FARM EXPOSURES AND PREVALENCE OF ATOPY AND ASTHMA AMONG SCHOOL-AGED CHILDREN LIVING IN AGRICULTURAL SETTINGS

A Dissertation Submitted to the College of
Graduate and Postdoctoral Studies
In Partial Fulfillment of the Requirements
For the Degree of
Doctor of Philosophy
In the Health Sciences Graduate Program
University of Saskatchewan
Saskatoon, SK Canada

By

MANH LUAN CHU

PERMISSION TO USE

In presenting this dissertation in partial fulfillment of the requirements for a Postgraduate degree from the University of Saskatchewan, I agree that the Libraries of this University may make it freely available for inspection. I further agree that permission for copying of this dissertation in any manner, in whole or in part, for scholarly purposes may be granted by the professor or professors who supervised my dissertation work or, in their absence, by the Head of the Department or the Dean of the College in which my dissertation work was done. It is understood that any copying or publication or use of this dissertation or parts thereof for financial gain shall not be allowed without my written permission. It is also understood that due recognition shall be given to me and to the University of Saskatchewan in any scholarly use which may be made of any material in my dissertation.

DISCLAIMER

Reference in this dissertation to any specific commercial products, process, or service by trade name, trademark, manufacturer, or otherwise, does not constitute or imply its endorsement, recommendation, or favoring by the University of Saskatchewan. The views and opinions of the author expressed herein do not state or reflect those of the University of Saskatchewan, and shall not be used for advertising or product endorsement purposes.
Requests for permission to copy or to make other uses of materials in this dissertation in whole or part should be addressed to:

Head of the Health Sciences Graduate Program, College of Medicine
107 Wiggins Road
University of Saskatchewan
Saskatoon, SK S7N 5E5
Canada.

OR

Dean
College of Graduate and Postdoctoral Studies
University of Saskatchewan
116 – 110 Science Place
Saskatoon, SK S7N 5C9
Canada.

ABSTRACT

Background: Asthma and atopic diseases are among the most common diseases afflicting children in Canada. The prevalence and impact of disease seems to differ between urban and rural locations but there are still many unknowns as to reasons for these differences. Some studies have shown an inverse association between farm dwelling and asthma, yet this association has not been always consistent Atopic/non-atopic asthma is one of the most common phenotypes considered in asthma classification yet there have been few studies examining the nature of the classification in with rural populations. Some of the inconsistency could result from differences in the exposure-outcome associations between atopic and non-atopic asthma phenotypes. In addition, there is little information as to what extent children with asthma and/or allergies are exposed to specific activities on the farm. Technology to accurately assess exposures is required such as the use of personal sampling techniques to determine levels of exposures including endotoxin, which has been inversely associated with asthma and atopy and occurs in higher levels in rural compared to urban levels. However, there is limited information about the utility and efficacy of environmental dust collection with personal samplers in children.

Objectives: The purpose of this dissertation is to examine the association between farm-related environmental factors and asthma and atopic disease in children. My specific objectives are: i) To determine if early life farm-related exposures are associated with atopy development in school-age children; ii) To estimate the prevalence of atopic and non-atopic asthma and identify risk factors for each phenotype among rural dwelling children; iii) To determine the prevalence of atopy and asthma and its associations with specific farm-related activities and exposures for both farm and non-farm school-age children; iv) To determine whether a personal air sampling pack

that we develop could be useful in future studies of lung health with regard to adequate dust collection and feasibility.

Methods: This thesis approaches the overall topic of environmental exposures in relation to atopy and asthma. First, it includes an updated systematic review to examine the association between early life exposures to a farm and the presence of atopy measured by skin-prick testing or IgE antibody by blood tests in school-age children. Second, it includes a cross-sectional survey analysis of a provincial dataset of rural dwelling children (The Saskatchewan Rural Health Study) to estimate the prevalence of atopic and non-atopic asthma and identify risk factors for each phenotype among rural dwelling children. Along with a questionnaire report of asthma, skin prick testing was used to asses atopy. Third to examine the associations between specific farm exposures and activities and the presence of asthma, atopy, and atopic asthma phenotypes in children we analyzed data from a separate cross-sectional survey dataset from a provincial sampling of rural dwelling children (The Saskatchewan Farm Injury Study). Fourth, the thesis includes the results of a pilot study to test the feasibility of personal exposure monitoring (PEM) to objectively measure environmental exposures in children.

Results: Fourteen studies met the inclusion criteria for the systematic review. The results consistently showed that early farm-related exposures can protect children from becoming atopic at school age. However, there was heterogeneity in the assessment of outcomes and exposures of interest.

From the Saskatchewan Rural Health Study, asthma prevalence was 14.7% of which 32.1% of cases were atopic. Location of residence (farm vs non-farm) was not associated with having either atopic or non-atopic asthma. Predictors of childhood asthma, regardless of atopic status were early respiratory illness and a family history of asthma. Being overweight and having a dog in the

home were associated with an increased risk of nonatopic asthma. Maternal history of smoking increased the risk of atopic asthma. Compared to those with nonatopic asthma, in the past 12 months, children with atopic asthma were more likely to report a sneezy, runny, or blocked nose or have shortness of breath, whereas those with nonatopic asthma were more likely to have parents who missed work. Those with nonatopic asthma had significantly lower forced expiratory volume in 1 second compared to those with atopic asthma.

From the Saskatchewan Farm Injury Study, asthma prevalence was 7.6% and atopy presence was 4.7%. Among those with asthma, 29.5% were atopic. Home location (farm vs non-farm) was not associated with asthma or asthma phenotype. Doing routine chores with large animals was associated with an increased risk of asthma [aOR=1.83(1.07-3.15); p=0.027], and atopic asthma [aOR= 2.37 (95%CI=1.04-5.40); p=0.04].

Using the PEM methods, we collected sufficient dust to detect endotoxin and β-(1 → 3)-D-glucan. Some correlations of these measures between personal (PEM) and settled (play area and mattresses) were observed but were not statistically significant. Evidence from our exit survey regarding the PEM wearing suggested that the design of the PEM device with a fanny pack should be modified to maximize convenience and suitability in order to make its use more practical.

Conclusions: Early-life farm exposures were found to be associated with a protective effect on objective markers of atopy. Exposures may contribute differentially to atopic and nonatopic asthma and result in differential clinical presentation or burden although not necessarily with farm dwelling. Those who did routine chores with large animals were at an increased risk of having asthma, specifically atopic asthma. The pilot study successfully showed that the PEM is an effective method to collect sufficient dust to detect endotoxin and β-(1 → 3)-D-glucan levels in children, although modifications should be made to it to make it more feasible in studies of

children. The results of this thesis suggest that exposure to a rural setting, including farm activities, can impact outcomes related to atopy and asthma. Future work should focus on refinement of data collection methods to help improve accuracy as well as future study of the characteristics of atopic and non-atopic asthma and its associations with environmental exposures is important for etiologic understanding and management decisions.

Key words: Farm exposures; children; asthma; atopy; children; agriculture

CO-AUTHORSHIP

This dissertation contains four separate manuscripts which were completed and written by Mr. Luan Manh Chu in collaboration with his supervisor, Dr. Joshua A. Lawson from the Canadian Centre for Health and Safety in Agriculture (CCHSA) and Department of Medicine, College of Medicine, University of Saskatchewan, and dissertation advisory committee members: Drs. Donna C. Rennie (CCHSA and College of Nursing, University of Saskatchewan), Donald Cockcroft (CCHSA and Department of Medicine, College of Medicine, University of Saskatchewan), John R. Gordon (CCHSA and Department of Medicine, College of Medicine, University of Saskatchewan) and Shelley Kirychuk, (CCHSA and Department of Medicine, College of Medicine, University of Saskatchewan). Other non-committee member co-authors include: Drs. William Pickett (Department of Public Health Sciences, Queen's University, Kingston, Ontario, Canada); James Dosman (CCHSA); Ms. Brooke Thompson (CCHSA); Mrs. Louise Hagel (CCHSA); Dr. Chandima P. Karunanayake (CCHSA); and Dr. Punam Pahwa (Department of Community Health and Epidemiology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada & CCHSA).

"Atopy risk among school-aged children in relation to early exposures to farm environments: A systematic review"

Mr. Chu conceptualized and designed the study, conducted data search and management, interpreted the data, and prepared and revised the manuscript; Dr. Joshua A. Lawson, Mr. Chu's PhD supervisor, contributed to the study concept, design, data search and management and interpretation, reviewed and revised the manuscript; Drs. Donna C. Rennie, Donald Cockcroft, John R. Gordon, Shelley Kirychuk contributed to the study methodology, results interpretation, reviewed and revised the manuscript.

"Prevalence, risk factors, and clinical outcomes of atopic and nonatopic asthma among rural children"

Mr. Chu conducted data management, interpreted data, prepared sections of the manuscript, and revised the manuscript. Dr. Joshua A. Lawson, Mr. Chu's PhD supervisor, contributed to the study concept, design, data management and interpretation, reviewed and revised the manuscript; Drs. Donna C. Rennie, Chandima P. Karunanayake, and Mrs. Louise Hagel contributed to the study methodology, results interpretation, reviewed and revised the manuscript. Drs James Dosman and Punam Pahwa were the PI and Co-PI on the grant used to collect the data.

"Farm exposures and allergic disease among children living in a rural setting"

Mr. Chu conceptualized and designed the study, conducted data management, interpreted the data, and prepared and revised the manuscript; Dr. Joshua A. Lawson contributed to the study concept, design, data management and interpretation, reviewed and revised the manuscript; Drs. Donna C. Rennie, Donald Cockcroft, John R. Gordon, Shelley Kirychuk, William Pickett, and James

Dosman contributed to the study methodology, results interpretation, reviewed and revised the manuscript. Drs Will Pickett and James Dosman were the PIs on the grant used to collect the data.

"An investigation of personal exposure monitoring to collect environmental exposure data in school-aged children"

Mr. Chu conceptualized and designed the study, conducted data management, interpreted the data, and prepared and revised the manuscript; Dr. Joshua A. Lawson, Mr. Chu's PhD supervisor, contributed to the study concept, design, data management and interpretation, reviewed and revised the manuscript; Drs. Donna C. Rennie, Donald Cockcroft, and John Gordon contributed to the study methodology, results interpretation, reviewed and revised the manuscript. Dr. Shelley Kirychuk and Ms. Brooke Thompson contributed to study methodology, sample preparation, laboratory analysis of dust samples, results interpretation, reviewed and revised the manuscript.

ACKNOWLEDGEMENTS

This research was supported by the Public Health and the Agricultural Rural Ecosystem (PHARE), the Saskatchewan Innovation and Opportunity Scholarship (SIOS), the Founding Chairs Fellowship at the Canadian Center for Health and Safety in Agriculture (CCHSA); and the College of Medicine Devolved Scholarship at the University of Saskatchewan. Thank you for making this project possible and a success.

I would like to thank my most amazing supervisor, Dr. Joshua A. Lawson–the 2017 University of Saskatchewan Life and Health Sciences Best Supervisor Award Winner. It is whole-heartedly appreciated that his immense support, expert guidance, understanding and great advice for my study proved monumental towards the success of this dissertation. To all members of my committee (Drs. Donna Rennie, Donald Cockcroft, John Gordon, and Shelley Kirychuk), I would like to recognize the invaluable assistance that you all provided during my study with direction, encouragements and timely feedback. I also would like to pay my special regards to Dr. William Picket and Dr. James Dosman who supported me during the manuscript writing. My sincere gratitude also goes to children, parents who took time to participate in the study. A special thanks to Ms. Brooke Thompson for assisting with planning and lab data analyses.

My appreciation also goes to all faculty and staff members in the Health Sciences program and the Canadian Centre for Health and Safety in Agriculture, University of Saskatchewan for their support, logistics and knowledge gained. My sincre thanks goes to Dr. Punam Pahwa and Dr. Chandima Karunanayake, for offering me support and provide me with invaluable advice towards my professional development.

Finally, I must express my very profound gratitude to my parents and my sister for providing me with unfailing support and continuous encouragement throughout my years of study. This accomplishment would not have been possible without them.

DEDICATION

This dissertation is dedicated to:

i) Children with asthma and other respiratory-related diseases, especially those who live and work in rural settings.

ii) All who are in search of knowledge.

TABLE OF CONTENTS

Content……	Page
Permission to use……	i
Disclaimer……	i
Abstract……	ii
Co-authorship……	v
Acknowledgements……	viii
Dedication……...……	ix
Table of Contents……	x
List of Tables…….	xv
List of Figures……...	xvii
List of Abbreviations……	xviii

CHAPTER 1: INTRODUCTION

1.1.Background……...	1
1.2. Purpose of the study…….	4
1.3. Organization of the dissertation ……..….	4
1.4. References……	5

CHAPTER 2: LITERATURE REVIEW

2.1.General scope of literature review……	14
2.2.Methods……	14
2.3.Measurement methods of atopy and asthma in children	15
2.3.1. Atopy……..	15

2.3.2. Asthma…… 16

2.3.2.1.Atopic and non-atopic asthma phenotype…… 17

2.4.Temporal and geographic patterns in condition prevalence …… 19

2.4.1. Atopy……. 19

2.4.2. Asthma……... 21

2.5.Rural dwelling or farming in children and its relationship with conditions…… 24

2.5.1. Atopy and farm vs. rural non-farm children…… 26

2.5.2. Asthma and farm vs. rural non-farm children…… 32

2.6.Farm-related activities and asthma and atopic disease……. 39

2.7.Microbial exposures and their relationship with atopy and asthma……. 41

2.8.Exposure measurement methodology and asthma and atopy in school-age children 54

2.9.Summary of literature review ……..….. 61

2.10. Research rationale……... 62

2.11. Research objectives and research questions…… 63

2.12. References……. 65

CHAPTER 3: ATOPY RISK AMONG SCHOOL-AGED CHILDREN IN RELATION TO EARLY EXPOSURES TO FARM ENVIRONMENTS: A SYSTEMATIC REVIEW (MANUSCRIPT 1)

3.1. Abstract…… 91

3.2. Introduction……. 92

3.3. Methods…….. 94

3.4. Results…… 97

3.5. Discussion…… 101

3.6. References…… 106

CHAPTER 4: PREVALENCE, RISK FACTORS, AND CLINICAL OUTCOMES OF ATOPIC AND NONATOPIC ASTHMA AMONG RURAL CHILDREN (MANUSCRIPT 2)

4.1. Abstract…… 129

4.2. Introduction……. 130

4.3. Methods…….. 132

4.4. Results…… 138

4.5. Discussion…… 140

4.6. References…… 145

CHAPTER 5: FARM EXPOSURES AND ATOPIC DISEASE AMONG CHILDREN LIVING IN A RURAL SETTING (MANUSCRIPT 3)

5.1. Abstract…… 159

5.2. Introduction…….. 161

5.3. Methods……... 163

5.4. Results……. 169

5.5. Discussion…… 171

5.6. References…… 176

CHAPTER 6: AN INVESTIGATION OF PERSONAL EXPOSURE MONITORING TO COLLECT ENVIRONMENTAL EXPOSURE DATA IN SCHOOL-AGE CHILDREN (MANUSCRIPT 4)

6.1. Abstract…… 190

6.2. Introduction…….. 191

6.3. Methods……... 194

6.4. Results…….. 199

6.5. Discussion…… 200

6.6. References…… 206

CHAPTER 7: DISCUSSION

7.1.Key findings……. 213

7.2.How the findings fit in literature…… 215

7.3.Validity of the study……. 220

7.3.1. Internal validity…….. 220

7.3.1.1.Selection bias……... 220

7.3.1.2.Information bias…….. 222

7.3.1.3.Confounding factors…… 224

7.3.2. External validity……. 225

7.4. References…… 227

CHAPTER 8: RECOMMENDATIONS AND CONCLUSIONS

8.1.Recommendations…… 234

8.2.Future research directions……. 234

8.3.Conclusions……... 236

8.4.References……. 236

CHAPTER 9: APPENDICES

9.1. Appendix A: MOOSE Checklist for Meta-analyses of Observational Studies…… 238

9.2. Appendix B: Quality assessment score……... 241

9.3. Appendix C: The Saskatchewan Farm Injury Cohort -Child's Components Study Questionnaire…… 243

9.4. Appendix D: Ethical approval for the secondary use of the Saskatchewan Farm Injury Cohort – Child's Component (Manuscript 3)…….... 258

9.5. Appendix E: Multivariate analyses of farm exposures and allergic disease among children 6-17 years old…… 259

9.6. Appendix F: Multivariate analyses of farm exposure s and allergic disease, splitting by age groups……. 260

9.7. Appendix G: Ethical approval for the PEM pilot study (Manuscript 4)…… 261

LIST OF TABLES

CHAPTER 2 Page

Table 2-1: Atopy prevalence among farmers' children and rural non-farmers' children at school age…… 28

Table 2-2: Asthma prevalence among school-age children living on a farm and rural non-farming residence…… 34

Table 2-3: Studies investigating the effect of microbial exposures and outcome of interest (atopy measured by Skin-prick testing or blood test measured overall and specific IgE)….. 43

Table 2-4: Studies investigating the effect of microbial exposures and asthma among school-age children……..… 49

Table 2-5: Levels of endotoxin between rural vs. urban and farm vs. non-farm settings by different measurement methods……..... 55

CHAPTER 3

Table 3-1: Associations between early-life farm exposures and atopic sensitization/atopy defined by total serum IgE, specific IgE, or skin prick test measured in children……. 119

CHAPTER 4

Table 4-1: Personal and Environmental Characteristics by Asthma Status…….. 154

Table 4-2: Results of Multivariate Analysis for Factors Related to Nonatopic and Atopic Asthma in Children……... 155

Table 4-3: Respiratory Symptoms, Allergic Symptoms, Health Care Use, and Morbidity and Indicators Specific to Asthma……. 156

CHAPTER 5

Table 5-1: Characteristics of the Saskatchewan Farm Injury Cohort – Child Cohort …….. 185

Table 5-2: Farm activities between farm and rural children ……..… 186

Table 5-3: Prevalence of asthma, allergies, SABA use, and asthma phenotypes by farm-related activities……. 187

CHAPTER 6

Table 6-1. Mean values (Arithmetic & geometric) and Median value of endotoxin and β-(1 → 3)-D-glucan in house dust from play area floor, mattress, and PEM samples……... 207

Table 6-2: Spearman correlation coefficients among different sources of dust……. 208

LIST OF FIGURES

CHAPTER 3

Figure 3-1. QUOROM flow chart to select articles to the systematic review……. 126

Figure 3-2: Summary of strengths of associations among included articles…….. 127

Figure 3-3: SPT vs. specific IgE measurement methods……. 128

Figure 3-4: Funnel plots of included articles (with main exposures)…….. 129

Figure 3-5: Funnel plots of included articles (with main exposures and other related exposures)……..... 129

CHAPTER 4

Figure 4-1. Mean lung function test results by asthma status……... 158

CHAPTER 5

Figure 5-1. Farm-related specific determinants among children 6-17 years old. … 190

Figure 5-2: Farm-related specific determinants of asthma phenotypes (N=1807) (Ref: No asthma) among children 6-17 years old……. 191

CHAPTER 6

Figure 6-1: The demonstration of a PEM fanny pack…… 208

LIST OF ABBREVIATIONS

BDG: Beta-(1→3)-D-Glucan

BMI: Body Mass Index

CCHSA: Canadian Center for Health and Safety in Agriculture

CI: Confidence interval

ECRHS: European Community Respiratory Health Survey

EPHPP: Effective public health practice project

ETS: Environmental tobacco smoke

EU: Endotoxin Units

GABRIEL: A Multidisciplinary Study to Identify the Genetic and Environmental Causes of Asthma in the European Community

GM: Geometric Means

GSD: Geometric Standard Deviation

IgE: Immunoglobulin E

ISAAC: International Study of Asthma and Allergies in Childhood

LAL: Limulus *Amoebocytes* Lysates

M-CAIS: Minority Farm Operator Childhood Agricultural Injury Survey

MOOSE: Meta-analysis of Observational Studies in Epidemiology

OR: Odds ratio

PARSIFAL: Prevention of Allergy Risk factors for Sensitization In children related to Farming and Anthroposophic Lifestyle

PEM: Personal exposure monitoring

QUORUM: Quality of Reporting of Meta-analyses

RRR: relative-risk ratio

SABAs: Short-Acting Beta-Agonists

SD: Standard Deviation

SPT: Skin Prick Test

SFIC: Saskatchewan Farm Injury Cohort

TLR: Toll-like receptor

TNF: Tumor necrosis factor

CHAPTER 1

INTRODUCTION

1.1.Background

Atopy is a condition that usually develops in childhood with familial tendency upon exposure to an allergen in which an individual will produce IgE antibodies.[1] The presence of atopy is usually determined by means of a skin-prick test or a blood test with specific or serum IgE measurements.[2-8] Most children with conditions such as rhinitis and dermatitis are atopic.[9-11] However, some children with atopy may not have atopic disease manifestations[12]. Atopic diseases have a significant impact on children's health, family burden and health care costs for parents.[13] According to a report from Health Canada, allergies are among the most common chronic conditions of Canadian children aged 12 years and older.[14] Asthma is a common chronic lung disease of the airways characterized by variable symptoms of wheeze, breathlessness, chest tightness and/or cough.[15] It is the most prevalent chronic respiratory disease globally and is the most common chronic disease in children.[16] Furthermore, asthma imposes a high lifetime burden on individuals and their families and is one of the main causes of hospitalization.[17]

Globally, asthma, atopy and atopic disease are among the most common chronic illnesses of childhood.[18] Atopy is also associated with asthma where the proportion of asthma attributable to atopy in children is estimated to be approximately 38% but there is considerable variation in the attributable proportions between studies (25–63%) based on a review by Pearce et al[10]. Many risk and protective factors for pediatric asthma and atopic diseases have been identified.[19,20] Atopy and asthma are complex with both a genetic and an environmental component. Genetic factors are clearly important, but cannot independently explain the increased prevalence of atopic diseases in

recent decades.[21,22] With the complexity of the relationship between asthma and atopy, the proportion of those with asthma who are atopic should be explored.

Recent evidence has suggested that changes in surrounding environments[23,24], modern dietary intakes[25,26], and lifestyle factors[27,28] have influenced the developing immune system and may contribute to the increasing prevalence of early childhood inflammatory disease including atopic disease.[29] Recent studies have shown that prenatal exposures, such as maternal smoking, diet and prematurity have effects on the development of atopic disorders.[30] Early childhood including the prenatal period has been highlighted as the period of greatest risk for developing allergies. A growing body of evidence indicates that atopy and atopic disease has fetal origins and the fetal time period is a critical window of immune system development.[31,32] It is possible that maternal exposure may affect the developing immune response through direct exposure of the fetus to antigens that cross the placental barrier.[33,34] Alternatively, maternal response to an environmental exposure may influence the in-utero environment for the developing fetus.[35] Maternal sensitization to allergens was associated with an elevated production of the Th2-like cytokine IL-13 in infants.[36] Recent reports indicate that the gut microbiota composition during the first months of life influences atopy development.[37,38] It is suggested that factors influencing the early maturation of the immune system play an important role in the presence of atopic disease.[39]

While early life is a very important period, there can be continued influence of various risk and protective factors at later stages of life as well. The natural history and risk factors of asthma and atopy vary considerably across the life course.[40] The development of asthma exacerbations and its phenotypes has been suggested to result from the interplay between the environment, biologic responses, immune systems, and genetic susceptibility.[41] The majority of chronic asthma begins in the first six years of life.[42-44] A number of prospective cohort studies have been conducted

to understand the natural history of asthma spanning childhood into adulthood.[45-47] Risk factors for asthma include infections, allergen exposure and sensitization, obesity, birth prematurity, environmental tobacco smoke exposure, and air pollution as well as genetic factors among others.[48] It is suggested that during a critical inception phase of lung development within the first 3 years of life, children are more likely to develop asthma.[49,50] By school-age, evidence has shown that allergen sensitization reaction to foods and inhalants which starts during infancy are important determinants of persistent asthma.[51] Many adults with asthma have childhood-onset disease that has persisted. Among these people, the factors that predict the persistence of asthma into adulthood include the severity of childhood disease, atopy, exposure to allergens, and a parental history of asthma[52], early age of disease onset with more severe symptoms, atopic status, cigarette smoking, gender, genetic and familial factors, and levels of allergen exposures, among others.[53,54]

Evidence has shown that environmental factors are associated with asthma and atopy. It has also been shown that there are large differences in environmental exposures between urban and rural locations including pollens and microbial exposures both in terms of their quantity and diversity.[55,56] Microbial exposures such as endotoxin is much higher in farming environments,[57,58] resulting in a possible protective effect on asthma and atopy.[59,62,63] The prevalence of childhood asthma and atopy has generally been suggested to be lower in rural children compared to the urban ones.[64-66] This trend has also been observed in Canada both at the national[67] and provincial levels[65,68]. One possible explanation for this difference is that rural children experience factors unique to their location that impact asthma development, farm exposures included. One of the explanations for the farm vs. rural non-farm childhood asthma prevalence differences has been that exposures to microbial components from a farm is key in mechanisms influencing asthma.[69] The role of these microbial agents in asthma and atopy development is complex.[69] However, the

protective effect of farm exposures with childhood asthma and atopy has been observed worldwide, yet has not always been consistent.[70-72]

1.2. Purpose of the study

The purpose of this dissertation is to examine the association between farm-related environmental factors and asthma and atopic disease in children. To better understand the relationship between the environment, specifically rural environments, and asthma and atopy in the greater context of what we know about asthma, this thesis was set up to look at those relationships in early life as well as in school age and to investigate a method to better measure environmental exposures. This was done through four major foci. First, a systematic review was conducted to explore the current literature around early life exposures to a farm and the development of atopy in school-age children. Second, a secondary analysis using province-wide cross-sectional data was conducted to estimate the prevalence of atopic and nonatopic asthma and identify risk factors for each phenotype among rural dwelling children. Third, a large sample of school-age children exposed to farm and non-farm environments in rural Saskatchewan was utilized to examine the associations between specific farm exposures and activities and atopy and asthma. Finally, personal exposure monitoring (PEM) was evaluated as a way of collecting objective measures of environmental exposures.

1.3. Organization of the dissertation

A manuscript-style approach was used for this dissertation where each major foci was investigated. Prior to the specific investigations, Chapter 2 details the relevant literature describing asthma and atopy in general, rural farm-nonfarm asthma/atopy differences and reported associated risk factors, associations between microbial exposures and outcomes of interest (asthma, atopy), and the environmental exposure measurement methodology. Chapters 3, 4, 5, and 6 present Manuscripts

1, 2,3, and 4 and describe the four major foci above, respectively. Chapter 7 includes a discussion based on the four manuscripts and brings the four manuscripts together. Finally, the recommendations and conclusions resulting from the study and future research directions are presented in Chapter 8.

1.4. References

1. Johansson SG, Bieber T, Dahl R, et al. Revised nomenclature for allergy for global use: Report of the Nomenclature Review Committee of the World Allergy Organization, October 2003. *J Allergy Clin Immunol.* May 2004;113(5):832-836.
2. Chinn S, Jarvis D, Luczynska CM, Lai E, Burney PG. Measuring atopy in a multi-centre epidemiological study. *European Journal of Epidemiology.* Apr 1996;12(2):155-162.
3. Salo PM, Arbes SJ, Jr., Crockett PW, Thorne PS, Cohn RD, Zeldin DC. Exposure to multiple indoor allergens in US homes and its relationship to asthma. *J Allergy Clin Immunol.* Mar 2008;121(3):678-684 e672.
4. Karakaya G, Ozturk AB, Kalyoncu AF. Prediction of atopy by skin prick tests in patients with asthma and/or persistent rhinitis. *Allergol Immunopathol (Madr).* Jan-Feb 2012;40(1):37-40.
5. Macneill SJ, Sozanska B, Danielewicz H, et al. Asthma and allergies: is the farming environment (still) protective in Poland? The GABRIEL Advanced Studies. *Allergy.* Apr 29 2013.
6. Illi S, Depner M, Genuneit J, et al. Protection from childhood asthma and allergy in Alpine farm environments-the GABRIEL Advanced Studies. *J Allergy Clin Immunol.* Jun 2012;129(6):1470-1477 e1476.

7. Hugg T, Jaakkola M, Ruotsalainen R, Pushkarev V, Jaakkola J. Exposure to animals and the risk of allergic asthma: a population-based cross-sectional study in Finnish and Russian children. *Environmental Health.* 2008/06/06 2008;7(1):1-8.

8. Alfven T, Braun-Fahrlander C, Brunekreef B, et al. Allergic diseases and atopic sensitization in children related to farming and anthroposophic lifestyle--the PARSIFAL study. *Allergy.* Apr 2006;61(4):414-421.

9. Duse M, Donato F, Porteri V, et al. High prevalence of atopy, but not of asthma, among children in an industrialized area in North Italy: the role of familial and environmental factors--a population-based study. *Pediatr Allergy Immunol.* May 2007;18(3):201-208.

10. Pearce N, Pekkanen J, Beasley R. How much asthma is really attributable to atopy? *Thorax.* Mar 1999;54(3):268-272.

11. Arshad SH, Tariq SM, Matthews S, Hakim E. Sensitization to common allergens and its association with allergic disorders at age 4 years: a whole population birth cohort study. *Pediatrics.* Aug 2001;108(2):E33.

12. Gold MS, Kemp AS. Atopic disease in childhood. *Med J Aust.* Mar 21 2005;182(6):298-304.

13. O'Connell EJ. The burden of atopy and asthma in children. *Allergy.* Aug 2004;59 Suppl 78:7-11.

14. Food Allergies and Intolerances. [Internet]. 2012; http://www.hc-sc.gc.ca/fn-an/securit/allerg/index-eng.php. Accessed Apr 5, 2013.

15. Global Initiative for Asthma Global Strategy for Asthma Management and Prevention. [Internet]. 2018; www.ginasthma.org.

16. Soriano JB, Abajobir AA, Abate KH, et al. Global, regional, and national deaths, prevalence, disability-adjusted life years, and years lived with disability for chronic obstructive pulmonary disease and asthma, 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015. *The Lancet Respiratory Medicine.* 2017;5(9):691-706.

17. Ferrante G, La Grutta S. The Burden of Pediatric Asthma. *Front Pediatr.* 2018;6:186.

18. Ballardini N, Kull I, Lind T, et al. Development and comorbidity of eczema, asthma and rhinitis to age 12 – data from the BAMSE birth cohort. *Allergy.* 2012;67(4):537-544.

19. Thomsen SF. Epidemiology and natural history of atopic diseases. *Eur Clin Respir J.* 2015;2.

20. Pyun BY. Natural history and risk factors of atopic dermatitis in children. *Allergy, asthma & immunology research.* 2015;7(2):101-105.

21. Reijmerink NE, Kerkhof M, Bottema RW, et al. Toll-like receptors and microbial exposure: gene-gene and gene-environment interaction in the development of atopy. *Eur Respir J.* 2011;38(4):833-840.

22. Ege MJ, Strachan DP, Cookson WO, et al. Gene-environment interaction for childhood asthma and exposure to farming in Central Europe. *J Allergy Clin Immunol.* Jan 2011;127(1):138-144, 144 e131-134.

23. Collier CH, Risnes K, Norwitz ER, Bracken MB, Illuzzi JL. Maternal infection in pregnancy and risk of asthma in offspring. *Matern Child Health J.* Dec 2013;17(10):1940-1950.

24. Wang IJ, Chen SL, Lu TP, Chuang EY, Chen PC. Prenatal smoke exposure, DNA methylation, and childhood atopic dermatitis. *Clin Exp Allergy.* May 2013;43(5):535-543.

25. Palmer DJ, Sullivan T, Gold MS, et al. Effect of n-3 long chain polyunsaturated fatty acid supplementation in pregnancy on infants' allergies in first year of life: randomised controlled trial. *BMJ.* 2012;344:e184.

26. Granell R, Heron J, Lewis S, Davey Smith G, Sterne JA, Henderson J. The association between mother and child MTHFR C677T polymorphisms, dietary folate intake and childhood atopy in a population-based, longitudinal birth cohort. *Clin Exp Allergy.* Feb 2008;38(2):320-328.

27. Harpsoe MC, Basit S, Bager P, et al. Maternal obesity, gestational weight gain, and risk of asthma and atopic disease in offspring: a study within the Danish National Birth Cohort. *J Allergy Clin Immunol.* Apr 2013;131(4):1033-1040.

28. Burke H, Leonardi-Bee J, Hashim A, et al. Prenatal and passive smoke exposure and incidence of asthma and wheeze: systematic review and meta-analysis. *Pediatrics.* Apr 2012;129(4):735-744.

29. Khan TK, Palmer DJ, Prescott SL. In-utero exposures and the evolving epidemiology of paediatric allergy. *Curr Opin Allergy Clin Immunol.* Oct 2015;15(5):402-408.

30. Herberth G, Bauer M, Gasch M, et al. Maternal and cord blood miR-223 expression associates with prenatal tobacco smoke exposure and low regulatory T-cell numbers. *J Allergy Clin Immunol.* Feb 2014;133(2):543-550.

31. Selgrade MK. Immunotoxicity: the risk is real. *Toxicol Sci.* Dec 2007;100(2):328-332.

32. Dietert RR. Developmental immunotoxicology: focus on health risks. *Chem Res Toxicol.* Jan 2009;22(1):17-23.

33. Warner JA, Warner JO. Early life events in allergic sensitisation. *Br Med Bull.* 2000;56(4):883-893.

34. Peters JL, Suglia SF, Platts-Mills TA, Hosen J, Gold DR, Wright RJ. Relationships among prenatal aeroallergen exposure and maternal and cord blood IgE: project ACCESS. *J Allergy Clin Immunol.* May 2009;123(5):1041-1046.

35. Folsgaard NV, Chawes BL, Rasmussen MA, et al. Neonatal cytokine profile in the airway mucosal lining fluid is skewed by maternal atopy. *Am J Respir Crit Care Med.* Feb 1 2012;185(3):275-280.

36. Kopp MV, Zehle C, Pichler J, et al. Allergen-specific T cell reactivity in cord blood: the influence of maternal cytokine production. *Clin Exp Allergy.* Oct 2001;31(10):1536-1543.

37. Abrahamsson TR, Jakobsson HE, Andersson AF, Bjorksten B, Engstrand L, Jenmalm MC. Low diversity of the gut microbiota in infants with atopic eczema. *J Allergy Clin Immunol.* Feb 2012;129(2):434-440, 440 e431-432.

38. Abrahamsson TR, Jakobsson HE, Andersson AF, Bjorksten B, Engstrand L, Jenmalm MC. Low gut microbiota diversity in early infancy precedes asthma at school age. *Clin Exp Allergy.* Jun 2014;44(6):842-850.

39. Prescott SL. Early origins of allergic disease: a review of processes and influences during early immune development. *Curr Opin Allergy Clin Immunol.* Apr 2003;3(2):125-132.

40. Trivedi M, Denton E. Asthma in Children and Adults—What Are the Differences and What Can They Tell us About Asthma? *Frontiers in Pediatrics.* 2019-June-25 2019;7(256).

41. Vercelli D. Gene-environment interactions in asthma and allergy: the end of the beginning? *Current opinion in allergy and clinical immunology.* 2010;10(2):145.

42. Bisgaard H, Bonnelykke K. Long-term studies of the natural history of asthma in childhood. *J Allergy Clin Immunol.* Aug 2010;126(2):187-197; quiz 198-189.

43. Morgan WJ, Stern DA, Sherrill DL, et al. Outcome of asthma and wheezing in the first 6 years of life: follow-up through adolescence. *Am J Respir Crit Care Med.* Nov 15 2005;172(10):1253-1258.

44. Phelan PD, Robertson CF, Olinsky A. The Melbourne Asthma Study: 1964-1999. *J Allergy Clin Immunol.* Feb 2002;109(2):189-194.

45. Trivedi M, Denton E. Asthma in Children and Adults–What Are The Differences and What Can They Tell Us About Asthma? *Frontiers in pediatrics.* 2019;7:256.

46. Sears MR, Greene JM, Willan AR, et al. A longitudinal, population-based, cohort study of childhood asthma followed to adulthood. *New England Journal of Medicine.* 2003;349(15):1414-1422.

47. Tai A. Strengths, Pitfalls, and Lessons from Longitudinal Childhood Asthma Cohorts of Children Followed Up into Adult Life. *BioMed research international.* 2016;2016.

48. Ross KR, Teague WG, Gaston BM. Life Cycle of Childhood Asthma: Prenatal, Infancy and Preschool, Childhood, and Adolescence. *Clinics in Chest Medicine.* 2019/03/01/ 2019;40(1):125-147.

49. Bisgaard H, Hermansen MN, Buchvald F, et al. Childhood asthma after bacterial colonization of the airway in neonates. *New England Journal of Medicine.* 2007;357(15):1487-1495.

50. Jackson DJ, Evans MD, Gangnon RE, et al. Evidence for a causal relationship between allergic sensitization and rhinovirus wheezing in early life. *American journal of respiratory and critical care medicine.* 2012;185(3):281-285.

51. Heymann PW, Carper HT, Murphy DD, et al. Viral infections in relation to age, atopy, and season of admission among children hospitalized for wheezing. *Journal of allergy and clinical immunology.* 2004;114(2):239-247.

52. Russell G. Asthma in the transition from childhood to adulthood. *Thorax.* Feb 2002;57(2):96-97.

53. Guilbert T, Krawiec M. Natural history of asthma. *Pediatric Clinics of North America.* 2003/06/01/ 2003;50(3):523-538.

54. Gustafsson PM, Kjellman B. Asthma from childhood to adulthood: course and outcome of lung function. *Respir Med.* May 2000;94(5):466-474.

55. Barnig C, Reboux G, Roussel S, et al. Indoor dust and air concentrations of endotoxin in urban and rural environments. *Letters in applied microbiology.* 2013;56(3):161-167.

56. Tyakht AV, Alexeev DG, Popenko AS, Kostryukova ES, Govorun VM. Rural and urban microbiota: to be or not to be? *Gut Microbes.* 2014;5(3):351-356.

57. Douwes J, Pearce N, Heederik D. Does environmental endotoxin exposure prevent asthma? *Thorax.* 2002;57(1):86-90.

58. von Mutius E, Braun-Fahrländer C, Schierl R, et al. Exposure to endotoxin or other bacterial components might protect against the development of atopy. *Clin Exp Allergy.* Sep 2000;30(9):1230-1234.

59. Ege MJ, Bieli C, Frei R, et al. Prenatal farm exposure is related to the expression of receptors of the innate immunity and to atopic sensitization in school-age children. *Journal of Allergy and Clinical Immunology.* 2006;117(4):817-823.

60. Schuijs MJ, Willart MA, Vergote K, et al. Farm dust and endotoxin protect against allergy through A20 induction in lung epithelial cells. *Science.* 2015;349(6252):1106-1110.

61. Williams LK, Ownby DR, Maliarik MJ, Johnson CC. The role of endotoxin and its receptors in allergic disease. *Annals of Allergy, Asthma & Immunology.* 2005;94(3):323-332.

62. Douwes J, Brooks C, Pearce N. Protective effects of farming on allergies and asthma: have we learnt anything since 1873? *Expert review of clinical immunology.* 2009;5(3):213-219.

63. Naleway AL. Asthma and atopy in rural children: is farming protective? *Clinical medicine & research.* 2004;2(1):5-12.

64. Kozyrskyj A, Becker A. Rural-urban differences in asthma prevalence: Possible explanations. *Journal of Allergy and Clinical Immunology.* 2004;113(2):S306.

65. Lawson JA, Rennie DC, Cockcroft DW, et al. Childhood asthma, asthma severity indicators, and related conditions along an urban-rural gradient: a cross-sectional study. *BMC pulmonary medicine.* 2017;17(1):4.

66. Mazur A, Szylling A, Bielecka T, Strzelak A, Kulus M. Is the “farm effect” hypothesis still current? Atopy and allergic diseases in rural and urban children in Poland. *Journal of Asthma.* 2018;55(10):1147-1155.

67. Parsons MA, Beach J, Senthilselvan A. Association of living in a farming environment with asthma incidence in Canadian children. *Journal of Asthma.* 2017;54(3):239-249.

68. Kozyrskyj A, Becker A. Rural-Urban Differences in Atopic And Non-Atopic Asthma in Children. *Epidemiology.* 2006;17(6):S276.

69. Wells AD, Poole JA, Romberger DJ. Influence of farming exposure on the development of asthma and asthma-like symptoms. *International immunopharmacology.* 2014;23(1):356-363.

70. Barry RJ, Pickett W, Rennie DC, et al. The role of farm operational and rural environments as potential risk factors for pediatric asthma in rural Saskatchewan. *Pediatric pulmonology.* 2014;49(9):842-851.

71. Barry RJ, Pickett W, Rennie DC, Senthilselvan A, Cockcroft DW, Lawson JA. Factors contributing to risks for pediatric asthma in rural Saskatchewan. *Annals of Allergy, Asthma & Immunology.* 2012;109(4):255-259.

72. Merchant JA, Naleway AL, Svendsen ER, et al. Asthma and farm exposures in a cohort of rural Iowa children. *Environmental health perspectives.* 2005;113(3):350-356.

CHAPTER 2

LITERATURE REVIEW

2.1.General scope of literature review

This chapter describes asthma and atopy as well as how they are assessed and the timing of exposures to a farm (from early life to adolescent) in relation to asthma and atopy presence. The differences in asthma prevalence between rural and urban, farmers' children and non-farmers' children at school age are also assessed. Methods to assess environmental exposures such as microbial compound exposures are discussed. In addition to this, characteristics of studies investigating associations between microbial exposures and childhood asthma and atopy are also provided.

2.1.Methods

The literature review for this study was conducted using information from multiple sources including peer-reviewed journal articles, textbooks, review articles, consensus guidelines, conference attendance, and internet resources. Updated searches were completed in June 2020 and the literature review was updated as appropriate. Searches were completed using PubMed, Embase, Google Scholar, Science Direct, Web of Science and the University of Saskatchewan Library search engines. Search terms included combinations of key words such as: "rural", "farming or agriculture", "activity", "toxin" "asthma", "atopy", "lung function", "children", "endotoxin", "beta-(1→3)-D-glucan", "fungal extracellular polysaccharides (EPSs)", "microbial compounds", "personal monitoring" among others as well as combinations of these. Selected articles were peer-reviewed articles but technical reports, executive summaries and proceedings were also considered if they contained important information. Selected articles were evaluated

based on the following criteria: 1) studies written in the English Language, 2) studies that include data and information pertinent to any of the research objectives, 3) studies that were published after 1990.

2.2.Measurement methods of atopy and asthma in children

2.2.1. Atopy

Atopy is defined as "a personal and/or familial tendency, usually in childhood or adolescence, to become sensitized and produce IgE antibodies in response to ordinary exposure to allergens, usually proteins".[1] The common methods used to determine the prevalence of atopy include skin prick tests (SPT), blood tests to detect total and allergen-specific IgE levels, the Phadiatop© test, and questionnaire reports. Each of these methods has its own advantages and disadvantages.[2] Among these methods, SPT, the Phadiatop©, and blood tests to detect IgE levels are considered standard methods because of their objective qualities. However, even for these objective methods, there can be definition issues such as determining the cut-off of wheal size for a positive SPT. In population-based epidemiologic studies where it may not be feasible to conduct these tests, questionnaire-based surveys are commonly used to estimate the presence of allergic disease including atopy by surrogate measure. There are advantages to using such tests including being noninvasive, low cost, and the provision of quick results.[3-5]

If questionnaire report is to be used in place of objective measures of SPT, we must ensure that questionnaire report of atopy accurately reflects the objective measure; therefore, comparisons of questionnaire responses with these objective measurements have been conducted.[6-10] It was found that the agreement between questionnaire report of hay fever symptoms and SPT has a wide range of sensitivity (28-76%) and specificity (21-94%).[6-10] Questions on rhinitis were highly specific and had high positive predictive values to detect atopy among children with symptoms, but not helpful for detecting atopy in a general population of children.[10] In a Canadian context,

there have been several childhood asthma and allergy studies conducted that compared the agreement between questionnaire report of atopy and skin pick test results.[5,11] In one study, it was found that questionnaire definitions of atopy based on parent-reported physician-diagnosed atopic conditions have moderate sensitivity (54.3%) and specificity (65.8%) among Canadian children.[11] Another study among schoolchildren (grades 1–8) from rural Saskatchewan conducted by our group found that the agreement between questionnaire report of allergic triggers and atopy measured by SPT was high (83.0–89.5%), while the agreement between atopy and report of allergic conditions ranged from 67.1% to 79.6%.[12] In that study, the sensitivity of the questionnaire variables of any allergy from questionnaire for atopy was generally low (47.3%), while the specificity was higher (80.4%). Thus, questionnaire report would aid in confirming or "ruling in" atopy. In epidemiologic studies that estimate prevalence, a highly sensitive test is preferable while more specific tests are recommended to estimate risk,[13] which are to be used in etiologic studies.

2.2.2. Asthma

There is no "gold standard" to diagnosis asthma in children. Guidelines have been developed to address the challenges of diagnosing asthma in children.[14-17] The diagnosis of asthma involves a thorough medical history, physical examination, and objective measures of lung function.[18] In epidemiological studies examining childhood asthma the two methods commonly used to assess the diagnosis and severity of asthma are questionnaire report and assessment of lung function. While questionnaire report incorporates information about asthma-related symptoms such as wheeze, cough, and shortness of breath to investigate the prevalence, incidence, and severity of asthma, objective measurement of lung function, such as the use of spirometry, is a frequently used tool to help confirm the diagnosis.[18] Spirometry is the preferred objective measure to assess airflow

limitation and excessive variability in lung function, and is recommended for individuals aged 6 years or older.[18]

Spirometry measures airflow parameters such as the forced vital capacity (FVC, the volume of air expired as forcefully as possible following full inspiration) and the forced expiratory volume in 1s (FEV_1, volume of air expired in one second of a forced expiratory manoeuvre). From these two measures, the FEV_1/FVC ratio can be computed. This measure helps identify restrictive vs obstructive lung disease and can help quantify the degree of obstruction in asthma. Other obtained lung function variables such as the peak expiratory flow rate (PEFR) and forced expiratory flow between 25% and 75% of FVC ($FEF_{25\%–75\%}$) are commonly used to quantify the degree of airflow impairment. These lung function variables can be expressed as absolute values and as a percentage of predicted values. The predicted values are obtained from a comparable population of healthy and asymptomatic subjects matched for age, gender, height, and, ethnicity. The degree of severity of the abnormality in airflow obstruction can be graded using the percent of predicted values of FEV_1 (e.g. FEV1 >80% = mild, 60% – 80% = moderate, and <60% = severe).[14] The FEV_1/FVC ratio in the general population is usually greater than 0.75-0.80 in adults and 0.90 in children.[15,16] Airflow obstruction/limitation is likely to occur when ration values are below this cutoff.

2.2.2.1.Atopic and nonatopic asthma phenotype

Asthma is a heterogeneous disease with multiple phenotypes,[19,20] in which patients with asthma may have distinct characteristics depending upon factors that trigger asthma attacks, the clinical presentation and patterns of inflammatory responses.[21,22] Atopic and nonatopic asthma are the most common phenotypes considered with this classification and is often based on positive skin prick tests to common allergens or the presence of specific IgE antibodies against common allergens.[23,24] Atopic asthma represents the most common form of asthma in the pediatric age group. [25-26] Data

from a large population-based study -The Third National Health and Nutrition Examination Survey- from the US among subjects aged 6 to 59 years suggested that 56.3% of asthma cases were attributable to atopy as defined by at least one positive allergen-specific test to 10 common allergens.[25] According to the 14-year follow-up analysis of an Australian community-based birth cohort, the proportion of asthma associated with atopy - defined by a positive skin-prick test assessed at the age of 6 years - was 52% overall.[26] Results from a review suggested that about 58% of children with asthma were skin prick test positive; however, about 29% of non-asthmatic children were also skin prick test positive.[27] This evidence suggests that not all children with asthma are atopic and many children with atopy do not develop asthma. Also, the proportion of asthma that can be attributable to atopy varies geographically.[28-30]

The severity of asthma by phenotype can differ where asthma is generally more severe in children with non-atopic status.[31-34] Atopic mechanisms have been implicated in 50–80% of patients with asthma and in approximately 50% of severe asthma.[35,36] Given the differences in severity between asthma phenotype, adjustments to treatment have been applied for each phenotype.[37]

Mechanisms are possibly related to the heterogeneity of asthma include allergic vs non-allergic, eosinophilic vs non-eosinophilic, and the clinical manifestations (e.g., severity, exacerbation frequencies).[38] Evidence from studies of eosinophilia and asthma are consistent with that from studies of atopy and asthma in that approximately 50% of asthma cases were attributable to allergic mechanisms.[39] This evidence suggests that there are other mechanisms rather than solely atopy to explain the asthma phenotypes. Since non-eosinophilic asthma is associated with neutrophilic responses associated with asthma severity scales (severe, moderate, and mild), it is hypothesized that neutrophilic airway inflammation may play a role in asthma phenotype

explanations. Most non-eosinophilic asthma is neutrophil mediated, whereas allergic asthma is eosinophil mediated.[39]

Atopic asthma is likely eosinophilic following the acquired immunity pathway, while non-atopic is more likely neutrophilic and follows the innate pathway of immunity.[40] The main feature of allergic asthma is likely related to eosinophilic inflammation. Other than eosinophils, interleukin (IL)-5 and possibly IL-4 can also stimulate the growth and activation of the eosinophils, leading to the IgE production as well as Th2 state in the naïve Th0 cells.[39] On the other hand, non-eosinophilic asthma is associated with increased neutrophil and IL-8 levels, suggesting the underlying mechanism for non-eosinophilic asthma is related to non-allergic neutrophil driven airways inflammation.[39]

In occupational populations and in the general population, neutrophils appear to be important in the pathophysiology of asthma.[39] This suggests a possible common mechanism related to innate immune responses.[39] However, many aspects of the pathogenesis of non-eosinophilic asthma and non-atopic asthma remain incompletely resolved.[41] For a better understanding of the underlying mechanisms of the increased prevalence of asthma worldwide as well as asthma causation, asthma phenotype investigation is needed.

2.3.Temporal and geographic patterns in condition prevalence

2.3.1. Atopy

In recent years, atopic related disease prevalence has increased in most developed countries for unknown reasons.[42-45] In children, there have been numerous studies examining the prevalence and factors contributing to atopy among school children worldwide, mostly in Europe.[46,47-49] For example, Ronmark et al[50] reported a significant increase from 21% in 1996 to 30% in 2006 ($P < 0.001$) in the prevalence of atopic sensitization which was defined by at least one positive reaction

to 10 common allergens in school children in northern Sweden. In another study, the prevalence of atopy, defined as a positive result from one of the eight most common inhalant allergens, almost doubled from 10% in 1987 to 19% in 1998 [RR=1.88; 95%CI:1.31-2.68].[49] Besides the increase in atopy prevalence reported in some countries, other studies also demonstrated a stable or decreasing pattern. Zollner and colleagues conducted 6 cross-sectional studies over 9 years (1992-2001) among 6762 school children (9-11 years old) in Germany.[51] In those studies, the prevalence of atopic sensitization, which was defined as having serum specific IgE antibodies ≥ 0.35 kU/l, remained stable over the 9 year study [Odds ratio for trend = 0.99 (0.97 to 1.02)]. Cross-sectional studies conducted in 1982, 1992, 2002 among school children aged 8-11 years in 1982 in Australia showed that the prevalence of atopy, defined by skin prick testing with a wheal size cut-off of 3mm, decreased 3.1% (from 39.3% in 1992 to 36.2% in 2002).[52] In Canada, data regarding the temporal trend of atopy measured by repeated objective tests such as skin prick testing or IgE tests among school children has not been reported.

In addition to temporal variation, geographic variations in the prevalence of atopic sensitization have also occurred. The International Study of Asthma and Allergies in Childhood (ISAAC)-Phase II study was conducted among schoolchildren in geographical areas worldwide.[53] An analysis of 31,759 children aged 8–12 years with skin-prick data was conducted. Skin-prick tests included locally relevant seasonal (mixed tree pollen and mixed grass pollen) and perennial (*Dermatophagoides pteronyssinus*, *D. Farinae* and cat hair) allergens. A positive skin-reaction was defined as a wheal diameter of ≥3 mm. In that study, the prevalence of sensitization to any of the perennial airborne allergens tested varied widely between centres, from 1.4% in Ghana to 45.2% in Hong Kong. Also, this study showed that the prevalence of skin-prick test sensitization to any of the seasonal allergens varied from 0.1% in Ghana to 25.8% in Tromsø (Norway).[54]

2.3.2. Asthma

There is substantial variation in asthma prevalence worldwide.[55,56] In general, over the past few decades, some evidence has shown the increasing trend of childhood asthma globally.[57] Lifetime prevalence of asthma remained the same or even decreased in high-income countries, whereas it increased in many low- and middle-income countries, especially in Eastern European, Latin American and Northern African countries.[56,58] To examine changes in the prevalence of symptoms of asthma and atopic disease, a worldwide study - the International Study of Asthma and Allergies in Childhood (ISAAC)- was conducted as Phase I from 1994 involving 156 collaborating centres in 56 countries with a total of 721,601 children. The Phase I survey was repeated after an interval of 5–10 years (Phase III) in 106 centres in 56 countries in children aged 13–14 years (n=304 679) and in 66 centres in 37 countries in children aged 6–7 years (n=193 404).[59] It was found that the time trends in asthma symptom prevalence showed different regional patterns with a reduction in current asthma symptom prevalence in English language countries (−0.51% and −0.09%). However, the overall percentage of children reported to have had asthma at some time in their lives increased by 0.28% per year in the 13–14-year age group and by 0.18% per year in the 6–7-year age group. Among the 13–14-year age group, the mean prevalence of "current wheeze" ("Have you had wheezing or whistling in your chest in the past 12 months?) increased only slightly from 13.2% to 13.7% (a mean increase of 0.06% per year), in which the highest increase was found in Latin America (+0.32% per year). Among the 6-7-year age group, the mean prevalence of wheeze in the last 12 months (current wheeze) increased only slightly from 11.1% to 11.6% (a mean increase of 0.13% per year), in which the highest increase was found in the Eastern Mediterranean (+0.79% per year). A population-based study in Taiwan, China in 2016 reported a substantial increase in annual prevalence of asthma among children aged 3-18 years from 2002 to 2008 (12.99% to

16.86%).[60] Using data from 4 cross-sectional studies performed in a town of Chorzow, Silesia, Poland in 1993 (n=1,130), 2002 (n=1,421), 2007 (n=1,661) and 2014 (n=1,698) in children aged 7-10 years, the authors found a statistically significant increase in the prevalence of physician-diagnosed asthma (1993-2002-2007- 2014) (3.4%-4.8%-8.6%-12.6%).[61] A recent report from the US using 2001–2013 National Health Interview Survey data for children ages 0 to 17 years showed that childhood asthma prevalence increased from 2001 to 2009[62] followed by a plateau then a decline in 2013.[63]

In Canada, asthma affects approximately 850,000 children under the age of 14 years.[64] Between ISAAC-Phase One and Phase Three, the prevalence of asthma symptoms in Canada was reported to increase in the 6-7 age-group from 14.1% (in 1997) to 18.2% (in 2006).[65] In another report, an increasing trend of Canadian childhood asthma has been reported among 0-5 years olds (8.4% in 1994/1995 to 9.9% in 2000/2001) and 10-11 years old (14.0% in 1994/1995 to 17.6 % in 2000/2001).[66] A recent report[67] (2010) from a survey in Ontario, Canada (more than one-third of Canada's population) showed that childhood asthma among 5-14 year olds increased from 1996 to 2001 (18.8% to 23.6%, respectively for the 5-9 year-old-group; and 13.1% to 21.7%, respectively for the 10-14-year-old-group).

In Saskatchewan, asthma prevalence increased in children from 1981 to 1990.[68] In the calendar year 1990, the prevalence of asthma was 5.1% in children < or = 4 years old, 4.4% in children 5 to 14 years old. [68] However, asthma prevalence was either stable or declining during the latter part of the 1990s.[69] It is noted that these prevalence estimates were from administrative data. Using primary data collected among children 6-17 years old in Humboldt, a city in the province of Saskatchewan, Canada, it was shown that there was an increased trend of asthma prevalence from 10.4% in the 1993 survey to 18.3% in the 2003 survey[70]. More recent results from 2013 by Lawson

et al[71] among 5-14-year-old children in various population densities of Saskatchewan, Canada showed asthma prevalence at 19.6%.

There is a large geographical variation in childhood asthma prevalence globally and regionally including results from the ISAAC Study. A high prevalence of childhood asthma ranging from 29% to 32% was found in South East Asia, North America and Latin America, whereas a lower prevalence of asthma was found in Asia, Northern Africa, Eastern Europe, and Eastern Mediterranean regions.[72,73] Significant geographic variations in asthma prevalence were also observed in Phase III of the ISAAC study conducted during 2000-2003 involving over 1,1000,000 school children from 98 countries.[59,74] The highest rates of asthma were observed in English-speaking countries and countries in Latin America, while lower rates were found in Africa, the Indian subcontinent and the Eastern Mediterranean.[74]

While global differences have been relatively clear based on the ISAAC studies, more regionally, within Europe, differences in childhood asthma prevalence also exist. A large population-based study of 79,000 children from two age groups (13-14 yrs and 6-7 yrs) in 18 study centres in urban and rural areas of Scandinavia and the formerly socialist countries of Eastern Europe[75] reported that the prevalence of wheezing among the 13–14-year-old children was 11.2–19.7% in Scandinavia (Finland and Sweden), 7.6–8.5% in countries with increasingly Westernized culture (Estonia, Latvia and Poland) and 2.6–5.9% in countries with the least amount of Western influence (Albania, Romania, Russia, Georgia and Uzbekistan (except Samarkand)). Using data from an international, multicenter, cross-sectional study of childhood asthma - the Belarus Ukraine Poland Asthma Study (BUPAS)- among children aged 7–13 years in Belarus, Ukraine and Poland, Brozek et al[76] found that asthma prevalence was different among these three countries [(Poland (rural, urban): 3.5 %, 4.1 %; Ukraine: 1.4 %, 2.1 %; Belarus: 1.4 %, 1.5 %]). Another report (2017)

by Lawson et al[76] using data from a cross-sectional survey study of children (5–15 years) from one urban centre in each of Canada (n=2414), Belarus (n=2766), Poland (n=1785), Republic of Georgia (Adjara) (n=3194), Republic of Macedonia (n=2310), and Ukraine (n=1168) revealed that the prevalence of ever asthma differed by country from 20.6% in Canada to 1.5% in Ukraine ($p<0.001$).

2.4.Rural dwelling or farming in children and its relationship with conditions

While geographic variation can be considered between political boundaries (e.g., international and national comparisons), it can also be considered more regionally such as between urban and rural locations. With regard to atopy, it is generally believed that the reported prevalence of atopy among children is lower in rural areas than in urban areas, yet this is not always consistent. A Polish study[77] among 400 schoolchildren aged 10-14 years from the capital city (n=223) and from a traditional rural part of the country (n=177) recruited from June to November 2011 showed that urban children had a higher overall prevalence of atopy, defined as at least one reaction to 14 inhalant allergens based on skin prick tests (SPTs) reactivity in 350 children, at least than children living in rural areas, 33.5% versus 20% ($p = 0.0045$). A cross-sectional study of children aged 9–12 years from a rural village, a rural town and an urban city in Korea[78] (2012) showed that the prevalence of atopic sensitization as indicated by SPT reactivity to 16 inhalant allergens was lower in children living in a rural village and a rural town compared to a urban area (35.5% vs. 38.8% vs.46.3%, $p<0.001$). However, another cross-sectional study with 738 children aged 6-18 years old in 2007 in Konya, Turkey[79] showed that that there were no statistically significant differences in the prevalence of atopy in rural and urban areas.

In Canada, very few studies have been conducted to determine if there are differences in prevalence and risk factors for atopy between urban vs. rural and farm vs. nonfarm children. One

of these studies was conducted in rural Saskatchewan, Canada in 2010 in 584 children (grades 1-8).[80] The reported prevalence of atopy, defined as a positive reaction to any of 6 allergens of at least 3 mm compared with the negative control, was 19.4% and findings were similar for farm and non-farm children (18.7% vs 20%, p = 0.71).

Similar to that of atopy, the reported prevalence of childhood asthma in urban areas tends to be higher than in rural areas, yet the results may vary across studies. For example, a cross-sectional study in China in 2009 among 7,077 school-age children (13-14 years old) showed that rural children had a significantly lower prevalence of physician's diagnosis of asthma than urban children, using the validated ISAAC (International Study of Asthma and Allergies in Childhood) questionnaire (1.1% vs 6.3%, p<0.001).[81] However, Pesek et al conducted a cross-sectional study in 6,376 children aged 4-17 years in the US (2010) and found that no apparent difference in provider-diagnosed asthma between urban and rural children (20% vs. 19%).[82] The insignificant difference in the prevalence of asthma between urban and rural location of residence was also observed another cross-sectional study in 2011 in Turkey among children aged 6-18 years (10.5% vs. 7.1%; *p*=0.16, respectively).[83]

Within Canada, regional variation in asthma has also been reported. Using data from a nationwide cross-sectional survey of schoolchildren (aged 11–15 years) - the Health Behaviour in School-aged Children study – it was reported that asthma prevalence was higher in urban metro areas compared to rural regions (17.6% vs. 14.8%).[84] Another report by Korzyskyj and Becker from two separate surveys among 3,564 children (7 year-old) in Manitoba, Canada showed that the prevalence of both atopic asthma and asthma were higher in children living in urban centers compared to children living in southern and northern parts of rural Manitoba (atopic asthma: 9%

in urban, 5% in southern rural, and 4% in northern rural;[85] asthma: 14% in urban, 10 % in southern rural, and 8% in northern rural).[86]

In Saskatchewan, several studies have been conducted to compare the prevalence of asthma between rural and urban school-age children.[87] Lawson et al[87] conducted a cross-sectional study among 3,509 children aged 5-14 years and found that prevalence of both ever asthma (15.1% vs. 20.7%) and current asthma (10.9% vs. 14.9%) were significantly lower in rural compared to urban children. This group also reported that rural children had a significantly reduced risk of current asthma compared to their urban counterparts (OR = 0.58; 95%CI: 0.42–0.99). The impact of the environment has been a focal point of the investigation to explain the differences between urban and rural children but still requires more investigation. Related to this environmental perspective, there have been several studies which focus on the relationship between farm dwelling, exposures, and activities related to atopic sensitization and asthma in children.

2.4.1. Atopy and farm vs. rural non-farm children

Table 2-1 summarizes studies looking at atopy prevalence among farmers' children and rural non-farmers' children at school age. Most studies have shown that in a rural context, there has been lower atopy prevalence among farming children compared to those not living on farms.[43,44,88-97]

In Europe, in general, evidence supports the farming protective effect on childhood atopy.[43,94,96] For example, one study with a large sample size of 14,893 children aged 5-13 years from a number of European countries including Austria, Germany, The Netherlands, Sweden and Switzerland was conducted by Alfven et al[96]. With the definition of atopy as Serum IgE $\geq$ 0.35 kU/l, the authors found that farm children had a lower likelihood of atopy than non-farm children [OR=0.53 (0.42-0.67)]. However, there is considerable heterogeneity between studies with respect

to the health outcomes (the definition of atopic outcome by SPT or IgE blood test) and with respect to the different type of farms between different regions which entail specific exposures.

With regard to atopy, in Canada (2000), Ernst et al[98] conducted a cross-sectional study in 1199 adolescents aged 12-19 years with skin prick test (SPT) positivity defined as a mean wheal diameter greater than 0 mm to any one of 24 common inhaled allergens. The results showed that children living on a farm were significantly less likely to be atopic compared to those not living on the farm [OR= 0.58 (95%CI=0.46-0.75)]. However, results from a study[99] conducted in 2010 among 584 school-age children in rural Saskatchewan, Canada showed that home location (farm vs non-farm) was not associated with atopic status where atopy was defined as a positive reaction of at least 3 mm to any of 6 allergens (local grasses, wheat dust, cat dander, house dust mite, *Alternaria* species, or *Cladosporium* species) compared with the negative control. A possible explanation was that there may be a lack of variability in exposures between farm and non-farm populations of these children in which the towns (ie, non-farm children) were small and adjacent to farming areas. However, in this study, livestock farming was found to be protective against atopy [aOR= 0.38 (95%CI=0.17-0.88)].

Table 2-1: Atopy prevalence among farmers' children and rural non-farmers' children at school age

Lead author (Year published) Country	Design	Study population (Age, sample size N, sub-N)	Definition of atopy (Objective tests/cut-off wheal size chosen)	Prevalence & Association with Atopy (OR; 95% CI)
Chu et al[99] (2014) Saskatchewan, Canada	CS	- Grade 1-8 - 584: rural Saskatchewan	A positive reaction to any of 6 allergens (local grasses, wheat dust, cat dander, house dust mite, Alternaria species, or Cladosporium species). Cut-off = 3mm	- Atopy prevalence= 19.4% - Home location (farm vs non-farm): n.s. - Livestock farming: (OR=0.38, 95% CI: 0.17-0.88)
Macneill et al[94] (2013) Poland and Alpine regions of Germany, Austria and Switzerland (Phase II only included Poland)	CS	- Grade 1-6 - 2,440	Specific serum IgE antibodies (specific IgE >=0.7 kU/l against *D. pteronyssinus*, cat or birch or a positive reaction (0.35 kU/l) to the grass mix.) - In Poland, skin prick testing (SPT) was performed using extracts from *D. pteronyssinus, D. farinae*, mixed grasses, birch and cat epithelia. Cut-off = 3mm	- Polish farm vs non-farm children: IgE: [aOR = 0.72 (0.57- 0.91)] and skin prick test [aOR = 0.65 (0.50- 0.86)]
Holbreich et al[91] (2012) Switzerland	CS	- Age: 6-12 years - Amish:157, Swiss farm children: 3006 and Swiss non-farm children: 10,912	- Any positive IgE lvel of 0.7 kU/L or more. - A positive skin prick test: greater than 3 mm (Cat, birch, mixed trees, mixed grasses, *Dematophagoides pteronyssinus*)	Amish: 7.2% (10/138), Swiss farm children: 25.2% (223/884) and Swiss non-farm children: 44.2% (281/628)
Illi et al[43] (2012) Austria, Germany, and Switzerland	CS	- Age: 6-12 years - 7,682	Serum IgE antibodies against inhalant (*Dermatophagoides pteronyssinus*, cat, grass mix [sweet vernal grass, rye	Children living on a farm were at significantly reduced risk of atopic sensitization [aOR=0.54;95%CI: 0.48-

			grass, timothy grass, cultivated rye, and velvet grass], birch, and mugwort) and food (egg white, cow's milk, fish, wheat, peanut, and soybean). Atopic sensitization was defined as specific IgE antibodies of at least 0.7 kU/L against *D pteronyssinus*, cat, or birch or a positive reaction (0.35 kU/L) to the grass mix.	0.61; p< 0.001) compared with nonfarm children.
Alfven et al[96] (2006) Austria, Germany, The Netherlands, Sweden and Switzerland	CS	- Age: 5-13 years - 14,893	Serum IgE ≥ 0.35 kU/l	Farm children vs. non-farm children: [OR=0.53; 95%CI: 0.42-0.67]
Perkin et al[100] (2006) Shropshire, England	2-stage cross-sectional study	- Rural primary schools (exact age not provided) - Stage 1: 4,767 Stage 2: 879	SPT (3mm). Any positive reactions to one of common aero-allergens: dog hair, cat hair, horse hair, cow hair, 6-grass mix, house dust mite, *Acarus siro, Lepidoglyphus destructor, Tyrophagus putrescentiae*	Compared with rural nonfarming children: adjusted [OR= 0.68;95%CI: 0.40-1.16; p = 0.15]
Remes et al[101] (2005) Eastern Finland	CS	- Age: 6-13 years - 710	SPT (3mm). Any positive reactions to one of common aero-allergens [birch, timothy grass and mugwort pollen, cat, dog, cow, and horse epithelial danders, cockroach (*Blatella germanica* , house dust mite (*Dermatophagoides pteronyssimus*), and storage mite (*Lepidoglyphus destructor*)].	Little difference was observed in sensitization against the other allergens between the farmers' (17.2%) and non-farmers (14.5%) children [aOR=1.11; 95%CI: 0.71-1.72]
Merchant et al[102] (2005) USA	Cohort	- Age: 0-17 years - 644	- SPT (3 mm). Any positive reactions to one of common aero-allergens (tree pollen mix, grass pollen mix, ragweed pollen, weed pollen mix, cockroach mix, mold mix, insect mix, caddis	- SPT: Born on farm vs not born on farm: [OR= 0.69; 95%CI: 0.34–1.40]; currently lives on farm vs not currently

			fly/moth/mayfly mix, cat pelt, dog hair, mouse and rat mix, and dust mite *Der f and Der p* mix. Farm aeroallergens included grain dust mix or grain smut mix, soybean dust or soybean wholegrain, cattle hair, horse hair, chicken feathers, and turkey feathers. - Total IgE cut-off was ≥ 60 kU/L	lives on farm: [OR=1.08; 95%CI: 0.57–2.06] - IgE: Born on farm vs not born on farm: [OR=0.57; 95%CI: 0.31–1.04]; currently lives on farm vs not currently lives on farm: [OR=0.76; 95%CI: 0.43–1.36]
Remes et al[103] (2003) Eastern Finland	CS	- Age: 6-13 years - 710	SPT (3 mm). Any positive reactions to 7common aero-allergens (birch, timothy grass and mugwort pollen, cat and dog epithelial danders and house dust mite (*Dermatophagoides pteronyssimus*)	Farmers' children vs. non-farmers' children [aOR=0.56; 95%CI: 0.40- 0.78]
Horak et al[104] (2002) Austria	Longitudinal design	- Age: 8-11 years - 844	SPT (2 mm). Any positive reactions to 6common aero-allergens (Cat, dog, birch, hazel, wheats, mites (*Dermatophagoides pternonyssinus, Dermatophagoides farinae*)	No farming: 12.2%, part-time farming: 6%, full-time farming: 2.2%; Farming vs. Non-farming: [OR= 0.34; 95%CI: 0.12- 0.98]
Wickens et al[105] (2002) New Zealand	CS	- Age: 7-10 years - 605 - 275 subjects underwent SPT	SPT (3mm). Any positive reactions to 8 allergens (*Dermatophagoides farinae, D. pteronyssinus,* mould mix, cockroach, rye grass, timothy grass, cat, dog)	First year of life farm residence vs non-farm: [OR= 1.0 (0.6–1.7)]; Current farm residence vs non-farm: [OR= 1.3; 95%CI: 0.8–2.3]
Klintberg et al[106] (2001) Sweden	Birth cohort	- Age: 7-8 years - 650	SPT (3mm). Any positive reactions to 6 ISAAC standardized allergens (pollens from birch and timothy, dander of cat, *A. alternata, D. pteronyssinus* and *D. farinae*)	Children of farmers and non-farmers: 30.7% and 32.7%. [RR = 0.94; 95%CI: 0.70 – 1.25]
Downs et al[97] (2001) Australia (two rural towns: Wagga	CS	- Age: 7-12 years - 1,500	SPT (3mm). Any positive reactions to 8 allergens (*D.pteronyssunus*, rye grass *pollen Lolium perenne*, cat dander, the fungus *Alternaria alternat*, the fugus	Lower risk of atopy in Wagga Wagga between farm vs non-farm living (adjusted odds ratio [aOR=0.47; 95%CI:

Wagga in a mixed farming region, and Moree in a crop farming region)		- 1,436 subjects underwent SPT	*Clasdosporium cladosporoides*, wheat wholegrain, grain mill dust and cotton lint)	0.32-0.72] but not in Moree [aOR= 0.97; 95%CI: 0.62-1.53].
Ernst et al[98] (2000) Canada	CS	- Age: 12-19 years - 1,199	SPT (3mm). Any positive reaction to any one of 24 common inhaled allergens (the dust mites, *Dermatophagoides pterynissinus and D. farinae* , cat, dog, ragweed, *Alternaria , Cladosporium* , tree mixture, maple, birch, oak, weed mixture, grass mixture, *Hormodendrum , Alternaria , Penicillium , Mucor , Aspergillus , Helminthosporium*, horse, cow, pig, and feathers).	Farm living vs non-farming living: [OR=0.58, 95%CI: 0.46-0.75]
Riedler et al[107] (2000) Austria	CS	- Age: 8-10 years - 2,001; - 1,006 subjects underwent SPT	SPT (3mm). Any positive reactions to 7 common allergens (*D.pteronyssunus, D. farinae*, cat fur, timothy grass, birch, *A.tenuis, C.herbarum)*	Farm vs non-farm living: 18.8% vs 32.7% (p = 0.001).

Abbreviations: CI, Confidence Interval; CS, cross-sectional; n.s., not significant

2.4.2. Asthma and farm vs. rural non-farm children

Table 2-2 summarizes studies examining asthma prevalence among school-age children residing in a farm and rural non-farm locations. Most studies were conducted in Europe or North America and the farming effect has been shown to be quite heterogeneous between countries. In Canada, results have been inconsistent regarding the difference in asthma prevalence between farm children compared to non-farm children. In a cohort study by Midodzi et al[108] in 2007 among 13,524 asthma-free children aged 0-11 years, the authors found that children from a farming environment had a reduced risk of asthma compared with children from a rural non-farming environment with odds ratios (OR) of 0.22 (0.07-0.74). In Europe, a study conducted in rural Austria, Germany, and Switzerland recruited 79,888 school-aged children to complete a questionnaire on farming and asthma. Using data from 7,682 children aged 6-13 years with specific farm exposures that they may have encountered in the past, Illli et al[46] reported that children residing in a farming environment had a significantly reduced risk of developing asthma (OR = 0.68; 95% CI: [0.59, 0.78]). However, the protective effect of farm living and asthma risk was not found in some studies. For example, results from a cross-sectional study conducted in rural Switzerland[109] suggested no evidence of the farm effect (OR = 1.17; 95% CI: [0.64, 2.13]). Studies in Saskatchewan, Canada also observed that there were no differences in asthma prevalence between farm and rural-nonfarm children.[110,111] The differences of the farm effects and asthma presence observed in these above studies were suggested to the differing farming practices between regions.[112]

Few studies have explored the importance of atopic and non-atopic status in the associations between farm living and asthma. With regard to studies involving asthma phenotype, distinct risk factors have been found to be associated with the two phenotypes (atopic and nonatopic) and may indicate different causal mechanisms.[113,114] Farming exposures can be

associated with asthma phenotype. Current evidence has suggested that farm exposures increase the risk of pediatric non-atopic asthma.[115] A Danish study of farming children reported that being exposed to swine and dairy confinements increased the risk of non-allergic asthma development [OR=3.37 (95% CI=1.63–6.97) and OR=2.47 (95% CI 1.14–5.34), respectively].[115]

Table 2-2: Asthma prevalence among school-age children living on a farm and rural non-farming residence

First author (Year published) Location(s)	Study design	Study population (Age, sample size N, sub-N)	Operational definition of asthma	Findings and strengths of associations
Parsons et al[116] (2017) Canada	Cohort study	10,941 children of ages 0 to 11 years who were free of asthma and wheeze at the baseline (1994-1995)	An incidence of asthma was obtained from health-professional diagnosed asthma reported either by the person most knowledgeable for children under 15 years or by the children themselves if they were of ages 16 years and over.	- The 14-year cumulative incidence of asthma among children living in farming environments was 10.18%, which was significantly lower than that observed for children living in rural non-farming (13.12%) and non-rural environments (16.50%). - A dose-response relationship was observed with children living in rural non-farming and farming environments having significantly reduced risk of asthma [hazard ratio (HR): 0.77; 95% confidence interval (CI): (0.60, 1.00); p = 0.047 and HR: 0.56; 95% CI: (0.41, 0.77); p < 0.001] in comparison to those living in non-rural environments.
Barry et al[110] (2013) Canada	Cross-sectional	2259 children in grades 1–12	Ever asthma was assessed by the question, “Has this child ever been diagnosed as having asthma by a doctor?”	Ever Asthma prevalence: Farm: 14.3 % (145/1024) vs small town: 16.4 (199/1235), p=0.18. Farm vs. town residence: Ever asthma (adjusted odds ratio [aOR, 95% CI],= 0.93 (0.71, 1.23)).
Illli et al[46] (2012)	Cross-sectional	79,888 school-aged children (phase I).	Asthma was defined as either current wheeze (parental reporting of wheeze in the past	Asthma prevalence:

Rural regions of Austria, Germany, and Switzerland		Of these, 9,668 were randomly selected for phase II by exposure stratum (ie, farm children, exposed nonfarm children, and unexposed nonfarm children), and 8,419 (87%) returned the detailed phase II questionnaire.	12 months), a positive answer to the question “Did your child ever use an asthma spray?” or a doctor's diagnosis of asthma at least once or of wheezy bronchitis more than once.	- Phase I: Farm children: 11.4%. Non-farm children: Exposed (15.8%) vs. non exposed (18.3) - Phase 2: Farm children: 14.1%. Non-farm children: Exposed (20.0%) vs. non exposed (22.2%) Children living on a farm were at significantly reduced risk of asthma (aOR= 0.68 (0.59-0.78); p< 0.001)
Farthing et al[111] (2009) Canada	Cross-sectional	553 school children aged 6-13	Asthma was defined as a report of asthma before or during the past 12 months that had been previously diagnosed by a doctor.	The prevalence of asthma and respiratory symptoms were 18.8% and 39.8%, respectively, and did not differ by home location (farm/nonfarm).
Ege et al[117] (2007) Rural areas in 5 European countries.	Cross-sectional	8263 school-age children	Children with reported physician-diagnosed asthma once or obstructive bronchitis more than once in their lifetime were defined as having asthma ever.	Inverse relations with a diagnosis of asthma were found for - pig keeping: aOR=0.57 (0.38-0.86), - farm milk consumption: aOR=0.77 (0.60-0.99), - frequent stay in animal sheds: aOR=0.71(0.54-0.95), - child's involvement in haying: aOR=0.56 (0.38-0.81), - Use of silage: aOR=0.55 (0.31-0.98) for non-atopic

				asthma) and in Germany for agriculture: aOR=0.34 (0.22-0.53).
Midodzi et al[108] (2007) Canada	Cohort	A total of 13 524 asthma-free children aged 0-11 years	An affirmative response to the question 'Does this child have asthma that has been diagnosed by a Health Professional?' was used to determine incidence of asthma.	- The 2-year cumulative incidence of asthma was 2.3%, 5.3% and 5.7% among children living in farming, rural non-farming and non-rural environments, respectively. - Children from a farming environment had a reduced risk of asthma compared with children from rural non-farming environment with odds ratios (OR) of 0.22 (0.07-0.74)
Alfven et al[118] (2006) Austria, Germany, the Netherlands, Sweden and Switzerland	Cross-sectional	Children aged 5–13 years N=14,893	A diagnosis of asthma established by a doctor on at least one occasion or a diagnosis of wheezy bronchitis established on more than one occasion.	- Asthma prevalence was lower in farm children compared to non-farm.: 6.3% (172/2750) vs 9.1 (484/5330). - Farm children was less likely to have asthma compared to non-farm children (aOR=0.74 (0.60–0.92).
Perkin et al[119] (2006) UK	Cross-sectional	4767 primary school children (Age not specified)	Parent-reported symptoms of asthma by using questions from the International Study of Asthma and Atopy in Childhood.	Prevalence: Farm children: 10.3% (52/503) vs rural non-farm 15.8% (592/3777). Compared with rural nonfarming children, farmers' children had significantly less current asthma symptoms (aOR=0.67; 95% CI, 0.49-0.91; P = .01)

Adler et al[120] (2005) Wisconsin, USA	Cross-sectional	36,500 rural kindergarten through 12th grade school children.	Asthma was defined as a report of asthma by the question "Have you (Has your child) ever had asthma?" –ISSAC questionnaire	- Farm-reared children were less likely to have had a diagnosis of asthma (22% vs 26%; P < 0.002). - Among the group as a whole, having lived on a farm was protective for having been diagnosed with asthma (aOR=0.80 (0.66-0.98)). - Farm residence was associated with a decreased likelihood of having had asthma among both younger (aged 9 years or less) [aOR=0.79 (0.62-1.02)] and adolescent age groups (10 years and older) [aOR=0.78 (0.59-0.98)].
Merchant et al[121] (2005) Rural Iowa, US	Population-based study	Children 8–17 years of age. N=644 (224 farm, 155 rural nonfarm, and 265 town)	Doctor-diagnosed asthma	Asthma prevalence: Currently lives on a farm vs Does not currently live on a farm [(11.9% (26/218) vs 11.7 % (47/402); p> 0.05].
Von Ehrenstein et al[122] (2001) Germany	Cross-sectional	5-7-year-old children N=10,163	The prevalence of doctor's diagnoses and symptoms asthma as assessed by parental report.	- Prevalence: Non-farm: 6.4 % (529/8235); Farming: 3.4 (39/1147); Part-time farming: 3.7% (26/709); and Full-time farming (3.0% (13/438). - Farming vs non-farm: aOR=0.65 (0.39-1.09); Part-time farming activity: aOR= 0.80 (0.45-1.40);

				Full time farming activity: aOR=0.38 (0.15-0.97).
Riedler et al[123] (2001) Rural areas of Austria, Germany, and Switzerland	Cross-sectional	6-13-year-old children N=2618	Doctor's diagnosis of asthma from the questionnaire	- Exposure of children younger than 1 year, compared with those aged 1-5 years, to stables and consumption of farm milk was associated with lower frequencies of asthma (1% [3/218] vs 11% [15/138]). - Continual long-term exposure to stables until age 5 years was associated with the lowest frequencies of asthma (0.8% [1/122] - Those being exposed to multiple factors including stables, farm life and unpasteurized milk had the most protection against development of asthma (OR: 0.14; 95% CI 0.14–0.48)
Riedler et al[124] (2000) Rural area in Austria	Cross-sectional	2283 children aged 8-10 years	A positive answer to “did a doctor ever diagnose “asthmatic”, “spastic” or “obstructive” bronchitis?”	A diagnosis of asthma was also lower (1.1 vs 3.9%, p=0.018), and the difference in asthma symptoms did not reach significance (4.7 vs 7.5%,p= 0.087). Similarly, a doctor's diagnosis of “asthmatic or spastic or obstructive bronchitis” was significantly lower in farmers' children (10.3 vs 15.2%, p=0.029).

2.5.Farm-related activities and asthma and atopic disease

Although studies examining associations between farm exposures and asthma and atopic disease among children in rural areas focus primarily on the location of the child's home (farm vs. non-farm), rural non-farm children can be exposed to farming activities and specific farm activities may differentially impact childhood asthma and atopy. One third of children in rural areas were exposed to farm animals on a regular basis (ie, at least once a week) in a study conducted in European countries including rural areas of Austria, Germany, The Netherlands, Sweden, and Switzerland.[125] In another study in the UK, 13% of rural non-farm children had regular contact with farm animals and 9% of them were playing in barns or stables once a week or more.[119] Similarly, in a population-based study in Saskatchewan, Canada, approximately one-third of children reported exposure to a farm or ranch in the past year.[126] In a large population-based study in Europe,[46] among rural non-farm children, those who were exposed to a farm and related farm activities had a lower likelihood of having asthma (defined as either current wheeze in the past 12 months, a positive answer to the question "Did your child ever use an asthma spray?," or a doctor's diagnosis of asthma at least once or of wheezy bronchitis more than once) compared to those who were not exposed to a farm (20.0% vs. 22.2%).[46] Because of these findings, studies should also take into account the sub-group who do not reside on a farm but who are exposed to a farm. It may be useful to examine specific farm activities such as riding horses, exposure to animal pens and sheds, and consumption of unprocessed cow's milk as well as farm-related activities among farm children and/or frequencies of farm visits and intensities of farm activities among nonfarm children.

There have been studies examining the associations between farm activities and pediatric asthma and atopic disease in children but results have been inconsistent. Farthing et al[127] (2009)

conducted a cross-sectional survey in rural Saskatchewan among 6-12-year-old-children. Farm activities that were found to be positively associated with respiratory symptoms included emptying and filling grain bins (OR=2.18, 95% CI: 1.03 to 4.62), playing on or near hay bales (OR=1.89, 95% CI: 1.19 to 3.01), cleaning pens (OR=2.70, 95% CI: 1.05 to 6.97), and exposure to haying activities (OR=2.08, 95% CI: 1.07 to 4.06).[127] However, opposite results were found in a cross-sectional study that examined reports from children living in five European countries (Austria, Germany, The Netherlands, Sweden, and Switzerland).[117] That is, an inverse association with a diagnosis of asthma was found for the child's involvement in haying (OR=0.56; 95% CI: 0.38 to 0.81). The discrepancy might be explained by different farm sizes and children's exposure as well as potential reverse causation in which active participation in farm activities might be avoided by children with asthma and/or allergies.

Dependent upon the types of farm activities and respiratory symptoms (ever wheeze, current asthma, ever asthma), the associations have shown mixed results. The farming effects on asthma and atopic disease may depend on the type of farming. While livestock farm exposure may protect children from asthma[107,128], grain farm exposures may increase the risk[127,129]. This finding might open up a discussion whether living on a livestock farm or actively participating in livestock feeding activities plays a role in the presence of childhood asthma. Chu et al[3] (2014) found that several farm related activities (feeding livestock in the past year, cleaning or playing in barns, cleaning or playing in pens or corrals) were associated with the increased risk of hay fever. Given that the farming practices in rural Saskatchewan might be different from other places,[97,130] it is necessary to examine these unique farm exposures in relation to asthma and atopic disease among school-age children in a more robust sample of children with detailed information of farm activities to which children might be exposed.

2.6.Microbial exposures and their relationship with atopy and asthma

The farming environment, which is rich in microbes, has been suggested to be protective against atopy and asthma in children. Animal sheds are found to contain a large variety of bacteria, fungi and their compounds.[131-133] Because of such high level of exposures, a farming environment offers an opportunity to evaluate the importance of microbial exposures on children's respiratory health and atopy.[134,135] Research has shown that early-life microbial exposure has been suggested to be important for a reduced risk of atopy and hay fever in farm children.[136] It is suggested that during a critical inception phase of lung development within the first 3 months of life, children are more likely to develop asthma.[137,138] Prenatal factors related to placental function including exposures to respiratory viruses, bacteria, and allergens are suggested to play a role.[138,139] However, there has been inconsistency in the current literature around the associations between farm-related environmental exposures in early life (from in-utero until 3 months of life) and atopy/atopic sensitization in school-age children and this question has never been brought together through a systematic review process. The evidence in the literature should be reviewed systematically.

It is also important to look at children after the early post-natal phase such as in school age. Several studies have assessed the health effects of microbial exposures by measuring the markers of bacterial and fungal exposures in mattress dust for atopy and asthma. More recently, protective associations have been observed for muramic acid, a marker primarily for gram-positive bacteria but can also be found in small quantities in gram-negative bacteria and extracellular polysaccharides (EPSs).[117,140,141] Recent studies looking at the association between these microbial exposures with atopy in school-age children are summarized in **Table 2-3**. In general, inconsistent results were found regarding the association between endotoxin exposure and atopy in children. Some studies found an increased risk[142-144] of atopic sensitization in school-age children, while

others showed an inverse association[145-148], and others did not show a statistically significant association[125]. Also, there is heterogeneity observed in exposure assessment of endotoxin, outcome definitions of atopy and allergies as well as study designs.

Table 2-3: Studies investigating the effect of microbial exposures and outcome of interest (atopy measured by Skin-prick testing or blood test measured overall and specific IgE)

Lead author (Year published)	Country	Design	Study population (Age, N, Sub-N)	Exposure assessment	Definition of atopy (Objective tests/cut-off wheal size chosen)	Prevalence & Association (OR; 95% CI)
Joanne et al[146] (2010)	Metropolitan Boston, the USA	Birth cohort	377 school-aged children	- Family room dust samples collected in infancy (dust vacuumed from the family room floor and an upholstered chair most often used by the parent while holding the infant). - House family room and bed samples from participating subjects at a mean age of 7 years (range 6.5 to 9.2 years).	Allergens used: common indoor (cat dander, dog dander, cockroach [*Blatella germanica*], dust mite [*Dermatophagoides pteronyssinus and Dermatophagoides farinae*], and mouse epithelial extract, and outdoor (ragweed, mixed trees, *Aspergillus*, *Alternaria*, mixed grasses, *Cladosporium*, and *Penicillium*) allergens. - Cut-off for Skin tests: ≥ 3 mm - Cut-off for IgE to specific allergens: ≥0.35 IU/mL.	- Exposure to gram-negative bacteria was inversely associated with atopic sensitization at school-age (for > median endotoxin: prevalence ratio [PR] =0.77 [95% CI=0.6 to 0.97] for atopic sensitization. - Eelevated gram-positive bacteria in the bed was not associated with atopic sensitization [POR=1.07 (0.8 to 1.4)]
Celedon et al[147] (2007)	Metropolitan Boston, the USA	Birth cohort	440 children with parental history of atopy. At a mean age of 7.4 years (range,	Dust samples: vacuuming the baby's bed, the baby's bedroom floor, the parent's bed (if the child slept there at least	The allergens tested: common indoor (cat dander, dog dander, cockroach [*Blatella germanica*], dust mite [*Dermatophagoides pteronyssinus and Dermatophagoides farinae*],	Exposure to endotoxin levels above the lowest quartile at age 2 to 3 months was associated with reduced odds of

			6.5-10.1 years), atopy skin testing was performed in 248 children, and IgEs specific to common allergens were measured in an additional 23 children.	half the time), the living room/family room (heretofore referred to as family room), and the kitchen floor.	and mouse epithelial extract) and outdoor (ragweed, mixed trees, *Aspergillus*, *Alternaria*, mixed grasses, *Cladosporium*, and *Penicillium*). - Sensitization to ≥ 1 allergen (atopy) was considered present at school age if there was at least 1 positive skin test or specific IgE to the allergens tested. Cut-off: ≥ 3 mm - IgEs to specific allergens were considered positive at a level ≥ 0.35 IU/mL.	atopy at school age [OR=0.5 (0.2-0.9)]
Gehring et al[148] (2007)	Germany, the Netherlands and Sweden	Nested case-control study	180 sensitised and 180 nonsensitised children were selected per country	House dust samples: vacuuming from the child's mattress and the living-room floor	Allergen panels differed between the cohorts, but specific IgE to egg white, milk, house dust mites, cat, tree and grass pollens were measured in all cohorts. - Atopic sensitization to any allergen was defined as specific IgE antibodies of ≥0.35 $kU \cdot L^{-1}$ for one of the allergens tested. - Atopic sensitization to inhalant and food allergens was defined as specific IgE antibodies of ≥0.35	Higher amounts of mattress dust and higher mattress dust loads of endotoxin, β (1, 3)-glucans and EPS were associated with a significantly decreased risk of sensitization to inhalant allergens. After mutual adjustment, only the protective effect of the amount of mattress dust

					$kU \cdot L^{-1}$ for one of the inhalant or food allergens tested.	remained significant [OR= 0.57(0.39–0.84)].
Ege et al[125] (2007)	Rural areas of Austria, Germany, The Netherlands, Sweden, and Switzerland	Cross-sectional	5-14-year-old rural children (N= 8263)	Levels of endotoxin, b(1/3)-glucan, and fungal extracellular polysaccharides (EPSs) were measured in mattress dust samples of 440 children.	- Blood analysis was performed in a subsample of 2086 children. - Cut-off: ≥ 0.35 kU/L for inhalant or food allergens.	No effect on atopic sensitization against inhalant and food allergens. The associations were less conclusive for exposure to (1,3)-β-D-glucan.
Antova et al[142] (2007)	Pool 12 cross-sectional studies	Russia, North America and 10 countries in Eastern and Western Europe	Children aged 6–12 years	Variable "indoor mold" is referred to any visible presence of mold in the household. For the study carried out in The Netherlands, it referred only to mold in the last 12 months, and, for the Italian study, only to mold in the child's bedroom.	Defined as "sensitivity to inhaled allergens"	Increased risk of sensitization against inhalant allergens in a pooled analysis of >58,000 children [aOR=1.33 (1.23 to 1.44)]
Jovanovic et al[149] (2004)	South-west Germany in two urban industrial regions	Cross-sectional	397 schoolchildren (age 9-11 years) (n = 199) and without (n =	- Home inspection (questionnaire) - Measurement of molds in air. Data on colony-forming units (CFU/m^3) and	Venous blood samples (5 ml) were taken from 352 children for determination of serum IgE against a mixture of moulds: *Penicillium notatum (chrysogenum),*	- No difference in concentrations and distribution of fungi species levels between children

	(Stuttgart, Mannheim)		198) atopic history were included	fungal composition in air were obtained - Measurement of molds in dust.	*Cladosporium herbarum, Aspergillus fumigatus, Candida albicans, Alternaria alternata, Helminthosporium halodes*). - Cutting point for positive samples: 0.35 kU/l	with and without atopic history - The sensitization rate against molds (IgE) was higher for children with atopic condition (9.2%) than in control children (4.4%) - No association with the fungi counts in the rooms
Jacob et al[150] (2002)	Germany	Two cross-sectional surveys in 1992–1993 and 1995–1996	German school children ages 5–14 years living in three areas of Saxony-Anhalt [n = 2,470 children (89.1%) and n = 2,814 (74.7%)	In each home, a dust sample was taken from the living room floor (97% were carpeted floors) by vacuuming an area of 1 m^2 for 2 min in a highly standardized manner using the same type of vacuum cleaner.	- Specific IgE for Dermatophagoides pteronyssinus (d1), cat allergens (e1), Cladosporium (m2), mixed grasses (g6), and birch (t3). - Atopic sensitization was defined as testing positive for at least one specific IgE (≥ 0.35 kU/L).	A higher risk of sensitization against inhalant allergens when exposed to *Cladosporium* an d *Aspergillus* (>35, 000 and 0–25,000 cfu·g^{-1} and above, respectively).
Schäfer et al[144] (1999)	Two West an d five East Ger man locations	Cross-sectional	1235 childre n (5-6 years old) had valid prick tests.	Questionnaire report from parents (dampness and visible mold)	Six common aero- (birch, grass, mugwort pollen, cat, HDM, alternaria) and two food allergens (egg, milk). Results were read after 15 minutes and assessed as positive if the wheal diameter was 2 mm or greater.	Exposure to visible mould was found to increase the risk of sensitization against mugwort, dust mites and cat [aOR=1.93 (1.19-3.12)]

Table 2-4 summarizes studies examining the associations between microbial exposures (e.g. endotoxin, EPSs, and beta-(1→3)-D-glucan)) and asthma among school-age children. Most studies were conducted in North America and Europe with various study designs which include cross-sectional, case-control, and longitudinal cohort studies. Overall, most studies used endotoxin levels as a main measure of microbial exposures, while less evidence for EPSs and beta-(1→3)-D-glucan have been presented. Inconsistent findings have been shown, where high endotoxin levels have been associated with the decreased risk of asthma, while others were not.

In the Canadian context, a case-control study among 422 children who participated in the population-based Study of Asthma, Genes and Environment (SAGE) birth cohort in Manitoba, Canada by Maheswaran et al[151] showed that children who were exposed to high level of beta-(1→3)-D-glucan in home dust at age 7–10 years developed persistent atopic asthma at age 11–14 years (OR = 1.79; 95%CI: 1.14–2.81).[151] A case-control study by Lawson et al[152] (2012) was conducted among children aged 6-18 years in a rural area in Saskatchewan, Canada. The authors found that among children aged 6-12 years, mattress endotoxin concentration (EU/mg) and load (EU/m^2) were inversely associated with being a case: aOR = 0.44 (0.20-0.98); and aOR = 0.38 (0.20-0.75), respectively]. However, Rennie at al[153] did not find these associations even though the study was also conducted in rural area of Saskatchewan.

Several studies have examined environmental exposures to indoor microbial products as assessed by the measurement of house dust endotoxin levels and its relationship with asthma phenotype. Findings from the Allergy and Endotoxin Study (ALEX) among children (aged 6–13 years) in Austria, Germany, and Switzerland suggested that endotoxin load in samples of dust derived from children's mattresses were inversely associated with atopic asthma.[154] A cross-

sectional study in Palestine among 6–12 year old children also found the medium and high (the second and third tertiles) endotoxin levels in play area floor dust to be inversely associated with atopic wheeze (report of wheeze in the past 12 months).[155] Similar findings were found in a Saskatchewan study with an inverse relationship between high endotoxin levels and atopic asthma, but the associations were limited to play area endotoxin levels.[156]

Table 2-4: Studies investigating the effect of microbial exposures and asthma among school-age children

Lead author (Year published) Country	Design	Study population (Age, Sample size)	Method of dust sample collection and levels of endotoxin exposure	Definition of asthma	Findings and strength of association
Mendy et al[157] (2020) Iowa, US	Cohort	Asthmatic school children (N = 104 from 89 homes) of Louisa and Keokuk counties in Iowa (aged 5-14 years)	Ambient air and surface sampling (child's bedroom bedding, the child's bedroom floor, the main play area floor, and the kitchen floor) for endotoxin analysis	Asthma was defined as doctor-diagnosed asthma ever and either having wheezed in the past year or having prescription medication for wheeze during the past year. Asthma symptoms were assessed using a questionnaire drawn from standard instruments.	Overall house dust endotoxin load reduction from baseline was associated with lower total asthma symptoms score (Odds ratio: 0.52, 95% confidence interval: 0.29-0.92).
Oluwole et al[156] (2018) Saskatchewan, Canada.	Cross-sectional	Children with asthma (n = 116) were identified from 335 schoolchildren (aged 7-17 years)	Levels of indoor endotoxin and beta-(1 → 3)-D-glucan were measured in dust samples obtained from play area floors and child's mattresses	Asthma severity based on recommended guidelines (continuous daytime asthma symptoms, frequent nighttime asthma symptoms, and ≤ 60% predicted FEV_1).	Higher mattress endotoxin concentration was associated with increased odds of moderate/severe asthma: aOR= 11.40 (1.45-89.43) Higher beta-(1 → 3)-D-glucan concentration [aOR = 0.16 (0.03-0.89)] and load: [aOR =0.10 (0.02-0.72)] in play areas were

					inversely associated with moderate/severe asthma.
Maheswaran et al[151] (2014) Canada	Prospective cohort	Children followed up from birth to age 14 years N= 422	Vacuum dust sampling from play area and mattress dust samples and were analyzed for beta-(1→3)-D-glucan levels.	Asthma (assessed at age 7–10 years and ages 11–14 years): Physician-diagnosed asthma confirmed by pediatric allergist according to the Canadian Asthma Consensus guidelines. Atopy asthma: Physician-diagnosed asthma plus positive test to at least 1 of 16 tested allergens.	At ages 7–10, beta-(1→3)-D-glucan levels in house increased the risk of asthma (OR = 1.15), and atopic asthma (OR = 1.21), albeit non-significant. However, after adjusting for potential confounders, including endotoxin exposure, beta-(1→3)-D-glucan exposure at age 7–10 was significantly associated with persistent atopic asthma (OR = 1.79).
Blatter et al[158] (2014) Puerto Rico	Case-control	6–14 years N = 317	Vacuum dust sampling from mattress surfaces, living room and kitchen areas and were analyzed for Beta-(1→3, 1→6)-D-glucan levels.	Asthma cases: Physician-diagnosed asthma and wheeze in the prior year Control: No asthma or wheeze.	No significant association between beta-(1→3, 1→6)-D-glucan and being a case for asthma.
Jacobs et al[159] (2013) The Netherlands	Case-control	6-12 years old Cases: 84 children with	Classroom and bedroom airborne settled dust was sampled using	Children were considered asthmatic when a positive answer was given to at least one of	Home endotoxin tended to be inversely associated with asthma symptoms, but only for

		asthma-like symptoms. Controls: 170 children without symptoms	electrostatic dust fall collectors analyzed for endotoxin.	following items: 1) wheezing or whistling in the chest in the past 12 months; 2) ever had asthma; 3) using any medicines, pills, inhalers or other medication for wheezing or asthma in the past 12 months; and 4) dry cough at night, apart from a cough associated with a cold or chest infection in the past 12 months.	atopic asthma (aOR= 0.87 (0.68–1.11)). School exposure was positively associated with nonatopic asthma symptoms (adjusted odds ratio = 1.11 (0.97–1.27)), but not with atopic asthma.
Lawson et al[152] (2012) Rural region of Humboldt, Canada.	Case-control	Children 6–18 years of age. Cases (n = 102) reported doctor-diagnosed asthma or wheeze in the past year. Controls (n = 208) were randomly selected from children without asthma or wheeze.	Vacuumed Play area and mattress for endotoxin.	Report of doctor-diagnosed asthma or wheeze in the past year	Among children aged 6-12 years, mattress endotoxin concentration (EU/mg) and load (EU/m^2) were inversely associated with being a case: aOR = 0.44 (0.20-0.98); and aOR = 0.38 (0.20-0.75), respectively]. These associations were not observed in older children or with play area endotoxin. Play area endotoxin concentration (OR = 1.64) and load (OR =

					1.10) increased the risk of being a case but not statistically significant.
Rennie et al[153] (2008) Southern Saskatchewan, Canada	Case-control	6-13 years old (n = 197 including 89 cases matched to 107 healthy controls based on age and sex)	Vacuumed dust samplings from play area and mattress and were analyzed for endotoxin levels.	Asthma case: Report of physician-diagnosed asthma and/or wheeze without a cold in the past 12 months Control: No asthma or wheeze	Mattress endotoxin (OR = 0.90) and play area (OR = 0.92) endotoxin concentration were not significantly associated with being a case.
Gehring et al[160] (2008) Multi-center study Albania, Italy, New Zealand, Sweden, and the United Kingdom	Cross-sectional	9–12 years old N = 840	Vacuumed dust sampling from living room floor dust was collected and analyzed for endotoxin.	Asthma ever was defined as a positive answer to the question 'Has your child ever had asthma?'.	Combined across countries, endotoxin levels were inversely associated with asthma ever: aOR=0.53 (0.29-0.96) for endotoxin levels per square meter of living room floor.
Ege et al[161] (2007) Rural areas of Austria, Germany, The Netherlands, Sweden, and Switzerland	Cross-sectional	5 to 13years N= 440	Levels of endotoxin, β(1→3)-glucan, and fungal extracellular polysaccharides (EPSs) were measured in mattress dust	Children with reported physician-diagnosed asthma once or obstructive bronchitis more than once in their lifetime were defined as having asthma ever.	EPSs were inversely related to asthma (p=0.0483) (No actually ORs were reported). Endotoxin: not statistically significant
Tavernier et al[162] (2005) The United Kingdom	Cross-sectional	4-17 years old. N=200 including 90 matched pairs of asthmatic and healthy controls.	House dust was obtained from the living room carpet, the child's bedroom carpet and the child's mattress	Those who had responded positively to three key questions on the questionnaire carried a positive predictive value of 84% for	Higher levels of endotoxin were found in the living room carpets, but not the bedroom carpet or mattresses of the asthma compared

		50 asthmatic and 45 non-asthmatic male children 55 asthmatic and 50 non-asthmatic female children.		meriting a trial of asthma medication[163]	with the control homes (aOR=1.88 (1.11-3.18); p=0.018).
Braun-Fahrländer et al[164] (2002) Rural areas of Germany, Austria, or Switzerland	Multicenter cross-sectional	6-13 years old. N=812	Dust was collected by vacuuming each mattress and was analyzed for endotoxin levels.	Atopic asthma: Report of physician-diagnosed asthma plus positive test for specific IgE ≥3.5 kU per liter otherwise, they are considered non-atopic asthma.	Mattress endotoxin loads was significantly associated with reduced risk of atopic asthma [aOR = 0.48; (0.28-0.81)], but not non-atopic asthma (OR = 1.13; 0.57-2.26) Reduced risks of atopic asthma was observed in those who were exposed to a farming environment during the first year of life and current endotoxin exposure [ORs = 0.42 (0.18-0.96) and 0.52 (0.30-0.90); respectively] but not non-atopic asthma.

2.7.Exposure measurement methodology and asthma and atopy in school-age children

Microbial exposures have been considered as a potential explanatory factor for geographic differences in asthma and atopy. With regard to microbial exposure measurements when looking at the relationship between the farm environment and asthma or atopy, endotoxin has been the most commonly investigated exposure.[165] **Table 2-5** summarizes recent studies measuring endotoxin levels in and around homes comparing rural vs. urban and farm vs. non-farm dwellings. As seen in **Table 2-5**, high levels of environmental indoor endotoxins were found in rural areas of developed countries and in farming communities.[166-170]

Table 2-5: Levels of endotoxin between rural vs. urban and farm vs. non-farm settings by different measurement methods. **(**GM= geometric mean, EU= endotoxin unit)

Authors[reference #] (Year published) Location	**Study population (sample size)**	**Comparison**	**Measurement of dust samplings**	**Endotoxin exposure and results**
Barnig et al[166] (2013) France	- 100 urban dwellings (50 apartments and 50 houses). -50 rural farmhouses -50 rural nonfarming references were matched to these farming samples.	Rural-urban Farm-nonfarm	- Vacuumed. Dust samples: from the floor and mattress. - Air samplers: The samplers were placed at the lapel of the person spending the longest time at home or close to him/her. Two 8-h samples with an airflow of 2 l min^{-1} were collected during daytime on two consecutive days.	- Endotoxin concentrations from floor and mattress dust samples did not differ between rural control dwellings and urban dwellings. - Endotoxin levels were significantly higher in floor (6600± 6100 vs. 3600± 5600 and 3800± 17000 ng/g; $p<0.001$) and mattress dust (2900± 4100 vs. 1100±2400 and 800 ± 2600 ng/g; $p<0.001$) from farmhouses compared to other rural and urban homes. - No difference between endotoxin concentrations in the air of urban and rural houses. - Endotoxin concentrations in the air samples were not significantly different between farming and nonfarming environments. - Airborne endotoxin levels did not correlate to dust endotoxin levels.
Lawson et al[152] (2012)	310 children 6–18 years old. Cases (n = 102) reported	Rural	Household settled dust: the floor of the room where the	Play area endotoxin: controls vs. cases

Humboldt, Canada	doctor-diagnosed asthma or wheeze in the past year. Controls (n = 208) were randomly selected from children without asthma or wheeze.		child spent most of his/her free time (play area) & the child's mattress (mattress)	- Concentration (EU/mg): 40.8 (35.7-46.6) vs 51.8 (42.0-63.9) - Load (EU/m^2): 817.3 (674.8-989.8) vs. 868.2 (672.9-1119.9) Mattress endotoxin: controls vs. cases - Concentration (EU/mg): 21.1 (15.6-24.8) vs. 19.6 (15.6-24.8) - Load (EU/m^2): 376.2 (324.4-436.2) vs. 240.5 (212.1-370.9) ($p < 0.05$)
Morcos et al[171] (2011) Egypt	40 rural and 40 urban school children (6-12 years).	Rural-urban schools	Settled dust samples were collected at schools.	Rural school dust contains significantly higher level of endotoxin (2-3 EU/mg) than urban school dust(0-0.1 EU/mg).
Hyvrinen et al[167] (2006) Eastern Finland	12 farming and 17 urban homes	Urban vs. farm	Endotoxin levels were determined from farming and urban homes using five different sample types: bed dust, floor dust, vacuum cleaner dust bag dust, settled dust and a filter sample collected from the air via stationery air samplers.	In farm homes (n=5). GM bed dust =7.2 EU/mg. Floor dust: GM=23.7 EU/mg; Dust bag dust GM=39.1 EU/mg; Settled dust GM=10.0 EU/mg. In urban homes (n=5). GM bed dust =2.4 EU/mg. Floor dust: GM=8.7 EU/mg; Dust bag dust GM=18.4 EU/mg; Settle dust GM=7.2 EU/mg. Air GM =0.43 EU/m^3
Waser et al[168] (2004) Rural areas of Austria, Germany and Switzerland	319 farmers' children and 493 children from non-farming families	Farm vs. non-farm	The living room floor and mattress were vacuumed.	Farmers' children: Living room GM =81.8 EU/mg; Mattress GM =37.8 EU/mg Non farmer's children: Living room GM=44.9EU/mg; Mattress GM =22.8EU/mg.

				Endotoxin levels in stables did not predict the amount of endotoxin measured in floors or mattresses in farming homes.
Gereda et al[169] (2000) USA, Peru and India	Homes from urban Denver (n=11), US farm houses (n=11), US farm barns (n=18); and rural homes in India and Peru (n=8)	Rural-urban Farm-nonfarm	Denver homes had dust vacuumed from living room floor, couch, kitchen floor, bedroom floor and subject's bed; however, there is unknown which samples were used for analysis.	Log endotoxin (EU/mL) from Denver homes=646 EU/ml; from US farm houses=2570 EU/ml; from rural homes in India and Peru=3981 EU/ml; and US farm barns=11,749 EU/mL.
Von Mutius et al[170] (2000) Baravia and Switzerland	Rural areas with 39 families living on a farm and 45 families not living on a farm (15 of these had exposure to livestock). 146 children aged 1–14 years (*n* = 119 in Bavaria and *n* = 27 in Switzerland)	Rural: farm vs. non-farm	Samples of settled dust were collected in stables, and settled dust indoors from kitchen floors and the children's mattresses.	- The endotoxin exposures per mg dust were significantly higher in dust samples from kitchen floors of farming families (GM=143 EU/mg) as compared to nonfarming families (children with livestock contact: GM=51 EU/mg, controls: GM=39 EU/mg. - Levels of endotoxin were also significantly higher in mattresses of farming families (GM=49479 EU/m^2) and marginally higher in families with children having regular contact to livestock (gm =23340 EU/m^2, as compared to the control group (GM=9383 EU/m^2.

Ideally, objective measures are used to determine exposure levels. In order to collect objective environmental exposures such as endotoxin, EPS, β-(1 → 3)-D-glucan, there are several sampling methods that can be used including vacuumed samples from the bed, play areas, and other floor areas as well as air samples. **Table 2-4** shows that the majority of studies that examine the association between asthma and atopy and microbial exposures in school age children was conducted with the collection of vacuumed dust.

One of the limitations in examining the association between environmental exposures and asthma and atopy in children in general and in rural dwellings specifically is the lack of comprehensive information about dust in the child's immediate environment. Questionnaire reports of exposures and collection of settled dust by vacuuming of homes, primarily floors and the child's mattress have been the main source of exposure information for children.[172-179] Dust measurement can provide information about the level and nature of microbial compounds, most commonly endotoxin, molds and β-(1 → 3)-D-glucan, in the relationship with asthma and atopic outcomes. These measurements provide an estimate of the domestic indoor exposures but may not account for the true exposure associated with the child's "personal cloud"[180] which can include both exposures in and outside of the home.

A few studies have been conducted to evaluate the associations between airborne endotoxin exposure (either ambient or personal) and asthma in schoolchildren. A recent case-control study that matched on age and class was conducted by Yen et al[181] (2020) to evaluate the association between the presence of asthma and atopy, and ambient airborne endotoxin in homes of school-age children in Kaohsiung City, Taiwan. The authors reported that household airborne endotoxin was associated with higher prevalence of asthma and atopy; OR = 4.88 (95% CI 1.16–20.55) for Quartile 3 (between 0.67 and 1.97 EU m^{-3}) vs. Quartile 1 (< 0.31 EU m^{-3}). Rabinovitch et al[180]

conducted a panel study in 2000 among 24 American children with asthma to investigate the relationship between day-to-day changes in personal endotoxin exposure and asthma severity. The authors monitored 24 children ages 6 to 13 years during school hours using personal exposure monitors (PEMs) operated at 2 L/min over 24 hours for 164 person-days. They found that those children with higher endotoxin levels from personal monitoring were more likely to experience asthma symptoms. PEM measured exposures did not correlate with stationary measurements suggesting that personal endotoxin exposure likely included substantial contributions from other particle sources from other environments. These findings demonstrate the importance of using personal monitoring to both measure and correlate endotoxin exposure with asthma severity. Another panel study was conducted by Delfino et al[182] in 2004 to characterize personal endotoxin exposures in 45 school children with asthma ages 9-18 years using 376 person-days of daily endotoxin data collected from $PM_{2.5}$ quartz filters. Using personal exposure monitors operated at 4 L/min. and daily ambient monitors, endotoxin measurements were collected from central ambient sites, and daily indoor and outdoor home endotoxin measurements in a subset of 14 children at 12 residential sites in Riverside and Whittier, California. Personal endotoxin was not associated with endotoxin measured from the floor inside the home or from research based stationary monitors located just outside the home but was associated with ambient endotoxin collected from central ambient sites within 10 km of homes in Riverside and 5 km of homes in Whittier (Stationary Site Air Monitoring) suggesting that daily fixed site measurements of endotoxin in the home environment may not predict daily personal exposure.

The findings for endotoxin collected by different sampling methods including vacuum sampling from home locations such as the floor or child's mattress, airborne sampling of home areas, and personal sampling of the subject often not correlated. The Spearman's correlation

coefficients between endotoxin from airborne monitoring and floor or mattress sampling in one study[183] were low (0.23 and 0.33, respectably) and no statistically significant results were found.

The magnitude of endotoxin's effect may also be underestimated using settled house-dust endotoxin as a surrogate for true exposure compared to airborne endotoxin measured by long-term or often-repeated personal breathing zone air samples[184]. In 2001, Park et al[184] found that exposure to elevated levels of endotoxin in family-room floor dust was observed to be significantly associated with increased wheeze in the first year of life among a cohort of 499 children in the Boston, Massachusetts, metropolitan area (RR=1.56, 95%CI 1.03-2.38). Family-room floor dust endotoxin was used as a surrogate for airborne endotoxin exposure. With the hypothesis that inhalation is the relevant route of exposure and by using the same dataset from the previous study[184], in 2006, Horick et al[185] conducted an evaluation to reexamine the relationship between exposure to endotoxin and wheeze in the first year of life, accounting for the measurement error associated with using house dust endotoxin measurements from the floor as surrogates for the suspected more relevant airborne exposure by comparing results from analyses using floor based endotoxin measures to airborne endotoxin measurements. By applying a measurement error correction technique, the authors found that the prevalence of "any wheeze" in the first year of life increases 6-fold for every 0.4 $\log_{10}(EU/m^3)$ increase in airborne endotoxin exposure (95% CI= 1.2-26) among the 360 children in households with dust endotoxin levels. This showed that only looking at house-dust endotoxin from floor samples and neglecting the role of airborne endotoxin can lead to bias in interpreting results.

While a major use of PEMs and focal point of asthma research has been exposures such as endotoxin, another set of exposures worth considering are allergens. In addition to detecting microbial compounds such as endotoxin and β-(1 → 3)-D-glucan present in the dust, personal

monitoring techniques have been used to assess allergens in the environment.[186] PEMs have been used to measure a number of allergens in the environment such as pollens (indoor and outdoor), dust mites, cockroaches and furred animals (indoor), and, in theory, could be used to measure others. Significant exposure to 'domestic allergens' also occurs in non-domestic location such as occupational, educational and public settings.[187,188] The collection of aeroallergens on filters using personal monitors is used routinely in occupational settings to measure personal exposure to allergens and microbials. [189-192] Very few studies conducted in the general population t have used PEMs to measure allergen levels. Using PEMs, one recent report[186] from a study in Australia aimed to determine the pattern of personal exposure to mite allergen bioaerosols over 24-hour periods and applied this in a small field study using 10 normal adults. In that study, indoor domestic exposures accounted for 59.5% of total exposure.

The above evidence shows the importance of applying a more advanced, precise, and acceptable device to capture information about exposures in the "personal cloud" that may occur in daily life and that these methods may be underused. More research should be conducted to test the feasibility or acceptability about the use of such a device, especially in children, before applying this technique in a larger population.

2.8.Summary of the literature review

The following points can be summarized from the evidence around asthma and atopic disease and environmental exposures, especially with regard to rural exposures.

- There is geographic variation in asthma and atopy prevalence. Lower asthma and atopy prevalence have usually been found in rural farm children compared to urban non-farm counterparts but there is some inconsistency which may be due to specific types of farming exposures or asthma phenotype.

- Current evidence suggests the importance of early life exposures, and their effect on the development of asthma during the life course from early school age, adolescent, to adulthood.
- Farming environments are heavy microbial environments and appear to confer the most protection against asthma and atopy.
- In Saskatchewan, previous work has suggested that there are no differences in atopy or asthma in school-age children when comparing farm and non-farm locations. Possible explanations were that there may be a lack of variability in exposures between farm and non-farm populations in which the towns (ie, non-farm children) were small and adjacent to farming areas. A more intensive analysis using a larger sample size with detailed information of farm exposures would be helpful to further examine this issue.
- Environmental exposures associated with atopic outcomes can be measured by objective measurements, but results can differ between techniques. There is limited use of personal sampling with children to access dust exposure.

2.9. Research rationale

In view of the above review of the literature:

- To date, there have not been studies which systematically summarize or review the associations between farm-related environmental exposures in early life (from in-utero until 1 year of age) and atopy/atopic sensitization measured by objective markers (Skin prick testing, serum and specific IgE measurements) in school-age children.
- Findings from studies point to an inverse association between farm dwelling and childhood asthma have been inconsistent. Some of the inconsistency could result from differences in the exposure-outcome associations between atopic and nonatopic asthma phenotypes, yet

little work has been conducted investigating asthma phenotypes and their risk factors in rural populations.

- Questionnaire report has often been used to collect information regarding farm exposures, yet there are few studies that have used more detailed questions to capture the intensity and types of exposures in a large sample of farm and non-farm children.
- There has been little information regarding to what extent non-farm children are exposed to a farm compared to the children living in a farm and to what extent these exposures affect the presence of atopic disease and asthma among children.
- There is a paucity of research with children assessing whether the levels of environmental exposures in dust collected by personal samplers are correlated with those obtained by vacuuming of settled house dust. More precise measurement of the exposures experienced by children could be obtained by personal sampling which could lead to more accurate assessment of microbial exposure for childhood atopy. Using personal monitoring has been suggested to be effective to capture the "personal cloud" of exposures.

2.10. Research objectives and research questions

The overall aim of this thesis is to examine the association between farm-related environmental factors and asthma and atopy in children. The following research objectives and questions were considered:

1. Research objective #1: To determine if early life farm-related exposures are associated with atopy development in school-age children.
    - Is there an association between early life farm-related exposures and atopy development in school-age children?

2. Research objective #2: To estimate the prevalence of atopic and nonatopic asthma and identify risk factors for each phenotype among rural dwelling children.
    - What is the prevalence of atopic and nonatopic asthma among school-age children?
    - Is there consistency in predictors between the 2 phenotypes?
    - What are the differences of clinical characteristics (symptoms and lung function) and burden of disease among children with atopic and nonatopic asthma?
3. Research objective #3: To determine the prevalence of atopy and asthma and its associations with farm-related exposures for both farm and non-farm school-age children.
    - Compared to children living on a farm, to what extent are children not living on a farm exposed to a farm-related environments and activities?
    - Compared to children with asthma and atopy, are children without these conditions exposed to a farm environment differently?
    - What are the potential risk and protective factors (e.g., types and/or intensities of farm exposures) associated with atopy and asthma conditions among farm and non-farm children in rural Saskatchewan?
4. Research objective #4: To determine whether a personal air sampling pack that we develop will be feasible in future studies of lung health.
    - Is there a correlation between each of the levels of environmental exposures in dust collected by personal samplers and by vacuuming of settled house dust?
    - Can the personal monitoring device effectively and conveniently be used in children?

The overall purpose of this thesis is to help understand the effect of farm exposures on children's atopy and asthma. This will be accomplished by approaching the problem from complementary perspectives through the four objectives stated above. First, I will explore the current literature on early farm exposures and the development of atopy in children through a systematic review. This will help determine the relationship between farming and atopic disease from the perspective of early life exposures. The second research objective will estimate the prevalence of atopic and nonatopic asthma and identify risk factors for each phenotype among rural dwelling children. The third research objective will examine the effect of living on a farm or visiting a farm on children's atopy/asthma. The second and third objectives will expand on the first research question by looking at more intensely at asthma phenotypes as well as current farm exposures and their relationship with atopic disease in a population of school age children. Finally, for the fourth research objective, I will extend the research by testing a methodological approach of personal exposure monitoring (PEM) to collect "personal cloud" exposures in children. These four research questions fall under the umbrella of the farm environment and its relationship with asthma and atopy in children Each of these research objectives will be described and presented in the form of a manuscript (Chapters 3, 4, 5, and 6, respectively).

2.11. References

1. Peebles RS, Church MK, Durham SR. 6 - Principles of allergy diagnosis. In: Holgate ST, Church MK, Broide DH, Martinez FD, eds. *Allergy (Fourth Edition)*. Edinburgh: W.B. Saunders; 2012:129-146.
2. Brand P. Allergy diagnosis: pros and cons of different tests, indications and limitations. *Breathe.* 2007;3(4):345-349.

3. Galimberti M, Passalacqua G, Incorvaia C, et al. Catching allergy by a simple questionnaire. *World Allergy Organization Journal.* 2015;8(1):1-7.
4. Sacchetti M, Baiardini I, Chini L, Moschese V, Bruscolini A, Lambiase A. Development and preliminary validation of a new screening questionnaire for identifying atopic children. *Pediatric Health, Medicine and Therapeutics.* 2017;8:99.
5. Chu L, Rennie D, Cockcroft D, et al. Agreement between questionnaire report of allergy-related outcomes in school-age children and objective measures of atopy: the Saskatchewan rural health study. *Clinical & Experimental Allergy.* 2015;45(8):1337-1345.
6. Dottorini M, Bruni B, Peccini F, et al. Skin prick-test reactivity to aeroallergens and allergic symptoms in an urban population of central Italy: a longitudinal study. *Clinical & Experimental Allergy.* 2007;37(2):188-196.
7. Annesi-Maesano I, Didier A, Klossek M, Chanal I, Moreau D, Bousquet J. The score for allergic rhinitis (SFAR): a simple and valid assessment method in population studies. *Allergy.* 2002;57(2):107-114.
8. Karakaya G, Ozturk A, Kalyoncu A. Prediction of atopy by skin prick tests in patients with asthma and/or persistent rhinitis. *Allergologia et immunopathologia.* 2012;40(1):37-40.
9. Gruchalla RS, Gan V, Roy L, et al. Results of an inner-city school-based asthma and allergy screening pilot study: a combined approach using written questionnaires and step testing. *Annals of Allergy, Asthma & Immunology.* 2003;90(5):491-499.

10. Braun-Fahrländer C, Wüthrich B, Gassner M, et al. Validation of a rhinitis symptom questionnaire (ISAAC core questions) in a population of Swiss school children visiting the school health services. *Pediatric allergy and immunology.* 1997;8(2):75-82.

11. Kwon S, Simons E, To T, Dell SD. How do questionnaire definitions of atopy status affect sample size calculations for asthma cohort studies in a population of Canadian children? *Allergy, Asthma, and Clinical Immunology : Official Journal of the Canadian Society of Allergy and Clinical Immunology.* 2011;7(Suppl 2):A12-A12.

12. Chu L, Rennie D, Cockcroft D, et al. Agreement between questionnaire report of allergy-related outcomes in school-age children and objective measures of atopy: the Saskatchewan rural health study. *Clinical & Experimental Allergy.* 2015;45(8):1337-1345.

13. Pekkanen J, Pearce N. Defining asthma in epidemiological studies. *Eur Respir J.* Oct 1999;14(4):951-957.

14. National Heart L, and Blood Institute. Guidelines for the Diagnosis and Management of Asthma (EPR-3). [Internet]. 2012; https://www.nhlbi.nih.gov/health-topics/guidelines-for-diagnosis-management-of-asthma. Accessed December 27, 2020.

15. Asthma GIf. Global strategy for asthma management and prevention. [Internet]. 2020; https://ginasthma.org/. Accessed September 27, 2020.

16. Lougheed MD, Lemiere C, Ducharme FM, et al. Canadian Thoracic Society 2012 guideline update: diagnosis and management of asthma in preschoolers, children and adults. *Canadian respiratory journal.* 2012;19.

17. Chung KF, Wenzel SE, Brozek JL, et al. International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. *European respiratory journal.* 2014;43(2):343-373.

18. Quirt J, Hildebrand KJ, Mazza J, Noya F, Kim H. Asthma. *Allergy Asthma Clin Immunol.* 2018;14(Suppl 2):50.

19. Martinez FD, Wright AL, Taussig LM, et al. Asthma and wheezing in the first six years of life. *New England Journal of Medicine.* 1995;332(3):133-138.

20. Wenzel SE. Asthma: defining of the persistent adult phenotypes. *The Lancet.* 2006;368(9537):804-813.

21. Fitzpatrick AM, Teague WG, Meyers DA, et al. Heterogeneity of severe asthma in childhood: confirmation by cluster analysis of children in the National Institutes of Health/National Heart, Lung, and Blood Institute Severe Asthma Research Program. *Journal of allergy and clinical immunology.* 2011;127(2):382-389. e313.

22. Konradsen JR, Nordlund B, Lidegran M, et al. Problematic severe asthma: a proposed approach to identifying children who are severely resistant to therapy. *Pediatric allergy and immunology.* 2011;22(1-Part-I):9-18.

23. Pekkanen J, Lampi J, Genuneit J, Hartikainen A-L, Järvelin M-R. Analyzing atopic and non-atopic asthma. *European journal of epidemiology.* 2012;27(4):281-286.

24. Romanet-Manent S, Charpin D, Magnan A, Lanteaume A, Vervloet D, Group EC. Allergic vs nonallergic asthma: what makes the difference? *Allergy.* 2002;57(7):607-613.

25. Arbes SJ, Jr., Gergen PJ, Vaughn B, Zeldin DC. Asthma cases attributable to atopy: results from the Third National Health and Nutrition Examination Survey. *The Journal of allergy and clinical immunology.* 2007;120(5):1139-1145.

26. Oddy W, De Klerk N, Sly P, Holt P. The effects of respiratory infections, atopy, and breastfeeding on childhood asthma. *European Respiratory Journal.* 2002;19(5):899-905.

27. Pearce N, Pekkanen J, Beasley R. How much asthma is really attributable to atopy? *Thorax.* 1999;54(3):268-272.

28. Sunyer J, Jarvis D, Pekkanen J, et al. Geographic variations in the effect of atopy on asthma in the European Community Respiratory Health Study. *Journal of Allergy and Clinical Immunology.* 2004;114(5):1033-1039.

29. Weinmayr G, Weiland SK, Bjorksten B, et al. Atopic sensitization and the international variation of asthma symptom prevalence in children. *American journal of respiratory and critical care medicine.* 2007;176(6):565-574.

30. Ronchetti R, Jesenak M, Rennerova Z, Barreto M, Ronchetti F, Villa MP. Relationship between atopic asthma and the population prevalence rates for asthma or atopy in children: atopic and nonatopic asthma in epidemiology. Paper presented at: Allergy & Asthma Proceedings2009.

31. Ulrik CS, Backer V, Dirksen A. A 10 year follow up of 180 adults with bronchial asthma: factors important for the decline in lung function. *Thorax.* 1992;47(1):14-18.

32. Sunyer J, Anto J, Kogevinas M, Soriano J, Tobias A, Munoz A. the Spanish group of the European Study of Asthma. Smoking and bronchial responsiveness in non-atopic and atopic young adults. *Thorax.* 1997;52(3):235-238.

33. Nieves A, Magnan A, Boniface S, et al. Phenotypes of asthma revisited upon the presence of atopy. *Respiratory Medicine.* 2005/03/01/ 2005;99(3):347-354.

34. Garcia G, Magnan A, Chiron R, et al. A proof-of-concept, randomized, controlled trial of omalizumab in patients with severe, difficult-to-control, nonatopic asthma. *Chest.* Aug 2013;144(2):411-419.

35. D'Amato G, Stanziola A, Sanduzzi A, et al. Treating severe allergic asthma with anti-IgE monoclonal antibody (omalizumab): a review. *Multidisciplinary respiratory medicine.* 2014;9(1):23.

36. Holgate ST. Stratified approaches to the treatment of asthma. *British journal of clinical pharmacology.* 2013;76(2):277-291.

37. Kuruvilla ME, Lee FE-H, Lee GB. Understanding Asthma Phenotypes, Endotypes, and Mechanisms of Disease. *Clinical reviews in allergy & immunology.* 2019;56(2):219-233.

38. Peters SP, Busse WW. New and Anticipated Therapies for Severe Asthma. *The Journal of Allergy and Clinical Immunology: In Practice.* 2017/09/01/ 2017;5(5, Supplement):S15-S24.

39. Douwes J, Gibson P, Pekkanen J, Pearce N. Non-eosinophilic asthma: importance and possible mechanisms. *Thorax.* 2002;57(7):643-648.

40. Murdoch JR, Lloyd CM. Chronic inflammation and asthma. *Mutat Res.* Aug 7 2010;690(1-2):24-39.

41. Pillai P, Corrigan CJ, Ying S. Airway epithelium in atopic and nonatopic asthma: similarities and differences. *ISRN allergy.* 2011;2011:195846-195846.

42. Sunyer J, Jarvis D, Pekkanen J, et al. Geographic variations in the effect of atopy on asthma in the European Community Respiratory Health Study. *J Allergy Clin Immunol.* 2004;114:1033 - 1039.

43. Illi S, Depner M, Genuneit J, et al. Protection from childhood asthma and allergy in Alpine farm environments-the GABRIEL Advanced Studies. *J Allergy Clin Immunol.* Jun 2012;129(6):1470-1477 e1476.

44. von Mutius E. 99th Dahlem conference on infection, inflammation and chronic inflammatory disorders: farm lifestyles and the hygiene hypothesis. *Clin Exp Immunol.* Apr 2010;160(1):130-135.

45. von Mutius E. Asthma and Allergies in Rural Areas of Europe. *Proceedings of the American Thoracic Society.* 2007/07/01 2007;4(3):212-216.

46. Illi S, Depner M, Genuneit J, et al. Protection from childhood asthma and allergy in Alpine farm environments—the GABRIEL Advanced Studies. *Journal of Allergy and Clinical Immunology.* 2012;129(6):1470-1477. e1476.

47. MacNeill S, Sozanska B, Danielewicz H, et al. Asthma and allergies: is the farming environment (still) protective in Poland? The GABRIEL Advanced Studies. *Allergy.* 2013;68(6):771-779.

48. Ronmark E, Bjerg A, Perzanowski M, Platts-Mills T, Lundback B. Major increase in allergic sensitization in schoolchildren from 1996 to 2006 in northern Sweden. *J Allergy Clin Immunol.* Aug 2009;124(2):357-363, 363.e351-315.

49. Krause TG, Koch A, Friborg J, Poulsen LK, Kristensen B, Melbye M. Frequency of atopy in the Arctic in 1987 and 1998. *The Lancet.* 2002;360(9334):691-692.

50. Rönmark E, Bjerg A, Perzanowski M, Platts-Mills T, Lundbäck B. Major increase in allergic sensitization in schoolchildren from 1996 to 2006 in northern Sweden. *Journal of allergy and clinical immunology.* 2009;124(2):357-363. e315.

51. Zollner IK, Weiland SK, Piechotowski I, et al. No increase in the prevalence of asthma, allergies, and atopic sensitisation among children in Germany: 1992-2001. *Thorax.* Jul 2005;60(7):545-548.

52. Toelle BG, Ng K, Belousova E, Salome CM, Peat JK, Marks GB. Prevalence of asthma and allergy in schoolchildren in Belmont, Australia: three cross sectional surveys over 20 years. *Bmj.* 2004;328(7436):386-387.

53. Weiland S, Björkstén B, Brunekreef B, Cookson W, Von Mutius E, Strachan D. Phase II of the International Study of Asthma and Allergies in Childhood (ISAAC II): rationale and methods. *European Respiratory Journal.* 2004;24(3):406-412.

54. Weinmayr G, Forastiere F, Weiland SK, et al. International variation in prevalence of rhinitis and its relationship with sensitisation to perennial and seasonal allergens. *European Respiratory Journal.* 2008;32(5):1250-1261.

55. Akinbami LJ, Moorman JE, Garbe PL, Sondik EJ. Status of Childhood Asthma in the United States, 1980–2007. *Pediatrics.* 2009;123(Supplement 3):S131-S145.

56. Asher MI, Montefort S, Björkstén B, et al. Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC Phases One and Three repeat multicountry cross-sectional surveys. *Lancet.* Aug 26 2006;368(9537):733-743.

57. Dharmage SC, Perret JL, Custovic A. Epidemiology of Asthma in Children and Adults. *Frontiers in pediatrics.* 2019;7:246-246.

58. Cruz Á A, Stelmach R, Ponte EV. Asthma prevalence and severity in low-resource communities. *Curr Opin Allergy Clin Immunol.* Jun 2017;17(3):188-193.

59. Pearce N, Aït-Khaled N, Beasley R, et al. Worldwide trends in the prevalence of asthma symptoms: phase III of the International Study of Asthma and Allergies in Childhood (ISAAC). *Thorax.* 2007;62(9):758-766.

60. Ma Y-C, Lin C-C, Li C-I, Chiang J-H, Li T-C, Lin J-G. Time-trend analysis of prevalence, incidence and traditional Chinese medicine use among children with asthma: a population-based study. *Journal of Public Health.* 2016;38(3):e263-e271.

61. Brozek G, Lawson J, Szumilas D, Zejda J. Increasing prevalence of asthma, respiratory symptoms, and allergic diseases: four repeated surveys from 1993-2014. *Respiratory medicine.* 2015;109(8):982-990.

62. Akinbami LJ, Moorman JE, Simon AE, Schoendorf KC. Trends in racial disparities for asthma outcomes among children 0 to 17 years, 2001-2010. *Journal of Allergy and Clinical Immunology.* 2014/09/01/ 2014;134(3):547-553.e545.

63. Akinbami LJ, Simon AE, Rossen LM. Changing Trends in Asthma Prevalence Among Children. *Pediatrics.* 2016;137(1):e20152354.

64. *Report from the Canadian Chronic Disease Surveillance System: Asthma and Chronic Obstructive Pulmonary Disease (COPD) in Canada.* Public Health Agency of Canada.;2018.

65. Asher MI, Montefort S, Björkstén B, et al. Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC Phases One and Three repeat multicountry cross-sectional surveys. *The Lancet.* 2006/08/26/ 2006;368(9537):733-743.

66. Garner R, Kohen D. Changes in the prevalence of asthma among Canadian children. *Health Rep.* Jun 2008;19(2):45-50.

67. Gershon AS, Guan J, Wang C, To T. Trends in Asthma Prevalence and Incidence in Ontario, Canada, 1996–2005: A Population Study. *American Journal of Epidemiology*. 2010;172(6):728-736.

68. Senthilselvan A. Prevalence of physician-diagnosed asthma in Saskatchewan, 1981 to 1990. *Chest.* Aug 1998;114(2):388-392.

69. Senthilselvan A, Lawson J, Rennie DC, Dosman JA. Stabilization of an increasing trend in physician-diagnosed asthma prevalence in Saskatchewan, 1991 to 1998. *Chest.* Aug 2003;124(2):438-448.

70. Rennie DC, Chen Y, Dosman JA, JA L. Changes in asthma prevalence in children: The 1993 and 2004 Humboldt Studies. *Eur Resp J.* 2007;30 (Suppl 51):298s.

71. Lawson JA, Rennie DC, Cockcroft DW, et al. Childhood asthma, asthma severity indicators, and related conditions along an urban-rural gradient: a cross-sectional study. *BMC Pulm Med.* Jan 5 2017;17(1):4.

72. Asher M, Keil U, Anderson H, et al. International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. *European respiratory journal.* 1995;8(3):483-491.

73. Beasley R, of Asthma TIS. Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema: ISAAC. *The Lancet.* 1998;351(9111):1225-1232.

74. Lai CK, Beasley R, Crane J, et al. Global variation in the prevalence and severity of asthma symptoms: phase three of the International Study of Asthma and Allergies in Childhood (ISAAC). *Thorax.* 2009;64(6):476-483.

75. Bjorksten B, Dumitrascu D, Foucard T, et al. Prevalence of childhood asthma, rhinitis and eczema in Scandinavia and Eastern Europe. *European Respiratory Journal.* 1998;12(2):432-437.

76. Brozek G, Lawson J, Shpakou A, et al. Childhood asthma prevalence and risk factors in three Eastern European countries-the Belarus, Ukraine, Poland Asthma Study (BUPAS): an international prevalence study. *BMC pulmonary medicine.* 2016;16(1):11.

77. Mazur A, Szylling A, Bielecka T, Strzelak A, Kulus M. Is the "farm effect" hypothesis still current? Atopy and allergic diseases in rural and urban children in Poland. *Journal of Asthma.* 2018;55(10):1147-1155.

78. Lee S, Kwon JW, Seo J, et al. Prevalence of atopy and allergic diseases in Korean children: associations with a farming environment and rural lifestyle. *International archives of allergy and immunology.* 2012;158(2):168-174.

79. Barnes M, Cullinan P, Athanasaki P, et al. Crete: does farming explain urban and rural differences in atopy? *Clinical & Experimental Allergy.* 2001;31(12):1822-1828.

80. Chu LM, Rennie DC, Cockcroft DW, et al. Prevalence and determinants of atopy and allergic diseases among school-age children in rural Saskatchewan, Canada. *Annals of Allergy, Asthma & Immunology.* 2014;113(4):430-439.

81. Ma Y, Zhao J, Han Z, Chen Y, Leung TF, Wong GW. Very low prevalence of asthma and allergies in schoolchildren from rural Beijing, China. *Pediatric pulmonology.* 2009;44(8):793-799.

82. Pesek RD, Vargas PA, Halterman JS, Jones SM, McCracken A, Perry TT. A comparison of asthma prevalence and morbidity between rural and urban schoolchildren in Arkansas. *Annals of Allergy, Asthma & Immunology.* 2010;104(2):125-131.

83. Guner S, Gokturk B, Kilic M, Ozkiraz S. The prevalences of allergic diseases in rural and urban areas are similar. *Allergologia et immunopathologia.* 2011;39(3):140-144.

84. Lawson JA, Janssen I, Bruner MW, Madani K, Pickett W. Urban-rural differences in asthma prevalence among young people in Canada: the roles of health behaviors and obesity. *Ann Allergy Asthma Immunol.* Sep 2011;107(3):220-228.

85. Kozyrskyj A, Becker A. Rural-Urban Differences in Atopic And Non-Atopic Asthma in Children. *Epidemiology.* 2006;17(6):S276.

86. Kozyrskyj A, Becker A. Rural-urban differences in asthma prevalence: Possible explanations. *Journal of Allergy and Clinical Immunology.* 2004;113(2):S306.

87. Lawson JA, Janssen I, Bruner MW, Hossain A, Pickett W. Asthma incidence and risk factors in a national longitudinal sample of adolescent Canadians: a prospective cohort study. *BMC pulmonary medicine.* 2014;14(1):51.

88. von Mutius E, Vercelli D. Farm living: effects on childhood asthma and allergy. *Nat Rev Immunol.* Dec 2010;10(12):861-868.

89. Wlasiuk G, Vercelli D. The farm effect, or: when, what and how a farming environment protects from asthma and allergic disease. *Curr Opin Allergy Clin Immunol.* Oct 2012;12(5):461-466.

90. SCALES N. ment process performed by &dquo; two-legged meter&dquo;(Stevens, 1958). *Educational and psychological measurement.* 1960;20(1).

91. Holbreich M, Genuneit J, Weber J, Braun-Fahrlander C, Waser M, von Mutius E. Amish children living in northern Indiana have a very low prevalence of allergic sensitization. *J Allergy Clin Immunol.* Jun 2012;129(6):1671-1673.

92. Fuchs O, Genuneit J, Latzin P, et al. Farming environments and childhood atopy, wheeze, lung function, and exhaled nitric oxide. *J Allergy Clin Immunol.* 2012;130(2):382-388.e386.

93. Lampi J, Canoy D, Jarvis D, et al. Farming environment and prevalence of atopy at age 31: prospective birth cohort study in Finland. *Clin Exp Allergy.* Jul 2011;41(7):987-993.

94. Macneill SJ, Sozanska B, Danielewicz H, et al. Asthma and allergies: is the farming environment (still) protective in Poland? The GABRIEL Advanced Studies. *Allergy.* Apr 29 2013.

95. Ma Y, Zhao J, Han ZR, Chen Y, Leung TF, Wong GW. Very low prevalence of asthma and allergies in schoolchildren from rural Beijing, China. *Pediatr Pulmonol.* Aug 2009;44(8):793-799.

96. Alfven T, Braun-Fahrlander C, Brunekreef B, et al. Allergic diseases and atopic sensitization in children related to farming and anthroposophic lifestyle--the PARSIFAL study. *Allergy.* Apr 2006;61(4):414-421.

97. Downs SH, Marks GB, Mitakakis TZ, Leuppi JD, Car NG, Peat JK. Having lived on a farm and protection against allergic diseases in Australia. *Clin Exp Allergy.* Apr 2001;31(4):570-575.

98. Ernst P, Cormier Y. Relative scarcity of asthma and atopy among rural adolescents raised on a farm. *Am J Respir Crit Care Med.* May 2000;161(5):1563-1566.

99. Chu LM, Rennie DC, Cockcroft DW, et al. Prevalence and determinants of atopy and allergic diseases among school-age children in rural Saskatchewan, Canada. *Ann Allergy Asthma Immunol.* Oct 2014;113(4):430-439.

100. Perkin MR, Strachan DP. Which aspects of the farming lifestyle explain the inverse association with childhood allergy? *J Allergy Clin Immunol.* Jun 2006;117(6):1374-1381.

101. Remes ST, Koskela HO, Iivanainen K, Pekkanen J. Allergen-specific sensitization in asthma and allergic diseases in children: the study on farmers' and non-farmers' children. *Clin Exp Allergy.* Feb 2005;35(2):160-166.

102. Merchant JA, Naleway AL, Svendsen ER, et al. Asthma and farm exposures in a cohort of rural Iowa children. *Environ Health Perspect.* Mar 2005;113(3):350-356.

103. Remes ST, Iivanainen K, Koskela H, Pekkanen J. Which factors explain the lower prevalence of atopy amongst farmers' children? *Clin Exp Allergy.* Apr 2003;33(4):427-434.

104. Horak F, Jr., Studnicka M, Gartner C, et al. Parental farming protects children against atopy: longitudinal evidence involving skin prick tests. *Clin Exp Allergy.* Aug 2002;32(8):1155-1159.

105. Wickens K, Lane JM, Fitzharris P, et al. Farm residence and exposures and the risk of allergic diseases in New Zealand children. *Allergy.* Dec 2002;57(12):1171-1179.

106. Klintberg B, Berglund N, Lilja G, Wickman M, van Hage-Hamsten M. Fewer allergic respiratory disorders among farmers' children in a closed birth cohort from Sweden. *Eur Respir J.* Jun 2001;17(6):1151-1157.

107. Riedler J, Eder W, Oberfeld G, Schreuer M. Austrian children living on a farm have less hay fever, asthma and allergic sensitization. *Clin Exp Allergy.* Feb 2000;30(2):194-200.

108. Midodzi WK, Rowe BH, Majaesic CM, Senthilselvan A. Reduced risk of physician-diagnosed asthma among children dwelling in a farming environment. *Respirology.* 2007;12(5):692-699.

109. TEAM S. Prevalence of hay fever and allergic sensitization in farmer's children and their peers living in the same rural community. *Clinical & Experimental Allergy.* 1999;29(1):28-34.

110. Barry RJ, Pickett W, Rennie DC, et al. The role of farm operational and rural environments as potential risk factors for pediatric asthma in rural Saskatchewan. *Pediatric pulmonology.* 2014;49(9):842-851.

111. Farthing P, Rennie D, Pahwa P, Janzen B, Dosman J. The association between farming activities and respiratory health in rural school age children. *Journal of agromedicine.* 2009;14(2):256-262.

112. Wells AD, Poole JA, Romberger DJ. Influence of farming exposure on the development of asthma and asthma-like symptoms. *International immunopharmacology.* 2014;23(1):356-363.

113. Moncayo AL, Vaca M, Oviedo G, et al. Risk factors for atopic and non-atopic asthma in a rural area of Ecuador. *Thorax.* 2010;65(5):409-416.

114. García-Marcos L, Castro-Rodríguez JA, Suarez-Varela MM, et al. A different pattern of risk factors for atopic and non-atopic wheezing in 9–12-year-old children. *Pediatric allergy and immunology.* 2005;16(6):471-477.

115. Omland Ø, Hjort C, Pedersen OF, Miller MR, Sigsgaard T. New-onset asthma and the effect of environment and occupation among farming and nonfarming rural subjects. *J Allergy Clin Immunol.* Oct 2011;128(4):761-765.

116. Parsons MA, Beach J, Senthilselvan A. Association of living in a farming environment with asthma incidence in Canadian children. *Journal of Asthma.* 2017;54(3):239-249.

117. Ege MJ, Frei R, Bieli C, et al. Not all farming environments protect against the development of asthma and wheeze in children. *J Allergy Clin Immunol.* May 2007;119(5):1140-1147.

118. Alfvén T, Braun-Fahrländer C, Brunekreef Bv, et al. Allergic diseases and atopic sensitization in children related to farming and anthroposophic lifestyle–the PARSIFAL study. *Allergy.* 2006;61(4):414-421.

119. Perkin MR, Strachan DP. Which aspects of the farming lifestyle explain the inverse association with childhood allergy? *Journal of Allergy and Clinical Immunology.* Jun 2006;117(6):1374-1381.

120. Adler A, Tager I, Quintero DR. Decreased prevalence of asthma among farm-reared children compared with those who are rural but not farm-reared. *Journal of allergy and clinical immunology.* 2005;115(1):67-73.

121. Merchant JA, Naleway AL, Svendsen ER, et al. Asthma and farm exposures in a cohort of rural Iowa children. *Environmental health perspectives.* 2005;113(3):350-356.

122. Ehrenstein V, Mutius V, Kries V. Reduced risk of hay fever and asthma among children of farmers. *Clinical & Experimental Allergy.* 2000;30(2):187-193.

123. Riedler J, Braun-Fahrländer C, Eder W, et al. Exposure to farming in early life and development of asthma and allergy: a cross-sectional survey. *The Lancet.* 2001;358(9288):1129-1133.

124. Riedler J, Krankenha KSs. Riedler, J., Eder, W., Oberfeld, G. & Schreuer, M. Austrian children living on a farm have less hay fever, asthma and allergic sensitization. Clin. Exp. Allergy 30, 194-200. *Clinical and experimental allergy.* 2000;30:194-200.

125. Ege MJ, Frei R, Bieli C, et al. Not all farming environments protect against the development of asthma and wheeze in children. *Journal of Allergy and Clinical Immunology.* 2007;119(5):1140-1147.

126. Pickett W, King N, Marlenga B, et al. Exposure to agricultural hazards among children who visit farms. *Paediatrics & child health.* 2018;23(7):e143-e149.

127. Farthing P, Rennie D, Pahwa P, Janzen B, Dosman J. The association between farming activities and respiratory health in rural school age children. *J Agromedicine.* 2009;14(2):256-262.

128. Dimich-Ward H, Chow Y, Chung J, Trask C. Contact with livestock--a protective effect against allergies and asthma? *Clin Exp Allergy.* Sep 2006;36(9):1122-1129.

129. Ege M, Frei R, Bieli C, et al. Not all farming environments protect against the development of asthma and wheeze in children. *J Allergy Clin Immunol.* 2007;119:1140 - 1147.

130. Chrischilles E, Ahrens R, Kuehl A, et al. Asthma prevalence and morbidity among rural Iowa schoolchildren. *J Allergy Clin Immunol.* 2004;113:66 - 71.

131. Perkin MR. Unpasteurized milk: health or hazard? *Clin Exp Allergy.* May 2007;37(5):627-630.

132. Peters M, Kauth M, Scherner O, et al. Arabinogalactan isolated from cowshed dust extract protects mice from allergic airway inflammation and sensitization. *Journal of Allergy and Clinical Immunology.* 2010;126(3):648-656. e644.

133. Seedorf J, Hartung J, Schröder M, et al. Concentrations and emissions of airborne endotoxins and microorganisms in livestock buildings in Northern Europe. *Journal of Agricultural Engineering Research.* 1998;70(1):97-109.

134. von Mutius E, Vercelli D. Farm living: effects on childhood asthma and allergy. *Nat Rev Immunol.* 12//print 2010;10(12):861-868.

135. Heederik D, von Mutius E. Does diversity of environmental microbial exposure matter for the occurrence of allergy and asthma? *J Allergy Clin Immunol.* Jul 2012;130(1):44-50.

136. von Mutius E. The microbial environment and its influence on asthma prevention in early life. *J Allergy Clin Immunol.* Mar 2016;137(3):680-689.

137. Bisgaard H, Hermansen MN, Buchvald F, et al. Childhood asthma after bacterial colonization of the airway in neonates. *New England Journal of Medicine.* 2007;357(15):1487-1495.

138. Jackson DJ, Evans MD, Gangnon RE, et al. Evidence for a causal relationship between allergic sensitization and rhinovirus wheezing in early life. *American journal of respiratory and critical care medicine.* 2012;185(3):281-285.

139. Rosas-Salazar C, Hartert TV. Prenatal exposures and the development of childhood wheezing illnesses. *Current Opinion in Allergy and Clinical Immunology.* 2017;17(2):110-115.

140. van Strien RT, Engel R, Holst O, et al. Microbial exposure of rural school children, as assessed by levels of N-acetyl-muramic acid in mattress dust, and its association with respiratory health. *J Allergy Clin Immunol.* May 2004;113(5):860-867.

141. Sordillo JE, Hoffman EB, Celedon JC, Litonjua AA, Milton DK, Gold DR. Multiple microbial exposures in the home may protect against asthma or allergy in childhood. *Clin Exp Allergy.* Jun 2010;40(6):902-910.

142. Antova T, Pattenden S, Brunekreef B, et al. Exposure to indoor mould and children's respiratory health in the PATY study. *Journal of Epidemiology and Community Health.* 2008;62(8):708-714.

143. Muller A, Lehmann I, Seiffart A, et al. Increased incidence of allergic sensitisation and respiratory diseases due to mould exposure: results of the Leipzig Allergy Risk children Study (LARS). *Int J Hyg Environ Health.* Feb 2002;204(5-6):363-365.

144. Schafer T, Kramer U, Dockery D, Vieluf D, Behrendt H, Ring J. What makes a child allergic? Analysis of risk factors for allergic sensitization in preschool children from East and West Germany. *Allergy Asthma Proc.* Jan-Feb 1999;20(1):23-27.

145. Karvonen A, Hyvärinen A, Gehring U, et al. Exposure to microbial agents in house dust and wheezing, atopic dermatitis and atopic sensitization in early childhood: a birth cohort study in rural areas. *Clinical & Experimental Allergy.* 2012;42(8):1246-1256.

146. Sordillo JE, Hoffman EB, Celedón JC, Litonjua AA, Milton DK, Gold DR. Multiple microbial exposures in the home may protect against asthma or allergy in childhood. *Clinical & Experimental Allergy.* 2010;40(6):902-910.

147. Celedón JC, Milton DK, Ramsey CD, et al. Exposure to dust mite allergen and endotoxin in early life and asthma and atopy in childhood. *Journal of allergy and clinical immunology.* 2007;120(1):144-149.

148. Gehring U, Heinrich J, Hoek G, et al. Bacteria and mould components in house dust and children's allergic sensitisation. *European Respiratory Journal.* 2007;29(6):1144-1153.

149. Jovanovic S, Felder-Kennel A, Gabrio T, et al. Indoor fungi levels in homes of children with and without allergy history. *International journal of hygiene and environmental health.* 2004;207(4):369-378.

150. Jacob B, Ritz B, Gehring U, et al. Indoor exposure to molds and allergic sensitization. *Environmental Health Perspectives.* 2002;110(7):647.

151. Maheswaran D, Zeng Y, Chan-Yeung M, et al. Exposure to Beta-(1, 3)-D-glucan in house dust at age 7–10 is associated with airway hyperresponsiveness and atopic asthma by age 11–14. *PLoS One.* 2014;9(6).

152. Lawson JA, Dosman JA, Rennie DC, et al. Endotoxin as a determinant of asthma and wheeze among rural dwelling children and adolescents: a case–control study. *BMC pulmonary medicine.* 2012;12(1):56.

153. Rennie DC, Lawson JA, Kirychuk SP, et al. Assessment of endotoxin levels in the home and current asthma and wheeze in school-age children. *Indoor Air.* Dec 2008;18(6):447-453.

154. Braun-Fahrländer C, Riedler J, Herz U, et al. Environmental exposure to endotoxin and its relation to asthma in school-age children. *N Engl J Med.* Sep 19 2002;347(12):869-877.

155. El-Sharif N, Douwes J, Hoet P, Nemery B. Childhood asthma and indoor aeroallergens and endotoxin in Palestine: a case-control study. *J Asthma.* Apr 2006;43(3):241-247.

156. Oluwole O, Rennie DC, Senthilselvan A, et al. The association between endotoxin and beta-(1→ 3)-D-glucan in house dust with asthma severity among schoolchildren. *Respiratory medicine.* 2018;138:38-46.

157. Mendy A, Metwali N, Perry SS, Chrischilles EA, Wang K, Thorne PS. Household endotoxin reduction in the Louisa Environmental Intervention Project for rural childhood asthma. *Indoor air.* 2020;30(1):88-97.

158. Blatter J, Forno E, Brehm J, et al. Fungal exposure, atopy, and asthma exacerbations in Puerto Rican children. *Annals of the American Thoracic Society.* 2014;11(6):925-932.

159. Jacobs JH, Krop EJ, de Wind S, Spithoven J, Heederik DJ. Endotoxin levels in homes and classrooms of Dutch school children and respiratory health. *European Respiratory Journal.* 2013;42(2):314-322.

160. Gehring U, Strikwold M, Schram-Bijkerk D, et al. Asthma and allergic symptoms in relation to house dust endotoxin: Phase Two of the International Study on Asthma and Allergies in Childhood (ISAAC II). *Clinical & Experimental Allergy.* 2008;38(12):1911-1920.

161. Ege MJ, Frei R, Bieli C, et al. Not all farming environments protect against the development of asthma and wheeze in children. *J Allergy Clin Immunol.* May 2007;119(5):1140-1147.

162. Tavernier G, Fletcher G, Francis H, et al. Endotoxin exposure in asthmatic children and matched healthy controls: results of IPEADAM study. *Indoor air.* 2005;15:25-32.

163. Frank T, Frank P, McNamee R, et al. Assessment of a simple scoring system applied to a screening questionnaire of asthma in children aged 5-15 yrs. *European Respiratory Journal.* 1999;14(5):1190-1197.

164. Braun-Fahrländer C, Riedler J, Herz U, et al. Environmental exposure to endotoxin and its relation to asthma in school-age children. *New England Journal of Medicine.* 2002;347(12):869-877.

165. Farokhi A, Heederik D, Smit LA. Respiratory health effects of exposure to low levels of airborne endotoxin–a systematic review. *Environmental Health.* 2018;17(1):14.

166. Barnig C, Reboux G, Roussel S, et al. Indoor dust and air concentrations of endotoxin in urban and rural environments. *Letters in applied microbiology.* 2013;56(3):161-167.

167. Hyvärinen A, Roponen M, Tiittanen P, Laitinen S, Nevalainen A, Pekkanen J. Dust sampling methods for endotoxin–an essential, but underestimated issue. *Indoor air.* 2006;16(1):20-27.

168. Waser M, Schierl R, Maisch S, et al. Determinants of endotoxin levels in living environments of farmers' children and their peers from rural areas. *Clinical & Experimental Allergy.* 2004;34(3):389-397.

169. Gereda JE, Leung DY, Liu AH. Levels of environmental endotoxin and prevalence of atopic disease. *Jama.* 2000;284(13):1652-1653.

170. von Mutius E, Braun-Fahrlander C, Schierl R, et al. Exposure to endotoxin or other bacterial components might protect against the development of atopy. *Clin Exp Allergy.* Sep 2000;30(9):1230-1234.

171. Morcos M, Morcos W, Ibrahim M, Shaheen M. Environmental exposure to endotoxin in rural and urban Egyptian school children and its relation to asthma and atopy. *Minerva pediatrica.* 2011;63(1):19-26.

172. Braun-Fahrlander C, Riedler J, Herz U, et al. Environmental exposure to endotoxin and its relation to asthma in school-age children. *N Engl J Med.* 2002;347:869-877.

173. Celedon JC, Milton D, Ramsey CD, et al. Exposure to dust mite allergen and endotoxin in early life and asthma and atopy in childhood. *J Allergy Clin Immunol.* 2007;120:144-149.

174. Ege MJ, Frei R, Bieli C, et al. Not all farming environments protect against the development of asthma and wheeze in children. *J Allergy Clin Immunol.* 2007;119:1140-1147.

175. El-Sharif N, Douwes J, Hoet P, Nemery B. Childhood asthma and indoor aeroallergens and endotoxin in Palestine: A case-control study. *Journal of Asthma.* 2006;43:241-247.

176. Lawson JA, Dosman JA, Rennie DC, et al. Endotoxin as a determinant of asthma and wheeze among rural dwelling children and adolescents: A case-control study. *BMC Pulmonary Medicine.* 2012;12:56 (10 pages).

177. Litonjua AA, Milton DK, Celedon JC, Ryan L, Weiss ST, Gold DR. A longitudinal analysis of wheezing in young children: the independent effects of early life exposure to house dust endotoxin, allergens and pets. *J Allergy Clin Immunol.* 2002;110:736-742.

178. Rennie DC, Lawson JA, Kirychuk S, et al. Assessment of endotoxin levels in the home and current asthma and wheeze in school-age children. *Indoor Air.* 2008;18:447-453.

179. Tavernier GOG, Fletcher GD, Francis HC, et al. Endotoxin exposure in asthmatic children and matched healthy controls: results of IPEADAM study. *Indoor Air.* 2005;15 (Suppl 10):25-32.

180. Rabinovitch N, Liu AH, Zhang L, et al. Importance of the personal endotoxin cloud in school-age children with asthma. *J Allergy Clin Immunol.* 2005;116:1053-1057.

181. Yen Y-C, Yang C-Y, Ho C-K, et al. Indoor ozone and particulate matter modify the association between airborne endotoxin and schoolchildren's lung function. *Science of The Total Environment.* 2020;705:135810.

182. Delfino RJ, Staimer N, Tjoa T. Personal endotoxin exposure in a panel study of school children with asthma. *Environ Health.* 2011;10:69.

183. Park J-H, Spiegelman DL, Gold DR, Burge HA, Milton DK. Predictors of airborne endotoxin in the home. *Environmental health perspectives.* 2001;109(8):859.

184. Park JH, Spiegelman DL, Gold DR, Burge HA, Milton DK. Predictors of airborne endotoxin in the home. *Environ Health Perspect.* Aug 2001;109(8):859-864.

185. Horick N, Weller E, Milton DK, Gold DR, Li R, Spiegelman D. Home endotoxin exposure and wheeze in infants: correction for bias due to exposure measurement error. *Environ Health Perspect.* Jan 2006;114(1):135-140.

186. Tovey ER, Liu-Brennan D, Garden FL, Oliver BG, Perzanowski MS, Marks GB. Time-based measurement of personal mite allergen bioaerosol exposure over 24 hour periods. *PLoS One.* 2016;11(5):e0153414.

187. Raja S, Xu Y, Ferro AR, Jaques PA, Hopke PK. Resuspension of indoor aeroallergens and relationship to lung inflammation in asthmatic children. *Environment international.* 2010;36(1):8-14.

188. Karlsson A-S, Andersson B, Renström A, Svedmyr J, Larsson K, Borres MP. Airborne cat allergen reduction in classrooms that use special school clothing or ban pet ownership. *Journal of allergy and clinical immunology.* 2004;113(6):1172-1177.

189. Hollander A, Van Run P, Spithoven J, Heederik D, Doekes G. Exposure of laboratory animal workers to airborne rat and mouse urinary allergens. *Clinical & Experimental Allergy.* 1997;27(6):617-626.

190. Gordon S, Tee R, Nieuwenhuijsen M, Lowson D, Harris J, Taylor AN. Measurement of airborne rat urinary allergen in an epidemiological study. *Clinical & Experimental Allergy.* 1994;24(11):1070-1077.

191. Tarlo SM, Sussman G, Contala A, Swanson MC. Control of airborne latex by use of powder-free latex gloves. *Journal of Allergy and Clinical Immunology*. 1994;93(6):985-989.

192. Houba R, Heederik D, Kromhout H. Grouping strategies for exposure to inhalable dust, wheat allergens and α-amylase allergens in bakeries. *The Annals of occupational hygiene.* 1997;41(3):287-296.

CHAPTER III

ATOPY RISK AMONG SCHOOL-AGED CHILDREN IN RELATION TO EARLY EXPOSURES TO A FARM ENVIRONMENT: A SYSTEMATIC REVIEW (MANUSCRIPT 1)

Authors: Luan M. Chu, MSc[1,2]; Donna C. Rennie, PhD[2]; Shelley Kirychuk, PhD[2,3]; Don Cockcroft, MD[4];John Gordon, PhD[4]; Joshua A. Lawson, PhD[2,4]

Affiliations: [1]Health Sciences Graduate Program, College of Medicine, University of Saskatchewan, Saskatoon, Saskatchewan, Canada

[2]Canadian Center for Health and Safety in Agriculture, University of Saskatchewan, Saskatoon, Saskatchewan, Canada

[3]College of Nursing, University of Saskatchewan, Saskatoon, Saskatchewan, Canada

[4]Department of Medicine, College of Medicine, University of Saskatchewan, Saskatoon, Saskatchewan, Canada

Corresponding author:

Luan Manh Chu, M.Sc, Ph.D. Candidate, Canadian Center for Health and Safety in Agriculture, University of Saskatchewan, Saskatoon, Saskatchewan, Canada, 104 Clinic Place PO Box 23, Saskatoon, SK, S7N 5E5, Canada; E-mail: cml779@mail.usask.ca

Reprinted from *Respir. Med.*, Chu LM, Rennie DC, Kirychuk S, et al. Atopy risk among school-aged children in relation to early exposures to a farm environment: A systematic review, 2021, 106378, ISSN 0954-6111, https://doi.org/10.1016/j.rmed.2021.106378 with permission from Elsevier. In Press.

Authors' contributions: Mr. Chu conceptualized and designed the study, conducted data search and management, interpreted the data, and prepared and revised the manuscript; Dr. Joshua A. Lawson, Mr. Chu's PhD supervisor, contributed to the study concept, design, data search and management and interpretation, reviewed and revised the manuscript; Drs. Donna C. Rennie, Donald Cockcroft, John Gordon, Shelley Kirychuk contributed to the study methodology, results interpretation, reviewed and revised the manuscript.

Conflict of interest: The authors have no conflict of interest related to this study to disclose.

Financial support: This research has been supported by a personal scholarship from the Public Health and the Agricultural Rural Ecosystem (PHARE) program, the Saskatchewan Innovation and Opportunity Scholarship (SIOS), the Founding Chairs Fellowship at the Canadian Center for Health and Safety in Agriculture (CCHSA); and the College of Medicine Devolved Scholarship at the University of Saskatchewan.

3.1.Abstract

Background and Objectives: Childhood atopy is a complex condition with both a genetic and an environmental component. This systematic review explored the current understanding of the importance of early life exposures to a farm in the development of atopy measured by objective markers of skin prick testing, and specific IgE measurements in school age children.

Methods: A systematic review was performed.

Results: Among 7285 references identified, 14 studies met the inclusion criteria (13 cross-sectional studies and 1 case-control study). The results were fairly consistent in that early farm-related exposures can protect children from becoming atopic at school age. In general, there was heterogeneity in the assessment of outcomes and exposures.

Conclusions: Early-life farm exposures are associated with a protective effect on childhood atopy as assessed by objective markers. Future work should focus on understanding specific farm exposures that may important in these associations between atopy and farm exposures in children.

Key words: farm exposure, atopic sensitization, atopy, childhood, early life, systematic review

3.2.Introduction

Atopy, the tendency to produce an exaggerated immunoglobulin E (IgE) immune response to a common allergen, usually measured by serum IgE and/or skin prick tests, and atopic diseases (atopic dermatitis, asthma, and hay fever) have become major public health issues, particularly in affluent societies,[1] including Canada. Atopic diseases diminish a patient's quality of life, have considerable socio-economic costs, cause patient morbidity, and lead to high healthcare utilization.[2-5] Atopic individuals are at increased risk of developing symptoms of asthma, atopic dermatitis, and atopic rhinoconjunctivitis.[6,7]Atopy and atopic diseases have become a major public health issue, particularly in affluent societies,[1] including Canada. Atopic diseases diminish a patient's quality of life, have considerable socio-economic costs, cause patient morbidity, and lead to high healthcare utilization.[2-5] Atopic individuals are at increased risk of developing symptoms of asthma, atopic dermatitis, and atopic rhinoconjunctivitis.[6,7]

The prevalence of atopy and atopic diseases has risen in recent decades in many nations around the world, predominantly in countries with a westernized lifestyle.[1,8] Atopy is likely caused by interactions between genetic and environmental factors. Genetic factors are clearly important, but cannot independently explain the increased prevalence of atopic diseases in recent decades.[9,10] Recent evidence links multiple changes in modern dietary intakes[11,12], lifestyle factors[13,14] and surrounding environments[15,16] to adverse influences on the developing immune system and increasing prevalence of early childhood inflammatory disease including atopic disease.[17]

Atopic related diseases, which can include atopic dermatitis, atopic rhinitis, allergies and asthma, are some of the most common chronic childhood diseases. The clinical definition of atopy is an

increased *Immunoglobulin E* (*IgE*) antibody response to an allergen and confirmation of sensitization with positive skin prick test (SPT) or presence of allergen-specific IgE antibodies identified by blood tests.[18] Atopy is characterized by an imbalance in the production of T-helper 1 (Th1) and T-helper 2 (Th2) cytokines through a Th-2 polarization and related IgE-mediated reactions[19] . Typically, individuals with atopy have excess production of IgE and Th2 cytokines.[20]

Early childhood, including the prenatal period, has been highlighted as a period of great risk for developing atopy. A growing body of evidence indicates that atopy and atopic disease has fetal origins and the fetal time period is a critical window of immune system development.[21,22] Earlier studies report immune dysregulation at birth suggesting that prenatal exposures may influence the programming of the neonatal immune response.[23] It is possible that maternal exposure may affect the developing immune response through direct exposure of the fetus to antigens that cross the placental barrier.[24,25] Alternatively, maternal response to an environmental exposure may influence the *in-utero* environment for the developing fetus.[26] Maternal sensitization to allergens has been shown to be associated with an elevated production of the Th2-relate cytokine interleukin (IL)-13 in the infant.[27] Fetal programming may occur through imprinting of the infant gut microbiota[28,29] or epigenetic regulation of immune function that begins in utero.[30] Recent reports indicate that the gut microbiota composition during the first months of life influences atopy development.[31,32] It has been suggested that factors influencing the early maturation of the immune system play an important role in the presence of atopic disease.[33]

The farming environment is rich in microbes. It has been suggested that this microbial exposure may be protective against atopy in children and partially explain the observed lower prevalence of

atopy and asthma in rural areas. Animal sheds, especially cow sheds, are found to contain a large variety of bacteria, fungi and their compounds[34-36]. Because of this high level of exposure, a farming environment offers an opportunity to evaluate the importance of microbial exposures to maturation of the immune system and children's respiratory health and atopy.[37,38] Research has shown that early-life microbial exposure may be important in reducing the risk of atopy and hay fever in farm children.[39] It has been suggested that during a critical inception phase of lung development within the first 3 years of life, children are more likely to develop asthma.[40,41] Prenatal factors related to placental function including exposures to respiratory viruses, bacteria, and allergens have been suggested to play a role.[41,42]

While there have been reviews looking at the association between farm exposure and asthma[43] or atopy[44,45], none have looked specifically at the relationship between early life farm exposures (pregnancy and the first year of life) with atopy/atopic sensitization measured by objective markers (skin prick testing, and serum specific IgE measurements) in children at school age (6-12 years old). The aim of this systematic review was to determine if farm-related exposures in early life protect children at school age from atopy as measured by objective markers.

3.3.Methods

This review was conducted following the Meta-analysis of Observational Studies in Epidemiology (MOOSE) guideline for meta-analysis of observational studies.[46] Despite the guideline also describing details specific to meta-analysis, we only applied criteria applicable to a systematic review from these guidelines because we did not conduct any meta-analysis techniques in this paper.

A systematic search was performed using search engines for medical literature including Medline through the PubMed interface, Embase, the Web of Science and the reference lists from studies. The literature was searched with the free text search terms such as the following query string: *child** or *childhood* or *early life* or *postnatal* or *prenatal* or *antenatal* or *neonatal* or *pregnant* or *pregnancy* or *in utero* or *infan** or *maternal exposure* or *newborn) AND (farm** or *agricultur** or *diary* or *grain* or *livestock* or *endotoxin* or *beta-glucan* or *lipopolysaccharide* or *mold) AND (atop** or *Skin prick test** or *Immunoglobulin E* or *IgE* or *atopic sensitization* or *hypersensitivity* or *allerg*).*

Electronic and manual searching was carried out in November 2019. The search was restricted to English language articles. Lists of studies identified from searching electronic databases were combined and duplicate abstracts removed. Papers were screened for relevance to the review based on the information contained in the title and abstract. Non-human studies were excluded based on the abstracts and not counted as search hits. Reference lists of all publications containing relevant content including reviews, editorials, commentaries, or letters were compared with the already retrieved hits. A publication was selected for further evaluation if it met inclusion criterion (below).

Inclusion/exclusion criteria

All retrieved abstracts were read, and a given publication was deemed relevant if (A) they captured exposures to a farm environment in early life (prenatal, and or the first year of life); (B) outcomes included objective markers of atopic sensitization defined in one of two ways: a positive skin prick test reaction to at least one specific allergen; or elevated specific IgE to at least one specific allergen and outcomes were measured in school-age children (C) the study design is either cross-sectional, case-control, or cohort; (D) the selected articles were peer-reviewed full-text; and (E) the publication date was after January 1, 2000. Publications that described only adult or occupational exposures to a farm were excluded. Original articles that did not describe data on atopy measured by objective markers in relation to exposures to a farm environment in early life were also excluded. Searches were restricted to all infant and non-food atopy report. Two reviewers (LC&JL) compared the search findings and discussed article inclusion.

Data extractions and assessment of quality

Data regarding study design, characteristics of the study population, measurements of exposures and outcome(s) together with 95% confidence intervals (95% CI) as well as the type of point estimate (e.g. odds ratio [OR])were extracted and presented in a table format. Quality assessment of included papers was carried out using the "Effective public health practice project (EPHPP) quality assessment tool for quantitative studies".[47] The EPHPP tool previously had the validity and inter-rater reliability evaluated.[48,49] The inter-rater reliability of the individual domains of the EPHPP tool is considered fair agreement among raters (the intra-class correlation coefficient (ICC)= 0.60).[48,49] This tool was used in a previous systematic review related to asthma and environmental exposures.[50] The quality assessment score used in this evaluation tool included:

selection bias, study design, confounders, blinding, data collection methods, withdrawals and drop-outs. We assessed validity based on the original tool as well as in a modified version. In the modified version, we did not include the "blinding" criteria due to the nature of included articles (observational where blinding is typically not mentioned). A global rating for each paper was then scored and categorized into one of the three categories (strong, moderate, or weak). Two of the authors (LC & JL) reviewed the articles for validity assessment then came to consensus when there were discrepancies. The quality of the included articles was based on the consensus decision and had a global rating score applied. An article was considered "strong" if there was no "weak" rating, while a "moderate" article had one "weak" rating, and a "weak" article had two or more "weak" ratings.

As part of our analysis, we also summarized the results of the studies based on sensitivity analyses around the methods used to assess atopy (Skin test vs. IgE measurements), study designs, and timing of exposures (during pregnancy vs. the first year of life).

Publication bias was evaluated by visualization of funnel plots.

3.4.Results

There were 7285 references identified from electronic databases. Initial screening produced 21 potentially relevant references and 14 studies met the inclusion criteria after further screening of full-text articles. Search results are summarized in **Figure 3-1** based on the Quality of Reporting of Meta-analyses QUORUM flow diagram. The summary of studies is located in **Table 3-1**. There were 13 cross-sectional studies and 1 case-control study. Most studies (9 of 14) were conducted in European countries. Most of the cross-sectional studies had a large sample size (sample size ranged between 293-8334). In contrast, the only case-control study had considerably fewer study subjects (n=103).

In general, there was heterogeneity in the assessment of outcomes and exposures of interest. Out of 14 studies, nine based atopy on skin testing; four used blood tests with specific IgE measurements; and one used both methods (**Table 3-1**). The choice of specific allergen extracts for skin prick testing varied widely between studies and was completed for a range of 6-15 allergens. A positive test reaction was defined similarly across SPT studies (the cut-off of ≥3 mm wheal). The threshold levels used to define a positive response using specific-IgE levels, however, were different between studies. These cut-offs included 0.35 kU/L, 0.7 kU/L or 3.5 kU/L (**Table 3-1**).

As shown in **Table 3-1**, the strengths of associations between early-life farm exposures and childhood atopy varied between studies. **Figure 3-2** shows the strengths of associations among the included articles. In **Figure 3-2**, 9 out of 13 studies (1 study did not report any OR) showed statistically significant protective effects, while 3 showed a non-statistically significant protective effect, and 1 study showed a non-statistically significant risk effect.

There was variation in definitions of type and timing of exposure [e.g. exposures to stables in the first year of life, farming in infancy, main line of production in infancy (dairy, livestock, plant, etc.), maternal exposure to stables during pregnancy, regular contact with farm animals ever, the child growing up on a farm, farmer's children identified by initial survey, early farm animal exposure, barn or stable exposure, parental farming at less than first year of age].

Of the 14 studies included, 13 were cross-sectional in design. Among these, ten studies showed a statistically significant protective effect, one showed mixed results, one showed a non- statistically significant protective effect, and one did not report the strength of association (e.g. OR). Overall, the strength of associations ranged from 0.18 to 1.3. There were three articles from a large multi-nations project (the Multidisciplinary Study to Identify the Genetic and Environmental Causes of Asthma in the European Community – the GABRIEL and GABRIEL Advanced surveys).[51-53]

Only one case-control study was found. Martikainen et al[54] (2015) conducted of French and Finnish children, including 65 children with asthma (26 farming and 39 non-farming) and 103 children without asthma (56 farming and 47 non-farming). The authors found no statistically significant association between early life exposures (e.g. staying in stable or barn) and the outcome of atopy-measured by specific IgE levels- at school age (at age 6).

SPT vs. IgE measurement methods

The use of specific IgE measurements showed more consistent results compared to the skin prick test method **(Figure 3-3)**. Five out of five specific IgE-based studies showed a protective effect of early life farm exposures and atopy at school age. Among nine SPT-based studies, 1 showed a

non-statistically significant increased risk, 6 showed statistically significant protective effects and 2 studies found no associations with early farm exposures.

Exposure timing (pregnancy vs first years) between farm-related exposures and atopy

The type and timing of exposure were not similar between studies. Some studies considered "early-life" farm exposures as parental farming in infancy or having been born and raised on a farm.[55,56] Others considered "early-life" farm exposures as exposure during the first years.[57,58]

Validity assessment

Using the original evaluation tool (with the "blinding' criteria) all of the included studies were classified as "weak". This was primarily due to the cross-sectional study design and not having blinding. Only two studies mentioned the act of blinding. Using the modified evaluation tool (without the "blinding' criterion), most (10 out of 14) of the included studies were in a "moderate" level, while 3 out of 14 studies were categorized in "weak" level, and one study was in the "strong" level.

For the specific validity criteria, there was some variability. For "Selection bias", 1 study was considered "weak", while 8 were considered "moderate" and 5 were considered "strong". For "Study design", 13 studies were considered "weak", while 1 was considered "moderate" and 0 was considered "strong". For "Confounders", 1 study was considered "weak", while 2 were considered "moderate" and 11 were considered "strong". For "Data Collection Methods", 13 studies were considered "strong", while 1 was considered "moderate', and 0 was considered "weak". For "Withdrawals and Drop-outs", 4 studies were considered "strong", while 8 were considered "moderate", and 2 were considered "weak".

Publication bias assessment

Figure 3-4 & 3-5 show the funnel plots for articles included. **Figure 3-4** depicted the odds ratios in relation to sample sizes of included articles with main exposures, while **Figure 3-5** depicted all estimates (Odds ratios) related to farm exposures and other related exposures mentioned in the included articles. Visually, both figures showed only minor asymmetry, suggesting a lower risk of publication bias.

3.5.Discussion

The aim of this systematic review was to determine if farm-related exposures in early life protect children at school age from atopy as measured by objective markers. Fourteen studies were selected in this systematic review. The results are fairly consistent in that early farm-related exposures can protect school-age children from being atopic.

A similarly focused review was completed by Campbell et al[44] although there were some notable differences. Campbell et al conducted a systematic review and meta-analysis of the research investigating associations between farm exposure and objective measures of atopy, that is serum IgE or skin prick tests results, at any age, including into adulthood while our current systematic review focuses explicitly on the farm exposures during the first year of life and a specific outcome which is atopy presence at school-age measured by serum IgE and/or skin prick testing. Campell et al concluded that exposure to farming in early childhood was associated with a protective effect on objective markers of atopy, while inconsistent findings were observed in adults with early life exposures. Our results extended that work by showing that early life exposures to a farm protect children from atopy at school-age specifically.

The microbial environment of the farm with high exposures to gram positive bacteria and gram negative bacteria during pregnancy and early childhood has been shown to be associated with a protection against atopy.[56,59-61] Of the farm-related factors, early-life and consistent contact to stables and the consumption of unpasteurized milk have been most strongly associated with decreased risk of IgE-sensitization.[62] In addition, prenatal exposure to animal sheds and hay has been reported to be inversely associated with cord blood IgE levels against seasonal allergens.[59] A variety of microbial products such as endotoxin, muramic acid, and [beta]-glucans[63-65] may play a

major role in protecting from asthma and atopy. Endotoxin levels are found to be higher in rural areas and a farming environment compared to the urban areas.[66-68] Maternal contact with farm animal species and barns and consumption of farm-produced butter during pregnancy enhanced the production of pro-inflammatory cord blood cytokines.[69] Most notably, maternal exposure to a higher number of farm animal species substantially enhanced the expression of the T-regulatory cell marker glucocorticoid-induced tumor necrosis factor (*TNF*) receptor and the secretion of interferon-gamma (IFN-γ) by cord blood cells in response to atogen and peptidoglycan,[70] which is associated with the protection against atopy in children.

There is an ongoing debate about whether atopic sensitization can occur prenatally[71-73] and whether maternal antibodies can influence cord blood IgE levels.[74] Epidemiological studies (including our findings in the current systematic review) indicate that mothers exposed to a farm environment during pregnancy protect against atopic sensitization in their children, whereas exposures during infancy alone or during the first years of life have weaker or no effect.[55,59] However, the mechanisms of prenatal atopy are complicated and not well-understood.

Recently, some studies have applied indicators of microbial exposure, such as a microbial diversity score to predict atopy and asthma and this may be a useful to describe microbial exposure[75]. In our systematic review, the definitions of farm exposures were limited to the "label" of exposures such as exposures to farm dwelling, poultry, livestock, farm milk, and stables, and so on. Future studies should look into "farm" exposures with a reliable and objective measurement to derive a better measure of what constitutes a farm exposures or how these should be grouped.

We found that the association between early life farm exposures and atopy at school-age was more consistent using the specific IgE tests compared to the skin prick testing. Blood tests detect IgE in the blood, while skin tests detect IgE response in the skin. There does not exist a so-called gold

standard to detect the presence of atopy. Both specific IgE and skin prick testing (SPT) have advantages and disadvantages. While SPT is cheap, quick, and sensitive, sIgE assays are more specific, but more expensive and results are not immediate. The comparison between the two methods has been evaluated and results have shown that SPTs are more sensitive than the IgE test, whereas the IgE test is more specific.[76,77] Moreover, the threshold to determine the presence of atopy in each method is different and can also differ between studies. While most studies use the cut-off of ≥ 3 mm wheal size using the skin prick tests, thresholds used in IgE tests may vary more often, ranging from 0.35 kU/l to 3.5 kU/l (as shown in our current systematic review. While the cut-offs and methods used are varied (either skin test or blood test), atopic status was used as a label and such a label has been commonly used to compare the outcomes of the two tests.

The majority of data included in this review were drawn from cross-sectional studies, and therefore the cause-effect relationship of the observed phenomena may be questioned. However, we looked exclusively at studies that used objective markers of atopy which can help minimize the misclassification of outcomes. Also, in our systematic review, we examined "quasi-longitudinal" exposures as the exposures in question, which were based on recall of their occurrence at early ages. This will help us limit issues around reverse-causation. Differential recall bias is not likely to occur because the outcomes (IgE levels and SPT results) were laboratory parameters. Furthermore, this systematic review is potentially influenced by submission bias (where some investigators do not submit papers that find no statistically significant associations), potentially resulting in some publication bias. However, we found that publication bias was not likely to occur based on our funnel plots.

In our current systematic review, most studies used questionnaire report to collect data on exposures. These detailed questionnaires not only assessed the type of exposure but also its time

period. In a previous study[51], when analyzing the association between timing and outcomes, the effects of exposures early in life (from pregnancy up to age 3 years) showed much stronger effects than current exposure at the time of outcome assessment.[51] This correlates with findings from other studies that observed an effect of farm exposure in pregnancy on specific IgE levels and cytokine responses in cord blood, indicating a protective farm effect as early as *in utero*.[59,69,70]

Until now, there has been no review on the association between early life farm exposures and atopy measured by objective markers such as IgE measurements and skin prick testing at school age according to systematic search criteria. We performed a reasonable and replicable systematic search using the electronic database PubMed, Embase, and other sources. However in this report we included only studies assessing the influence of early farm exposures on objective markers of atopic sensitization, and not the studies assessing a particular atopic disease (asthma, atopic eczema, hay fever) as an outcome. This approach had permitted us to assess the influence of early life farm exposures on the immune system more objectively with particular respect to the early mechanisms of atopic immune response.

One of the main limitations of the original studies' outcome definitions was the difference in the quantity and type of allergen extracts as well as cut-off used to identify positive IgE and skin prick test results. In reality, it is difficult to have complete uniformity in the use of exact procedures and panels of allergens. Therefore, cautions are needed when interpreting results as well as comparing findings between studies. Another limitation is the definition of type and timing of farm exposures. As shown in **Table 1**, early life farm exposures were considered differently in studies such as parental farming in infancy or having been born and raised on a farm[55,78] or farm-related exposures up to one year of age[58,79]. Most of the included results are generalizable within a European context because most of the studies were from Europe, many studies were population-based, and they were

conducted in a variety of countries with a diverse range of farming practices. However, not all countries or continents were represented by the included studies. Also, there were three studies in this systematic review originating from a large project: GABRIEL and GABRIEL Advanced surveys[51-53]. The generalization at a global perspective may be limited because not all countries or continents were represented by the included studies. Furthermore, the validity of articles included in this systematic review was moderate. We used an evaluation tool which previously had the validity and inter-rater reliability evaluated.[48,49] The scores for the included articles in our current systematic review were low due to the use of cross-sectional studies. Ideally, a meta-analysis would have been performed. However, it is recommended not to conduct a meta-analysis when there is considerable variation in results and inconsistency in the direction of effect, potentially misleading the overall effect of interest.[80] In our current systematic review, since there was a great deal of heterogeneity in the definitions of both exposures and outcomes, a meta-analysis was not performed.

In conclusion, we found a consistent association between early farm-related exposures and a protective effect against atopy in school age children. Future work should focus on higher quality studies that report larger samples, prospective prenatal cohort designs with clear and specific definitions of farm exposures along with increased use of objective markers for these exposures and disease outcomes. This will help investigators to isolate key factors in the prevention of atopic diseases; and better indicators of the total quantity and diversity of microbial exposures on a farm.

3.6.References

1. Asher MI, Montefort S, Bjorksten B, et al. Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC Phases One and Three repeat multicountry cross-sectional surveys. *Lancet.* Aug 26 2006;368(9537):733-743.
2. O'Connell EJ. The burden of atopy and asthma in children. *Allergy.* Aug 2004;59 Suppl 78:7-11.
3. Kim DH, Li K, Seo SJ, et al. Quality of life and disease severity are correlated in patients with atopic dermatitis. *J Korean Med Sci.* Nov 2012;27(11):1327-1332.
4. Anandan C, Gupta R, Simpson CR, Fischbacher C, Sheikh A. Epidemiology and disease burden from allergic disease in Scotland: analyses of national databases. *J R Soc Med.* Oct 2009;102(10):431-442.
5. Barbeau M, Bpharm HL. Burden of Atopic dermatitis in Canada. *Int J Dermatol.* Jan 2006;45(1):31-36.
6. Arruda LK, Solé D, Baena-Cagnani CE, Naspitz CK. Risk factors for asthma and atopy. *Current Opinion in Allergy and Clinical Immunology.* 2005;5(2):153-159.
7. Bantz SK, Zhu Z, Zheng T. The atopic march: progression from atopic dermatitis to allergic rhinitis and asthma. *Journal of clinical & cellular immunology.* 2014;5(2).
8. Schmitz R, Atzpodien K, Schlaud M. Prevalence and risk factors of atopic diseases in German children and adolescents. *Pediatr Allergy Immunol.* Dec 2012;23(8):716-723.
9. Reijmerink NE, Kerkhof M, Bottema RW, et al. Toll-like receptors and microbial exposure: gene-gene and gene-environment interaction in the development of atopy. *Eur Respir J.* 2011;38(4):833-840.

10. Ege MJ, Strachan DP, Cookson WO, et al. Gene-environment interaction for childhood asthma and exposure to farming in Central Europe. *J Allergy Clin Immunol.* Jan 2011;127(1):138-144, 144 e131-134.

11. Palmer DJ, Sullivan T, Gold MS, et al. Effect of n-3 long chain polyunsaturated fatty acid supplementation in pregnancy on infants' allergies in first year of life: randomised controlled trial. *BMJ.* 2012;344:e184.

12. Granell R, Heron J, Lewis S, Davey Smith G, Sterne JA, Henderson J. The association between mother and child MTHFR C677T polymorphisms, dietary folate intake and childhood atopy in a population-based, longitudinal birth cohort. *Clin Exp Allergy.* Feb 2008;38(2):320-328.

13. Harpsoe MC, Basit S, Bager P, et al. Maternal obesity, gestational weight gain, and risk of asthma and atopic disease in offspring: a study within the Danish National Birth Cohort. *J Allergy Clin Immunol.* Apr 2013;131(4):1033-1040.

14. Burke H, Leonardi-Bee J, Hashim A, et al. Prenatal and passive smoke exposure and incidence of asthma and wheeze: systematic review and meta-analysis. *Pediatrics.* Apr 2012;129(4):735-744.

15. Collier CH, Risnes K, Norwitz ER, Bracken MB, Illuzzi JL. Maternal infection in pregnancy and risk of asthma in offspring. *Matern Child Health J.* Dec 2013;17(10):1940-1950.

16. Wang IJ, Chen SL, Lu TP, Chuang EY, Chen PC. Prenatal smoke exposure, DNA methylation, and childhood atopic dermatitis. *Clin Exp Allergy.* May 2013;43(5):535-543.

17. Khan TK, Palmer DJ, Prescott SL. In-utero exposures and the evolving epidemiology of paediatric allergy. *Curr Opin Allergy Clin Immunol.* Oct 2015;15(5):402-408.

18. Johansson SG, Bieber T, Dahl R, et al. Revised nomenclature for allergy for global use: Report of the Nomenclature Review Committee of the World Allergy Organization, October 2003. *J Allergy Clin Immunol.* May 2004;113(5):832-836.

19. Singh SP, Gundavarapu S, Pena-Philippides JC, et al. Prenatal secondhand cigarette smoke promotes Th2 polarization and impairs goblet cell differentiation and airway mucus formation. *J Immunol.* Nov 1 2011;187(9):4542-4552.

20. Ker J, Hartert TV. The atopic march: what's the evidence? *Annals of Allergy, Asthma & Immunology.* 10// 2009;103(4):282-289.

21. Selgrade MK. Immunotoxicity: the risk is real. *Toxicol Sci.* Dec 2007;100(2):328-332.

22. Dietert RR. Developmental immunotoxicology: focus on health risks. *Chem Res Toxicol.* Jan 2009;22(1):17-23.

23. Martino D, Prescott S. Epigenetics and prenatal influences on asthma and allergic airways disease. *Chest.* Mar 2011;139(3):640-647.

24. Warner JA, Warner JO. Early life events in allergic sensitisation. *Br Med Bull.* 2000;56(4):883-893.

25. Peters JL, Suglia SF, Platts-Mills TA, Hosen J, Gold DR, Wright RJ. Relationships among prenatal aeroallergen exposure and maternal and cord blood IgE: project ACCESS. *J Allergy Clin Immunol.* May 2009;123(5):1041-1046.

26. Folsgaard NV, Chawes BL, Rasmussen MA, et al. Neonatal cytokine profile in the airway mucosal lining fluid is skewed by maternal atopy. *Am J Respir Crit Care Med.* Feb 1 2012;185(3):275-280.

27. Kopp MV, Zehle C, Pichler J, et al. Allergen-specific T cell reactivity in cord blood: the influence of maternal cytokine production. *Clin Exp Allergy.* Oct 2001;31(10):1536-1543.

28. Russell SL, Finlay BB. The impact of gut microbes in allergic diseases. *Curr Opin Gastroenterol.* Nov 2012;28(6):563-569.

29. Julia V, Macia L, Dombrowicz D. The impact of diet on asthma and allergic diseases. *Nat Rev Immunol.* May 2015;15(5):308-322.

30. de Planell-Saguer M, Lovinsky-Desir S, Miller RL. Epigenetic regulation: the interface between prenatal and early-life exposure and asthma susceptibility. *Environ Mol Mutagen.* Apr 2014;55(3):231-243.

31. Abrahamsson TR, Jakobsson HE, Andersson AF, Bjorksten B, Engstrand L, Jenmalm MC. Low diversity of the gut microbiota in infants with atopic eczema. *J Allergy Clin Immunol.* Feb 2012;129(2):434-440, 440 e431-432.

32. Abrahamsson TR, Jakobsson HE, Andersson AF, Bjorksten B, Engstrand L, Jenmalm MC. Low gut microbiota diversity in early infancy precedes asthma at school age. *Clin Exp Allergy.* Jun 2014;44(6):842-850.

33. Prescott SL. Early origins of allergic disease: a review of processes and influences during early immune development. *Curr Opin Allergy Clin Immunol.* Apr 2003;3(2):125-132.

34. Perkin MR. Unpasteurized milk: health or hazard? *Clin Exp Allergy.* May 2007;37(5):627-630.

35. Peters M, Kauth M, Scherner O, et al. Arabinogalactan isolated from cowshed dust extract protects mice from allergic airway inflammation and sensitization. *Journal of Allergy and Clinical Immunology.* 2010;126(3):648-656. e644.

36. Seedorf J, Hartung J, Schröder M, et al. Concentrations and emissions of airborne endotoxins and microorganisms in livestock buildings in Northern Europe. *Journal of Agricultural Engineering Research.* 1998;70(1):97-109.

37. von Mutius E, Vercelli D. Farm living: effects on childhood asthma and allergy. *Nat Rev Immunol.* 12//print 2010;10(12):861-868.

38. Heederik D, von Mutius E. Does diversity of environmental microbial exposure matter for the occurrence of allergy and asthma? *J Allergy Clin Immunol.* Jul 2012;130(1):44-50.

39. von Mutius E. The microbial environment and its influence on asthma prevention in early life. *J Allergy Clin Immunol.* Mar 2016;137(3):680-689.

40. Bisgaard H, Hermansen MN, Buchvald F, et al. Childhood asthma after bacterial colonization of the airway in neonates. *New England Journal of Medicine.* 2007;357(15):1487-1495.

41. Jackson DJ, Evans MD, Gangnon RE, et al. Evidence for a causal relationship between allergic sensitization and rhinovirus wheezing in early life. *American journal of respiratory and critical care medicine.* 2012;185(3):281-285.

42. Rosas-Salazar C, Hartert TV. Prenatal exposures and the development of childhood wheezing illnesses. *Current Opinion in Allergy and Clinical Immunology.* 2017;17(2):110-115.

43. Genuneit J. Exposure to farming environments in childhood and asthma and wheeze in rural populations: a systematic review with meta-analysis. *Pediatric Allergy and Immunology.* 2012;23(6):509-518.

44. Campbell B, Lodge C, Lowe A, Burgess J, Matheson M, Dharmage S. Exposure to 'farming'and objective markers of atopy: a systematic review and meta-analysis. *Clinical & Experimental Allergy.* 2015;45(4):744-757.

45. Poole JA. Farming-associated environmental exposures and atopic diseases. *Annals of allergy, asthma & immunology: official publication of the American College of Allergy, Asthma, & Immunology.* 2012;109(2):93.

46. Stroup DF, Berlin JA, Morton SC, et al. Meta-analysis of observational studies in epidemiology: a proposal for reporting. *Jama.* 2000;283(15):2008-2012.

47. Project EPHP. Quality Assessment Tool For Quantitative Studies. Hamilton, ON: Effective Public Health Practice Project. [Internet]. 1998; https://merst.ca/ephpp/. Accessed November 1, 2019.

48. Thomas B, Ciliska D, Dobbins M, Micucci S. A process for systematically reviewing the literature: providing the research evidence for public health nursing interventions. *Worldviews on Evidence-Based Nursing.* 2004;1(3):176-184.

49. Armijo-Olivo S, Stiles CR, Hagen NA, Biondo PD, Cummings GG. Assessment of study quality for systematic reviews: a comparison of the Cochrane Collaboration Risk of Bias Tool and the Effective Public Health Practice Project Quality Assessment Tool: methodological research. *Journal of evaluation in clinical practice.* 2012;18(1):12-18.

50. Dick S, Friend A, Dynes K, et al. A systematic review of associations between environmental exposures and development of asthma in children aged up to 9 years. *BMJ open.* 2014;4(11):e006554.

51. Illi S, Depner M, Genuneit J, et al. Protection from childhood asthma and allergy in Alpine farm environments-the GABRIEL Advanced Studies. *J Allergy Clin Immunol.* Jun 2012;129(6):1470-1477 e1476.

52. Loss G, Apprich S, Waser M, et al. The protective effect of farm milk consumption on childhood asthma and atopy: the GABRIELA study. *Journal of Allergy and Clinical Immunology.* 2011;128(4):766-773. e764.

53. MacNeill S, Sozanska B, Danielewicz H, et al. Asthma and allergies: is the farming environment (still) protective in P oland? The GABRIEL Advanced Studies. *Allergy.* 2013;68(6):771-779.

54. Martikainen MV, Kääriö H, Karvonen A, et al. Farm exposures are associated with lower percentage of circulating myeloid dendritic cell subtype 2 at age 6. *Allergy.* 2015;70(10):1278-1287.

55. Riedler J, Braun-Fahrlander C, Eder W, et al. Exposure to farming in early life and development of asthma and allergy: a cross-sectional survey. *Lancet.* Oct 6 2001;358(9288):1129-1133.

56. Ege MJ, Bieli C, Frei R, et al. Prenatal farm exposure is related to the expression of receptors of the innate immunity and to atopic sensitization in school-age children. *J Allergy Clin Immunol.* Apr 2006;117(4):817-823.

57. Perkin MR, Strachan DP. Which aspects of the farming lifestyle explain the inverse association with childhood allergy? *J Allergy Clin Immunol.* Jun 2006;117(6):1374-1381.

58. von Hertzen L, Makela MJ, Petays T, et al. Growing disparities in atopy between the Finns and the Russians: a comparison of 2 generations. *J Allergy Clin Immunol.* Jan 2006;117(1):151-157.

59. Ege MJ, Herzum I, Buchele G, et al. Prenatal exposure to a farm environment modifies atopic sensitization at birth. *J Allergy Clin Immunol.* Aug 2008;122(2):407-412, 412 e401-404.

60. Loss G, Bitter S, Wohlgensinger J, et al. Prenatal and early-life exposures alter expression of innate immunity genes: the PASTURE cohort study. *J Allergy Clin Immunol.* Aug 2012;130(2):523-530.e529.

61. Batool T, Reece PL, Schulze KM, et al. Prenatal and early-life predictors of atopy and allergic disease in Canadian children: results of the Family Atherosclerosis Monitoring In earLY life (FAMILY) Study. *Journal of Developmental Origins of Health and Disease.* 2016;7(6):665-671.

62. Alfven T, Braun-Fahrlander C, Brunekreef B, et al. Allergic diseases and atopic sensitization in children related to farming and anthroposophic lifestyle--the PARSIFAL study. *Allergy.* Apr 2006;61(4):414-421.

63. Braun-Fahrlander C, Riedler J, Herz U, et al. Environmental exposure to endotoxin and its relation to asthma in school-age children. *N Engl J Med.* Sep 19 2002;347(12):869-877.

64. Schram-Bijkerk D, Doekes G, Douwes J, et al. Bacterial and fungal agents in house dust and wheeze in children: the PARSIFAL study. *Clin Exp Allergy.* Oct 2005;35(10):1272-1278.

65. van Strien RT, Engel R, Holst O, et al. Microbial exposure of rural school children, as assessed by levels of N-acetyl-muramic acid in mattress dust, and its association with respiratory health. *J Allergy Clin Immunol.* May 2004;113(5):860-867.

66. von Mutius E. The environmental predictors of allergic disease. *Journal of Allergy and Clinical Immunology.* 2000;105(1):9-19.

67. Adhikari A, Gupta J, Wilkins III JR, et al. Airborne microorganisms, endotoxin, and (1→3)-β-D-glucan exposure in greenhouses and assessment of respiratory symptoms among workers. *Annals of occupational hygiene.* 2011;55(3):272-285.

68. Lee A, Sangsupawanich P, Ma S, et al. Endotoxin levels in rural Thai and urban Singaporean homes. *International archives of allergy and immunology.* 2006;141(4):396-400.

69. Pfefferle PI, Buchele G, Blumer N, et al. Cord blood cytokines are modulated by maternal farming activities and consumption of farm dairy products during pregnancy: the PASTURE Study. *J Allergy Clin Immunol.* Jan 2010;125(1):108-115 e101-103.

70. Schaub B, Liu J, Hoppler S, et al. Maternal farm exposure modulates neonatal immune mechanisms through regulatory T cells. *J Allergy Clin Immunol.* Apr 2009;123(4):774-782 e775.

71. Prescott SL. Maternal allergen exposure as a risk factor for childhood asthma. *Curr Allergy Asthma Rep.* Feb 2006;6(1):75-80.

72. Rowe J, Kusel M, Holt BJ, et al. Prenatal versus postnatal sensitization to environmental allergens in a high-risk birth cohort. *J Allergy Clin Immunol.* May 2007;119(5):1164-1173.

73. Hagendorens MM, Ebo DG, Bridts CH, Van de Water L, De Clerck LS, Stevens WJ. Prenatal exposure to house dust mite allergen (Der p 1), cord blood T cell phenotype and cytokine production and atopic dermatitis during the first year of life. *Pediatr Allergy Immunol.* Aug 2004;15(4):308-315.

74. Bønnelykke K, Pipper CB, Bisgaard H. Transfer of maternal IgE can be a common cause of increased IgE levels in cord blood. *Journal of Allergy and Clinical Immunology.* 2010/09/01/ 2010;126(3):657-663.

75. Karvonen A, Hyvärinen A, Rintala H, et al. Quantity and diversity of environmental microbial exposure and development of asthma: a birth cohort study. *Allergy.* 2014;69(8):1092-1101.

76. Choi IS, Koh YI, Koh JS, Lee MG. Sensitivity of the skin prick test and specificity of the serum-specific IgE test for airway responsiveness to house dust mites in asthma. *J Asthma.* Apr 2005;42(3):197-202.

77. Bignardi D, Comite P, Mori I, et al. Allergen-specific IgE: comparison between skin prick test and serum assay in real life. *Allergologie select.* 2019;3(1):9-14.

78. Braun-Fahrländer C, Riedler J, Herz U, et al. Environmental Exposure to Endotoxin and Its Relation to Asthma in School-Age Children. *New England Journal of Medicine.* 2002;347(12):869-877.

79. Remes ST, Iivanainen K, Koskela H, Pekkanen J. Which factors explain the lower prevalence of atopy amongst farmers' children? *Clin Exp Allergy.* Apr 2003;33(4):427-434.

80. Deeks JJ HJ, Altman DG (editors). Chapter 10: Analysing data and undertaking meta-analyses. In: Higgins JPT, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, Welch VA (editors). *Cochrane Handbook for Systematic Reviews of Interventions version 6.2 (updated February 2021)*: Cochrane; 2021.

81. Klintberg B, Berglund N, Lilja G, Wickman M, van Hage-Hamsten M. Fewer allergic respiratory disorders among farmers' children in a closed birth cohort from Sweden. *Eur Respir J.* Jun 2001;17(6):1151-1157.

82. Wickens K, Lane JM, Fitzharris P, et al. Farm residence and exposures and the risk of allergic diseases in New Zealand children. *Allergy.* Dec 2002;57(12):1171-1179.

83. Zekveld C, Bibakis I, Bibaki-Liakou V, et al. The effects of farming and birth order on asthma and allergies. *European Respiratory Journal.* 2006;28(1):82-88.

84. Waser M, Michels K, Bieli C, et al. Inverse association of farm milk consumption with asthma and allergy in rural and suburban populations across Europe. *Clinical & Experimental Allergy.* 2007;37(5):661-670.

Table 3-1: Associations between early-life farm exposures and atopic sensitization/atopy defined by total serum IgE, specific IgE, or skin prick test measured in children

First author reference # (Year published) Location	Study Design	Study population (Sample size)	Type & timing of exposure	Outcome assessment	Outcome & strength of association
Cross-sectional studies					
Riedler et al[55] (2001) Rural areas of Austria, Germany, and Switzerland.	Cross-sectional	N = 812; 6–13 years old	Exposure to stables in the first year of life, farm milk consumption	Atopic sensitization was defined as at least one positive specific IgE test result of 3.5 kU/L or greater for the six aeroallergens (house dust mite, storage mite, grass and birch pollen, cat dander, and cow epithelium).	-Exposure to stables and farm milk in the 1st year of life (n=218): [aOR=0.32 (0.17-0.62)] -Exposure to stables, but not farm milk in the 1st year of life: [aOR=0.56 (0.25-1.27)] -Farm milk but no stables [aOR=0.43(0.24-0.77) -Pregnant mother active daily on farm vs. pregnant mother not active daily on farm and child not exposed to stable and farm milk in 1st year [aOR=0.18 (0.06-0.56)] -Having above average exposure (more than 20 minutes in the stables) in the 1st year of life (n=133) [aOR=0.28 (0.12–0.66)] -Having above median aged <5 years (n=122) [aOR=0.23 (0.10–0.53)]

Klintberg et al[81] (2001) The island of Gotland, in the Baltic Sea	Cross-sectional	N= 650 children born in 1989 (356 boys and 351 girls) was divided into two groups of exposures, all either living in a farming environment or never having lived in such an environment. -range 7–8 years old	Living in a farming environment during the first year of life .	The skin reactivity to standardized allergen extracts: pollen (birch, timothy, mugwort), animal dander (cat, dog, horse), *Alternaria alternata* and food allergens (egg, hazelnut, peanut), house dust mites (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*) and storage mites (*Lepidoglyphus destructor*, *Tyrophagus putrescentiae* and *Acarus siro*). Atopy was defined as a mean wheal size ≥3 mm.	-32% of the children had at least one positive test. -Sensitization to any allergen panel (Birch, Timothy, Cat, A. alternata, Dermatophagoides pteronyssinus, Dermatophagoides farina): [aRR=0.94 (0.70 – 1.25)]
Braun-Fahrlander et al[63] (2002) Rural areas of Germany, Austria, or Switzerland	Cross-sectional	N = 812; 6–13 years old	Exposure to farming during the first year of life	Atopy was defined by at least one positive test for specific IgE indicating a titer of at least 3.5 kU per liter for one or more of the six airborne all ergens (house dust mites, storage mites, grass pollen, birch pollen, cat dander, and cow epithelium).	-Exposure to farming in the first year of life showed a strong inverse association with atopy [aOR = 0.45 (0.30–0.68)]
Wickens et al[82], (2002) New Zealand	Cross-sectional	N= 293; 7-10 years old	-Farm exposures in the first year of life	Skin prick tests to common allergens (Dermatophagoides farinae, D. pteronyssinus, mould mix, ockroach, rye grass, timothy grass, cat, dog). A positive SPT reaction (SPT +) was defined according to the presence of a mean wheal diameter of 3 mm or	-Exposure in the first year of life: +Farm abode:[aOR=1.3 (0.5-3.6)] +Regular poultry:[aOR=1.1 (0.4-3.5)] +Regular pig:[aOR=0.2 (0.1-0.9)]

				more to at least one of the allergens.	
Remes et al[79] (2003) Finland	Cross-sectional	N=714; 6-13 years old	-Mother farming in infancy, main line of production in infancy (dairy, livestock, plant,..)	Any reaction (SPT ≥ 3mm) to one or more of allergens: birch, timothy grass and mygwort pollens, cat and dog epithelial danders, and house dust mite (Dermatophagoides pteronyssinus).	-Farming in infancy: [aOR=0.68 (0.48-0.95)] -Main line of production in infancy: +Dairy farming (ref.: no farming):[aOR= 0.68 (0.46-1.00)] +Other livestock (ref.: no farming):[aOR=0.51 (0.28-0.93)] +Plant farming (ref.: no farming):[aOR=0.74 (0.42-1.32)] +Other or nor defined:[aOR=0.46 (0.09-2.38)]
Zekveld et al[83] (2005) rural Crete	Cross-sectional	N=797 Schoolchildren aged 7–18 yrs	Children's exposure to and contact with farm animals or pets and consumption of farm milk at three different stages of life: infancy, at 5 years of age, and during the past year.	Skin-prick tests involving a series of 10 common aeroallergens: grass pollen (Mediterranean mix), *Parietaria*, olive blossom, cat fur, *Dermatophagoides pteronyssinus* and *D. farinae*, goat epithelium, *Cladosporium*, cockroach, and poultry. The negative and positive controls were saline and histamine respectively. Atopy was defined as a mean wheal size ≥3 mm	Children with regular animal contact, at any stage of life, were equally likely to be atopic or have current wheeze than children with less frequent or no contact. For example, the prevalence of atopy among sheep/goats exposures at infancy (daily, weekly, never) were 33%, 20%, 23% (p-trend=0.49)

Ege et al[56] (2006) Multi-country European study (Austria, Germany, the Netherlands, Sweden, and Switzerland)	Cross-sectional	N=2086; 5-13 years old	-Maternal exposure to stables during pregnancy	Allergen-specific IgE for common inhalant. Atopy was defined as IgE values of 3.5 kU/L or greater	-Maternal exposure to stables during pregnancy [aOR=0.58 (0.39-0.86)]
Perkin et al[57] (2006) UK	Cross-sectional	N=879; mean age 8.11 years	-Early farm animal exposure or barn or stable exposure	Skin prick testing: a panel of allergens (dog hair, cat hair, horse hair, cow hair, 6-grass mix, house dust mite (Dermatophagoides pteronyssinus), Acarus siro, Lepidoglyphus destructor, Tyrophagus putrescentiae) and positive and negative controls. A positive response was a 3-mm or larger mean wheal diameter was defined as atopy.	-Early farm animal exposure aOR=0.53 (0.29-0.99)
von Hertzen et al[58] (2006) Finland & Russia	Cross-sectional	N=732;7–16 years old	Parental farming at less than first year of age	A standard set of 9 airborne and food allergens (birch timothy grass, mugwort, cat, dog, horse, cow, Dermatophagoides pteronyssinus, and fish). Atopy was defined as a mean wheal size ≥3 mm.	Parental farming at less than first year of age vs. currently: - In Finland:[aOR=0.45 (0.22-0.91); - In Russia: [aOR=0.47 (0.22-1.03) - Both 2 countries: [aOR=0.50 (0.31-0.81)]
Waser et al[84] (2006) Austria, Germany, the Netherlands,	Cross-sectional	N= 3979 Children aged 5–13 years	Farm milk consumption in the first year of life	-Allergen-specific IgE measurements -A mix of common inhalant allergens (birch, timothy, mugwort, *Dermatophagoides*	-Using a cut-off level of 0.35 kU/L, a significant inversed association was found for a sensitization to horse dander 0.50 (0.28–0.87).

Sweden, and Switzerland.				*pteronyssinus* and *farinae*, cat, dog and hose epithelium and *Cladosporium herbarum*) Atopic sensitization was defined as allergen-specific IgE ≥ 0.35 kU/L. In addition, a cut-off value of 3.5kU/L was also considered for the analyses. Pollen sensitization was defined as positive grass pollen mix and/or positive tree pollen mix.	-Using a cut-off level of ≥3.5 kU/L, the negative association with farm milk consumption for pollen sensitization [aOR=0.67 (0.47–0.96)]
Loss et al[52] (2011) rural regions of Germany, Austria, and Switzerland	Cross-sectional	N= 8334 6- to 12-year-old school children	Farm milk consumption by questionnaire	Atopy was defined as positive test results for specific IgE antibodies against *Dermatophagoides pteronyssinus*, cat, or birch (cutoff, 0.7 kU/L) or against a grass mix (cutoff, 0.35 kU/L)	-First unboiled farm milk <1 y OR=0.72 (0.61-0.85) -First unboiled farm milk >1 y OR=0.78 (0.63-0.97)
Illi et al[51] (2012) Rural areas of southern Germany (Bavaria and Baden-Wurttemberg Switzerland Austria and Poland)	Cross-sectional	N= 7682 Mean age: 8.7± 1.4 years	Farm exposures (pregnancy to age 3 years)	Serum IgE antibodies against inhalant (Dermatophagoides pteronyssinus, cat, grass mix [sweet vernal grass, rye grass, timothy grass, cultivated rye, and velvet grass], birch, and mugwort) and food (egg white, cow's milk, fish, wheat, peanut, and soybean) allergens. Atopic sensitization was defined as specific IgE antibodies of at least 0.7 kU/L against D pteronyssinus, cat, or birch or a positive reaction (0.35 kU/L) to the grass mix.	Farm exposures (pregnancy to age 3 years) -Contact with animals +Cow:[aOR= 0.75 (0.65-0.88)] +Poultry:[aOR= 0.76 (0.64-0.91)] +Horses:[aOR= 0.79 (0.63-0.99)] -Stay in cow sheds:[aOR= 0.78 (0.67-0.92)] -Contact with animal feed: +Hay:[aOR= 0.74 (0.63-0.86)] +Grain:[aOR= 0.72(0.61-0.86)] +Corn:[aOR=0.78(0.64-0.95)]

					+Corn silage:[aOR=0.79(0.65-0.95)] -Stay in barn:[aOR= 0.70 (0.59-0.82)] -Stay in fodder storage room: aOR=[0.79 (0.64-0.98)]
Macneil et al[53] (2013) Poland	Cross-sectional	N=2586 6- to 12-year-old school children	Early exposures (pregnancy to age 3 years) to a farm or farm-related	-Specific serum immunoglobulin E (IgE) antibodies measured to: *D.pteronyssinus*, cat dander, grass mix and common silver birch. Atopy was defined by specific IgE≥ 0.7 kU/l against *D. pteronyssinus*, cat or birch or a positive reaction (0.35 kU/l) to the grass mix. -Skin prick testing (SPT) was performed using extracts from *D. pteronyssinus*, *D. farinae*, mixed grasses, birch and cat epithelia with histamine and saline controls (ALK-Abelló, Hungerford, UK). Atopy was defined as a mean wheal size ≥3 mm.	- Early-life contact with grain was inversely related to the risk of atopy measured by IgE [aOR = 0.66 (0.47- 0.92)] -Early-life contact with poultry was inversely related to the risk of atopy measured by SPT [aOR = 0.54 (0.38-0.77)]
Case-control study					
Martikainen et al[54] (2015) French and Finnish children from the PASTURE study	Case–control	65 asthmatic (26 farming and 39 nonfarming) and 103 nonasthmatic (56 farming and 47	Prenatal maternal consumption of farm milk, prenatal exposures to the stable and barn.	Specific IgE levels in serum against 6 food (hen's egg, cow's milk, peanut, hazelnut, carrot, and wheat flour) and 13 inhalant (*Dermatophagoides pteronyssius*; *Dermatophagoides farinae*; cat; horse; dog; *Alternaria* species; mugwort, plantain, alder, birch, hazel, and rye pollen; and grass	Farmer during pregnancy [aOR= 0.81 (0.38-1.72)]. Stay in stable [aOR= 1.05(0.48-2.26)]; Stay in barn [aOR=0.9 (0.42-1.93)]

		nonfarming) children.		pollen mix) allergens were assessed at age 6 by using the Allergy Screen test panel for atopy. Positive sensitization against perennial, seasonal, or food allergens was defined by using a cutoff for specific IgE of 0.70 IU/ml or greater.	

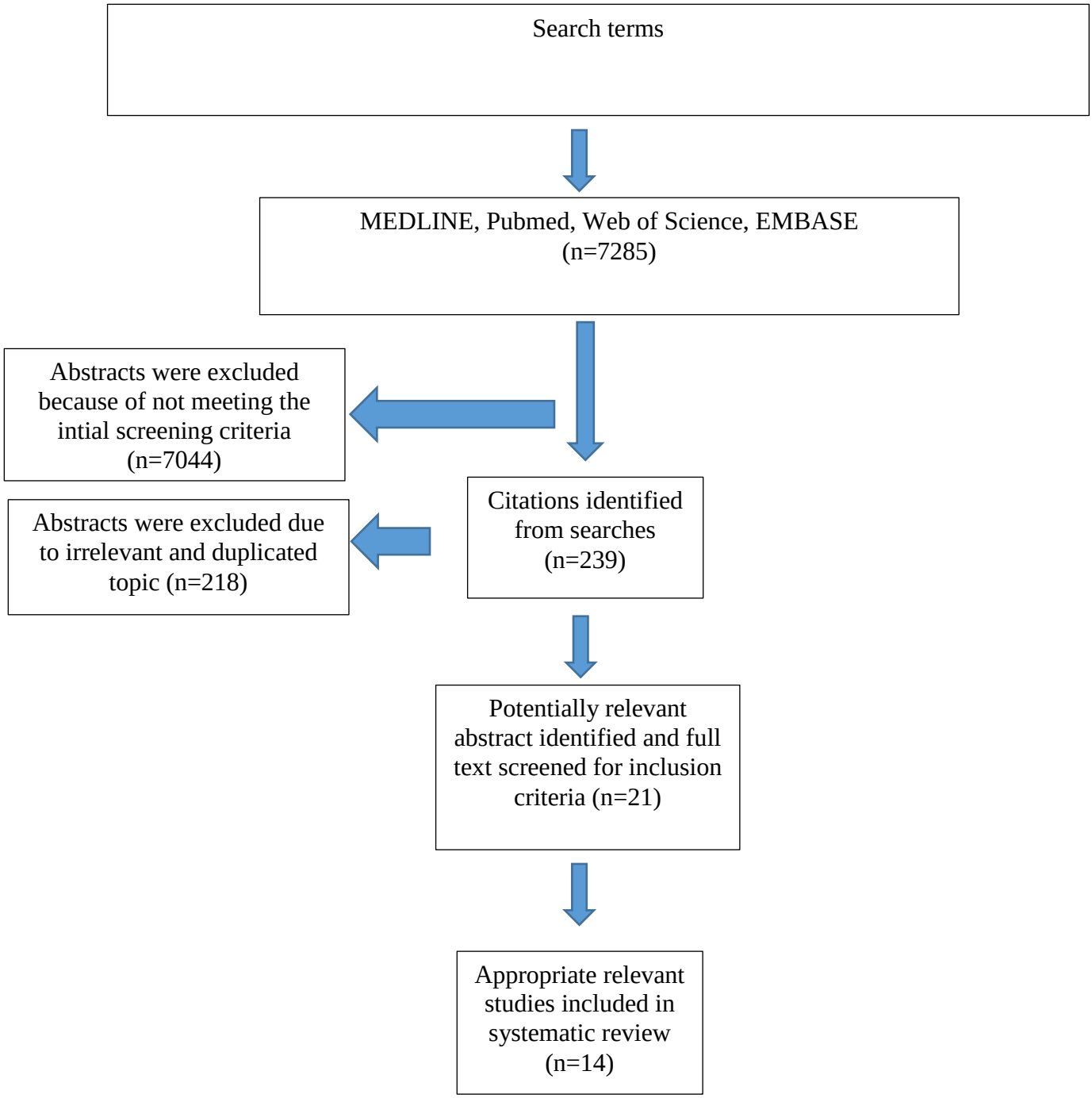

Figure 3-1. QUOROM flow chart to select articles to the systematic review

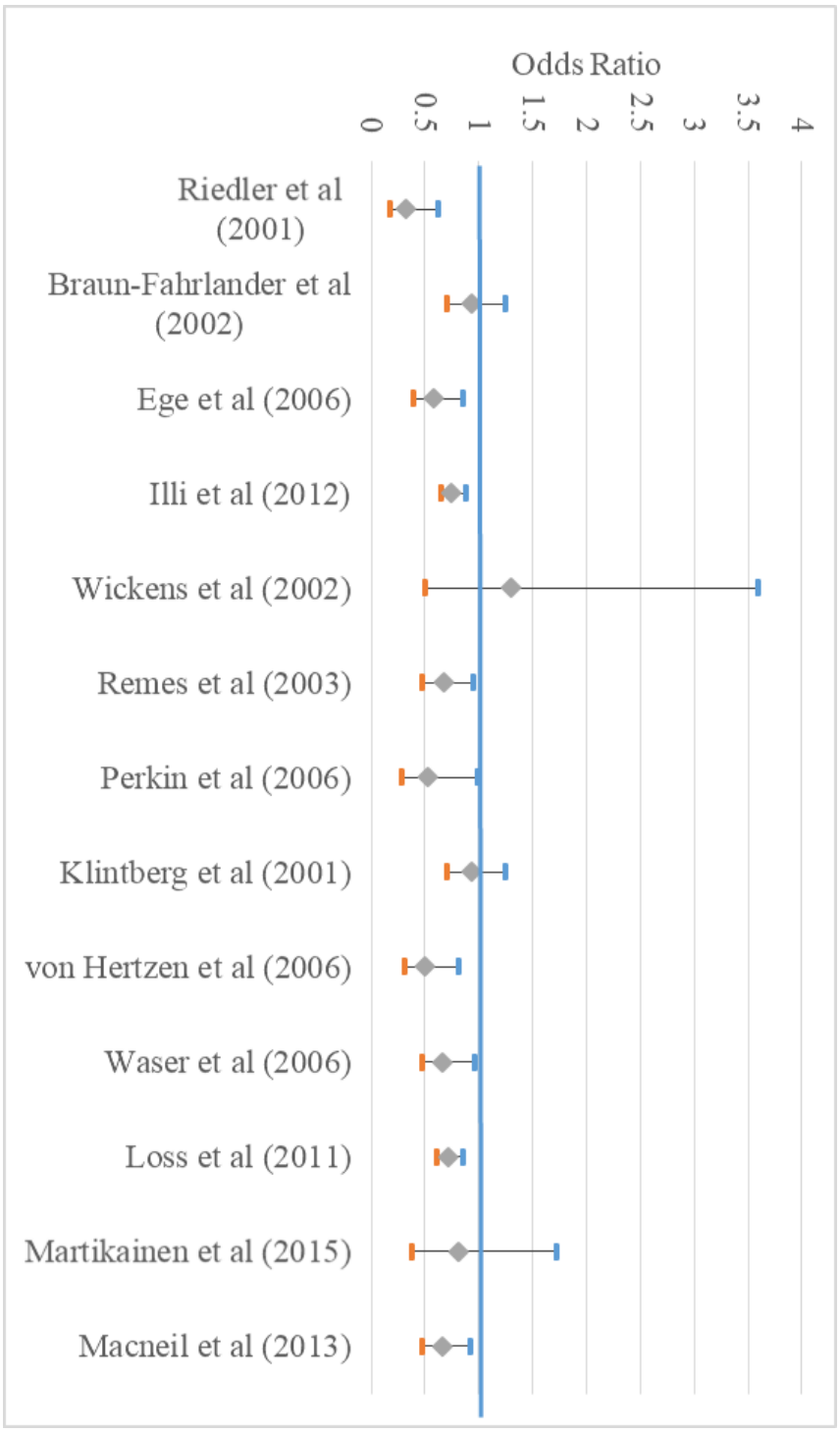

Figure 3-2: Summary of strengths of associations among included articles

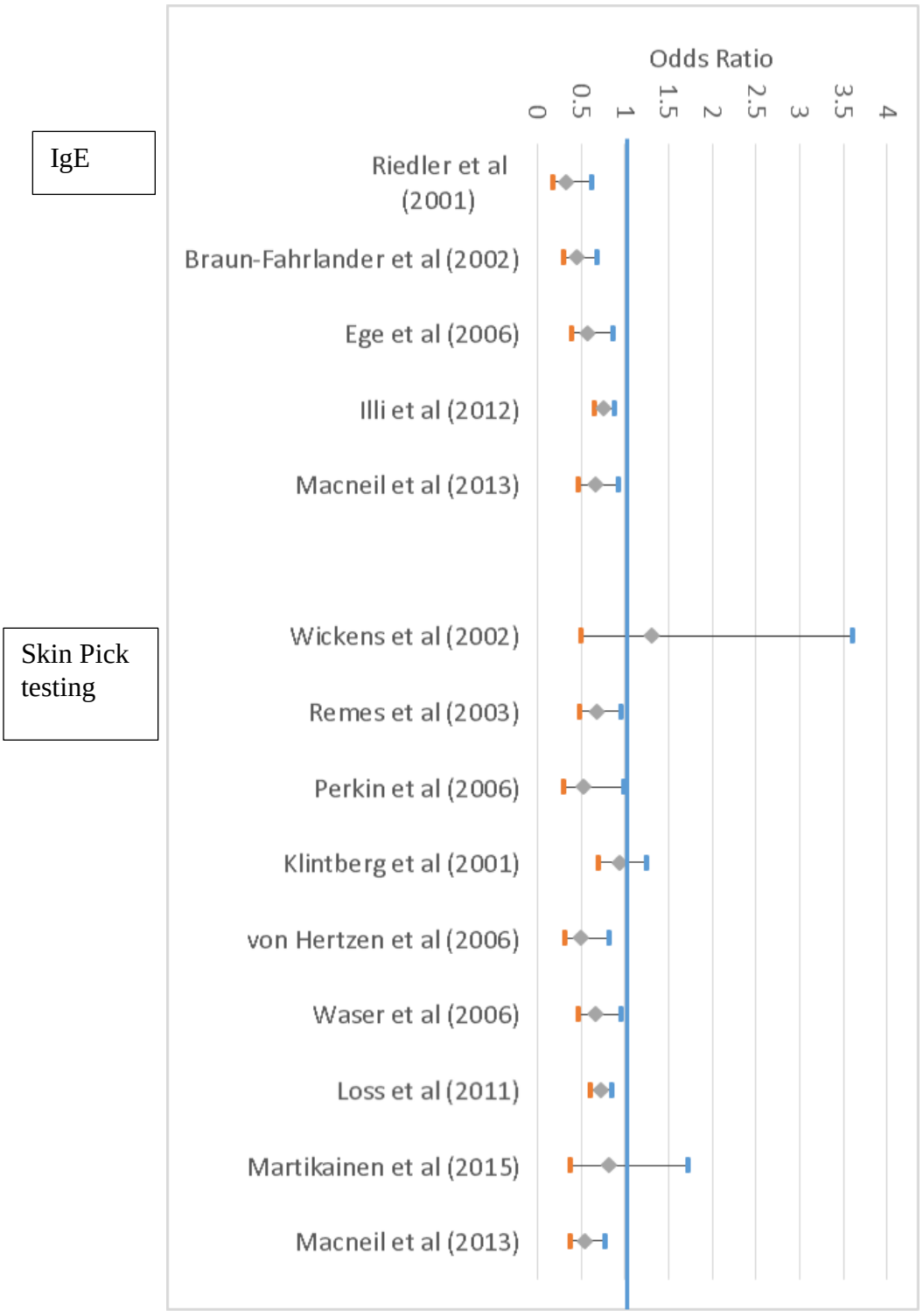

Figure 3-3. SPT vs. specific IgE measurement methods

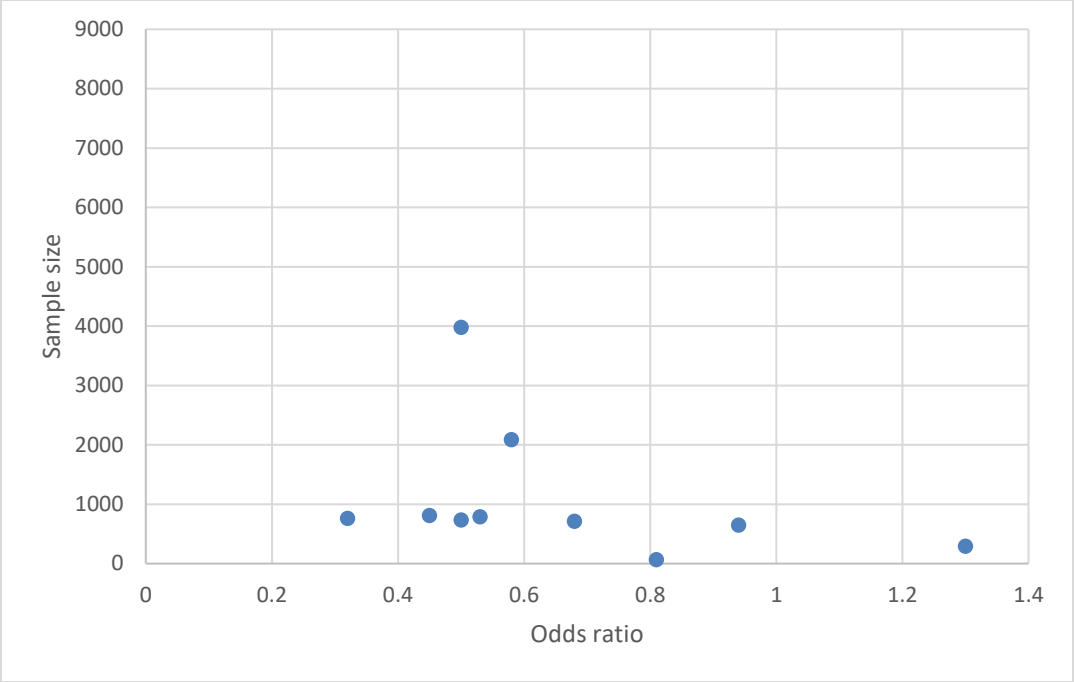

Figure 3-4: Funnel plots of included articles (with main exposures)

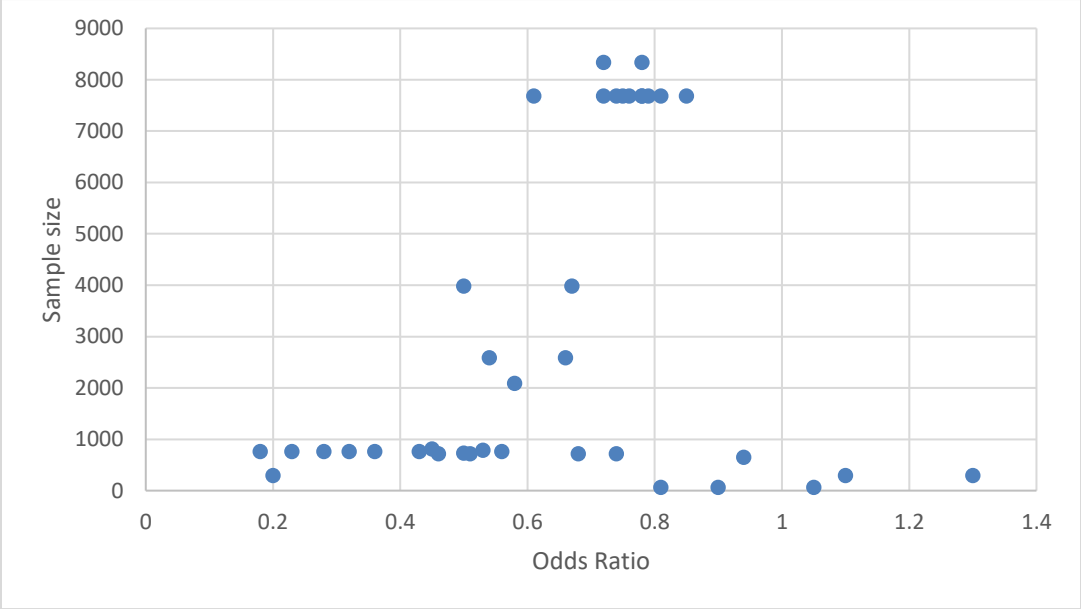

Figure 3-5: Funnel plots of included articles (with main exposures and other related exposures)

CHAPTER IV

PREVALENCE, RISK FACTORS, AND CLINICAL OUTCOMES OF ATOPIC AND NONATOPIC ASTHMA AMONG RURAL CHILDREN

(MANUSCRIPT 2)

Authors: Joshua A. Lawson, PhD[1,2]; Luan M. Chu, MSc[2,3]; Donna C. Rennie, PhD[2,4]; Louise Hagel, MSc[2]; Chandima P. Karunanayake, PhD[2]; Punam Pahwa, PhD[2,5]; James A. Dosman, MD[2]

Affiliations: [1]Department of Medicine, University of Saskatchewan, Saskatoon, Saskatchewan, Canada

[2]Canadian Center for Health and Safety in Agriculture, University of Saskatchewan, Saskatoon, Saskatchewan, Canada

[3]Health Sciences Program, College of Medicine, University of Saskatchewan, Saskatoon, Saskatchewan, Canada

[4]College of Nursing, University of Saskatchewan, Saskatoon, Saskatchewan, Canada

[5]Department of Community Health and Epidemiology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada

Reprinted from *Ann Allergy Asthma Immunol.*, Lawson JA, Chu LM, Rennie DC, et al, Prevalence, risk factors, and clinical outcomes of atopic and nonatopic asthma among rural children, 118(3):304-310, Mar 2007, with permission from Elsevier.

4.1. Abstract

Background: Because of time and cost constraints, objective classification of atopic and nonatopic asthma has been limited in large epidemiologic studies. However, as we try to better understand exposure-outcome associations and ensure appropriate treatment of asthma, it is important to focus on phenotype-defined asthma classification.

Objective: To compare atopic and nonatopic asthma in rural children with regard to risk factors and clinical outcomes.

Methods: We conducted a cross-sectional study in rural Saskatchewan, Canada, in 2011. Parents of 6- to 14-year-old children completed a health and exposure survey. Skin prick tests were completed in a subsample of 529 children. Asthma was based physician diagnosis. Asthma status was defined as no asthma, nonatopic asthma, and atopic asthma.

Results: Asthma prevalence was 14.7% of which 32.1% of cases were atopic. After adjustment, early respiratory illness and a family history of asthma were predictors of childhood asthma, regardless of atopic status ($P < .05$). A mother with a history of smoking increased the risk of atopic asthma ($P= .01$). Compared with those with nonatopic asthma, in the past 12 months, children with atopic asthma were more likely to report a sneezy, runny, or blocked nose or have shortness of breath (odds ratio >2), whereas those with nonatopic asthma were more likely to have parents who missed work (odd ratio >3). Those with nonatopic asthma had significantly lower forced expiratory volume in 1 second compared with those with atopic asthma.

Conclusion: Exposures may contribute differentially to atopic and nonatopic asthma and result in differential clinical presentation or burden. The study of these characteristics is important for etiologic understanding and management decisions.

4.2. Introduction

Asthma is one of the most prevalent chronic respiratory disorders[1] and costs hundreds of millions of dollars annually in Canada.[2] It is a heterogeneous condition with several phenotypes[3] and most common among children. Atopic/non-atopic asthma is one of the most common phenotypes considered with this classification often based on skin tests or blood tests.[4-6] Due to time and cost constraints, objective classification of atopic/non-atopic asthma has been limited in large epidemiological studies. However, as we try to better understand exposure-outcome relationships and ensure appropriate treatment of asthma, it is important to focus on phenotype-defined asthma classification. Distinct patterns of risk factors for atopic and nonatopic asthma in children have been identified through epidemiological studies[4,5,7] and may indicate different causal mechanisms.[6,8] While both atopic and non-atopic asthma phenotypes are associated with a family history of asthma[5,7], other risk factors vary between the two[5].

In addition, findings from studies point to an inverse association between farm dwelling and asthma.[9-11] However, these results are still inconsistent.[12,13] Some of the inconsistency in the relationship between rural exposures and asthma could result from differences in the exposure-outcome associations between atopic and non-atopic asthma phenotypes yet very little work has been conducted investigating asthma phenotypes and their risk factors in rural populations.[6] While a recent study has suggested the impact of exposures rich in microbes on shaping innate immunity,[14] it is still important to consider clinical profiles of those with atopic and nonatopic asthma.

Our objectives were to estimate the prevalence of atopic and nonatopic asthma and identify risk factors for each phenotype among rural dwelling children. As part of this, we sought to determine if there is consistency of predictors between the 2 phenotypes and to compare clinical

characteristics (symptoms and lung function) and burden of disease among children with atopic and nonatopic asthma.

4.3. Methods

Study design

Data for this study were from the child component of a rural health study.[15-17] Methods of this study have been described previously.[15-17] This is a longitudinal study of children and adults being completed in a rural area. The children's baseline data were collected by two methods. The first was a survey phase. The second was a clinical testing phase in a subsample of children in winter and spring of 2011. The current analysis was completed using the children's component of the baseline data.

Study population, selection, and recruitment

This study was conducted in four rural quadrants of a rural region. Rural was defined as living in a small town (<5,000 people) or rural municipality, a definition in alignment with Statistics Canada's definitions.[18] The participant must also have lived more than 60 km away from an urban area.[15] Initially, meetings were held with the school board directors to discuss the project, followed by communication with the principals of schools within a school division agreeing to participate. All 10 school divisions approached agreed to participate. Of the 43 schools approached, 39 agreed to participate.

Study packages, which included an information letter, survey, consent forms, and assent forms were distributed to schools. All children aged 6-18 years attending selected schools were eligible to participate in the survey phase. Study packages were sent home through the schools to parents for completion and to be returned to the school for retrieval by the study team. Surveys from 2,382 students were returned (response rate: 42%).

A subset of students attending Grades 1 to 8 (ages 6-14 years) were selected for clinical testing. Schools included in clinical testing were selected based on higher numbers of survey

participation at that school in order to maximize efficiency. Clinical testing included allergen skin prick tests (SPTs), anthropometric measures, and lung function testing with spirometry. One school division refused to allow clinical measurements in the school. There were 16 schools taking part in the clinical testing with 1,768 students being approached. In total, 584 students completed the clinical testing and survey. These students are included in the current analysis.

This study was approved by the University of Saskatchewan Biomedical Research Ethics Board. Before clinical testing, parents were required to sign a consent form, whereas children completed an assent form. All school divisions involved approved of the study.

Data collection

Parent-completed surveys

Surveys were distributed through schools to parents for self-completion. These surveys were based on standardized questionnaires, such as the International Study of Allergy and Asthma in Childhood (ISAAC) questionnaire,[19] American Thoracic Society Children's Respiratory Disease questionnaire,[20] and questionnaires used previously in other lung studies.[21,22] Information was collected on lung and general health, indoor environment, health behaviours, and sociodemographics.

Asthma was defined by the survey question, "Has this child ever been diagnosed as having asthma by a doctor?" Following previous definitions,[16,17] location of residence was based on the question, "Where is your home located?" The child was considered to live on a farm if he/she reported living on a farm or acreage. If the child reported living in town, he/she was considered to live in a small town. Current parental smoking status was defined as a report of parental smoking for the mother or father at the time of questionnaire completion. Early respiratory illness was considered to have occurred if there was a report of any 1 of the following 4 conditions in the first

2 years of life: bronchitis, pneumonia, whooping cough or croup. Farming activities were based on the question, “In the past 12 months, on average, how often has this child spent 1 hour near or in the following activities?” The farming activities listed were haying or moving or playing with hay bales, feeding livestock, cleaning or playing in barns, emptying or filling grain bins, cleaning or playing in pens or corrals, and riding horses. The responses of everyday, at least once a week, at least once a month were coded as regular activity, whereas those who responded less than once a month or never were classified as irregular activity.[16]

For physical activity levels, participants were designated to the high activity group based on the average response to the following 2 questions: 1) “Over the past 7 days, on how many days were you physically active for a total of at least 60 minutes per day?” and 2) “Over a typical or usual week, on how many days are you physically active for a total of at least 60 minutes per day?” [23]. The mean number of physically active days have been found to be reliable for classifying participants as meeting or not meeting physical activity guidelines of 60 minutes of physical activity on 5 days or more per week.[24] On the basis of these guidelines, participants were categorized as physically active if they were active for 5 days or more per week

Clinical characteristics included respiratory symptoms and allergic symptoms and conditions based on the response to the survey. Respiratory symptoms included wheeze, shortness of breath, and wheeze with exercise. Allergic symptoms and conditions included hay fever and eczema and their associated symptoms.

Burden was based on indicators of care, management, and morbidity and was assessed from the survey. These indicators included breathing medication use, parents missing work, the child missing school, and physical activity levels.

Skin prick testing

All SPTs were completed at the school. SPTs were completed on the forearm and measured after 15 minutes following international standards.[25] The panel of allergens selected was chosen because they were considered the most common allergens in the region. These included 6 allergens: *Alternaria* (mold), *Cladosporium* (mold), cat dander, local grasses, wheat dust, and house dust mite (ALK-Abelló Pharmaceuticals, Inc, Mississauga, Ontario, Canada). Histamine (10 mg/ml) and saline solution (0.9%) were used as positive and negative controls, respectively. We were interested in the outcome of the presence of atopic and nonatopic asthma. Atopy was defined as a 3-mm or greater wheal formed for any of the allergens compared to the negative control on SPTs. A 3-level categorical asthma status variable was defined based on the previously described definitions of atopy and asthma for which children were defined as having no asthma (no asthma), nonatopic asthma (no atopy but yes to asthma), and atopic asthma (yes to atopy and asthma).

Anthropometric measurements

Height and weight were measured objectively as part of the pulmonary function testing. Height was measured with the patient against a wall using a fixed tape measure with patients standing in socks on a hard floor. Weight was measured using a calibrated spring scale with patients in socks and dressed in normal indoor clothing. From these measures, body mass index (BMI) was calculated as the weight in kilograms divided by square of height in meters.[26] Overweight/obesity categories were derived from objectively measured BMI using age and sex cut-off values described by Cole et al.[27] These base the cut-off value as the predicted adult equivalent of a BMI of 25 for overweight and of 30 for obese at the age of 18 years. We categorized our BMI status variable as not overweight vs overweight. Overweight also includes obese. As part of the reference group (neither overweight nor obese), we included those who would be classified as underweight.

Lung function testing

Pulmonary function was assessed through spirometry using the forced expiratory maneuver. A dry-rolling seal spirometer (Sensormedics model 922; Sensor medics Corporation, Anaheim, CA) was used to conduct the testing. American Thoracic Society guidelines were followed.[28,29] Children completed the testing seated while wearing a nose clip. At least 3 and no more than 7 maneuvers were attempted. Before testing each day, calibration of the spirometer was performed using a 3-L syringe. The spirometer was recalibrated at least once if there was an extended length of time of testing or if the temperature changed by 2C or more. From these tests, we were able to assess forced vital capacity (FVC), forced expiratory volume in 1 second (FEV_1), FEV_1/FVC ratio, and forced expiratory flow between 25% and 75% ($FEF_{25\%-75\%}$).

Statistical Analysis

Analyses were completed using SPSS statistical software, version 23 (SPSS Inc, Chicago, Illinois). Statistical significance was based on an α of .05. Initially, descriptive statistics using means (SD) for continuous data and frequencies for categorical data were completed. The variable of primary interest was asthma status (no asthma, nonatopic asthma, atopic asthma). Prevalence of asthma status was calculated. The prevalence of clinical characteristics and risk factors was also calculated for each of the 3 asthma status groups with statistical comparisons completed using the independent-samples χ^2 test and where appropriate, the independent-samples t test. After the descriptive analyses, a risk factor analysis was conducted using multiple polychotomous logistic regression to adjust for potential confounders for which asthma status was the outcome. Variables were selected to the final model based on statistical significance ($p < 0.05$), biological importance, and the effect that the removal of the variable had on the beta (β) coefficients (>15% change) of the remaining independent variables in the model. The strengths of associations were based on the

odds ratios (ORs) and 95% confidence intervals (CIs). Differences in lung function among the 3 asthma status categories were assessed using analysis of covariance. The outcomes included FVC, FEV_1, FEV_1/FVC ratio, and $FEF_{25\%-75\%}$. Each of these analyses was completed after adjustment for age, sex, height, and asthma status.

4.4. Results

Overall, the prevalence of asthma was 14.7%. Of the children with asthma, 31.2% were considered to be atopic. Among the no asthma group, 17.7% were atopic. As such, the prevalence of atopic asthma was 4.7%, whereas the prevalence of nonatopic asthma was 10.0%. The prevalence of asthma was not statistically different between farm and nonfarm dwellers (13.2% vs 15.8%) or between those with atopic vs nonatopic asthma (32.3% in farm dwellers vs 30.4% in nonfarm dwellers).

The descriptive statistics for personal and environmental variables stratified by asthma status are presented in **Table 4-1**. There was a statistically higher prevalence of children with a report of an early respiratory illness, family history of asthma, and a maternal smoking history among children with atopic or nonatopic asthma compared with those with no asthma. In addition, those with nonatopic asthma were more likely to be overweight, own a dog in the past 12 months, and have a paternal history of smoking than those without asthma. Those with atopic asthma were more likely to be born at a low birth weight and have a cat at home in the past 12 months compared with those with no asthma. There were no statistically significant differences between the nonatopic asthma and atopic asthma groups with regard to the variables considered in **Table 4-1**.

After adjusting the analysis for potential confounders, the presence of early respiratory illness and family history of asthma were associated with asthma, regardless of atopic status (**Table 4-2**). Although the findings were not statistically significant, a dog at home in the past 12 months ($p=0.07$) and being overweight ($p=0.06$) were significantly associated with nonatopic asthma. Similarly, mother being an ex-smoker was associated with atopic asthma, although this finding was not statistically significant ($p=0.07$).

When comparing the prevalence of respiratory symptoms among the 3 asthma status groups, as expected, those with asthma consistently had a higher prevalence of symptoms considered (**Table 4-3**). **Table 4-3** also presents the results of comparisons of burden among the asthma status groups. No difference was found in the proportion of those reporting high activity among the 3 groups. Regardless of nonatopic or atopic asthma status, those with asthma had a higher proportion of reporting using breathing medications and missing school compared with those without asthma. However, only those with nonatopic asthma had a higher proportion of children with parents missing work because of their child's breathing problems. Although some differences between those with nonatopic and atopic asthma were large, these differences were only statistically significant ($P < .05$) for ever reported shortness of breath, sneezing in the past 12 months, and report of hay fever. Differences were also not statistically significant ($P < .10$) for parents having to miss work, report of sleep disturbance in the past 12 months, and everyday or nearly every day medication use. With the exception of parents missing work, in each of these cases, children with atopic asthma had a higher proportion of the outcome being reported.

Figure 4-1 presents the results comparing lung function across the asthma status groups. Those in the nonatopic asthma group consistently had the lowest lung function. When compared with the no asthma or the atopic asthma group, the nonatopic asthma group had a statistically significant lower FEV_1 compared with the atopic asthma group. In addition, when compared with the no asthma group, the nonatopic asthma group also had significantly ($P < .05$) lower FEV_1/FVC ratio and $FEF_{25\%-75\%}$.

4.5. Discussion

A major finding of the present study was that asthma was common among these rural dwelling children (14.7%) and that most of those with asthma had nonatopic asthma. Although personal factors of early respiratory illness and family history of asthma were associated with both phenotypes, only trends were seen between other independent risk factors for atopic and nonatopic asthma specifically. In addition, those with atopic asthma generally had a higher proportion reporting symptom and burden indicators, whereas those in the nonatopic asthma group consistently had the lowest lung function.

The prevalence of asthma in our study among these rural dwellers was similar to that seen among other rural dwelling children which has ranged between 5.3%[30] and 14.8%[31]. In addition, only a small proportion of children with asthma in our study population had evidence of allergen skin test reactivity that was lower compared to results from a study by Moncayo et al[6] in Ecuador in 2007 among 6- to 16 year-old children (4.7% vs. 14.4%). In the study from Ecuador[6], atopy was defined by a different SPT panel than ours, and wheeze in the past 12 months was used as a surrogate for asthma classification, also differing from our study.

We found a higher prevalence of nonatopic than atopic asthma, supporting previous evidence[32-34] and in contrast to the general perception that childhood asthma is generally an atopic disease. The proportion of asthma attributable to atopy in children has been estimated to be 38%, but there is considerable variation between studies (25% to 63%).[33] Data from ISAAC phase 2 surveys found that a higher fraction of recent wheezing was attributable to atopy (measured by allergen skin test reactivity) in affluent (41%) compared with nonaffluent countries (20%)[32]. Among Australian children aged 8 to 10 years, Ponsonby et al[34] found that 33% of asthma cases were attributable to atopy (defined as skin test positivity to any of 10 aeroallergens). A study in

rural Poland[35] among school-aged children found that only 22% of children with asthma were sensitized to indoor allergens (cat or dust) and 17% to outdoor allergens (grass mix or birch pollens). These findings indicate that childhood asthma being an atopic disease may be overemphasized.

The prevalence of asthma and the proportion of atopic asthma were not statistically different between farm and nonfarm dwellers. Our findings support previous work that did not find any effect of farming exposures.[6,7] In terms of nonatopic asthma, inconsistent results have been reported recently.[36-38] For example, the PARSIFAL (Prevention of Allergy Risk factors for Sensitization In children related to Farming and Anthroposophic Lifestyle) study team in Europe found that pig farming was notably protective of asthma and atopy.[38] In our study, it is possible that agricultural exposures do not vary appreciably between rural Saskatchewan town and farm dwellers where the towns were small and adjacent to farming areas. Despite this, a recent study of different farm lifestyles has shown that there are differences where there is lower asthma and varied innate immunity response with increased microbial exposure.[14] Type of farming may be important to consider. In our study, among those who lived on a farm, the most common commodities reported were grain (76.8%) and beef (70.8%). Type of farming was not associated with asthma prevalence and did not influence associations with other variables in the model (data not shown). Our findings indicate that although FEV_1 was lower among children with nonatopic asthma compared with atopic asthma, other lung function measures did not differ significantly between the 2 groups. In addition, those with nonatopic asthma had lower FEV_1, FEV_1/FVC ratio, and $FEF_{25\%-75\%}$ than controls, whereas those with atopic asthma were not statistically different from controls. In our study population, it is possible that children with atopic asthma are more recognizable or expected within the age group and therefore are more treated, as observed as a

trend in our current study, whereas children with nonatopic asthma may be overlooked and undertreated.

Our results add to the literature regarding differences in lung function between atopic asthma groups, although the overall association between lung function and atopic asthma status still remains unclear. In a previous case-control study conducted between 1995 and 1997 with 61 patients with asthma aged 5 to 15 years in Turkey, children with atopic asthma had lower lung function (FEV_1, FVC, and peak expiratory flow rate) than children with nonatopic asthma.[39] However In a separate study conducted between 2002 and 2009 in Santiago, Chile[40] among 136 school-children (mean [SD] age, 9.07 [2.5] years) with a diagnosis of asthma or recurrent wheezing, there was no difference in FEV_1 between the atopic and nonatopic groups based on SPTs. We found that most measures of current morbidity among those with asthma did not vary significantly by atopic status. When measures of morbidity varied by atopic status, they were worse in the atopic asthma group. The exception to this was lung function, as described above. Given the atopic asthma status, a higher prevalence of sneezing and hay fever among children with atopic asthma is plausible. In addition, increasing asthma severity has been associated with increasing atopy.[41]

Aside from personal factors, we found trends of differing associations for nonatopic and atopic asthma. We must, however, be cautious of these findings given the lack of statistical significance. A dog at home in the past 12 months and being overweight were both associated with nonatopic asthma, whereas mother being an ex-smoker was associated with atopic asthma. Meta-analyses of pet ownership studies have indicated inverse associations with sensitization to aeroallergens and suggested positive associations with nonatopic asthma.[42] Being overweight or obese has been shown to increase the risk of asthma in nonatopic children.[43,44] It has been

suggested that these effects may be mediated through systemic inflammation, which may be a key factor in mediating the effects of overweight and/or obesity.[45] In our study, the association between ex-smoking mothers and asthma but not current smoking mothers may reflect changes in smoking behaviour of the mother after the diagnosis of asthma.

There are some limitations that are worth noting. First, participation rates in this study should be considered. However, when compared with children who did not take part in the clinical testing, those who took part were similar with regard to most characteristics, including history of asthma or report of allergic disease. Exceptions were those who took part were significantly more likely to be male (54% vs 48%) and younger (mean age, 9.6 years vs. 10.3 years), although the importance of each of these differences is questionable. This participation rate cannot rule out the possibility of a selection bias.

Second, asthma status can be misclassified given that the history of diagnosis is based on parent report. Nevertheless, questionnaires have been widely used to define respiratory outcomes in epidemiologic studies in children and have generally been accurate.46 The issue of disease misclassification was reduced when categorizing asthma phenotypes because atopy was measured objectively. We also consider lung function as an objective measure, further increasing the strength of our study. The principal methodologic limitations of our study were its cross-sectional design and the lack of temporal assessment to establish a causal association.

In summary, we found no associations between farm residential status and asthma phenotypes (atopic and nonatopic asthma) among school-aged children in this rural region. Although personal characteristics were associated with atopic and nonatopic asthma consistently, other observed differences in associations between risk factors and asthma status should be

interpreted with caution. Lung function measured by spirometry was generally lower in the nonatopic asthma group compared with other groups.

4.6. References

1. Asher I, Pearce N. Global burden of asthma among children. *Int J Tuberc Lung Dis.* Nov 2014;18(11):1269-1278.
2. Masoli M, Fabian D, Holt S, Beasley R. The global burden of asthma: executive summary of the GINA Dissemination Committee report. *Allergy.* May 2004;59(5):469-478.
3. Agache I, Akdis C, Jutel M, Virchow JC. Untangling asthma phenotypes and endotypes. *Allergy.* Jul 2012;67(7):835-846.
4. Ronmark E, Jonsson E, Platts-Mills T, Lundback B. Different pattern of risk factors for atopic and nonatopic asthma among children--report from the Obstructive Lung Disease in Northern Sweden Study. *Allergy.* Sep 1999;54(9):926-935.
5. Kurukulaaratchy R, Fenn M, Matthews S, Arshad S. Characterisation of atopic and non-atopic wheeze in 10 year old children. *Thorax.* 2004;59(7):563-568.
6. Moncayo AL, Vaca M, Oviedo G, et al. Risk factors for atopic and non-atopic asthma in a rural area of Ecuador. *Thorax.* May 2010;65(5):409-416.
7. Garcia-Marcos L, Castro-Rodriguez JA, Suarez-Varela MM, et al. A different pattern of risk factors for atopic and non-atopic wheezing in 9-12-year-old children. *Pediatr Allergy Immunol.* Sep 2005;16(6):471-477.
8. Stein RT, Martinez FD. Asthma phenotypes in childhood: lessons from an epidemiological approach. *Paediatr Respir Rev.* Jun 2004;5(2):155-161.
9. Von Ehrenstein O, Von Mutius E, Illi S, Baumann L, Bohm O, von Kries R. Reduced risk of hay fever and asthma among children of farmers. *Clin Exp Allergy.* 2000;30:187 - 193.

10. Riedler J, Braun-Fahrlander C, Eder W, et al. Exposure to farming in early life and development of asthma and allergy: a cross-sectional survey. *Lancet.* Oct 6 2001;358(9288):1129-1133.

11. Riedler J, Eder W, Oberfeld G, Schreuer M. Austrian children living on a farm have less hay fever, asthma and allergic sensitization. *Clin Exp Allergy.* Feb 2000;30(2):194-200.

12. Merchant J, Naleway A, Svendsen E, et al. Asthma and farm exposures in a cohort of rural Iowa children. *Environ Health Perspect.* 2005;113:350 - 356.

13. Wickens K, Lane JM, Fitzharris P, et al. Farm residence and exposures and the risk of allergic diseases in New Zealand children. *Allergy.* Dec 2002;57(12):1171-1179.

14. Stein MM, Hrusch CL, Gozdz J, et al. Innate immunity and asthma risk in Amish and Hutterite farm children. *New England Journal of Medicine.* 2016;375:411-421.

15. Pahwa P, Karunanayake CP, Hagel L, et al. The Saskatchewan rural health study: an application of a population health framework to understand respiratory health outcomes. *BMC Res Notes.* 2012;5:400.

16. Barry RJ, Pickett W, Rennie DC, et al. The role of farm operational and rural environments as potential risk factors for pediatric asthma in rural Saskatchewan. *Pediatr Pulmonol.* Sep 2014;49(9):842-851.

17. Chu LM, Rennie DC, Cockcroft DW, et al. Prevalence and determinants of atopy and allergic diseases among school-age children in rural Saskatchewan, Canada. *Ann Allergy Asthma Immunol.* Oct 2014;113(4):430-439.

18. Du Plessis V, Beshiri R, Bollman R, Clemenson H. *Definitions of rural.* Ottawa, Ontario, Canada2002.

19. The International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee. Worldwide Variations in the Prevalence of Asthma Symptoms: The International Study of Asthma and Allergies in Childhood (ISAAC). *Eur Respir J.* 1998;12:315-335.

20. Ferris Jr. B. Epidemiology Standardization Project. *Am Rev Respir Dis.* 1978;118:36-47.

21. Rennie DC, Lawson JA, Cockcroft DW, Senthilselvan A, McDuffie HH. Differences in respiratory symptoms and pulmonary function in children in 2 Saskatchewan communities. *Ann Allergy Asthma Immunol.* Jan 2004;92(1):52-59.

22. Lawson JA, Dosman JA, Rennie DC, et al. Endotoxin as a determinant of asthma and wheeze among rural dwelling children and adolescents: a case-control study. *BMC Pulm Med.* 2012;12(56):1471-2466.

23. Prochaska J, Sallis J, Long B. A physical activity screening measure for use with adolescents in primary care. *Arch Pediatr Adolesc Med.* 2001;155:554-559.

24. Biddle S, Sallis J, Cavill N. Policy framework for young people and health-enhancing physical activity. In: S B, Sallis J, N C, eds. *Young and active? Young People and Health-Enhancing Physical Activity: Evidence and Implications.* London, United Kingdom: Health Education Authority; 1998:3-16.

25. Heinzerling L, Mari A, Bergmann KC, et al. The skin prick test - European standards. *Clin Transl Allergy.* 2013;3(1):3.

26. Centers for Disease Control. Percentiles for Body Mass Index. 2000; http://www.cdc.gov/growthcharts.

27. Cole TJ, Bellizzi MC, Flegal KM, Dietz WH. Establishing a standard definition for child overweight and obesity worldwide: an international survey. *BMJ.* 2000;320:1240-1243.

28. Miller A. Lung function testing: selection of reference values and interpretative strategies. *Am Rev Respir Dis.* Nov 1992;146(5 Pt 1):1368-1369.

29. Standardization of Spirometry, 1994 Update. American Thoracic Society. *Am J Respir Crit Care Med.* Sep 1995;152(3):1107-1136.

30. Midodzi WK, Rowe BH, Majaesic CM, Senthilselvan A. Reduced risk of physician-diagnosed asthma among children dwelling in a farming environment. *Respirology.* Sep 2007;12(5):692-699.

31. Lawson JA, Janssen I, Bruner MW, Madani K, Pickett W. Urban-rural differences in asthma prevalence among young people in Canada: the roles of health behaviors and obesity. *Ann Allergy Asthma Immunol.* Sep 2011;107(3):220-228.

32. Weinmayr G, Weiland SK, Bjorksten B, et al. Atopic sensitization and the international variation of asthma symptom prevalence in children. *Am J Respir Crit Care Med.* Sep 15 2007;176(6):565-574.

33. Pearce N, Pekkanen J, Beasley R. How much asthma is really attributable to atopy? *Thorax.* Mar 1999;54(3):268-272.

34. Ponsonby AL, Gatenby P, Glasgow N, Mullins R, McDonald T, Hurwitz M. Which clinical subgroups within the spectrum of child asthma are attributable to atopy? *Chest.* Jan 2002;121(1):135-142.

35. Macneill SJ, Sozanska B, Danielewicz H, et al. Asthma and allergies: is the farming environment (still) protective in Poland? The GABRIEL Advanced Studies. *Allergy.* Apr 29 2013.

36. Pavilonis BT, Sanderson WT, Merchant JA. Relative exposure to swine animal feeding operations and childhood asthma prevalence in an agricultural cohort. *Environ Res.* Apr 2013;122:74-80.

37. Braun-Fahrlander C, Riedler J, Herz U, et al. Environmental exposure to endotoxin and its relation to asthma in school-age children. *N Engl J Med.* Sep 19 2002;347(12):869-877.

38. Ege MJ, Frei R, Bieli C, et al. Not all farming environments protect against the development of asthma and wheeze in children. *J Allergy Clin Immunol.* May 2007;119(5):1140-1147.

39. Gurkan F, Davutog Lu M, Bilici M, Sincar N, Haspolat K. Pulmonary functions in atopic and nonatopic asthmatic children. *Allergol Immunopathol (Madr).* Mar-Apr 2002;30(2):70-73.

40. Castro-Rodriguez JA, Navarrete-Contreras P, Holmgren L, Sanchez I, Caussade S. Bronchial hyperreactivity to methacholine in atopic versus nonatopic asthmatic schoolchildren and preschoolers. *J Asthma.* Oct 2010;47(8):929-934.

41. Carroll WD, Lenney W, Child F, et al. Asthma severity and atopy: How clear is the relationship? *Arch Dis Child.* 2006;91:405-409.

42. Takkouche B, González-Barcala FJ, Etminan M, Fitzgerald M. Exposure to furry pets and the risk of asthma and allergic rhinitis: a meta-analysis. *Allergy.* 2008;63(7):857-864.

43. Kelley CF, Mannino DM, Homa DM, Savage-Brown A, Holguin F. Asthma phenotypes, risk factors, and measures of severity in a national sample of US children. *Pediatrics.* 2005;115(3):726-731.

44. Visness CM, London SJ, Daniels JL, et al. Association of childhood obesity with atopic and nonatopic asthma: results from the National Health and Nutrition Examination Survey 1999-2006. *J Asthma.* Sep 2010;47(7):822-829.

45. Sideleva O, Black K, Dixon AE. Effects of obesity and weight loss on airway physiology and inflammation in asthma. *Pulm Pharmacol Ther.* Aug 2013;26(4):455-458.

46. Yang CL, To T, Foty RG, Stieb DM, Dell SD. Verifying a questionnaire diagnosis of asthma in children using health claims data. *BMC Pulm Med.* 2011;11:52.

Table 4-1. Personal and Environmental Characteristics by Asthma Status[a]

Characteristics	No asthma (n=451)	Non-atopic asthma (n=53)	Atopic asthma (n=25)
Patient characteristics			
Age, mean (SD), y	9.6 (2.2)	9.6 (2.1)	10.0 (2.5)
Female	47.7	49.1	32.0
Nonwhite	7.2	11.3	16.0
With early respiratory illness (<2 years of age)	26.2	60.4[b]	48.0[c]
Overweight or obese	24.4	43.4[b]	36.0
Breastfed	82.0	82.7	88.0
Born premature	9.4	15.1	8.3
Low birthweight (<2,500g)	5.5	9.6	16.7[c]
Firstborn	39.2	35.8	48.0
Day care attendance	57.8	50.9	68.0
Farm-based exposure			
Currently live on a farm	45.5	39.6	41.7
Lived on a farm in first year of life	31.0	34.0	20.0
Mother lived on farm while pregnant	32.1	34.6	20.0
Haying or moving or playing with bales regularly[d]	25.2	25.5	20.0
Feeding livestock regularly[d]	29.1	27.5	20.0
Playing or cleaning in barns regularly[d]	24.5	27.5	12.0
Emptying or filling grain bins regularly[d]	9.2	3.9	0.0
Cleaning or playing in pens or corrals regularly[d]	24.4	17.6	12.0
Riding horses regularly[d]	9.2	9.8	4.0
Family Characteristics			
Mother smoking			
Never smoker	64.0	47.2[b]	36.0[c]
Ex-smoker	16.8	26.4	36.0
Current smoker	19.2	26.4	28.0
Father smoking			
Never smoker	55.8	39.6[b]	48.0
Ex-smoker	17.7	26.4	12.0
Current smoker	26.5	34.0	40.0
Mother smoked during pregnancy	19.2	32.1	29.2
Maternal education with any postsecondary	64.0	57.7	52.2
Paternal education with any postsecondary	47.7	47.2	33.3
Family history of asthma	12.2	32.1[b]	28.0[c]
Home exposure			
Dog at home in last 12 months	40.6	56.6[b]	40.0
Dog in first year of life	28.7	37.3	34.8
Cat in the home in last 12 months	35.9	39.6	56.0[c]
Cat in first year of life	27.0	35.3	30.4
Home built prior to 1980	64.1	65.4	81.0
With mould or mildew smell	24.8	24.0	12.0

With home mould	21.1	22.6	28.0
Heating type			
Natural gas	71.8	62.3	76.0
Coal or wood	11.3	17.0	4.0
Other	16.9	20.8	20.0
With major home renovations in last 12 months	27.9	26.4	16.7

[a]Data are reported as percentage of patients unless otherwise indicated. The percentages reported are column percentages, indicating the proportion of children within the status group (column) who experienced the exposure listed. In the last 12 months refers to exposure at any point in the past 12 months.
[b]$P < .05$ comparing no asthma and nonatopic asthma based on independent samples χ^2 tests.
[c]$P < .05$ comparing no asthma with atopic asthma based on independent-samples χ^2 tests.
[d]Regularly is defined by a positive response to at least 1 hour at a time taking part in the listed activities every day, at least once a week, or at least once a month.

Table 4-2. Results of Multivariate Analysis for Factors Related to Nonatopic and Atopic Asthma in Children[a]

	Non-atopic asthma (Ref: No asthma)		Atopic asthma (Ref: No asthma)	
	OR (95% CI)	p-value	OR (95% CI)	p-value
Age	1.02 (0.88-1.19)	.78	1.17 (0.94-1.44)	.15
Female (ref: Male)	1.05 (0.56-2.01)	.88	0.54 (0.21-1.41)	.21
Mother with more than high school (ref: high school or less)	0.87 (0.45-1.70)	.69	0.75 (0.29-1.94)	.56
Early respiratory illness (ref: none)	4.20 (2.19-8.06)	<0.001	3.10 (1.23-7.75)	.02
Family history of asthma (ref: none)	3.27 (1.55-6.85)	.002	3.29 (1.10-9.80)	.03
Farm dwelling (ref: not farm)	0.94 (0.45-1.97)	.87	1.43 (0.51-3.97)	.50
Mother ex-smoker (ref: never)	1.46 (0.60-3.54)	.56	3.13 (0.92-10.61)	.07
Mother current smoker (ref: never)	1.06 (0.34-3.36)	.92	2.12 (0.47-9.71)	.33
Smoking during pregnancy (ref: none)	1.84 (0.68-5.00)	.23	1.11 (0.31-4.05)	.87
Dog at home in the past 12 months (ref: no dog)	1.84 (0.96-3.51)	.07	0.77 (0.30-1.99)	.59
Cleaning or playing in pens or corrals regularly[b] (ref: irregular)	0.63 (0.25-1.56)	.32	0.43 (0.11-1.68)	.22
Overweight or obese (ref: Not overweight or obese)	1.88 (0.97-3.66)	.06	1.80 (0.70-4.59)	.22

Abbreviations: CI, confidence interval; OR, odds ratio.

[a] Adjusted for each variable in the table. Results in bold indicate P < .05.

[b] Regularly is defined by a positive response to at least 1 hour at a time taking part in the listed activities every day, at least once a week, or at least once a month.

Table 3. Respiratory Symptoms, Allergic Symptoms, Health Care Use, and Morbidity and Indicators Specific to Asthma[a]

Outcome	Patients, %			p-value[b]		
	No asthma (n=451)	Nonatopic asthma (n=53)	Atopic asthma (n=25)	No asthma vs Nonatopic	No asthma vs Atopic	Nonatopic vs atopic asthma
Respiratory symptoms						
Wheeze in the past 12 months	5.5	43.4	56.0	<0.001	<0.001	.30
Shortness of breath ever	4.5	37.7	62.5	<0.001	<0.001	.04
Shortness of breath in the past 12 months	2.0	18.9	36.0	<0.001	<0.001	.10
Wheeze with exercise ever	7.2	52.8	58.3	<0.001	<0.001	.65
Wheeze with exercise in the past 12 months	4.2	37.7	36.0	<0.001	<0.001	.88
Allergic symptoms and conditions						
Itchy rash ever	13.8	28.3	24.0	.006	.16	.69
Rash in the last 12 months	13.7	28.3	24.0	.005	.15	.69
Eczema	25.7	35.8	40.0	.12	.12	.72
Sneezing ever	25.2	54.7	68.0	<0.001	<0.001	.27
Sneezing in the past 12 months	21.8	35.8	64.0	.02	<0.001	.02
Hay fever	6.5	11.8	52.4	.17	<0.001	<0.001
Health care utilization and morbidity						
Use of breathing medications	1.6	48.1	50.0	<0.001	<0.001	.88
Parent miss work	5.6	24.5	8.0	<0.001	.61	.08
3 days missed school from chest illness	6.2	20.8	24.0	<0.001	.001	.75
High activity in a week	63.4	60.4	64.0	.66	.95	.76
Indicators specific to asthma						
Mean age of diagnosis (SD), years		3.7 (3.0)	3.1 (2.4)			.40
Visited a doctor for asthma in the past 12 months		40.4	50.0			.43

With a written action plan	15.7	30.4	.14
No. of asthma episodes in the past 12 months			
None	37.2	39.1	
1-2	30.2	21.7	
> 2	32.6	39.1	.74
Asthma medication use in the past 12 months			
Not every day or nearly every day	71.7	52.0	
Every day or nearly every day	28.3	48.0	.09
Missed school due to asthma	25.0	31.8	.56
Sleep disturbance in the past 12 months	42.3	64.0	.08

[a] Data are reported as percentage of patients unless otherwise indicated. The percentages reported are column percentages, indicating the proportion of children within the status group (column) who experienced the exposure listed. In the last 12 months refers to exposure at any point in the past 12 months.

[b] On the basis of independent-samples χ^2 tests for categorical comparisons and independent-samples t test for age of diagnosis.

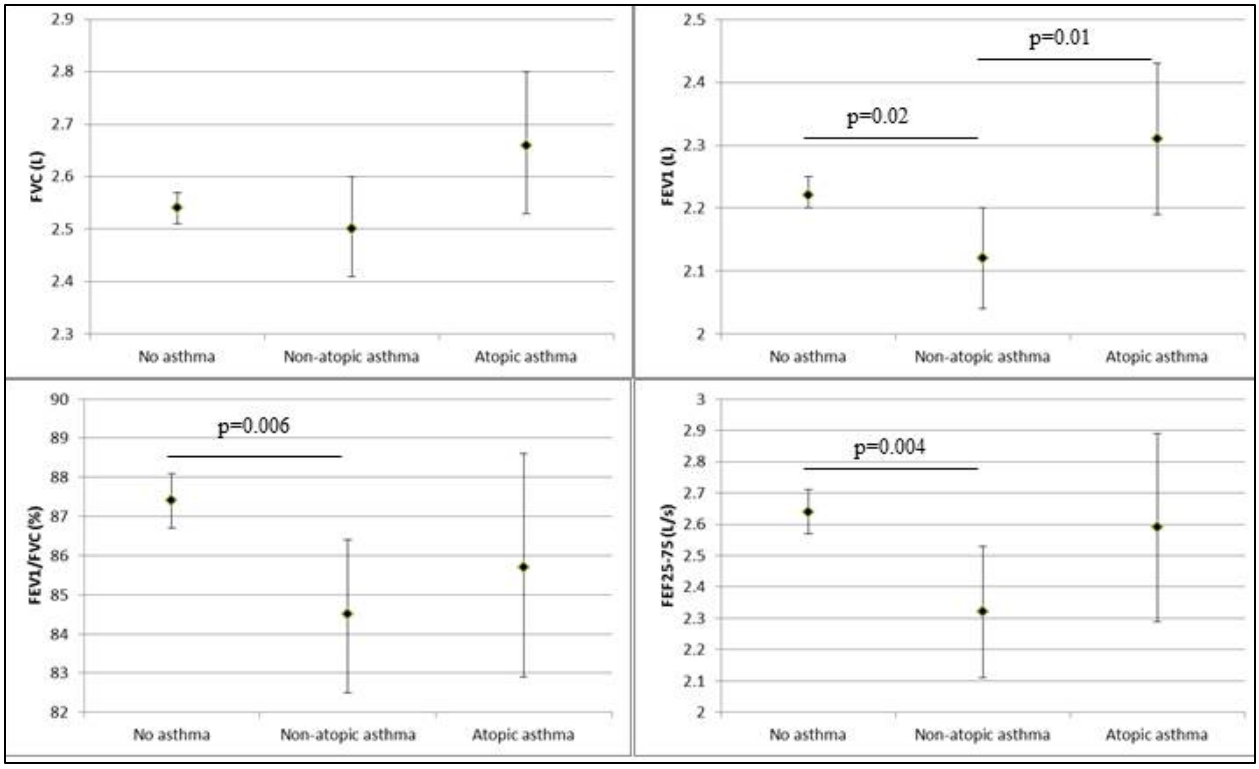

Figure 4-1. Mean lung function test results by asthma status. Statistical comparisons are based on analysis of covariance after adjustment for age, height, sex, and asthma status. Error bars indicate SDs. $FEF_{25\%-75\%}$, forced expiratory flow between 25% and 75%; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity.

CHAPTER 5

FARM EXPOSURES AND ATOPIC DISEASE AMONG CHILDREN LIVING IN A RURAL SETTING

(MANUSCRIPT 3)

Authors: Luan M. Chu, MSc[1,2]; Donna C. Rennie, PhD[2]; Shelley Kirychuk, PhD[2,3]; Don Cockcroft, MD[4]; John Gordon, PhD[4]; William Pickett, PhD[5]; James Dosman, MD[2]; Joshua A. Lawson, PhD[2,4]

Affiliations: [1]Health Sciences program, College of Medicine, University of Saskatchewan, Saskatoon, Saskatchewan, Canada

[2]Canadian Center for Health and Safety in Agriculture, University of Saskatchewan, Saskatoon, Saskatchewan, Canada

[3]College of Nursing, University of Saskatchewan, Saskatoon, Saskatchewan, Canada

[4]Department of Medicine, College of Medicine, University of Saskatchewan, Saskatoon, Saskatchewan, Canada

[5]Department of Public Health Sciences, Queen's University, Kingston, Ontario, Canada

Corresponding author: Luan Manh Chu, M.Sc, Ph.D. Candidate, Canadian Center for Health and Safety in Agriculture, University of Saskatchewan, Saskatoon, Saskatchewan, Canada, 104 Clinic Place PO Box 23, Saskatoon, SK, S7N 5E5, Canada; E-mail: cml779@mail.usask.ca

Contributor statement: LC conceptualized and designed the study, carried out the initial analyses and interpretation of the data, drafted the initial manuscript, reviewed and revised the manuscript and approved the final manuscript. DR, SK, DC, JG, WP, JD critically reviewed the manuscript and approved the final manuscript. JL designed the recruitment strategy, coordinated and supervised data collection, critically reviewed the manuscript and approved the final manuscript. WP and JD are co-investigators for the Saskatchewan Farm Injury Cohort.

All authors accept accountability for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The corresponding author confirms full access to all aspects of the research and writing process and takes final responsibility for this paper.

Funding sources: This research was conducted with support from Canadian Institutes of Health Research Operating Grant 200109MOP-230156 – PH1-CEDA-56847 'Saskatchewan Farm Injury Cohort – Phase 2'. *Financial disclosure*: The authors have indicated they have no financial relationships relevant to this article to disclose.

5.1.Abstract

BACKGROUND: A lower prevalence of atopic disease and asthma have been reported in farm children compared to their non-farm counterparts. While living on a farm is an overall indicator of agricultural exposures, investigation of specific exposures that may be associated with asthma, atopy, and asthma phenotypes is important to help understand the etiology of these conditions. This study aims to examine the associations between such exposures and asthma, atopy and asthma phenotypes in farm children.

METHODS: This was a cross-sectional analysis of data collected from across the province of Saskatchewan, Canada in 2014. Surveys were completed by parents of 2275 rural dwelling children (farm and non-farm) aged 0 to 17 years within 46 rural schools. Questionnaires were distributed through schools for parents to complete. Parents reported on the child's conditions, including asthma and atopy, as well as the child's related occupational and environmental exposures. Children aged 6-17 years with information about farm activities were included in multivariate analyses. Information on asthma and atopy were combined to define asthma phenotype (i.e. atopic vs non-atopic asthma).

RESULTS: Asthma prevalence was 7.6% while the prevalence of atopy was 4.7%. Among those with asthma, 29.5% were atopic. The prevalence of asthma, atopy, and asthma phenotype did not differ by location of dwelling (farm vs non-farm). Farm children participated in farm-related activities/work more frequently than their non-farm counterparts (range of participation among farm children: 5.5% to 36.8%; among non-farm children 0.4% to 6.8%; $p<0.05$). After adjustment for potential confounders, home location (farm vs non-farm) was not associated with asthma, atopy, or asthma phenotype. Doing farm work in the summer was associated with an increased

risk of asthma [adjusted OR (aOR) =1.71 (1.02-2.88); p=0.041]. Doing routine chores with large animals was associated with an increased risk of asthma [aOR=1.83 (1.07-3.15); p=0.027], and atopic asthma [aOR= 2.37 (95%CI=1.04-5.40); p=0.04].

CONCLUSION: The present study showed that the prevalence of asthma, atopy, and asthma phenotypes were similar between farm and non-farm rural children. However, completion of some farm activities was associated with an increased risk of asthma. In rural areas, characterisitcs of farm activities may be a better indicator of exposure than location of dwelling.

Keywords: Farm activities, children, atopic disease, asthma, rural settings

5.2.Introduction

Over the past few decades, childhood asthma and atopy prevalence have been increasing worldwide.[1,2] These conditions have also been shown to vary geographically,[3-7] including a reportedly lower prevalence in farming locations.[3,6] While most studies from Europe have found a lower prevalence of asthma and atopic disease between farm and non-farm children living in rural areas,[8-12] recent findings from Canadian studies including from the province of Saskatchewan, have suggested that there may not be differences in atopy or asthma between farm and non-farm locations in rural dwelling school-age children.[13-15] A possible explanation for the similarities found could be a lack of variability in exposures between farm and non-farm children since those non-farm children living adjacent to farming areas could readily take part in farm activities. Therefore, using the location of residence as a surrogate for farm exposures might not be an accurate measure of agricultural exposure. It may be more useful to examine specific farm exposures and the intensities of activities undertaken.

There have been studies examining the associations between farm activities as opposed to location of residence (i.e., farm vs non-farm) and asthma and atopic disease in children; however, results have been inconsistent. Some studies have found protective effects of farm activities,[8,10,16] while others have not.[14,17] Although there are some inconsistencies, the farming effects on asthma and atopic disease could depend on different types of farming practices.[13,17-20] For example, in European studies conducted in rural regions of Germany, Austria, and Switzerland among school-age children, the authors found a protective effect of living on a farm (often where farming activities are close to the homes) against asthma, hay fever, and atopy,[8,12] while some studies in the United States (e.g. large-scale hog confinements) showed an opposite effect.[20,21] In other

locations, it has been found that while livestock farms may protect from asthma[19,22], grain or dust exposures may increase the risk[17,18].

The purpose of this study was to examine the association between farm-related environmental factors and asthma, atopy and asthma phenotypes in children while highlighting the farming exposure experiences of non-farm rural children in an agricultural province of Canada. The following research questions were considered: 1) Is there a difference in the prevalence of asthma, atopy, and asthma phenotypes between farm and non-farm rural children? 2) Compared to children living on a farm, to what extent are children not living on a farm but living in rural areas exposed to farm-related environments and activities? 3) What are the potential agricultural risk and protective factors associated with asthma, atopy, and asthma phenotypes among children in rural Saskatchewan? This study is important because it moves beyond the investigation of farm dwelling and its relationship with asthma, atopy, and asthma phenotypes and more into the specific farm activities in a large representative population of children living in a rural setting including both farm and non-farm children.

5.3.Methods

The Saskatchewan Farm Injury Cohort (SFIC)[23] is a prospective cohort study which aimed to understand the determinants of health among rural dwellers. This includes individual risk factors related to farm exposures as well as contextual and environmental factors related to farm work environments. While the study primarily investigated the etiology of farm injury, secondary foci included several other health conditions common to children. The SFIC Phase 1 began in January 2007 and was designed as a multistage, stratified sample of farms. Clusters of farms were nested within rural municipalities across the province of Saskatchewan. A limitation, however, was that very little information on children was collected. To address this, in 2014, the SFIC – Child Cohort was established.[23] This cohort aimed to study the determinants of health specifically among children in rural areas. The current analysis is based on data from the 2014 SFIC-Child Cohort.

Study population and procedures

Baseline data from the Saskatchewan Farm Injury Cohort Study (SFICS)-Child Cohort[23] were used. The sampling frame and data collection approach have been described elsewhere.[23-25] In brief, this cohort was designed to target a population that included families (farm and non-farm) with children attending rural schools in Saskatchewan. Families from 46 schools nested within 7 randomly selected school divisions were included. Questionnaires were distributed through schools for parents to complete. One questionnaire was completed for all children in the family with a section in the questionnaire designated specifically to each child. Baseline data collection was from January to April 2014. Protocols for the SFIC component were reviewed and approved by the Behavioural Research Ethics Board at the University of Saskatchewan (BEH #12-265) and Queen's University Health Sciences and Affiliated Teaching Hospital Research Ethics Board (EPID 358-11).

Two versions of the questionnaires were distributed based on the location of residence of the children (farm vs nonfarm). Both questionnaires included identical questions about the children's demographics, health history, farm exposures and activities, and health behaviours. The 'farm family version' included additional questions about the farm on which the child lived, while the 'rural family version' asked the same questions but about a farm that the child had visited most often.

Operational definitions

The **primary outcome variables** of asthma, atopy, and asthma phenotype were based on the following question, which was asked for each child: "Has a doctor diagnosed any of the following long term conditions for this child:...(Check all that apply)". One response option was "asthma" while another was "respiratory allergies (e.g. hayfever)". The latter response option was used to define the presence or absence of atopy. From these two response options about asthma and atopy, three outcomes were used: asthma present (Yes/No); atopy present (Yes/No); and asthma phenotypes [(atopic asthma (yes to asthma and yes to respiratory allergy); non-atopic asthma (yes to asthma and no to respiratory alleryg); and no asthma)]. Parental responses to a questionnaire report of doctor diagnosis for asthma have been shown to have high validity when compared to physician assessment of asthma[26,27] and have been previously used in epidemiologic studies with pediatric populations[28,29]. Questionnaire report from parents of 5-9 year olds using "Has your child ever had asthma?" and "Was this diagnosed by a doctor?" has been validated using health claims data showing low sensitivity (59.0%) but high specificity (95.9%) for asthma.[27] A 2015 validation study of schoolchildren (grades 1–8) from rural Saskatchewan found that the percentage of agreement between questionnaire and SPTs ranged between 74.0 to 89.5%.[31] In epidemiologic studies that estimate prevalence, a highly sensitive test is preferable while more specific tests are

recommended to estimate risk,[30] which are to be used in etiologic studies and is therefore more appropriate for our current study.

The **primary independent variables** were the residence locations (farm vs. non-farm), and farm work/activities/exposures. Exposures to farm activities and work included whether each child had *"*ever worked on a farm or ranch?*"* and *"*how many hours per week*"* the child spent *"*doing farm work*"* for each season (fall, summer, spring, and winter) during the past year. Information on how often the child spent at least 1 hour taking part in the following (in the past year): *"*haying/moving/ playing with hay bale*"*, *"*feeding livestock*"*, *"*cleaning or playing in barns*"*, *"*cleaning grain bins*"*, *"*cleaning or playing in pens/corals*" was* collected. Children were considered to perform an activity regularly if they selected "At least once a month" or more as has been used previously[14,32]. Information on how often (days per year and hours per day) the children did the following (in the past year): "routine chores with large animals? (e.g., cattle or pigs)", "routine chores with small animals? (e.g., chickens)", "herd maintenance activities? (e.g., branding, vaccinating", "veterinary activities? (e.g., medications, breeding, birthing)". Dichotomous variables (Yes/No) for each activity were also created.

Potential confounding variables included: Age groups *("*0–5 years", *"*6–12 years", *"*13–17 years"); SexMales/Female); Smoking status (To your knowledge, does this child smoke cigarettes on a daily basis? Yes/No); Physical activity ["Over a typical or usual week, on how many days is this child physically active for a total of at least 60 minutes per day?" (Less than 2 days/week/3-5 days/week/6-7 days/week")]; Environmental tobacco smoke (ETS) (Does anyone in this household smoke regularly inside the house? Yes/No); and Parental Education levels (Less or completed High School/ High School or more). Asthma medication use was based on the question: "Does the child take any of the following prescribed medication on a regular basis?

Check all that apply,Ventolin (inhalers for asthma) (Yes/No). Ventolin is the trade name for a commonly prescribed bronchdilator to treat asthma exacerbations in the class of short-acting beta-agonists (SABAs).[33] We will use the term SABA for the remainder of this report to describe their use.

Statistical analyses

We used SAS Version 9.4 (SAS Institute, Cary, NC, 2012), SPSS statistical software Version 26 (SPSS Inc, Chicago, Illinois), and STATA/SE Version 15.1 (StataCorp LLC, College Station, Texas). Initially, descriptive analyses using frequencies for categorical data and mean values and standard deviations (SD) for continuous data were completed. The prevalence of outcomes of interest included asthma, atopy, and asthma phenotypes (no asthma, non-atopic asthma, and atopic asthma) were calculated. The prevalence of characteristics and risk factors was calculated for each outcome with statistical comparisons between outcome categories completed using Rao-Scott chi-square tests adjusting for clustering by families.

After the descriptive analyses, a series of binary ("PROC GENMOD" command in SAS) logistic regression models for the outcomes of asthma and atopy and multinomial logistic regression models ("mlogit" command in STATA) for the outcome of asthma phenotype using a generalized estimating equations approach were used to develop the model to account for clustering by farms/families. Among young children (0-5 years old), there was a very low prevalence of those who participated in farm activities. Also, because our sampling frame was school lists, young families participating in this study who did not have children in school yet may be missed, biasing the under 6 population in the study. Because of this, we conducted multivariate analyses only in children aged 6-17 years. Our independent variables of primary interest were the residence locations (farm vs. non-farm), and farm work/activities/exposures. Our outcome

variables were asthma, atopy, and asthma phenotype. We included the following variables in the regression models as they were considered to be potential confounders: age, sex, ETS, parental education, and physical activity. First, we fitted a base model that included location of residence and potential confounders for each outcome. Then we added each variable representing a farm work/activity to the aforementioned base models for each outcome. We tested each activity variable separately in the models for each outcome to avoid co-linearity and small cell sizes.

After fitting the base models, a mediation analysis approach was undertaken. This was done to examine how much of the association between location of residence (farm vs non-farm) and each outcome was explained by a specific farm activity. Each variable representing one specific farm activity *was added to the base model which included the location of residence. The potential mediation effect of each variable was assessed by:* $([OR_{\text{full model without mediator}} - OR_{\text{full model}}]/ OR_{\text{full model}})*100$. For mediation to occur, a statistically significant association between the primary exposure (farm vs. rural non-farm status) and the outcomes (asthma, atopy, and asthma phenotypes) must be smaller and no longer statistically significant when the mediator is included.[34] A 5% change in the association is considered to be a meaningful difference.

A stratified analysis was conducted to limit the effect of different age groups when looking at the relationship between exposures and outcomes. Guidelines have been developed to recommend when children should be exposed to different farm activities.[35] In addition, children take on greater responsibilities on the farm through different amounts and types of work as they age. Because of these things, it is important to consider the analyses by age group.

We also conducted two sensitivity analyses. First, based on our definition of asthma phenotype, atopic children without asthma would be part of our control group. In order to examine any potential impact of having this group remain in the control population (i.e., no asthma group),

we re-ran all of the multinomial logistic regression modeling to look at asthma phenotype after removing those children in the control group who were atopic. Second, we conducted a sensitivity analysis with a more specific definition of asthma which required an affirmative response to the question around a physician-diagnosis of asthma as well as a positive response to the question regarding SABA use. Here, we reran all the binary logistic regression analyses using this modified asthma outcome in place of the original asthma outcome.

Odds ratios (ORs) and 95% confidence intervals (CIs) were used to present the strength of the associations between farm exposures with asthma, atopy, and asthma phenotype. For asthma phenotype, we used the relative-risk ratios (RRRs) (equivalent to ORs) and 95% CIs as suggested by the Stata Manual.[36,37] However, throughout this manuscript, the term ORs will be used for consistency.

5.4.Results

The final sample included 2328 children from 1094 families, of which 1737 children from 818 families were nonfarm residents. The response rate was 31%. Overall, the prevalence of asthma among children 0-17 years old was 7.6% (95% CI=6.5-8.7). The prevalence of atopy was 4.7% (95% CI=3.8-5.6). Of the children with asthma, 29.5% (95% CI=23.1-36.7) were atopic. Among the no asthma group, 2.6% (95% CI=2.0-3.4) were atopic. The prevalence of asthma, atopy, and asthma phenotypes were not statistically different between farm and rural non-farm dwellers (**Table 5-1**).

Overall, 75.6% among farm dwelling children participated in a farming activity compared to 27.8% among rural non-farm dwelling children. Farm children also participated in all specific farm-related activities/work more frequently than did their non-farm counterparts (range of participation among farm children: 5.5% to 36.8%; among non-farm children 0.4% to 6.8%; $p<0.05$) (**Table 5-2**). Overall, 53.9% of the population were rural non-farm dwelling children who did not take part in farm activities, 20.7% were rural non-farm dwelling children who took part in farm activities, and 25.4% lived on a farm.

Those who did routine chores with large animals (e.g., cattle, pigs) were more likely to have asthma (12.1% vs. 7.1%; p=0.026) than those who did not. Asthma and atopy were more prevalent among 13-17 years old compared to the other age groups (**Table 5-3**) and boys were more likely to have these conditions.

After adjustment for potential confounders, doing farm work in the summer was associated with an increased risk of asthma [adjusted OR (aOR)=1.71 (95%CI=1.02-2.88); p=0.041] (**Figure 5-1**). Doing routine chores with large animals was associated with an increased risk of asthma [aOR=1.83 (1.07-3.15); p=0.027] (**Figure 5-1**), and atopic asthma [aOR= 2.37 (95%CI=1.04-

5.40); p=0.04] (**Figure 5-2**). We observed no other statistically significant associations between farm-related exposures and the outcomes considered (**Figures 5-1 and 5-2**).

The following farm activities changed the association between the location of residence and outcomes of interests by more than 5% in mediation analyses: 1) Asthma (cleaning or playing in barns, 7.7%); 2) Respiratory allergies (routine chores with large animals, 10.5%; performing haying/moving/playing with hay bales; feeding livestock; cleaning or playing in barns; cleaning grain bins; cleaning or playing in pens/corals; 9.6-22.1%); Atopic and non-atopic asthma (farm work in the summer, 19.3%; tractor operation, 12.5%; combine operation, 5.08%; routine chores with large animals, 15.5%; and doing veterinary activities, 8.5%). However, regardless of the change, mediation the original associations between the location of residence and the outcomes were not statistically significant.

Results from the stratified analyses showed that among children 6-12 years of age, routine chores with large animals were associated with an increased risk of asthma [aOR=2.18 (95%CI=1.05-4.51); p=0.034], and specifically, atopic asthma [aOR=3.41 (95%CI=1.35-8.62); p=0.009]. These associations were not observed in the older age group **(Appendix E & F).**

When we considered asthma presence defined by an affirmative response to both "physician-diagnosed asthma" and "the use of SABA", the overall prevalence of the combination was 5.2 %, in which 1.7% were considered atopic. Although not statistically significant, the associations between farm exposures and outcomes (asthma, atopy, and asthma phenotypes) were generally in the same direction as those when using only the definition of physician diagnosed asthma alone (data not shown). Also, in another sensitivity analysis, very few children did not have asthma but had allergies, the removal of these children did not affect the results.

5.5.Discussion

The main purpose of this study was to examine the association between farm-related environmental factors and asthma, atopy and asthma phenotypes in children while highlighting the farming exposure experiences of non-farm rural children in an agricultural province of Canada. Our most important findings were: The prevalence of asthma was 7.6%, the prevalence of atopy was 4.7% and that most of those with asthma had non-atopic asthma; The majority of children living on a farm were engaged in farm work while a third of children living in a non-farm rural setting performed farm work; Those who did routine chores with large animals were more likely to have asthma, specifically with atopic asthma; and these associations were observed in children aged 6-12 years but were not observed in adolescents aged 13-17 years.

The prevalence of asthma in the current study was in the range of values consistent with the literature which has ranged from 5.3%[38] to 15.4%[13]. The prevalence of atopy was lower (4.7%) compared to a previous finding from rural areas of the province of Saskatchewan (8.8%),[15] possibly due to the differences in definitions of allergy used between studies where the earlier study used skin prick testing. Our finding, however, contradicts the common thought that childhood asthma is generally an atopic disease. We found a higher prevalence of non-atopic asthma compared to atopic asthma. This was observed in a previous study in rural Saskatchewan children where 32.1%[32] of those with asthma were atopic based on skin prick testing. The proportion of those with atopic asthma is also in the range observed from other studies[39-41] (ranging from 4% to 63%[39] for atopic asthma).

The prevalence of asthma, atopy, and atopic asthma was not statistically different between farm and nonfarm dwellers. This observation is also in line with a previous finding from rural Saskatchewan[32] with a possible explanation of homogeneity of farm exposures between farm and small-town dwellers[14,15,32]. In the current study, we found a high proportion of non-farm dwellers

who were exposed to farming activities. This was based on completing actual activities so is likely an underestimate as to how much rural-non farm children are exposed to farms since these children would likely visit some farms without being involved in activities such as to visit relatives who farm in the area. However, our finding is inconsistent with several other studies, mostly from European countries, examining this relationship where protective effects of exposures to a farm have been consistently observed.[8-12] This has been partly explained by high exposures to micro-organisms, in particular, bacterial endotoxin[8-12] where it has been shown that household endotoxin was significantly elevated in house dust in homes of farm children and in non-farm children with regular contact with farm animals.[42]

The associations between farm activities and childhood allergies and asthma as opposed to farm dwelling have been examined previously, although less frequently and with inconsistent results.[3,8,12,16] Data from large European studies including the Prevention of Allergy – Risk Factors for Sensitization in Children Related to Farming and Anthroposophic Lifestyle (PARSIFAL) and the multidisciplinary Study to Identify the Genetic and Environmental Causes of Asthma in the European Community (GABRIEL)[8-12] generally demonstrated a protective effect of participating in farm work and allergies or asthma in children. However, some specific farm-related activities including keeping hares and rabbits and the use of pressed hay was positively associated with childhood asthma.[18]

In the USA, a child cohort study (birth through 17 years of age) by Merchant et al[20] in 2005 found that a higher risk of asthma was associated with farm residence when swine was raised and even more so when swine was raised and antibiotics were added to animal feed. Findings from the United States 2008 Minority Farm Operator Childhood Agricultural Injury Survey[21] (M-CAIS) suggested that children (ages 0–19 years) who were working on their household farms had a

significantly increased prevalence of asthma (13%) compared to children who were not participating in the farming operations (9%). Exposures to specific types of farms such as beef cattle farms and ranches with animal and animal products (dander, fur, and body wastes) have been associated with asthma and other respiratory symptoms.[43] In Saskatchewan, the associations between farm activities and respiratory illness have not always been protective.[14,17] Children who were exposed to emptying and filling of grain bins had higher odds of asthma and reports of playing on or near hay bales and cleaning pens were associated with increased respiratory symptoms.[17]

The increased risk of pediatric asthma in relation to some farm activities observed in Saskatchewan and the United States might be explained partly by the similarity in farm practices and environmental layouts North American farms. Environmental irritants and allergens in the farming environments that North American children are exposed could also different from those found in European farms. . Our findings are similar to those found by others in North America about allergy and asthma and associations with farm activities. We found that 6-12-year-old children who did routine chores with large animals were at an increased risk of asthma. This finding was, in part, in line with previous studies in Saskatchewan that showed feeding livestock was a risk factor for wheeze[14] and an increased risk of respiratory symptoms was reported in children who cleaned large animal pens.[17] Emissions from livestock operations that are in close proximity to children's living areas might be a source for respiratory exposures and potentially associated with the respiratory health outcomes.

There are some potential limitations of our study. First, the response rates in this study were modest (31%). Second, recall error might have occurred regarding information about various farm exposures in the past year collected by questionnaire. However, similar questions have been

used in previous studies[13,14,17] in the same province, making the results comparable. The results should be interpreted with caution when comparing to other studies in different regions (e.g., Europe) where these specific questions were not used and farming practices may be quite different. Third, there may be some residual confounding present. This study was originally designed to collect data on farm injury. Some important information related to asthma and allergy was not collected (e.g. family history of asthma or allergies, mould presence, and anti-inflammatory medication use, etc.). Fourth, the information on atopy was not objectively measured, leading to potential misclassification. The use of parental report of physician-diagnosed asthma or allergic diseases in questionnaires is acceptable and commonplace in epidemiological studies.[44] A previous validation study[44] was conducted in 2006 in 2884 Canadian school children aged 5 to 9 years to compare to the agreement between "questionnaire diagnosis" (affirmative responses to the questions "Has your child ever had asthma?" and "Was this diagnosed by a doctor?") and a previously validated "health claims data diagnosis". The authors observed that questionnaire diagnosis was insensitive (59.0%) but specific (95.9%) for asthma and moderate agreement (kappa = 0.60). It is noted that in epidemiologic studies that estimate prevalence, a highly sensitive test is preferable to calculate prevalence while more specific tests are required to estimate risk,[30] which are to be used in etiologic studies. In addition, to evaluate the agreement between questionnaire responses and skin prick testing (SPT) to assess atopy in rural children, a validation study[31] (2015) that included 480 schoolchildren (grades 1–8) from rural Saskatchewan found that the percentage of agreement between questionnaire and SPTs ranged between 74.0 to 89.5%. Finally, the limitations of a cross-sectional study, as in our study, such as temporality cannot be ignored.

Our study also has several strengths. First, data from this study was from a strong sampling technique with stratification and random selection at the municipality level. It also covered a broad

range of the province and included a heterogeneous degree of exposures allowing for a generalizable sample of rural-dwelling children. Second, specific and detailed farm exposures were collected, allowing us to examine these exposures in association with not only farm injuries but also other health outcomes, (i.e., asthma and allergies). Third, we conducted several analyses to increase our confidence in the results. We included only children 6-17 years of age for the multivariate analyses and omitted information for children 0-5 years of age due to lack of exposures to farm activities within this age group and the potential to miss to include this group in this study. We also tested the results using a more robust definition of asthma (asthma and SABA use). The use of this robust asthma definition did not change the results appreciably when it comes to its associations with farm exposures strengthening the evidence from our study.

We were unable to collect datas about corticosteroid use or antihistamine use which could further our understanding of what contributed the association founf=c between asthma amd large animals which likely included such actictivites as milking and feeding cows and riding, feeding and grooming horses.

CONCLUSIONS

The present study showed that the prevalence of asthma, atopy, and asthma phenotypes were similar between farm and non-farm children. Those who did routine chores with large animals were at an increased risk of asthma, specifically with atopic asthma. There did not appear to be differential involvement in farming activities between those with and without asthma.

I am still having issues here with assessing the relative importance of children who live off the farm but visit it and participate in farming activites. I think you had a question that identified the number who visited farm and then also have a number of those who participated in activities.

Could you not run the analysis excluding the non-farm kids to determine the relative importance of their role in farming exposures? Maybe I am missing something here.

Also mechanisims x=could have been discussed here.

5.6.References

1. Anandan C, Nurmatov U, van Schayck OC, Sheikh A. Is the prevalence of asthma declining? Systematic review of epidemiological studies. *Allergy.* Feb 2010;65(2):152-167.
2. Platts-Mills TA, Commins SP. Increasing prevalence of asthma and allergic rhinitis and the role of environmental factors. UpToDate. www. uptodate. com [Accessed January 2019]; 2018.
3. Naleway AL. Asthma and atopy in rural children: is farming protective? *Clinical medicine & research.* 2004;2(1):5-12.
4. Ege MJ, Mayer M, Normand A-C, et al. Exposure to Environmental Microorganisms and Childhood Asthma. *New England Journal of Medicine.* 2011;364(8):701-709.
5. House JS, Wyss AB, Hoppin JA, et al. Early-life farm exposures and adult asthma and atopy in the Agricultural Lung Health Study. *Journal of Allergy and Clinical Immunology.* 2017;140(1):249-256.e214.
6. Fall T, Lundholm C, Örtqvist AK, et al. Early exposure to dogs and farm animals and the risk of childhood asthma. *JAMA pediatrics.* 2015;169(11):e153219-e153219.
7. Adler A, Tager I, Quintero DR. Decreased prevalence of asthma among farm-reared children compared with those who are rural but not farm-reared. *Journal of allergy and clinical immunology.* 2005;115(1):67-73.
8. Illi S, Depner M, Genuneit J, et al. Protection from childhood asthma and allergy in Alpine farm environments-the GABRIEL Advanced Studies. *J Allergy Clin Immunol.* Jun 2012;129(6):1470-1477 e1476.

9. Macneill SJ, Sozanska B, Danielewicz H, et al. Asthma and allergies: is the farming environment (still) protective in Poland? The GABRIEL Advanced Studies. *Allergy.* Apr 29 2013.

10. Lampi J, Canoy D, Jarvis D, et al. Farming environment and prevalence of atopy at age 31: prospective birth cohort study in Finland. *Clin Exp Allergy.* Jul 2011;41(7):987-993.

11. Wjst M. Another explanation for the low allergy rate in the rural Alpine foothills. *Clin Mol Allergy.* Jun 5 2005;3:7.

12. Alfven T, Braun-Fahrlander C, Brunekreef B, et al. Allergic diseases and atopic sensitization in children related to farming and anthroposophic lifestyle--the PARSIFAL study. *Allergy.* Apr 2006;61(4):414-421.

13. Barry RJ, Pickett W, Rennie DC, Senthilselvan A, Cockcroft DW, Lawson JA. Factors contributing to risks for pediatric asthma in rural Saskatchewan. *Ann Allergy Asthma Immunol.* Oct 2012;109(4):255-259.

14. Barry RJ, Pickett W, Rennie DC, et al. The role of farm operational and rural environments as potential risk factors for pediatric asthma in rural Saskatchewan. *Pediatr Pulmonol.* Sep 2014;49(9):842-851.

15. Chu LM, Rennie DC, Cockcroft DW, et al. Prevalence and determinants of atopy and allergic diseases among school-age children in rural Saskatchewan, Canada. *Annals of Allergy, Asthma & Immunology.* 2014;113(4):430-439.

16. MacNeill S, Sozanska B, Danielewicz H, et al. Asthma and allergies: is the farming environment (still) protective in P oland? The GABRIEL Advanced Studies. *Allergy.* 2013;68(6):771-779.

17. Farthing P, Rennie D, Pahwa P, Janzen B, Dosman J. The association between farming activities and respiratory health in rural school age children. *J Agromedicine.* 2009;14(2):256-262.

18. Ege M, Frei R, Bieli C, et al. Not all farming environments protect against the development of asthma and wheeze in children. *J Allergy Clin Immunol.* 2007;119:1140 - 1147.

19. Riedler J, Eder W, Oberfeld G, Schreuer M. Austrian children living on a farm have less hay fever, asthma and allergic sensitization. *Clin Exp Allergy.* Feb 2000;30(2):194-200.

20. Merchant JA, Naleway AL, Svendsen ER, et al. Asthma and farm exposures in a cohort of rural Iowa children. *Environmental health perspectives.* 2005;113(3):350-356.

21. Syamlal G, Hendricks K, Mazurek JM. Asthma among household youth on racial minority operated farms—United States, 2008. *Journal of agromedicine.* 2018;23(2):144-153.

22. Dimich-Ward H, Chow Y, Chung J, Trask C. Contact with livestock--a protective effect against allergies and asthma? *Clin Exp Allergy.* Sep 2006;36(9):1122-1129.

23. Pickett W, Day L, Hagel L, et al. The Saskatchewan farm injury cohort: rationale and methodology. *Public health reports.* 2008;123(5):567-575.

24. Pickett W, King N, Marlenga B, et al. Exposure to agricultural hazards among children who visit farms. *Paediatrics & Child Health.* 2018;23(7):e143-e149.

25. Marlenga B, King N, Pickett W, et al. Impact of sleep on injury risk among rural children. *Paediatrics & Child Health.* 2017;22(4):211-216.

26. Cornish RP, Henderson J, Boyd AW, Granell R, Van Staa T, Macleod J. Validating childhood asthma in an epidemiological study using linked electronic patient records. *BMJ Open.* 2014;4(4):e005345.

27. Yang CL, To T, Foty RG, Stieb DM, Dell SD. Verifying a questionnaire diagnosis of asthma in children using health claims data. *BMC Pulmonary Medicine.* 2011/11/22 2011;11(1):52.

28. Oluwole O, Rennie DC, Senthilselvan A, et al. Asthma diagnosis among children along an urban-rural gradient. *Journal of Asthma.* 2018/11/02 2018;55(11):1242-1252.

29. El-Sharif N, Abdeen Z, Qasrawi R, Moens G, Nemery B. Asthma prevalence in children living in villages, cities and refugee camps in Palestine. *Eur Respir J.* Jun 2002;19(6):1026-1034.

30. Pekkanen J, Pearce N. Defining asthma in epidemiological studies. *Eur Respir J.* Oct 1999;14(4):951-957.

31. Chu L, Rennie D, Cockcroft D, et al. Agreement between questionnaire report of allergy-related outcomes in school-age children and objective measures of atopy: the Saskatchewan rural health study. *Clinical & Experimental Allergy.* 2015;45(8):1337-1345.

32. Lawson JA, Chu LM, Rennie DC, et al. Prevalence, risk factors, and clinical outcomes of atopic and nonatopic asthma among rural children. *Annals of Allergy, Asthma & Immunology.* 2017/03/01/ 2017;118(3):304-310.

33. Lougheed MD, Lemière C, Dell SD, et al. Canadian Thoracic Society Asthma Management Continuum–2010 Consensus Summary for children six years of age and over, and adults. *Canadian Respiratory Journal.* 2010;17.

34. Frazier PA, Tix AP, Barron KE. Testing moderator and mediator effects in counseling psychology research. *Journal of counseling psychology.* 2004;51(1):115.

35. (CASA) CASA. North American Guidelines for Children's Agricultural Tasks – Driving a Farm Tractor No Implement Attached. [Internet]. 2019; https://www.casa-acsa.ca/en/safetyshop-library/north-american-guidelines-for-childrens-agricultural-tasks-driving-a-farm-tractor-no-implement-attached/. Accessed November 24, 2020.

36. Skalamera J, Hummer RA. Educational attainment and the clustering of health-related behavior among US young adults. *Preventive medicine.* 2016;84:83-89.

37. StataCorp. Stata 15 Base Reference Manual. College Station, TX: Stata Press. 2017.

38. Midodzi WK, Rowe BH, Majaesic CM, Senthilselvan A. Reduced risk of physician-diagnosed asthma among children dwelling in a farming environment. *Respirology.* 2007;12(5):692-699.

39. Sunyer J, Jarvis D, Pekkanen J, et al. Geographic variations in the effect of atopy on asthma in the European Community Respiratory Health Study. *J Allergy Clin Immunol.* Nov 2004;114(5):1033-1039.

40. Pearce N, Pekkanen J, Beasley R. How much asthma is really attributable to atopy? *Thorax.* 1999;54(3):268-272.

41. Comberiati P, Di Cicco ME, D'Elios S, Peroni DG. How Much Asthma Is Atopic in Children? *Frontiers in pediatrics.* 2017;5:122-122.

42. Mutius V. Exposure to endotoxin or other bacterial components might protect against the development of atopy. *Clinical & Experimental Allergy.* 2000;30(9):1230-1234.

43. Heederik D, Sigsgaard T, Thorne PS, et al. Health Effects of Airborne Exposures from Concentrated Animal Feeding Operations. *Environmental Health Perspectives.* 2007;115(2):298-302.

44. Yang CL, To T, Foty RG, Stieb DM, Dell SD. Verifying a questionnaire diagnosis of asthma in children using health claims data. *BMC pulmonary medicine.* 2011;11:52-52.

Table 5-1. Characteristics of the Saskatchewan Farm Injury Cohort – Child Cohort (N=2275)

		Total (N=2275)		**Rural (n=1698)**		**Farm (n=577)**		**p-value***
		n	%	n	%	n	%	
Location of residence	Rural	1698	74.6					
	Farm	577	25.4					
Age groups	0-5 years	468	20.6	371	21.8	97	16.8	**0.015**
	6-12 years	1192	52.4	892	52.5	300	52	
	13-17 years	615	27.0	435	25.6	180	31.2	
Sex	Male	1103	48.5	843	49.8	260	45.3	0.068
	Female	1163	51.1	849	50.2	314	54.7	
	Missing	9	0.4					
BMI	Non-overweight	1062	46.7	755	68.7	307	69.1	0.126
	Overweight	299	13.1	225	20.5	74	16.7	
	Obese	182	8.0	119	10.8	63	14.2	
	Missing	732	32.2					
Physical activity	Less than 2 days	258	11.3	216	12.9	42	7.4	
	3-5 days	1147	50.4	860	51.5	287	50.6	**0.008**
	6-7 days	831	36.5	593	35.5	238	42	
	Missing	39	1.7					
Household smoking exposures	Yes	163	7.2	126	7.5	37	6.5	0.59
	No	2092	92.0	1556	92.5	536	93.5	
	Missing	20	0.9					
Parental education	Less or completed high school	466	20.5	393	23.3	73	12.8	**<0.001**
	More than high school	1788	78.6	1292	76.7	496	87.2	
	Missing	21	0.9					
Atopy	No	2169	95.3	1621	95.5	548	95	0.68
	Yes	106	4.7	77	4.5	29	5	
Asthma	No	2102	92.4	1568	92.3	534	92.5	0.88
	Yes	173	7.6	130	7.7	43	7.5	
SABA (inhalers for asthma)	No	2141	94.1	1604	94.5	537	93.1	0.28
	Yes	134	5.9	94	5.5	40	6.9	
Asthma phenotypes	Atopic asthma	51	2.2	41	2.4	10	1.7	0.61
	Non atopic asthma	122	5.4	89	5.2	33	5.7	
	Non asthma	2102	92.4	1568	92.3	534	92.5	

*p-values were based on Rao-Scott chi-square tests adjusting for clustering by families.

Table 5-2. Farm activities between farm and rural children (N=2275)

	Rural (n=1698)	Farm (n=577)
	%	%
Farm work in the spring (Ref: No)	3.7	44.2
Farm work in the summer (Ref: No)	5.2	43.5
Farm work in the fall (Ref: No)	4.0	44.9
Farm work in the winter (Ref: No)	2.5	29.8
Tractor Operation (Ref: No)	2.3	18.4
Combines Operation (Ref: No)	0.4	9.2
Grain augers Operation (Ref: No)	0.3	6.4
Routine chores with large animals (e.g. cattle or pigs) (Ref: No)	4.4	25.6
Routine chores with small animals (e.g. chickens) (Ref: No)	2.9	20.3
Doing herd maintenance activities (e.g. branding, vaccinating) (Ref: No)	2.1	12.3
Doing veterinary activities (e.g. medications, breeding, birthing) (Ref: No)	0.9	12.5
Regularly* performing haying/moving/playing with hay bales (Ref: Not regularly)	6.4	30.2
Regularly feeding livestock (Ref: Not regularly)	6.8	36.8
Regularly cleaning or playing in barns (Ref: Not regularly)	5.6	26.4
Regularly cleaning grain bins (Ref: Not regularly)	0.4	5.5
Regularly cleaning or playing in pens/corals (Ref: Not regularly)	5.1	23.3

*‘‘Regular’’ is defined as at least once a month

Table 5-3. Prevalence of asthma, atopy, and asthma phenotypes by farm-related activities

		Asthma		Atopy		Atopic asthma	Non atopic asthma	
		Yes (n=173)		Yes (n=106)		Yes (n=51)	Yes (n=122)	
		%	p-value*	%	p-value *	%	%	p-value*
Age groups	0-5 years	4.5	<0.001	2.6	0.052	1.3	3.2	0.046
	6-12 years	7.8		5.0		2.2	5.6	
	13-17 years	9.6		5.5		3.1	6.5	
Sex	Male	9.1	**0.013**	5.6	**0.033**	2.8	6.3	**0.041**
	Female	6.2		3.8		1.7	4.5	
Body Mass Index	Not overweight	6.7	0.11	4.4	0.21	2.4	4.2	0.09
	Overweight	6.4		4.3		0.7	5.7	
	Obese	11.0		7.7		3.8	7.1	
Physical Activity	Less than 2 days	6.6	0.37	5.8	0.68	2.7	3.9	0.37
	3-5 days	8.4		4.4		2.1	6.3	
	6-7 days	6.7		4.7		2.3	4.5	
Parental Education	Less or completed high school	6.4	0.38	2.6	**0.028**	1.3	5.2	0.41
	More than high school.	7.8		5.2		2.5	5.4	
Household smoking exposures	Yes	6.7	0.75	2.5	0.24	1.2	5.5	0.64
	No	7.5		4.8		2.3	5.2	
Haying/moving/playing with hay bales	Regular	8.3	0.68	5.1	0.72	1.8	6.5	0.63
	Not regular	7.6		4.5		2.3	5.2	
Feeding livestock	Regular	8.1	0.77	5.0	0.71	2.5	5.6	0.93
	Not regular	7.6		4.5		2.2	5.4	
Cleaning or playing in barns	Regular	7.0	0.69	4.5	0.97	2.2	5.0	0.92
	Not regular	7.7		4.6		2.2	5.5	
Cleaning grain bins	Regular	10.5	0.55	2.6	0.56	2.6	7.9	0.76
	Not regular	7.6		4.6		2.2	5.4	

	Regular	7.5	0.91	4.7	0.98	1.9	5.6	0.92
Cleaning or playing in pens/corals	Not regular	7.7		4.6		2.3	5.4	
	No	7.4	0.39	4.5	0.55	2.1	5.3	0.65
Doing farm work in Spring	Yes	8.8		5.4		2.8	6.0	
	No	7.1	**0.058**	4.5	0.43	2.1	5.0	0.09
Doing farm work in Summer	Yes	10.6		5.6		3.2	7.4	
	No	7.3	0.27	4.6	0.64	2.2	5.2	0.54
Doing farm work in Fall	Yes	9.2		5.2		2.8	6.4	
	No	7.4	0.22	4.5	0.35	2.1	5.2	0.42
Doing farm work in Winter	Yes	9.8		6.0		3.3	6.5	
	No	7.6	0.77	4.7	0.75	2.3	5.3	0.56
Operating tractors	Yes	8.3		4.1		1.4	6.9	
	No	7.6	0.8	4.7	0.57	2.3	5.4	0.95
Operating combines	Yes	6.8		3.4		1.7	5.1	
	No	7.5	0.37	4.7	0.97	2.2	5.3	0.45
Operating grain augers	Yes	11.9		4.8		2.4	9.5	
	No	7.1	**0.026**	4.4	0.2	2.1	5.0	**0.028**
Doing routine chores with large animals	Yes	12.1		6.7		3.6	8.5	
	No	7.5	0.5	4.7	0.5	2.2	5.3	0.77
Doing routine chores with small animals	Yes	9.0		3.6		2.4	6.6	
	No	7.5	0.33	4.7	0.61	2.2	5.3	0.54
Doing herd maintenance activities	Yes	10.3		3.7		2.8	7.5	
	No	7.5	0.43	4.7	0.97	2.3	5.2	0.3
Doing vet activities	Yes	10.3		4.6		1.1	9.2	
	No	6.7	**0.0064**	6.4	0.14	2.0	8.2	**0.0062**
Has this child completed any farm safety education?	Yes	11.7		4.3		3.6	4.7	
	No	7.3	0.17	4.6	0.54	2.2	5.1	0.38

Has this child completed any agriculture courses or training?	Yes	11.2		5.9		3.3	7.9	

*p-values were based on Rao-Scott chi-square tests adjusting for clustering by families.

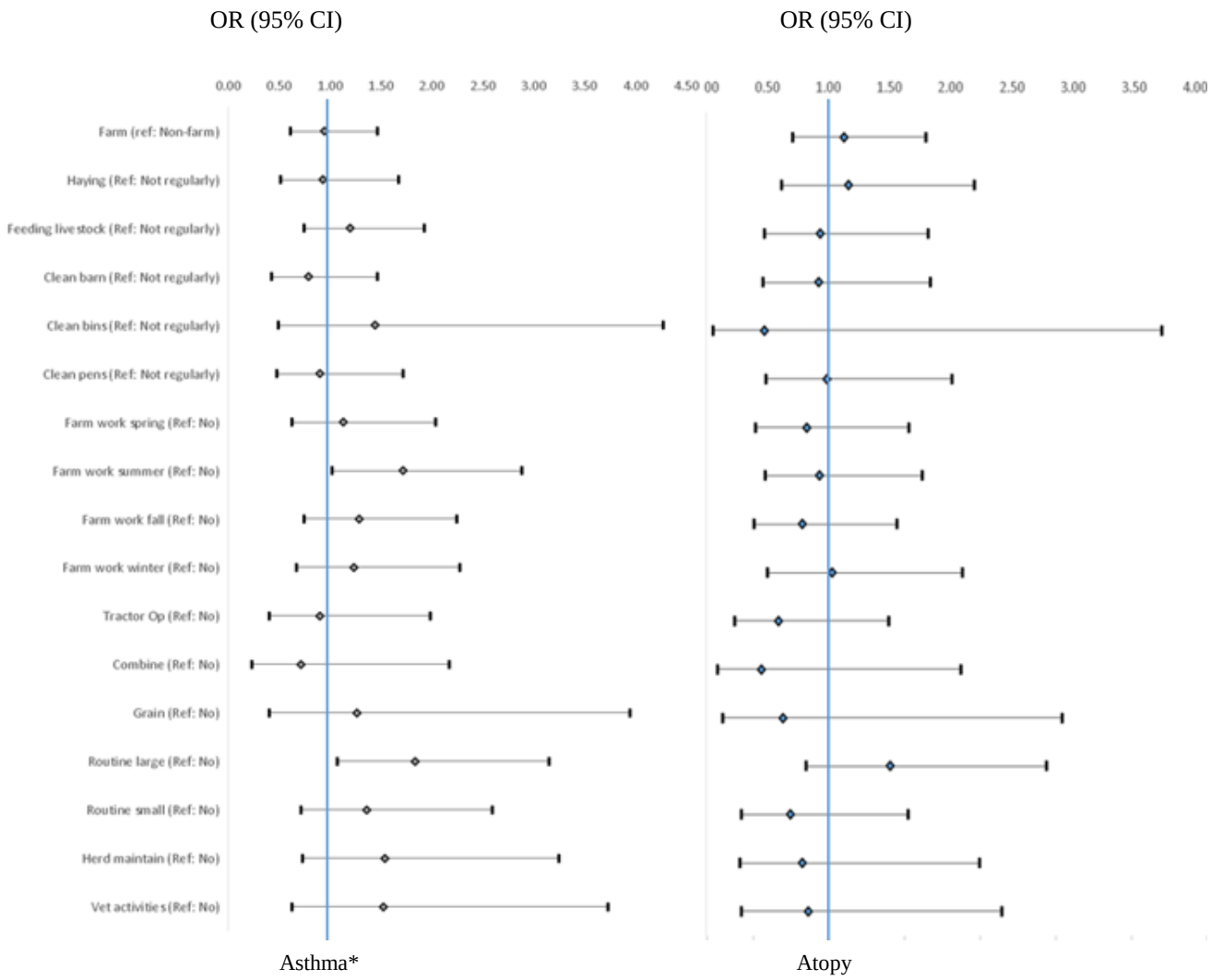

Figure 5-1. Farm-related specific determinants among children 6-17 years old. Adjusted ORs with 95% CIs additionally adjusted for sex, age, parental education, physical activity, household smoking. Each farm activity was added singularly in the final model with these above confounders/co-variates together with the location of residence (farm vs. non-farm) in the model.

*Included "Atopy" variable in the model

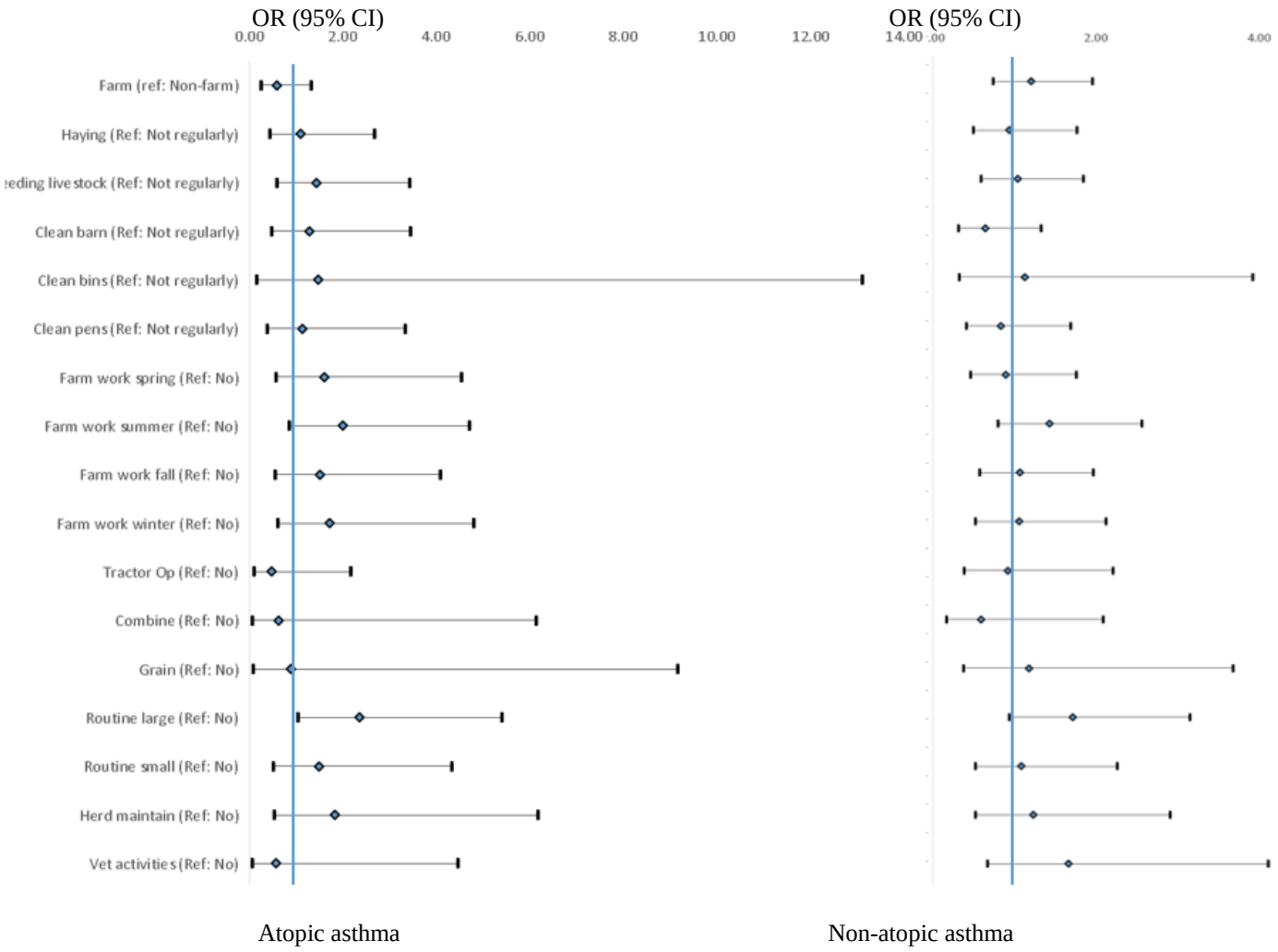

Figure 5-2. Farm-related specific determinants of asthma phenotypes (N=1807) (Ref: No asthma) among children 6-17 years old. Adjusted ORs with 95% CIs additionally adjusted for sex, age, parental education, physical activity, household smoking. Each farm activity was added singularly in the final model with these above confounders/co-variates together with the location of residence (farm vs. non-farm) in the model.

CHAPTER 6

AN INVESTIGATION OF PERSONAL EXPOSURE MONITORING TO COLLECT ENVIRONMENTAL EXPOSURE DATA IN SCHOOL-AGE CHILDREN (MANUSCRIPT 4)

Authors: Luan M. Chu, MSc[1,2]; Shelley Kirychuk, PhD[2]; Donna C. Rennie, PhD[2,3]; John Gordon, PhD[4]; Donald Cockcroft, MD[4]; Brooke Thompson, BSc[2]; Joshua A. Lawson, PhD[2,4]

Affiliations: [1]Health Sciences program, College of Medicine, University of Saskatchewan, Saskatoon, Saskatchewan, Canada, 103 Hospital Drive, Saskatoon SK S7N 0W8, Canada

[2]Canadian Center for Health and Safety in Agriculture, University of Saskatchewan, Saskatoon, Saskatchewan, Canada, 104 Clinic Place PO Box 23, Saskatoon, SK, S7N 5E5, Canada

[3]College of Nursing, University of Saskatchewan, Saskatoon, Saskatchewan, Canada, 104 Clinic Place Saskatoon, SK, S7N 2Z4, Canada

[4]Department of Medicine, College of Medicine, University of Saskatchewan, Saskatoon, Saskatchewan, Canada, 103 Hospital Drive, Saskatoon SK S7N 0W8, Canada

Corresponding author: Luan Manh Chu, M.Sc, Ph.D. Candidate, Canadian Center for Health and Safety in Agriculture, University of Saskatchewan, Saskatoon, Saskatchewan, Canada, 104 Clinic Place PO Box 23, Saskatoon, SK, S7N 5E5, Canada; E-mail: cml779@mail.usask.ca

6.1. Abstract (word count=270)

Background: In epidemiological studies, an objective, reliable, and replicable tool to measure personal exposures in the living environment is crucial, especially when drawing associations between these exposures and health outcomes, including asthma and allergies. To date there is limited information of the utility and efficacy of environmental dust collection with personal samplers in children. The aims of this pilot study were (1) to determine if a personal exposure monitoring (PEM) device can be used to effectively collect representative environmental samples in children; (2) to determine whether personal environmental levels correlate with that of settled area samples; and (3) whether the PEM can be effectively and conveniently used in children.

Methods: This was a cross-sectional pilot study conducted in 2016. A purposeful sampling technique was used to select and recruit participants. This pilot study included 5 children in Kindergarten to Grade 8 living in and around Saskatoon, Canada. The data collection methods included: survey, personal dust sampling, daily activity monitoring, and floor and mattress dust sampling. A comparison of the environmental levels identified from the floor, mattress, and personal monitoring was made using correlations.

Results: Using the PEM methods, we collected sufficient dust to detect endotoxin and β glucan. Some correlations of these measures between personal (PEM) and settled (play area and mattresses) were observed, however, were not significant.

Conclusion: This pilot sucessfully showed that PEM is an effective method for collecting sufficient dust to detect endotoxin and β-(1 → 3)-D-glucan levels in children. Our results can be used as the starting point for future sample size estimates and how to adapt the PEM to be more suitable, especially for children.

Keywords: Personal exposure monitoring, children, endotoxin, β-(1 → 3)-D-glucan, pilot

6.2. Introduction

Current research has suggested that the environment plays a role in the development of childhood asthma and allergies. Furthermore, the prevalence of these conditions is generally lower in rural compared to urban populations.[1-4] Microbial components such as endotoxin and β-(1 → 3)-D-glucan which have been shown to be higher in rural areas[2] have been suggested as an explanation for the inverse associations with the development of atopy, asthma, wheeze, and respiratory diseases in rural populations.[2,3]

In epidemiological studies, an objective, reliable, and replicable tool to measure personal environmental exposures is crucial to examining associations between these exposures and health outcomes, including asthma and allergies. Studies assessing the role of endotoxin and childhood asthma or atopy have relied heavily on the collection and analysis of settled dust from floors and mattresses of the homes of children[5-12] as surrogates for personal exposure. Although using such samples is convenient and makes large sample collections in epidemiological studies more feasible, it is not clear how well these surrogate exposures reflect the actual relevant personal exposure. While these methods can provide some estimate of domestic personal exposure, it may not account for the true exposure associated with the child's "personal cloud",[13] given that children spend much of the time at school or in outdoor environments including both rural and urban environments (e.g. at a playground, on a farm, etc). Endotoxin levels collected by different sampling methods including air-borne sampling of home areas, and vacuum sampling from home locations such as the floor or child's mattress have not always correlated.[14] The true relationship between ambient air quality and health oucomes are likely underestimated because exposure misclassification may occur when area measurements from central ambient air quality monitors are used to assign personal exposures instead of using personal monitoring devices.[15] It has been observed that the use of settled dust

may also lead to biased associations which underestimate the true strength of associations with respiratory outcomes (e.g. wheeze) when compared to air sampling.[16]

Personal air sampling methods have mostly been used in adults in occupational-based settings.[17-22] There have been few studies conducted in children to examine associations between inhalable dust concentration and asthma using personal samplers[23,24] or airborne endotoxin at the home of pediatric subjects.[25,26] There has also been limited discussion around the utility and/or efficacy of these methods.[27-29] Investigation of personal air sampling methods may be especially beneficial in studies of childhood asthma and allergy and exposures in a rural or farming environment. Because there are differences in the prevalence of asthma and allergy between rural and urban areas[30-32] and typology and amounts of microbial exposures involved in these associations appear to also differ between these locations,[33-37]accurate exposure assessment will be critical to reducing bias in these investigations. In addition to this, there has been some inconsistency in these associations when comparing rural non-farm and farm children[38-40] with a potential explanation being homogeneity in exposures between groups. Personal air sampling in children, giving a more accurate assessment of the child's exposure while both in and outdoors would contribute to the understanding of the association between the farming environment and asthma or allergies. Using PEM in agricultural settings will help our understanding of the unique exposures for children in these areas and aid in recommendations for improved respiratory health.

Pilot studies using methodologic advancements are important to advancing knowledge and improving evidence in a study area.[41-43] Along these lines, we must continue to develop, test and use tools that will more precisely assess environmental exposures if we are to accurately investigate their associations with health outcomes. In the current pilot study, we aimed to test the feasibility and convenience of using a personal exposure monitoring (PEM) device to collect

airborne dust samples from children, and to test the correlation of these measures to concurrent settled dust samples using traditional vacuuming methods. Specificially, our aims were to determine if (1) the PEM device could collect sufficient levels of dust to detect endotoxin and β-(1 → 3)-D-glucan in these samples, (2) whether these microbial compounds correlate with that of settled dust samples, and (3) whether the PEM can be used effectively and conveniently in children.

6.3.Methods

Study design and population

This was a cross-sectional pilot study conducted in 2016. A purposeful sampling technique was used to select and recruit participants. This pilot study included 5 children in Grades 1 to 3 living in and around Saskatoon, Canada. Children who were recruited were known to the researchers through friends or family. Children and parents provided written assent and consent, respectively, prior to home dust and personal dust collection. The study was approved by the University of Saskatchewan Biomedical Research Ethics Board (Bio #:15-320).

Data collection

The data collection methods included: an initial health and household survey, daily activity monitoring diary, personal environmental monitoring (PEM), floor and mattress settled dust sampling, and an exit interview.

Survey and Diary

An initial health and household survey was completed by each participant/parent to collect demographic information, child health, environmental exposures, housing characteristics, and health behaviours. Completion and return of the survey implied voluntary consent for the questionnaire portion.

During the monitoring period, parents of participants, in consultation with participants, kept a written record (diary) of all activities undertaken during the time when the monitor was in operation. The monitor was in "off" mode when sleeping. Instructions of how to use diaries were provided within each diary provided to the particpants.

Personal environmental monitoring

The personal environmental monitors were active air samplers worn in a fanny pack over 2 consecutive days**.** To measure the particulate within the breathing zone of participants, we used a personal sampling pump (AirChek® TOUCH Sample Pump. Cat. No. 220-Series) and a Parallel Particle Impactor (PPI) (Thoracic cut-point (10 μm), run at 2 L/min. The sampling rate was calibrated prior to sampling. PPIs were fit with 37 mm Polyvinyl Chloride filters (PVC, 5-μm pore size), supporting pad, and substrates (SKC Inc., PA, USA). Filters were desiccated and acclimitized before weighing and after sampling according to a standard operating procedure. Filters were pre- and post-weighed on a calibrated microbalance (MX5, Mettler Toledo, Mississauga, CA).

Each subject wore a portable personal exposure monitoring (PEM) pack (**Figure 6-1**) during waking hours and kept the PEM in close proximity off the ground (e.g. nightstand) when it was not possible to wear. The PPI for the PEM was pinned to the shoulder strap, close to the breathing zone when worn. The PEM was worn for 2 consecutive days to collect personal air samples.

The pack was sound insulated and had an extra compartment to hold the diary. The weight of the PEM device (including the bag) was approximately 20 ounces (567 grams). Parents recorded information regarding any potential issues with the PEM as well as activities while the children wore the PEMs.

Floor and mattress dust sampling

Two settled samples of dust were collected from the home to measure endotoxin (lipopolysaccharides) and β-(1 → 3)-D-glucan. The first sample was collected from the room where the child spent most of their free time in the home. The second sample was from the mattress

where the child slept. Dust was collected by vacuuming (S514 Solaris vacuum, 900 W, Vaughan, Canada), adhering to recommended standardized protocols[44] using pre-weighed X-Cell-100 filter socks with a pore size of approximately 4.0–12.3 microns (Midwest Filtration LLC, OH, USA). Collection of dust was completed by inserting the sock filter into the end of the vacuum extension and securing it with a clean crevice tool. Floors with wall-to-wall carpet had $2m^2$ vacuumed for 4 minutes. Smooth floor with at least $4m^2$ of carpet had $2m^2$ vacuumed for 4 minutes. Completely smooth floor or floor with one or two small rugs had $4m^2$ vacuumed for 4 minutes. Dust collection from the bed was completed after all duvets, blankets, and sheets that the child slept under were removed. The sheets that the child slept on were left to be vacuumed. The length and width of the bed were measured and the whole area of the bed was sampled for 2 minutes. Temperature and humidity were collected at the home at the time of vacuuming. The sampling was done at the convenience of the participants. No specific instructions were provided to the participants prior to sampling such as cleaning or changing the child's sheets, etc. Filters were desiccated and acclimitized before weighing and after sampling according to a standard operating procedure. Settled dust samples were then sieved using the 300 μm sieve.

Lab analyses

Post sampling PEM and settled dust samples were stored in a desiccator at 4 °C until post - weighing, extraction, and analysis. Samples were then brought to room temperature and 10 mg of sieved dust was weighed out for extraction. Filters and sieved dust samples were extracted with 10 mL 0.05% Tween 20 (Fisher Scientific, Mississauga, ON, Canada) in pyrogen-free water[45] (GE Healthcare Bio-Science, Mississauga, ON, Canada) and shaken at 325 revolutions per minute (RPM) for 2 h. The samples were centrifuged (Sorvall ST 16R, Thermo Fisher Scientific, Mississauga, ON, Canada) at 1000×g for 15 min to obtain supernatant and 1 mL aliquots were

stored at −80 °C until analysis for endotoxin analysis. To the tube containing the dust, 1 mL of 3M sodium hydroxide (NaOH) was added to give a final concentration of 0.3M. Samples were vortexed briefly and shaken at 325 rpm for 10 minutes. The samples were centrifuged (Sorvall ST 16R, Thermo Fisher Scientific, Mississauga, ON, Canada) at 1000×*g* for 15 min to obtain supernatant and 1 mL aliquots were stored at −80 °C until analysis for water-soluble β-(1→3)-D-glucan analysis. The chromogenic *Limulus* Amoebocyte Lysate (LAL) Kinetic QCL assay according to manufacturer's specifications (Lonza, Walkersville, MD, USA) was used to analyze samples to measure endotoxin. The water soluble β-(1 → 3)-D-glucans were measured using the Glucatell assay kit Kinetic Onset Time protocol according to manufacturer's specifications (Associate of Cape Cod, East Falmouth, MA, USA)[46].

To quantify endotoxin and β-(1 → 3)-D-glucan levels, the absorbance of endotoxin and β-(1 → 3)-D-glucan was monitored at 405 nm for 2 h and 1 h, respectively, using the Biotek ELx808 plate reader (BioTek, Winooski, VT, USA). Values were compared to standard curves prepared for endotoxin (0.005EU/mL–50 EU/mL) and β-(1 → 3)-D-glucan (3.125 pg/mL–100 pg/mL).

For all dust samples, endotoxin was reported as the endotoxin in the air that was sampled (EU/ m^3), the endotoxin in the floor or mattress area that was sampled (EU/ m^2), and the endotoxin in the dust sample that was collected (EU/mg). Similarly, β-(1 → 3)-D-glucan levels were reported as the β-(1 → 3)-D-glucan in the air that was sampled (ug/m^3), the β-(1 → 3)-D-glucan in the floor or mattress area that was sampled (ug/m^2), and the β-(1 → 3)-D-glucan in the dust sample that was collected (μg/g).

Exit Survey

After 2 days of using the PEM, researchers/technicians collected PEMs and diaries and conducted exit interviews with parents/guardians and their children together to determine the convenience of

using this device and identify areas for improvement. All exit interview were recorded for future playback and to facilitate note taking regarding the monitoring experience. For "Convenience of the PEM", we asked the question:"On a scale of 0-10, how do you score the convenience of wearing the PEM?". For "Suitability of the PEM", the following question was asked:"On a scale of 0-10, how do you score the suitability of size or weight PEM?" was used?" For "noise associated with the PEM" we asked:"On a scale of 0-10, how do you score the noise from the PEM?" For "Convenience" and "suitability", 0 represented the lowest, and 10 represented the highest values, but for "noise", 0 represented the highest, and 10 represented the lowest values.

Statistical analyses

All analyses were completed using the Statistical Package for the Social Science (SPSS) Version 26 (SPSS Inc. Armonk, NY: IBM Corp.). Descriptive analyses using frequencies and proportions for categorical variables and means with standard deviations for continuous variables (or medians and inter-quartile ranges if the distributions are not normal) were conducted. The relationship between environmental levels identified from the floor, mattress, and personal monitoring was tested using Spearman's correlations. Statistical significance was set at alpha=0.05. Qualitative data from interviews was presented to support findings related to the convenience/acceptability characteristics issues of the PEM equipment. Mean scores of each acceptability characteristics issue were calculated.

6.4.Results

Of the 6 children approached, 5 participated. These children were aged 7-9 years old (1 male, 4 females). One sample from the PEM was out of detectable range for endotoxin (too high). The mean time of wearing PEM among participants was 17.78 hours (min-max=12-22.17).

The arithmetic mean (min-max) as well as geometric mean (GM) and geometric standard deviation (GSD) of endotoxin and β-(1 → 3)-D-glucan in house dust from the play area floor and mattress and from the PEM filter are shown in **Table 6-1**. The levels of endotoxin in the floor dust (EU/m^2) were higher than that of mattress dust [GM (GSD)=18,390 (2.46) versus 9,061 (2.48)]. The GM level of endotoxin in the air samples was 313.04 EU/m^3 (4.20). The GM levels of β-(1 → 3)-D-glucan in the floor dust (ug/m^2) was lower than that of mattress dust (1.79 (4.17) versus 2.75 (2.74)) while the value for air samples was 0.35 ug/m^3 (1.60).

The correlation between each of the levels of environmental exposures in dust collected by personal samplers and by vacuuming of settled house dust is shown in **Table 6-2**. None of these correlations were statistically significant. Despite this, several of the correlations were found to be strong including PEM vs. floor dust and PEM vs. mattress dust.

We looked at indicators of acceptability. Specifically, the mean scores for "convenience" and "suitability" were both 5.5. Regarding "noise", the mean score was 0.3. Some comments included: "A little heavy for long time use, especially while playing" or "The tube is a little uncomfortable and hard to keep it in place. Some suggestions were given such as "The use of a backpack instead" or "Switch position from in front to the back" or "Inconvenient for most activities – Noise is an issue".

6.5.Discussion

In our pilot study, using the PEM, we were able to collect sufficient dust to detect endotoxin and β-(1 → 3)-D-glucan. Correlations of these measures between PEM dust and play area dust as well as mattresses dust were strong, but not statistically significant. Evidence from our exit survey on comfort of PEM wearing suggested that the design of the PEM device with a fanny pack should be modified to maximize convenience and suitability in order to make it more user friendly.

Using comparable method and data collection tools, the detectable amount of endotoxin level (geometric mean value) in our pilot study was 35.89 (2.51) EU/mg in the play area which was lower than that of a previous study (ranging from 49.2 to 58.7 EU/mg)[47] using the same vacuums and methods as well as being from the same province. With regard to the levels of β-(1 → 3)-D-glucan in the play area, they were higher than that from a previous study (20.91 ug/g vs. 7.1-9.7 ug/g,[47] with the same trend was found in the mattress samples.

With regard to the PEM sampling results, it is challenging to compare our results to other studies of children due to the differences between studies of the types of filters used, pump speed, durations of wearing.[23,24] For example, the durations of wearing a PEM were different between studies such as school-based endotoxin measurements during 2 intervals (ten consecutive schooldays and two consecutive schooldays repeated 3 times) by Rabinovitch et al[24] in comparison to the home-based endotoxin assessment periods of 10 days duration used by Delfino et al in.[23] Also, personal exposure monitors were operated at 4 L/min by Delfino et al[23] and 2 L/min by Rabinovitch et al[24]. Our results suggest that PEM operated at 2 L/min over 17 hours should be sufficient for dust collection of endotoxin and beta glucan.

Our findings regarding the correlations between dust samples from different sources (play area, mattress, and personal dust sampling using the PEM) were not always consistent with

previous findings.[14] We observed mixed correlations (high and low in some pairs) that were not statistically significant, likely due to the small sample size, resulting in low statistical power. Similar to our findings, no statistically significant results were found for the correlations between endotoxin measured by floor vacuumed dust and by stationary airborne samplers in one study conducted by Park et al (2001)[14]. However, the airborne samplers in the study by Park et al were not in the form of a PEM, and the correlation coefficients between endotoxin from airborne monitoring and floor or mattress sampling were low (0.23 and 0.33, respectively).

While not statistically significant, we observed a strong correlation (rho=) $\rho \geq 0.8$) between PEM and floor endotoxin levels. We speculate that this correlation might be due to a higher level of endotoxin on the floor surface samples compared to mattress samples, The participants could have spent more time in the living room areas, particularly on the floor since PEMs were turned off during sleep. Unfortunately, we could not determine if they spend more time in the living room versus bedroom despite having a diary. We also observed a strong correlation between PEM vs. mattress β-(1 → 3)-D-glucan ((rho) $\rho=0.8$). We do not have a concrete explanation of why this occurred, but it is possible that there were factors that affected levels of β-(1 → 3)-D-glucan levels while the child wore the PEM during the daytime. These factors might include temperature and humidity of the living room or bedroom in which β-(1 → 3)-D-glucan is present.[48,49] In this study, temperature ranged from 21.3 to 26.6 degree Celsius and humidity ranged from 20 to 40%.

There were limitations to this pilot study that should be mentioned. First, we only enrolled a small group of participants given this was a pilot study. However, similar pilot studies with small sample sizes have been published in the literature.[41-43] It is important to disseminate the findings from pilot studies, even with small sample sizes, to build knowledge around methodological advances on which to improve in the future (e.g., protocols and technology for more accurate dust

collection). Second, the different collection methods (sock collection versus filter collection) are likely to influence the results. The size of collected particles are different in which much smaller particles are collected by the filters than by the socks.[50,51] It is likely that different sized particles could have different affinities for endotoxin and beta glucan,[50-52] thereby impacting the correlations. Third, we could not draw associations between the microbial levels and respiratory illnesses, due to a small sample size. Fourth, a convenience sampling technique was used for this pilot study, raising the issue of a lack of generalizability. However, we tried to include participants from varied living conditions including urban and rural areas.

There were some strengths to this pilot study. There has been little research into the use of PEMs as well as the suitability of PEMs among children. Our results could aid in the future design of PEMs which could be used in a larger groups of participants. Second, using the PEM, we could collect objective measures for exposures such as "personal cloud" dust sampling, which may be an improvement over the use of traditional methods of collecting dust samples from play areas and mattress of the children to provide a better assessment of the personal cloud associated with children's daily respirable exposure. Third, settled dust samples were collected by trained technicians using standardized protocols,[44] which should minimize measurement bias.

In conclusion, this pilot showed that it was possible to use PEM to collect sufficient dust to detect endotoxin and β-(1 → 3)-D-glucan over a relatively short period of time (2 days). Our results can be used as the starting point for future sample size estimates and point to equipment improvements making the PEM more suitable for use in children. For example, issues around comfort, noise, and ability to collect dust through the night could be addressed. Future work will include redesigning the PEM device and testing its use in larger populations as well as comparing

results looking at the associations between environmental exposures and health outcomes collected by PEMs to those collected by settled dust and ambient air collection methods.

Table 6-1. Mean values (Arithmetic & geometric) and Median value of endotoxin and β-(1 → 3)-D-glucan in house dust from play area floor, mattress, and PEM samples (n=5)

	Play area (Floor)	Mattress	PEM filter
	Arithmetic mean (min-max)		
Dust (mg)	106.25 (13-295)	78.8 (17-135)	0.3076 (0.083-0.456)
Endotoxin in the air (EU/m^3)	-	-	696.18 (73.55-2207.05)*
Endotoxin in the floor or mattress area (EU/m^2)	23592.59 (4383.94-41069.50)	12858.38 (3504.05-33836.59)	-
β-(1 → 3)-D-glucan in the air (ug/m^3)	-	-	0.380 (0.167-0.622)
β-(1 → 3)-D-glucan in the floor or mattress area (ug/m^2)	0.985 (0.054-2.669)	0.532 (0.121-1.062)	-
Endotoxin (EU/mg)	52.40 (9.00-82.00)	26.60 (11.00-66.00)	255.66 (35.35-795.50)
β-(1 → 3)-D-glucan (ug/g)	21.83 (16.75-36.06)	15.01 (7.53-23.50)	252.32 (80.63-638.23)
	Median (*Interquartile Range-IQR)*		
Endotoxin in the air (EU/m^3)	-	-	252.06 (1633.23)
Endotoxin in the floor or mattress area (EU/m^2)	21467.97 (22945.02)	10580.78 (25142.54)	-
β-(1 → 3)-D-glucan in the air (ug/m^3)	-	-	0.37 (0.24)
β-(1 → 3)-D-glucan in the floor or mattress area (ug/m^2)	0.83 (1.45)	0.28 (0.90)	-
Endotoxin (EU/mg)	56.00 (39.00)	22.50 (45.00)	95.90 (576.04)
β-(1 → 3)-D-glucan (ug/g)	19.12 (10.16)	13.94 (9.90)	217.84 (340.50)
	Geometric mean (Geometric standard deviation)		
Endotoxin in the air (EU/m^3)	-	-	313.04 (4.20)*
Endotoxin in the floor or mattress area (EU/m^2)	18,390 (2.46)	9,061 (2.48)	-
β-(1 → 3)-D-glucan in the air (ug/m^3)	-	-	0.350 (1.600)
β-(1 → 3)-D-glucan in the floor or mattress area (ug/m^2)	1.79 (4.17)	2.75 (2.74)	-
Endotoxin (EU/mg)	35.89 (2.51)	23.90 (2.21)	126.32 (3.73)
β-(1 → 3)-D-glucan (ug/g)	20.91 (1.36)	14.06 (1.51)	190.85 (2.25)

EU: Endotoxin Unit; *n=4 due to 1 measurement is out of detection range; "-: Not applicable

Table 6-2: Spearman correlation coefficients among different sources of dust

	endotoxin			β-(1 → 3)-D-glucan		
	n	ρ	p-value	n	ρ	p-value
PEM vs. Floor	3*	0.86	0.33	4	- 0.31	0.68
PEM vs. Mattress	3	0.50	0.66	4	0.80	0.20
Mattress vs. Floor	4	0.31	0.68	4	- 0.63	0.36

*One sample was not included because of the different type of filter

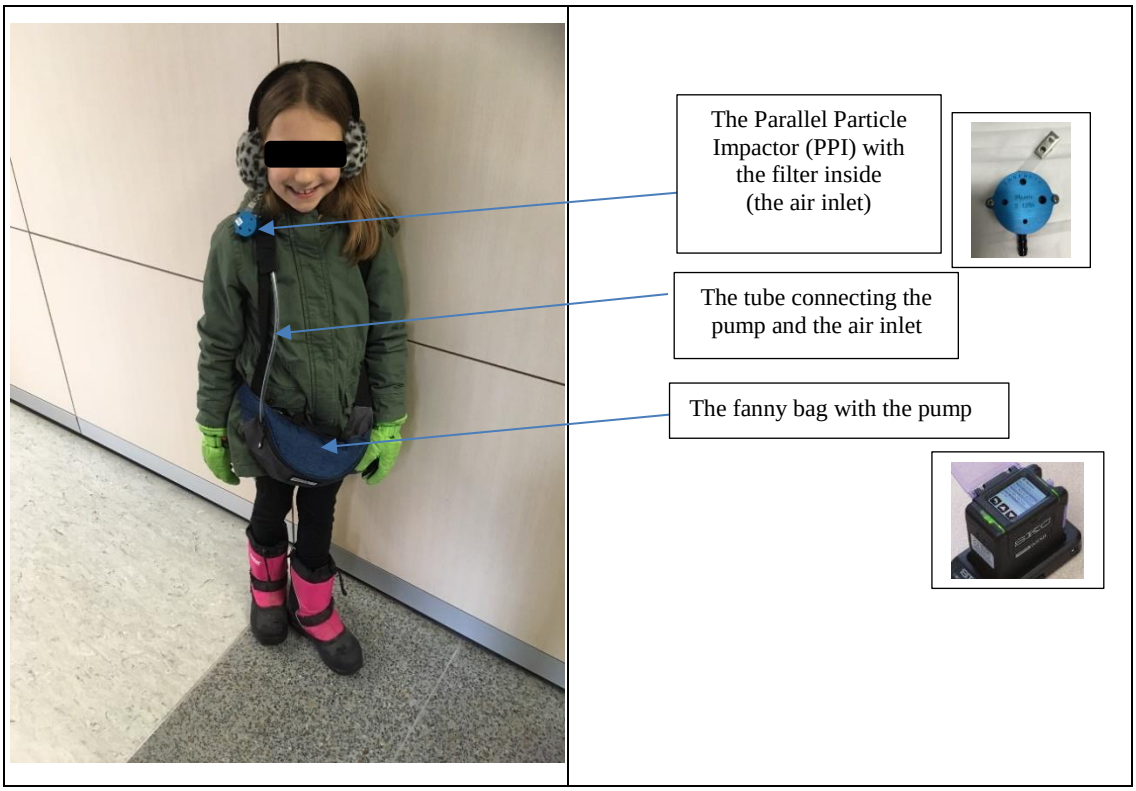

Figure 6-1: The demonstration of a PEM fanny pack

6.6 References

1. Lawson JA, Rennie DC, Cockcroft DW, et al. Childhood asthma, asthma severity indicators, and related conditions along an urban-rural gradient: a cross-sectional study. *BMC Pulmonary Medicine.* 2017/01/05 2017;17(1):4.
2. Barnig C, Reboux G, Roussel S, et al. Indoor dust and air concentrations of endotoxin in urban and rural environments. *Letters in applied microbiology.* 2013;56(3):161-167.
3. Mutius V. Exposure to endotoxin or other bacterial components might protect against the development of atopy. *Clinical & Experimental Allergy.* 2000;30(9):1230-1234.
4. von Mutius E. Asthma and allergies in rural areas of Europe. *Proceedings of the American Thoracic Society.* 2007;4(3):212-216.
5. Braun-Fahrlander C, Riedler J, Herz U, et al. Environmental exposure to endotoxin and its relation to asthma in school-age children. *N Engl J Med.* 2002;347:869-877.
6. Celedon JC, Milton D, Ramsey CD, et al. Exposure to dust mite allergen and endotoxin in early life and asthma and atopy in childhood. *J Allergy Clin Immunol.* 2007;120:144-149.
7. Ege MJ, Frei R, Bieli C, et al. Not all farming environments protect against the development of asthma and wheeze in children. *J Allergy Clin Immunol.* 2007;119:1140-1147.
8. El-Sharif N, Douwes J, Hoet P, Nemery B. Childhood asthma and indoor aeroallergens and endotoxin in Palestine: A case-control study. *Journal of Asthma.* 2006;43:241-247.

9. Lawson JA, Dosman JA, Rennie DC, et al. Endotoxin as a determinant of asthma and wheeze among rural dwelling children and adolescents: A case-control study. *BMC Pulmonary Medicine.* 2012;12:56 (10 pages).

10. Litonjua AA, Milton DK, Celedon JC, Ryan L, Weiss ST, Gold DR. A longitudinal analysis of wheezing in young children: the independent effects of early life exposure to house dust endotoxin, allergens and pets. *J Allergy Clin Immunol.* 2002;110:736-742.

11. Rennie DC, Lawson JA, Kirychuk S, et al. Assessment of endotoxin levels in the home and current asthma and wheeze in school-age children. *Indoor Air.* 2008;18:447-453.

12. Tavernier GOG, Fletcher GD, Francis HC, et al. Endotoxin exposure in asthmatic children and matched healthy controls: results of IPEADAM study. *Indoor Air.* 2005;15 (Suppl 10):25-32.

13. Rabinovitch N, Liu AH, Zhang L, et al. Importance of the personal endotoxin cloud in school-age children with asthma. *J Allergy Clin Immunol.* 2005;116:1053-1057.

14. Park J-H, Spiegelman DL, Gold DR, Burge HA, Milton DK. Predictors of airborne endotoxin in the home. *Environmental health perspectives.* 2001;109(8):859.

15. Johannesson S, Rappaport SM, Sallsten G. Variability of environmental exposure to fine particles, black smoke, and trace elements among a Swedish population. *Journal of exposure science & environmental epidemiology.* 2011;21(5):506-514.

16. Horick N, Weller E, Milton DK, Gold DR, Li R, Spiegelman D. Home endotoxin exposure and wheeze in infants: correction for bias due to exposure measurement error. *Environ Health Perspect.* 2006;114:135-140.

17. Halstensen AS, Nordby KC, Wouters IM, Eduard W. Determinants of microbial exposure in grain farming. *Ann Occup Hyg.* 2007;51:581-592.

18. Lee SA, Adhikari A, Grinshpun SA, McKay R, Shukla R, Reponen T. Personal exposure to airborne dust and microorganisms in agricultural environments. *J Occ Env Hyg.* 2006;3:118-130.

19. Nieuwenhuijsen MJ, Noderer KS, Schenker MB, Vallyathan V, Olenchock SA. Personal exposure to dust, endotoxin and crystalline silica in California agriculture. *Ann Occup Hyg.* 1999;43:35-42.

20. Harrison R, Thornton C, Lawrence R, Mark D, Kinnersley R, Ayres JG. Personal exposure monitoring of particulate matter, nitrogen dioxide, and carbon monoxide, including susceptible groups. *Occupational and environmental medicine.* 2002;59(10):671-679.

21. Wheeler AJ, Xu X, Kulka R, et al. Windsor, Ontario exposure assessment study: design and methods validation of personal, indoor, and outdoor air pollution monitoring. *Journal of the Air & Waste Management Association.* 2011;61(2):142-156.

22. Koehler KA, Peters TM. New methods for personal exposure monitoring for airborne particles. *Current environmental health reports.* 2015;2(4):399-411.

23. Delfino RJ, Staimer N, Tjoa T. Personal endotoxin exposure in a panel study of school children with asthma. *Environmental Health.* 2011;10(1):69.

24. Rabinovitch N, Liu AH, Zhang L, et al. Importance of the personal endotoxin cloud in school-age children with asthma. *Journal of Allergy and Clinical Immunology.* 2005;116(5):1053-1057.

25. Park J-H, Spiegelman DL, Gold DR, Burge HA, Milton DK. Predictors of airborne endotoxin in the home. *Environmental health perspectives.* 2001;109(8):859-864.

26. Tager IB, Lurmann FW, Haight T, Alcorn S, Penfold B, Hammond SK. Temporal and spatial patterns of ambient endotoxin concentrations in Fresno, California. *Environmental health perspectives.* 2010;118(10):1490-1496.

27. Raaschou-Nielsen O, Lohse C, Thomsen BT, Skov H, Olsen J. Ambient air levels and the exposure of children to benzene, toluene and xylenes in Denmark. *Environmental Research.* 1999;75:149-159.

28. Sagona JA, Shalat SL, Wang Z, et al. Evaluation of particle resuspension in young children′ s breathing zone using stationary and robotic (PIPER) aerosol samplers. *Journal of aerosol science.* 2015;85:30-41.

29. Sagona JA, Shalat SL, Wang Z, et al. Comparison of particulate matter exposure estimates in young children from personal sampling equipment and a robotic sampler. *Journal of exposure science & environmental epidemiology.* 2017;27(3):299-305.

30. Priftis KN, Mantzouranis EC, Anthracopoulos MB. Asthma symptoms and airway narrowing in children growing up in an urban versus rural environment. *J Asthma.* Apr 2009;46(3):244-251.

31. Ma Y, Zhao J, Han ZR, Chen Y, Leung TF, Wong GW. Very low prevalence of asthma and allergies in schoolchildren from rural Beijing, China. *Pediatr Pulmonol.* Aug 2009;44(8):793-799.

32. Lawson JA, Rennie DC, Cockcroft DW, et al. Childhood asthma, asthma severity indicators, and related conditions along an urban-rural gradient: a cross-sectional study. *BMC pulmonary medicine.* 2017;17(1):4-4.

33. Naleway AL. Asthma and atopy in rural children: is farming protective? *Clinical medicine & research.* 2004;2(1):5-12.

34. von Mutius E. The microbial environment and its influence on asthma prevention in early life. *Journal of Allergy and Clinical Immunology.* 2016;137(3):680-689.

35. Lin X, Ren X, Xiao X, et al. Important Role of Immunological Responses to Environmental Exposure in the Development of Allergic Asthma. *Allergy Asthma Immunol Res.* 0/ 2020;12.

36. Ege MJ, Mayer M, Normand A-C, et al. Exposure to environmental microorganisms and childhood asthma. *New England Journal of Medicine.* 2011;364(8):701-709.

37. Murrison LB, Brandt EB, Myers JB, Hershey GKK. Environmental exposures and mechanisms in allergy and asthma development. *The Journal of clinical investigation.* 2019;129(4):1504-1515.

38. Lawson JA, Chu LM, Rennie DC, et al. Prevalence, risk factors, and clinical outcomes of atopic and nonatopic asthma among rural children. *Ann Allergy Asthma Immunol.* Mar 2017;118(3):304-310.

39. Adler A, Tager I, Quintero DR. Decreased prevalence of asthma among farm-reared children compared with those who are rural but not farm-reared. *Journal of Allergy and Clinical Immunology.* 2005/01/01/ 2005;115(1):67-73.

40. Illi S, Depner M, Genuneit J, et al. Protection from childhood asthma and allergy in Alpine farm environments—the GABRIEL Advanced Studies. *Journal of Allergy and Clinical Immunology.* 2012;129(6):1470-1477. e1476.

41. Julian TR, Pickering AJ. A pilot study on integrating videography and environmental microbial sampling to model fecal bacterial exposures in peri-urban Tanzania. *PLoS One.* 2015;10(8):e0136158.

42. Takeuchi A, Ogawa Y, Nishinoiri O, Kanno S, Shimizu H. Development of a method for monitoring personal exposure to benzyl violet 4B and direct blue 15 in workplace air. *Journal of occupational health.* 2017:17-0190-BR.

43. Rohlman D, Dixon HM, Kincl L, et al. Development of an environmental health tool linking chemical exposures, physical location and lung function. *BMC public health.* 2019;19(1):854.

44. Weiland S, Björkstén B, Brunekreef B, Cookson W, Von Mutius E, Strachan D. Phase II of the International Study of Asthma and Allergies in Childhood (ISAAC II): rationale and methods. *European Respiratory Journal.* 2004;24(3):406-412.

45. Gereda JE, Leung DYM, Liu AH. Levels of environmental endotoxin and prevalence of atopic disease [6]. *Journal of the American Medical Association.* 2000;284(13):1652-1653.

46. Cherid H, Foto M, Miller JD. Performance of Two Different Limulus Amebocyte Lysate Assays for the Quantitation of Fungal Glucan. *Journal of Occupational and Environmental Hygiene.* 2011/09/01 2011;8(9):540-543.

47. Oluwole O, Rennie DC, Senthilselvan A, et al. The association between endotoxin and beta-(1 → 3)-D-glucan in house dust with asthma severity among schoolchildren. *Respiratory Medicine.* 2018/05/01/ 2018;138:38-46.

48. Hwang SH, Yoon CS, Park JB. Outdoor (1→ 3)-β-D-glucan levels and related climatic factors. *Journal of Preventive Medicine and Public Health.* 2014;47(2):124.

49. Hwang S, Park DU, Yoon C. Seasonal variation in (1→3)-β-D-glucan levels with health risk assessment and related factors in indoor environments of microbiology laboratories. *Human and Ecological Risk Assessment: An International Journal.* 2019/07/04 2019;25(5):1096-1106.

50. Kozajda A, Jeżak K, Cyprowski M, Szadkowska-Stańczyk I. Inhalable dust, endotoxins and (1–3)-β-d-glucans as indicators of exposure in waste sorting plant environment. *Aerobiologia.* 2017;33(4):481-491.

51. Paba E, Tranfo G, Corsetti F, Marcelloni AM, Iavicoli S. Indoor exposure to airborne endotoxin: a review of the literature on sampling and analysis methods. *Industrial health.* 2013:MS1325.

52. Kirychuk SP, Reynolds SJ, Koehncke NK, et al. Endotoxin and dust at respirable and nonrespirable particle sizes are not consistent between cage-and floor-housed poultry operations. *Annals of occupational hygiene.* 2010;54(7):824-832.

CHAPTER 7

DISCUSSION

7.1.Key findings

The goal of this thesis was to examine the association between farm-related environmental factors and asthma and atopic disease in children. This thesis included a systematic review which examined the association between early life exposures to a farm and the presence of atopy measured by skin-prick testing/IgE antibody by blood tests in school-age children; two cross-sectional analyses examining the associations between farm exposures and the presence of asthma and atopic disease among children in a rural setting; and a pilot study to test the feasibility of using a personal monitoring technique to objectively measure environmental exposures in children. All four objectives of the thesis fall under the umbrella of farm exposures and their relationship with asthma and atopy in children and have been written as manuscripts.

The main results of the four manuscripts are as follows:

Manuscript 1: "Atopy risk among school-aged children in relation to early exposures to farm environments: A systematic review"

- Results of the systematic review showed a fairly consistent inverse association between early farm-related exposures and atopy in school age children.
- In general, there is heterogeneity in the assessment of outcomes (atopy) and exposures of interest (farm exposures) in the literature.
- Based on targeted sensitivity analyses, the use of specific IgE measurements showed more consistent results compared to the skin prick test method. The "early-life" farm exposures

during pregnancy and in infancy showed a stronger association with atopy at school age compared to the exposures in the first year of life.

Manuscript 2: “Prevalence, risk factors, and clinical outcomes of atopic and nonatopic asthma among rural children”

- No associations between farm residential status and asthma phenotypes (atopic and nonatopic asthma) were observed among school-aged children in the rural region of Saskatchewan, Canada.
- Personal characteristics such as early respiratory illness and a family history of asthma were predictors of childhood asthma, regardless of atopic status.
- Compared to those with nonatopic asthma, in the past 12 months, children with atopic asthma were more likely to report a sneezy, runny, or blocked nose or have shortness of breath.
- Those with nonatopic asthma were more likely to have parents who missed work.
- Lung function measured by spirometry was generally lower in the nonatopic asthma group compared with the other groups.

Manuscript 3: “Farm exposures and allergic disease among children living in a rural setting”

- Using a large sample of school-age children exposed to farm and non-farm environments in rural Saskatchewan, the prevalence of asthma and asthma phenotypes were similar between farm and non-farm children.
- There did not appear to be differential involvement in farming activities between those with and without asthma.

- Among children 6-12 years of age, routine chores with large animals were associated with an increased risk of asthma and atopic asthma.

Manuscript 4: "An investigation of personal exposure monitoring to collect environmental exposure data in school-aged children"

- Results from the pilot suggested that objective measures of environmental exposures from PEMs could collect sufficient dust to detect endotoxin and beta-(1 → 3)-D-glucan over a relatively short period of time (17 hours).
- Evidence from our exit survey on the comfort of PEM wearing suggested that the design of the PEM device with a fanny pack should be modified to a back pack to maximize convenience and suitability in order to make its use more practical. Noise from the PEM was a problem for all participants.

7.2.How the findings fit in literature

This dissertation showed that early life exposures to a farm confers protection against atopy in school age children. This "farm effect" is supported by recent evidence that the level of exposure to environmental or microbial biodiversity in early life may impact the subsequent risk of atopy and atopic outcomes.[1-4] Exposure to increased loads of microbes such as viral, bacterial and parasitic agents associated with farming environments may contribute to the protective effects.[1-4] The microbial environment of the farm with high exposures to gram-positive bacteria and gram-negative bacteria during pregnancy and early childhood has been shown to be associated with a protection against atopy. [5,6-8] Of the farm-related factors, early-life and consistent contact to stables and the consumption of farm milk have been associated with decreased risk of IgE-sensitization.[9] This finding was confirmed from the results of the systematic review in this thesis.

This protective effect of a farming environment was not found in Manuscripts 2 and 3. In Manuscript 2, no associations between farm residential status and asthma phenotypes (atopic and nonatopic asthma) among school-aged children in the rural region of Saskatchewan, Canada were found. A major finding was that asthma was common among these rural dwelling children (14.7%). Furthermore, most of those with asthma had nonatopic asthma, which was in line with a previous study in the same province.[10] However, when we compared personal and clinical characteristics among those in specific asthma phenotypes, results showed that whilepersonal factors of early respiratory illness and family history of asthma were associated with both phenotypesonly trends were seen between other independent risk factors for atopic and nonatopic asthma specifically. Being overweight and having a dog in the home were associated with an increased risk of nonatopic asthma, while a mother with a history of smoking increased the risk of atopic asthma. We also found that FEV_1 was lower among children with nonatopic asthma compared with atopic asthma, whereas other lung function measures did not differ significantly between the 2 groups. Lower values of FEV_1, FEV_1/FVC ratio, and $FEF_{25\%-75\%}$ were also observed in those with nonatopic asthma compared to controls (those without asthma). However, these values were not statistically different between atopic asthma versus controls. Possible explanations may be that children with atopic asthma are more recognizable and properly treated while children with nonatopic asthma are overlooked and possibly undertreated. This finding was corroborated from the literature suggesting the unclear associations between lung function and atopic asthma status in children.[11,12]

In Manuscript 3, routine chores with large animals were found to be ssociated with an increased risk of asthma and atopic asthma. Data from large European studies[9,13-16] have generally

demonstrated a protective effect of participating in farm work and allergies or asthma in children. However, some specific farm-related activities including keeping hares and rabbits and the use of pressed hay was positively associated with childhood asthma.[17] In the USA, a cohort study by Merchant et al[18] in 2005 in Iowa of children from birth through 17 years of age found that a higher risk of asthma was associated with farm residence when swine was raised and even more so when swine was raised and antibiotics were added to animal feed. Findings from the United States 2008 Minority Farm Operator Childhood Agricultural Injury Survey[19] (M-CAIS) suggested that children (ages 0–19 years) who were working on their household farms had a significantly increased prevalence of asthma (13%) compared to children who were not participating in the farming operations (9%). The higher prevalence of asthma was suggested to be confounded by ethnic background.[19] In that study, exposures to specific types of farms such as beef cattle farms and ranches with animal and animal products (dander, fur, and body wastes) have been associated with asthma and other respiratory symptoms.[20]

In Saskatchewan, the associations between farm activities and respiratory illness have not been consistent.[21,22] While protective effects of doing farm work were generally found for asthma, inconsistency exists for respiratory symptoms (e.g. wheeze, cough).[22] Children who were exposed to emptying and filling of grain bins had higher odds of asthma and reports of playing on or near hay bales and cleaning pens were associated with increased respiratory symptoms.[21] The increased risk of pediatric asthma in relation to some farm activities observed in Saskatchewan and the US might be explained partly by the similarity in farm practices and environmental layouts in North American farms compared to European farms, leading to similar environmental irritants and

allergens on the farm that North American children might be exposed to but would not be exposed to in Europe.

Diverse microbial exposure early in life stimulates immune tolerance, protecting against atopy to common antigens throughout life,[23,24] with the strongest protection when exposures to farm animals and hay or grain products occur from pregnancy to school age.[25] The possible immunological mechanism for the protective effect of early exposures to the farming environment with a wide range of microbes on atopic disease are still unclear, possibly by promoting type 1 T-helper (Th) lymphocytes, inhibiting atopic Th2 response.[26] This protection seems to extend beyond childhood as it has been recently shown that a farm-upbringing is associated with decreased atopy and atopic disease in adults.[27,28] However, results from Manuscript 3 with regard to the increased risk of asthma among children aged 6-12 years who performed routine chores with large animals in the past year conflicted with the immune response mechanism as described. It may be differences in the immune response between early life and later childhood. This may be because childhood asthma is complex with a strong interaction of genetic, epigenetic, and environmental factors.[29]

Results of this thesis added to the literature that has shown the inconsistent associations between farm exposures and asthma and allergies, possibly because of differences in definitions and measurement of environmental exposures. There is a heterogeneity of "farm" measurements which varies from type of farming, intensity of farm work involvement, and timing of farm exposures (e.g. early life or later in life).[3] The inconsistent definitions and measurements of farm and farm-related activities hamper the efforts to compare results from different studies.

With regard to the personal dust sampling results, it is challenging to compare our results to other studies of children due to the difference of types of filters used, pump speed, durations of wearing.[30,31] For example, the durations of wearing a PEM were different between studies such as school-based endotoxin measurements during 2 intervals (ten consecutive schooldays and two consecutive schooldays repeated 3 times) by Rabinovitch et al[30] in comparison to the home-based endotoxin assessment periods of 10 days duration used by Delfino et al in.[31] Also, personal exposure monitors were operated at 4 L/min by Delfino et al[31] and 2 L/min by Rabinovitch et al[30]. Therefore, it is challenging to compare our results to others given the differences in operations. Our results suggest that PEM operated at 2 L/min over 17 hours should be sufficient for dust collection.

Our findings regarding the correlations between dust samples from different sources (play area, mattress, and personal dust sampling using the PEM) were not always consistent with previous findings. We observed mixed correlations (high and low in some pairs) that were not statistically significant, likely due to the small sample size, resulting in lower statistical power. Similar to our findings, no statistically significant results were found for the correlations between endotoxin measured by floor vacuumed dust and by stationary airborne samplers in one study conducted by Park et al (2001).[32] However, the airborne samplers in that study are not from a PEM, and the Spearman's correlation coefficients between endotoxin from airborne monitoring and floor or mattress sampling were low (0.23 and 0.33, respectably). While the methods were different in the previous study, we also found a low correlation.

For the past few decades, different methods have been used to collect "dust" samples to quantify microbial compounds such as endotoxin, LPS which are ubiquitous at elevated levels in

a farm environment. Due to some limitations of such measurements, it is suggested that a PEM can be used in epidemiological studies to account for "personal cloud" in which each person is exposed differently to an environment. For example, this method will be useful to accurately measure "farm" exposures in which children are exposed or actively participated in farm activities as parts of their daily routine or occasional events.

We found that the atopic asthma was more prevalent compared to non-atopic asthma in Manuscripts 2&3, which did not support the common perception about the dominance of atopic asthma in children. There are several explanations for this observation. First, the proportion of asthma that can be attributable to atopy varies geographically.[33] Atopic asthma is more common than nonatopic asthma in which atopy was defined as the presence of allergic sensitization, specifically among school-aged children in the USA, other countries with a Western lifestyle,[33] and recently Puerto Rico.[34] In contrast, non-atopic asthma is found to be more common in some Latin American countries such as Brazil and Ecuador, and in Canada.[10,35-37] ISAAC phase II study centers in Latin America reported that only 11% of asthma was attributable to atopy.[33] In a cross-sectional study among 3960 children aged 6–16 years living in rural communities in Ecuador, the population fraction of asthma attributable to atopy was extremely low (2.4%).[35] In that study, atopy was defined by allergen skin prick reactivity. In a cross-sectional survey in Saskatchewan, Canada, the prevalence of atopic asthma was 21.1% among 351 school-age children living on two rural reserve communities, in which atopy was defined by a greater or equal to 3-mm wheal response to any of six respiratory allergens upon skin prick testing.[10]

Literature has suggested that while sharing common risk factors such as family history of asthma/allergic diseases, factors that affect atopic and non-atopic asthma presence can be

different.[38] For example, potentially modifiable risk factors for atopic asthma (eg, BMI), nonatopic asthma (eg, daycare attendance), or both (eg, an unhealthy diet) were found in Puerto Rican children.[34] However, socioeconomic levels have been suggested to be associated with the higher prevalence of nonatopic asthma/wheeze compared to atopic phenotype. Heavy *Trichuris trichiura* infections were found to be strongly inversely associated with atopic wheeze in one study in 2007 among 3960 children aged 6–16 years living in rural communities in Ecuador.[35] Such infections are highly prevalent in underprivileged populations and are hypothesized that such factors may attenuate allergic asthma. Since clinical features are similar between atopic and non-atopic asthma, and the overlapping of the risk factors between the two, future studies should consider examining asthma phenotypes by different methods rather than based on atopic and non-atopic mechanisms.

Another explanation is that other exposures that may lead to irritant induced asthma that may be non-atopic.[39] Intensive livestock operations may be releasing air pollutants into the atmosphere, which consist of a mixture of gases and particulate matter (PM) contaminated with toxins/pollutants such as endotoxins,[20] hydrogen sulfide (H_2S), and ammonia, among many others.[40] Such variation in livestock air pollution emissions is associated with asthma-like symptoms, and lung function deficits in both farming and nonfarming residents.[41] An increased incidence of higher non-IgE-mediated asthma was reported in children living in a rural setting in some studies.[42,43] Possible explanations for such observations were that high endotoxin levels and the proinflammatory effects of endotoxin might exacerbate already existing asthma.[44,45] It has been of interest to understand the effect of residential proximity to agricultural activities, e.g., large swine animal feeding operations, on respiratory health outcomes,[46] specifically asthma-like symptoms without the allergic mechanisms involved. Future epidemiological and occupational

studies should expand on exploring such possible exposures to better understand the etiology of asthma phenotypes in children, regardless of their location of residence (children living in towns or rural non-farming locales or farming children). In the domestic environment, significant concentrations of endotoxin occur from pets kept indoors, carpeting, as well as air conditioning.[47,48] Endotoxin levels between such areas have been shown to vary. House dust endotoxin appears to be higher in common household places such as the family or living room floor, the kitchen floor, and the bedroom sofa.[49] Endotoxin from dust sampled from mattresses have better reproducibility and less variation than that from floors. Studies have reported that household endotoxin levels within the same location depends upon seasonality,[50] with higher concentrations during the summer. However, the seasonal variations of endotoxin levels have not been consistently detected.[48,51] The predictors of household endotoxin have been shown to vary and include environmental tobacco smoke, infrequent dusting, carpeted floor, the number of children and occupants of the home, dogs present in the home, living in poverty, evidence of cockroach infestation, humidity, moisture and dampness, home burning biomass fuels, and the absence of indoor ventilation systems.[52,53] Such different sources of endotoxin may result in different associations with respiratory health outcomes.[42,52]

Endotoxin from different sources may contain different molecular composition. A study by Park et al[54] found that LPS within homes were qualitatively different depending upon different locations in the home. That study also found that different types of bacteria were observed in different areas of the home. It has been suggested that variations in structure of LPS may control endotoxin activity, potentially affecting human health. This notion was supported by a study by Zhao et al[55]. In that study, when taking into account the differences in length of LPS, an inversed

association between endotoxin measured from schools and attacks of breathlessness was found with a shorter length of LPS, while an opposite association was observed with a longer length of LPS.

In this current thesis, results from two different studies in Saskatchewan using similar but different methods to assess asthma and atopy among children support the same patterns in that higher prevalence of non-atopic asthma compared to atopic asthma. Environmental exposures may contributes to explain these findings. Better exposure monitoring and investigation of charactization of micobial compounds (e.g., endotoxin) as well as its interaction with different allergens in the environment are needed to undertand the underlying mechanisms of such high prevalence of childhood non-atopic asthma.

7.3.Validity of the study

7.3.1. Internal validity

In epidemiological studies, it is important to address factors or alternative explanations for the findings. The process to rule out such alternate explanations includes the assessment of internal validity, in which research design and/or operational procedures for the study are evaluated. In this chapter, given the differences in study types for each manuscript (a systematic review, cross-sectional designs and a pilot study), it is difficult to put these study types under one type of validity assessment. Therefore, most of the evaluations regarding internal validity will be available for the first 3 manuscripts. Because Manuscript 4 is a pilot study, the evaluation will be limited.

7.3.1.1.Selection bias

In epidemiological studies, selection bias occurs when there is a systematic difference between the characteristics of those selected and not selected for the study.

One source of selection bias in Manuscript 1 (the systematic review) is that not all available data on the topic may have been identified throughout the process, raising the concern of publication bias. One source of publication bias is that data from statistically significant studies are more likely to be published than those that are not. In our systematic review, two authors simultaneously conducted a rigorous search using multiple search engines (e.g. PubMed, EMBASE), then agreed through consensus which articles would be included. Articles that appeared in the reference list of each individual study were also considered. Despite this process, there still could be articles missed. To help assess publication bias, the relationship between effect estimates (the magnitude of the exposure's effect on the outcome (the ORs)) and sample size of studies included in the systematic review graphically were plotted. The visual symmetry and funnel shape observed in our findings suggested a low risk of publication bias. To address selection bias of primary studies included in the systematic review, a validity checklist was used to consider this issue.Lack of meta-analysis to determine a common effect due to heterogeneity of studies.

In Manuscript 2, the response rate was modest, which limits generalizing the results and presents a potential for selection bias. For the survey portion, 5667 children were approached with 2383 children taking part for a response rate of 42%. A total of 584 out of 1768 students from 16 schools who were approached, took part in clinical testing. These 1768 were from among the 2383 who took part. We conducted an analysis to compare characteristics of children who took part in clinical testing and those who did not. It was found that the two groups were similar in most characteristics such as history of asthma or report of allergic disease. There was a slight difference in those who took part in that they were more likely to be male (54% vs. 48%, respectively) and younger (mean age, 9.6 years vs. 10.3 years) compared to those who did not.

In Manuscript 3, the SFIC used a multistage sampling technique with stratification and random selection at the municipality level together with survey methods to recruit school age children. The participation rate was 31%, resulting in a total of 2328 school students participating in this study. This modest participation rate raised the concern of possible selection biases, in which prevalence and risk factors of farm exposures and outcomes of interest (asthma, atopy) may not be representative of the overall rural child population of Saskatchewan. Since the primary focus of the SFIC study was to investigate farm-related injuries among children, if selection bias occurs, it will likely be non-differential among those those with asthma or allergies.

In Manuscript 4, we only included 5 participants and recruited these participants by using a convenience sampling technique, which might result in issues of selection bias. However, this was a pilot study. Future studies using the PEM should follow a more rigorous epidemiological approach to ensure selection bias to be minimized.

7.3.1.2.Information bias

Information bias, also called measurement bias, is a distortion in the measure of associations between exposures and outcomes due to a lack of accurate measurements of key study variables.

In Manuscript 1, despite the heterogeneity in the studies included in the systematic review with regard to exposures and outcomes, objective measures of outcomes (atopy by skin prick testing and IgE antibody by blood tests) were used, leading to a lower likelihood of measurement bias from the outcome perspective. Information about early life exposures was based on parental questionnaires and was collected retrospectively, raising the issues of recall bias. In epidemiological studies, objective measures are highly recommended to reduce bias. It would be

optimal to collect dust samples with subsequent measurements of microbial compounds (e.g. endotoxin) as opposed to asking questions via questionnaire.

In Manuscript 2, information about asthma status was drawn from parent report, which can be misclassified. However, in epidemiological studies, questionnaires have been commonly and widely used to define respiratory outcomes, asthma included, and have generally been accurate.[56] In this study, atopy status was objectively measured using skin prick testing, which should reduce the likelihood of misclassification when categorizing asthma phenotypes. Also, lung function was objectively measured using spirometry and increased the strength of the findings in this study. Due to the nature of the cross-sectional design used, the issue of temporality to establish a casual association should be cautiously interpreted.

In Manuscript 3, a questionnaire was used to collect information about farm exposures and asthma and atopy. One of the limitations of the use of questionnaires was the introduction of recall bias. However, this method has been commonly used in epidemiological research. Evidence of how accurately such measures capture the truth or so called "test validity" with regard to farm exposures has been limited. For example, farm activities (e.g. feeding livestock) are measured in the past 12 months with an estimation of intensities (every day, at least once a week, or never), which may be difficult for a participant to accurately estimate, especially for parents to report for their children on their behalf. It is difficult to recall, in detail, all of the farm activities of a child during different seasons of the year. Also, the definition of physician-diagnosed asthma and or atopy used in this study were self-reported. The information on physician-diagnosed asthma has often been used in epidemiological studies,[57-59] since there is not a gold standard to diagnose

asthma.[60-62] To date, this method has been the optimal approach in epidemiological research.[63,64] Moreover, the definition of atopy in this study was from a self-reported questionnaire. Since there is not a so-called gold standard to identify atopic individuals, better measurements with the combination of objective measures using skin-prick testing or specific IgE measurements and questionnaires may be considered to collect medical information, particularly historical information, in a large study sample.[65] Finally, the focus of the SFIC was not atopy, but farm-related injuries, leading to a lack of potentially important information about asthma and atopic disease such as severity, history, management, healthcare utilization, and so on.

In Manuscript 4, dust samples were collected objectively using recommended protocols[66] and appropriate analytical procedures based on the quantitative kinetic chromogenic LAL assay (endotoxin) and the Kinetic Onset Time Glucatell assay [beta-(1→3)-D-glucan]. By doing so, the possibility of misclassification of the quantity of microbial compounds (endotoxin and beta-(1→3)-D-glucan) might be minimized. Another source of misclassification bias could be from the method of collecting feedback of the comfort of PEM using. The influence of the face-to-face approach to ask for your children's feedback might affect the true evaluations using the scale 0-10 towards the comfort of PEM using.

7.3.1.3.Confounding factors

Confounding is a distortion in the estimated measure of association between an exposure and an outcome that occurs when the primary exposure of interest is biased because of other factors that are associated with the outcome.[67]

In Manuscript 1, confounders were adjusted for in each individual study included in the systematic review through multivariate modeling analyses. However, the number and types of

confounding factors were different in each study. In the systematic review, stratification analyses were utilized to help consider these differences. We stratified by exposure timing (pregnancy vs first years) between farm-related exposures and atopy as well as SPT vs. IgE measurement methods.

In Manuscript 2, a wide range of potential confounders was collected using questionnaire report. These variables were used in previous studies with similar topics (e.g., farm exposures and asthma in children).[13,14,68-70] These confounders were appropriately adjusted for in the multivariate analyses following a well-established purposeful selection technique.[71]

In Manuscript 3, a series of multivariate modelling techniques and stratification was used to adjust for confounders in the associations between exposures and outcomes. However, it is possible that there are some unmeasured potential confounders (residual confounders) such as information on household income, healthcare, and age at which asthma was diagnosed as well as indoor environmental information such as mold, cat dander, etc. Such residual confounders might then bias effect estimates towards or away from the null, limiting the interpretation of the findings and may explain inconsistencies across studies. Due to the secondary nature of the analyses, only variables/confounders available in the questionnaire were used in the multivariate analyses.

In Manuscript 4, no control of confounding was conducted. Future studies utilizing information from a PEM and examining associations between microbial compounds from the dust samples should consider to adjust for potential confounders. Some factors that can confound these associations include dog and cat ownership, number of people in the home, carpet (percentage, age, and regularity of cleaning), observed cockroaches, observed rodents, flooding damage, surface mold or mildew, livestock, central air-conditioning, to name a few.[31,72]

7.3.2. External validity

In Manuscript 1, the results could be generalized to the population included in the systematic review of which the majority of the primary studies were from North America or Europe. However, the results might not necessarily be applicable to those from different continents such as Asia or Africa. Also, the systematic review also applied a clear set of inclusion and exclusion criteria. Thus, generalizability should be considered within this context. For example, results from the systematic review might not be generalized to food atopy because the skin tests or blood tests did not include food allergens.

For Manuscript 2, the modest participation rate (42%) may raise the issue of a lack of generalizability. However, our findings can be generalized to the non-clinical study population because the characteristics between the clinical and non-clinical populations were similar.

For Manuscript 3, the response rates for the SFIC study are low, with 21.5% of school-age children participating. As a result, the prevalence of outcomes of interest (asthma and atopic disease) and farm exposures might not be representative of the Saskatchewan rural farm and non-farm population, leading to the issue of over- and under-report of subject matters such as exposures and outcomes of interest. However, the SFIC was not solely designed to be representative in terms of asthma and atopic disease prevalence. Instead, it was designed to capture a heterogeneous range of exposures, especially to a farm. Also, a complex stratified sampling technique was used to maximize participant rates across stratifications.[73] Generalizability of the findings should be interpreted cautiously. Specifically, findings with regard to descriptive or multivariate associations between farm exposures and asthma and atopic disease found in Manuscript 2 may not be applicable to populations outside of the age range studied (6–17 years old). Despite stratified

analyses being conducted among different age groups (0-17 years old), very limited farm exposure in the age group of 0-5 years made it hard to draw any conclusions around the associations between exposures and outcomes if any.

Results from Manuscript 4 suggested that it was feasible to collect dust samples using PEM in a general group of children. Since there are some modifications suggested such as using a backpack instead of a fanny bag, future studies elsewhere should also consider that, especially in school age children. School-children have relatively fixed time-schedule patterns such as activities in classroom, indoors and outdoors at home. Therefore, the use of PEM will take into account the movements in different locations and capture the "personal cloud" that children expose themselves.

7.4.References

1. von Mutius E, Vercelli D. Farm living: effects on childhood asthma and allergy. *Nat Rev Immunol.* Dec 2010;10(12):861-868.
2. Braun-Fahrlander C, Riedler J, Herz U, et al. Environmental exposure to endotoxin and its relation to asthma in school-age children. *N Engl J Med.* Sep 19 2002;347(12):869-877.
3. von Mutius E, Braun-Fahrlander C, Schierl R, et al. Exposure to endotoxin or other bacterial components might protect against the development of atopy. *Clin Exp Allergy.* Sep 2000;30(9):1230-1234.
4. Schram-Bijkerk D, Doekes G, Douwes J, et al. Bacterial and fungal agents in house dust and wheeze in children: the PARSIFAL study. *Clin Exp Allergy.* Oct 2005;35(10):1272-1278.

5. Ege MJ, Bieli C, Frei R, et al. Prenatal farm exposure is related to the expression of receptors of the innate immunity and to atopic sensitization in school-age children. *Journal of Allergy and Clinical Immunology.* 2006;117(4):817-823.

6. Ege MJ, Herzum I, Buchele G, et al. Prenatal exposure to a farm environment modifies atopic sensitization at birth. *J Allergy Clin Immunol.* Aug 2008;122(2):407-412, 412 e401-404.

7. Loss G, Bitter S, Wohlgensinger J, et al. Prenatal and early-life exposures alter expression of innate immunity genes: the PASTURE cohort study. *J Allergy Clin Immunol.* Aug 2012;130(2):523-530 e529.

8. Batool T, Cyr MM, Denburg JA, Schulze K, Anand S, Teo K. Pre-natal and early life predictors of atopy in canadian children: Results of the family study. *Journal of Allergy and Clinical Immunology.* February 2014;1):AB235.

9. Alfven T, Braun-Fahrlander C, Brunekreef B, et al. Allergic diseases and atopic sensitization in children related to farming and anthroposophic lifestyle--the PARSIFAL study. *Allergy.* Apr 2006;61(4):414-421.

10. Rennie DC, Karunanayake CP, Lawson JA, et al. Domestic Risk Factors for Atopic and non-Atopic Asthma in First Nations Children Living in Saskatchewan, Canada. *Children (Basel).* Apr 27 2020;7(5).

11. Carroll WD, Lenney W, Child F, et al. Asthma severity and atopy: how clear is the relationship? *Archives of disease in childhood.* 2006;91(5):405-409.

12. Gürkan F, Davutog M, Bilici M, Sincar N, Haspolat K. Pulmonary functions in atopic and nonatopic asthmatic children. *Allergologia et immunopathologia.* 2002;30(2):70-73.

13. Illi S, Depner M, Genuneit J, et al. Protection from childhood asthma and allergy in Alpine farm environments-the GABRIEL Advanced Studies. *J Allergy Clin Immunol.* Jun 2012;129(6):1470-1477 e1476.

14. Macneill SJ, Sozanska B, Danielewicz H, et al. Asthma and allergies: is the farming environment (still) protective in Poland? The GABRIEL Advanced Studies. *Allergy.* Apr 29 2013.

15. Lampi J, Canoy D, Jarvis D, et al. Farming environment and prevalence of atopy at age 31: prospective birth cohort study in Finland. *Clin Exp Allergy.* Jul 2011;41(7):987-993.

16. Wjst M. Another explanation for the low allergy rate in the rural Alpine foothills. *Clin Mol Allergy.* Jun 5 2005;3:7.

17. Ege M, Frei R, Bieli C, et al. Not all farming environments protect against the development of asthma and wheeze in children. *J Allergy Clin Immunol.* 2007;119:1140 - 1147.

18. Merchant JA, Naleway AL, Svendsen ER, et al. Asthma and farm exposures in a cohort of rural Iowa children. *Environmental health perspectives.* 2005;113(3):350-356.

19. Syamlal G, Hendricks K, Mazurek JM. Asthma among household youth on racial minority operated farms—United States, 2008. *Journal of agromedicine.* 2018;23(2):144-153.

20. Heederik D, Sigsgaard T, Thorne PS, et al. Health Effects of Airborne Exposures from Concentrated Animal Feeding Operations. *Environmental Health Perspectives.* 2007;115(2):298-302.

21. Farthing P, Rennie D, Pahwa P, Janzen B, Dosman J. The association between farming activities and respiratory health in rural school age children. *J Agromedicine.* 2009;14(2):256-262.

22. Barry RJ, Pickett W, Rennie DC, et al. The role of farm operational and rural environments as potential risk factors for pediatric asthma in rural Saskatchewan. *Pediatr Pulmonol.* Sep 2014;49(9):842-851.

23. Rutkowski K, Sowa P, Rutkowska-Talipska J, Sulkowski S, Rutkowski R. Allergic diseases: the price of civilisational progress. *Postepy Dermatol Alergol.* May 2014;31(2):77-83.

24. Von Hertzen L, Hanski I, Haahtela T. Natural immunity. *EMBO reports.* 2011;12(11):1089-1093.

25. Douwes J, Cheng S, Travier N, et al. Farm exposure in utero may protect against asthma, hay fever and eczema. *Eur Respir J.* Sep 2008;32(3):603-611.

26. Schuijs MJ, Willart MA, Vergote K, et al. Farm dust and endotoxin protect against allergy through A20 induction in lung epithelial cells. *Science.* Sep 4 2015;349(6252):1106-1110.

27. Campbell B, Raherison C, Lodge CJ, et al. The effects of growing up on a farm on adult lung function and allergic phenotypes: an international population-based study. *Thorax.* Mar 2017;72(3):236-244.

28. Elholm G, Linneberg A, Husemoen LL, et al. The Danish urban-rural gradient of allergic sensitization and disease in adults. *Clin Exp Allergy.* Jan 2016;46(1):103-111.

29. Krusche J, Basse S, Schaub B. Role of early life immune regulation in asthma development. Paper presented at: Seminars in Immunopathology2019.

30. Rabinovitch N, Liu AH, Zhang L, et al. Importance of the personal endotoxin cloud in school-age children with asthma. *Journal of Allergy and Clinical Immunology.* 2005;116(5):1053-1057.

31. Delfino RJ, Staimer N, Tjoa T. Personal endotoxin exposure in a panel study of school children with asthma. *Environmental Health.* 2011;10(1):69.

32. Park J-H, Spiegelman DL, Gold DR, Burge HA, Milton DK. Predictors of airborne endotoxin in the home. *Environmental health perspectives.* 2001;109(8):859.

33. Weinmayr G, Weiland SK, Björkstén B, et al. Atopic sensitization and the international variation of asthma symptom prevalence in children. *Am J Respir Crit Care Med.* Sep 15 2007;176(6):565-574.

34. Landeo-Gutierrez J, Han YY, Forno E, et al. Risk factors for atopic and nonatopic asthma in Puerto Rican children. *Pediatr Pulmonol.* Sep 2020;55(9):2246-2253.

35. Moncayo AL, Vaca M, Oviedo G, et al. Risk factors for atopic and non-atopic asthma in a rural area of Ecuador. *Thorax.* May 2010;65(5):409-416.

36. da Silva TM, Fiaccone RL, Kehdy FSG, et al. African biogeographical ancestry, atopic and non-atopic asthma and atopy: A study in Latin American children. *Pediatr Pulmonol.* Feb 2019;54(2):125-132.

37. Castro-Rodriguez JA, Ramirez AM, Toche P, et al. Clinical, functional, and epidemiological differences between atopic and nonatopic asthmatic children from a

tertiary care hospital in a developing country. *Annals of Allergy, Asthma & Immunology.* 2007/03/01/ 2007;98(3):239-244.

38. Dharmage SC, Perret JL, Custovic A. Epidemiology of Asthma in Children and Adults. *Frontiers in pediatrics.* 2019;7:246-246.

39. May S, Romberger D, Poole J. Respiratory Health Effects of Large Animal Farming Environments. *Journal of toxicology and environmental health. Part B, Critical reviews.* 11/01 2012;15:524-541.

40. Guidry VT, Kinlaw AC, Johnston J, Hall D, Wing S. Hydrogen sulfide concentrations at three middle schools near industrial livestock facilities. *J Expo Sci Environ Epidemiol.* Mar 2017;27(2):167-174.

41. Borlée F, Yzermans J, Aalders B, et al. Air Pollution from Livestock Farms Is Associated with Airway Obstruction in Neighboring Residents. *American journal of respiratory and critical care medicine.* 05/10 2017;196.

42. Lawson JA, Dosman JA, Rennie DC, Beach J, Newman SC, Senthilselvan A. The association between endotoxin and lung function among children and adolescents living in a rural area. *Can Respir J.* Nov-Dec 2011;18(6):e89-94.

43. LAWSON JA, DOSMAN JA, RENNIE DC, BEACH J, NEWMAN SC, SENTHILSELVAN A. Relationship of endotoxin and tobacco smoke exposure to wheeze and diurnal peak expiratory flow variability in children and adolescents. *Respirology.* 2011;16(2):332-339.

44. Cleave J, Willson P, Town J, Gordon J. Fractionation of Swine Barn Dust and Assessment of Its Impact on the Respiratory Tract Following Repeated Airway Exposure. *Journal of toxicology and environmental health. Part A.* 06/24 2010;73:1090-1101.

45. Reed CE, Milton DK. Endotoxin-stimulated innate immunity: A contributing factor for asthma. *J Allergy Clin Immunol.* Aug 2001;108(2):157-166.

46. Pavilonis BT, Sanderson WT, Merchant JA. Relative exposure to swine animal feeding operations and childhood asthma prevalence in an agricultural cohort. *Environ Res.* Apr 2013;122:74-80.

47. Singh J, Schwartz DA. Endotoxin and the lung: Insight into the host-environment interaction. *J Allergy Clin Immunol.* Feb 2005;115(2):330-333.

48. Gereda JE, Klinnert MD, Price MR, Leung DYM, Liu AH. Metropolitan home living conditions associated with indoor endotoxin levels. *Journal of Allergy and Clinical Immunology.* 2001/05/01/ 2001;107(5):790-796.

49. Chinn IN, Williams LW. Endotoxin Exposure Is a Risk Factor for Asthma: The National Survey of Endotoxin in United States Housing. *Pediatrics.* 2007;120(Supplement 3):S130-S131.

50. Salonen H, Duchaine C, Létourneau V, et al. Endotoxin levels and contribution factors of endotoxins in resident, school, and office environments — A review. *Atmospheric Environment.* 2016/10/01/ 2016;142:360-369.

51. Madsen AM. Airborne endotoxin in different background environments and seasons. *Ann Agric Environ Med.* 2006;13(1):81-86.

52. Thorne PS, Cohn RD, Mav D, Arbes SJ, Zeldin DC. Predictors of Endotoxin Levels in U.S. Housing. *Environmental Health Perspectives.* 2009;117(5):763-771.

53. Lawson JA, Dosman JA, Rennie DC, et al. Endotoxin as a determinant of asthma and wheeze among rural dwelling children and adolescents: a case-control study. *BMC pulmonary medicine.* 2012;12:56-56.

54. Park J-H, Szponar B, Larsson L, Gold DR, Milton DK. Characterization of lipopolysaccharides present in settled house dust. *Applied and environmental microbiology.* 2004;70(1):262-267.

55. Zhao Z, Sebastian A, Larsson L, Wang Z, Zhang Z, Norbäck D. Asthmatic symptoms among pupils in relation to microbial dust exposure in schools in Taiyuan, China. *Pediatr Allergy Immunol.* Aug 2008;19(5):455-465.

56. Yang CL, To T, Foty RG, Stieb DM, Dell SD. Verifying a questionnaire diagnosis of asthma in children using health claims data. *BMC pulmonary medicine.* 2011;11(1):52.

57. Asher M, Anderson H, Stewart A, Crane J. International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee. Worldwide variations in the prevalence of asthma symptoms: the International Study of Asthma and Allergies in Childhood (ISAAC). *Eur Respir J.* 1998;12:315-335.

58. Asher M, Keil U, Anderson H, et al. International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. *European respiratory journal.* 1995;8(3):483-491.

59. Mascarenhas JMO, Silva RdCR, Assis AMOd, Pinto EdJ, Conceição JS, Barreto ML. Symptoms of asthma and associated factors in adolescents from Salvador, Bahia. *Revista Brasileira de Epidemiologia.* 2016;19:181-193.

60. Saglani S, Menzies-Gow AN. Approaches to asthma diagnosis in children and adults. *Frontiers in pediatrics.* 2019;7:148.

61. Kainu A, Pallasaho P, Piirilä P, Lindqvist A, Sovijärvi A, Pietinalho A. Increase in prevalence of physician-diagnosed asthma in Helsinki during the Finnish Asthma Programme: improved recognition of asthma in primary care? A cross-sectional cohort study. *Primary Care Respiratory Journal.* 2013;22(1):64-71.

62. Lawson JA, Rennie DC, Cockcroft DW, et al. Childhood asthma, asthma severity indicators, and related conditions along an urban-rural gradient: a cross-sectional study. *BMC pulmonary medicine.* 2017;17(1):4.

63. Remes ST, Pekkanen J, Remes K, Salonen RO, Korppi M. In search of childhood asthma: questionnaire, tests of bronchial hyperresponsiveness, and clinical evaluation. *Thorax.* Feb 2002;57(2):120-126.

64. Jenkins MA, Clarke JR, Carlin JB, et al. Validation of questionnaire and bronchial hyperresponsiveness against respiratory physician assessment in the diagnosis of asthma. *Int J Epidemiol.* Jun 1996;25(3):609-616.

65. Hoppin JA, Jaramillo R, Salo P, Sandler DP, London SJ, Zeldin DC. Questionnaire predictors of atopy in a US population sample: findings from the National Health and Nutrition Examination Survey, 2005–2006. *American journal of epidemiology.* 2011;173(5):544-552.

66. Weiland S, Björkstén B, Brunekreef B, Cookson W, Von Mutius E, Strachan D. Phase II of the International Study of Asthma and Allergies in Childhood (ISAAC II): rationale and methods. *European Respiratory Journal.* 2004;24(3):406-412.

67. Skelly AC, Dettori JR, Brodt ED. Assessing bias: the importance of considering confounding. *Evidence-based spine-care journal.* 2012;3(01):9-12.

68. Horak F, Jr., Studnicka M, Gartner C, et al. Parental farming protects children against atopy: longitudinal evidence involving skin prick tests. *Clin Exp Allergy.* Aug 2002;32(8):1155-1159.

69. Riedler J, Eder W, Schreuer M, Waser M, Maisch S. Exposure to farming in early life and development of asthma and allergy: a cross-sectional survey. *Lancet.* 2001;358(9288):1129-1133.

70. Chrischilles E, Ahrens R, Kuehl A, et al. Asthma prevalence and morbidity among rural Iowa schoolchildren. *J Allergy Clin Immunol.* Jan 2004;113(1):66-71.

71. Bursac Z, Gauss CH, Williams DK, Hosmer DW. Purposeful selection of variables in logistic regression. *Source code for biology and medicine.* 2008;3(1):17.

72. Zhang L, Guo C, Jia X, et al. Personal exposure measurements of school-children to fine particulate matter (PM2. 5) in winter of 2013, Shanghai, China. *PloS one.* 2018;13(4).

73. Pickett W, King N, Marlenga B, et al. Exposure to agricultural hazards among children who visit farms. *Paediatrics & child health.* 2018;23(7):e143-e149.

CHAPTER 8

RECOMMENDATIONS AND CONCLUSIONS

8.1.Recommendations

Some recommendations for research and practice can be drawn from the results from this dissertation.

From an early age, children encounter a large number of exposures in the environment. One of the findings from this thesis regarding the increased risk of asthma and atopic disease among those who performed routine chores with large animals suggested that children should be aware of the potential risks while working on a farm.

The nonatopic phenotype was the most common presentation of childhood asthma in our study populations in a rural setting in Saskatchewan, Canada. It will be important to consider asthma in nonatopic children, particularly in children living in a rural area.

Findings from this dissertation suggest that a novel approach (PEM) to collect environmental exposures data from children might help assess the accuracy of childhood exposures. In doing so, estimates of associations between environmental exposures and outcomes such as respiratory health in epidemiological studies could be more reliable.

8.2.Future research directions

Given that the systematic review identified primarily cross-sectional studies, future work should focus on higher quality studies such as prospective prenatal cohort designs, together with clear and specific definitions of farm exposures along with increased use of objective markers for these exposures and disease outcomes. By doing this, it will help investigators to isolate key factors in

the prevention of atopic diseases; and better indicators of the total quantity and diversity of microbial exposures on a farm.

Second, findings from this thesis have shown that associations between farm and nonfarm rural children with asthma and allergies may vary depending on phenotype and specific exposures. Future work should focus on these specific issues by enhancing common definitions of asthma such as questionnaires and the use of objective markers to establish asthma phenotypes. With regard to specific exposures, this thesis has suggested that geographical locations impose different manifestations of asthma and respiratory diseases. Also, future work should include activities with large animals as a specific category when assessing farm activities/exposures in children.

Third, our results can be used as the starting point for future sample size estimates and point to equipment improvements making the PEM more suitable for use in children. Future work should include redesigning the PEM device and testing its use in larger populations as well as comparing results looking at the associations between environmental exposures and health outcomes collected by PEMs to those collected by settled dust and ambient air collection methods.

Fourth, future research should consider using the PEM device as one of the methods for sampling and allergen quantification in various exposure settings (e.g. home-based or workplace-based). Allergen monitoring can be an important tool for exposure assessment to measure the patterns of daily personal exposure to aeroallergens or other bioaerosols (fungi, endotoxin, microflora, viral aerosols etc). By using a PEM device, we could assess the “personal cloud” in the environment to ascertain exposures and investigate their specific associtations with both allergic and non-allergic asthma and potentially identify which exposures are responsible or contribute to the risk factors of non-allergic asthma while working with large animals in a farm.

Dust could also be analyzed to look at specific consistuents and relate these to the specific outcomes.

Along this line, future work may also take into account the use of cell phone-based exposure monitoring technology (e.g. global positioning system (GPS), sensor technology) to pinpoint geographic location and/or track real-time personal environmental exposures.[1]

8.3.Conclusions

Results of this dissertation support the importance of early-life exposures on a farm in the etiology of atopic disease and asthma at school-age. During the period from early life to adolescence, environmental exposures to a farm may be different and affect the development of respiratory illnesses such as asthma and allergies, among others. By understanding these associations/relationships, detrimental effects on children's health might be mitigated or tackled via public health interventions including education. In addition to this, this dissertation suggested a novel approach to collect dust samples in a farm or surrounding environments in which children might be exposed to (the PEM). In epidemiological studies, objective measures are important to assess the associations between environmental exposures and respiratory outcomes in children.

8.4.References

1. De Nazelle A, Seto E, Donaire-Gonzalez D, et al. Improving estimates of air pollution exposure through ubiquitous sensing technologies. *Environmental pollution.* 2013;176:92-99.

CHAPTER 9: APPENDICES

9.1. Appendix A

MOOSE Checklist for Meta-analyses of Observational Studies

Item No	Recommendation	Reported on Page No
Reporting of background should include		
1	Problem definition	93
2	Hypothesis statement	95
3	Description of study outcome(s)	95
4	Type of exposure or intervention used	95
5	Type of study designs used	95
6	Study population	95
Reporting of search strategy should include		
7	Qualifications of searchers (eg, librarians and investigators)	Title page
8	Search strategy, including time period included in the synthesis and key words	96
9	Effort to include all available studies, including contact with authors	-
10	Databases and registries searched	96
11	Search software used, name and version, including special features used (eg, explosion)	-
12	Use of hand searching (eg, reference lists of obtained articles)	96
13	List of citations located and those excluded, including justification	97
14	Method of addressing articles published in languages other than English	-
15	Method of handling abstracts and unpublished studies	97
16	Description of any contact with authors	-

Reporting of methods should include		
17	Description of relevance or appropriateness of studies assembled for assessing the hypothesis to be tested	97
18	Rationale for the selection and coding of data (eg, sound clinical principles or convenience)	-
19	Documentation of how data were classified and coded (eg, multiple raters, blinding and interrater reliability)	97
20	Assessment of confounding (eg, comparability of cases and controls in studies where appropriate)	97
21	Assessment of study quality, including blinding of quality assessors, stratification or regression on possible predictors of study results	97
22	Assessment of heterogeneity	98
23	Description of statistical methods (eg, complete description of fixed or random effects models, justification of whether the chosen models account for predictors of study results, dose-response models, or cumulative meta-analysis) in sufficient detail to be replicated	-
24	Provision of appropriate tables and graphics	-
Reporting of results should include		
25	Graphic summarizing individual study estimates and overall estimate	Figures 3-1, 3-2, 3-3, 3-4, 3-5 (Pages 126-129)
26	Table giving descriptive information for each study included	Table 3-2 (page 127)
27	Results of sensitivity testing (eg, subgroup analysis)	100-101
28	Indication of statistical uncertainty of findings	-
Reporting of discussion should include		
29	Quantitative assessment of bias (eg, publication bias)	102
30	Justification for exclusion (eg, exclusion of non-English language citations)	-
31	Assessment of quality of included studies	101

Reporting of conclusions should include		
32	Consideration of alternative explanations for observed results	-
33	Generalization of the conclusions (ie, appropriate for the data presented and within the domain of the literature review)	107
34	Guidelines for future research	107
35	Disclosure of funding source	NA
From: Stroup DF, Berlin JA, Morton SC, et al, for the Meta-analysis Of Observational Studies in Epidemiology (MOOSE) Group. Meta-analysis of Observational Studies in Epidemiology. A Proposal for Reporting. *JAMA*. 2000;283(15):2008-2012. doi: 10.1001/jama.283.15.2008		

9.2. Appendix B

Quality assessment score. Global rating 1=Weak; 2= Moderate; 3= Strong

No.	Study Ref	Selection Bias	Study Design	Confounders	Blinding	Data Collection Methods	Withdrawals and Drop-outs	Global rating (without blinding)	Global rating (with blinding)
1	Riedler et al (2001)	2	1	3		3	3	2	1
2	Klintberg et al (2001)	1	1	3		3	2	1	1
3	Braun-Fahrlander et al (2002)	2	1	3		3	3	2	1
4	Wickens et al (2002)	3	1	3		3	2	2	1
5	Remes et al (2003)	2	1	3		3	2	2	1
6	Xekveld et al (2005)	3	1	1	3*	3	1	1	1

7	Ege et al (2006)	2	1	3		3	3	2	1
8	Perkin et al (2006)	2	1	2		3	2	2	1
9	von Hertzen et al (2006)	2	1	3		3	2	2	1
10	Waser et al (2006)	2	1	3		3	3	2	1
11	Loss et al (2011)	3	1	3	2*	3	1	1	1
12	Illi et al (2012)	3	1	3		2	2	2	1
13	Macneil et al (2013)	3	1	2		3	2	2	1
14	Martikainen et al (2015)	3	2	1		1	2	2	1

*Studied looked at blinding component and was scored

9.3. Appendix C

The Saskatchewan Farm Injury Cohort -Child's Components Study Questionnaire

SASKATCHEWAN FARM INJURY PROJECT

Phase 2

Farm Child and Young People's Survey

UNIVERSITY OF SASKATCHEWAN

Does your family currently operate a farm?

If **YES**, Please complete the Green "Farm Child and Young People's Survey"

If **NO**, Please complete the Blue "Rural Child and Young People's Survey"

Dear Parents:

We ask that one responsible person complete the questionnaire on behalf of every child or young person in your home. **If more than one child brings this questionnaire home, please only complete one questionnaire, but ensure that you enter information for each child or young person living in the home.** Please try to answer all of the questions, but remember you don't have to answer any questions if you choose not to. When you have finished please place the questionnaire in the envelope and return it to your child's school.

Instructions

1. In Section B of this questionnaire we have asked questions about each child or young person in your family. We have included enough space in this booklet for 4 children.
2. Please read each question carefully.
3. Answer each question by placing a check mark in the box provided. For some questions you will write in the space provided.
4. Please be sure to complete the contact information on the last page.
5. **Thank you for taking part in this important study.**

The University of Saskatchewan

Sponsored by the Canadian Institutes of Health Research
(Canada's main funder of medical research)

PART A YOUR FARM OR RANCH

A-1 From the list below, please check each commodity that is produced for sale on your farm or ranch. (Check all that apply)

1. Grain crops [1]☐
(e.g., cereal, pulse, oil seeds, forage crops)
2. Cattle (beef) [2]☐
3. Cattle (dairy) [3]☐
4. Pigs [4]☐
5. Poultry [5]☐
6. Vegetables/Fruit [6]☐
7. Other animals [7]☐

A-2 What is the total area of land in your farming or ranching operation? (Exclude land rented to others)

Total Acres: _____

A-3 How many of these types of livestock are typically raised on your farm or ranch?

1. No Livestock [1]☐
2. Cattle (beef) _____ (number)
3. Cattle (dairy) _____ (number)
4. Swine _____ (number)
5. Poultry _____ (number)
6. Horses _____ (number)
7. Other _____ (number)

A-4 What is the operating arrangement of your farm?

Individual family farm [1]☐
Partnership (with or without a written agreement) [2]☐
Family corporation [3]☐
Other type [4]☐

A-5 Did you have any custom workers on your farm during 201? (Check all that apply)

No custom workers [1]☐
Seeding [2]☐
Combining [3]☐
Spraying [4]☐
Trucking [5]☐
Other: *specify*_____ [6]☐

A-6 Did you have any hired workers on your farm during 2013? (Exclude custom workers)

Yes [1]☐ No [2]☐

If YES, how many _____

A-7 Is the farm your family's main place of residence?

Yes, all family members on the farm [1]☐
Yes, some family members on the farm [2]☐
No family members live on the farm [3]☐

A-8 Does your farm have a designated fenced play area?

Yes [1]☐ No [2]☐

A-9 Does anyone in this household smoke regularly inside the house?

Yes [1]☐ No [2]☐

A-10 Highest level of education completed by mother:

Less than High School [1]☐
Completed High School [2]☐
Completed University [3]☐
Technical/Community College [4]☐
Don't know [5]☐
Not applicable [99]☐

A-11 Highest level of education completed by father:

Less than High School [1]☐
Completed High School [2]☐
Completed University [3]☐
Technical/Community College [4]☐
Don't know [5]☐
Not applicable [99]☐

A-12 For each season during the past year, on average, how many hours per week did you (parents of children in A-15) spend doing farm work?

	Spring	Summer	Fall	Winter	Not applicable
Mother	_____ hrs/wk	_____ hrs/wk	_____ hrs/wk	_____ hrs/wk	99☐
Father	_____ hrs/wk	_____ hrs/wk	_____ hrs/wk	_____ hrs/wk	99☐

A-13 During the past year, on average, how many hours per week did you (parents of children in A-15) spend working away from the farm?

Mother _____ hrs/wk Job Type: _____ Not applicable 99☐

Father _____ hrs/wk Job Type: _____ Not applicable 99☐

A-14 Do you have any of the following located near your home?

	Distance from Home			
	Yes, < 100 yards	Yes, 100- 300 yards	Yes, > 300 yards	No
Indoor (barn) intensive livestock operation (building)	1☐	2☐	3☐	4☐
Outdoor feedlot or corrals	1☐	2☐	3☐	4☐
Grain bins	1☐	2☐	3☐	4☐
Sewage pond or manure lagoon	1☐	2☐	3☐	4☐
Balestack or bales	1☐	2☐	3☐	4☐
Water source (e.g., dugout, slough)	1☐	2☐	3☐	4☐
Machine shop	1☐	2☐	3☐	4☐
Machinery storage	1☐	2☐	3☐	4☐

A-15 In the table below please identify all of the children (under the age of 18) who currently live in your home.

	Child's Initials	Age	Sex	School (if attending)	Homeroom Teacher	Grade
Example	C M S	8	M 1☐ F 2☒	Greystone Heights School	Mrs. Turner	3
Child 1	___		M 1☐ F 2☐			
Child 2	___		M 1☐ F 2☐			
Child 3	___		M 1☐ F 2☐			
Child 4	___		M 1☐ F 2☐			
Child 5	___		M 1☐ F 2☐			
Child 6	___		M 1☐ F 2☐			

Complete PART B for each child listed in A-15. This booklet has space for 4 children.

If you have more than 4 children listed in A-15, fill in the pages for 4 children and check the box. 1☐

PART B CHILDREN ON YOUR FARM

PLEASE COMPLETE THE NEXT 4 PAGES FOR THE FIRST CHILD LISTED IN A-15

Child 1 – Page 1

Name: _____
(Please print initials, including middle initial)

B-1 Date of Birth: Month_____ Day_____ Year_____

B-2 Sex: Male [1]☐ Female [2]☐

B-3 Child's height: Inches_____ OR cm_____

B-4 Child's weight: Pounds_____ OR kg_____

B-5 Has this child attended off farm daycare, child care or nursery school in the past year?

Off farm, Full time [1]☐
Off farm, Part time [2]☐
No [3]☐

B-6 Has this child completed any farm safety education? (e.g., Farm Safety Day)

Yes [1]☐ No [2]☐

If **YES**, Please specify: _____

_____

B-7 Has this child completed any agriculture courses or training? (e.g., 4H)

Yes [1]☐ No [2]☐

If **YES**, Please specify: _____

_____

B-8 On school days this child usually goes to bed at

_____ pm and gets up at _____ am.

On weekends and holidays this child usually goes to bed at _____ pm and gets up at _____ am.

B-9 Does this child snore?

Yes [1]☐ No [2]☐

B-10 Has this child ever fallen asleep in class?

Yes [1]☐ No [2]☐

B-11 This child's eyesight using both eyes (with glasses or contact lenses, if used) is:

Good [1]☐ Fair [2]☐ Poor [3]☐

B-12 Does this child complain of aches and pains?

Yes [1]☐ No [2]☐ Don't know [3]☐

If **YES**, where? _____

B-13 Has a doctor diagnosed any of the following long term conditions for this child: (Check all that apply)

a. Respiratory Allergies (e.g., hay fever)..... ☐
b. Asthma..... ☐
c. Stomach or intestinal problems..... ☐
d. Anxiety disorder..... ☐
e. Depression..... ☐
f. Mood Disorder..... ☐
g. Migraines..... ☐
h. Attention deficit disorder..... ☐
(with or without hyperactivity)
i. Autism Spectrum Disorder..... ☐
j. Hearing loss..... ☐
k. Sleep Apnea..... ☐
l. Other: *specify* _____ ☐

m. **None of the above**..... ☐

B-14 Does he/she take any of the following prescribed medication on a regular basis: (Check all that apply)

a. Antihistamines..... ☐
b. Ventolin (inhalers for asthma)..... ☐
c. Stomach remedies or laxatives..... ☐
d. Anxiety medication..... ☐
e. Antidepressants..... ☐
f. Tranquilizers or nerve pills..... ☐
g. Pain medication..... ☐
h. Ritalin or similar medications..... ☐
i. Antipsychotics..... ☐
j. Anticonvulsants..... ☐
k. Other: *specify* _____ ☐

l. **No medication**..... ☐

B-15 To your knowledge, does this child drink alcohol at least once per week?

Yes [1]☐ No [2]☐

B-16 To your knowledge, does this child smoke cigarettes on a daily basis?

Yes [1]☐ No [2]☐

Child 1 – Page 2

Physical activity is any activity that increases your heart rate and makes you get out of breath some of the time. Physical activity can be done in sports, school activities, playing with friends, or walking to school.
For these next two questions, add up all the time this child spends in physical activity each day.

B-17 Over the past 7 days, on how many days was this child physically active for a total of at least 60 minutes per day?

[1] ☐ 0 days [2] ☐ 1 day [3] ☐ 2 days [4] ☐ 3 days [5] ☐ 4 days [6] ☐ 5 days [7] ☐ 6 days [8] ☐ 7 days

B-18 Over a typical or usual week, on how many days is this child physically active for a total of at least 60 minutes per day?

[1] ☐ 0 days [2] ☐ 1 day [3] ☐ 2 days [4] ☐ 3 days [5] ☐ 4 days [6] ☐ 5 days [7] ☐ 6 days [8] ☐ 7 days

B-19 Has this child had an off-farm job in the last 12 months?

Seasonal Job [1] ☐ Regular Part-time Job [2] ☐ No Job [3] ☐

If YES, Please specify the number of hours per week that they work at their off- farm job: _____ hrs/wk

Job Industry (e.g., retail): _____

Job Type (e.g., paper boy): _____

For each season during the past year, on average, how many hours per week did this child spend:

	Spring	Summer	Fall	Winter	Not applicable
B-20 Present in the farm worksite? (do not include time spent in home)	_____ hrs/wk	_____ hrs/wk	_____ hrs/wk	_____ hrs/wk	[99] ☐
B-21 Doing Farm work?	_____ hrs/wk	_____ hrs/wk	_____ hrs/wk	_____ hrs/wk	[99] ☐

During 2013, on average how many hours per week did this child:

	Spring	Summer	Fall	Winter	Not applicable
B-22 Operate all-terrain vehicles?	_____ hrs/wk	_____ hrs/wk	_____ hrs/wk	_____ hrs/wk	[99] ☐
B-23 Ride horses?	_____ hrs/wk	_____ hrs/wk	_____ hrs/wk	_____ hrs/wk	[99] ☐
B-24 Operate snowmobiles?	_____ hrs/wk	_____ hrs/wk	_____ hrs/wk	_____ hrs/wk	[99] ☐

Child 1 – Page 3

B-25 How often does this child wear a **helmet** when operating an all-terrain vehicle?

Always 1☐ Sometimes 2☐ Never 3☐ Not applicable 99☐

B-26 How often does this child wear a **helmet** when riding a horse?

Always 1☐ Sometimes 2☐ Never 3☐ Not applicable 99☐

B-27 How often does this child wear a **helmet** when operating a snowmobile?

Always 1☐ Sometimes 2☐ Never 3☐ Not applicable 99☐

B-28 **In the past 12 months**, on average, how often has this child spent at least 1 hour taking part in the following activities: (Check the box that best applies)

	Every day	At least once a week	At least once a month	Less than once a month	Never
Haying/moving/playing with hay bales	1☐	2☐	3☐	4☐	5☐
Feeding livestock	1☐	2☐	3☐	4☐	5☐
Cleaning or playing in barns	1☐	2☐	3☐	4☐	5☐
Cleaning grain bins	1☐	2☐	3☐	4☐	5☐
Cleaning or playing in pens/corals	1☐	2☐	3☐	4☐	5☐

During 2013, on average how many days per year and hours per day did this child:

			Not applicable
B-29 Operate tractors?	_____ days/yr	_____ hrs/day	99☐
B-30 Operate combines?	_____ days/yr	_____ hrs/day	99☐
B-31 Operate grain augers?	_____ days/yr	_____ hrs/day	99☐
B-32 Do routine chores with **large animals**? (e.g., cattle or pigs)	_____ days/yr	_____ hrs/day	99☐
B-33 Do routine chores with **small animals**? (e.g., chickens)	_____ days/yr	_____ hrs/day	99☐
B-34 Do **herd maintenance activities**? (e.g., branding, vaccinating)	_____ days/yr	_____ hrs/day	99☐
B-35 Do **veterinary activities**? (e.g., medications, breeding, birthing)	_____ days/yr	_____ hrs/day	99☐

Child 1 – Page 4

The questions in this column ask about **farm injuries**. We are interested in all injuries that occurred in a farm environment whether the child or young person was working or not. This includes injuries that occurred off farm but involved farm work (e.g., driving a tractor on public road). This also includes being poisoned or burned. If a child had more than one farm injury, think about the **one most serious injury** that occurred during 2013.

B-36 Has this child had a farm injury during 2013?

Yes [1] No [2]

If YES, how many injuries? _____

If this child did not have a farm injury during 2013, please skip to the grey box to the right.

B-37 In what month did the one most serious farm injury happen?

[1] Jan | [2] Feb | [3] Mar | [4] Apr | [5] May | [6] Jun | [7] Jul | [8] Aug | [9] Sep | [10] Oct | [11] Nov | [12] Dec

B-38 Was the injured child working on the farm at the time of the injury?

Yes [1] No [2]

B-39 In the space below, please provide details about how the injury occurred.

What was the child doing? _____

_____

_____

_____

_____

Where did it happen? _____

_____

_____

How did it happen? _____

_____

_____

_____

What went wrong? _____

_____

_____

_____

Type of injury & body part injured; (e.g., broken arm, cut leg)

_____

B-40 Where was this one most serious farm injury treated? (Check all that apply)

- Self-treated [1]
- Doctor's office/health clinic [2]
- Emergency Room at hospital [3]
- Hospital admission overnight/longer [4]
- Other: *specify* _____ [5]

The next questions ask about **non-farm injuries**. We are interested in all other injuries that occurred, that **were not sports injuries**. If a child had more than one **non-farm injury**, think about the **one most serious injury** that occurred during 2013.

B-41 Has this child had a non-farm injury during 2013?

Yes [1] No [2]

If YES, how many injuries? _____

If this child did not have an off-farm injury during 2013, please skip to the grey box below.

B-42 In what month did the one most serious non-farm injury happen?

[1] Jan | [2] Feb | [3] Mar | [4] Apr | [5] May | [6] Jun | [7] Jul | [8] Aug | [9] Sep | [10] Oct | [11] Nov | [12] Dec

B-43 In the space below, please provide details about how the injury occurred.

What was the child doing? _____

_____

_____

Where did it happen? _____

_____

How did it happen? _____

_____

_____

What went wrong? _____

_____

Type of injury & body part injured; (e.g., broken arm, cut leg)

_____

B-44 Where was this one most serious non-farm injury treated? (Check all that apply)

- Self-treated [1]
- Doctor's office/health clinic [2]
- Emergency Room at hospital [3]
- Hospital admission overnight/longer [4]
- Other: *specify* _____ [5]

If you have another child listed in A-15, continue on to the next page. Otherwise go to Part C.

PART C

YOUNG CHILDREN ON YOUR FARM

C-1 Please describe your farm with respect to young children less than 7 years of age:
(Please answer each of the questions below)

a) Young children have ever lived on the farm	Yes [1]☐	No [2]☐
b) Young children have lived on the farm in the last 10 years	Yes [1]☐	No [2]☐
c) Young children currently visit the farm	Yes [1]☐	No [2]☐
d) Young children currently live on the farm	Yes [1]☐	No [2]☐

Please think about your activities when children were present on your farm most recently.
When answering the following questions **think about the summer months.**

C-2 How often were young children present on your farm worksite?

Every day	*At least once a week*	*At least once a month*	*Less than once a month*	*Never*
[1]☐	[2]☐	[3]☐	[4]☐	[5]☐

C-3 How often did young children ride in a cabbed tractor or cabbed combine with an adult operator?

Every day	*At least once a week*	*At least once a month*	*Less than once a month*	*Never*
[1]☐	[2]☐	[3]☐	[4]☐	[5]☐

C-4 How often were young children present in the farm worksite while adults are working nearby?

Every day	*At least once a week*	*At least once a month*	*Less than once a month*	*Never*
[1]☐	[2]☐	[3]☐	[4]☐	[5]☐

C-5 How often were young children assigned small jobs to assist with the farm operation?

Every day	*At least once a week*	*At least once a month*	*Less than once a month*	*Never*
[1]☐	[2]☐	[3]☐	[4]☐	[5]☐

You are more than half-way through!
We really appreciate your time and effort!

YOUNG WORKERS ON YOUR FARM

C-6 Please describe your farm with respect to young workers (aged 12-16 years):
(Please answer each of the questions below)

a) Young workers have ever worked on the farm — Yes [1]☐ No [2]☐
b) Young workers have worked on the farm in the last 10 years — Yes [1]☐ No [2]☐
c) Young workers have worked on the farm in the last 12 months — Yes [1]☐ No [2]☐

Please think of the young worker (aged 12-16 years) who worked the most on your farm in the last 10 years.
When answering the following questions **think about the summer months** when this young worker first started working on your farm.

C-7 Boy [1]☐ Girl [2]☐ C-8 Age: _____
(started working)

C-9 How often did that young worker operate a tractor greater than 20 hp?

Every day	*At least once a week*	*At least once a month*	*Less than once a month*	*Never*
[1]☐	[2]☐	[3]☐	[4]☐	[5]☐

C-10 How often did that young worker operate a tractor without rollover protection structures?

Every day	*At least once a week*	*At least once a month*	*Less than once a month*	*Never*
[1]☐	[2]☐	[3]☐	[4]☐	[5]☐

C-11 How often did that young worker operate farm equipment that is more than 20 years old?

Every day	*At least once a week*	*At least once a month*	*Less than once a month*	*Never*
[1]☐	[2]☐	[3]☐	[4]☐	[5]☐

C-12 How often was that young worker exposed to working at heights?
(e.g., climbing grain bins or large equipment)

Every day	*At least once a week*	*At least once a month*	*Less than once a month*	*Never*
[1]☐	[2]☐	[3]☐	[4]☐	[5]☐

C-13 How often was that young worker involved in large animal work such as branding, calving, feeding or transporting?

Every day	*At least once a week*	*At least once a month*	*Less than once a month*	*Never*
[1]☐	[2]☐	[3]☐	[4]☐	[5]☐

C-14 The farm work tasks this young worker did most often were: (select one)

Their favorite tasks	*Assigned by owner-operator*	*Tasks most capable of*	*Negotiated on a daily basis*	*Other*	*Not Applicable*
[1]☐	[2]☐	[3]☐	[4]☐	[5]☐	[99]☐

THE MAIN FARM OWNER/OPERATOR ON YOUR FARM

Please think about the main operator of your farm.
When answering the following questions think of the **summer months**.

C-15 Man [1]☐ Woman [2]☐

C-16 Age: _____

C-17 How often does he/she operate a tractor greater than 20 hp?

Every day	*At least once a week*	*At least once a month*	*Less than once a month*	*Never*
[1]☐	[2]☐	[3]☐	[4]☐	[5]☐

C-18 How often does he/she operate a tractor without rollover protection structures?

Every day	*At least once a week*	*At least once a month*	*Less than once a month*	*Never*
[1]☐	[2]☐	[3]☐	[4]☐	[5]☐

C-19 How often does he/she operate farm equipment that is more than 20 years old?

Every day	*At least once a week*	*At least once a month*	*Less than once a month*	*Never*
[1]☐	[2]☐	[3]☐	[4]☐	[5]☐

C-20 How often is he/she exposed to working at heights? (e.g., climbing grain bins or large equipment)

Every day	*At least once a week*	*At least once a month*	*Less than once a month*	*Never*
[1]☐	[2]☐	[3]☐	[4]☐	[5]☐

C-21 How often is he/she involved in large animal work such as branding, calving, feeding or transporting?

Every day	*At least once a week*	*At least once a month*	*Less than once a month*	*Never*
[1]☐	[2]☐	[3]☐	[4]☐	[5]☐

SAFETY PRACTICES OF THE OWNER/OPERATOR

This series of questions deals with actual safety practices of the **main farm operator** on your farm.

C-22 When operating farm machinery he/she keeps all safety shields and guards in place.

Always	*Often*	*Occasionally*	*Never*	*Don't Know*
1☐	2☐	3☐	4☐	5☐

C-23 When using hand tools, such as grinders or drills, he/she wears eye protection.

Always	*Often*	*Occasionally*	*Never*	*Don't Know*
1☐	2☐	3☐	4☐	5☐

C-24 When he/she is applying agricultural chemicals such as fertilizers, pesticides or herbicides, he/she wears protective devices such as gloves or a respirator.

Not applicable	*Always*	*Often*	*Occasionally*	*Never*	*Don't Know*
99☐	1☐	2☐	3☐	4☐	5☐

C-25 When working around animals and/or machinery he/she wears steel toed work boots or shoes.

Always	*Often*	*Occasionally*	*Never*	*Don't Know*
1☐	2☐	3☐	4☐	5☐

C-26 In noisy working conditions he/she wears hearing protection.

Always	*Often*	*Occasionally*	*Never*	*Don't Know*
1☐	2☐	3☐	4☐	5☐

C-27 He/she trains and observes workers prior to operating a piece of equipment.

Not applicable	*Always*	*Often*	*Occasionally*	*Never*	*Don't Know*
99☐	1☐	2☐	3☐	4☐	5☐

C-28 He/she trains and observes workers prior to taking on a new job involving large animals.

Not applicable	*Always*	*Often*	*Occasionally*	*Never*	*Don't Know*
99☐	1☐	2☐	3☐	4☐	5☐

WORK HABITS OF THE OWNER/OPERATOR

C-29 He/she often undertakes hazardous farm activities without thinking about the possible consequences.

Always	*Often*	*Occasionally*	*Never*	*Don't Know*
1☐	2☐	3☐	4☐	5☐

C-30 He/she usually finds that there aren't enough hours in a day to get the work completed on the farm.

Always	*Often*	*Occasionally*	*Never*	*Don't Know*
1☐	2☐	3☐	4☐	5☐

C-31 Most of the time he/she works at a fairly leisure pace.

Always	*Often*	*Occasionally*	*Never*	*Don't Know*
1☐	2☐	3☐	4☐	5☐

C-32 While doing farm work how often does he/she experience a "near miss" that under different circumstances might have resulted in personal injury or property loss?

Always	*Often*	*Occasionally*	*Never*	*Don't Know*
1☐	2☐	3☐	4☐	5☐

SAFETY INVESTMENTS OF THE OWNER/OPERATOR

C-33 When necessary he/she invests **time** to improve safety conditions on the farm.

Always	*Often*	*Occasionally*	*Never*	*Don't Know*
1☐	2☐	3☐	4☐	5☐

C-34 When necessary he/she invests **money** to improve safety conditions on the farm.

Always	*Often*	*Occasionally*	*Never*	*Don't Know*
1☐	2☐	3☐	4☐	5☐

SAFETY ATTITUDES OF THE OWNER/OPERATOR

C-35 Any good farmer who is actively involved in his/her operation will invariably have an accident sometime in his/her career.

Strongly agree	*Agree*	*Disagree*	*Strongly disagree*
1☐	2☐	3☐	4☐

C-36 Farm safety should have the highest priority on every agricultural operation.

Strongly agree	*Agree*	*Disagree*	*Strongly disagree*
1☐	2☐	3☐	4☐

C-37 He/she doesn't worry much about being hurt when he/she is working.

Strongly agree	*Agree*	*Disagree*	*Strongly disagree*
1☐	2☐	3☐	4☐

C-38 The number of farm accidents could be best prevented by (select one).

Educating people	*Better design of equipments and buildings*	*Safety regulations on farms*	*None of these*
1☐	2☐	3☐	4☐

FARM ECONOMICS

Here are three statements about your farm and its financial health.
Please choose <u>one</u> response for each statement.

C-39 During 2013, how often were cash flow shortages a source of worry on your farm?

Every day	*At least once a week*	*At least once a month*	*Less than once a month*	*Never*
1☐	2☐	3☐	4☐	5☐

C-40 During 2013, how often was debt a source of worry on your farm?

Every day	*At least once a week*	*At least once a month*	*Less than once a month*	*Never*
1☐	2☐	3☐	4☐	5☐

C-41 At the end of the most recent fiscal year did your farm operation have?

Large Deficit	*Small Deficit*	*Break Even*	*Small Surplus*	*Large Surplus*
1☐	2☐	3☐	4☐	5☐

PART D THE FARM ENVIRONMENT

The following questions are about the general condition of **your farm**. (If not applicable write "0")

D-1 How many tractors are in use on the farm?..... _____

How many are equipped with a rollover protection structure?..... _____

How many were manufactured before 1984?..... _____

D-2 How many combines are in use on the farm?..... _____

How many have all safety shields and guards in place?..... _____

D-3 How many augers are in use on the farm?..... _____

How many have all safety shields and guards in place?..... _____

D-4 How many grain bins are on the farm?..... _____

How many are equipped with a ladder cage?..... _____

D-5 How many corrals are in use on your farm?..... _____

How many have a man escape?..... _____
(in addition to a gate for the animals)

D-6 How many ladders are in use on your farm?..... _____
(include movable ladders and those attached to buildings and machines)

How many have all the rungs in place and are free of debris at all times? _____

D-7 How many water sources are located on your farm?..... _____
(e.g., sewage lagoons, dugouts, wells, cisterns)

How many have a barrier around them or are in a penned area?..... _____

D-8 Would you say the safety conditions and practices on your farm are:

Excellent [1]☐ Good [2]☐ Fair [3]☐ Poor [4]☐

FOLLOW- UP

CONTACT INFORMATION

- As part of this project, we will be contacting your farm by mail 4 times over two years.
- We will do this to see whether children on your farm have experienced any injuries.
- We will ask you to complete a 1 page form on each occasion.
- Typically, this will take less than 5 minutes of your time.

PLEASE PRINT

Name: _____ Age: _____
(Name of person completing the survey)

Relationship to children:_____

Address: _____

_____

Email address:_____

Telephone Numbers (check most preferred):

Work _____ 1☐
Home _____ 2☐
Cell _____ 3☐

This is the end of the survey.

Thank you very much for your help.

Please place the completed questionnaire in the envelope and return it to your child's school.

9.4. Appendix D

Ethical approval for the secondary use of the Saskatchewan Farm Injury Cohort – Child's Component

UNIVERSITY OF SASKATCHEWAN

Behavioural Research Ethics Board (Beh-REB) 15/May/2020

Certificate of Approval

Application ID: 1920

Principal Investigator: Joshua Lawson Department: Department of Medicine

Locations Where Research Activities are Conducted: University of Saskatchewan, Canada

Student(s): Luan Chu

Funder(s):

Sponsor:

Title: Farm Exposures and Allergic Disease among Children Living in a Rural Setting

Approved On: 15/May/2020

Expiry Date: 14/May/2021

Approval Of: Behavioural Research Ethics Application

Acknowledgment Of: Beh ID 12-265 Certificate of Approval

Beh ID 11-270 Certificate of Approval

Review Type: Delegated Review

CERTIFICATION

The University of Saskatchewan Behavioural Research Ethics Board (Beh-REB) is constituted and operates in accordance with the current version of the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans (TCPS 2 2014). The University of Saskatchewan Behavioural Research Ethics Board has reviewed the above-named project. The proposal was found to be acceptable on ethical grounds. The principal investigator has the responsibility for any other administrative or regulatory approvals that may pertain to this project, and for ensuring that the authorized project is carried out according to the conditions outlined in the original protocol submitted for ethics review. This Certificate of Approval is valid for the above time period provided there is no change in experimental protocol or consent process or documents.

Any significant changes to your proposed method, or your consent and recruitment procedures should be reported to the Chair for Research Ethics Board consideration in advance of its implementation.

ONGOING REVIEW REQUIREMENTS

In order to receive annual renewal, a status report must be submitted to the REB Chair for Board consideration within one month prior to the current expiry date each year the project remains open, and upon project completion. Please refer to the following website for further instructions: https://vpresearch.usask.ca/researchers/forms.php.

Digitally Approved by Vivian Ramsden
Vice-Chair, Behavioural Research Ethics Board
University of Saskatchewan

1 / 1

9.5. Appendix E

Multivariate analyses of farm exposures and allergic disease among children 6-17 years old

			Ventolin		Asthma		Respitarory allergies		Allergic asthma	p-value	Non allergic asthm	p-value
			OR (95%CI)	p-value	OR (95%CI)	p-value	OR (95%CI)	p-value	OR (95%CI)		OR (95%CI)	
	Male (ref: Female)		1.61 (1.07-2.41)	0.021	1.57 (1.10-2.22)	0.011	1.33 (0.86-2.04)	0.18	1.39 (0.75-2.56)	0.29	1.65 (1.09-2.50)	0.017
	13-17 years old (ref: 6-12 years old)		1.28 (0.84-1.94)	0.24	1.31 (0.91-1.87)	0.14	1.12 (0.72-1.76)	0.59	1.57 (0.84-2.95)	0.15	1.20 (0.78-1.84)	0.38
	Parental education								0.49 (0.19-1.26)	0.14	0.93 (0.55-1.56)	0.78
	Less or completed high school (ref: h		0.95 (0.56-1.60)	0.84	0.78 (0.49-1.24)	0.29	0.48 (0.24-0.94)	0.034				
	Physical activity (ref: 6-7 days)											
		Less than 2	0.88 (0.40-1.92)	0.76	1.08 (0.58-1.99)	0.8	1.38 (0.71-2.70)	0.34	0.81 (0.31-2.09)	0.81	1.02 (0.46-2.24)	0.95
		3-5 days/w	1.28 (0.82-2.01)	0.26	1.19 (0.81-1.75)	0.37	0.84 (0.52-1.35)	0.48	0.66 (0.27-1.60)	0.36	1.43 (0.69-2.95)	0.33
	Household smoking (Ref: No)		0.79 (0.34-1.87)	0.6	0.97 (0.49-1.92)	0.94	0.61 (0.22-1.70)	0.34	0.61 (0.14-2.59)	0.5	1.14 (0.53-2.43)	0.73
Main effect model	Farm (ref: Non farm)		1.50 (0.98-2.31)	0.059	0.99 (0.67-1.47)	0.96	1.13 (0.70-1.80)	0.61	0.59 (0.27-1.30)	0.19	1.20 (0.76-1.88)	0.42
	Farm work spring	(Ref: No)	0.94 (0.51-1.74)	0.86	1.06 (0.60-1.85)	0.84	0.82 90.41-1.63)	0.58	1.62 (0.59-4.41)	0.34	0.89 (0.46-1.72)	0.74
	Farm work summer	(Ref: No)	1.32 (0.75-2.31)	0.333	1.58 (0.95-2.60)	0.073	0.92 (0.49-1.76)	0.81	2.01 (0.83-4.84)	0.11	1.43 (0.79-2.56)	0.23
	Farm work fall	(Ref: No)	1.10 (0.60-2.00)	0.75	1.18 (0.68-2.03)	0.55	0.78 (0.39-1.55)	0.49	1.52 (0.57-4.09)	0.4	1.06 (0.56-2.00)	0.84
	Farm work winter	(Ref: No)	1.35 (0.73-2.49)	0.32	1.23 (0.68-2.16)	0.51	1.03 (0.50-2.10)	0.93	1.71 (0.61-4.81)	0.3	1.05 (0.53-2.07)	0.87
	Tractor Op	(Ref: No)	0.44 (0.18-1.04)	0.064	0.79 (0.40-1.58)	0.51	0.59 (0.23-1.50)	0.27	0.48 (0.10-2.20)	0.34	0.92 (0.42-1.98)	0.83
	Combine	(Ref: No)	0.67 90.22-2.03)	0.48	0.60 (0.20-1.77)	0.35	0.45 (0.10-1.99)	0.29	0.63 (0.07-5.18)	0.66	0.59 (0.17-2.05)	0.4
	Grain	(Ref: No)	0.64 (0.18-2.26)	0.49	1.10 (0.39-3.04)	0.85	0.63 (0.14-2.84)	0.55	0.89 (0.10-7.42)	0.91	1.18 (0.38-3.67)	0.77
	Routine large	(Ref: No)	1.64 (0.94-2.87)	0.079	1.89 (1.15-3.11)	0.011	1.51 (0.81-2.80)	0.194	2.37 (1.01-5.56)	0.047	1.71 (0.95-3.08)	0.07
	Routine small	(Ref: No)	0.92 (0.45-1.88)	0.83	1.19 90.64-2.20)	0.57	0.69 (0.28-1.67)	0.41	1.49 (0.49-4.52)	0.47	1.09 (0.53-2.23)	0.81
	Herd maintain	(Ref: No)	1.06 (0.46-2.43)	0.87	1.39 (0.68-2.81)	0.36	0.78 (0.27-2.25)	0.65	1.83 (0.53-6.31)	0.33	1.23 (0.54-2.83)	0.61
	Vet activities	(Ref: No)	1.39 (0.62-3.12)	0.41	1.38 (0.64-2.96)	0.4	0.83 (0.28-2.45)	0.75	0.58 (0.07-4.56)	0.61	1.66 (0.73-3.77)	0.22
	Haying	(Ref: Not r	1.13 (0.62-2.04)	0.68	0.98 90.56-1.69)	0.95	1.16 90.61-2.22)	0.64	1.09 (0.14-2.64)	0.52	0.93 (0.49-1.76)	0.83
	Feeding livestock	(Ref: Not r	1.29 (0.74-2.39)	0.36	1.15 (0.69-1.90)	0.58	0.93 (0.48-1.80)	0.84	1.43 (0.58-3.50)	0.42	1.04 (0.58-1.88)	0.88
Each variable was added	Clean barn	(Ref: Not r	0.83 (0.43-1.61)	0.59	0.80 (0.43-1.46)	0.46	0.92 (0.45-1.88)	0.83	1.30 (0.47-3.54)	0.61	0.64 90.31-1.35)	0.24
singularly in the main model	Clean bins	(Ref: Not r	1.01 (0.29-3.53)	0.98	1.20 90.40-3.61)	0.73	0.47 (0.06-3.65)	0.47	1.48 (0.18-12.11)	0.71	1.12 (0.32-3.94)	0.85
with the main variable "farm"	Clean pens	(Ref: Not r	0.94 (0.48-1.86)	0.87	0.91 (0.49-1.70)	0.78	0.99 (0.47-2.07)	0.98	1.15 (0.38-3.41)	0.8	0.83 (0.39-1.73)	0.62

9.6. Appendix F

Multivariate analyses of farm exposure s and allergic disease, splitting by age groups

			6-12 years old										13-17 years old							
			Ventolin		Asthma		Respitarory allergies		Allergic asthma	p-value	Non aller	p-value	Ventoline		Asthma		Respitarory allergie	Allergic a	p-value	Non aller	p-value
			OR (95%CI)	p-value	OR (95%CI	p-value	OR (95%CI)	p-value	OR (95%CI)		OR (95%CI)		OR (95%CI)	p-value	OR (95%CI	p-value	OR (95%CI	p-value	OR (95%CI)		OR (95%CI)	
	Male (ref: Female)		1.58 (0.94-2.66)	0.083	1.69 (1.08-	0.022	1.68 (0.97-2.91)	0.063	2.12 (0.90-5.03)	0.085	1.56 (0.92-	0.095	1.64 90.86-3.1	0.13	1.40 (0.80-	0.23	0.93 (0.46-	0.84	0.83 (0.32-	0.7	1.81 (0.92-	0.084
	Parental education (Less or		1.22 (0.65-2.29)	0.52	0.30 (0.53-	0.79	0.44 (0.18-1.05)	0.067	0.53(0.15-1.82)	0.31	1.10 (0.59-	0.74	0.60 90.22-1.5	0.3	0.56 (0.24-	0.17	0.49 (0.16-	0.2	0.44 (0.10-	0.28	0.63 90.23-	0.63
	Physical activity (ref: 6-7 days)						0.63 (0.18-2.15)	0.46									2.23 (0.83-	0.1				
		Less than	1.10 (0.36-3.32)	0.86	1.06 (0.42-	0.9	0.85 (0.49-1.49)	0.58	0.87 (0.18-4.17)	0.87	0.97 (0.32-	0.97	0.72 (0.23-2.19	0.56	1.15 (0.47-	0.75	0.86 (0.35-	0.75	0.96 (0.27-	0.96	0.85 (0.25-	0.79
		3-5 days/v	1.42 (0.82-2.48)		1.19 (0.74-	0.46			0.89 (0.19-4.06)	0.88	1.24 (0.43-	0.68	1.09 (0.52-2.31	0.8	1.22 (0.61-	0.56			0.54 (0.17-	0.3	1.54 (0.57-	0.39
	Household smoking (Ref: No		0.74 (0.22-2.45)	0.62	1.41 (0.62- | 0.4 | 0.61 90.14-2.58)	0.5	0.75 (0.10-5/73)	0.78	1.65 (0.68-	0.26	0.88 (0.25-3.00	0.84	0.55 (0.16-	0.34	0.58 (0.13-	0.47	0.48 (0.06-	0.48	0.59 (0.13-	0.48
Main effect model	Farm (ref: Non-farm)						1.10 (0.60-2.01)	0.75	0.77 (0.28-2.10)	0.61	1.19 (0.66-	0.55					1.25 (0.58-	0.56	0.42 (0.11-	0.17	1.23 (0.60-	0.55
	Farm work spring	(Ref: No)	0.88 (0.39-2.00)	0.77	1.17 (0.55-	0.66	0.88 (0.35-2.20)	0.8	1.75 (0.45-6.83)	0.41	1.01 (0.42-	0.97	1.02 (0.39-2.47	0.96	0.94 (0.39-	0.89	0.77 (0.26-	0.64	1.47 (0.36-	0.59	0.72 (0.25-	0.54
	Farm work summe	(Ref: No)	1.13 (0.53-2.43)	0.73	1.53 (0.78-	0.21	0.99 (0.42-2.32)	0.99	2.24 (0.67-7.48)	0.18	1.33 (0.60-	0.48	1.58 (0.68-3.70	0.28	1.67 (0.80-	0.17	0.90 (0.33-	0.84	1.83 (0.53-	0.34	1.57 (0.64-	0.31
	Farm work fall	(Ref: No)	0.97 (0.43-2.16)	0.94	1.23 (0.59-	0.56	0.80 (0.32-1.99)	0.64	1.61 (0.41-6.24)	0.48	1.12 (0.48-	0.78	1.29 (0.51-3.24	0.58	1.12 (0.49-	0.78	0.79 (0.28-	0.67	1.38 (0.34-	0.64	0.97 (0.36-	0.96
	Farm work winter	(Ref: No)	1.19 (0.50-2.82)	0.68	1.41 (0.64-	0.39	1.09 (0.41-2.89)	0.85	1.94 (0.46-8.12)	0.36	1.26 (0.50-	0.62	1.57 (0.65-3.81	0.31	1.05 (0.45-	0.91	1.10 (0.38-	0.85	1.48 (0.35-	0.59	0.85 (0.31-	0.75
	Tractor Op	(Ref: No)	0.31 (0.04-2.42)	0.26	0.83 (0.24-	0.77	0.79 (0.17-3.55)	0.76	Not converged		1.16 (0.32-	0.82	0.45 (0.16-1.26	0.13	0.79 (0.33-	0.59	0.57 (0.17-	0.36	0.77 (0.15-	0.75	0.74 (0.27-	0.6
	Combine	(Ref: No)	Not converged		Not converged		1.40 (0.16-11.8)		Not converged		Not converged		0.82 (0.25-2.70	0.74	0.78 (0.24-	0.67	0.28 (0.03-	0.23	1.05 (0.11-	0.96	0.68 (0.18-	0.58
	Grain	(Ref: No)	Not converged		Not converged		Not converged		Not converged		Not converged		0.71 (0.18-2.71	0.62	1.43 (0.47-	0.52	0.93 (0.18-	0.93	1.58 (0.16-	0.69	1.31 (0.37-	0.66
	Routine large	(Ref: No)	2.49 (1.21-5.12)	0.013	2.34 (1.20-	0.012	1.97 (0.90-4.32)	0.088	3.41 (10.10-10.6	0.034	2.01 (0.91-	0.082	0.98 (0.39-2.41	0.96	1.53 (0.72-	0.264	1.05 (0.37-	0.92	1.48 (0.39-	0.56	1.53 (0.63-	0.34
	Routine small	(Ref: No)	1.29 (0.56-2.97)	0.54	1.43 (0.68-	0.34	0.96 (0.35-2.61)	0.94	1.94 (0.51-7.34)	0.33	1.27 (0.53-	0.59	0.43 (0.09-1.91	0.27	0.82 90.27-	0.73	0.27 (0.03-	0.21	0.80 (0.10-	0.84	0.82 (0.23-	0.76
	Herd maintain	(Ref: No)	1.44 (0.57-3.64)	0.44	1.48 (0.63-	0.36	0.91 (0.26-3.11)	0.88	1.87 (0.40-8.75)	0.42	1.35 (0.49-	0.55	0.38 (0.05-3.01	0.36	1.14 (0.32-	0.83	0.58 (0.07-	0.61	1.64 (0.20-	0.64	0.93 (0.20-	0.93
	Vet activities	(Ref: No)	1.57 90.50-4.93)	0.43	2.39 90.88-	0.085	0.87 (0.19-3.96)	0.85	1.61 (0.18-14.36	0.66	2.65 (0.89-	0.078	1.23 (0.39-3.86	0.72	0.74 (0.21-	0.64	0.83 (0.18-	0.81	Not converged		1.08 (0.30-	0.9
	Haying	(Ref: Not r	1.39 (0.68-2.84)	0.36	1.30 (0.68-	0.43	1.67 (0.79-3.51)	0.17	1.45 (0.45-4.66)	0.53	1.24 (0.58-	0.57	0.68 90.22-2.1	0.51	0.55 (0.18-	0.28	0.52 (0.11-	0.39	0.52 (0.06-	0.54	0.54 (0.15-	0.34
Each variable was	Feeding livestock	(Ref: Not r	1.30 (0.64-2.64)	0.47	1.21 (0.63-	0.55	1.26 (0.58-2.76)	0.55	1.87 (0.61-5.70)	0.27	1.01 (0.47-	0.96	1.23 (0.51-2.95	0.64	1.08 (0.48-	0.85	0.53 (0.15-	0.33	0.85 (0.17-	0.85	1.13 (0.45-	0.79
added singularly in the	Clean barn	(Ref: Not r	1.08 (0.50-2.30)	0.84	1.12 (0.56-	0.73	1.29 (0.58-2.86)	0.53	2.28 (0.75-6.86)	0.14	0.81 (0.34-	0.64	0.40 (0.09-1.77	0.23	0.31 (0.07-	0.12	0.26 (0.03-	0.2	Not converged		0.43 (0.10-	0.27
main model with the	Clean bins	(Ref: Not r	Not converged		Not converged		Not converged		Not converged		Not converged		1.37 (0.36-5.17	0.64	1.69 90.52-	0.37	0.71 90.08-	0.75	2.69 (0.29-	0.38	1.38 (0.36-	0.63
variable "farmrural"	Clean pens	(Ref: Not r	1.13 (0.52-2.48)	0.74	1.08 90.52-	0.83	1.32 90.57-3.01)	0.5	1.94 (0.60-6.30)	0.26	0.82 (0.33-	0.67	0.52 (0.11-2.30	0.38	0.63 90.18-	0.46	0.33 (0.04-	0.29	Not converged		0.97 (0.27-	0.96

9.7. Appendix G

Ethical approval for the PEM pilot study

UNIVERSITY OF SASKATCHEWAN

Biomedical Research Ethics Board (Bio-REB) 01-Apr-2020

Certificate of Re-Approval

Ethics Number: 15-320

Principal Investigator: Josh Lawson Department: University of Saskatchewan

Locations Where Research Activities are Conducted: University of Saskatchewan, Canada

Student(s): Luan Chu

Funder(s): Saskatchewan Health Research Foundation

Sponsor:

Title: Personal Exposure Monitoring in Children - An Investigation of the Usefulness of Personal Exposure Monitoring to Collect Environmental Exposures Data in School-Age Children

Protocol Number:

Approved On: 23/03/2020

Expiry Date: 22/03/2021

Acknowledgment Of: n/aÂ

Review Type: Delegated Review

IRB Registration Number: Not Applicable

* This study, inclusive of all previously approved documents, has been re-approved until the expiry date noted above

CERTIFICATION

The University of Saskatchewan Biomedical Research Ethics Board (Bio-REB) has reviewed the above-named project.Â Theproject is acceptable on scientific and ethical grounds. Â Theprincipal investigator has the responsibility for any other administrative or regulatory approvals that may pertain to this project, and for ensuring that the authorized project is carried out according to governing law. This approval is valid for the specified period provided there is no change to the approved project.

FIRST TIME REVIEW AND CONTINUING APPROVAL

The University of Saskatchewan Research Ethics Boards review above minimal projects at a full-board (face-to-face) meeting. Â Ifa project has been reviewed at a full board meeting, a subsequent project of the same protocol may be reviewed through the delegated review process. Any research classified as minimal risk is reviewed through the delegated (subcommittee) review process. Â Theinitial Certificate of Approval includes the approval period the REB has assigned to a study. Â TheStatus Report form must be submitted within one month prior to the assigned expiry date. The researcher shall indicate to the REB any specific requirements of the sponsoring organizations (e.g. requirement for full-board review and approval) for the continuing review process deemed necessary for that project.

REB ATTESTATION

In respect to clinical trials, the University of Saskatchewan Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Part 4 of the Natural Health Products Regulations and Part C Division 5 of the Food and Drug Regulations and carries out its functions in a manner consistent with Good Clinical Practices. Â Membersof the Bio-REB who are named as investigators, do not participate in the discussion related to, nor vote on such studies when presented to the Bio-REB. This approval and the views of this REB have been documented in writing. The University of Saskatchewan Biomedical Research Ethics Board is constituted and operates in accordance with the current version of the Tri-Council Policy Statement: Â EthicalConduct for Research Involving Humans (TCPS 2 2018).Â

Digitally Approved by Dr. Gordon McKay, Ph.D.
Chair, Biomedical Research Ethics Board
University of Saskatchewan