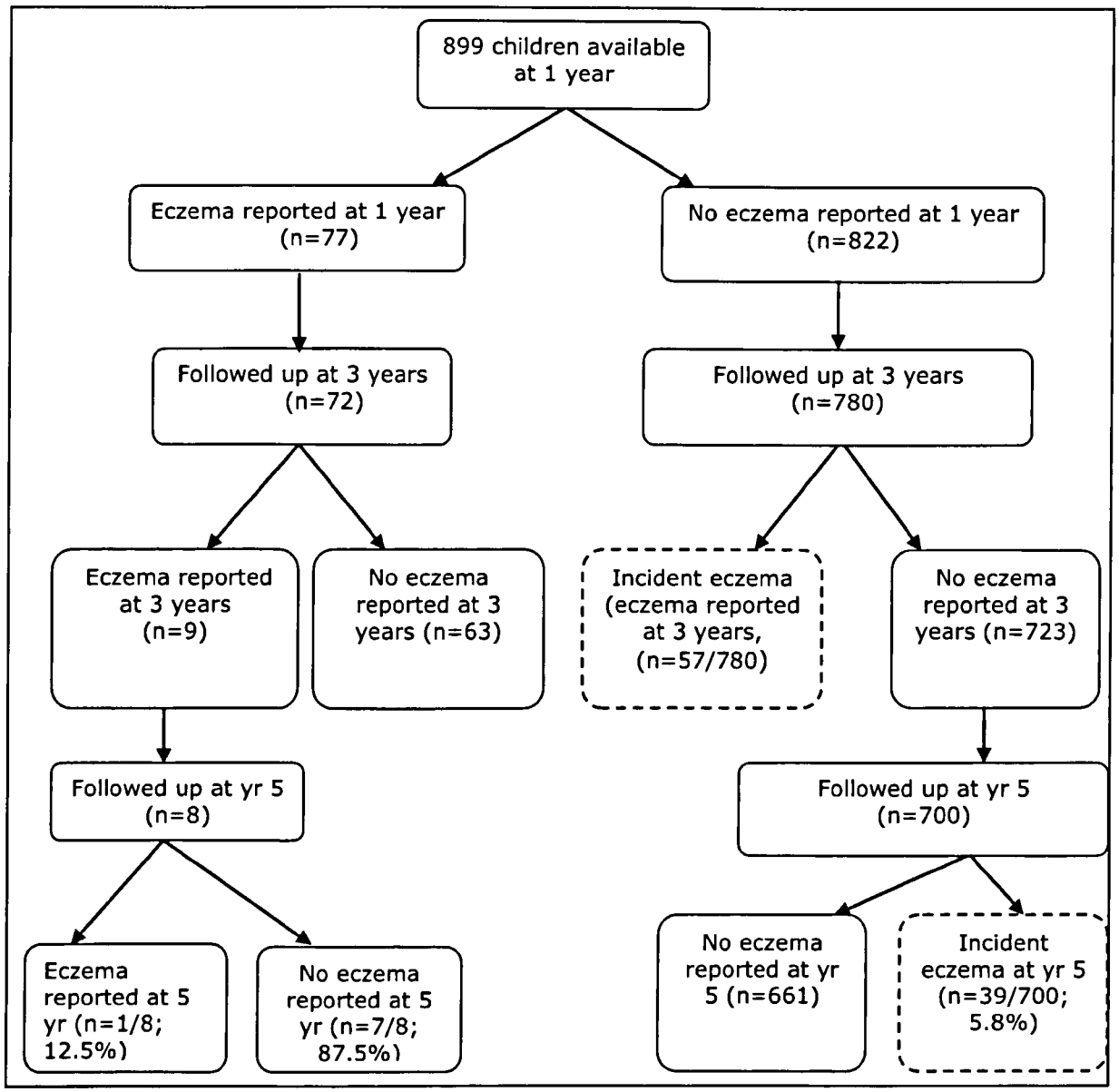

Figure 5.2 Eczema reporting between one and five years

Again, of the rhinitis-free cohort at age three who were followed up to age five (n=798), incident rhinitis was reported in 3.9% (31/798) of the children. Of the 50 children with reported rhinitis ever at age three who were successfully followed-up, 9 (18%) still had persistent rhinitis and 41 (82%) did not (Figure 5.3).

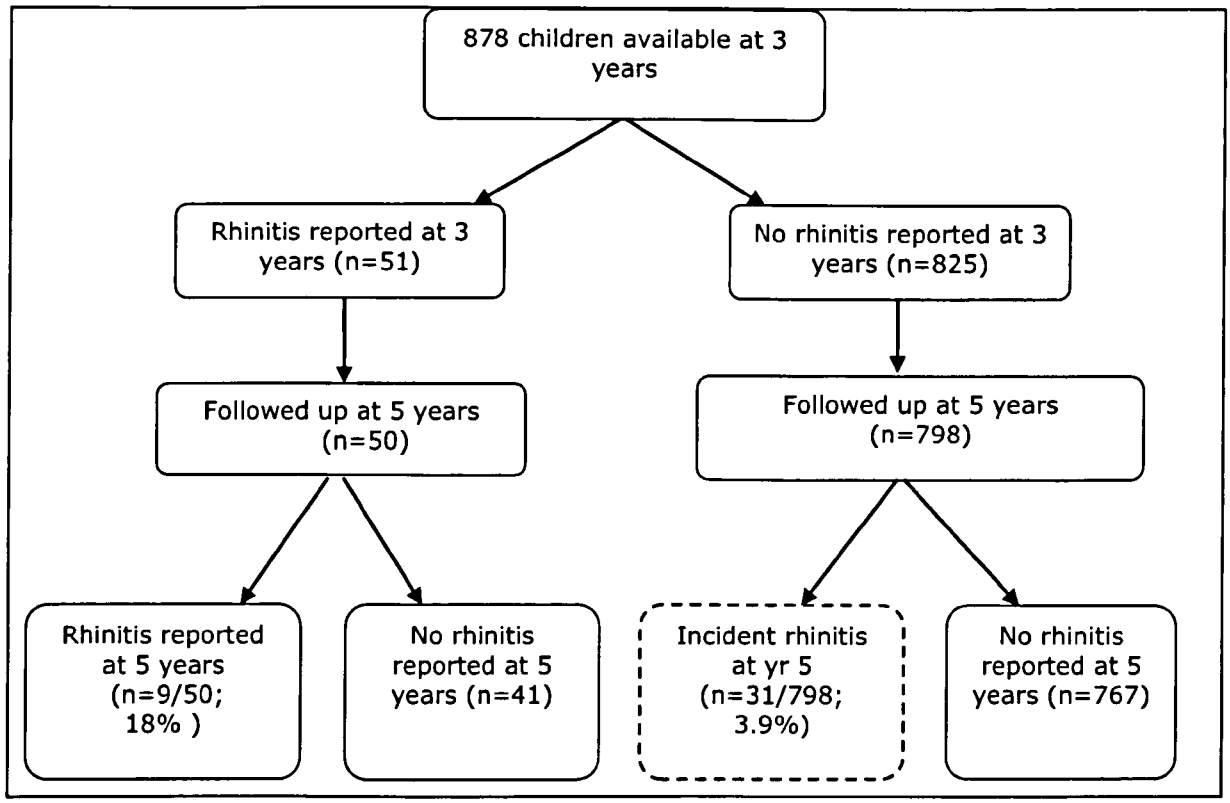

Figure 5.3 Rhinitis reporting between three and five years

Similarly, of the children with no sensitization at the age of three followed up at age five (n=766), incident sensitization to either *D. pteronyssinus* or cockroach allergen between ages three and five was found in 2.0% (15/766) (Figure 5.4). However, of the 72 children who were skin test positive for these allergens at age three, and were successfully followed-up, only 1 (1.4%) remained sensitized at age five and the rest 71 (98.6%) were not (Figure 5.4).

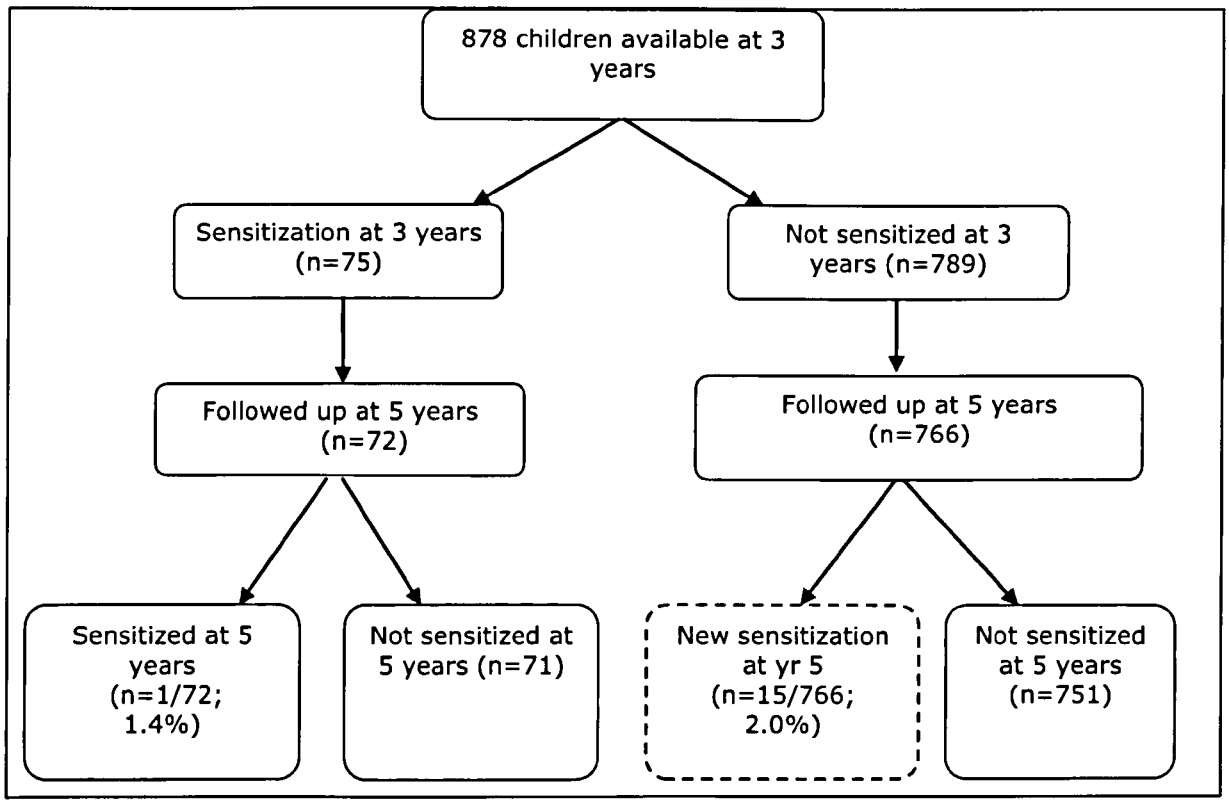

Figure 5.4 Patterns of skin sensitisation between three and five years

5.2.3 Associations between early sensitization and incident allergic symptoms

Sensitization at the age of three increased the risk of incident wheeze, eczema and rhinitis between ages three and five, and reached statistical significance for new onset eczema (OR, 2.49; 95% CI, 1.04, 5.95, p=0.03) and rhinitis (OR, 2.54; 95% CI, 1.00, 6.45, p=0.04) (Table 5.1). The risk of incident wheeze was increased (OR, 1.45; 95% CI, 0.54, 3.84, p=0.46), but the confidence interval was wide and significance not reached (Table 5.1).

Table 5.1 OR for incident wheeze, eczema and rhinitis in relation to skin sensitization at the age of three

	Any sensitization at year three†			
New symptoms at age 5	Over all N (%)	Yes n (%)	Crude OR (95%CI)	P-value
Wheeze	63 (9.2)	5 (8.2)	1.45 (0.54,3.84)	0.46
Eczema	61 (8.9)	7 (11.5)	2.49 (1.04,5.95)	0.03
Rhinitis	71 (9.0)	6 (8.5)	2.54 (1.00,6.45)	0.04

†Sensitization to either *D. pteronyssinus* or cockroach allergen

5.2.4 Associations between urban and rural residence and incidence of outcomes

The reported new-onset symptoms and sensitization to any allergen did not differ by place of residence, however, slightly more rural than urban children reported incident outcomes between ages three and five, none of these comparisons, however, reached statistical significant (Table 5.2).

Table 5.2 Incidence of the outcomes by area of residence

Incident Outcome	Overall n (%)	Urban Yes n (%)	Rural Yes n (%)	Crude OR (95% CI)	p-value
Wheeze (N=676)	40 (5.9)	2 (2.6)	38 (6.4)	0.39 (0.09,1.64)	0.18
Eczema (N=700)	39 (5.8)	2 (2.4)	37 (6.0)	0.38 (0.09,1.62)	0.17
Rhinitis (N=798)	31 (3.9)	2 (2.0)	29 (4.2)	0.48 (0.11,2.03)	0.30
Any sensitization[†] (N=766)	15 (2.0)	1 (1.1)	14 (2.1)	0.54 (0.07,4.15)	0.55

[†] Sensitization to either *D. pteronyssinus* or cockroach allergen

5.2.5 Distribution of potential confounders with incident disease outcomes

5.2.5.1 Association between demographic and lifestyle risk factors with incident wheeze, eczema, rhinitis and sensitization

Table 5.3 to Table 5.6 show the distribution of various potential early life confounders measured in the first year of life on the incidence of wheeze, eczema, rhinitis and sensitization between ages three and five. Borderline significant inverse associations were seen between incident wheeze and increasing household size (ORs [95% CIs], 0.68 [0.30, 1.58] for 4-6 people and 0.37 [0.13, 1.04] for 7+ people, compared to 1-3 number of people; p for trend=0.05), but not with the other outcomes (Table 5.3). The other early life risk factors including demographic variables, parental history of allergic diseases, child's birth weight, breast feeding and vaccination status, indoor cooking and other household and life style risk factors were not associated with any of the incident reported outcomes, or sensitization (Table 5.3 to Table 5.6).

Table 5.3 Distribution of potential confounders in the first year of life in relation to incident wheeze between ages 3 and 5

Variables	Wheeze never up to age 3 (N=676)			
	Overall N (%)	n (%)new wheeze	Crude OR (95% CI)	P-value
Residence				
Urban	78 (11.5)	2 (2.6)	0.39 (0.09,1.64)	0.18
Rural	598 (88.5)	38 (6.4)	1	
Child's gender				
Male	336 (49.7)	24 (7.1)	1.56 (0.81,2.99)	0.18
Female	340 (50.3)	16 (4.7)	1	
Maternal education				
Formal	121 (17.9)	10 (8.3)	1.58 (0.75,3.32)	0.23
Non formal	555 (82.1)	30 (5.4)	1	
Low birth weight				
Low birth weight	28 (6.2)	3 (10.3)	2.44 (0.68,8.81)	0.16
Normal	427 (93.9)	20 (4.7)	1	
Cough reported at yr 1				
Yes	390 (57.7)	32 (8.2)	3.11 (1.40,6.87)	<0.01
No	286 (42.3)	8 (2.8)	1	
Fast breathing at yr 1				
Yes	218 (32.3)	21 (9.6)	2.46 (1.29,4.70)	<0.01
No	458 (67.8)	19 (4.2)	1	
Fever reported at yr 1				
Yes	513 (75.9)	38 (7.4)	6.44 (1.52,27.27)	<0.01
No	163 (24.1)	2 (1.2)	1	
Exclusive breast feeding at 2 mo				
Yes	565 (83.8)	36 (6.4)	1.79 (0.62,5.13)	0.27
No	109 (16.2)	4 (3.7)	1	
Vaccination at 2 mo				
Yes	393 (58.3)	15 (5.3)	1.20 (0.62,2.33)	0.58
No	281 (41.7)	25 (6.4)	1	
Parental allergic history				
Yes	33 (4.9)	1 (3.0)	0.48 (0.06,3.64)	0.47
No	643 (95.1)	39 (6.1)	1	
Insecticide use in the home				
Yes	559 (82.7)	34 (6.1)	1.20 (0.49,2.92)	0.69
No	117 (17.3)	6 (5.1)	1	

Table 5.3 (continued)

Variables	Wheeze never up to age 3 (N=676)			
	Overall N (%)	n (%)new wheeze	Crude OR (95% CI)	P-value
Household size				0.14
1-3	87 (12.9)	8 (9.2)	1	0.05†
4-6	370 (54.7)	24 (6.5)	0.68 (0.30,1.58)	
7+	219 (32.4)	8 (3.7)	0.37 (0.13,1.04)	
No of older siblings				0.13
0	96 (14.2)	6 (6.3)	1	0.14†
1-3	366 (54.1)	27 (7.4)	1.19 (0.48,2.99)	
4-10	214 (31.7)	7 (3.3)	0.51 (0.17,1.56)	
Child's sleeping place				0.15
Bed/platform	52 (7.7)	3 (5.8)	1	
Floor	291 (43.1)	23 (7.9)	1.40 (0.40,4.86)	
Grass matting	332 (49.2)	14 (4.2)	0.72 (0.20,2.60)	
Indoor cooking				
Yes	549 (81.2)	33 (6.0)	1.10 (0.47,2.54)	0.83
No	127 (18.8)	7 (5.5)	1	
Indoor kerosene use				
Yes	77 (11.4)	6 (7.8)	1.40 (0.57,3.47)	0.46
No	599 (88.6)	34 (5.7)	1	
Smoking in the house at yr 3				
Yes	85 (12.6)	2 (2.4)	0.35 (0.08,1.49)	0.14
No	591 (87.4)	38 (6.4)	1	
Antibiotic use at yr 3				
Yes	183 (27.1)	11 (6.0)	1.02 (0.50,2.09)	0.95
No	493 (72.9)	29 (5.9)	1	
Type of roof				
Thatched roof	528 (78.1)	31 (5.9)	0.96 (0.45,2.07)	0.92
corrugated iron sheet	148 (21.9)	9 (6.1)	1	

† p value for trend

Table 5.4 Distribution of potential confounders in the first year of life in relation to incident eczema between ages 3 and 5

Variables	Eczema never up to age 3 (N=700)			
	Overall N (%)	n (%)new eczema	Crude OR (95% CI)	P-value
Residence				
Urban	84 (12.0)	2 (2.4)	0.38 (0.09,1.62)	0.17
Rural	676 (88.0)	37 (6.0)	1	
Child's gender				
Male	362 (51.7)	20 (5.9)	0.88 (0.46,1.68)	0.70
Female	338 (48.3)	19 (5.3)	1	
Maternal education				
Formal	133 (19.0)	8 (6.0)	1.11 (0.50,2.47)	0.80
Informal	567 (81.0)	31 (5.5)	1	
Low birth weight (<2.5kg) vs. normal				
Low birth weight	37 (7.8)	2 (5.4)	0.78 (0.18,3.41)	0.74
Normal	440 (92.2)	30 (6.8)	1	
Cough reported at yr 1				
Yes	436 (62.3)	26 (6.0)	1.22 (0.62,2.43)	0.56
No	264 (37.7)	13 (4.9)	1	
Fast breathing at yr 1				
Yes	264 (37.7)	16 (6.1)	1.16 (0.60,2.24)	0.66
No	436 (62.3)	23 (5.3)	1	
Fever reported at yr 1				
Yes	550 (78.6)	33 (6.0)	1.53 (0.63,3.73)	0.34
No	150 (21.4)	6 (4.0)	1	
Exclusive breast feeding at 2 mo				
Yes	592 (84.7)	31 (5.2)	0.79 (0.34,1.84)	0.58
No	107 (15.3)	7 (6.5)	1	
Vaccination at 2 mo				
Yes	415 (59.4)	20 (4.8)	0.75 (0.39,1.44)	0.38
No	284 (40.6)	18 (6.3)	1	
Insecticide use in the home				
Yes	581 (83.0)	36 (6.2)	2.55 (0.77,8.46)	0.11
No	119 (17.0)	3 (2.5)	1	
Household size				0.33
1-3	81 (11.6)	6 (7.4)	1	0.70†
4-6	403 (57.6)	18 (4.5)	0.58 (0.22,1.52)	
7+	216 (30.9)	15 (6.9)	0.93 (0.35,2.50)	

Table 5.4 (Continued)

Variables	Eczema never up to age 3 (N=700)			
	Overall N (%)	n (%)new eczema	Crude OR (95% CI)	P-value
No of older siblings				0.84
0	94 (13.4)	4 (4.3)	1	0.68†
1-3	397 (56.7)	23 (5.8)	1.38 (0.47,4.11)	
4-10	209 (29.9)	12 (5.7)	1.37 (0.43,4.38)	
Child's sleeping place				0.43
Bed/platform	52 (7.4)	1 (1.9)	1	
Floor	309 (44.2)	16 (5.2)	2.78 (0.36,21.6)	
Grass matting	338 (48.4)	21 (6.2)	3.38 (0.44,25.8)	
Indoor cooking				
Yes	568 (81.1)	34 (6.0)	1.62 (0.62,4.22)	0.32
No	132 (18.9)	5 (3.8)	1	
Indoor kerosene use				
Yes	77 (11.0)	3 (3.9)	0.66 (0.20,2.20)	0.50
No	623 (89.0)	36 (5.8)	1	
Smoking in the house at yr 3				
Yes	89 (12.7)	3 (3.4)	0.56 (0.17,1.85)	0.33
No	611 (87.3)	36 (5.9)	1	
Antibiotic use at yr 3				
Yes	200 (28.6)	16 (8.0)	1.80 (0.93,3.50)	0.08
No	500 (71.4)	23 (4.6)	1	
Type of roof				
Thatched roof	542 (77.4)	32 (5.9)	1.35 (0.59,3.13)	0.48
corrugated iron sheet	158 (22.6)	7 (4.4)	1	

† p value for trend

Table 5.5 Distribution of potential confounders in the first year of life in relation to incident rhinitis between ages 3 and 5

Variables	Rhinitis never at the age of 3 (N=798)			
	Overall N (%)	n (%)new rhinitis	Crude OR (95% CI)	p-value
Residence				
Urban	99 (12.4)	2 (2.0)	0.48 (0.11,2.03)	0.31
Rural	699 (87.6)	29 (4.2)	1	
Child's gender				
Male	406 (50.9)	18 (4.4)	1.35 (0.65,2.80)	0.41
Female	392 (49.1)	13 (3.3)	1	
Maternal education				
Formal	146 (18.3)	3 (2.1)	0.47 (0.14,1.56)	0.21
Informal	652 (81.7)	28 (4.3)	1	
Low birth weight				
Low birth weight	39 (7.2)	1 (2.6)	0.71 (0.09,5.48)	0.74
Normal	504 (92.8)	18 (3.6)		
Cough reported at yr 1				
Yes	485 (60.8)	22 (4.5)	1.60 (0.73,3.54)	0.24
No	313 (39.2)	9 (2.9)		
Fast breathing at yr 1				
Yes	296 (37.1)	14 (4.7)	1.42 (0.69,2.92)	0.34
No	502 (62.9)	17 (3.4)	1	
Fever reported at yr 1				
Yes	625 (78.3)	29 (4.6)	4.16 (0.98,17.70)	0.04
No	173 (21.7)	2 (1.2)	1	
Exclusive breast feeding at 2 mo				
Yes	670 (84.3)	26 (3.9)	0.97 (0.36,2.57)	0.95
No	125 (15.7)	5 (4.0)		
Vaccination at 2 mo				
Yes	470 (59.1)	17 (3.6)	0.83 (0.40,1.72)	0.62
No	325 (40.9)	14 (4.3)	1	
Parental allergic history				
Yes	48 (6.0)	3 (6.3)	1.71 (0.50,5.88)	0.38
No	750 (94.0)	28 (3.7)	1	
Insecticide use in the home				
Yes	647 (83.3)	22 (3.4)	0.54 (0.23,1.24)	0.14
No	130 (16.7)	8 (6.2)	1	

Table 5.5 (Continued)

Variables	Rhinitis never at the age of 3 (N=798)			
	Overall N (%)	n (%)new rhinitis	Crude OR (95% CI)	p-value
Household size				0.17
1-3	94 (12.1)	4 (4.3)	1	0.23†
4-6	435 (56.0)	12 (2.8)	0.64 (0.20,2.03)	
7+	248 (31.9)	14 (5.7)	1.35 (0.43,4.21)	
No of older siblings				0.17
0	105 (13.5)	3 (2.9)	1	0.10†
1-3	431 (55.5)	13 (3.0)	1.06 (0.30,3.78)	
4-10	241 (31.0)	14 (5.8)	2.10 (0.59,7.49)	
Child's sleeping place				0.13
Bed/platform	61 (7.9)	2 (3.3)	1	
Floor	329 (42.4)	18 (5.5)	1.71 (0.38,7.58)	
Grass matting	386 (49.7)	10 (2.6)	0.78 (0.17,3.68)	
Indoor cooking				
Yes	630 (81.1)	24 (3.8)	0.93 (0.37,2.32)	0.88
No	147 (18.9)	6 (4.1)	1	
Indoor kerosene use				
Yes	87 (11.2)	5 (5.8)	1.62 (0.60,4.36)	0.33
No	690 (88.8)	25 (3.6)	1	
Smoking in the house at yr 3				
Yes	99 (12.4)	4 (4.0)	1.05 (0.36,3.06)	0.93
No	699 (87.6)	27 (3.9)	1	
Antibiotic use at yr 3				
Yes	225 (28.2)	9 (4.0)	1.04 (0.47,2.30)	0.92
No	573 (71.8)	22 (3.8)	1	
Type of roof				
Thatched roof	609 (78.0)	24 (3.9)	1.14 (0.46,2.82)	0.79
corrugated iron sheet	172 (22.0)	6 (3.5)	1	

† p value for trend

Table 5.6 Distribution of potential confounders in the first year of life in relation to incident sensitization between ages 3 and 5

Variables	Not sensitized up to age 3 (N=766)			
	Overall N (%)	n (%) new sensitization	Crude OR (95% CI)	p-value
Residence				
Urban	89 (11.6)	1 (1.1)	0.54 (0.07,4.15)	0.55
Rural	677 (88.4)	14 (2.1)	1	
Child's gender				
Male	390 (50.9)	8 (2.1)	1.10 (0.40,3.08)	0.85
Female	376 (49.1)	7 (1.9)	1	
Maternal education				
Formal	146 (19.1)	2 (1.4)	0.65 (0.14,2.91)	0.57
Informal	620 (80.9)	13 (2.1)	1	
Cough reported at yr 1				
Yes	474 (62.0)	8 (1.7)	0.70 (0.25,1.94)	0.49
No	291 (38.0)	7 (2.4)	1	
Fast breathing at yr 1				
Yes	283 (37.0)	6 (2.1)	1.14 (0.40,3.24)	0.80
No	483 (63.1)	9 (1.9)	1	
Fever reported at yr 1				
Yes	607 (79.4)	9 (1.5)	0.38 (0.13,1.09)	0.06
No	158 (20.7)	6 (3.8)	1	
Exclusive breast feeding at 2 mo				
Yes	648 (84.9)	11 (1.7)	0.64 (0.18,2.35)	0.50
No	115 (15.1)	3 (2.6)	1	
Vaccination at 2 mo				
Yes	448 (58.7)	8 (1.8)	0.94 (0.32,2.73)	0.90
No	315 (41.3)	6 (1.9)	1	
Parental allergic history				
Yes	49 (6.4)	1 (2.0)	1.05 (0.13,8.13)	0.97
No	717 (93.6)	14 (2.0)	1	
Insecticide use in the home				
Yes	617 (82.9)	14 (2.3)	2.93 (0.38,22.52)	0.28
No	127 (17.1)	1 (0.8)	1	
Household size				0.46
1-3	88 (11.8)	1 (1.1)	1	0.23†
4-6	415 (55.8)	7 (1.7)	1.49 (0.18,12.32)	
7+	241 (32.4)	7 (2.9)	2.60 (0.31,21.59)	

Table 5.6 (continued)

Variables	Not sensitized up to age 3 (N=766)			
	Overall N (%)	n (%) new sensitization	Crude OR (95% CI)	p-value
No of older siblings				0.76
0	98 (13.2)	2 (2.0)	1	0.62†
1-3	411 (55.2)	7 (1.7)	0.83 (0.17,4.07)	
4-10	235 (31.6)	6 (2.8)	1.26 (0.25,6.36)	
Child's sleeping place				0.96
Bed/platform	57 (7.7)	1 (1.8)	1	
Floor	315 (42.3)	6 (1.9)	1.09 (0.13,9.23)	
Grass matting	372 (50.0)	8 (2.2)	1.23 (0.15,10.05)	
Indoor cooking				
Yes	607 (81.6)	12 (2.0)	0.90 (0.25,3.24)	0.87
No	137 (18.4)	3 (2.2)	1	
Indoor kerosene use				
Yes	81 (10.9)	1 (1.2)	0.58 (0.08,4.47)	0.60
No	663 (89.1)	14 (2.1)	1	
Smoking in the house at yr 3				
Yes	102 (13.4)	2 (2.0)	1.00 (0.22,4.49)	0.99
No	662 (86.7)	13 (2.0)	1	
Antibiotic use at yr 3				
Yes	221 (28.9)	4 (1.8)	0.89 (0.28,2.83)	0.85
No	543 (71.1)	11 (2.0)	1	
Type of roof				
Thatched roof	584 (78.0)	12 (2.2)	1.86 (0.41,8.32)	0.41
corrugated iron sheet	165 (22.0)	2 (1.2)	1	

† p value for trend

5.2.5.2 Early life symptoms of respiratory tract infections and association with incident wheeze, eczema, and rhinitis

Table 5.7 shows the distribution of the potential confounders, early life symptoms of respiratory infections (cough, fever and fast breathing), and their association with incident wheeze, eczema and rhinitis between ages three and five. Incident wheeze was significantly increased in those who reported cough (OR, 3.11; 95% CI, 1.40, 6.87, $p<0.01$), fever (OR, 6.44; 95% CI, 1.52, 27.27, $p<0.01$), and fast breathing (OR, 2.46; 95% CI, 1.29, 4.70, $p<0.01$) in the first year of life (Table 5.7). These symptoms of respiratory tract infections in the first year of life were all positively related, though not significantly so, with incident eczema (Table 5.7). Only reported fever at year one was significantly associated with incident rhinitis (OR, 4.16; 95% CI, 0.98, 17.70, $p=0.04$); the symptoms of cough and fast breathing were associated with slightly increased risks of incident rhinitis although ORs were not significant (Table 5.7).

Table 5.7 Symptoms of respiratory tract infections in the first year of life in relation to incident wheeze, eczema and rhinitis

Variables	Wheeze never up to age 3 (N=676)			
	Overall N (%)	n (%)new wheeze	Crude OR (95% CI)	P-value
Cough reported at yr 1				
Yes	390 (57.7)	32 (8.2)	3.11 (1.40,6.87)	<0.01
No	286 (42.3)	8 (2.8)	1	
Fast breathing at yr 1				
Yes	218 (32.3)	21 (9.6)	2.46 (1.29,4.70)	<0.01
No	458 (67.8)	19 (4.2)	1	
Fever reported at yr 1				
Yes	513 (75.9)	38 (7.4)	6.44 (1.52,27.27)	<0.01
No	163 (24.1)	2 (1.2)	1	
Variables	Eczema never up to age 3 (N=700)			
	Overall N (%)	n (%)new eczema	Crude OR (95% CI)	P-value
Cough reported at yr 1				
Yes	436 (62.3)	26 (6.0)	1.22 (0.62,2.44)	0.56
No	264 (37.7)	13 (4.9)	1	
Fast breathing at yr 1				
Yes	264 (37.7)	16 (6.1)	1.16 (0.60,2.24)	0.66
No	436 (62.3)	23 (5.3)	1	
Fever reported at yr 1				
Yes	550 (78.6)	33 (6.0)	1.53 (0.63,3.73)	0.34
No	150 (21.4)	6 (4.0)	1	
Variables	Rhinitis never at the age of 3 (N=798)			
	Overall N (%)	n (%)new rhinitis	Crude OR (95% CI)	p-value
Cough reported at yr 1				
Yes	485 (60.8)	22 (4.5)	1.60 (0.73,3.54)	0.24
No	313 (39.2)	9 (2.9)	1	
Fast breathing at yr 1				
Yes	296 (37.1)	14 (4.7)	1.42 (0.69,2.92)	0.34
No	502 (62.9)	17 (3.4)	1	
Fever reported at yr 1				
Yes	625 (78.3)	29 (4.6)	4.16 (0.98,17.70)	0.04
No	173 (21.7)	2 (1.2)	1	

Early life symptoms of respiratory tract infections and association with sensitization Table 5.8 shows associations between symptoms of respiratory tract infections as measured in the first year of life in the disease-free cohort at age three (negative skin tests at age 3), and incident sensitization between ages three and five. Reported fever at year one was negatively associated (borderline significant) with new sensitization (OR, 0.38; 95% CI, 0.13, 1.09, p=0.06), however symptoms of cough, and fast breathing were not significantly associated with sensitization (Table 5.8).

Table 5.8 Symptoms of respiratory tract infections in the first year of life in relation to incident sensitization

Variables	Not sensitized at age 3 (N=766)			
	Overall N (%)	n (%) new sensitization	Crude OR (95% CI)	p-value
Cough reported at yr 1				
Yes	474 (62.0)	8 (1.7)	0.70 (0.25,1.94)	0.49
No	291 (38.0)	7 (2.4)	1	
Fast breathing at yr 1				
Yes	283 (37.0)	6 (2.1)	1.14 (0.40,3.24)	0.80
No	483 (63.1)	9 (1.9)	1	
Fever reported at yr 1				
Yes	607 (79.4)	9 (1.5)	0.38 (0.13,1.09)	0.06
No	158 (20.7)	6 (3.8)	1	

5.2.6 Longitudinal associations between paracetamol use and incidence of allergic outcomes

5.2.6.1 Paracetamol exposure up to age three

Paracetamol use in the first three years of life was commonly reported, with 18% reported use at age one but not three, 23% at age three but not one and 21% at both time points. In terms of early life paracetamol dose, almost 7% of the children were classified as heavily exposed (use in past month at both time points), and 33% of them were exposed to medium dose (use in past month at age one or three).

5.2.6.2 Incident wheeze in relation to paracetamol use

In univariate analysis, incident wheeze was increased in relation to persistent exposure (reported use at ages one and three), (crude OR, 95% CI, 2.25; 0.98, 5.18), and exposure at year three but not at year one (crude OR, 95% CI, 1.77; 0.79, 3.98) compared with non users, but overall significance was not reached (overall p=0.11) (Table 5.9). A significantly increased risk, however, was seen between early life paracetamol dose and incident wheeze (crude OR, 95% CI, 4.14; 1.56, 11.06 for heavy exposure compared to low exposure in past month, overall p=0.04, p trend=0.06) (Table 5.9).

Table 5.9 Univariate analysis of incident wheeze in relation to early life exposure to paracetamol

Exposure at ages 1 and 3	Wheeze never up to age 3 (N=676)			
	Overall N (%)	n (%)new wheeze	Crude OR (95% CI)	P-value
Early life paracetamol use*				0.11‡
Never exposed	287 (42.5)	13 (4.5)	1	
Exposed at year 1, but not at yr 3	120 (17.8)	4 (3.3)	0.73 (0.23,2.78)	
Exposed at year 3, but not at yr 1	155 (22.9)	12 (7.7)	1.77 (0.79,3.98)	
Persistently exposed	114 (16.9)	11 (9.7)	2.25 (0.98,5.18)	
Early life paracetamol dose†				0.04‡
Low exposure	436 (64.5)	23 (5.3)	1	0.06¶
Medium exposure	208 (30.8)	11 (5.3)	1.00 (0.48,2.10)	
Heavy exposure	32 (4.7)	6 (18.8)	4.14 (1.56,11.06)	

‡Overall p-value (likelihood ratio test).

¶ P value for trend, computed for dose of paracetamol use in the past month: low exposure (never reported use in the past month), medium exposure (use in past month at age one or three), heavy exposure (use in past month at both time points).

* Early life paracetamol use: refers to use prior to the disease outcomes (i.e. first three years of life).

† Early life paracetamol dose: refers to dose of exposure in past month in first three years of life prior to disease outcomes.

In multivariate analysis following adjustment for *a priori* confounders, associations were little changed. Overall, the association with the early life paracetamol use variable was not significant (p=0.18). Further adjustment for symptoms of respiratory tract infections and potential confounders in Table 5.3 brought the odds ratios for exposed at age three not one, and for persistent exposed, slightly closer to one, and the overall p value was 0.26 (Table 5.10). The positive association between early life paracetamol dose and incident wheeze remained significant with ORs little changed following control for *a priori* confounders (adjusted OR, 95% CI, 4.08; 1.51, 11.07, for heavy exposure versus low exposure in past month, overall p=0.04, and p trend=0.05) (Table

5.10). Further adjustment for infant respiratory tract infections slightly reduced the OR for heavy exposure (adjusted OR, 95% CI, 3.64; 1.34, 9.90, relative to low exposure) and there was no significant trend (p trend=0.11), but overall significance remained (p=0.05) (Table 5.10). Further adjustment for potential confounders in Table 5.3 did not materially alter the strength and the overall significance of the associations.

Table 5.10 Multivariate analysis of incident wheeze in relation to early life use of paracetamol

Exposure at ages 1 and 3	Wheeze never up to age 3 (N=676)			
	Adjusted OR* (95% CI)	P-value	Further adjusted OR† (95% CI)	P-value
Early life paracetamol use£		0.18‡		0.26‡
Never exposed	1		1	
Exposed at year 1, but not at yr 3	0.73 (0.23,2.31)		0.62 (0.20,1.96)	
Exposed at year 3, but not at yr 1	1.63 (0.72,3.70)		1.49 (0.65,3.39)	
Persistently exposed	2.09 (0.90,4.86)		1.72 (0.74,4.03)	
Early life paracetamol dose$		0.04‡		0.05‡
Low exposure	1	0.05¶	1	0.11¶
Medium exposure	1.04 (0.50,2.19)		0.92 (0.44,1.95)	
Heavy exposure	4.08 (1.51,11.07)		3.64 (1.34,9.90)	

*ORs adjusted for child's gender, area of residence and maternal education.

†ORs adjusted for child's gender, area of residence and maternal education and additionally adjusted for symptoms of respiratory infections in the first year of life.

‡Overall p-value (likelihood ratio test).

¶ P value for trend computed for dose of paracetamol use in the past month: low exposure (never reported use in the past month), medium exposure (use in past month at age one or three), heavy exposure (use in past month at both time points).

£Early life paracetamol use: refers to use prior to the disease outcomes (i.e. first three years of life).

$Early life paracetamol dose: refers to dose of exposure in past month in first three years of life prior to disease outcomes

5.2.6.3 Incident eczema in relation to paracetamol use

In the univariate analysis, early life paracetamol use was significantly associated with incident eczema between ages three and five such that ORs for all exposed groups were significantly increased relative to the never exposed (overall $p<0.01$) (Table 5.11). The highest OR was in relation to persistent exposure (crude OR, 95% CI, 4.17; 1.53, 11.36), and almost equally high for exposure at year three but not at year one (OR=3.94; 95% CI 1.47, 10.58), and exposure at year one but not year three (OR=3.12; 95% CI 1.06, 9.19) compared with never users, overall $p<0.01$) (Table 5.11). The early life paracetamol dose variable was also associated significantly with increased risk of incident eczema (crude OR, 95% CI, 1.73; 0.49, 6.19 for heavy dose exposure, 2.42; 1.23, 4.77 for medium dose exposure, overall $p=0.04$, p trend=0.04), with higher effects seen for medium exposure than heavy (Table 5.11).

Table 5.11 Univariate analysis of incident eczema in relation to early life use of paracetamol

Exposure at ages 1 and 3	Eczema never up to age 3 (N=700)			
	Overall N (%)	n (%)new eczema	Crude OR (95% CI)	P-value
Early life paracetamol use*				<0.01‡
Never exposed	275 (39.3)	6 (2.2)	1	
Exposed at year 1, but not at yr 3	123 (17.6)	8 (6.5)	3.12 (1.06,9.19)	
Exposed at year 3, but not at yr 1	161 (23.0)	13 (8.1)	3.94 (1.47,10.58)	
Persistently exposed	141 (20.1)	12 (8.5)	4.17 (1.53,11.36)	
Early life paracetamol dose†				0.04‡
Low exposure	423 (60.4)	16 (3.8)	1	0.04¶
Medium exposure	230 (32.9)	20 (8.7)	2.42 (1.23,4.77)	
Heavy exposure	47 (6.7)	3 (6.4)	1.73 (0.49,6.19)	

‡Overall p-value (likelihood ratio test).

¶ P value for trend computed for dose of paracetamol use in the past month: low exposure (never reported use in the past month), medium exposure (use in past month at age one or three), heavy exposure (use in past month at both time points).

* Early life paracetamol use: refers to use prior to the disease outcomes (i.e. first three years of life).

† Early life paracetamol dose: refers to dose of exposure in past month in first three years of life prior to disease outcomes.

After adjusting for *a priori* confounders, paracetamol use in the first three years of life was still significantly associated with an increased risk of incident eczema (overall p=0.01;Table 5.12). The odds ratio was still highest in the persistently exposed category, (adjusted OR, 3.96; 95% CI, 1.44, 10.89) and still significantly increased in those exposed at age three but not one (adjusted OR 3.75; 95% CI, 1.39, 10.12) and in those exposed at age one but not age three (adjusted OR 3.11; 95% CI, 1.05, 9.18) compared with those never exposed (Table 5.12). Adjustment for respiratory tract infections only marginally reduced the strength of the associations, and overall statistical significance remained (overall p=0.02) (Table 5.12). Control for *a priori* confounders had very little

effect on the associations with early life paracetamol dose, with ORs little changed and overall significance unchanged (p=0.04) (Table 5.12). Further adjustment for symptoms of respiratory tract infections in the first year of life slightly reduced the strength of the associations, and overall non-significant (p=0.08) (Table 5.12).

Further adjustment for demographic, environmental and lifestyle variables in the first years of life, in Table 5.4, made little change to the odds ratio or the level of significance (Table 5.12).

Table 5.12 Multivariate analysis of incident eczema in relation to early life paracetamol use

Exposure at age 1 and 3	Eczema never up to age 3 (N=700)			
	Adjusted OR* (95% CI)	P-value	Further adjusted OR† (95% CI)	P-value
Early life paracetamol use£		0.01‡		0.02‡
Never exposed	1		1	
Exposed at year 1, but not at yr 3	3.11 (1.05,9.18)		3.01 (1.00,9.04)	
Exposed at year 3, but not at yr 1	3.75 (1.39,10.12)		3.70 (1.37,10.01)	
Persistently exposed	3.96 (1.44,10.89)		3.82 (1.36,10.73)	
Early life paracetamol dose$		0.04‡		0.08‡
Low exposure	1	0.05¶	1	0.06¶
Medium exposure	2.39 (1.21,4.71)		2.31 (1.16,4.60)	
Heavy exposure	1.65 (0.46,5.93)		1.59 (0.44,5.74)	

*ORs adjusted for child's gender, area of residence and maternal education

†ORs adjusted for child's gender, area of residence and maternal education and additionally adjusted for symptoms of respiratory infections in the first year of life

‡Overall p-value (likelihood ratio test)

¶ P value for trend computed for dose of paracetamol use in the past month: low exposure (never reported use in the past month), medium exposure (use in past month at age one or three), heavy exposure (use in past month at both time points).

£Early life paracetamol use: refers to use prior to the disease outcomes (i.e. first three years of life).

$Early life paracetamol dose: refers to dose of exposure in past month in first three years of life prior to disease outcomes

5.2.6.4 Incident rhinitis in relation to paracetamol use

In the univariate analysis, early life paracetamol use was also associated with an increased risk of incident rhinitis between ages three and five. The magnitude of the odds ratios, all compared with never users, were higher for heavily exposed children (crude OR, 95% CI, 3.77; 1.24, 11.44) and those exposed at age three but not age one (OR, 95% CI, 4.04; 1.38, 11.82) than exposure at age one but not age three (OR, 95% CI, 2.85; 0.86, 9.52), (overall p=0.03; Table 5.13).

There was also a significant positive trend between paracetamol dose and incident rhinitis (crude OR, 95% CI, 2.08; 0.96, 4.50 for medium exposure, and 2.85; 0.90, 9.06 for heavy exposure vs. low exposure, p trend 0.03) (Table 5.13).

Table 5.13 Univariate analysis of incident rhinitis in relation to early life use of paracetamol

Exposure at ages 1 and 3	Rhinitis never at the age of 3 (N=798)			
	Overall N (%)	n (%) new rhinitis	Crude OR (95% CI)	P-value
Early life paracetamol use*				0.03‡
Never exposed	319 (40.0)	5 (1.6)	1	
Exposed at year 1, but not at yr 3	138 (17.3)	6 (4.4)	2.85 (0.86,9.52)	
Exposed at year 3, but not at yr 1	182 (22.8)	11 (6.0)	4.04 (1.38,11.82)	
Persistently exposed	159 (19.9)	9 (5.7)	3.77 (1.24,11.44)	
Early life paracetamol dose†				0.09‡
Low exposure	485 (60.8)	13 (2.7)	1	0.03¶
Medium exposure	258 (32.3)	14 (5.4)	2.08 (0.96,4.50)	
Heavy exposure	55 (6.9)	4 (7.3)	2.85 (0.90,9.06)	

‡Overall p-value (likelihood ratio test).

¶ P value for trend computed for dose of paracetamol use in the past month: low exposure (never reported use in the past month), medium exposure (use in past month at age one or three), heavy exposure (use in past month at both time points).

* Early life paracetamol use: refers to use prior to the disease outcomes (i.e. first three years of life).

† Early life paracetamol dose: refers to dose of exposure in past month in first three years of life prior to disease outcomes.

Adjusting for the *a priori* confounders made little change on the ORs for early life paracetamol use and overall significance remained (overall p=0.04) (Table 5.14). Further adjustment for symptoms of respiratory tract infections in the first year of life also reduced the odds ratios (adjusted OR, 95% CI, 3.10; 1.00,

9.57 for persistent exposure, 3.74; 1.27, 11.04 for exposure at age three but not one and 2.42; 0.72, 8.14 for exposure at age one but not three) compared to never users, and overall significance became borderline (overall p=0.07) (Table 5.14).

Following adjustment for *a priori* confounders, the significant dose-dependent trend between early life paracetamol use and incident rhinitis persisted (p trend=0.04) with little change to the ORs (Table 5.14). Further adjustment for respiratory tract infections, however, slightly reduced the strength of associations, and the significant trend became borderline (p for trend=0.07) (Table 5.14). Further adjustment for various potential confounders presented in Table 5.5 did not alter the odds ratio materially (Table 5.14).

Table 5.14 Multivariate analysis of incident rhinitis in relation to paracetamol exposure early in life

Exposure at ages 1 and 3	Rhinitis never at the age of 3 (N=798)			
	Adjusted OR* (95% CI)	P-value	Further adjusted OR† (95% CI)	P-value
Early life paracetamol use£		0.04‡		0.07‡
Never exposed	1		1	
Exposed at year 1, but not at yr 3	2.75 (0.82,9.20)		2.42 (0.72,8.14)	
Exposed at year 3, but not at yr 1	3.96 (1.35,11.65)		3.74 (1.27,11.04)	
Persistently exposed	3.60 (1.18,11.03)		3.10 (1.00,9.57)	
Early life paracetamol dose$		0.10‡		0.18‡
Low exposure	1	0.04¶	1	0.07¶
Medium exposure	2.07 (0.95,4.47)		1.90 (0.87,4.12)	
Heavy exposure	2.61 (0.82,8.39)		2.31 (0.72,7.46)	

*ORs adjusted for child's gender, area of residence and maternal education

†ORs adjusted for child's gender, area of residence and maternal education and additionally adjusted for symptoms of respiratory infections in the first year of life

‡Overall p-value (likelihood ratio test)

¶ P value for trend computed for dose of paracetamol use in the past month: low exposure (never reported use in the past month), medium exposure (use in past month at age one or three), heavy exposure (use in past month at both time points).

£Early life paracetamol use: refers to use prior to the disease outcomes (i.e. first three years of life).

$Early life paracetamol dose: refers to dose of exposure in past month in first three years of life prior to disease outcomes

5.2.6.5 Incident sensitization in relation to paracetamol use

In univariate analysis, the effect of early life use of paracetamol on incident sensitization was not significant, with a non-significant increased risk seen in those exposed persistently compared to never users (crude OR, 95% CI, 1.78; 0.51, 6.25). However, the ORs for exposure at age three but not one (crude OR, 95% CI, 0.99; 0.23, 4.19) and exposure at age one but not three (crude OR,

95% CI, 0.87; 0.17, 4.54) were slightly closer to 1, and the overall p value was 0.76 (Table 5.15). Similarly, there was no significant dose-dependent association between early life paracetamol dose and incident sensitization, though higher odds ratios were found for the heavily exposed category (crude OR, 95% CI, 2.11; 0.44, 10.18) than for the medium exposed category (crude OR, 95% CI, 1.06; 0.34, 3.26) compared to low exposure, overall p=0.69, and p trend=0.48) (Table 5.15).

Table 5.15 Univariate analysis of sensitization in relation to paracetamol exposure early in life

Exposure at ages 1 and 3	Not sensitized up to age 3 (N=766)			
	Overall N (%)	n (%) new atopy	Crude OR (95% CI)	P-value
Early life paracetamol dose[†]				0.76[‡]
Never exposed	290 (38.0)	5 (1.7)	1	
Exposed at year 1, but not at yr 3	133 (17.4)	2 (1.5)	0.87 (0.17,4.54)	
Exposed at year 3, but not at yr 1	176 (23.0)	3 (1.7)	0.99 (0.23,4.19)	
Persistently exposed	165 (21.6)	5 (3.0)	1.78 (0.51,6.25)	
Early life paracetamol dose[†]				0.69[‡]
Low exposure	446 (58.4)	8 (1.8)	1	0.48[¶]
Medium exposure	264 (34.6)	5 (1.9)	1.06 (0.34,3.26)	
Heavy exposure	54 (7.1)	2 (3.7)	2.11 (0.44,10.18)	

[‡]Overall p-value (likelihood ratio test).

[¶] P value for trend computed for dose of paracetamol use in the past month: low exposure (never reported use in the past month), medium exposure (use in past month at age one or three), heavy exposure (use in past month at both time points).

[*] Early life paracetamol use: refers to use prior to the disease outcomes (i.e. first three years of life).

[†] Early life paracetamol dose: refers to dose of exposure in past month in first three years of life prior to disease outcomes.

In multivariate analyses adjusting for *a priori* confounders, the ORs for early life paracetamol use changed little (overall p=0.78) (Table 5.16). On further adjusted analysis for symptoms of respiratory tract infection at year one, the odds ratios increased for all exposure categories, in the expected direction, with higher effects seen for persistent exposure (further adjusted OR, 95% CI, 2.48; 0.63, 9.76) than exposure at age one or three alone. The confidence intervals however were wide and significance was not reached (overall p=0.59) (Table 5.16).

The ORs for early life paracetamol dose were also changed little on adjustment for *a priori* confounders, and the overall p was 0.75 (Table 5.16). Adjustment for respiratory tract infections markedly increased the odds ratio for both heavy (further adjusted OR, 95% CI, 2.59; 0.50, 13.29) and medium exposure categories (further adjusted OR, 95% CI, 1.26; 0.40, 4.04) compared to low, but the association remained non-significant (overall p=0.57, and p trend=0.31) (Table 5.16).

Adjustment for potential confounders in Table 5.6 did not alter the strength of the associations, or the level of significance (Table 5.16).

Table 5.16 Multivariate analysis of sensitization in relation to paracetamol exposure early in life

Exposure at ages 1 and 3	Not sensitized up to age 3 (N=766)			
	Adjusted OR* (95% CI)	P-value	Further adjusted OR† (95% CI)	P-value
Early life paracetamol use£		0.78‡		0.59‡
Never exposed	1		1	
Exposed at year 1, but not at yr 3	0.85 (0.16,4.42)		1.13 (0.21,6.23)	
Exposed at year 3, but not at yr 1	0.98 (0.23,4.17)		1.09 (0.25,4.70)	
Persistently exposed	1.73 (0.49,6.11)		2.48 (0.63,9.76)	
Early life paracetamol dose$		0.72‡		0.57‡
Low exposure	1	0.51¶	1	0.31¶
Medium exposure	1.05 (0.34,3.26)		1.26 (0.40,4.04)	
Heavy exposure	1.99 (0.41,9.70)		2.59 (0.50,13.29)	

*ORs adjusted for child's gender, area of residence and maternal education

†ORs adjusted for child's gender, area of residence and maternal education and additionally adjusted for symptoms of respiratory infections in the first year of life

‡Overall p-value (likelihood ratio test)

¶ P value for trend computed for dose of paracetamol use in the past month: low exposure (never reported use in the past month), medium exposure (use in past month at age one or three), heavy exposure (use in past month at both time points).

£Early life paracetamol use: refers to use prior to the disease outcomes (i.e. first three years of life).

$Early life paracetamol dose: refers to dose of exposure in past month in first three years of life prior to disease outcomes

5.2.7 Cross-sectional analyses between paracetamol and allergic outcomes at age five

5.2.7.1 Prevalence of wheeze, eczema, rhinitis and sensitization at age five

Wheeze was reported at age five in 4.3% (37/852) of children, eczema in 4.2% (36/852), and rhinitis in 3.6% (31/852). Sensitization to any allergen (*D. pteronyssinus* and cockroach allergen) was found in 2.0% (17/855) of children at age five (Table 5.17). Only one child was sensitized to cockroach allergen, and merging was necessary with *D. Pteronyssinus* sensitization (Table 5.17). Wheeze was reported slightly more frequently in urban (4.8%) than rural (4.3%) areas, whilst eczema (3.8% in urban vs. 4.3% in rural), rhinitis (2.9% in urban vs. 3.8% in rural), and sensitization (1.9% in urban vs. 2.0% in rural) were more likely to be reported in rural than urban children. However, none of these differences were statistically significant (Table 5.17).

Table 5.17 Prevalence of allergic symptoms and sensitization by area of residence

Outcomes	Over all N (%)	Urban n (%)	Rural n (%)	Crude OR (95%CI)	P-value
Wheeze	37 (4.3)	5 (4.8)	32 (4.3)	1.12 (0.43,2.94)	0.82
Eczema	36 (4.2)	4 (3.8)	32 (4.3)	0.88 (0.31,2.56)	0.82
Rhinitis	31 (3.6)	3 (2.9)	28 (3.8)	0.76 (0.23,2.53)	0.65
Any sensitization[†]	17 (2.0)	2 (1.9)	15 (2.0)	0.96 (0.22,4.27)	0.96

N=852
[†]Sensitization to either *D. pteronyssinus* or cockroach allergen (N=855)

Figure 5.5 below summarises the prevalence of allergic symptoms and sensitization between age one and five and shows that the prevalence of each of the reported outcomes, and sensitization gradually decreased as the child grew up (Figure 5.5).

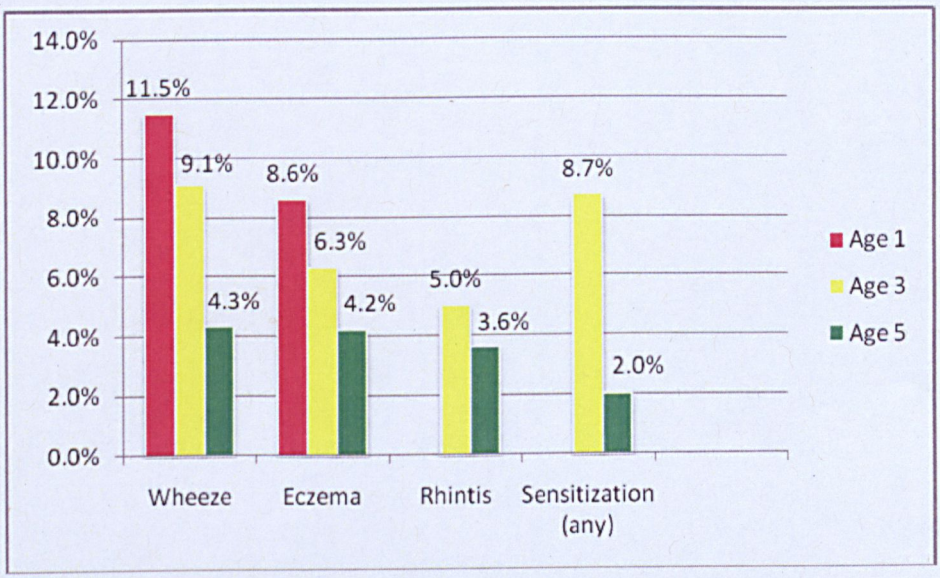

Figure 5.5 Prevalence of wheeze and allergic outcomes by age

5.2.7.2 Cross-sectional associations between allergic symptoms and sensitization at age five

Sensitization at age five increased the risk of reported eczema and rhinitis (OR, 95% CI, 3.11; 0.68, 14.21, p=0.12 for eczema, and 3.67; 0.80, 16.88, p=0.07 for rhinitis), and reached borderline significance for rhinitis but not eczema. No children with wheeze were sensitized, and therefore ORs could not be computed or further analysis performed (Table 5.18).

Table 5.18 OR for wheeze and allergic outcomes in relation to sensitization at age five

Reported symptoms*	Any sensitization (N=855)†			
	Over all N (%)	Yes n (%)	Crude OR (95%CI)	P-value
Wheeze	17 (2.0)	0 (0.0)	-	-
Eczema	17 (2.0)	2 (11.7)	3.11 (0.68,14.21)	0.12
Rhinitis	17 (2.0)	2 (11.8)	3.67 (0.80,16.88)	0.07

†Sensitization to either *D. pteronyssinus* or cockroach allergen

*2 missing data on reported symptoms outcome

5.2.7.3 Distribution of potential confounders and allergic symptoms and sensitization at age five

The distribution of potential confounders measured at age five and their association with the prevalence of allergic symptoms and sensitization are shown in Table 5.19 to Table 5.22. The *a priori* confounders residence, child's gender and maternal education were not significantly associated with wheeze, eczema, rhinitis or sensitization. Maternal history of allergy was significantly associated with an increased risk of the reported outcome sensitization (OR, 95% CI, 5.37; 2.37, 12.18, p<0.01 for wheeze, 5.58; 2.45, 12.69, p<0.01 for eczema, 6.89; 2.96, 16.04, p<0.01 for rhinitis), and a borderline significant

increased risk of sensitization (OR, 95% CI, 3.26; 0.91, 11.77, p=0.06) (Table 5.19 to Table 5.22). Similarly, paternal history of allergy increased the risks of all outcomes, significantly so for eczema (OR, 95% CI, 5.91; 2.38, 14.67, p<0.01), and rhinitis (OR, 95% CI, 4.45; 1.60, 12.39, p<0.01) (Table 5.19 to Table 5.22). Child's use of antibiotic also increased the risks of wheeze, eczema, rhinitis and sensitization, and reached statistical significance for eczema, and borderline significance for rhinitis (OR, 95% CI, 2.47; 1.16, 5.28, p=0.02 for eczema, and 2.20; 0.96, 5.06, p=0.06 for rhinitis) (Table 5.19 to Table 5.22). The other potential confounders measured at year five including household size, siblingship, child's sleeping place, insecticide use at home, indoor cooking and charcoal use, smoking in the residential house, and type of roof were not significantly associated with any of the outcomes (Table 5.19 to Table 5.22).

Table 5.19 Distribution of potential confounders measured at the age of five in relation to reported wheeze at year five

Variables	Overall N (%)	Wheeze Yes n (%)	**Wheeze** Crude OR (95% CI)	P-value
Residence*				
Urban	105 (12.3)	5 (4.8)	1.12 (0.43,2.94)	0.82
Rural	747 (87.9)	32 (4.3)	1	
Child's gender*				
Male	434 (50.9)	23 (5.3)	1.61(0.82,3.19)	0.16
Female	418 (49.1)	14 (3.4)	1	
Maternal education*				
Formal	160 (18.8)	7 (4.4)	1.01 (0.45,2.34)	0.98
Informal	692 (81.2)	30 (4.3)	1	
Maternal history of allergy				
Yes	55 (6.5)	9 (16.4)	5.37 (2.37,12.18)	<0.01
No	797 (93.5)	28 (3.5)	1	
Paternal history of allergy				
Yes	39 (4.6)	3 (7.7)	1.90 (0.56,6.52)	0.29
No	813 (95.4)	34 (4.2)	1	
Household size				
1-6	484 (56.8)	22 (4.6)	1	0.74
7+	368 (43.2)	15 (4.1)	0.89 (0.46,1.75)	
No of older siblings				0.94
0	122 (14.3)	6 (4.9)	1	0.74‡
1-3	463 (54.3)	20 (4.3)	0.87 (0.34,2.23)	
4-10	267 (31.3)	11 (4.1)	0.83 (0.30,2.30)	
Child's sleeping place				0.19
Bed	44 (5.2)	1 (2.3)	1	
Mattress	198 (23.2)	13 (6.6)	3.02 (0.38,23.95)	
Grass matting	610 (71.6)	23 (3.8)	1.68 (0.22,12.80)	
Insecticide use in the home				
Yes	570 (66.9)	24 (4.2)	0.91 (0.46,1.82)	0.79
No	282 (33.1)	13 (4.6)	1	

Table 5.19 (continued)

Variables	Overall N (%)	Wheeze Yes n (%)	**Wheeze** Crude OR (95% CI)	P-value
Indoor cooking				
Yes	630 (73.9)	30 (4.8)	1.54 (0.66,3.55)	0.31
No	222 (26.1)	7 (3.2)	1	
Indoor charcoal use				
Yes	160 (18.8)	7 (4.4)	1.01 (0.44,2.34)	0.98
No	692 (81.2)	30 (4.3)	1	
Smoking in the house				
Yes	103 (12.1)	4 (3.9)	0.88 (0.30,2.53)	0.81
No	749 (87.9)	33 (4.4)	1	
Antibiotic use				
Yes	120 (14.1)	7 (5.8)	1.45 (0.62,3.38)	0.39
No	732 (85.9)	30 (4.1)	1	
Roof type				
Thatched roof	648 (77.8)	26 (4.0)	0.66 (0.32,1.37)	0.26
Corrugated iron sheet	185 (22.2)	11 (6.0)	1	

N=852

*Demographic variables collected at during pregnancy and at birth

ǂ p value for trend

Table 5.20 Distribution of potential confounders measured at the age of five in relation to reported eczema at year five

Variables	Overall N (%)	Eczema Yes n (%)	Eczema Crude OR (95% CI)	p-value
Residence*				
Urban	105 (12.3)	4 (3.8)	0.88 (0.31,2.56)	0.82
Rural	747 (87.7)	32 (4.3)	1	
Child's gender*				
Male	434 (50.9)	15 (3.5)	0.68 (0.34,1.33)	0.26
Female	418 (49.1)	21 (5.0)	1	
Maternal education*				
Formal	160 (18.8)	5 (3.1)	0.69 (0.26,1.80)	0.44
Informal	692 (81.2)	31 (4.5)	1	
Maternal history of allergy				
Yes	55 (6.5)	9 (16.4)	5.58 (2.45,12.69)	<0.01
No	797 (93.5)	27 (3.4)	1	
Paternal history of allergy				
Yes	39 (4.6)	7 (18.0)	5.91 (2.38,14.67)	<0.01
No	813 (95.5)	29 (3.6)	1	
Household size				
1-6	484 (56.8)	18 (3.7)	1	0.40
7+	368 (43.2)	18 (4.9)	1.33 (0.68,2.60)	
No of older siblings				0.30
0	122 (14.3)	2 (1.6)	1	0.31*
1-3	463 (54.3)	22 (4.8)	2.99 (0.69,12.96)	
4-10	267 (31.3)	12 (4.5)	2.82 (0.62,12.89)	
Child's sleeping place				0.50
Bed	44 (5.2)	1 (2.3)	1	
Mattress	198 (23.2)	11 (5.6)	2.53 (0.32,20.28)	
Grass matting	610 (71.6)	24 (3.9)	1.76 (0.23,13.36)	
Insecticide use in the home				
Yes	570 (66.9)	25 (4.4)	1.13 (0.55,2.33)	0.74
No	282 (33.1)	11 (3.9)	1	
Indoor cooking				
Yes	630 (73.9)	27 (4.3)	1.06 (0.49,2.29)	0.88
No	222 (26.1)	9 (4.1)	1	

Table 5.20 (continued)

Variables	Overall N (%)	Eczema Yes n (%)	**Eczema** Crude OR (95% CI)	p-value
Indoor charcoal use				
Yes	160 (18.8)	4 (2.5)	0.53 (0.18,1.52)	0.23
No	692 (81.2)	32 (4.6)	1	
Smoking in the house				
Yes	103 (12.1)	2 (1.9)	0.42 (0.10,1.76)	0.22
No	749 (81.9)	34 (4.5)	1	
Antibiotic use				
Yes	120 (14.1)	10 (8.3)	2.47 (1.16,5.28)	0.02
No	732 (85.9)	26 (3.6)	1	
Roof type				
Thatched roof	648 (77.8)	30 (4.6)	1.45 (0.59,3.54)	0.41
Corrugated iron sheet	185 (22.2)	6 (3.2)	1	

N=852

* Demographic variables collected at during pregnancy and at birth

‡ p value for trend

Table 5.21 Distribution of potential confounders measured at the age of five in relation to reported rhinitis at year five

Variables	Overall N (%)	Rhinitis Yes n (%)	**Rhinitis** Crude OR (95% CI)	p-value
Residence*				
Urban	105 (12.3)	3 (2.9)	0.76 (0.23,2.53)	0.65
Rural	747 (87.7)	28 (3.8)	1	
Child's gender*				
Male	434 (50.9)	18 (4.2)	1.35 (0.65,2.79)	0.42
Female	418 (49.1)	13 (3.1)	1	
Maternal education*				
Formal	160 (18.8)	6 (3.8)	1.04 (0.42,2.58)	0.93
Informal	692 (81.2)	25 (3.6)	1	
Maternal history of allergy				
Yes	55 (6.5)	9 (16.4)	6.89 (2.96,16.04)	<0.01
No	797 (93.5)	22 (2.8)	1	
Paternal history of allergy				
Yes	39 (4.6)	5 (12.8)	4.45 (1.60,12.39)	<0.01
No	813 (95.4)	26 (3.2)	1	
Household size				
1-6	484 (56.8)	13 (2.7)	1	0.09
7+	368 (43.2)	18 (4.9)	1.86 (0.90,3.86)	
No of older siblings				0.23
0	122 (14.3)	4 (3.3)	1	0.19*
1-3	463 (54.3)	13 (2.8)	0.85 (0.27,2.66)	
4-10	267 (31.3)	14 (5.2)	1.63 (0.52,5.08)	
Child's sleeping place				0.85
Bed	44 (5.2)	1 (2.3)	1	
Mattress	198 (23.2)	8 (4.0)	1.81 (0.23,14.95)	
Grass matting	610 (71.6)	22 (3.6)	1.61 (0.21,12.25)	
Insecticide use in the home				
Yes	570 (66.9)	21 (3.7)	1.04 (0.48,2.24)	0.92
No	282 (33.1)	10 (3.6)	1	
Indoor cooking				
Yes	630 (73.9)	21 (3.3)	0.73 (0.34,1.58)	0.42
No	222 (26.1)	10 (4.5)	1	

Table 5.21 (continued)

Variables	Overall N (%)	Rhinitis Yes n (%)	**Rhinitis** Crude OR (95% CI)	p-value
Indoor charcoal use				
Yes	160 (18.8)	8 (5.0)	1.53 (0.67,3.49)	0.31
No	692 (81.2)	23 (3.3)	1	
Smoking in the house				
Yes	103 (12.1)	3 (2.9)	0.77 (0.23,2.59)	0.67
No	749 (87.9)	28 (3.7)	1	
Antibiotic use				
Yes	120 (14.1)	8 (6.7)	2.20 (0.96,5.06)	0.06
No	732 (85.9)	23 (3.1)	1	
Roof type				
Thatched roof	648 (77.8)	23 (3.6)	0.94 (0.39,2.22)	0.88
Corrugated iron sheet	185 (22.2)	7 (3.8)	1	

N=852

* Demographic variables collected at during pregnancy and at birth

‡ p value for trend

Table 5.22 Distribution of potential confounders measured at the age of five in relation to sensitization at year five

Variables	Overall N (%)	Sensitization Yes n (%)	**Sensitization** Crude OR (95% CI)	P-value
Residence*				
Urban	104 (12.2)	2 (1.9)	0.96 (0.22,4.27)	0.96
Rural	751 (87.8)	15 (2.0)	1	
Child's gender*				
Male	434 (50.8)	9 (2.1)	1.09 (0.42,2.86)	0.86
Female	421 (49.2)	8 (1.9)	1	
Maternal education*				
Formal	162 (19.0)	2 (1.2)	0.57 (0.13,2.50)	0.45
Informal	693 (81.0)	15 (2.2)	1	
Maternal history of allergy				
Yes	55 (6.5)	3 (5.6)	3.26 (0.91,11.7)	0.06
No	797 (93.5)	14 (1.8)	1	
Paternal history of allergy				
Yes	38 (4.5)	2 (5.3)	2.93 (0.64,13.4)	0.14
No	807 (95.5)	15 (1.9)	1	
Household size				
1-6	479 (56.7)	10 (2.1)	1	0.86
7+	366 (43.3)	7 (1.9)	0.91 (0.34,2.43)	
No of older siblings				0.68
0	120 (14.2)	2 (1.7)	1	0.73‡
1-3	459 (54.3)	11 (2.4)	1.45 (0.32,6.64)	
4-10	266 (31.5)	4 (1.5)	0.90 (0.16,5.00)	
Child's sleeping place				
Bed/mattress	240 (28.4)	6 (2.5)	1	0.52
Grass matting	605 (71.6)	11 (1.8)	0.72 (0.26,1.98)	
Insecticide use in the home				
Yes	564 (66.8)	13 (2.3)	1.63 (0.53, 5.06)	0.39
No	281 (33.3)	4 (1.4)	1	
Indoor cooking				
Yes	624 (73.9)	12 (1.9)	0.85 (0.29,2.43)	0.76
No	221 (26.2)	5 (2.3)	1	

Table 5.22 (continued)

Variables	Overall N (%)	Sensitization Yes n (%)	**Sensitization** Crude OR (95% CI)	P-value
Indoor charcoal use				
Yes	159 (18.8)	3 (1.9)	0.92 (0.26,3.25)	0.90
No	686 (81.2)	14 (2.0)	1	
Smoking in the house				
Yes	103 (12.1)	1 (1.0)	0.44 (0.06,3.40)	0.42
No	749 (87.9)	16 (2.2)	1	
Antibiotic use				
Yes	119 (14.1)	3 (2.5)	1.32 (0.37,4.65)	0.67
No	726 (85.9)	14 (1.9)	1	
Roof type				
Thatched roof	650 (77.8)	14 (2.2)	1.34 (0.38,4.73)	0.65
Corrugated iron sheet	186 (22.3)	3 (1.6)	1	

N=855

* Demographic variables collected at during pregnancy and at birth

‡ p value for trend

5.2.7.4 Paracetamol exposure up to age five

Paracetamol use in the past 12 months at year five was reported by 305 of 848 (36%) of the children in the birth cohort. Lifetime use of paracetamol (persistent use at ages one, three and five) was reported by 11.6% of the children, and 30.4% were never users up to age five. Using the lifetime dose indicator variable, 33.7% were classified as lightly exposed, 22.4% medium exposed and 13.4% heavily exposed.

5.2.7.5 Cross-sectional association between paracetamol and wheeze

In the univariate analysis in Table 5.23, lifetime use of paracetamol (use up to

the age of five) was associated significantly and in a dose-dependent manner with increased risk of reported wheeze at age five. The odds ratio was highest in relation to persistent use (use at ages one, three and five), and that for current exposure at age five was larger than past exposure (crude OR, 95% CI, 9.71; 3.08, 30.59 for persistent exposure, 3.22; 1.00, 10.43 for current exposure with or without past exposure, and 2.31; 0.72, 7.45 for past exposure but not current, compared to never users, overall $p<0.01$) (Table 5.23). Similarly, in relation to the dose variable, children who were exposed to heavy and medium doses of paracetamol were nearly nine and four times more likely to report wheezing symptoms than never exposed groups respectively (crude OR, 95% CI, 8.89; 2.86, 27.66 for heavy exposure, 3.90; 1.22, 12.45 for medium exposure and 1.83; 0.0.54, 6.14 for light exposure) than never exposed (overall $p<0.01$ and p trend <0.01) (Table 5.23).

Table 5.23 Univariate analysis of wheeze in relation to paracetamol exposure up to the age of 5 year

Exposure at ages 1, 3 and 5 (N=852)	Overall N (%)	Wheeze N (%)	Crude OR (95% CI)	P-value
Lifetime paracetamol use*				<0.01‡
Never exposed	258 (30.4)	4 (1.6)	1	
Past exposure but not current	285 (33.6)	10 (3.5)	2.31 (0.72,7.45)	
Current exposure ± past exposure	207 (24.4)	10 (4.8)	3.22 (1.00,10.43)	
Persistent exposure	98 (11.6)	13 (13.3)	9.71 (3.08,30.59)	
Lifetime paracetamol dose†				<0.01‡
Never exposed	258 (30.4)	4 (1.6)	1	<0.01¥
Light exposure	286 (33.7)	8 (2.8)	1.83 (0.54,6.14)	
Medium exposure	190 (22.4)	11 (5.8)	3.90 (1.22,12.45)	
Heavy exposure	114 (13.4)	14 (12.3)	8.89 (2.86,27.66)	

‡Overall p-value (likelihood ratio test).

¥P value for trend as dose of paracetamol computed using a composite score of paracetamol exposure from age 1, 3, and 5 yr as: Never exposed, Light exposure, Medium exposure, and Heavy exposure.

*Lifetime paracetamol use: refers to use of paracetamol up to the age of 5 (i.e. ages 1, 3 and 5).

†Lifetime paracetamol dose: refers to dose of paracetamol exposure up to the age of 5 (i.e. ages 1, 3 and 5 yr).

In the multivariate analyses, after adjusting for *a priori* confounders, the effects of lifetime paracetamol use and dose of exposure on wheeze outcome were little changed, and remained highly statistically significant (overall p<0.01, p trend <0.01) (Table 5.24). Adjustment for respiratory tract infections reduced the magnitude of the association, particularly for persistent and current exposures, but overall significance remained (p=0.01 and p trend<0.01) (Table 5.24). Further adjustment for potential confounders as reported at year five in Table 5.19 did not materially alter the associations (Table 5.24).

Table 5.24 Multivariate analysis of wheeze in relation to paracetamol exposure up to the age of 5 years

Exposure at ages 1, 3 and 5 (N=852)	Adjusted OR* (95% CI)	P-value	Further adjusted OR† (95% CI)	P-value
Lifetime paracetamol use£		<0.01‡		0.01‡
Never exposed	1		1	
Past exposure but not current	2.38 (0.73,7.75)		2.14 (0.65,7.04)	
Current exposure ± past exposure	3.14 (0.97,10.20)		1.81 (0.54,6.05)	
Persistent exposure	10.29 (3.19,33.19)		5.87 (1.76,19.62)	
Lifetime paracetamol dose$		<0.01‡		0.01‡
Never exposed	1	<0.01¥	1	<0.01¥
Light exposure	1.83 (0.54,6.20)		1.50 (0.44,5.13)	
Medium exposure	3.90 (1.22,12.47)		2.55 (0.78,8.35)	
Heavy exposure	8.96 (2.85,28.23)		5.03 (1.54,16.37)	

*ORs adjusted for child's gender, area of residence and maternal education

†ORs adjusted for child's gender, area of residence and maternal education and additionally adjusted for symptoms of respiratory infections as reported at age of 5

‡Overall p-value (likelihood ratio test).

¥P value for trend as dose of paracetamol computed using a composite score of paracetamol exposure from age 1, 3, and 5 yr as: Never exposed, Light exposure, Medium exposure, and Heavy exposure.

£Lifetime paracetamol use: refers to use of paracetamol up to the age of 5 (i.e. ages 1, 3 and 5).

$Lifetime paracetamol dose: refers to dose of paracetamol exposure up to the age of 5 (i.e. ages 1, 3 and 5).

5.2.7.6 Cross-sectional association between paracetamol and eczema

As in the findings of wheeze at year five, lifetime paracetamol use was significantly associated with an increased risk of reported eczema symptoms (Table 5.25). The odds ratios were almost equally high for persistent exposure and current exposure, and much lower for exposure in the past (crude OR, 95% CI, 5.64; 1.66, 19.20 for persistent exposure, 5.68; 1.88, 17.16 for current

exposure with or without past exposure, and 1.60; 0.46, 5.53 for past exposure versus never users, overall $p<0.01$) (Table 5.25).

There was also a significant dose-dependent trend between reported eczema and lifetime paracetamol dose. The odds ratios increased in the heavy and medium exposure categories compared to light and low exposure groups (crude OR, 95% CI, 7.47; 2.35, 23.70 for heavy exposure, 3.16; 0.96, 10.41 for medium exposure, and 2.54; 0.80, 8.08 for light exposure compared with never exposed category, overall $p<0.01$, and p trend<0.01) (Table 5.25).

Table 5.25 Univariate analysis of eczema in relation to paracetamol exposure up to the age of 5 year

Exposure at ages 1, 3 and 5 (N=852)	Overall N (%)	Eczema N (%)	Crude OR (95% CI)	P-value
Lifetime paracetamol use*				<0.01‡
Never exposed	258 (30.4)	4 (1.6)	1	
Past exposure but not current	285 (33.6)	7 (2.5)	1.60 (0.46,5.53)	
Current exposure ± past exposure	207 (24.4)	17 (8.2)	5.68 (1.88,17.16)	
Persistent exposure	98 (11.6)	8 (8.2)	5.64 (1.66,19.20)	
Lifetime paracetamol dose†				<0.01‡
Never exposed	258 (30.4)	4 (1.6)	1	<0.01¥
Light exposure	286 (33.7)	11 (3.9)	2.54 (0.80,8.08)	
Medium exposure	190 (22.4)	9 (4.7)	3.16 (0.96,10.41)	
Heavy exposure	114 (13.4)	12 (10.5)	7.47 (2.35,23.70)	

‡Overall p-value (likelihood ratio test)

¶ P value for trend (computed for dose of paracetamol use in the past month: None/Light (never exposed in the past month or exposed in the past year), Medium exposure (≥ 1 tablets/month at age 1 or 3), Heavy exposure (≥ 1 tablets/month persistently at age 1 and 3)

¥ P value for trend as dose of paracetamol computed using a composite score of paracetamol exposure from age 1, 3, and 5 yr as: Never exposed, Light exposure, Medium exposure, and Heavy exposure.

*Lifetime paracetamol use: refers to use of paracetamol up to the age of 5 (i.e. ages 1, 3 and 5).

†Lifetime paracetamol dose: refers to dose of paracetamol exposure up to the age of 5 (i.e. ages 1, 3 and 5).

Following adjustment for *a priori* confounders and further adjustment for potential confounders in Table 5.20, the magnitude and level of significance of the associations between lifetime use of paracetamol and dose of exposure on eczema did not change materially (Table 5.26). Further controlling for respiratory tract infections slightly reduced the strength of the associations but the overall significance and trend remained (overall $p<0.01$, p trend<0.01) (Table 5.26).

Table 5.26 Multivariate analysis of eczema in relation to paracetamol exposure up to the age of 5 year

Exposure at ages 1, 3 and 5 (N=852)	Adjusted OR* (95% CI)	P-value	Further adjusted OR† (95% CI)	P-value
Lifetime paracetamol use£		<0.01‡		<0.01‡
Never exposed	1		1	
Past exposure but not current	1.66 (0.48,5.76)		1.61 (0.46,5.61)	
Current exposure ± past exposure	6.00 (1.98,18.19)		5.29 (1.67,16.80)	
Persistent exposure	6.12 (1.77,21.13)		5.40 (1.50,19.45)	
Lifetime paracetamol dose$		<0.01‡		0.01‡
Never exposed	1	<0.01¥	1	<0.01¥
Light exposure	2.71 (0.85,8.66)		2.53 (0.79,8.13)	
Medium exposure	3.26 (0.98,10.77)		2.78 (0.82,9.45)	
Heavy exposure	8.27 (2.57,26.58)		6.70 (1.99,22.54)	

*ORs adjusted for child's gender, area of residence and maternal education

†ORs adjusted for child's gender, area of residence and maternal education and additionally adjusted for symptoms of respiratory infections as reported at age of 5

‡Overall p-value (likelihood ratio test)

¥ P value for trend as dose of paracetamol computed using a composite score of paracetamol exposure from age 1, 3, and 5 yr as: Never exposed, Light exposure, Medium exposure, and Heavy exposure.

£Lifetime paracetamol use: refers to use of paracetamol up to the age of 5 (i.e. ages 1, 3 and 5).

$Lifetime paracetamol dose: refers to dose of paracetamol exposure up to the age of 5 (i.e. ages 1, 3 and 5).

5.2.7.7 Cross-sectional association between paracetamol and rhinitis

In the univariate analysis, the risks of rhinitis were higher for persistent lifetime users of paracetamol and current users than users in the past, and these associations were significant (crude OR, 95% CI, 12.94; 2.74, 61.05 for persistent exposure, 7.18; 1.57, 32.78 for current exposure with or without past exposure, and 4.17; 0.89, 19.50 for past exposure vs. non-users, overall $p<0.01$) (Table 5.27). In terms of dose-response association, the risks were

higher in those exposed to heavy or medium doses than those exposed to light doses of paracetamol (crude OR, 95% CI, 15.06; 3.31, 68.47 for heavy dose, 7.11; 1.54, 32.84 for medium dose, and 3.21; 0.66, 15.60 for light exposure compared with non-users, overall p<0.01, and p trend<0.01) (Table 5.27).

Table 5.27 Univariate analysis of rhinitis in relation to paracetamol exposure up to the age of 5 years

Exposure at ages 1, 3 and 5 (N=852)	Overall N (%)	Rhinitis n (%)	Crude OR (95% CI)	P-value
Lifetime paracetamol use*				<0.01‡
Never exposed	258 (30.4)	2 (0.9)	1	
Past exposure but not current	285 (33.6)	9 (3.2)	4.17 (0.89,19.50)	
Current exposure ± past exposure	207 (24.4)	11 (5.3)	7.18 (1.57, 32.78)	
Persistent exposure	98 (11.6)	9 (9.2)	12.94 (2.74,61.05)	
Lifetime paracetamol dose†				<0.01‡
Never exposed	258 (30.4)	2 (0.9)	1	<0.01¥
Light exposure	286 (33.7)	7 (2.5)	3.21 (0.66,15.60)	
Medium exposure	190 (22.4)	10 (5.3)	7.11 (1.54,32.84)	
Heavy exposure	114 (13.4)	12 (10.5)	15.06 (3.31,68.47)	

‡Overall p-value (likelihood ratio test).

¥ P value for trend as dose of paracetamol computed using a composite score of paracetamol exposure from age 1, 3, and 5 yr as: Never exposed, Light exposure, Medium exposure, and Heavy exposure.

*Lifetime paracetamol use: refers to use of paracetamol up to the age of 5 (i.e. ages 1, 3 and 5).

†Lifetime paracetamol dose: refers to dose of paracetamol exposure up to the age of 5 (i.e. ages 1, 3 and 5).

In multivariate analyses controlling for *a priori* confounders and further adjusting for the various early life confounders included in Table 5.21, the magnitude and significance of the associations for both paracetamol variables were little changed (Table 5.28). Adjustment for symptoms of respiratory tract infections, however, reduced the odds ratios for lifetime use, and overall

significance became borderline (p=0.07) (Table 5.28). In regards to dose-response association controlling for respiratory tract infections, though the magnitude of the associations substantially reduced, the overall significance and trend remained (adjusted OR, 95% CI, 7.65; 1.63, 35.97 for heavy exposure, 4.36; 0.93, 20.55 for medium exposure and 2.48; 0.50, 12.25 for light exposure versus never, overall p=0.01, p trend<0.01) (Table 5.28).

Table 5.28 Multivariate analysis of rhinitis in relation to paracetamol exposure up to the age of 5 years

Exposure at age 1, 3 and 5 (N=852)	Adjusted OR* (95% CI)	P-value	Further adjusted OR† (95% CI)	P-value
Lifetime paracetamol use£		<0.01‡		0.07‡
Never exposed	1		1	
Past exposure but not current	4.10 (0.87,19.21)		3.60 (0.76,17.01)	
Current exposure ± past exposure	7.09 (1.55,32.38)		3.82 (0.82,17.82)	
Persistent exposure	12.58 (2.63,60.02)		6.59 (1.34,32.35)	
Lifetime paracetamol dose$		<0.01‡		0.01‡
Never exposed	1	<0.01¥	1	<0.01¥
Light exposure	3.16 (0.65,15.38)		2.48 (0.50,12.25)	
Medium exposure	7.01 (1.52,32.44)		4.36 (0.93,20.55)	
Heavy exposure	14.66 (3.20,67.10)		7.65 (1.63,35.97)	

*ORs adjusted for child's gender, area of residence and maternal education

†ORs adjusted for child's gender, area of residence and maternal education and additionally adjusted for symptoms of respiratory infections as reported at age of 5

‡Overall p-value (likelihood ratio test)

¥ P value for trend as dose of paracetamol computed using a composite score of paracetamol exposure from age 1, 3, and 5 yr as: Never exposed, Light exposure, Medium exposure, and Heavy exposure.

£Lifetime paracetamol use: refers to use of paracetamol up to the age of 5 (i.e. ages 1, 3 and 5).

$Lifetime paracetamol dose: refers to dose of paracetamol exposure up to the age of 5 (i.e. ages 1, 3 and 5).

5.2.7.8 Cross-sectional association between paracetamol and sensitization

In the univariate analysis, lifetime use of paracetamol was associated with a non-significant increased risk of sensitization at age five, with higher odds ratios seen for persistently exposed groups compared to the other categories. However, none of these associations were statistically significant (crude OR, 95% CI; 2.66; 0.65, 10.85 for persistent exposure, 1.24; 0.31, 5.01 for current exposure with or without past exposure, 1.12; 0.30, 4.23 for past exposure versus never users, overall p=0.57) (Table 5.29). There was however a borderline significant trend between lifetime paracetamol dose and sensitization outcome, with increasing risk in heavy dose exposure (crude OR, 95% CI, 3.50; 0.97, 12.67) followed by medium exposure (crude OR, 95% CI, 1.01; 0.22, 4.56), and light exposure (crude OR, 95% CI, 0.89; 0.22, 3.59) compared with never users (p for trend=0.07), but not to the level of overall significance (p=0.15) (Table 5.29).

Table 5.29 Univariate analysis of sensitization in relation to paracetamol exposure up to the age of 5 years

Exposure at ages 1, 3 and 5 (N=841)	Overall N (%)	Sensitization n (%)	Crude OR (95% CI)	P-value
Lifetime paracetamol use*				0.57‡
Never exposed	254 (30.2)	4 (1.6)	1	
Past exposure but not current	283 (33.7)	5 (1.8)	1.12 (0.30,4.23)	
Current exposure ± past exposure	206 (24.5)	4 (1.9)	1.24 (0.31,5.01)	
Persistent exposure	98 (11.7)	4 (4.1)	2.66 (0.65,10.85)	
Lifetime paracetamol dose†				0.15‡
Never exposed	254 (30.2)	4 (1.6)	1	0.07¥
Light exposure	285 (33.9)	4 (1.4)	0.89 (0.22,3.59)	
Medium exposure	189 (22.5)	3 (1.6)	1.01 (0.22,4.56)	
Heavy exposure	113 (13.4)	6 (5.3)	3.50 (0.97,12.67)	

‡Overall p-value (likelihood ratio test)

¥P value for trend as dose of paracetamol computed using a composite score of paracetamol exposure from age 1, 3, and 5 yr as: Never exposed, Light exposure, Medium exposure, and Heavy exposure.

*Lifetime paracetamol use: refers to use of paracetamol up to the age of 5 (i.e. ages 1, 3 and 5).

†Lifetime paracetamol dose: refers to dose of paracetamol exposure up to the age of 5 (i.e. ages 1, 3 and 5).

In multivariate analyses, adjusted for *a priori* confounders, the odds ratios for lifetime use of paracetamol and dose of exposure on sensitization, and the overall level of significance remained unchanged (Table 5.30). Adjustment for respiratory tract infections for lifetime paracetamol use slightly increased the magnitude of associations, all in the expected direction, but remained non-significant (overall p=0.46) (Table 5.30). In terms of dose-response association, however, on controlling for respiratory tract infection, the trend became statistically significant (adjusted OR, 95% CI, 4.80; 1.17, 19.68 for heavy exposure, 1.20; 0.25, 5.64 for medium exposure, 0.99; 0.24, 4.06 for never exposure versus never, p trend=0.04, overall p=0.09) (Table 5.30). Further

adjustment for potential confounders in Table 5.22 made little change to the magnitude of the odds ratio or level of significance (Table 5.30).

Table 5.30 Multivariate analysis of sensitization in relation to paracetamol exposure up to the age of 5 years

Exposure at age 1, 3 and 5 (N=841)	Adjusted OR* (95% CI)	P-value	Further adjusted OR† (95% CI)	P-value
Lifetime paracetamol use£		0.54‡		0.46‡
Never exposed	1		1	
Past exposure but not current	1.15 (0.30,4.39)		1.19 (0.31,4.53)	
Current exposure ± past exposure	1.26 (0.31,5.12)		1.48 (0.34,6.44)	
Persistent exposure	2.83 (0.68,11.86)		3.35 (0.74,15.11)	
Lifetime paracetamol dose$		0.13‡		0.09‡
Never exposed	1	0.06¥	1	0.04¥
Light exposure	0.93 (0.23,3.78)		0.99 (0.24,4.06)	
Medium exposure	1.01 (0.22,4.58)		1.20 (0.25,5.64)	
Heavy exposure	3.71 (1.01,13.68)		4.80 (1.17,19.68)	

*ORs adjusted for child's gender, area of residence and maternal education

†ORs adjusted for child's gender, area of residence and maternal education and additionally adjusted for symptoms of respiratory infections as reported at age of 5

‡Overall p-value (likelihood ratio test)

¥ P value for trend as dose of paracetamol computed using a composite score of paracetamol exposure from age 1, 3, and 5 yr as: Never exposed, Light exposure, Medium exposure, and Heavy exposure.

£Lifetime paracetamol use: refers to use of paracetamol up to the age of 5 (i.e. ages 1, 3 and 5).

$Lifetime paracetamol dose: refers to dose of paracetamol exposure up to the age of 5 (i.e. ages 1, 3 and 5).

5.2.8 Indications for use of paracetamol at age five

5.2.8.1 Availability and preference of paracetamol use at age five

This information was not available at ages one and three, but at year five, paracetamol was the analgesic and antipyretic drug of choice for 716 (84%) of the birth cohort mothers[d] (Figure 5.6). It was also reported to be readily available 644 (75.6%) and affordable (access with low cost) 785 (92.1%) (Figure 5.7). Seventy eight percent of the mothers were able to differentiate paracetamol from aspirin (Figure 5.7). Aspirin avoidance was reported by only 1.4% of the mothers because of asthma, and 1.5% due to other allergic diseases (Figure 5.7).

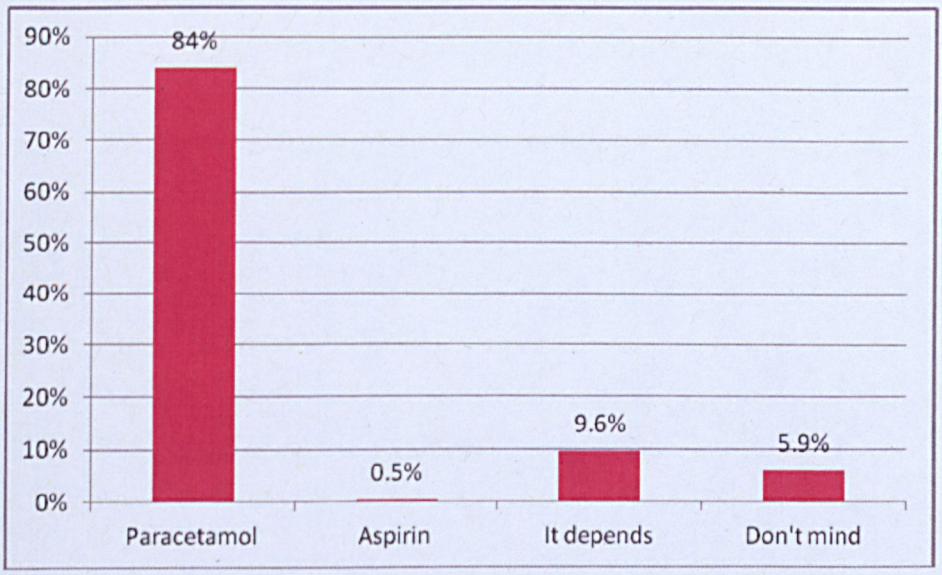

Figure 5.6 Preference for child's use of paracetamol at the age of five

[d] Based on responses to the question: do you prefer to give aspirin or paracetamol for your child?

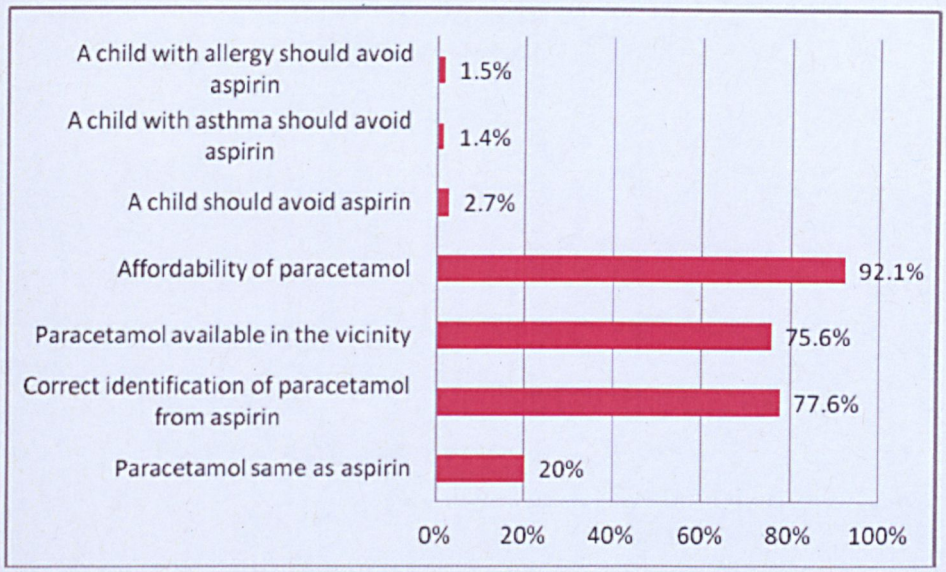

Figure 5.7 Maternal perceptions of paracetamol and aspirin use

5.2.8.2 Indications for use of paracetamol at age five

The most common indications for use of paracetamol at year five included non-respiratory illness – headache, malaria, and any allergy (25.2%); respiratory illness – wheezing illness, coughing episode and common cold (8.5%); and fever of any origin (31%) (Figure 5.8).

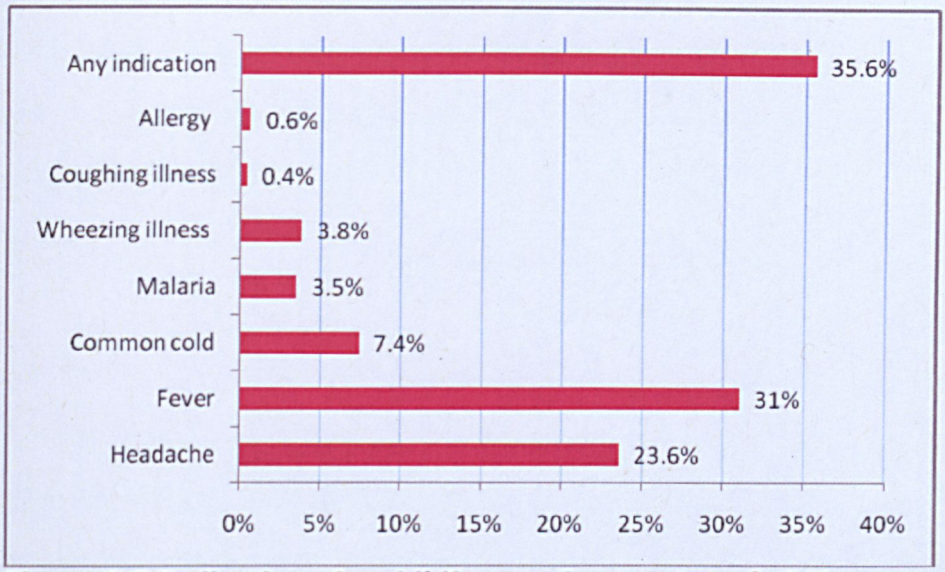

Figure 5.8 Indications for child's use of paracetamol at the age of five

*Any indication refers to use of paracetamol for one or more symptoms or illnesses.

5.3 SUMMARY

In summary, this chapter which further explores the role of paracetamol on allergic outcomes at a later follow up found significant positive dose-response effects of early life use of paracetamol on the incidence and prevalence of the reported allergic outcomes. The current study was conducted as children reached their 5th birthdays, possibly increasing the chance of capturing an intermediate and late-onset wheezing phenotype that correlated with asthma later in childhood.[16]

Consistent with the previous follow up of the birth cohort in chapter three, early life use and dose of paracetamol in the first three years of life was seen to increase the risk of incident wheeze, eczema and rhinitis between ages three and five (data on rhinitis were not available at age one). The associations were dose-dependent and independent of infant respiratory tract infections, and

numerous other potential confounders. The effects of early life paracetamol use and dose on incident sensitization outcomes were also in the expected direction, with a non-significant increased risk seen in the persistent and heavy dose category. However confidence intervals were wide and significance not reached.

Significant dose-dependent cross-sectional associations have also been demonstrated between both lifetime use of paracetamol (use up until the age of five), and lifetime paracetamol dose (dose up to the age of five), and increased risk of wheeze, eczema, and rhinitis at the age of five. These findings were independent of the potential confounders measured. A non-significant increased risk of sensitization was seen among lifetime paracetamol users, but like the longitudinal analysis the confidence interval was wide, which could be due to the low prevalence of sensitization in the birth cohort. However, a significant dose-dependent increasing trend was also observed between sensitization and lifetime dose exposure, higher risks being in the heavily exposed category. The study has also shown that persistent and heavy use of paracetamol appears to increased the risk of allergic diseases and sensitization more than irregular use or exposure at a lower dose.

At year five, it was also found that paracetamol was easily accessible and affordable in the vicinity, and that it was the analgesic and antipyretic drug of choice of most mothers. Furthermore, over a third of the mothers could correctly differentiate paracetamol from aspirin, and fever of any origin, headache, common cold and malaria illness were the most common indications for use of paracetamol.

A detailed discussion of the strengths and weaknesses of this study, and consistency with previous studies along with the evaluation of the evidence against causal inference will be made in chapter seven.

6 EXPOSURE TO GASTRO-INTESTINAL INFECTIONS AND THE PREVALENCE AND INCIDENCE OF WHEEZE AND ALLERGIC OUTCOMES AT AGE FIVE

6.1 INTRODUCTION

In this chapter, analyses conducted following the five year follow-up relating to gastro-intestinal infections, the other main exposure of interest, and their associations on allergic disease outcomes are presented.

Data on geohelminth infection were available at ages one and three; and it is therefore possible to investigate the longitudinal effects of infection early in life on the incidence of allergic disease and sensitization between ages three and five. The effects of *Helicobacter pylori* and commensal microflora exposure at age three on the incidence of these outcomes were also investigated (these exposures were not measured at year one). Further cross-sectional analyses were conducted to explore the effects of current and recent infection with geohelminth and *H. pylori* at age five (data on intestinal microflora were not available at year five) on the prevalence of the outcomes; as these analyses are on larger sample sizes than the longitudinal analyses, and benefit from greater statistical power.

The aims of this chapter are therefore to:

- Determine the independent effects early life infection with geohelminths, *Helicobacter pylori,* and commensal microflora on the incidence of wheeze, eczema, rhinitis and sensitization between ages three and five.
- Determine the independent effects of geohelminth and *H. pylori* infection up to the age of five on the prevalence of allergic diseases and sensitization at age five.

In the next sections, results from longitudinal and cross-sectional analyses will be presented followed by a brief summary of the main findings.

6.2 RESULTS

6.2.1 The birth cohort at age five

The birth cohort between ages three and five has been described in chapter five (section 5.3.1) and in chapter two (Fig 2.3). In brief, 863 singleton children were available for study at year five. Of these, 852 reported disease outcome information (and geohelminth and *H. pylori* data were available in 847 of these), while 855 provided skin test data (and of these, geohelminth and *H. pylori* data were available for 854).

6.2.2 H. pylori and geohelminth infection at age five

The prevalence of each of the geohelminth parasites at ages one and three, and

of *H. pylori* and commensal infections at age three are described in chapter four. At year five, hookworm, *Ascaris lumbricoides,* and *T. trichiura* were detected in 6.3% (53/847), 4.4% (37/847), and 0.5% (4/847) of children, respectively, with an overall prevalence of any infection of 10.4% (88/847), and infections prevalence increase with age. Infection with *H. pylori* was found in 43.7% (370/847) of the children (Figure 6.1).

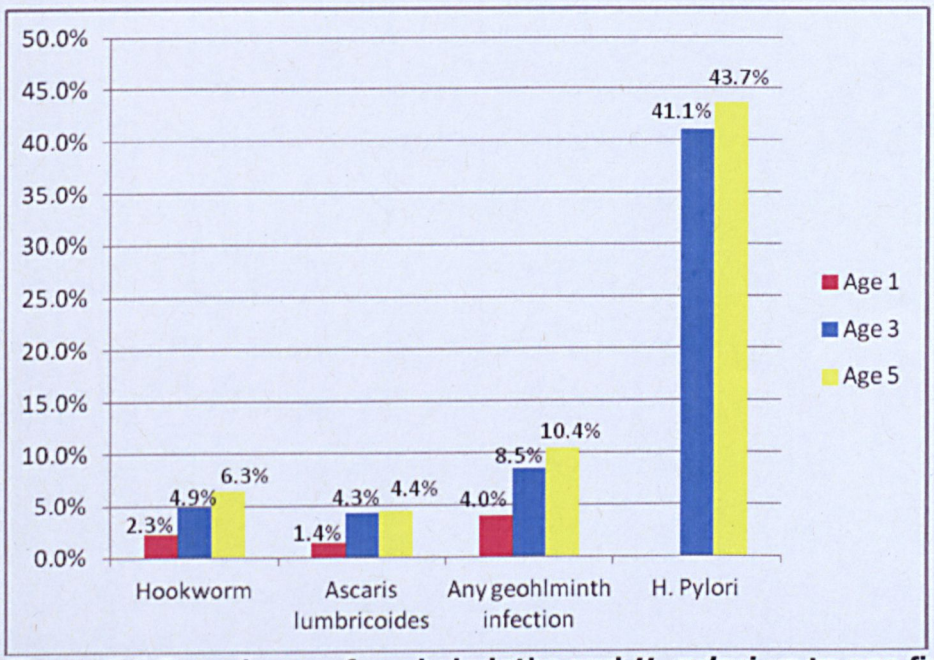

Figure 6.1 Prevalence of geohelminths and *H. pylori* up to age five

6.2.3 Longitudinal associations between H. pylori, geohelminth infection and commensal bacteria and incidence of allergic outcomes

6.2.3.1 Incident wheeze in relation to geohelminth infection, H. pylori infection and commensal bacteria

In the univariate analysis, early life exposure (infection at age one or three or both) to hookworm, *A. lumbricoides* and 'any geohelminth' infection (7%, 6.1% and 12.7% respectively) was associated with a non-significant increased risk of incident wheeze (Table 6.1). For hookworm, the risk of incident wheeze was higher in those infected at ages one and three (6.5%) than uninfected (6.0%), and a similar pattern was seen for *A. lumbricoides* (9.8% vs. 5.7%), and for any geohelminth (8.1% vs. 5.6%) (Table 6.1). In multivariate analysis, adjusting for *a priori* confounders (residence, child's age and maternal education), the risk of incident wheeze was increased with geohelminth exposure, but none of these associations reached statistical significance (adjusted OR, 95%, CI, 1.06; 0.31, 3.60, p=0.93 for hookworm, 1.89; 0.63, 5.66, p=0.29 for *A. lumbricoides*, and 1.48; 0.63, 3.49, p=0.38, for any geohelminth for exposure at any age up to year three vs. never), and the confidence intervals were wide.

The relation between *H. pylori* infection at age three and incident wheeze between ages three and five was non-significant, with higher risk found among infected (6.5%) than uninfected (4.4%) children. In multivariate analysis adjusted for *a priori* confounders, *H. pylori* infection increased the risk of

incident wheeze non-significantly (adjusted OR, 95%, CI, 1.56; 0.69, 3.51, p=0.28) (Table 6.1).

Similarly, none of the intestinal microflora present at age three were significantly associated with incident wheeze between ages three and five (Table 6.1). In multivariate analysis adjusted for *a priori* confounders, the effects were negatively related but none of these reached statistical significance (adjusted OR, 95%, CI, 0.80; 0.33, 1.95, p=0.62, for enterococci, 0.84; 0.32, 2.19, p=0.71 for lactobacilli, and 0.40; 0.09, 1.75 p=0.17 for bifidobacterium) (Table 6.1).

Further adjustment for other potential confounders in Table 5.3 made little difference to the odds ratios in relation to each exposure (Table 6.1).

Table 6.1 Univariate and multivariate analysis of gastro-intestinal infection in relation to incident wheeze between ages 3 and 5

Exposures	Wheeze never up to age 3 (N=676)				
	Overall N (%)	n (%) new wheeze	Crude OR (95% CI)	Adjusted OR* (95% CI)	P-value‡
Early life exposure to hookworm‡ (N=668)					
Never exposed	622 (93.1)	37 (6.0)	1	1	0.93
Exposed at any age up to yr 3	46 (7.0)	3 (6.5)	1.10 (0.33,3.72)	1.06 (0.31,3.60)	
Early life exposure to *Ascaris lumbricoides*‡ (N=668)					
Never exposed	627 (93.9)	36 (5.7)	1	1	0.29
Exposed at any age up to yr 3	41 (6.1)	4 (9.8)	1.77 (0.60,5.25)	1.89 (0.63,5.66)	
Early life exposure to any geohelminth† (N=676)					
Never exposed	590 (87.3)	33 (5.6)	1	1	0.38
Exposed at any age up to yr 3	86 (12.7)	7 (8.1)	1.50 (0.64,3.50)	1.48 (0.63,3.49)	
Helicobacter pylori exposure at year 3 (N=474)					
Yes	201 (42.4)	13 (6.5)	1.50 (0.67,3.37)	1.56 (0.69,3.51)	0.28
No	273 (57.6)	12 (4.4)	1	1	
Intestinal microflora exposure at year 3 (N=414)					
Enterococci					
Yes	162 (39.1)	8 (4.9)	0.82 (0.34,1.98)	0.80 (0.33,1.95)	0.62
No	252 (60.9)	15 (6.0)	1	1	
Lactobacilli					
Yes	122 (29.5)	6 (4.9)	0.84 (0.32,2.18)	0.84 (0.32,2.19)	0.71
No	292 (70.5)	17 (5.8)	1	1	
Bifidobacteria					
Yes	79 (19.1)	2 (2.5)	0.39 (0.09,1.69)	0.40 (0.09,1.75)	0.17
No	335 (80.9)	21 (6.3)	1	1	

*ORs adjusted for child's gender, area of residence and maternal education

‡Overall p-value (likelihood ratio test); †Hookworm, *A. lumbricoides,* or *Trichuris trichiura*

‡Early life exposure to hookworm, *A. Lumbricoides* or any geohelminth: refers to exposure prior to disease outcome (i.e. first three years of life)

6.2.3.2 Incident eczema in relation to geohelminth infection, H. pylori infection and commensal bacteria

In univariate analysis, incident eczema was more common amongst those with early lifeinfection with hookworm (7.8% among infected vs. 5.4% in uninfected), *A. lumbricoides* (12.2% among infected vs. 5.2% in uninfected), and any geohelminth (9.2% among infected vs. 5.2% in uninfected) but odds ratios were not statistically significant (Table 6.2). After adjustment for *a priori* confounders, hookworm, *A. lumbricoides*), and any geohelminth were all still associated with an increased risk of incident eczema, but only infection with *A. lumbricoides* reached borderline significance (adjusted OR, 95% CI, 2.86; 1.04, 7.86, p=0.07) (Table 6.2).

Early life exposure to *H. pylori* infection was inversely and significantly associated with incident eczema between ages three and five (2.4% among exposed and 6.1% among non-exposed, p=0.02) (Table 6.2). In multivariate analysis adjusted for *a priori* confounders and additionally for breastfeeding history in the first year of life, *H. pylori* infection was still associated with a significantly reduced risk of incident eczema (adjusted OR, 95% CI, 0.31; 0.10, 0.94, p=0.02) (Table 6.2).

None of the commensal microflora studied at year three were significantly associated with incident eczema between ages three and five (Table 6.2). In multivariate analysis, however, the odds ratios were consistently protective (adjusted OR, 95% CI, 0.59; 0.24, 1.45, p=0.24 for enterococci, 0.83; 0.34, 2.05, p=0.68 for lactobacilli, and 0.57; 0.16, 1.95 p=0.34 for bifidiobacterium),

but none of these associations reached significance (Table 6.2). Adjustment for potential confounders in the first year of life presented in Table 5.4 made little change to the odds ratio (Table 6.2).

Table 6.2 Univariate and multivariate analysis of gastro-intestinal infection in relation to incident eczema between ages 3 and 5

Exposures	Eczema never up to age 3 (N=700)				
	Overall N (%)	n (%) new eczema	Crude OR (95% CI)	Adjusted OR* (95% CI)	P-value‡
Early life exposure to hookworm‡ (N=694)					
Never exposed	643 (92.7)	35 (5.4)	1	1	0.52
Exposed at any age up to yr 3	51 (7.4)	4 (7.8)	1.48 (0.50,4.34)	1.45 (0.49,4.28)	
Early life exposure to *Ascaris lumbricoides*‡ (N=694)					
Never exposed	653 (94.1)	34 (5.2)	1	1	0.07
Exposed at any age up to yr 3	41 (5.9)	5 (12.2)	2.53 (0.93,6.85)	2.86 (1.04,7.86)	
Early life exposure to any geohelminth† (N=700)					
Never exposed	613 (87.6)	31 (5.1)	1	1	0.12
Exposed at any age up to yr 3	87 (12.4)	8 (9.2)	1.90 (0.84,4.28)	1.97 (0.87,4.47)	
Helicobacter pylori exposure at year 3 (N=498)					
Yes	205 (41.2)	5 (2.4)	0.38 (0.14,1.05)	0.31 (0.10,0.94)	0.02£
No	293 (58.8)	18 (6.1)	1	1	
Intestinal microflora exposure at year 3 (N=439)					
Enterococci					
Yes	171 (39.0)	7 (4.1)	0.59 (0.24,1.45)	0.59 (0.24,1.45)	0.24
No	268 (61.1)	18 (6.7)	1	1	
Lactobacilli					
Yes	134 (30.5)	7 (5.2)	0.88 (0.36,2.16)	0.83 (0.34,2.05)	0.68
No	305 (69.5)	18 (5.9)	1	1	
Bifidobacteria					
Yes	81 (18.5)	3 (3.7)	0.59 (0.17,2.01)	0.57 (0.16,1.95)	0.34
No	358 (81.6)	22 (6.2)	1	1	

*ORs adjusted for child's gender, area of residence and maternal education

£ Additionally adjusted for reported breast feeding history in the first years of life

‡Overall p-value (likelihood ratio test); †Hookworm, *A. lumbricoides,* or *Trichuris trichiura*

‡Early life exposure to hookworm, *A. lumbricoides* or any geohelminth: refers to exposure prior to disease outcome (i.e. first three years of life)

6.2.3.3 Incident rhinitis in relation to geohelminth infection, H. pylori infection and commensal bacteria

Early life exposure to hookworm, *A. lumbricoides* and any geohelminth, were all positively related to incident rhinitis between ages three and five with ORs ranging from 1.19 to 2.66, but none of these associations were significant (Table 6.3). After adjusted analysis, the effects were similar to those in the univariate analysis, with a non-significantly increased risk of incident rhinitis in relation to each infection variable (adjusted OR, 95% CI, 2.44; 0.89, 6.68, p=0.11 for hookworm, 1.21; 0.28, 5.28, p=0.81 for *A. lumbricoides,* and 2.12; 0.88, 5.09, p=0.11 for any geohelminth) (Table 6.3).

H. pylori infection at age three was also positively, though not significantly, related to incident rhinitis between ages three and five (4.4% among infected and 3.9% among non-infected children) (Table 6.3). After adjustment, the association did not change (adjusted OR, 95% CI, 1.07; 0.45, 2.57, p=0.88) (Table 6.3).

The association between incident rhinitis and presence of commensal microflora at age three did not reach significance, although a higher risk was seen among colonized than non-colonized children (Table 6.3). In the adjusted analysis, the effects were similar to those in the univariate analysis (adjusted OR, 95% CI, 1.63; 0.69, 3.86, p=0.27 for enterococci, 1.57; 0.65, 3.77, p=0.32 for lactobacilli, and 1.32; 0.47, 3.71 p=0.60 for bifidobacteria) (Table 6.3). Adjustment for potential confounders in Table 5.5 did not alter any of the associations materially (Table 6.3).

Table 6.3 Univariate and multivariate analysis of gastro-intestinal infection in relation to incident rhinitis between ages 3 and 5

Exposures	Rhinitis never at the age of 3 (N=798)				
	Overall N (%)	n (%)new rhinitis	Crude OR (95% CI)	Adjusted OR* (95% CI)	P-value‡
Early life exposure to Hookworm‡ (N=773)					
Never exposed	716 (92.6)	25 (3.5)	1	1	0.11
Exposed at any age up to yr 3	57 (7.4)	5 (8.7)	2.66 (0.98,7.23)	2.44 (0.89,6.68)	
Early life exposure to *Ascaris lumbricoides*‡ (N=773)					
Never exposed	729 (94.3)	28 (3.8)	1	1	0.81
Exposed at any age up to yr 3	44 (5.7)	2 (4.6)	1.19 (0.27,5.17)	1.21 (0.28,5.28)	
Early life exposure to any geohelminth† (N=781)					
Never exposed	683 (87.5)	23 (3.4)	1	1	0.11
Exposed at any age up to yr 3	98 (12.6)	7 (7.1)	2.21 (0.92,5.29)	2.12 (0.88,5.09)	
Helicobacter pylori exposure at year 3 (N=559)					
Yes	225 (40.3)	10 (4.4)	1.15 (0.49,2.67)	1.07 (0.45,2.57)	0.88£
No	334 (59.8)	13 (3.9)	1	1	
Intestinal microflora exposure at year 3 (N=491)					
Enterococci					
Yes	189 (38.5)	11 (5.8)	1.63 (0.69,3.85)	1.63 (0.69,3.86)	0.27
No	302 (61.5)	11 (3.6)	1	1	
Lactobacilli					
Yes	153 (31.2)	9 (5.9)	1.56 (0.65,3.74)	1.57 (0.65,3.77)	0.32
No	338 (68.8)	13 (3.9)	1	1	
Bifidobacteria					
Yes	93 (18.9)	5 (5.4)	1.27 (0.46,3.54)	1.32 (0.47,3.71)	0.60
No	398 (81.1)	17 (4.3)	1	1	

*ORs adjusted for child's gender, area of residence and maternal education

£ Additionally adjusted for Indoor cooking; ‡Overall p-value (likelihood ratio test)

†Hookworm, *A. lumbricoides,* or *Trichuris trichiura*

‡Early life exposure to hookworm, *A. lumbricoides* or any geohelminths: refers to exposure prior to disease outcome (i.e. first three years of life)

6.2.3.4 *Incident sensitization in relation to geohelminth infection, H. pylori infection and commensal bacteria*

Effects of infection with the geohelminth parasites hookworm and *A. lumbricoides* in the first three years of life on new-onset sensitization to any allergen (*D. pteronyssinus* and cockroach allergen) between ages three and five were not significant, although incident sensitization was more common among infected than uninfected children (3.6% vs. 1.9% for hookworm, 2.9% vs. 2.0% for *A. lumbricoides*, and 3.5% vs. 1.8% for any geohelminth infection) (Table 6.4). In the adjusted multivariate analysis in Table 6.4, the odds ratios remained increased but not significant for hookworm (adjusted OR, 95% CI, 1.83; 0.40, 8.38, p=0.47), *A. lumbricoides* (OR, 95% CI, 1.47; 0.19, 11.67, p=0.73), and any geohelminth (OR, 95% CI, 1.88; 0.52, 6.84, p=0.37).

The effects of *H. pylori* infection at age three on incident sensitization to any allergen were similar to the geohelminth effects above, with higher risks observed among infected (2.6%) than uninfected (2.2%) children (Table 6.4). In the adjusted analysis, the effects were the same as in the univariate analysis, with a non-significant increased risk seen among exposed compared to unexposed children (adjusted OR, 95% CI, 1.23; 0.40, 3.73, p=0.72) (Table 6.4).

Similarly, the effects of the commensal microflora on incident sensitization to any allergen were non-significant. In the multivariate analysis, the effects of enterococci (adjusted OR, 95% CI, 0.72; 0.22, 2.37, p=0.58) and bifidobacteria (adjusted OR, 95% CI, 0.34; 0.04, 2.69, p=0.24) were protective, while

colonization with the commensal microflora lactobacilli (adjusted OR, 95% CI, 1.98; 0.65, 6.03, p=0.24) increased the risks of incident sensitization (Table 6.4).

Adjustment for various potential confounders in Table 5.6 made little effect on the associations (Table 6.4).

Table 6.4 Univariate and multivariate analysis of gastro-intestinal infection in relation to incident sensitization between ages 3 and 5

Exposures	Sensitization never at age 3 (N=766)				
	Overall N (%)	n (%) new atopy	Crude OR (95% CI)	Adjusted OR* (95% CI)	P-value‡
Early life exposure to Hookworm‡ (N=746)					
Never exposed	690 (92.5)	13 (1.9)	1	1	0.47
Exposed at any age up to yr 3	56 (7.5)	2 (3.6)	1.93 (0.42,8.77)	1.83 (0.40,8.38)	
Early life exposure to *Ascaris lumbricoides*‡ (N=746)					
Never exposed	711 (95.3)	14 (2.0)	1	1	0.73
Exposed at any age up to yr 3	35 (4.7)	1 (2.9)	1.46 (0.19,11.46)	1.47 (0.19,11.67)	
Early life exposure to any geohelminth† (N=749)					
Never exposed	662 (88.4)	12 (1.8)	1	1	0.37
Exposed at any age up to yr 3	87 (11.6)	3 (3.5)	1.93 (0.53,7.00)	1.88 (0.52,6.84)	
Helicobacter pylori exposure at year 3 (N=552)					
Yes	234 (42.4)	6 (2.6)	1.17 (0.39,3.53)	1.23 (0.40,3.37)	0.72
No	318 (57.6)	7 (2.2)	1	1	
Intestinal microflora exposure at year 3 (N=485)					
Enterococci					
Yes	185 (38.1)	4 (2.2)	0.71 (0.23,2.35)	0.72 (0.22,2.37)	0.58
No	300 (61.9)	9 (3.0)	1	1	
Lactobacilli					
Yes	149 (30.7)	6 (4.0)	1.97 (0.65,5.97)	1.98 (0.65,6.03)	0.24
No	336 (69.3)	7 (2.1)	1	1	
Bifidobacteria					
Yes	94 (19.4)	1 (1.1)	0.34 (0.04,2.64)	0.34 (0.04,2.69)	0.24
No	391 (80.6)	12 (3.1)	1	1	

*ORs adjusted for child's gender, area of residence and maternal education

‡Overall p-value (likelihood ratio test)

†Hookworm, *A. lumbricoides,* or *Trichuris trichiura*

‡Early life exposure to hookworm, *A. Lumbricoides* or any geohelminths: refers to exposure prior to disease outcome (i.e. first three years of life)

6.2.4 Cross-sectional analysis of H. pylori and geohelminth infection, and prevalence of allergic outcomes at age five

H. pylori infection was found in 17% of the children at age three but not age five, 21% at age five but not at age three, and 25% persistently exposed at both ages. The cumulative prevalences of hookworm, *A. lumbricoides* and any geohelminth infection up to age five were 12.9%, 9.1% and 21% respectively.

6.2.4.1 Effects of geohelminth infection and H. pylori on wheeze

The effects of lifetime exposure (exposure at age one, three and five) to hookworm, *A. lumbricoides* or any geohelminth on the prevalence of wheeze were non-significant, with higher risks seen among infected than uninfected children (5.7% vs. 4.4% for hookworm, and 5.4% vs. 4.5% for *A. lumbricoides*) (Table 6.5). In analyses adjusted for the *a priori* confounders residence, child's gender and maternal education, the effects were similar to those in the univariate analysis, with a non-significant increased risk of wheeze found among lifetime exposed children vs. never (adjusted OR, 95% CI, 1.30; 0.53, 3.23, p=0.58 for hookworm, 1.23; 0.42, 3.61, p=0.71 for *A. lumbricoides*, and 1.41; 0.67, 2.97, p=0.38 for any geohelminths) (Table 6.5).

The analysis of the four level *H. pylori* infection variable representing timing of infection revealed the lowest prevalence of wheeze was among children exposed at age three but not at age five (3.0%), and the highest amongst the those infected at age 3 and 5 (6.9%), but overall the differences were not significant (adjusted OR, 95% CI, 0.62; 0.16, 2.30) (Table 6.5). In the univariate and

adjusted analyses of effects of current infection, *H. pylori* infection at year five was associated with a small but non-significant increased risk of wheeze (adjusted OR, 95% CI, 1.37; 0.71, 2.65, p=0.35) (Table 6.5).

Further adjustment for potential confounders in Table 5.19 made little change to the odds ratios (Table 6.5).

Table 6.5 Univariate and multivariate analysis of wheeze in relation to geohelminth and *H. pylori* infection up to the age of 5

Exposures	Overall N (%)	Wheeze Yes n (%)	Crude OR (95% CI)	Adjusted OR* (95% CI)	P-value ‡
Lifetime exposure to Hookworm‡ (N=816)					
Never exposed	711 (87.1)	31 (4.4)	1	1	0.58
Exposed at any age up to yr 5	105 (12.9)	6 (5.7)	1.33 (0.54,3.27)	1.30 (0.53,3.23)	
Lifetime exposure to *Ascaris lumbricoides*‡ (N=816)					
Never exposed	742 (90.9)	33 (4.5)	1	1	0.71
Exposed at any age up to yr 5	74 (9.1)	4 (5.4)	1.23 (0.42,3.57)	1.23 (0.42,3.61)	
Lifetime exposure to any geohelminth† (N=824)					
Never exposed	651 (79.0)	27 (4.2)	1	1	0.38
Exposed at any age up to yr 5	173 (21.0)	10 (5.8)	1.42 (0.67,2.99)	1.41 (0.67,2.97)	
Helicobacter pylori exposure at age 5 (N=847)					
Yes	370 (43.7)	19 (5.1)	1.38 (0.71,2.67)	1.37 (0.71,2.65)	0.35
No	477 (56.3)	18 (3.8)	1	1	
Helicobacter pylori exposure at ages 3 and 5 (N=593)					
Never exposed	222 (37.4)	10 (4.5)	1	1	0.52
Exposed at age 3 but not at age 5	101 (17.0)	3 (3.0)	0.65 (0.17,2.42)	0.62 (0.16,2.30)	
Exposed at age 5 but not at age 3	126 (21.3)	5 (4.0)	0.88 (0.29,2.63)	0.89 (0.30,2.68)	
Persistent exposure	144 (24.3)	10 (6.9)	1.58 (0.64,3.91)	1.52 (0.61,3.77)	

*ORs adjusted for child's gender, area of residence and maternal education

‡Overall p-value (likelihood ratio test)

†Hookworm, *A. lumbricoides,* or *Trichuris trichiura*.

‡Lifetime exposure to hookworm, *A. lumbricoides* or any geohelminths: refers to exposure at ages 1, 3 and 5.

6.2.4.2 Effects of geohelminth infection and H. pylori on eczema

The relation between lifetime exposure to hookworm, *A. lumbricoides* or any geohelminth and the prevalence of eczema was very similar to that seen for wheeze, with a non-significant increased risk among children exposed at any age compared to non-exposed children (Table 6.6). The odds ratios were little changed when adjusted for *a priori* confounders (adjusted ORs, 95% CI, 1.10; 0.41, 2.91, p=0.85 for hookworm, 1.62; 0.60, 4.36, p=0.36 for *A. Lumbricoides*, and 1.45; 0.69, 3.09, p=0.34 for any geohelminth) (Table 6.6). In univariate and multivariate analyses, the effects of *H. pylori* infection were generally protective with the lowest risks seen in those exposed at age three but not age five (adjusted OR, 95% CI, 0.29; 0.06, 1.28) and in persistently exposed children vs. never exposed (adjusted OR, 95% CI, 0.40; 0.13, 1.24), however, overall these associations did not reach statistical significance (p=16) (Table 6.6). Similarly for current *H. pylori* infection, a non-significant decreased risk of eczema was seen on exposure to *H. pylori* infection at age five in both univariate and multivariate analyses (adjusted OR, 95% CI, 0.63; 0.31, 1.28, p=0.19) (Table 6.6).

Further adjustment for variables in Table 5.20 did not materially alter the results (Table 6.6).

Table 6.6 Univariate and multivariate analysis of eczema in relation to geohelminths and *H. pylori* infection up to the age of 5

Exposures	Overall N (%)	Eczema Yes n (%)	Crude OR (95% CI)	Adjusted OR* (95% CI)	P-value‡
Lifetime exposure to Hookworm‡ (N=816)					
Never exposed	711 (87.1)	31 (4.4)	1	1	0.85
Exposed at any age up to yr 5	105 (12.9)	5 (4.8)	1.10 (0.42,2.89)	1.10 (0.41,2.91)	
Lifetime exposure to *Ascaris lumbricoides*‡ (N=816)					
Never exposed	742 (90.9)	31 (4.2)	1	1	0.36
Exposed at any age up to yr 5	74 (9.1)	5 (6.8)	1.66 (0.63,4.42)	1.62 (0.60,4.36)	
Lifetime exposure to any geohelminth† (N=824)					
Never exposed	651 (79.0)	26 (4.0)	1	1	0.34
Exposed at any age up to yr 5	173 (21.0)	10 (5.8)	1.47 (0.70,3.12)	1.45 (0.69,3.09)	
Helicobacter pylori exposure at age 5 (N=847)					
Yes	370 (43.7)	12 (3.2)	0.63 (0.31,1.28)	0.63 (0.31,1.28)	0.19
No	477 (56.3)	24 (5.0)	1	1	
Helicobacter pylori exposure at age 3 and 5 (N=593)					
Never exposed	222 (37.4)	15 (6.8)	1	1	0.16
Exposed at age 3 but not at age 5	101 (17.0)	2 (2.0)	0.28 (0.06,1.26)	0.29 (0.06,1.28)	
Exposed at age 5 but not at age 3	126 (21.3)	5 (4.0)	0.57 (0.20,1.61)	0.54 (0.19,1.54)	
Persistent exposure	144 (24.3)	4 (2.8)	0.39 (0.13,1.22)	0.40 (0.13,1.24)	

*ORs adjusted for child's gender, area of residence and maternal education

‡Overall p-value (likelihood ratio test)

†Hookworm, *A. lumbricoides,* or *Trichuris trichiura.*

‡Lifetime exposure to hookworm, *A. lumbricoides* or any geohelminths: refers to exposure at ages 1, 3 and 5.

6.2.4.3 Effects of geohelminth infection and H. pylori on rhinitis

Lifetime exposure to hookworm was associated with an increased risk of rhinitis at age five (6.7% among exposed vs. 3.1% in unexposed), whereas *A. lumbricoides* was associated with decreased risk (2.7% among exposed vs. 3.6% in unexposed), but none of these differences were statistically significant (Table 6.7). In adjusted analysis, the associations changed only slightly (adjusted OR, 95% CI, 2.13; 0.88, 5.15, p=0.12 for hookworm, 0.79; 0.18, 3.41, p=0.75 for *A. lumbricoides*, and 1.65; 0.74, 3.68, p=0.24 for any geohelminth) (Table 6.7). As in the wheeze and eczema findings, the relationship between *H. pylori* infection at ages three and five and prevalence of rhinitis was generally protective (exposure at age three but not age five vs. never exposed children, adjusted OR, 95% CI, 0.73; 0.19, 2.76), however none of these assications were to the point of significance (p=0.83) (Table 6.7). In an adjusted analysis, current *H. pylori* infection also reduced the risk of rhinitis, but did not reach significance (adjusted OR, 95% CI, 0.94; 0.45, 1.94, p=0.86) (Table 6.7).

Further adjustment for potential confounders in Table 5.21 did not alter the results (Table 6.7).

Table 6.7 Univariate and multivariate analysis of rhinitis in relation to geohelminths and *H. pylori* infection up to the age of 5

Exposures	Overall N (%)	Rhinitis Yes n (%)	Crude OR (95% CI)	Adjusted OR* (95% CI)	P-value‡
Lifetime exposure to hookworm‡ (N=816)					
Never exposed	711 (87.1)	22 (3.1)	1	1	0.12
Exposed at any age up to yr 5	105 (12.9)	7 (6.7)	2.24 (0.93,5.39)	2.13 (0.88,5.15)	
Lifetime exposure to *Ascaris lumbricoides*‡ (N=816)					
Never exposed	742 (90.9)	27 (3.6)	1	1	0.75
Exposed at any age up to yr 5	74 (9.1)	2 (2.7)	0.74 (0.17,3.16)	0.79 (0.18,3.41)	
Lifetime exposure to any geohelminth† (N=824)					
Never exposed	651 (79.0)	21 (3.2)	1	1	0.24
Exposed at any age up to yr 5	173 (21.0)	9 (5.2)	1.65 (0.74,3.67)	1.65 (0.74,3.68)	
Helicobacter pylori exposure at age 5 (N=847)					
Yes	370 (43.7)	13 (3.5)	0.93 (0.45,1.92)	0.94 (0.45,1.94)	0.86
No	477 (56.3)	18 (3.8)	1	1	
Helicobacter pylori exposure at age 3 and 5 (N=593)					
Never exposed	222 (37.4)	9 (4.1)	1	1	0.83
Exposed at age 3 but not at age 5	101 (17.0)	3 (3.0)	0.72 (0.19,2.74)	0.73 (0.19,2.76)	
Exposed at age 5 but not at age 3	126 (21.3)	3 (2.4)	0.56 (0.15,2.18)	0.60 (0.16,2.26)	
Persistent exposure	144 (24.3)	6 (4.2)	1.03 (0.36,2.96)	1.05 (0.36,3.03)	

*ORs adjusted for child's gender, area of residence and maternal education

‡Overall p-value (likelihood ratio test)

†Hookworm, *A. lumbricoides,* or *Trichuris trichiura*.

‡Lifetime exposure to hookworm, *A. lumbricoides* or any geohelminth: refers to exposure at ages 1, 3 and 5.

6.2.4.4 Effects of geohelminth infection and H. pylori on sensitization

The risk of sensitization was higher in those infected with hookworm at any age up to year five (3.8%) than in those not infected (1.8%), and there was a similar higher risk in those exposed to *A. lumbricoides* at any age (2.7%) than in unexposed children (2.0%) (Table 6.8). However, none of these differences were statistically significant. Adjusted analysis showed a comparable finding, with non-significant increased risk of sensitization seen among children with hookworm, *A. lumbricoides*, or any geohelminth (adjusted OR, 95% CI, 2.08; 0.66, 6.57, p=0.24 for hookworm, 1.31; 0.29, 5.96, p=0.73 for *A. Lumbricoides*, and 2.04; 0.74, 5.60, p=0.17 for any geohelminth) (Table 6.8).

In the univariate analysis, the risk of sensitization was lower in those currently infected with *H. pylori* (0.8%), than uninfected (2.9%) (Table 6.8), and the difference was statistically significant. In multivariate analysis adjusted for *a priori* confounders, the odds ratio was unchanged from the univariate, and showed a significantly decreased risk of sensitization in those exposed to *H. pylori* compared to unexposed children (adjusted OR, 95% CI, 0.26; 0.07, 0.92, p=0.02) (Table 6.8). The effect of previous exposure to *H. pylori* up to age five on sensitization outcome was also protective, but less stronger than current infection (adjusted OR, 95% CI, 0.62; 0.21, 1.76, p=36) (Table 6.8).

Further adjustment for potential confounders in Table 5.22 made little change to the odds ratios (Table 6.8).

Table 6.8 Univariate and multivariate analysis of sensitization in relation to geohelminth and *H. pylori* infection up to the age of 5

Exposures	Overall N (%)	Any sensitization Yes n (%)	Crude OR (95% CI)	Adjusted OR* (95% CI)	P-value‡
Lifetime exposure to hookworm† (N=822)					
Never exposed	717 (87.2)	13 (1.8)	1	1	0.24
Exposed at any age up to yr 5	105 (12.8)	4 (3.8)	2.14 (0.69,6.71)	2.08 (0.66,6.57)	
Lifetime exposure to *Ascaris lumbricoides*‡ (N=822)					
Never exposed	748 (91.0)	15 (2.0)	1	1	0.73
Exposed at any age up to yr 5	74 (9.0)	2 (2.7)	1.36 (0.30,6.05)	1.31 (0.29,5.96)	
Lifetime exposure to any geohelminth† (N=830)					
Never exposed	656 (79.0)	11 (1.9)	1	1	0.17
Exposed at any age up to yr 5	174 (21.0)	6 (3.5)	2.09 (0.76,5.74)	2.04 (0.74,5.60)	
Helicobacter pylori exposure at age 5 (N=854)					
Yes	375 (43.9)	3 (0.8)	0.27 (0.08,0.94)	0.26 (0.07,0.92)	0.02
No	479 (56.1)	14 (2.9)	1	1	
Helicobacter pylori exposure at age 3 and 5 (N=597)					
Never exposed	224 (37.5)	7 (3.1)	1	1	0.36
Exposed at any age up to yr 5	373 (62.5)	7 (1.9)	0.59 (0.21,1.71)	0.61 (0.21,1.76)	

*ORs adjusted for child's gender, area of residence and maternal education

‡Overall p-value (likelihood ratio test)

†Hookworm, *A. lumbricoides,* or *Trichuris trichiura*.

‡Lifetime exposure to hookworm, *A. lumbricoides* or any geohelminth: refers to exposure at ages 1, 3 and 5.

6.3 SUMMARY

This study provides important insights into the aetiologies of allergic disease and sensitization. As in the findings from the follow up at year three, the study demonstrated evidence of a protective effect of *H. pylori* on eczema and sensitization in young children in a low-income birth cohort in which confounding by social advantage or antibiotic therapy are unlikely to play a role. In the longitudinal analysis, infection with *H. pylori* was associated with a 69% decreased risk of incident eczema, and in the cross-sectional analysis, current exposure to *H. pylori* infection at year five was associated with a 74% decreased risk of sensitization.

The effects of the geohelminths hookworm and *A. lumbricoides,* either as 'early life exposure' (exposure prior to disease onset), or 'lifetime exposure' (exposure up to age five) were not found to be associated significantly with either of the reported allergic outcomes or sensitization. In both longitudinal and cross-sectional analyses the effects were largely positive, and none of these reached statistical significance. However, in the former analysis, early life exposure to *A. lumbricoides* infection was associated with a borderline-significant increased risk of incident eczema. The power of the study in relation to these exposures was low due to the mass-deworming program continued throughout the study period, which is reflected in the wide confidence interval of the findings, and may have contributed to the lack of significant associations.

Similarly, the study found no evidence of the role of the commensal microflora enterococci, lactobacilli, and bifidobacteria. Broadly, particularly in the

longitudinal analysis, the effects of the commensal microflora were protective; however, none of these differences reached significance.

A detailed discussion of the findings along with the consistencies of the findings with other epidemiological studies will be presented in chapter seven.

7 DISCUSSION

In this section, the principal findings of the study will be summarized, by exposure of interest. The strengths and weaknesses of the study will be dealt with in more detail, and comparison of the study results with the available epidemiological studies, along with alternative explanations will be presented. The final section will draw an overall conclusion, and assessed, where appropriate, against the Bradford Hill criteria for causality. This will be followed by suggestions for possible future studies.

7.1 PRINCIPAL FINDINGS

7.1.1 Wheeze, allergic disease and sensitization

The findings from this study suggest that the prevalence and incidence of wheeze, eczema, rhinitis and sensitization in this young developing country population is low, with estimates all less than 10%. This can be contrasted with a 2 to 3-fold higher prevalence in developed countries,[32] however it is comparable with previous studies in under five children in Ethiopia.[97, 258] The study also showed that sensitization at the age of three increased the risk of incident wheeze (OR=1.44), eczema (OR=2.48) and rhinitis (OR=2.54), and reached statistical significance for new onset eczema and rhinitis, but not wheeze. Similarly, sensitization at age five and the reported prevalence of eczema and rhinitis were also positively associated and reached borderline significance for rhinitis but not eczema.

7.1.2 Effects of paracetamol

Paracetamol was commonly used in the study population (use in first year of life reported in over one third of infants), and significantly increased the risk of incident wheeze in a dose dependent manner between ages one and three. The effects were higher for use of more than four tablets per month, compared to never-use, with an adjusted odds ratio of 7.3. The risk of incident eczema was also increased, in the expected direction, but not to the point of significance, with an adjusted odds ratio of 1.7 for those taking more than one tablet per month compared to never users. Data on rhinitis and sensitization were not available in the first year of life; however, further, more detailed analysis of the effects of paracetamol including effects on rhinitis and sensitization and investigation of timing of exposure were explored during subsequent follow up.

In the follow up analyses of effects of early life use of paracetamol (ages one and three) on incident outcomes between ages three and five, similar significant positive associations were seen. Use in the first three years of life increased the risk of incident wheeze, eczema and rhinitis between ages three and five in a dose-dependent manner, independent of potential confounders. The effect on incident wheeze at this age was slightly weaker than the one seen between ages one and three, however that for eczema was stronger, and for incident rhinitis reached borderline significance. Early life paracetamol use and incident sensitization were positively related, and in the expected direction, but did not reach significance and the number of incident cases was very small. For all incident outcomes, there was no indication that a certain window of age, for example, exposure in the first year of life or later in life, was more critical than another.

Cross-sectional analyses were also performed, and whilst inferior to the longitudinal analyses as unable to assess temporality, they provided greater statistical power. The findings were in accordance with those from the longitudinal analyses, and showed a significant positive association between lifetime use of paracetamol (exposure at ages one, three and five) and the prevalence of the outcomes wheeze, eczema and rhinitis at age five, with adjusted odds ratios ranging up to six. The risk of sensitization at age five was also increased with lifetime exposure to paracetamol, with an adjusted odds ratio of 4.8. The associations were all dose-dependent with higher trends seen for prevalence of reported outcomes than sensitization.

Data on indications for use of paracetamol were not available at ages one and three, only at year five. Paracetamol was the analgesic and antipyretic drug of choice for 84% of the birth cohort mothers, and was readily available and affordable. Seventy eight percent of the mothers were able to differentiate paracetamol from aspirin. The most common indications for use of paracetamol at year five include headache, malaria, common cold, and fever of any origin. Use of paracetamol for wheezing illness, coughing episodes, and allergy-related diseases accounted for less than 5%. Whilst low, it was important to further explore the possibility of confounding by indication, particularly that arising from paracetamol being taken by children for respiratory tract infections in early life, who are also more susceptible to the subsequent development of allergic diseases.[254] Analyses were therefore adjusted for the main symptoms of respiratory infections (cough, fast breathing, and fever), as reported during the child's first year of life, and the wheeze, eczema and rhinitis association reduced in magnitude but remained statistically significant. The reduction was more pronounced for prevalent outcomes than incident outcomes. However,

adjustment of incident sensitization for respiratory infection slightly increased the odds ratio.

7.1.3 Effects of H. pylori

In this population-based birth cohort of young Ethiopian children, *H. pylori* infection was common with 41% of them infected at age three, and 44% at age five. A pattern of sero-conversion and sero-reversion with *H. pylori* infection was found such that 17% of children were infected at age three but not five, 21% at age five but not three years, and 25% at both ages, consistent with previous prevalence surveys in children in Butajira.[259] In the longitudinal analysis, a significant inverse association between early life exposure to *H. pylori* infection (exposure at age three), and the risk of incident eczema between ages three and five was found (OR, 95% CI, 0.31; 0.10, 0.94), and a similar effect was seen cross-sectionally at age three (OR, 95% CI, 0.49; 0.24, 1.01). In cross sectional analysis, a borderline-significant decreased risk of *D. pteronyssinus* sensitization was found in relation to *H. pylori* infection at age three (OR, 95% CI, 0.42; 0.17, 1.08), and a significant inverse association between current exposure to *H. pylori*, and any sensitization at age five (OR, 95% CI, 0.26; 0.07, 0.92). However, no significant associations, cross-sectionally or longitudinally, were seen for wheeze and rhinitis, and the directions of the ORs were inconsistent. No suggestion that exposure at age three was more important than current exposure or exposure after three years of life was found. This observation is a novel one in children of this age from a developing country with a high prevalence of early *H. pylori* infection.

7.1.4 Effects of geohelminth infection

The prevalence and intensity of geohelminth infection was low in the children in the birth cohort compared to previous studies within Ethiopia.[104, 203] The cumulative prevalence of any geohelminth infection up to age three was 12%, with median infection intensity of 7 eggs (interquartile range 3-23). The prevalence of this infection however increased with age, probably as mobility and cross-contamination increased, but remained low most likely because of the deworming program in the study area.[246] Whilst the risk of new onset wheeze was lower in those infected with any geohelminth infection in the first year of life than in those not infected, the 95% CI for this risk estimate was wide and significance not reached. No children with geohelminth infection in the first year of life reported incident eczema at age three, and therefore ORs could not be computed or further analysis performed. Similarly, no significant associations between any geohelminth infection and incident wheeze, eczema, rhinitis or sensitization between the ages of three and five were seen. However, a borderline-significant increased risk of incident eczema was seen in those exposed to *A. lumbricoides* infection in the first three years of life. The study found no indication that early exposure was more important than exposure later in life, but again, such analyses were limited by insufficient statistical power.

7.1.5 Effects of commensal bacteria

Children in this cohort at age three were found to be commonly colonized with enterococci (38%), lactobacilli (31%) and bifidobacteria (19%). In the cross sectional analysis, the presence of enterococci, lactobacilli and bifidobacteria in children's stools tended to be associated with an increased risk of allergic

symptoms and decreased risk of allergic sensitization, but none of the observed associations were statistically significant. Moreover, in the longitudinal analysis, no significant association was found between any of the commensal bacteria and incident wheeze, eczema, rhinitis or sensitization between ages three and five.

7.2 STRENGTHS AND WEAKNESSES OF THE STUDY

7.2.1 Birth cohort design

The use of a low income setting prospective birth cohort in assessing early life determinants of wheeze and allergic diseases has several strengths. The prospective design provides information on temporal relations between the exposure and outcome, and permitted analysis of the effects of early exposures on later development of allergic diseases. Reverse causation is therefore unlikely to have played a role in this study since the longitudinal analyses were based on those children who had never reported the outcomes early in life. Furthermore, the birth cohort design meant it was possible to explore the timing of exposures, and association with allergic diseases, which has barely been explored in most previous studies; in particular, to explore the importance of exposure in the first year of life which is thought to be the important window of life in terms of immune development.[140] The use of an unselected birth cohort to make very early measurements of potential exposures and allergic outcomes, and approaching the mothers during pregnancy minimized recall and reporting bias.

Selection bias is not likely to be a major issue in this study primarily because the study was based on unselected population based cohort of children. The study

also demonstrated very good retention of the surviving mother-child dyads up to the age of five years, with less than 6% lost to follow-up between birth and five. The majority of the losses to follow up were due to high infant and under-5 mortality rate in Butajira,[238] and Ethiopia as a whole.[227] Moreover, bias associated with non-response in this birth cohort was unlikely as only five women declined to participate in the study.

7.2.2 Representativeness of the cohort

The extent of comparable data on key variables between national figures[227] and this birth cohort suggests that it is highly representative of the wider population. For instance, the infant mortality rate in the birth cohort is 64 deaths per 1000 live births which compares with 59 per 1000 births for Ethiopia[227], while the under-5 mortality rate of 83 deaths per 1,000 live births compares with 88 per 1,000 live births for Ethiopia.[227] The demographic data including the urban-rural distribution, the proportion of literate mothers, distribution of environmental characteristics and lifestyle factors from the birth cohort are very similar to the wider Ethiopian population,[220] and compare well with the source population – the Butajira DSS.[236]

7.2.3 The study power

The sample size of the study was originally determined to address objectives other than this thesis.[240, 241, 251, 252] Post-hoc power calculation showed that the study has good power to detect an association between the exposures and the outcomes.[242, 253, 260] It was demonstrated that for an outcome with 8%

prevalence, our sample of children provided approximately 80% power at the 5% significance level to detect an odds ratio of around 0.45 for gastro-intestinal exposure,[253] and 2.00 for use of paracetamol.[242] However, relying on retrospective power calculation may introduce a degree of bias, and it may be that low study power disguised the effects seen, particularly for geohelminth infections, and other exposures related to sensitization outcomes, as seen by wide confidence interval.

7.2.4 Measurement error and information bias

7.2.4.1 Outcome reporting

Whilst the sensitization outcome was measured objectively, measures of wheeze, eczema and rhinitis were based on maternal questionnaire reporting, and are hence susceptible to reporting or information bias. The questions asked came from the widely used and validated ISAAC symptoms questionnaire,[25] which was administered by individuals known to the mothers, and which used common local terms (for example *'sit sit'* for wheezing symptoms, an onomatopoeic local term), all of which should reduce the degree of reporting error. In this low-income birth cohort, it was difficult to collect outcome and exposure data using daily diaries as the literacy level of many mothers was low (over 80% of the mothers had no formal education). Instead mothers were asked to recall symptoms, and poor recall may have led to a degree of error in the wheeze, eczema and rhinitis variables. Even though reported questionnaire-based symptoms were used, since most practical for a population based epidemiological study,[261] the lack of objective measures of asthma like BHR, lung function test and fractional exhaled NO (FeNO) is a limitation. Inclusion of

such measures would have likely reduced information bias, but these could not be included due to logistical issues in such a rural setting. The observation of a large proportion of children with reported wheeze ever at age one having a negative response to wheeze at ages three and five is likely to be explained by mothers not remembering wheeze symptoms experienced by their child at an early age which then resolved. This transient wheeze phenotype is thought to be infection-related and more common in those born with small airways,[262] and in our previous risk factor analysis of the cohort at age one, evidence of such a phenotype was found (positively associated with household size, and analgesic use during infancy).[242] The implications of poor recall on the findings are that some incident cases may have been missed if symptoms only occurred early in the one to three year follow-up period and did not persist up to the age of five, although such non-persistent cases are likely to be those with mild disease.

Reporting bias could also be a problem for the rhinitis outcome, particularly as a result of misclassification of symptoms of common cold and conjunctivitis. However, to reduce this bias this outcome was only measured from age three onwards and not measured at age one, consistent with other studies in very young children.[197, 199] Moreover, the observation that symptom reporting was associated with the sensitization outcome, albeit not perfectly, suggests relatively little misclassification.

Similarly, for reported eczema, itchy skin manifestations such as scabies or other skin conditions may be misclassified as 'eczema symptoms'. Whilst it has previously shown during the one year follow up of the cohort that the symptoms being reported appear to be linked to an allergic aetiology (positively related to

parental allergy, and negatively to sleeping on the floor and large household size),[242] some degree of misclassification cannot be ruled out. It was also seen in our group's previous validation work in Jimma in young Ethiopian children that the ISAAC criteria for eczema have a high negative predictive value (91%), taking clinical examination as a reference, but a lower positive predictive value (49%), suggesting the positive responses to the eczema question are not always true eczema.[258] However analysis of risk factors for eczema in the same survey population resulted in similar findings (direction and size of effects) regardless of whether eczema was defined using the ISAAC question or clinical examination.[103]

In general, even though the symptoms questionnaire was not validated in this cohort of children, the study utilized the same ISAAC questionnaires which have been successfully used by our group's previous work in adults in Butajira,[45] in under five children,[97, 263] and in older age groups in Jimma, Ethiopia.[43, 104] Moreover, incidence and prevalence of these outcomes in the study appeared to be positively related to sensitization; albeit weakly with wheeze, and also with parental allergy, suggesting that some of these are markers of allergy. Altogether, although it is difficult to completely exclude the possibility of reporting and misclassification bias in the study, which is inherent to use of symptoms-based questionnaires;[9] it is likely that this bias is non differential,[9] and so unlikely to affect the study findings.

Sensitization in the study was measured using skin prick testing to common household allergens previously found in Ethiopia.[104] However, the observation that a significant proportion of children were sensitized at ages three but not at

age five may indicate errors in measuring the wheal diameters. Reversal of sensitization status in early childhood has been reported in a range of studies.[264-267] However, the prevalence of reversal in this study was substantially greater than that seen by Barbee *et al,*[264] though reversal prevalences as large as 50% have been reported elsewhere.[265] Measurement error may introduce a degree of misclassification, though this bias is likely to be random, and the interrater agreement[247] of those individuals performing the test was reasonably good (kappa=0.75 for any sensitization).

7.2.4.2 Paracetamol reporting

Paracetamol use was also ascertained by self report in which mothers were asked about use in the past year, and additionally about consumption in the past month. Therefore maternal report of child's use of paracetamol is an issue owing to reporting bias, particularly those arising from medication taken by other siblings. However, the maximum time of recall was one year, and one month for dose exposure, designed to minimize recall bias, which has affected studies in which subjects were asked about first year paracetamol use several years later.[189]

Another issue is misclassification of the exposure due to the availability of multiple formulations of paracetamol and other non-aspirin nonsteroidal anti-inflammatory drugs (NSAIDs): in developed countries, for example in the UK, over 64 brands contain paracetamol.[218] This is unlikely to be a source of bias in this rural setting as multiple formulations of this drug are uncommon. Our group's previous qualitative and quantitative work in the same setting has

shown that the study participants in Butajira were able to distinguish different analgesics, and that the majority of the general population in the study area know paracetamol to be different from aspirin, [203, 213] Similarly, in this birth cohort it was found that over 78% of the mothers can distinguish between strips of paracetamol and aspirin, indicating that misclassification of drugs is unlikely.

Bias arising from arbitrary classification of frequency of paracetamol use is another issue related to paracetamol exposure. This is possible, since there is no standard classification of dose of paracetamol, however we have utilized broadly similar categorization to the previous multicenter studies.[189, 190] Since there is no reason that allergic subjects would be more or less likely to misreport paracetamol use, any reporting error is likely to be non-differential and would therefore result in the odds ratios being biased towards the null value; which contrasts with the large odds ratio observed in the study.

7.2.4.3 Geohelminth, H. pylori, and commensal bacteria measurement

Measurement of geohelminth intensity level using the sedimentation method, unlike the Kato-Katz technique,[268] is liable to miss eggs, though the method is generally sensitive in identifying true positives.[268] This may be due to eggs being trapped by debris or floating, resulting in eggs being lost during the sedimentation process. However, in view of the fact that the intensity of infection in the study area was generally low,[45] the chances of missing helminth eggs are small. The sediment of the concentration was also examined in three slides per sample, which should have increased the yield of the test.

H. pylori was measured objectively using stool antigen testing, an acceptably reliable means to determine current infection status in young children,[249] although we were unable to specifically measure the strains of *H. pylori*. Other studies have shown protective associations to be particularly strong for *H. pylori* possessing cagA strains.[269]

The detection of the faecal microflora was based on stool bacteriological culture identified to the genus level using a microscope and analysed qualitatively. Even under optimal laboratory conditions with good anaerobic bacteriological techniques, only 25 to 40% of these commensals are cultivable and identified microscopically.[270] The non-cultivable bacteria can only be identified using molecular techniques.[271] Furthermore, the selective culture media used in the study may not be completely selective, in particular, the culture of bifidobacteria using this method may underestimate the bacterial population,[272] However, it is important to note that previous epidemiological studies using similar approaches have shown differences in microbial composition (particularly lactobacilli and bifidobacteria species) between allergic and non allergic subjects.[144, 150, 152] Moreover, in the study it was only measured selected commensal bacteria commonly hypothesized in the literature; and since the gut is colonized by a large number of micro-organims,[273] the approach may miss other commensals.

In addition, the study has no information on the exposure status of the children younger than three years, particularly for *H. pylori* and commensal bacteria, and it is possible that considering all infections (incident and prevalent) as incident has led to an underestimation of the effects of these infections. The implication of this is that the study might have missed the effects of these exposures in the

first year of life. One other issue in relation to the analysis of *H. pylori* exposure is the issue of multiple testing (the probability that one can reject the null hypothesis among multiple analyis.[274]) Even though, this may be considered in the interpretation of the findings, the consistency of the results on both cross-sectional and longitudinal analysis, and that these associations were seen among different outcomes at different time points, made the likelihood of bias associated with multiple testing less likely.

7.2.5 Residual confounders

Unmeasured confounders may play a role in the study, though the study was designed to try to include most hypothesized early life risk factors common to developing countries and linked to asthma and allergy in the published literature. The collection of these potential confounders, including markers of socio-economic status and dust allergens from child's bedding, allowed an adjusted analysis to be explored. Of particular concern was confounding by socioeconomic status, as this may influence access to healthcare and health seeking behaviour. This marker was based on maternal education, and area of residence, as there is no previously validated measure of socio-economic index in the area. However, none of our markers of socioeconomic status were associated with the outcomes or with child's use of paracetamol, which make socioeconomic differences unlikely to explain these findings.

Although we have not collected information on pollution and dietary habits, both the urban district of the study area (less than 15% of the study population), and the rural districts (over 85% of the study population) have no industry, little

motorized transport, and a diet based on locally produced crops. Therefore the effects seen are unlikely to be due to residual confounding by risk factors including air pollution and heterogeneity in dietary habits. Furthermore, we have no information on several exposures previously linked with asthma and allergy,[196, 275] particularly geohelminth infection and paracetamol, during pregnancy, and hence were unable to adjust for possible prenatal exposures.

In relation to paracetamol exposure, one issue in previous studies has been confounding by indication: paracetamol may be given for a respiratory illness that increases the risk of a child developing asthma and allergic conditions.[212, 276] The study was unable to prospectively record respiratory infection episodes, or specifically measure indications for use of paracetamol during infancy due to difficulty in ascertaining this information in this cohort. We have, however, data on symptoms of infantile respiratory tract infection in the first year of life and these effects were adjusted for in the multivariate analysis. Furthermore, information on use of non steroidal anti inflammatory drugs (NSAIDs) and use of aspirin that may be linked to wheeze and allergic diseases[201] were not collected early in life as they are not readily available or affordable in this rural community.

7.3 CONSISTENCY OF THE FINDINGS

7.3.1 Paracetamol, wheeze and allergic diseases

Most studies, that have focused on wheeze or asthma, though primarily cross-sectional, have demonstrated an increased risk of asthma and allergic conditions

among paracetamol users.[187, 189, 190, 192, 193, 195, 197-206] This study from a low-income population based birth cohort has confirmed these previous observations, and found that persistent and heavy exposure of paracetamol increases the risk of wheeze in a dose-dependent manner, independent of symptoms of respiratory tract infections. The current findings fit with a recent meta-analysis of studies reporting a 63% increased risk of asthma associated with paracetamol use in children and adults (OR, 95% CI, 1.63; 1.46, 1.77).[193] These findings were also consistent with the ISAAC multicountry study, the largest study so far, on use of paracetamol in the first year of life, exposure later in childhood and risks of asthma at the age of six and seven years, although ORs were not as large as those seen here (OR for asthma=1.46 for use vs. non users).[189] Similar exposure-dependent increased risks for current use of paracetamol and symptoms of asthma, also fitting with the current study, were reported from the ISAAC study in adolescents aged 13- to 14-years.[190] Despite several strengths of these studies including their multinational nature, their power, and the consistency of findings across centres, the cross-sectional nature and retrospective recall of the exposure introduce a degree of bias;[189, 190] and probably accounted for the difference in the risk estimate. The current study also concurs with our group's previous study in older children and adults in the same Butajira study area which reported a similar level of paracetamol use (42%), and a significant dose-dependent association with wheeze.[203]

Prospective evidence has been limited to a handful of studies showing a positive relation between paracetamol exposure in the intrauterine environment, particularly in late pregnancy, and the risk of allergic diseases in childhood.[197-200, 211, 215] The Avon Longitudinal Study of Parents and Children (ALSPAC) study, for example, showed significant and dose-dependent associations between

frequent paracetamol use in late pregnancy (most days/daily) and wheezing in children at age 30-42 months and later at age 69-81 months, although the ORs were not as large as those seen here (2.10 and 1.86, respectively).[197] A recent meta-analysis of the available observational studies reporting exposure *in utero* and risk of childhood wheeze demonstrated a pooled OR of 1.21 (95% CI, 1.02, 1.44), irrespective of gestational age,[196] which also fits with the previous meta-analysis that reported a pooled odds ratio of 1.50.[193]

The Melbourne Atopy longitudinal Cohort Study has also reported finding relating personal consumption of paracetamol with risk of asthma in children that are broadly in line with our findings.[212] This study followed 620 children with a family history of allergic disease and documented paracetamol use from birth to two years and outcome measured at six and seven years.[212] The study showed a crude increase in the risk of wheeze among children exposed to paracetamol early in life. However, no association remained after adjustment for respiratory tract infection (otitis media, tonsillitis and throat infection), unlike the current study in which a significant association remained. Since the study adjusted for multiple infections, bias associated with over adjustment in the model is difficult to exclude. Moreover, since the study was conducted in children with a family history of allergy, there is the possibility that parents with asthma may avoid giving their child aspirin and hence an association arises because children with genetic susceptibility are more likely to have superimposed infections, and be given paracetamol. However, in the current study in Butajira, it has previously been established that only 1% of the population in the study area reported avoidance of aspirin due to asthma risk.[213] The study also fits with the findings of a prospective study in adults that reported a positive relation between paracetamol use and adult-onset asthma in women.[206]

The current study also fits with the sole US-based RCT of the use of paracetamol compared to ibuprofen in febrile asthmatic children aged 6 months to 12 years, which showed an increased rate of hospitalization and out patient visits for asthma in the paracetamol arm vs. ibuprofen, reaching significance for outpatient visits (OR, 95% CI, 1.79; 1.05, 2.94).[201] However, since the study did not include a placebo arm, it is impossible to determine whether the effect came from a relative efficacy of ibuprofen or due to an adverse role paracetamol as acknowledged by the authors.[201]

The study has also demonstrated a dose-dependent increased risk of eczema symptoms among children exposed to paracetamol early in life. In agreement with the current findings, studies, mostly cross-sectional, have reported similar positive associations between paracetamol use and eczema.[189, 190, 203] The ISAAC multicountry study in children[189] reported that use of paracetamol for fever during infancy significantly increased the risk of eczema later in life (OR=1.35, for use vs. never). The same ISAAC Phase Three investigators also recently reported that use of paracetamol in the past 12 months was associated with an increased risk of eczema. In both studies, the associations were found in most major regions and adjusted for main confounders. The magnitudes of the ORs reported by the ISAAC studies, however, were not as large as seen in our study. This difference might be due to the different level of paracetamol intake, presence of residual confounders, for example, confounding by indication or difference in the windows of exposure.

Longitudinal studies exploring the link between paracetamol and eczema symptoms are scarce. The Melbourne Birth Cohort Study found no significant

association between use of paracetamol up to two years on eczema among children aged five to seven years controlling for infections.[212] The ALSPAC cohort studies in pregnancy also showed no associations between paracetamol exposure *in utero* and the risk of eczema.[197, 219] The reason why these prospective studies have not found independent associations between paracetamol use and eczema, unlike the current study, may be attributed to low statistical power or misclassification of eczema symptoms, or there may be no true effect and underlying mechanisms relate specifically to the lung.

The study also found that use of paracetamol increased the risk of rhinitis; however, the association was not as strong as seen for wheeze and eczema. Other studies have also reported similar positive associations between rhinitis and paracetamol exposure in children.[189, 190] The ISAAC cross-sectional study demonstrated a dose-dependent increased risk of first year of life use of paracetamol and the risk of rhinoconjunctivitis in children aged six to seven years (OR=1.48, for use vs. never).[189] A similar dose-dependent increased risk was reported among children exposed to paracetamol aged 13 to-14 years (OR=2.39, high dose vs. never). A previous cross-sectional study in the same study population in Butajira also found a 2.5-fold increased risk of rhinitis in those using over 3 tablets per month.[203]

Like the other outcomes in the study, only a few longitudinal studies have explored the associations between use of paracetamol and rhinitis, and have reported conflicting findings.[212, 219] The Melbourne Birth Cohort Study demonstrated a borderline increased risk of rhinitis in children exposed to paracetamol early in life.[212] However, the association disappeared once adjusted

for frequency of infections;[212] unlike the current study where the association remained significant. The prenatal longitudinal study exploring *in utero* exposure and the risk of off-spring asthma reported weak evidence between paracetamol use in late pregnancy and risk of rhinitis.[219]

The study also demonstrated a borderline significant, albeit less strong, association between sensitization at age five, and lifetime exposure to paracetamol at ages one, three and five. There are few, and conflicting, reports of epidemiological studies that have investigated the effect of paracetamol on atopy.[202, 203, 212, 219, 276, 277] Among the cross-sectional studies, an ecological study of paracetamol sales in children and adults reported some evidence of a positive association with atopy but not with total IgE.[202] Our group's own previous study in Butajira showed increased risk of cockroach sensitization, but not *D. pteronyssinus* sensitization with exposure to paracetamol in children and adults.[203]

Among the prospective studies, a study by Wickens[277] showed paracetamol exposure in the first year of life to be associated with a 3-fold increased risk of atopy at the age of six years. However, they found no association between current use and atopy at six years of life.[277] Although the current study found no significant associations between early exposure to paracetamol and incident sensitization, the effects seen were large and in the expected direction. This longitudinal analysis however was likely affected by power, as the incidence of sensitization in the study was very low (15 incident cases).

In conclusion, the findings of this study fit with a range of epidemiological studies in children and adults implicating exposure to paracetamol and the risk of wheeze and other allergic diseases. Unlike the longitudinal study by Lowe and colleagues,[212] on controlling for symptoms of respiratory tract infection, the association between paracetamol and wheeze, eczema or rhinitis reduced in magnitude, but overall significance remained. Confounding by indication is therefore less likely to have been responsible for the overall consistent association seen between paracetamol and our allergic outcomes. However, as an observational epidemiological study, the possibility that residual confounding, particularly by respiratory infection at an early age, is difficult to exclude. If present, the implication for this in the study findings are that the odds ratios reported may overestimate the actual risk.

7.3.2 Gastro-intestinal infections

7.3.2.1 H. pylori, wheeze and allergic diseases

Most studies investigating the link between *H. pylori* infection and asthma and allergic disease, to date, are cross-sectional[121-128] or case-control.[58, 119, 129-133] Studies in young children, particularly those from developing countries, are remarkably scarce, with only three studies reporting links in children.[121, 122]

In this study, a significant inverse association between early life exposure to *H. pylori* infection and the risk of incident eczema between ages three and five (adjusted OR=0.31) was found and a similar effect was seen cross-sectionally at age three (adjusted OR=0.49). These findings are in agreement with the

available cross-sectional studies in children.[121, 122] Amongst the US based studies in children (age 3-19 years), the National Health and Nutrition Examination Survey (NHANES IV), reported significant inverse association between *H. pylori* seropositivity and eczema (adjusted OR=0.73).[121] Our finding is also consistent with data reported by Herbarth *et al*[122] from Germany who found a 63% reduction in doctor-diagnosed eczema in children (mean age 6.3 years) exposed to *H. pylori*. The difference in size of the ORs could be due to variation in age, outcome ascertainment, level of infection, for example the prevalence of *H. pylori* in these studies was <10%[121, 122] compared with 41% in ours, and difference in measurement of infection status (serology vs. rapid stool antigen test used in the current study).

Alternative explanations for the association seen in this study, such as reverse causation, are unlikely to explain the effects of *H. pylori* on eczema. Although reverse causation may be an issue in the cross-sectional analyses, replication of findings in the longitudinal analyses excludes this possibility. One other issue could be that *H. pylori* status is a proxy indicator of other infections or socio-economic conditions.[122, 135, 278] To explore such a possibility, the findings were controlled for markers of socio-economic status which may have confounded the previous study,[126] and adjusted for other infections including geohelminth infections, and commensal bacteria, but no evidence was found to support this argument.

This study also provides some evidence, though cross-sectional, for an effect of *H. pylori* on sensitization at age three (adjusted OR=0.42) and five (adjusted OR=0.26). Most of the available observational studies in children did not

specifically explore the effects of *H. pylori* on sensitization.[122] [121] In a study comparing Finnish and Russian children, the prevalence of atopic sensitization was higher in Finland than Russia, and it appeared that this was due to an inverse association with *H. pylori* infection.[133] Even though it is difficult to draw direct inference from studies in adults, our findings fit with cross-sectional studies in these age groups.[58, 123, 130] The NHANES III survey in the US demonstrated an inverse association with sensitization to pollen and moulds, with a greater effect seen in younger (median age <43 years) and cagA$^+$ subjects (OR=0.69).[123] However, the same group of investigators in another study reported no association between *H. pylori* infection and serum IgE.[130]

One explanation for these findings might be confounding by use of antibiotics, in that use for asthma or allergy may in turn affect *H. pylori* infection, and hence reverse causation. Even though, this remains a possibility, in the current study in children from a low-income cohort with limited access to standard antibiotics and no *H. pylori* eradication program, this was less likely to be a source of bias. Moreover, though age at acquisition was not assessed in this study, the fact that *H. pylori* infection occurs in early childhood[259, 279] and persists for life,[280] makes it unlikely that sensitization precedes *H. pylori* infection.

The study however did not detect an effect of *H. pylori* on wheeze or rhinitis in this cohort of young children on both cross-sectional and longitudinal analyses. One previous study in children, the NHANES IV study, showed reduction in ever having had asthma (OR=0.69) and allergic rhinitis (OR=0.60) in *H. pylori* infected subjects.[121] Another US based study in adults also reported inverse associations, and it appeared that the effects were strong in younger adults

(median age <43 yr), for asthma and rhinitis cases with onset during childhood (≤15 yr), and in those infected by cagA$^+$ strains.[123] Contradictory findings have also been reported from most case-control studies.[58, 119, 131-133] Two UK based case-control studies by Jarvis *et al*[124] and Bodner *et al*[132] found no association between *H. pylori* infection and wheezing, rhinitis or atopy, including with *H. pylori* cagA$^+$ strains.[131] However, these inconsistencies might also be due to small number of cases and controls included in the studies or could be due to bias associated with the selection of cases and controls.

The lack of associations with the reported wheeze and rhinitis in the this study may be due to mechanisms other than those related to wheeze or asthma physiopathology, or to outcomes partly unrelated to allergic phenotype.[14-16, 281] Another explanation may be that the effects relate mainly to *H. pylori* cagA$^+$ strains, as this was reported to have strong effect on asthma,[123, 135] and this study has no data on cagA serology. However, a previous study in Ethiopian dyspeptic patients has shown that cagA genes were detected in 79% of the study subjects,[282] suggesting this may be the dominant strain in the population, and perhaps also explaining the strong protective effects seen in the study.

In conclusion, this study from a low-income birth cohort in young children provides evidence for a protective role *H. pylori* on eczema and sensitization, both of which have been previously reported to be strongly linked to the risk of childhood asthma.[23, 283]

7.3.2.2 Geohelminth, wheeze and allergic diseases

Evidence from epidemiological studies on the hypothesis that geohelminth infections may protect against asthma and allergic diseases has been summarized in two meta-analyses.[108, 109] The first one found a strong, intensity-related reduction in asthma risk with hookworm infection (pooled OR, 0.50; 0.28, 0.90), but a significant increased risk with *A. lumbricoides*.[108] The second meta-analysis showed a 31% reduction in atopy among those infected with *A. lumbricoides*, and a similar reduction with *T. trichiura* and weakly associated with hookworm.[109] In contrast to these earlier observations, in this study however, early life exposure to geohelminth infections was not associated with incidence or prevalence of our allergic outcomes. Although, the risk of new onset wheeze was lower in those infected with any geohelminth infection in the first year of life, the 95% CI was wide and significance not reached.

Whilst this study showed no significant associations, the analyses had limited statistical power as a result of the low prevalence and intensity of geohelminth infection early in life, and small numbers with the outcomes amongst the infected children. Geohelminth infection prevalence was much lower than our group's previous studies both in Butajira, [45] and Jimma in south west Ethiopia,[97] (prevalence up to year three: any geohelminth infection of 12.2% compared with 33.8% in Butajira five year and more olds,[45] and 69% in Jimma one to four years old children[97]). Intensity of infection was also much lower (median number of eggs per gram of faeces among those positive for hookworm was 6 (range 1-134), and for *A. lumbricoides* 12 (range 1-475), vs. 30 (range 3-895) and 167 (range 2-31,378) in Jimma young children). In both children[97] and adults[104] in Jimma, a strong protective effect of hookworm infection was reported in relation

to wheeze (in adults, OR= 0.48, 95% CI; 0.24, 0.93[104]) and a dissociation of allergic sensitization from the risk of wheeze was seen due a protective effect of high intensity geohelminth infection.[104] Whilst these findings have not been replicated in the current study, the current findings are consistent with our group's work in Butajira, southern Ethiopia, in a similar study population, with a relatively lower prevalence and intensity of geohelminth infection[45] than Jimma,[97, 104] and showed no significant protective effect of geohelminth infection against wheeze or asthma, but a weak inverse association with cockroach sensitization.[45]

Possible explanations for the low prevalence of geohelminth infection in the study area include: (1) a biannual mass de-worming programme, the Enhanced Outreach Strategy for Child Survival (EOS),[284] a campaign similar to Child Health Days in other countries, that started in 2006, shortly after the cohort established, in under-five year olds.[255] Indeed, almost half of the children reported having taken at least one dose of anthelminthic medication in the past 6 months at age five; (2) siblings in the household may have received the treatment which may also diminish cross-transmission and; (3) the study participants were provided with free medical care, in keeping with the Ethiopian ethics requirements, which might have promoted treatment seeking behaviour which in turn affects infection prevalence. Overall, the main consequence of de-worming, which tended to be non-differential and coupled with low outcome prevalence, would be to diminish the ability to measure any effect of geohelminth infection on allergic outcomes or sensitization.

The other explanations for the lack of an association in the study may be that

the study had no data to assess the effect of *in utero* exposure to geohelminth infection on childhood allergy and sensitization. Even though it did not appear that exposure in the first year of life was more important than exposure later in life, this may have been important since infection with parasite antigens often starts *in utero*,[285] affecting early immune programming. An intervention study during pregnancy has shown some indication that this is the case.[286]

Despite this observation, however, the study showed a borderline significant, albeit with wide CIs, increase in eczema risk in those infected with *A. lumbricoides* infection, and is consistent with a previous meta analysis that showed positive association with asthma.[108] Even though, this may a chance occurrence, the reason for this apparent increased risk of eczema symptoms is not clarified in the study. A possible explanation may relate to the effect of *A. lumbricoides* infection, particularly at low intensity, in triggering symptoms of asthma,[107] and inducing the release of inflammatory mediators.[287] It has been documented that low intensity and transient infection with *A. lumbricoides*, also the case in the present study, may promote allergenicity, partly through stimulation of IgE synthesis,[288] and partly through an increase in the number of circulating Th2 cytokines.[289] High intensity and more persistent infection, in contrast, may stimulate excess polyclonal IgE capable of blocking an allergen response,[288] and this is the case in our group's previous study in Jimma.[104]

The lack of a protective association in the present study also fits with the findings of intervention studies among previously exposed subjects by Cooper *et al*[105] and Elliott *et al*[275] and among unexposed individuals by Feary *et al.*[106] Cooper and colleagues conducted a randomized controlled trial looking at the

effect of albendazole treatments on the prevalence of atopy in school children and found no evidence to support the hypothesis that deworming may increase atopy or clinical allergy.[105] Elliot *et al*'s[275] study in Uganda reported that albendazole treatment during pregnancy was associated with a non significant increased risk of infantile eczema up to 15 months of age. The first hookworm trial in the UK also showed no difference in airway responsiveness, markers of asthma morbidity or atopy between the intervention and the placebo arm.[106] A number of factors may explain the discrepancies in the findings between the meta-analysis and intervention studies. Firstly, consistent inverse associations between geohelminth infection and atopy,[108] and less consistent associations with clinical allergy[290] have been reported mainly from cross-sectional studies in which residual confounding may partly explain these associations. The second explanation may be reverse causality: allergic disease may confer protection against helminth infection. Studies have shown that atopic children produce higher levels of IL-4 and IL-5 in response to *A. lumbricoides* infection,[289] and are more resistant to hookworm infection.[291] However, longitudinal analysis, like this study, makes reverse causality less likely to be the explanation for this argument. However, other explanations including choice of the target group[106] (with or without established disease or with or without disease onset), target parasite and infection endemicity (particularly relevant for eradication studies)[105] and other residual confounders cannot be ruled out.

In conclusion, the evidence on the hypothesis that geohelminth infection protects against wheeze and allergic disease is conflicting; with strong support from observational studies, but most interventional studies to date are disappointing. The current birth cohort study does not prove or refute this hypothesis due to small numbers infected resulting in insufficient power to

determine independent effects, requiring careful interpretation.

7.3.2.3 Commensal bacteria, wheeze and allergic diseases

The 'microflora hypothesis' first debated by Sepp and colleagues[143] about two decades ago, has since received support and been reviewed by Penders *et al.*[140] Despite high prevalence of microflora colonization, our study found no significant associations between the commensals enterococci, lactobacilli or bifidobacteria and the incidence or prevalence of our allergic outcomes, but the ORs were broadly protective. The largest observational studies so far are a birth cohort study by Bisgaard *et al*[153] and a multicenter comparative cross-sectional study by Ege *et al.*[88] The former included 411 children followed for six years and showed significant inverse associations between diverse bacterial colonization, but not specific bacterial strains, during infancy, and allergic sensitization, blood eosinophil count, and allergic rhinitis, but found no association with asthma or atopic eczema.[153] The multicenter study compared children living on farms with the reference group, and a diversity of bacterial and fungal genera were found to be protective of asthma with an odds ratios of 0.37 and 0.57 respectively.[88] Even though both studies did not identify specific microbes, the findings support a more recent notion that it is the dynamic bacterial diversity rather than a particular bacterial strain that plays a role in allergic disease. The European birth cohort study of infants followed up to 18 months in the UK, Italy and Germany however showed no association between colonization by commensal bacteria early in life and atopy or eczema.[161]

Several issues may have accounted for lack of significant association in the

current study or conflicting findings in the literature. Primarily, most of the studies that have shown a difference in microbial composition among allergic and non allergic individuals have measured exposure during the neonatal period,[150, 154] or in the first year of life.[140, 144, 151, 152, 155] It has been suggested that the first year of life is a critical window period for immune regulation,[154] and the first three months are often suggested as optimal timing for analyzing commensal bacteria.[140] Even though there exist legitimate arguments[159] about gut microbiota composition (also dependent on several factors including breastfeeding),[140] they remain a potential explanation for this study as the study lacks this data during infancy. Secondly, our detection of the faecal microflora based on stool culture might miss non-cultivable bacteria, particularly anaerobes which could be identified better using molecular techniques.[271] Thirdly, the other issue may relate to only investigating certain commensal bacteria as suggested in the literature.[144] Given the complex nature of the gut microbiota,[292] and the fact that the gut is colonized with more than 10^{14} micro-organisms,[273] the study approach may have missed other important commensals. For example, the Danish large-scale birth cohort study found no significant association, like ours, between colonization with bifidobacteria in the first months of life and seroatopy and eczema at ages two,[154] which was also replicated in a nested study by the same group.[160] However, the study did find that *Escherichia coli* (*E. coli)* and *Clostridium difficile* (*C. difficile)* were positively associated with increased atopy and eczema, respectively.[154] Fourth, it may be that we have no data at species level, and some studies have shown that the effect of microflora on allergy may depend on the species within the genera. For example, Suzuki *et a*[155] reported different species of bifidobacteria that have distinct roles in allergy. Finally, the alternative explanation may relate to confounders, particularly those arising from antibiotic use,[293] difference in dietary habits including formula feeding,[141]

and mode of delivery[161] all of which, in one way or another, determine colonization pattern. However, these confounders are relatively unimportant in this rural low income cohort, and since the multivariate model was adjusted for potential confounders, this is an unlikely source of bias here.

Overall, this study showed no associations between selected commensal bacteria and allergic diseases in an Ethiopian birth cohort, whilst the existing literature from available large scale studies provide conflicting findings as to the role of a specific bacterial strain, and suggest bacterial diversity may be important.

7.4 CONCLUSIONS AND CLINICAL RELEVANCE

7.4.1 Paracetamol, wheeze and allergic disease

This thesis prospectively explored the effects of paracetamol, and provides evidence for its role in the pathogenesis of wheeze and allergic diseases in children. Together with previous studies and taking into account the Bradford Hill criteria,[294] this study may suggest a cause-effect relationship.

First, the reported strength of association is large with an odds ratio of up to four. Secondly, the evidence for this association is remarkably consistent *in utero*, during infancy, in childhood, and in adults irrespective of differences in socio-economic and cultural variations.[189-191, 202] The increased risk was also seen consistently with wheeze, eczema, rhinitis and to a lesser extent with sensitization, and consistency was seen across different analyses at different

time points. Third, the association found in this study (particularly for lifetime paracetamol exposure), and elsewhere by others,[189, 190] is dose-dependent with higher risk seen among frequent and heavier users which support the biological gradient. Fourth, the longitudinal nature of this study excludes, albeit not perfectly, the possibility of reverse causation and suggests that exposure precedes the outcome. This is also consistent with the findings that suggest *in utero*[197-199, 214, 215] exposure to paracetamol increases the risk of asthma. Fifth, the finding supports the hypothesis that the temporal increase in asthma[32] corresponds to widening use of paracetamol in most developed countries,[187, 189-191] and elsewhere in Africa.[47]

Finally, the finding fits with plausible mechanisms from *in vivo* and *in vitro* investigations. Therapeutic doses of paracetamol have been shown to reduce serum antioxidant capacity within 14 days,[295] possibly through a reduction in Glutathione (GSH) levels,[216, 295] with the lung bearing the brunt of this depletion.[296] Glutathione is an antioxidant that metabolizes N-acetyl-p-benzoquinoneimine (NAPQI) - an active free-radical or reactive oxygen-producing compound of paracetamol,[297] which contributes to oxidative stress, and inflamation.[298, 299] Glutathione depletion may also cause a shift from Th_1 to Th_2 cytokine production, favouring allergic disorders.[300] Other observations have shown that therapeutic concentrations of paracetamol decrease GSH levels in human pulmonary macrophages *in vitro*, and in animal alveolar macrophages, and type 2 pneumocytes.[217] Paracetamol may also contribute to the increased risk of asthma, via NAPQI through transient receptor potential ankyrin-1 (TRPA1) channel, and neurogenic pathways.[301] This study showed that NAPQI-dependent TRPA1 releases proinflamatory cytokines not only in the airways, but also in the skin, suggesting involvement beyond the airways, for instance, in

eczema.[301] A study has also shown that glutathione is present in nasal fluid, and therefore depletion via active metabolites of paracetamol (NAPQI), could explain the increased risk of rhinitis and rhinoconjuctivitis.[302]

Overall, this study provides further and much needed prospective evidence that paracetamol may be involved in wheeze and allergic disease aetiology. The findings are congruent with other studies which are larger, and making a causal explanation increasingly likely.

7.4.2 H. pylori, wheeze and allergic disease

Among the studied gastro-intestinal infections, this study provides some evidence for a protective effect of *H. pylori* on allergic disease but careful consideration is necessary in drawing causality. This finding is consistent with a range of epidemiological studies that showed inverse associations between *H. pylori* and allergic diseases,[135] and with a secular trend that as *H. pylori* has declined,[118, 119, 303] asthma prevalence has risen.[32] But, most of the findings to date are derived from cross-sectional or case control studies, and based in developed countries, in which reverse causation is difficult to exclude, and hence are less coherent. Moreover, this study did not demonstrate an effect on wheeze or rhinitis and therefore lacks specificity among Hill's criteria.[294] The large odds ratios found in this study, however, suggest a strong association, and are unlikely to have arisen by chance or due to alternative explanations including confounding by social advantage or antibiotic therapy. Moreover, the use of a birth cohort and the longitudinal analyses provide evidence that exposure to *H. pylori* precedes eczema.

Plausible mechanisms in support of these observations also exist. Studies have shown that *H. pylori*-induced inflammatory response is associated with Th1-mediated cellular responses,[137, 269, 304, 305] with higher expression of interferon-γ (IFN-γ),[137] IL-10, and IL-12.[304] Particularly strong effects were seen in those possessing the pathogenic cagA$^+$ strains.[269, 304] Work by D'Elios and colleagues has shown that administration of purified neutrophil-activating protein of *H. pylori* (HP-NAP) can inhibit Th2 responses, eosinophilia and IgE in an OVA mouse allergy model.[134] The protective effects of *H. pylori* against allergy are also mediated by secretion of regulatory T-cells (Tregs)[269, 306] that suppress immunity and inflammation via bystander effects of IL-10.[138, 306, 307]

Overall, the observation is a novel one in children of this age from a developing country with a high prevalence of early *H. pylori* infection, and suggests that *H. pylori* may be involved in the aetiology of allergic disease, and also warrant further investigation.

7.4.3 Geohelminth, wheeze and allergic diseases

The study however found no significant association between early life exposure to geohelminth infections and incidence and prevalence of allergic disease and sensitization; and has not been able to much advance our knowledge on this relation. The prevalence and intensity of geohelminth infection in these young children were much lower than levels previously reported to confer protection against asthma,[104] and the observation that infection with *A. lumbricoides* marginally increased the risk of eczema symptoms supports the notion that low infection intensity may induce symptoms,[107] while heavy and chronic exposure

may promote protection against asthma and allergy.[104] Therefore, it is possible that the lack of a significant association in this study can be attributed to its low statistical power, probably due to the de-worming programme, and a case for future study.

7.4.4 Commensal bacteria, wheeze and allergic diseases

Similarly, the study found no evidence to support the etiological role of the intestinal microflora enterococci, lactobacilli and bifidobacteria in allergic diseases or sensitization. However, given the timing of the measurements of these commensal bacteria in the study (age three), and the methodological limitations of a culture-dependent bacteriological technique; the relation between commensal bacteria and allergic disease remains worthy of further investigation.

7.5 SUGGESTIONS FOR FUTURE RESEARCH

The role of paracetamol in the aetiology of asthma is intriguing, and this enigma in asthma clearly merits further investigation. As most of the evidence to date comes from observational studies, a randomized clinical trial (RCT) is needed to generate definitive evidence before reviewing public use in children. The questions that need to be addressed in RCTs are whether paracetamol causes asthma, and whether paracetamol exacerbates asthma. Designs for these might present ethics dilemmas, since the former ideally requires a controlled trial in pregnancy (or in children) and the latter a design in which the hypothesis is that active treatment makes asthma worse; the alternative might therefore be trial

designs involving withdrawal of paracetamol in relevant groups using non-aspirin NSAIDs analgesics, for instance codeine phosphate, as placebo. However, such a trial should be adequately powered with good length of follow-up to allow accumulation of the primary outcomes. Study of mechanisms and genetic variants would be important additional areas of investigation, since studies have reported involvement of GSTP1 genetic variants in the causal pathway.[215, 308]

Even though a RCT would provide important insight into the role of paracetamol in the development of asthma, if it is impossible to take forward due to ethical concerns, the study findings need to be replicated in a well-powered birth cohort study primarily aiming at investigating such a link. Such a birth cohort should consider first, prospective documentation of indications for use of paracetamol, including respiratory tract infections. Second, the length of follow-up should be longer, to account for different wheezing phenotype since it has now been widely acknowledged that transient wheezing early in life may represent an infection phenotype, and may not be a predictor of later asthma.[14-16] There should also be stringent ascertainment of outcomes, using combinations of methods including objective measurements. Finally, the possibility of confounding by other NSAIDs should be minimised, through using a population unlikely to access them or through following prescription and over-the-counter use patterns.

The inverse association between *H. pylori* and allergy observed in the study and elsewhere also clearly requires further investigation. Almost exclusively the evidence to date comes from cross-sectional and case-control studies, and reverse causation is difficult to exclude. Hence, future investigation using a prospective birth cohort study is warranted. Such a birth cohort should be

adequately powered and include information on: (1) *H. pylori* virulence including cagA$^+$ strains; (2) age at acquisition of *H. pylori* infection; (3) objective measures of asthma, and allergy, and (4) potential confounders including antibiotic use which could help to exclude confounding by indication. One other possible study could be a quasi-experiment comparing areas receiving *H. pylori* eradication therapy and areas without the program. However, the problem of this kind of intervention study is that antibiotics may be given for other infections unrelated to *H. pylori*, for example upper and lower respiratory tract infections.[309] Further investigations of the immunological mechanisms behind this effect might have therapeutic and preventive value.

The role of geohelminth infections in asthma and atopy also requires further investigation. Further detailed prospective birth cohort studies, possibly followed starting from pregnancy, as infection with parasite antigens often starts *in utero*;[285] would provide important insight into this relationship. These studies should take into account deworming programmes (and areas with sufficient prevalence) and potential confounders, and that also include objective outcome measurements. Longer follow-up of a birth cohort may help for two reasons: firstly, infection with geohelminths increases with age, particularly hookworm,[310] adding to the power of the study and; second, the longer follow-up may help to delineate the late-onset asthma phenotype from the infection-related phenotype. An intervention study in a developing country might also be undertaken by comparing areas with a parasite eradication program and those without, although lack of baseline data on potential confounders, and contamination of the reference group might limit interpretation. However, a randomised controlled trial among previously uninfected study subjects, with less advanced disease, with adequate power and longer periods of follow-up,

along with measurement of the immunological mediators, might provide better evidence.

Even if this study has provided no support for the 'microflora hypothesis,' given the dynamic and complex nature of the gut microbiota, further study is required. As described previously, the best way to gain more insight into this relationship is through a well powered birth cohort study using culture-independent molecular technique, and objective assessment of the outcomes, and collecting data on potential confounders. It seems likely that the timing of exposure assessment is critical, and future studies should aim to assess exposure from the neonatal period through to infancy. Periodic examination of faecal samples may provide additional information particularly in regard to the role of persistent colonization on allergic outcomes.

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APPENDICES

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Appendix II Year three follow up questionnaire, Butajira birth cohort, Ethiopia

Section I: Child characteristics

G01	Has your child ever had wheezing or whistling in their chest?	Yes	1	→ G02	WHZL3A
		No	2	→ G05	
G02	In the past 12 months, has your child had wheezing or whistling in their chest?	Yes	1		WHZ3A
		No	2		
G03	When was the first time that your child had wheezing or whistling in their chest?	[ ] [ ] month(s)			WHZAGE3A
G04	How many times in the last year has your child had an attack of wheezing?	0	1		WHZFRQ3A
		1-3	2		
		4-12	3		
		>12	4		
G05	Has your child ever had Asthma?	Yes	1	→ G06	ASTL3A
		No	2	→ G08	
G06	Has your child had ASTHMA in the last year?	Yes	1		AST3A
		No	2	→ G08	

G07	Has this been confirmed by a doctor?	Yes	1		ASTHDR3A
		No	2		
G08	Has Your child ever had an itchy skin rash which has affected the skin creases (eg, the folds of the elbow or behind the knees)?	Yes	1	→ G09	RASHL3A
		No	2	→ G10	
G09	In the last 1 year, has your child had an itchy skin rash which has affected the skin creases (eg, the folds of the elbow or behind the knees)?	Yes	1	→G09A	RASH3A
		No	2	→ G10	
G09A	IF YES, has this rash affected any of the following places? (Multiple Answer possible)	The elbow folds	1		RASHL3AA
			2		
		Behind the knees	1		RASHL3AB
			2		
		In front of the ankles	1		RASHL3AC
			2		

		Under the buttocks	1	RASHL3AD
			2	
		Around the neck	1	RASHL3AE
			2	
		Around the eyes/ears	1	RASHL3AF
			2	

G10	Has your child ever had hay fever or persistent sneezing attacks?	Yes	1	→ G11	HAYFL3A
		No	2	→ G12	
G11	In the last year, has the child had hay fever or persistent sneezing attacks?	Yes	1		HAYF3A
		No	2		
G12	Has your child taken any paracetamol/panadol in the last year?	Yes	1	→ G13	PARA3A
		No	2	→ G14	
G13	How many tablets of paracetamol/panadol have your child took in the last month?	[ ] [ ]			PARAFR3A
G14	Has your child taken any drug prescribed by the health institution for any illnesses other than paracetamol/panadol?	Yes	1	→ G15	ANTIB3A
		No	2	→ G16	
G15	If yes, please observe the drug and write the name and type of the drug.				ANTIBM3A
G1	How many people were living in your home now?	[ ] [ ]			PEOP3A
G1	How many older brothers/sisters does your child have who are alive?	[ ] [ ]			SIBS3A

G18A	Is there anyone who smoke cigarette in your home?	Yes	1	→ G18B	HCIGR3A
		No	2	→ G19	
G18B	If yes, please write the total number of people who smoke cigarette?	[ ]			HCIGRN3A
G19	What does your child sleep on?	Bed	1		CHSLP3A
		Medeb	2		
		Floor	3		

		'Jibba'	4	
		'Sigaja'	5	
		Other (Specify)	9	
G20	What does your child bed made of?	Iron metal	1	CHBED3A
		Wood	2	
		Flat metal	3	
		Rope	4	
		leather	5	
		No bed	6	
		Other (Specify)	9	
G20A	What does your child mattress made of?	Cotton	1	CHMAT3A
		Sponge	2	
		Greass	3	
		Kapoak	4	
		No matress	5	
		Other (Specify)	9	
G20 B	What does your child pillow made of?	Cotton	1	CHPIL3A
		Sponge	2	
		Grass	3	
		kapoak	4	
		Cloth	5	
		No pillow	6	
		Other (Specify)	9	

Section 2: Maternal characteristics

G21	Have you had asthma in the last 1 year?	Yes	1	MOAS3A
		No	2	
G21A	Was this confirmed by a doctor?	Yes	1	MOASSDR3A
		No	2	

G22	Has the baby's father had asthma in the last 1 year?	Yes	1	FAAS3A
		No	2	
		NA	9	
G22A	Was this confirmed by a doctor?	Yes	1	FAASDR3A
		No	2	
G23	In the last 1 year have you had hay fever?	Yes	1	MOHAY3A
		No	2	
G24	In the last 1 year has the baby's father had hay fever?	Yes	1	FAHAY3A
		No	2	
		NA	9	
G25	Have you had eczema in the last 1 year?	Yes	1	MOEZC3A
		No	2	
G26	Has the baby's father had eczema in the last 1 year?	Yes	1	FAEZC3A
		No	2	
		NA	9	
G27	Have you taken paracetamol/panadol in the last year?	Yes	1	MOPAR3A
		No	2	
G28	How many tablets of paracetamol/panadol have you taken in the last month?	[ ] [ ]	1	MOPAF3A
G29	Have you taken any drug prescribed by the health institution for any illnesses other ther than paracetamol/panadol)	Yes	1	GANTIB3A
		No	2	
G30	If yes, please observe the drug and write name and type drug.			GANBTY3A

Section 3: Housing characteristics

G32	What type of roof does your house have?	Thatched	1	GROOF3A
		Corrugated iron	2	
		Other (specify)	9	
G33	What are the walls of your house made of?	Wood	1	GWALL3A
		Wood and grass	2	
		Cement	3	

		Brocket	4	
		Bricks	5	
		Corrugated iron	6	
		Other (specify)_____	9	
G34	How many rooms your house had (observe and fill)	[ ]		GROOM3A
G35	What type of floor does your house have?	Cement	1	GFLOOR3A
		Wood	2	
		Bricks	3	
		Mud	4	
		Other (specify)_____	9	
G35A	Is the floor covered?	Yes	1	GCOVER3A
		No	2	

G36	Where do you do most of your cooking? (tick one that apply)	Inside the house in the main living area	1	GCOOK3A
		Inside the house in a room other than the main living area	2	
		Outside the house in a separate building	3	
		Outside the house in the open air	4	

G37 How often do you use the following for cooking? — GFUEL3A

Fuel	Never	Sometimes	Every day
1. Charcoal	1	2	3
2. Wood	1	2	3
3. Leaves	1	2	3
4. Dung	1	2	3
5. Nafta/Lanba	1	2	3
6. Gas	1	2	3

		7. Electricity	1	2	3	
		9. Other	1	2	3	

G37A How often do you use the following inside the house for purposes other than cooking (e.g. heating, lighting)? — GFUELA3A

Fuel	Never	Sometimes	Every day
1. Charcoal	1	2	3
2. Wood	1	2	3
3. Leaves	1	2	3
4. Dung	1	2	3
5. Nafta/Lanba	1	2	3
6. Gas	1	2	3
7. Electricity	1	2	3
8. A locally made battery	1	2	3
9. Other	1	2	3

G38 Which of the following animals do you or your household keep? (Multiple answers possible) — GANIM3A

ANIMAL	Not available	Inside	Outside
1. Cat	1	2	3
2. Dog	1	2	3
3. Hen	1	2	3
4. Cow/ox	1	2	3
5. Sheep	1	2	3
6. Horse	1	2	3
7. Pig	1	2	3
8. Goat	1	2	3

		10. Mule/donkey	1	2	3	
		9 Other	1	2	3	

G39	What is your main source of drinking water? (Tick one that apply)	Piped into compound	1	GWATER3A
		Piped outside compound	2	
		Open well or spring	3	
		Covered well or spring	4	
		River, pond or dam	5	
		Rainwater	6	
G40	What type of toilet facility do you use? (Tick one which apply)	Flush toilet	1	GTOILET3A
		Ventilated improved pit	2	
		Traditional pit	3	
		None/bush/field	4	
G41	How do you dispose your waste?	Pit	1	GSAND3A
		Open field	2	
		Burning	3	
		Garbage bin	4	
		Other(Specify)_____	9	
G42	Do you use any of the following insecticides in your house? (Multiple answer possible)	DDT	Yes 1 / No 2	GINSE3AA
		Malathion	Yes 1 / No 2	GINSE3AB
		Flit	Yes 1 / No 2	GINSE3AC
		Application of dung	Yes 1 / No 2	GINSE3AD
		Other(specify)	Yes 1	GINSE3AE

			No	2	
G43	Where do you place insecticides in your house? (observe)	Reach out of children		1	PROT3A
		Reach of children		2	

Appendix III Year three follow up questionnaire (Amharic translated), Butajira birth cohort, Ethiopia

ክፍል አንድ

ልጁን ሰዶዎ ስላጋጠመው የደረት ህመምና ተመሳሳይነት ያላቸውን ጉዳዮች እጠይቅዎታለሁ። እባክዎን ባለፉት ጊዜያቶች ውስጥ ያጋጠመውንና ከዚህ ጋር ተያያዥነት ያላቸው ችግሮች በማስታወስ ይንገሩኝ።

1.1 ህፃኑን/ኗን የተመለከተ

G101	ህፃኑ/ኗ ከተወለደ/ች ጀምሮ በየትኛውም ጊዜ ቢሆን በደረቱ/ትዋ ውስጥ ሲጥ ሲጥ የሚል ወይም የፉጨት ድምፅ ናሮት ያውቃል/ታውቃለች?	አዎን	1		WHEZEVE36
		የለም	2		
G102	ባለፉት 12 ወራት ዉስጥ በሕፃኑ/ኗ ደረት ውስጥ ሲጥ ሲጥ የሚል ወይም የፉጨት ድምፅ ተሰምቶ ያውቃል/ታውቃለች?	አዎን	1		WHEEZE36
		የለም	2		
G103	ህፃኑ/ኗ በደረቱ/ትዋ ውስጥ ሲጥ ሲጥ የሚል ወይም የፉጨት ድምፅ ለመጀመሪያ ጊዜ የታየበት/ባት መቼ ነበር? (ይህንን ጥያቄ በተቻለ መጠን ጊዜውን እንዲያስታውሱ በመርዳት ወሩን ወይም አመቱን እንዲነግሩሽ አድርጊ)	[ ] [ ] አመት [ ] [ ] ወር			WEZEAGE36
G104	በአለፈው ዓመት ህፃኑ/ኗ ደረት ውስጥ ሲጥ ሲጥ የሚል ወይም የፉጨት ድምፅ ተሰምቶ የነበረዉ ስንት ጊዜ ነበር?	0			WHZFRQ36
		1-3			
		4-12			
		ከ13 በላይ			
G105	ህፃኑ/ኗ ከተወለደ/ች ጀምሮ በየትኛውም ጊዜ ቢሆን አስም ናሮት ያውቃል/ታውቃለች?	አዎን	1		ASTHEVE36
		የለም	2		
G106	ባለፉት 12 ወራት ዉስጥ ህፃኑ/ኗ አስም ናሮት ያውቃል/ታውቃለች?	አዎን	1	→ G106	ASTHMA36
		የለም	2	→ G107	
G107	ህፃኑ/ኗ አስም እንዳለበት/ባት በሐኪም ተረጋጧል?	አዎን	1		ASTHDR36
		የለም	2		
G108	ህፃኑ/ኗ ከተወለደ/ች ጀምሮ በየትኛውም ጊዜ ቢሆን በአጥንት መታጠፊያ ቦታዎቹ(ጀ) (በክርን መታጠፍያ፤ከጉልበቱ ኋላ ባለዉ መታጠፍያ) የሚያሳክክ ሽፍታ ወጥቶበት(ወጥቶባት) ነበር?	አዎን	1		RASHEVE36
		የለም	2		
G109	ባለፉት 12 ወራት ዉስጥ ልጁ(ልጅቷ) በአጥንት መታጠፊያ ቦታዎቹ(ጀ) (በክርን መታጠፍያ፤ከጉልበቱ ኋላ ባለዉ መታጠፍያ) የሚያሳክክ ሽፍታ ወጥቶበት(ወጥቶባት) ነበር?	አዎን	1		RASH36
		የለም	2	→ G110	
G110	መልሱ አዎን ከሆነ፡ ሽፍታዉ የነበረዉ በየትኛ ዎቹ ቦታዎች ላይ ነዉ? (መልሱ ይነበብ፤ ከአንድ በላይ መልሰ መስጠት ይቻላል)	በክርን መታጠፊያ		1	RASHLOC36
		ከጉልበትም ኋላ		2	
		በቁርጭምጭሚት ፊትለፊት		3	

		ከመቀመጫ በታች	4		
		በእንገትዎ ዙሪያ	5		
		በኢይንና በጀሮዎች ዙሪያ	6		
G111	ህፃኑ/ኗ ከተወለደ/ች ጀምሮ በየትኛውም ጊዜ ቢሆን ንፍጥ የሚያበዛ ጉንፋን፤የማያቋርጥ ማስነጠስ፤አፍንጫ ወይም ዐይንን የሚያቃጥል ሕመም ነበረበት(ባት)?	አዎን	1		CHAYEVE36
		የለም	2		
G112	ባለፉት 12 ወራት ዉስጥ ልጅዎ ንፍጥ የሚያበዛ ጉንፋን፤የማያቋርጥ ማስነጠስ፤አፍንጫ ወይም ዐይንን የሚያቃጥል ሕመም ነበረበት(ባት)?	አዎን	1		CHAY36
		የለም	2		
G113	ሕጻኑ(ኗ) ባለፈዉ ዓመት ፓራሲታሞል/ፓናዶል ወስዶ(ዳ) ያዉቃል(ታዉቃለች)?	አዎን	1		PARACET36
		የለም	2		
G114	ህፃኑ/ኗ በላፊው ወር ስንት የፓራሲታሞል ወይም ፓናዶል ኪኒኖች ወሰዶዋል? (በቁጥር ይገለፅ)	[ ] [ ] ወስዶዋል/ችለች።			PARAFRQ36
G115	ህፃኑ/ኗ ሰሳንባ (ደረት) በሽታ የሚሰጥ ማንኛውም መድኃኒት በሕክምና በቅርብ ጊዜ ታዞለት/ላት ያውቃል(ለች)?	አዎን	1		ANTIB36
		የለም	2		
G116	መልስው አዎ ከሆነ የሚወስዱትን መድሃኒት ኢይነቱንና ስሙን በማየት ይሞላ				ANTIBTY36
G117	በቤት ውስጥ ምን ያህል ሰዎች ይኖራሉ?	[ ][ ]			PEOP36
G118	ህፃኑ/ኗ ስንት ታላቅ ወንድምና እህቶች በሕይወት አሉት/አሏት?	[ ][ ]			SIBS36
G119	ህፃኑ/ኗ በሚኖርበት/በምትኖርበት ቤት ውስጥ ሲጋራ/ትምባሆ የሚያጨስ ሰው አለ?	አዎን	1		HCIGR36
		የለም	2		

1.2 የህፃኑ/ኗ እናትን/እፃትን የተመለከተ

G120	ባለፉት 12 ወራት አስም ነበረብዎት?	አዎ	1	MOAS36
		የለም	2	
G121	ባለፉት 12 ወራት የልጁ(ልጅቷ) አባት አስም ነበረባቸዉ?	አዎ	1	FAAS36
		የለም	2	
G122	ባለፉት 12 ወራት ዉስጥ ንፍጥ የበዛበት ጉንፋን፤ የማያቋርጥ ማስነጠስ፤ አፍንጫ ወይም ዓይን ማቃጠል ነበረብዎት?	አዎ	1	MOHAY36
		የለም	2	
G123	ባለፉት 12 ወራት ዉስጥ የልጁ(ልጅቷ) አባት፤ ንፍጥ የበዛበት ጉንፋን፤ የማያቋርጥ ማስነጠስ፤ አፍንጫ ወይም ዓይን ማቃጠል ነበረባቸዉ?	አዎ	1	FAHAY36
		የለም	2	
G124	ባለፉት 12 ወራት የሚያላክክና ፤በተለይም የአጥንት መታጠፊያ አካባቢዎች ያሉትን የሰውነት ክፍሎችን/ለምሳሌ የክንድ፤ ከጉልበት በስተኋላ ታጣፊ ቆዳዎችን/ የሚያጠቃ የቆዳ ሽፍታ ነበረብዎት?	አዎ	1	MOEZC36
		የለም	2	
G125	ባለፉት 12 ወራት የልጁ(ልጅቷ) አባት ፤በተለይም የአጥንት መታጠፊያ አካባቢዎች ያሉትን	አዎ	1	FAEZC36

	የሰውነት ክፍሎችን/ለምሳሌ የክንድ፤ ከጉልበት በሰተኋላ ታጣፊ ቆዳዎችን/ የሚያጠቃ የቆዳ ሽፍታ ነበረባቸዉ?	የለም	2	
G126	ባላፈው ዓመት ፓራሴታሞል/ፓናዶል ወስደው ያውቃሉ?	አዎ	1	MOPAR36
		የለም	2	
G127	በላፊው ወር ስንት የፓራሴታሞል ወይም ፓናዶል ኪኒኖች ዉጠዋል?	[ ] [ ] ዘስዳሰት።		MOPAFR36
G128	ለሳንባ (ደረት) በሽታ የሚሰጥ ማንኛውም መድኃኒት በሕክምና በቅርብ ጊዜ ታዞልዎት ያውቃል?	አዎን	1	MANTIB36
		የለም	2	
G129	መልስዎ አዎ ከሆነ የሚወስዱትን መድሃኒት አይነቱንና ስሙን በማየት ይሞላ።			MANBTY36

ክፍል ሁለት፡ ቤትዎን የተመለከተ

G201	የቤትዎ ጣራ የተሰራው ከምንድን ነው?	ሣር		1	GROOF36
		ቆርቆሮ		2	
		ሌላ (ይገለጽ)		9	
G202	ግድግዳው ከምን የተሠራ ነው?	እንጨትና ሞቃ		1	GWALL36
		እንጨት፣ ሞራሮና ሳር		2	
		ድንጋይና ሲሚንቶ		3	
		ብሎኬት		4	
		ጡብ		5	
		ቆርቆሮ		6	
		ሌላ (ይገለጽ)		9	
G203	የህፃኑ/ኗ መኖሪያ ቤት ወለል የተሰራው ከምንድን ነው?		ተሸፍኗል	አልተ ሸፈነም	GFLOOR36
		1. ከሲሚንቶ	1	2	
		2. ከጣውላ ወይም እንጨት	1	2	
		3. ከሸክላ	1	2	
		4. ከአፈር	1	2	
		9. ሌላ(ይግለጹ)	1	2	
G204	ህፃኑ/ኗ በምን ላይ ነዉ የሚተኛ ዉ(የምትተኛዉ)?	አልጋ		1	CHSLP36
		መደብ		2	
		ወለል		3	
		ጆባ		4	
		ስጋጃ		5	

		ሌላ (ይግለጹ)	9	
G205	ህፃኑ/ኗን ለመኝታ የሚጠቀሙዉ/የምትጠቀመዉ አልጋ ከሆነ የተሠራው ከምንድ ነው?	ከሸቦ	1	CHBED 36
		ከእንጨት	2	
		ከቦንዳ	3	
		ከገመድ	4	
		ከቁርበት	5	
		አልጋ የለኝም	6	
		ሌላ (ይግለጹ)	9	
G205A	ህፃኑ/ኗን ለመኝታ የሚጠቀሙዉ/የምትጠቀመዉ ፍራሽ ከሆነ የተሠራው ከምንድ ነው?	ከጥጥ	1	CHMAT 36
		ከስፖንጅ	2	
		ከሳር	3	
		ከአበባ የሚገኝ ጥጥ መሰል ነገር	4	
		ፍራሽ የለኝም	5	
		ሌላ (ይግለጹ	9	
G205B	ህፃኑ/ኗን ለመኝታ የሚጠቀሙዉ/የምትጠቀመዉ ትራስ ከሆነ የተሠራው ከምንድ ነው?	ከጥጥ	1	CHPIL36
		ከስፖንጅ	2	
		ከሳር	3	
		ከአበባ የሚገኝ ጥጥ መሰል ነገር	4	
		ፈልፍቅ ወይም ልብስ	5	
		ከፈልፍቅ የተሰራ ትራስ	6	
		ፍራሽ የለኝም	7	
		ሌላ (ይግለጹ	9	
G206	ቤተሰቡ ምግብ በብዛት የሚያበስሉት የት ነው? **(አንዱ ላይ ብቻ ምልክት አድርጊ)**	በዋናው ቤት ውስጥ	1	GCOOK36
		እቤት ውስጥ ሆኖ ከዋናው ቤት ሌላ	2	
		ከቤት ውጭ ማዕድ ቤት	3	
		ከቤት ውጭ ክፍት ቦታ	4	
G206A	የህፃኑ/ኗን ቤተሰብ ከሚከተሉት የማገዶ ዓይነቶች ምግብ ለማብሰል በየስንት ጊዜው እንድሚጠቀሙ ቢገልፁልኝ?			FUEL36

ማገዶ/ነዳጅ	አልጠቀምም	አንዳንድ ጊዜ	በየቀኑ
1. ከሠል	1	2	3

		2. እንጨት	1	2	3	
		3. ቅጠል	1	2	3	
		4. ኩበት	1	2	3	
		5. ናፍጣ/ሳንባ	1	2	3	
		6. ቡታጋዝ	1	2	3	
		7. መብራት/ኮርንቲ	1	2	3	
		9. ሌላ (ይግለጹ)	1	2	3	
G206B	የህጭ/ኗ ቤተሰብ ከሚከተሉት የማገዶ ዓይነቶች ምግብ ከማብሰል ውጭ ለሌላ ጉዳይ ይጠቀማል (ላምሳሌ ለሙቀትና ለመብራት)?					FUELOH36
		ማገዶ/ነዳጅ	አልፎቀምም	አንዳንድ ጊዜ	ምየቀኑ	
		1. ከሠል	1	2	3	
		2. እንጨት	1	2	3	
		3. ቅጠል	1	2	3	
		4. ኩበት	1	2	3	
		5. ናፍጣ/ሳንባ	1	2	3	
		6. ቡታጋዝ	1	2	3	
		7. ባትሪ ድንጋይ	1	2	3	
		8. መብራት/ኮርንቲ	1	2	3	
		9. ሌላ (ይግለጹ)	1	2	3	
G207	የህጭ/ኗ ቤተሰብ ከሚከተሉት እንስሳትና ከብቶች ዓይነቶች የትኞቹ አሏችው?					ANIM36
		እንስሳ	በቤት ውስጥ ይጠበቃሉ/ያድራሉ	ከቤት ውጪ ይጠበቃሉ		
		1. ድመት	1	2		
		2. ውሻ	1	2		
		3. ዶሮ	1	2		
		4. ላም/በሬ	1	2		
		5. በግ	1	2		
		6. ፈረስ	1	2		

		7. አሳማ	1	2	
		8. ፍየል	1	2	
		10. በቅሎ/አህያ	1	2	
		9. ሌላ	1	2	
G208	የህፃኑ/ኗን ቤተሰብ የመጠጥ ውሃ በዋነኝነት የሚያገኙት ከየት ነው?	በግቢው ውስጥ ከሚገኝ ቧንቧ	1		WAT36
		ከግቢ ውጪ ከሚገኝ ቧንቧ	2		
		ከጉድጓድ ወይም ምንጭ	3		
		ከተጠበቀ ጉድጓድ ወይም ምንጭ	4		
		ከወንዝ፣ ከኩሬ፣ከጉድጓድ	5		
		ከዝናብ ውሃ	6		
G209	የህፃኑ/ኗን ቤተሰብ የሚገለገልበት መፀዳጃ ቤት ምን አይነት ነው?	በውሃ የሚሰራ ሽንት ቤት	1		SANIT36
		ሽታ አልባ መጸዳጃ	2		
		የተሰመደ ዓይነት የሸንት ቤት ጉድጓድ	3		
		ሜዳ ላይ ወይም ጫካ	4		
G210	አሁን ቤትዎ ውስጥ ሳሙና አለዎት? እንዲያሳዩኝ ጠይቂያቸው	አዎ	1		SOAP36
		የለም	2		
G211	ምን ያህል ጊዜ ነው ሳሙና የምትጠቀሙት? ሳሙና የሚጠቀሙት ለማንኛውም ዓይነት የንጽህና መጠበቅ ሊሆን ይችላል	አልጠቀምም	1		SOAPFR36
		በየቀኑ	2		
		ቢያንስ በሳምንት አንድ ጊዜ	3		
		ቢያንስ በ15 ቀን አንድ ጊዜ	4		
		በበዓል ቀን ወይንም ለየት ባለ ቀን	5		
		አላውቅም	6		
		መልስ መስጠት አልፈለጉም	7		
G212	የህፃኑ/ኗ ቤተሰብ ከሚከተሉት የተባይ ማጥፊያ መድኃኒቶች በቤቱ ውስጥ የትኛውን ይጠቀማሉ? (ከአንድ በላይ መልሰ መስጠት ይቻላል) (መልሱ ይነበብ)	ዲዲት	1		GINSECT36
		ማላታይን	2		
		ፍሊት	3		
		ሌላ (ይግለጹ)	9		
G213	እንደ ፊሊት የመሳሰሉ ጸረተባይ መድሀኒቶች የት ነው የሚያስቀምጡት?(ስፍራዉን	ልጆች ሊደርሱበት በሚችሉበት ቦታ	1		PROT36

	እንዲያሳዩሽ ጠይቂያቸዉ) አሁን ቤት ውስጥ ጸረተባይ መድኃኒቶች ስሌላቸው ቢኖራቸው ኖሮ የት እንደሚያስቀምጡ ጠይቂያቸው	ልጆች ሲደርሱበት በማይችሉበት ቦታ	2	

Appendix IV Year five follow up questionnaire, Butajira birth cohort, Ethiopia

Section 1: Child characteristics

G01	Has your child ever had wheezing or whistling in their chest?	Yes	1		WHZL6A
		No	2		
G02	In the last 2 years, has your child had wheezing or whistling in their chest?	Yes	1		WHZT6A
		No	2		
G03	In the last 1 year, has your child had wheezing or whistling in their chest?	Yes	1	→ G04	WHZ6A
		No	2	→ G05	
G04	How many times in the last year has your child had an attack of wheezing?	0	1		WHZFRQ6A
		1-3	2		
		4-12	3		
		>12	4		
G05	Has your child ever had Asthma?	Yes	1		ASTL6A
		No	2		
G06	In the last 2 years, has your child had Asthma?	Yes	1		ASTT6A
		No	2		
G07	Has your child had Asthma in the last year?	Yes	1	→ G08	AST6A
		No	2	→ G09	

G08	Has this been confirmed by a doctor?	Yes	1		ASTHDR6A
		No	2		
G09	Has your child ever had an itchy skin rash which has affected the skin creases (eg, the folds of the elbow or behind the knees)?	Yes	1		RASHL6A
		No	2		
G10	In the last 2 years, has your child had an itchy skin condition affecting the skin creases (front of the elbow, behind the knees, the front of the ankles, around the neck, or around the eyes?	Yes	1		RASHT6A
		No	2		
G11	In the last 1 year, has your child had an itchy skin condition affecting the skin creases (front of the elbow, behind the knees, the front of the ankles, around the neck, or around the eyes?	Yes	1	→G11A	RASH6A
		No	2	→ G12	
G11A	IF YES, has this rash affected any of the following places?	The elbow folds		1	RASHL6AA
				2	

	(Multiple Answers possible)	Behind the knees	1		RASHL6AB
			2		
		In front of the ankles	1		RASHL6AC
			2		
		Under the buttocks	1		RASHL6AD
			2		
		Around the neck	1		RASHL6AE
			2		
		Around the eyes/ears	1		RASHL6AF
			2		

G12	Has your child ever had hay fever or persistent sneezing attacks?	Yes	1		HAYFL6A
		No	2		
G13	In the last 2 years, has your child had hay fever or persistent sneezing with sneezing or running nose (excluding colds or flu), or problems with itchy watery eyes?	Yes	1		HAYFT6A
		No	2		
G14	In the last year, has your child had hay fever or persistent sneezing with sneezing or running nose (excluding colds or flu), or problems with itchy watery eyes?	Yes	1		HAYF6A
		No	2		
G15	How many people are living in your home now? [ ] [ ]	[ ] [ ]			PEOP6A
G16	How many older brothers/sisters does your child have who are alive now?	[ ] [ ]			SIBS6A

G17	Is paracetamol the same as aspirin?	Yes	1	→ G18	PARASP6A
		No	2	→ G19	
G18	Can you tell me which one of these is paracetamol and which aspirin? **(show medication strip)**	Correct identification	1		PARADIF6A
		Incorrect identification	2		
G19	Has your child taken any paracetamol/panadol in the last year?	Yes	1	→ G20	PARA6A
		No	2	→ G21	
G20	How many tablets of paracetamol/panadol has your child taken in the last month?	[ ] [ ]			PARAFR6A

G21	Can you name any symptoms for which you have given your child paracetamol? **(Multiple answers possible)**	Headache	Yes	1		PAHED6A
			No	2		
		Fever	Yes	1		PAFEV6A
			No	2		
		Malaria	Yes	1		PAMAL6A
			No	2		
		Common cold	Yes	1		PACOLD6A
			No	2		
		Birrd	Yes	1		PABIRD6A
			No	2		
		Wheeze	Yes	1		PAWHEZ6A
			No	2		
		Cough	Yes	1		PACOU6A
			No	2		
		Shortness of breath	Yes	1		PASOB6A
			No	2		
		Sneezing/running nose/itchy eyes	Yes	1		PASNEZ6A
			No	2		
		Skin rash in the creases	Yes	1		PARASH6A
			No	2		
		Other (specify)				PAOTHE6A
G22	Is paracetamol available close to where you live?	Yes	1			PAVAIL6A
		No	2			
G23	Is paracetamol affordable to you?	Yes	1			PAFORD6A
		No	2			
G24	Do you avoid giving your child aspirin?	Yes	1			ASAVOD6A
		No	2			
G25	Should any people NOT take aspirin? **(PW – people with....)**	Children	Yes	1		ASCHIL6A
			No	2		
		PW gastritis	Yes	1		ASGAS6A
			No	2		
		PW asthma	Yes	1		ASASTH6A
			No	2		
		PW hay fever	Yes	1		ASHAY6A
			No	2		
		Don't know			9	ASAVDK6A
G26	Do you prefer to give aspirin or paracetamol for your child?	Aspirin	1			ASPREF6A
		Paracetamol	2			
		Depends	3			
		Don't mind	4			

G27	Has your child taken any drug prescribed by the health institution for any illnesses currently? (Other than paracetamol/panadol/aspirin)	Yes	1	→ G27A	ANTIB6A
		No	2	→ G28	
G27A	If yes, please observe the drug and write the name and type the child currently taking.	----------			ANTIBM6A
G28	Has your child taken any de-worming medication in the last 6 months? **(De-worming refers to antihelmintics treatment given by the heath office free of charge without stool examination)**	Yes	1		DEWOR6A
		No	2		

G29	Is there anyone who smokes cigarettes in your home?	Yes	1	→ G29A	HCIGR6A
		No	2	→ G30	
G29A	If yes, please write the total number of people who smoke cigarettes in the home where the child living?	[ ]			HCIGRN6A
G30	What does your child sleep on?	Bed	1		CHSLP6A
		Medeb	2		
		Floor	3		
		'Jibba'	4		
		'Sigaja'	5		
		Other (Specify)	9		
G31	What is your child's bed made of?	Iron metal	1		CHBED6A
		Wood	2		
		Flat metal	3		
		Rope	4		
		leather	5		
		No bed	6		
		Other (Specify)	9		
G32	What is your child's mattress made of?	Cotton	1		CHMAT6A
		Sponge	2		
		Grass	3		
		Kapok	4		
		No mattress	5		
		Other (Specify)	9		
G33	What is your child's pillow	Cotton	1		CHPIL6A

	made of?	Sponge	2	
		Grass	3	
		kapok	4	
		Cloth	5	
		No pillow	6	
		Other (Specify)	9	

Section 2: Maternal characteristics

G34	Have you had wheezing or whistling in your chest in the last 1 year?	Yes	1	→ G35	MOWHZ6A
		No	2	→ G36	
G35	How many times in the last year have you had an attack of wheezing?	0	1		MOWHFR6A
		1-3	2		
		4-12	3		
		>12	4		
G36	Have you had asthma in the last 1 year?	Yes	1	→ G37	MOAS6A
		No	2	→ G38	
G37	Was this confirmed by a doctor?	Yes	1		MOASSDR6A
		No	2		
G38	Has the baby's father had wheezing or whistling in the chest in the last 1 year?	Yes	1		FAWHEZ6A
		No	2		
G39	Has the baby's father had asthma in the last 1 year?	Yes	1	→ G40	FAAS6A
		No	2	→ G41	
		NA	9		
G40	Was this confirmed by a doctor?	Yes	1		FAASDR6A
		No	2		
G41	In the last 1 year have you had hay fever?	Yes	1		MOHAY6A
		No	2		
G42	In the last 1 year has the baby's father had hay fever?	Yes	1		FAHAY6A
		No	2		
		NA	9		
G43	Have you had eczema in the last 1 year?	Yes	1		MOEZC6A
		No	2		
G44	Has the baby's father had eczema in the last 1 year?	Yes	1		FAEZC6A
		No	2		
		NA	9		
G45	Have you taken paracetamol/Panadol in the last year?	Yes	1		MOPAR6A
		No	2		
G46	How many tablets of paracetamol/Panadol have you	[ ]	1		MOPAF6A

	taken in the last month?			
G47	Have you taken any drug prescribed by the health institution for any problem currently? (Other than Paracetamol/Panadol)	Yes	1	GANTIB6A
		No	2	
G48	If Yes, please observe the drug and write the name and type the mother currently taking.	----------		GANBTY6A

Section 3: Housing characteristics

G49	What type of roof does your house have?	Thatched	1	GROOF6A
		Corrugated iron	2	
		Other (specify)	9	
G50	What are the walls of your house made of?	Wood	1	GWALL6A
		Wood and grass	2	
		Cement	3	
		Brocket	4	
		Bricks	5	
		Corrugated iron	6	
		Other (specify)_____	9	
G51	How many rooms does your house have? (observe and fill the no of rooms)	[ ]		GROOM6A
G52	What type of floor does your house have?	Cement	1	GFLOOR6A
		Wood	2	
		Bricks	3	
		Mud	4	
		Other (specify)_____	9	
G53	Is the floor covered by any material?	Yes	1	GCOVER6A
		No	2	

G54	Where do you do most of your cooking? (tick one that applies)	Inside the house in the main living area	1	GCOOK6A
		Inside the house in a room other than the main living area	2	
		Outside the house in a separate building	3	
		Outside the house in the open air	4	
G55	How often do you use the following for cooking?			GFUEL6A

		Fuel	Never	Sometimes	Every day
		1. Charcoal	1	2	3
		2. Wood	1	2	3
		3. Leaves	1	2	3
		4. Dung	1	2	3
		5. Nafta/Lanba	1	2	3
		6. Gas	1	2	3
		7. Electricity	1	2	3
		9. Other	1	2	3
G56	How often do you use the following inside the house for purposes other than cooking (e.g. heating, lighting)?				GFUELA6A
		Fuel	Never	Sometimes	Every day
		1. Charcoal	1	2	3
		2. Wood	1	2	3
		3. Leaves	1	2	3
		4. Dung	1	2	3
		5. Nafta/Lanba	1	2	3
		6. Gas	1	2	3
		7. Electricity	1	2	3
		8. A locally made battery	1	2	3
		9. Other	1	2	3
G57	Which of the following animals do you or your household keep? **(Multiple answers possible)**				GANIM6A
		Animal	Not available	Inside	Outside
		1. Cat	1	2	3
		2. Dog	1	2	3
		3. Hen	1	2	3
		4. Cow/ox	1	2	3
		5. Sheep	1	2	3
		6. Horse	1	2	3
		7. Pig	1	2	3
		8. Goat	1	2	3
		10. Mule/donkey	1	2	3
		9 Other	1	2	3

G58	What is your main source of drinking water? (Tick one	Piped into compound	1	GWATER6A
		Piped outside compound	2	
		Open well or spring	3	

	which applies)	Covered well or spring	4	
		River, pond or dam	5	
		Rainwater	6	
G59	What type of toilet facility do you use? (Tick one which applies)	Flush toilet	1	GTOILET6A
		Ventilated improved pit	2	
		Traditional pit toilet	3	
		None/bush/field	4	
G60	How do you dispose your waste?	Pit	1	GSAND6A
		Open field	2	
		Burning	3	
		Garbage bin	4	
		Other(Specify)_____	9	
G61	Do you use any of the following insecticides in your house? (Multiple answers possible)	DDT	Yes 1 / No 2	GINSE6AA
		Malathion	Yes 1 / No 2	GINSE6AB
		Flit	Yes 1 / No 2	GINSE6AC
		Application of dung	Yes 1 / No 2	GINSE6AD
		Other(specify)	Yes 1 / No 2	GINSE6AE
G61	Where do you place insecticides in your house? (observe)	Out of reach of children	1	PROT6A
		Within reach of children	2	

Appendix V Year five follow up questionnaire (Amharic translated), Butajira birth cohort, Ethiopia

ስቡን ሰጅም ሰላጋጠመው የደረት ሄመምና ተመሳሳይነት ያላቸውን ጉዳዮች እጠይቅዎታለሁ። እባክዎን ባለፉት ጊዜያቶች ውስጥ ያጋጠሙዎትን ከዚህ ጋር ተያያጅነት ያላቸው ችግሮች በማስታወስ ይንገሩኝ።

G01	ህፃኑ/ኗ ከተወለደ/ች ጀምሮ በየትኛውም ጊዜ ቢሆን በደረቱ/ትዋ ውስጥ ሲጥ ሲጥ የሚል ወይም የፉጨት ድምፅ ኖሮት ያውቃል/ታውቃለች?	እምን የለም	1 0		WHZL6A
G02	ባለፉት ሁለት አመታት በየትኛውም ጊዜ ቢሆን በደረቱ/ትዋ ውስጥ ሲጥ ሲጥ የሚል ወይም የፉጨት ድምፅ ኖሮት ያውቃል/ታውቃለች?	እምን የለም	1 0		WHZT6A
G03	ባለፉት 12 ወራት ዉስጥ በሕፃኑ/ኗ ደረት ውስጥ ሲጥ ሲጥ የሚል ወይም የፉጨት ድምፅ ተሰምቶ ያውቃል/ታውቃለች?	እምን የለም	1 0	→ G04 → G05	WHZ6A
G04	ባለፉት 12 ወራት ህፃኑ/ኗ ደረት ውስጥ ሲጥ ሲጥ የሚል ወይም የፉጨት ድምፅ ተሰምቶ የነበረዉ ስንት ጊዜ ነበር?	0 1-3 4-12 ከ13 በላይ	0 1 2 3		WHZFRQ6A
G05	ህፃኑ/ኗ ከተወለደ/ች ጀምሮ በየትኛውም ጊዜ ቢሆን አስም ኖሮት ያውቃል/ታውቃለች?	እምን የለም	1 2		ASTL6A
G06	ባለፉት ሁለት አመታት በየትኛውም ጊዜ ቢሆን ህፃኑ/ኗ አስም ኖሮት/ሯት ያውቃል/ታውቃለች?	እምን የለም	1 0		ASTT6A
G07	ባለፉት 12 ወራት ዉስጥ ህፃኑ/ኗ አስም ኖሮት ያውቃል/ታውቃለች?	እምን የለም	1 0	→G08	AST6A
G08	ህፃኑ/ኗ አስም እንዳለበት/ባት በሐኪም ተረጋግጧል?	እምን የለም	1 0		ASTHDR6A
G09	ህፃኑ/ኗ ከተወለደ/ች ጀምሮ በየትኛውም ጊዜ ቢሆን በአጥንት መታጠፊያ ቦታዎቹ(ጀ) (በክርን መታጠፍያ፤ከጉልበቱ ጏላ ባለዉ መታጠፍያ፤በቁርጭምጭሚት ፌት ለፌት፤ በእንገት ዙሪያ፤ እና በአይን አካባቢ) የሚያሳክክ ሽፍታ ወጥቶበት (ወጥቶባት) ነበር?	እምን የለም	1 0		RASHL6A
G10	ባለፉት ሁለት አመታት በየትኛውም ጊዜ ቢሆን ህፃኑ/ኗ በአጥንት መታጠፊያ ቦታዎቹ(ጀ) (በክርን መታጠፍያ፤ከጉልበቱ ጏላ ባለዉ መታጠፍያ፤በቁርጭምጭሚት ፌት ለፌት፤ በእንገት ዙሪያ፤ እና በአይን አካባቢ) የሚያሳክክ ሽፍታ ወጥቶበት (ወጥቶባት) ነበር?	እምን የለም	1 0		RASHT6A
G11	ባለፉት 12 ወራት ዉስጥ ልጁ(ልጅቷ) በአጥንት መታጠፊያ ቦታዎቹ(ጀ) (በክርን መታጠፍያ፤ከጉልበቱ ጏላ ባለዉ መታጠፍያ፤በቁርጭምጭሚት ፌት ለፌት፤ በእንገት ዙሪያ፤ እና በአይን አካባቢ) የሚያሳክክ ሽፍታ ወጥቶበት (ወጥቶባት) ነበር?	እምን የለም	1 0	→G11A	RASH36
G11A	መልሱ አምን ከሆነ፡ ሽፍታዉ የነበረዉ በየትኛዎቹ ቦታዎች ላይ ነዉ? (መልሱ ይነበብ፤ ከአንድ በላይ መልሰ መስጠት	በክርን መታጠፊያ ከጉልበትም ጏላ	1 0 1		RASH6AA RASH6AB

	ይቻላል)			0		
		በቁሮጭምጭሚት ፊትለፊት		1		RASH6AC
				0		
		ከመቀመጫ በታች		1		RASH6AD
				0		
		በእንጥልም ዙሪያ		1		RASH6AE
				0		
		በእይንና በጆሮዎች ዙሪያ		1		RASH6AF
				0		
G12	ህፃኑ/ኗ ከተወለደ/ች ጀምሮ በየትኛውም ጊዜ ቢሆን ንፍጥ የሚያበላ ጉንፋን፤የማያቋርጥ ማስነጠስ፤አፍንጫ ወይም ዐይንን የሚያቃጥል ሕመም ነበረበት(ባት)? (እነኚህ ምልክቶች የታዩት ህፃኑ በጉንፋን ላይያዝ መሆን አለበት)።	አዎን	1			HAYFL6A
		የለም	0			
G13	ባለፉት ሁለት አመታት በየትኛውም ጊዜ ቢሆን ህፃኑ/ኗ ንፍጥ የሚያበላ ጉንፋን፤የማያቋርጥ ማስነጠስ፤አፍንጫ ወይም ዐይንን የሚያቃጥል ሕመም ነበረበት(ባት)? (እነኚህ ምልክቶች የታዩት ህፃኑ በጉንፋን ላይያዝ መሆን አለበት)።	አዎን	1			HAYFT6A
		የለም	0			
G14	ባለፉት 12 ወራት ዉስጥ ልጅዎ ንፍጥ የሚያበላ ጉንፋን፤የማያቋርጥ ማስነጠስ፤አፍንጫ ወይም ዐይንን የሚያቃጥል ሕመም ነበረበት(ባት)? (እነኚህ ምልክቶች የታዩት ህፃኑ በጉንፋን ላይያዝ መሆን አለበት)።	አዎን	1			HAYF6A
		የለም	0			
አሁን ህፃኑን/ኗ በታመሙ ጊዜ ፓራሲታሞልና የመሳሰሉትን የህመም ማስታገሻ እንደወሰዱ እጠይቅዎታለሁ።						
G15	ፓራሲታሞል ከአስፕሪን ጋር አንድ ነው?	አዎን	1			PADIF6A
		የለም	0	→G17		
G16	ከነዚህ ከሁለቱ መካከል ፓራሲታሞልና አስፕሪንን ልትለይልን ትችያለሽ ?	ትክክለኛ መለያ	1			PADFA6A
		የተሳሳተ መለያ	0			
G17	ሕጻኑ(ኗ) ባለፈዉ ዓመት ፓራሲታሞል/ፓናዶል ወስዶ(ዳ) ያዉቃል(ታዉቃለች)?	አዎን	1	→G18		PARA6A
		የለም	0	→G20		
G18	ህፃኑ/ኗ በሳፈው ወር ስንት የፓራሲታሞል ወይም ፓናዶል ኪኒኖች ወሰዶዋል? (በቁጥር ይገለፅ)	[ ] [ ]				PARAFR6A
G19	ፓራሲታሞል ለሕጻኑ(ኗ) የሰጡበት የሕመም ምልክቶች/በሽታዎች ከሚከተሉት የትኞቹ ናቸው? (መልሱ ይነበብ፤ ከአንድ በላይ መልስ መስጠት ይቻላል)	ራስ ምታት	አዎን	1		PAHED6A
			የለም	0		
		ትኩሳት	አዎን	1		PAFEV6A
			የለም	0		
		ወላ	አዎን	1		PAMAL6A
			የለም	0		
		ጉንፋን	አዎን	1		PACOLD6A
			የለም	0		
		ብርድ	አዎን	1		PABIRD6A
			የለም	0		
		ሳል	አዎን	1		PACOU6A
			የለም	0		
		ሲጥ ሲጥ ሲልበት/ባት	አዎን	1		PAWHEZ6A
			የለም	0		
		የትንፋሽ	አዎን	1		PASOB6A

		ማጠር	የለም	0	
		ማስነጠስ/ እንደ ንፍጥ ያለ በአፍንጫ ሲወርድ/የአይን ማሳከክ ሲኖር	አዎን	1	PASNEZ6A
			የለም	0	
		የቆዳ ሽፍታ በመታጠፊያ አካባቢዎች ሲኖር	አዎን	1	PARASH6A
			የለም	0	
		ሌላ (ይገለፅ) _____			PAOTHE6A
G20	በሚኖሩብት አካባቢ ፓራሴታሞልን በቅርበት ያገኘ ታል?	አዎን	1		PARAV6A
		የለም	0		
G21	አርስዎ ፓራሴታሞልን ለመግዛት ዋጋውን ይችሉታል?	አዎን	1		PAFORD6A
		የለም	0		
G22	አስፕሪን ለልጅዎ ላለመስጠት ይሞክራሉ?	አዎን	1		ASAVOD6A
		የለም	0		
G23	አስፕሪን መውሰድ የሌለባቸው ሰዎች አሉ? (መልሱ ይነበብ፤ ከአንድ በላይ መልስ መስጠት ይቻላል)	ሕፃናት	አዎን	1	ASCHIL6A
			የለም	0	
		ጨጓራ ያለባቸው	አዎን	1	ASGAS6A
			የለም	0	
		አስም ያለባቸው	አዎን	1	ASASTH6A
			የለም	0	
		የአፍንጫ አስም ያለባቸው	አዎን	1	ASHAY6A
			የለም	0	
		አላውቅም	አዎን	1	ASAVDK6A
			የለም	0	
		ሌላ (ይገለፅ)_____			
G24	አርስዎ ለልጅዎ ለመስጠት የሚመርጡት አስፕሪንን ነው ወይንስ ፓራሴታሞልን?	አስፕሪን	1		ASPREF6A
		ፓራሴታሞል	2		
		እንደ ሁኔታው	3		
		ምንም ምርጫ የለኝም	4		
G25	ህፃኑ/ኗ ለማንኛውም አይነት መድኃኒት ለየትኛውም አይነት በሽታ በሕክምና በቅርብ ጊዜ ታዘለት/ላት ያውቃል(ለች)? (ይህን ጥያቄ ለማንኛውም አይነት በሽታ የታዘዘለትን/ላትን መድሃኒት ያካትታል ነገር ግን ፓራሴንታሞል/ፓናዶልን ወይም አስፕሪንን አይጨምርም።)	አዎን	1	→G25A	ANTIB6A
		የለም	0	→G26	
G25A	መልስም አም ከሆነ የሚወስዱትን መድሃኒት አይነቱንና ስሙን በማየት ይሞላ።	1.----------			BANTA6A
		2.----------			BANTB6A
		3.----------			BANTC6A

G26	ህፃኑ/ኗ ባለፉት ስድስት ወራት ለሆድ ትላትል መከላከያ መድሃኒት ወስዶአል? (የሆድ ትላትል መከላከያ ሲባል በዋነኛነት በጤና ባለሙያ በአመት ሁለት ጊዜ ቤት ለቤት በነፃ የሚታደል ማለት ነው::)	አዎን	1			DEWOR6A
		የለም	0			
G27	በቤት ውስጥ ምንያህል ሰዎች ይኖራሉ?	[ ] [ ]				PEOP6A
G28	ህፃኑን/ኗ ስንት ታላቅ ወንድምና እህቶች በሕይወት አሉት/አሏት?	[ ] [ ]				SIBS6A
G29	ህፃኑ/ኗ በሚኖርበት/በምትኖርበት ቤት ውስጥ ሲጋራ/ትምባሆ የሚያጨስ ሰው አለ?	አዎን	1	→G29A		HCIGR6A
		የለም	0	→G30		
G29A	መልሱ አዎ ከሆነ የሚያጨስ ሰው ብዛት ጠይቀሽ መዝግቢ	[ ] [ ]				HCIGRN6A
G30	ህፃኑ/ኗ በምን ላይ ነዉ የሚተኛ ዉ(የምትተኛዉ)?	አልጋ		1		CHSLP6A
		መደብ		2		
		ወለል		3		
		ጀባ		4		
		ሲጋጃ		5		
		ሌላ (ይግለጹ)		9		
G31	ህፃኑ/ኗን ለመኝታ የሚጠቀሙዉ/የምትጠቀመዉ አልጋ ከሆነ የተሠራው ከምንድ ነው?	ከሸቦ		1		CHBED6A
		ከእንጨት		2		
		ከቦንዳ		3		
		ከገመድ		4		
		ከቁርበት		5		
		አልጋ የለኝም		6		
		ሌላ (ይግለጹ)		9		
G32	ህፃኑ/ኗን ለመኝታ የሚጠቀሙዉ/የምትጠቀመዉ ፍራሽ ከሆነ የተሠራው ከምንድ ነው?	ከጥጥ		1		CHMAT6A
		ከስፖንጅ		2		
		ከሳር		3		
		ከአበባ የሚገኝ ጥጥ መሰል ነገር		4		
		ፍራሽ የለኝም		5		
		ሌላ (ይግለጹ		9		
G33	ህፃኑ/ኗን ለመኝታ የሚጠቀሙዉ/የምትጠቀመዉ ትራስ ከሆነ የተሠራው ከምንድ ነው?	ከጥጥ		1		CHPIL6A
		ከስፖንጅ		2		
		ከሳር		3		
		ከአበባ የሚገኝ ጥጥ መሰል ነገር		4		
		ጨርቅ ወይም ልብስ		5		
		ከጨርቅ የተሰራ ትራስ		6		
		ትራስ የለውም/ላትም		7		
		ሌላ (ይግለጹ		9		

1.2 የህፃኑ/ኗ እናትን/አባትን የተመለከተ

G34	ባለፉት 12 ወራት በደራትም ዉስጥ ሲጥ ሲጥ የሚል ወይም የፉጨት ድምፅ ነበረብዎት?	አዎ	1	→G35	MWHZ6A
		የለም	0	→G36	
G35	ባለፉት 12 ወራት በደራትም ዉስጥ ሲጥ ሲጥ የሚል ወይም የፉጨት ድምፅ ተሰምቶዎት የነበረዉ ስንት ጊዜ ነበር?	0	0		MWHFR6A
		1-3	1		
		4-12	2		
		ከ13 በላይ	3		

G36	ባለፉት 12 ወራት አስም ነበረብዎት?	አዎ	1	→G37	MOAS6A
		የለም	0	→G38	
G37	እርስዎ አስም እንዳለብዎት በሐኪም ተረጋግጧል?	አዎ	1		MASDR6A
		የለም	0		
G38	ባለፉት 12 ወራት የልጁ(ልጅቷ) አባት በደረታቸው ዉስጥ ሲጥ ሲጥ የሚል ወይም የፉጨት ድምፅ ነበረባቸው?	አዎ	1		FWHZ6A
		የለም	0		
		አይመለከትም	9		
G39	ባለፉት 12 ወራት የልጁ(ልጅቷ) አባት አስም ነበረባቸዉ?	አዎ	1	→G40	FAAS6A
		የለም	0	→G41	
		አይመለከትም	9		
G40	የልጁ(ልጅቷ) አባት አስም እንዳለባቸው በሐኪም ተረጋግጧል?	አዎ	1		FASDR6A
		የለም	0		
G41	ባለፉት 12 ወራት ዉስጥ ንፍጥ የበዛበት ጉንፋን፤ የማያቋርጥ ማስነጠስ፤ አፍንጫ ወይም ዓይን ማቃጠል ነበረብዎት?	አዎ	1		MOHAY6A
		የለም	0		
G42	ባለፉት 12 ወራት ዉስጥ የልጁ(ልጅቷ) አባት፤ ንፍጥ የበዛበት ጉንፋን፤ የማያቋርጥ ማስነጠስ፤ አፍንጫ ወይም ዓይን ማቃጠል ነበረባቸዉ?	አዎ	1		FAHAY6A
		የለም	0		
		አይመለከትም	9		
G43	ባለፉት 12 ወራት የሚያሳክክና ፤በተለይም የአጥንት መታጠፊያ አካባቢዎች ያሉትን የሰውነት ክፍሎችን/ለምሳሌ የክንድ፤ ክንልበት በሉተኋላ ታጣፊ ቆዳዎችን/ የሚያጠቃ የቆዳ ሽፍታ ነበረብዎት?	አዎ	1		MOEZCA6A
		የለም	0		
G44	ባለፉት 12 ወራት የልጁ(ልጅቷ) አባት ፤በተለይም የአጥንት መታጠፊያ አካባቢዎች ያሉትን የሰውነት ክፍሎችን/ለምሳሌ የክንድ፤ ክንልበት በሉተኋላ ታጣፊ ቆዳዎችን/ የሚያጠቃ የቆዳ ሽፍታ ነበረባቸዉ?	አዎ	1		FAEZC6A
		የለም	0		
		አይመለከትም	9		
G45	በላፈው ዓመት ፓራሲታሞል/ፓናዶል ወስደው ያውቃሉ?	አዎ	1		MOPAR6A
		የለም	0		
G46	በላፈው ወር ሰንት የፓራሲታሞል ወይም ፓናዶል ኪኒኖች ዉጠዋል?	[ ] [ ] ¨eÇK<::			MOPAFR6A
G47	ማንኛውም አይነት መድኃኒት ለየትኛውም አይነት በሽታ በሕክምና በትርብ ጊዜ ታዘልዎት ያውቃል? (ይህን ጥያቄ ለማንኛውም አይነት በሽታ የታዘዘልዎትን መድሃኒት ያካትታል ነገር ግን ፓራሲንታሞል/ፓናዶልን ወይም አስፕሪንን አይጨምርም::)	አዎን	1		MANTIB6A
		የለም	0		
G48	መልስዎ አዎ ከሆነ የሚወስዱትን መድሃኒት አይነቱንና ስሙን በማየት ይሞላ::	1.----------			MANTA6A
		2.----------			MANTB6A
		3.----------			MANTC6A

1.3 ቤትዎን የተመለከተ

G49	የቤትዎ ጣራ የተሰራው ከምንድን ነው?	ሣር	1	GROOF6A
		ቆርቆሮ	2	
		ሌላ (ይገለጽ)	9	
G50	ግድግዳው ከምን የተሠራ ነው?	እንጨትና ጭቃ	1	GWALL6A
		እንጨት፣ ጭራሮና ሳር	2	
		ድንጋይና ሲሚንቶ	3	
		ብሎኬት	4	
		ጡብ	5	
		ቆርቆሮ	6	
		ሌላ (ይገለጽ)	9	
G51	የህፃኑ/ኗ መኖሪያ ቤት ወለል የተሰራው ከምንድን ነው?			GFLOOR6A
		ከሲሚንቶ	1	
		ከጣውላ ወይም እንጨት	2	
		ከሸክላ	3	
		ከአፈር	4	
		ሌላ(ይግለጹ)	9	
G52	የህፃኑ/ኗ መኖሪያ ቤት ወለል በምንጣፍ ወይም በሌላ ነገር ተሸፍኗል?	አዎን	1	GCOVER6A
		የለም	0	

G53	ቤተሰቡ ምግብ በብዛት የሚያበስሉት የት ነው? **(አንዱ ላይ ብቻ ምልክት አድርጊ)**	በዋናው ቤት ውስጥ	1	GCOOK6A
		እቤት ውስጥ ሆኖ ከዋናው ቤት ሌላ	2	
		ከቤት ውጭ ማዕድ ቤት	3	
		ከቤት ውጭ ክፍት ቦታ	4	

G54 የህፃኑ/ኗን ቤተሰብ ከሚከተሉት የማገዶ ዓይነቶች ምግብ ለማብሰል በየስንት ጊዜው እንድሚጠቀሙ ቢጠቁሙ?

ማገዶ/ነዳጅ	አልፎቀምም	አንዳንድ ጊዜ	ምየቀኑ	
1. ከሠል	1	2	3	GFUEL6AA
2. እንጨት	1	2	3	GFUEL6AB
3. ቅጠል	1	2	3	GFUEL6AC
4. ከብት	1	2	3	GFUEL6AD
5. ናፍጣ/ሳንባ	1	2	3	GFUEL6AE
6. ቡታጋዝ	1	2	3	GFUEL6AF
7. መብራት/ኮርንቲ	1	2	3	GFUEL6AG
9. ሌላ (ይግለጹ)	1	2	3	GFUEL6AH

G55 የህፃኑ/ኗን ቤተሰበ ከሚከተሉት የማገዶ ዓይነቶች ምግብ ከማብሰል ውጭ ለሌላ ጉዳይ ይጠቀማል (ለምሳሌ ለሙቀትና ለመብራት)?

ማገዶ/ነዳጅ	አልጠቀምም	አንዳንድ ጊዜ	በየቀኑ	
1. ከሠል	1	2	3	GFUEL6AAA
2. እንጨት	1	2	3	GFUEL6AAB
3. ቅጠል	1	2	3	GFUEL6AAC
4. ከብት	1	2	3	GFUEL6AAD
5. ናፍጣ/ሳንባ	1	2	3	GFUEL6AAE
6. ቡታጋዝ	1	2	3	GFUEL6AAF
7. ባትሪ ድንጋይ	1	2	3	GFUEL6AAG
8. መብራት/ኮርንቲ	1	2	3	

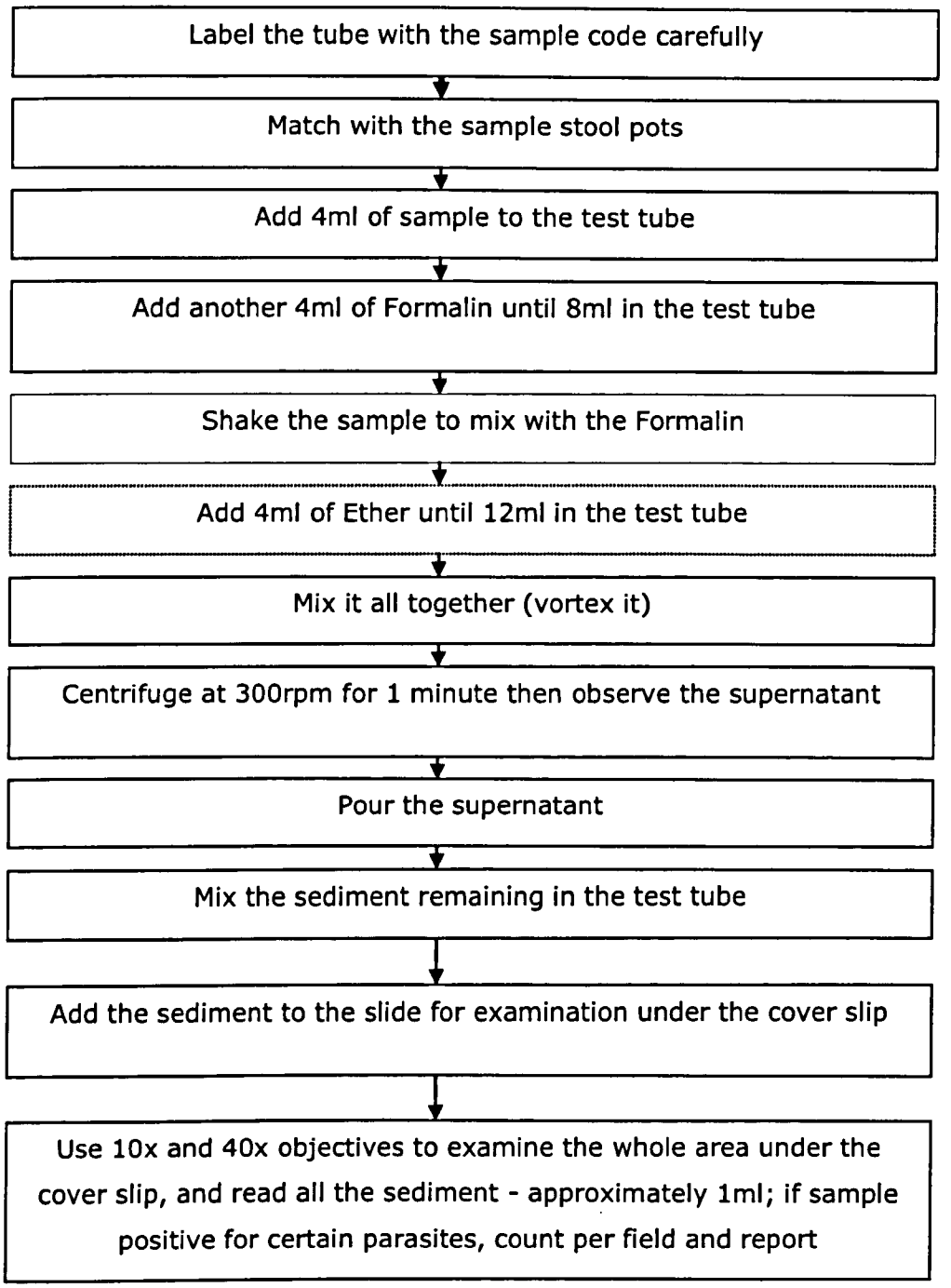
	9. ሌላ (ይግለጹ)	1	2	3	GFUEL6AAH GFUEL6AAI

G56	የህፃኑ/ኗን ብተሰብ ከሚከተሉት እንሳሳትና ከብቶች ዓይነቶች የትኞቹ አሏችው?			
		አንስሳ	በቤት ውስጥ ይጠበቃሉ/ያድራሉ	ከቤት ውጪ ይጠበቃሉ
		1. ድመት	1	2
		2. ውሻ	1	2
		3. ዶሮ	1	2
		4. ሳም/በሬ	1	2
		5. በግ	1	2
		6. ፈረስ	1	2
		7. ፍየል	1	2
		8. በቅሎ/አህያ	1	2
		9. ሌላ	1	2

G56				GANIM6AA GANIM6AB GANIM6AC GANIM6AD GANIM6AE GANIM6AF GANIM6AG GANIM6AH GANIM6AI
G57	የህፃኑ/ኗን ቤተሰብ የመጠጥ ውሃ በዋንኝነት የሚያገኙት ከየት ነው?	በግቢው ውስጥ ከሚገኝ ቧንቧ	1	WAT6A
		ከግቢ ውጪ ከሚገኝ ቧንቧ	2	
		ከጉድጓድ ወይም ምንጭ	3	
		ከተጠበቀ ጉድጓድ ወይም ምንጭ	4	
		ከወንዝ፤ ከኩሬ፤ከጉድጓድ	5	
		ከዝናብ ውሃ	6	
G58	የህፃኑ/ኗን ቤተሰቡ የሚገለገልበት መፀዳጀ ቤት ምን አይነት ነው?	በውሃ የሚሰራ ሽንት ቤት	1	SANIT6A
		ሽታ አልባ መጸዳጃ	2	
		የተለመደ ዓይነት የሽንት ቤት ጉድጓድ	3	
		ሜዳ ላይ ወይም ጫካ	4	
		በየቀኑ	2	
		ቢያንስ በሳምንት አንድ ጊዜ	3	
		ቢያንስ በ15 ቀን አንድ ጊዜ	4	
		በበዓል ቀን ወይንም ለየት ባለ ቀን	5	
		አሳውቅም	6	
		መልስ መስጠት አልፈለኩም	7	
G59	የህፃኑ/ኗ ቤተሰብ ቆሻሻ ለመጣያነት የሚገለገሉበት ምንድን ነው?	በጉድጓድ ውስጥ	1	GSAND6A
		ሜዳ ላይ	2	
		አንድ ላይ ሰብስቦ በማቃጠል	3	
		በቆሻሻ መጣያ እቃ	4	
		ሌላ (ይገለፅ)	9	
G60	የህፃኑ/ኗ ቤተሰብ ከሚከተሉት የተባይ ማጥፊያ መድኃኒቶች በቤቱ ውስጥ የትኛውን ይጠቀማሉ? (ከአንድ በላይ መልስ መስጠት ይቻላል) (መልሱ ይነበብ)	ዲዲት	1	GINSE6AA
			0	
		ማላታይን	1	GINSE6AB
			0	
		ፍሊት	1	GINSE6AC
			0	
		ሌላ (ይግለጹ)	1	GINSE6AD
			0	
G61	እንደ ፊሊት የመሳሰሉት ጸረተባይ መድሀኒቶች የት ነው የሚያስቀምጡት?(ስፍራዉን	ልጆች ሊደርሱበት በሚችሉበት ቦታ	1	PROT6A

	እንዲያሳዩሽ ጠይቂያቸዉ) አሁን ቤት ውስጥ ጸረተባይ መድኃኒቶች ከሌላቸው ቢኖራቸው ኖሮ የት እንደሚያስቀምጡ ጠይቂያቸው	ልጆች ሊደርሱበት በማይችሉበት ቦታ	0	

Appendix VI Formol Ether Concentration technique for geohelminth analysis, Butajira birth cohort, Ethiopia



Appendix VII Rapid test on card to determine Helicobacter pylori antigen in the stool sample, Butajira birth cohort, Ethiopia

Procedures:

1. Liquid stool: Open the bottle with the extraction liquid and drop 6-7 drops of the stool sample in the bottle, and for solid stool use 3 or 4 grains of rice.
2. Mix the stool thoroughly (vortex mixer can be used).
3. Wait at least 5 minutes after dissolving well.
4. Drop 4 drops of the mixture in to the sampling window.
5. Read within 2-3 minutes (maximum within 10 minutes).
6. Report using *H. pylori* reporting format

Interpretation:

1. Negative: Only one transversal BLUE line (CONTROL) appears in the test window.
2. Positive: Beside the control transversal blue line, there will be another transversal RED line in the area near the test window.

Appendix VIII Field skin test protocol for sensitization test, Butajira birth cohort, Ethiopia

Skin Test Protocol

1. Seat the study subject as comfortable as possible and ask them to hold their left forearm towards you, resting it on their leg.
2. Explain that you are going to measure the skin's response to some solutions, which this will not hurt but that occasionally it can be itchy.
3. Use a biro and the ruler to draw an 8cm line longways down the middle of the palmar side of the fore-arm.
4. Label sections from the top: S (saline); D (Dermatophagoides); H (histamine); C (cockroach).
5. Use the dropper in the bottle to put a TINY drop of each solution at the correct label.
6. Place the skin prick lancet into the drop of solution almost parallel with the skin, press the tip in lightly so it just catches, then lift the skin for 2 seconds and release. Use a skin prick lancet for each drop of solution, or the solution from one may contaminate the next.
7. Wait 15 minutes. You may use this time to start filling in the questionnaire.
8. For each section, use the ruler to measure the BIGGEST diameter where the skin is raised as well as red. Record this as 'D1' on the sheet. Then measure the diameter at right angles to the first. Record this as 'D2'. Don't forget to record the participant's project identification number.

Appendix IX Ethics approval certificate, Ethiopian Science and Technology Ministry, Addis Ababa University Institutional Review Board and University of Nottingham, UK.

በኢትዮጵያ ፌዴራላዊ ዴሞክራሲያዊ ሪፑብሊክ
የሳይንስና ቴክኖሎጂ ሚኒስቴር
The Federal Democratic Republic of Ethiopia
Ministry of Science and Technology

ቁጥር ፀ/ሚ/ቴ\65-91\2010
Ref. No. 4 የካቲት 2002
ቀን
Date

Addis Ababa University, Faculty of Medicine
Office of Associate Dean,
Postgraduate Program and Research
Addis Ababa--Ethiopia

Re: Renewal and Extension of Ethical Approval

We would like to appreciate and recognize your research undertaking in "Effects of Geohelminth infection on the incidence of Wheeze and Allergic in an Ethiopian Birth cohort" for which you request to the National Ethics Review Committee for project extension/renewal.

It is our pleasure to inform you that; since the aforementioned project proposal has no any major changes (the study participants remain the same; the study methodology is identical; the study instruments are the same; the same outcomes are to be measured) the Secretariat office of NERC has evaluated the submitted progress report and the renewed project proposal and approved the extension/renewal request by extending the project life to July, 2011.

Worth mentioning this; we are confident that the principal investigator shall comply with the standard International and National scientific and ethical implementation guidelines. It is also strictly recommend that the host institute to monitor the ethical implementation of the project as it was stipulated in the original approved document.

With regards,

Feleke Kibret
Secretary of NERC

The Federal Democratic Republic of Ethiopia Ministry of Science and Technology

C.C.
Gail Davey (Dr)
Addis Ababa University,
Faculty of Medicine, Addis Ababa

ማነጋገር ቢያስፈልግዎ
You may Contact

ፖ.ሳ.ቁ P.O.Box 2490 | አዲስ አበባ ኢትዮጵያ Addis Ababa Ethiopia | ስልክ Tel 251-011-5-56 21 55 | ፋክስ Fax 251-011-1-56 27 49
E-mail most@ethionet.et Web site:- http:/www.most.gov.et

Material Transfer Agreement

This Material Transfer Agreement (MTA) has been prepared for use by The University of Nottingham, represented in this instance by its School of Community Health Sciences ("The Recipient") and Addis Ababa University, P.O. Box 9086 Addis Ababa, Ethiopia, in this instance represented by its School of Public Health ("The Provider"), and relates to the transfer of dust samples arising from the project entitled: "Effects of geohelminth infection on the incidence of wheeze and allergic sensitization in an Ethiopian birth cohort".

1. The Provider agrees to transfer to the Recipient a maximum of 1000 dust samples collected from the houses of research participants living in and around Butajira, southern Ethiopia in 2008/2009, and a maximum of 1000 dust samples collected in 2010/2011. The research material will only be used for research purposes as described in the protocol attached hereto in the designated laboratory, under suitable containment conditions. The research material will not be used for commercial purposes such as screening, production or sale for which a commercial licence may be required. The Recipient agrees to comply with all National and International guidelines and regulations applicable to the research project and to the handling of the research material.

a) Are the research materials of human origin?

Yes ☐ No ☒

b) If Yes, are the materials collected according to the details in the protocol and in adherence to National Health Research Ethics Review Committee (NERC) recommendations? (N/A)

Yes ☐ No ☐

2. This research material and its derivatives will be used by the Recipient investigators solely in connection with the project "Effects of geohelminth infection on the incidence of wheeze and allergic sensitization in an Ethiopian birth cohort", as described in the protocol.

3. all presentations or written publications concerning the research project, the Recipient will seek agreement of the Provider and acknowledge the Provider's contribution of this research material unless requested otherwise.

4. This research material represents a significant contribution on the part of the Provider and is considered proprietary to the Provider. The Recipient therefore agrees to retain control over this research material and further agrees not to transfer it to other people not under his/her direct supervision without advance written approval of the Provider. The research material will be disposed of as agreed at the end of the Project.

5. The Provider does not take any responsibility for loss, damage, wastage or spoilage of the research material during or after shipment to the address provided by the Recipient under conditions of shipment agreed to in the attached protocol. This research material is provided as a service to the research community. It is being supplied to the Recipient with no warranties, express or implied, including any warranty of merchantability or fitness for a particular purpose. The Provider makes no representations that the use of the research material will not infringe any patent or proprietary right of third parties.

6. The Recipient shall notify the Provider in writing of any invention, improvement, modification, discovery or development to the material or the information arising from it, herein after referred to as the Invention. Nothing in the agreement shall however be construed as conveying to the Provider any rights under any patents related to such an Invention. At its option, the Provider shall be entitled to receive samples of materials derived from the research materials for its own research and evaluation purposes only.

7. The under-signed Provider and Recipient expressly certify that the contents of any statements made here are truthful and accurate.

8. Any additional terms: N/A

9. The Provider maintains ownership right of the research material and its derivatives unless stated otherwise.

Signature Page

For the **Recipient**:

Recipient Investigator
(Name) Professor John Britton
(Signature) .....
Date 6.10.08
Mailing Address:
University of Nottingham
School of Community Health Sciences
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Nottingham
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United Kingdom
Tel:0115 823 1708
Fax:0115 823 1946

Duly Authorized
(Name) Mr. Paul N Cartledge
(Signature) .....
Date 1/10/08
Mailing Address:
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Research Innovation Services
King's Meadow Campus
Lenton Lane
Nottingham
NG7 2NR
United Kingdom

Tel: 0115 951 5679
Fax:0115 951 3633

For the **Provider**:

Provider Investigator
(Name) Dr Fikre Enqueselassie
(Signature) .....
Date .....
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Addis Ababa, Ethiopia
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Duly Authorized
(Name) Dr Damen HaileMariam
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Date .....
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ANNEX 3
Form AAUMF 03-008

IRB's Decision

Meeting No: 020/2009 Date (D/M/Y): August 05/2009
Protocol number: 077/09/SPH Assigned No.:.....

Protocol Title: **Effects of Geohelminth infection on the incidence of Wheeze and Allergic symptoms in a Ethiopia Birth cohort.**		
Principal Investigators:	Dr. Gale Davey	
Institute:	AAU –MF School of Public Health	
Elements Reviewed (AAUMF 01-008) :	☑ Attached	☐ Not attached
Review of Revised Application ☐ Yes ☐ No	Date of Previous review:	
Decision of the meeting:	☑ **Approved** ☐ Approved with Recommendation ☐ Resubmission ☐ Disapproved	

I. Elements approved- 1. Protocol Version No. .....
2. Protocol Version Date.....
3. Informed consent Version No. .....
4. Informed Consent Version Date .....

II. Obligations of the PI-
1. Should comply with the standard international & national scientific and ethical guidelines
2. All amendments and changes made in protocol and consent form needs IRB approval
3. The PI should report SAE within 10 days of the event
4. End of the study, including manuscripts and thesis works should be reported to the IRB

III. TO ESTM ☑

Institution Review Board (IRB) Approval: Period from **17/08/09 to 16/08/2011**

Follow up report expected in

3 Months ___ 6 months ✓ 9 months_____ one year____

Chairperson, IRB
Dr. Yeweyenhareg Feleke
Signature _____
Date: 17/08/09

Institutional Review Board Office (IRB) Faculty of Medicine ADDIS ABABA UNIVERSITY

Associate Dean for Postgraduate and Research
Signature _____
Date 19/08/09

Dean's Office Faculty of Medicine ADDIS ABABA UNIVERSITY

The University of Nottingham

Faculty of Medicine and Health Sciences

Medical School Research Ethics Committee
Division of Therapeutics & Molecular Medicine
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Queen's Medical Centre
Nottingham
NG7 2UH

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Please quote ref no: **G/9/2007**

Direct line/e-mail
+44 (0) 115 8231063
Louise.Sabir@nottingham.ac.uk

Professor John Britton
Head of Division of Epidemiology and PH
Division of Epidemiology and Public Health
Clinical Sciences Building
City Hospital Campus
Nottingham University Hospitals Trust
Hucknall Road
NG5 1PB

19 November 2007

Dear Professor Britton

Ethics Reference No: G/9/2007 - *Please quote this number on all correspondence*
Study Title: Effects of intestinal parasite infection on the incidence of wheeze and allergic sensitization in an ethopian birth cohort.
Lead Investigator: Professor John Britton, Head of Division of Epidemiology and PH
Co Investigators: Dr Gail Davey, Associate Professor/Hon Senior Lecturer Division of Epidemiology & PH UoN, Dept of Community Health, Addis Ababa University Ethiopia, Dr Charlotte Hanlon, Wellcome Trust Research Training Fellow, Dept of Health Services Research Institute of Psychiatry, Kings College London, Dr Andrea Venn, Associate Professor in Epidemiology, Division of Epidemiology & Public Health, School of Community Health Sciences.

Thank you for your letter 15th October responding to the issues raised by the Committee and were considered at tits meeting on 15th November 2007. The following revised documents were reviewed:

- Informtion sheet (English) Aug 2007

The Committee felt reassured by your responses and this study was approved.

Approval is given on the understanding that the Conditions of Approval set out below are followed.

Conditions of Approval

You must follow the protocol agreed and any changes to the protocol will require prior Ethic's Committee approval.

This study is approved for the period of active recruitment requested. The Committee also provides a further 5 year approval for any necessary work to be performed on the study which may arise in the process of publication and peer review.

You promptly inform the Chairman of the Ethic's Committee of

(i) Deviations from or changes to the protocol which are made to eliminate immediate hazards to the research subjects.

(ii) Any changes that increase the risk to subjects and/or affect significantly the conduct of the research.

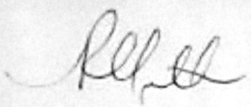
(iii) All adverse drug reactions that are both serious and unexpected.

(iv) New information that may affect adversely the safety of the subjects or the conduct of the study.

Statement of Compliance (from May 2004 only)

The University of Nottingham Medical Research Ethics Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

Yours sincerely



Professor R C Spiller
Chairman, Nottingham University Medical School Ethics Committee