PREVALENCE AND DETERMINANTS OF ATOPY AMONG SCHOOL-AGE CHILDREN IN RURAL SASKATCHEWAN, CANADA

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Abstract

Background & Objectives: There has been few investigation of the association between the farming related activities or specific characteristics and atopic disease in rural Canadian children. In population-based studies, assuring the quality of information from questionnaires is of concern. We conducted this study in order to: first, identify the prevalence and risk factors of atopy and allergic conditions among school-age children in a rural region of Canada. Also, we sought to evaluate the validity and reliability of a questionnaire report of allergy to assess in this population.

Methods: As part of a longitudinal study of lung health in rural residents, we conducted a cross-sectional baseline study in rural Saskatchewan, Canada. This included an initial survey phase followed by a clinical testing phase. A sub-sample of 584 children (grades 1-8) completed skin prick testing to assess atopic status. Of these, 480 children completed a questionnaire report of allergy and atopic outcomes and participated in skin prick testing (SPT). Atopy was defined as a positive reaction to any of 6 allergens (local grasses, wheat dust, cat dander, house dust mite, Alternaria, Clasdosporium)≥3mm compared to the negative control. Agreement between questionnaire report and objective measures of atopy was considered overall and between the specific allergens tested on SPT and those assessed on questionnaire. We considered percent concordance, Kappa, sensitivity, specificity, and the positive and negative predictive values of reported allergies or allergic conditions in comparison to SPT as the gold standard.

Results: The prevalence of atopy as well as allergen-specific sensitizations was similar between farm and non-farm children but supported the notion that livestock farming is protective against atopy. Also, we found that 25.0% of children reported a history of allergic conditions by questionnaire and 19.4% were atopic detected by skin pick test. In our study,

the agreement between questionnaire report of specific allergic triggers and atopy measured by SPT was high (83.0% - 89.5%).

Conclusion: In children, livestock exposure has a protective effect on SPT positivity.The agreement between questionnaire report of allergic symptoms and atopy measured by SPT was high and the agreement between atopy and report of allergic conditions was moderate.

Co-Authorship

This thesis contains two manuscripts which was done by Manh Luan Chu in collaboration with his supervisor, Dr. Joshua A. Lawson from the University of Saskatchewan, as well as co-authors of the Saskatchewan Rural Health Study team.

"Prevalence and determinants of atopy and allergic diseases among school-age children in rural Saskatchewan, Canada"

Mr. Chu, with the supervision of Dr. Lawson, was involved in the formation of the research questions as well as the completion of the data analysis, interpretation of results and preparation and revision of the manuscript. Dr. Lawson provided suggestions, guidance and editorial input into the creation of the manuscript. Dr. Rennie, Dr. Cockcroft, Dr. Pahwa, Dr. Dosman, Ms. Hagel, Dr. Karunanayake, Dr. Pickett were involved with the conception, design and data collection of the children's portion of the Saskatchewan Rural Health Study and reviewed this manuscript as members of the Saskatchewan Rural Health Study team.

"Validation of Questionnaire Report of Atopic Outcomes in School-age Children: The Saskatchewan Rural Health Study"

Mr. Chu constructed the formation of the research questions and completed the data analysis, interpreted of results and prepared and revised the manuscript. Dr. Lawson provided suggestions, guidance and editorial input into the creation of the manuscript. Dr. Rennie, Dr. Cockcroft, Dr. Pahwa, Dr.Dosman, Ms. Hagel, Dr. Karunanayake were involved with the conception, design and data collection of the children's portion of the Saskatchewan Rural Health Study and reviewed this manuscript as the Saskatchewan Rural Health Study team.

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Table of Contents

List of Tables

Chapter 2

Chapter 3

Chapter 4

List of Figures

List of Acronyms

CCHSA	Canadian Centre for Health and Safety in Agriculture CI Confidence Interval
CI	Confidence Interval
CS	Cross-sectional
OR	Odds ratio
SRHS	Saskatchewan Rural Health Study
SPSS	Statistical Package for Social Sciences
SPT	Skin Prick Test
US	United States

Chapter 1

Introduction

1.1. Background

Atopy is defined as "the personal or familial tendency to produce IgE antibodies in response to low allergen doses, and to develop typical conditions such as asthma, rhinitis or eczema"[1]. Atopic diseases have a significant impact on children's health as well as burden and health care costs for parents.[2] In Canada, according to a report from Health Canada, allergies are among the most common chronic conditions of Canadian children aged 12 years and older.[3] The results from Phase III in 2003 of the International Study of Asthma and Allergies in Childhood (ISAAC) conducted in 6-7 years old Canadian children showed that 10.8% of Canadian children suffered from allergic rhino-conjunctivitis symptoms, 18.2% with asthma symptoms, and 12.0% with eczema symptoms.[4]

Over the past century, the prevalence of allergic diseases and atopic sensitization has increased rapidly,[5-8] and varied between countries. Genetic factors are not likely the answer for the increase in prevalence given the short time period. Therefore, environmental factors are a candidate to explain the temporal and geographic variation in atopy prevalence.[9] Despite the obvious consequences of allergic diseases for children's health and their standard of living, the etiology of allergic diseases in children is complicated and controversial. It is suggested that the cause of allergic disease is rooted not only in the genetic and environmental factors involved, but interaction between those genetic and environmental factors.[10]

There have been numerous studies examining the prevalence and factors contributing to atopy among school children worldwide, mostly in Europe.[11-16] Most findings showed the trend of lower prevalence of atopy among rural and farming children compared to urban and non-farming children, although there are some discrepancies.[17,18] The prevalence of allergic diseases and allergic sensitization is lower in certain populations namely, those living on a farm, for both adults and children.[19,20] In farming populations, most findings also indicate that

a lower prevalence of atopy has been found among farming children compared to non-farming children.[16,21-27] The farm environment has been shown to reduce the occurrence of atopic sensitization in children[25,28] and growing up on a farm is suggested to protect from both sensitization and allergic diseases in childhood.[21] Having had a farming childhood appears to contribute to a lower risk of sensitization to common allergens as assessed by skin prick testing (SPT) and IgE serology.[29] Those who were self-employed farmers,[30] had farming parents, or when the child's mother had contact with farm animals during pregnancy[31] had a lower prevalence of SPT positivity.

In Canada, few studies have reported to determine the prevalence and risk factors for atopic sensitization among adults,[19,32] adolescents,[33] and children.[34,35] Furthermore, studies related to farming exposure are scarce.[36] Studies demonstrated a lower prevalence of atopic sensitization in farming residence versus non-farm residence. However, another study among 8-20 years old from a farming residence[36] did not find the clear evidence for the protective effect of farm living and livestock contact on atopic sensitization. It is still unknown about the objective measure of atopy prevalence and risk factors related to it among rural Canadian children.

The mechanisms that may explain the lower prevalence of atopic sensitization among farm residence are still obscure. It is suggested that specific farm exposures and the great diverstiy of microbial exposures on the farm may confer protection on atopic sensitization.[37] Possible exposures that may explain the low prevalence for atopic sensitization among farm children include farm milk consumption, livestock contact, and animal feed.[36,38]

Farming exposures in Canadian children may differ from those in European countries given the different farming practices among those countries. Literature calls for research in rural Canada to preliminarily identify risk and protective factors related to atopy among rural children, given the limited amount of Canadian study among this population.

Questionnaires are an effective tool in epidemiological studies which are usually conducted among large population-based studies.[39] Thus the proficiency and adequacy of questionnaire report are important, but whether or not that questionnaire reflects the true results shown in objective measures such as skin testing for allergy should be taken into account because it affects the valid assessment of the outcome of interest. In the case of atopic diseases and allergy, in large-scale epidemiologic studies, researchers have commonly relied on reported allergic conditions or exposure reported by questionnaire. In order to clinically facilitate diagnosis of allergic status or atopic conditions, objective measures such as skin-prick test positivity, elevated total IgE, and specific IgE are often used. The validity, accuracy and agreement of questionnaire report compared to the results from objective tests are, therefore, of concern.[39-42]

In line with the aforementioned gaps in terms of atopy information (prevalence and risk factors) in Canada generally and Saskatchewan specifically among rural children, I propose to conduct the research described herein to address this issue by using data from the Saskatchewan Rural Health Study (SRHS).

1.2. Rationale

There is paucity of information on the prevalence and risk factors for atopy among school children in rural Canada in general and in rural Saskatchewan specifically. Farming practices in Canada can differ compared to other countries which could affect atopy prevalence and associations with it. Also, according to the Statistics Canada (2011), Census of Agriculture and Census of the Population, the proportion of the rural population in Saskatchewan is high (33%)[43] compared to total rural population of Canada as a whole (19%),[44] making this an ideal environment for studies of atopy in a rural population.

Epidemiological studies require the use of survey methods for cost-efficient data collection. However, there is little work validating the questions used regarding allergic disease and their relationship to atopy, especially in Canada. Given that the questions

regarding atopic diseases used in this study was compiled from different sources, it is important to assess the validity and agreement of atopy detected by skin prick testing with reported allergic status from the questionnaire.

1.3. Research questions

The aims of this study were to determine the prevalence of atopy in rural children in Saskatchewan and identify the protective and risk factors associated with atopy in rural Saskatchewan school children Grade 1-8. As part of this aim, I examined the agreement between an objective measure of atopy and questionnaire reports of atopic diseases including atopy, hay fever, current allergic rhinitis symptoms, and eczema to inform conduct in future studies.

The following 3 research questions were included:

1. What is the prevalence of atopy in rural Saskatchewan children and is there a difference in atopy prevalence between farming and non-farming residential status?

2. What are the individual and environmental factors associated with atopy in Grade 1-8 rural Saskatchewan children with a focus on farming exposures?

3. What is the agreement between atopy assessed by skin prick testing and allergic disease based on questionnaire report?

1.4. Thesis Organization and Outline

A manuscript style approach was undertaken to this thesis. This thesis includes two separate manuscripts. Manuscript 1 aims to: first, identify the prevalence of atopy (the primary outcome of interest) and hay fever, hay fever symptoms, eczema (the secondary outcomes of interest) among children in a rural region of Canada; and second, to identify the risk factors of atopy, hay fever, current allergic rhinitis symptoms, and eczema specific to a rural environment. Manuscript 2 described the agreement between atopy measured by SPT and allergy measured by the questionnaire report, the agreement between atopy measured by

SPT and atopic outcomes measured by the questionnaire, and evaluates the predictive values of the questionnaire to assess atopic outcomes.

Relevant literature review will be presented in Chapter 2 describing different methods of atopy measurements and prevalence, risk factors relating to atopy in Grade 1-8 rural farm and rural non-farm children. Chapter 3 is Manuscript 1, and Chapter 4 is Manuscript 2. Chapter 5 will include the summary and conclusion of the key findings as based of Chapter 3 and 4.

1.5. Data source for this thesis

The data in this thesis were based on the baseline survey and clinical visit from the children's component of the Saskatchewan Rural Health Study conducted in 2010. This is a longitudinal study (2010-2015) examining the health outcomes of rural adults and children in the Canadian province of Saskatchewan with a focus on lung disease and related conditions. Below is a brief description of the methods for this study. Methods specific to each manuscript are presented in their respective chapters.

1.5.1. Study population and data collection

In the Saskatchewan Rural Health Study, four rural quadrants of Saskatchewan (Northwest, Northeast, Southwest and Southeast) were selected by a multistage stratified random sampling strategy.[45] With the definition of rural area being located at least 60 kilometers from an urban center,[46] a sample of 9 rural municipalities (RMs) was randomly selected for each quadrant from a purposeful sample of 48 RMs (12 from each quadrant). Overall, 32/36 (89%) RMs and 15/16 (94%) small towns participated.

Based on these selected four quadrants of the adult study, schools located within the four quadrants were considered the target schools for the child study. Ten school divisions in the 4 quadrants agreed to participate and 43 schools within these divisions were approached accordingly. Of the 43 selected schools, 39 schools agreed to take part in this study.

The study team prepared packages that included an information letter, questionnaire, consent, and assent forms. Following approval from the school district boards, all schoolchildren in Grades 1-12 were sent a study package containing a questionnaire for parents to complete. Classroom teachers distributed the packages to students. Completed questionnaires were sent back to the school. For the survey portion, 5667 children were approached with 2383 children taking part for a response rate of 42%.

A subset of students in Grades 1-8 attending from 16 pre-selected schools received a study package containing the questionnaire and a request to participate in clinical assessments including spirometry and skin testing for allergens. The schools where clinical assessments were conducted were selected based on school participation numbers in the survey in order to maximize efficiencies and reduce costs. We also excluded schools with high First Nations enrollments. One school division refused to allow clinical assessments in their schools. A total of 1768 students from 16 schools were approached for clinical testing. Of these, 584 took part. It is this group of students who are included in the analysis for this thesis.

1.5.2. Cross-sectional survey

The cross-sectional questionnaire was based on questions from standardized questionnaires including: the American Thoracic Society's 1979 Children's Respiratory Disease Questionnaire,[47] questionnaires used in a study in Estevan, Saskatchewan in 2000 and 2003,[48,49] the International Study of Asthma and Allergies in Childhood Study (ISAAC) questionnaire,[50] and a questionnaire used in a study conducted in Humboldt, Saskatchewan[51]. The questionnaire includes information on socio-demographics, the respiratory and general health of the child, allergic disease, life style, home environment, and early life exposures.

1.5.3. Skin prick testing (SPT)

A panel of allergens that are most common in Saskatchewan was used including *Alternaria* (mold), *Cladosporium* (mold), cat dander, local grasses, wheat dust and house dust

mite (ALK – Abello Pharmaceuticals, Inc., Ontario, Canada). Histamine (10mg/ml) and saline solution (0.9%) were used as positive and negative controls, respectively. Skin prick testing for atopy was conducted according to international standards for testing.[52,53]

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Chapter 2

Literature Review

2.1. Scope of literature review

The purpose of the literature review in this thesis is to explore existing literature to describe and discuss what is already known about the epidemiology of atopy or allergic diseases among children, especially those at school age. The methods to assess atopy, the prevalence of atopy and the current knowledge and evidence of risk and protective factors that contribute to atopy, especially between urban and rural dwellers and between farming and non-farming residential status, as well as the methods to validate information from a questionnaire report and corresponding results from objective measure (skin prick testing) were described.

2.2. Methods

The literature search was conducted between the periods of December 2012 to June 2014. Some common search engines were used such as Pubmed, Google Scholar and the Saskatchewan's University Library literature search to look for current scientific publications and reports. Also, additional relevant scientific articles were considered if they appeared in the reference section of a selected article. A filter used in the search were if the papers were published after the year of 1990. Research terms included "atopy", "atopic sensitization", "school age children", "farming exposure", "risk factors", "skin prick testing", and combinations of these. Also other research terms included were "agreement", "concordance", "questionnaire", "objective measures" and combinations of these. The search was limited to humans only.

2.3. Rural living and farming in Canada and Saskatchewan

According to the Statistics Canada (2011), Census of Agriculture and Census of the Population, the proportion of the rural population in Saskatchewan is high (33%)[1] compared to total rural population of Canada as a whole (19%).[2] As cited from Statistics Canada,[3] the

number of farms across Canada is decreasing from 246,923 (2001) to 229,373 (2006), but the total farming area has been stable from 1956 to 2006. During that time, the proportion of field crops has been the highest (41 % in 2001 and 39.8% in 2006) followed by "beef cattle and feedlots" (27.5% in 2001 and 26.6% in 2006).

In Saskatchewan, based on the report of the Census of Agriculture 2001-2006, the total number of farms decreased (50,598 in 2001 and 44,329 in 2006).[4] Among specific farming types, beef cattle ranching and farming, including feedlots accounted for the largest amount of farming (24.1 % in 2001 and 27.6% in 2006). The second was wheat farming (18.4% in 2001 and 15.7% in 2006). Unknown and other farms including other grain farming accounts for 31.2% in 2001 and 27.2 % in 2006. According to Census of Agriculture 2011, Saskatchewan had 38.5% of the total farm area in Canada in 2011 and had the second largest cattle herd in the country after Alberta, with 20.7% of the national total.[5]

Also, the Rural Health and Northern Health Research Initiative (2004) reported that rural population health is considered one of the national research priorities.[6] Along with that, the Government of Canada, through the Canadian Institutes of Health Research (CIHR) increased investment from $733,054 (2000-2001) to $10 million (2004-05) in rural and remote health research.[6]

2.4. Immunology of atopy

Allergic atopic disorders, such as rhinitis, asthma, and atopic dermatitis, are the result of a systemic inflammatory reaction triggered by Type 2 T-helper (Th2) cell-mediated immune responses against 'innocuous' antigens (allergens) of complex genetic and environmental origin.[7] The term ''atopy'' describes the tendency to become IgE-sensitized to common allergens but do not have a prolonged IgE antibody response.[8]

2.5. Measurement of atopy

2.5.1. Methods to assess atopic status

Allergic diseases are diagnosed by certain objective tests namely total serum IgE, specific IgE,[9] the skin prick test (SPT),[10,11], and Phadiatop.[12] Each has its own advantages and disadvantages[9,13,14] which are listed in **Table 1**.

Table 1: Characteristics of different tests of allergic diseases

Name of the test	Advantage	Disadvantage
Skin prick testing (SPT)	- *in vivo* - Is cheap, quick, efficient, more accurate than other tests, - visual - Rarely induces anaphylaxis. - are virtually painless, minimal discomfort - Standardized allergens available	- Not all allergens are available for skin testing - Skin prick testing takes approximately 20 minutes or less to perform at a community laboratory. - Less quantifiable - Dermagraphism - Histamine will cause itchiness
Blood test (Total serum IgE, specific IgE - "RAST" tests (**R**adio-**A**llergo**S**orbent **T**est))	- *in vitro* - Better for those with widespread eczema, or at risk of severe allergic reaction, such as anaphylaxis. - can test on anti-histamines, quantifies the amount of free specific IgE antibody.	- Invasive - Is only reliable in 80% of cases and causes many to get false negatives or false positives - Does not provide some essential information, as tests fail to show the severity level of a child's allergy symptoms. - Expensive - High IgEs skew results - Results take time - Are usually difficult to interpret the results.
Phadiatop	- Is a multi-allergen screen for aero-allergens - has a sensitivity of >90%, but somewhat lower sometimes than a single allergen - can test for regional allergens	- Expensive and invasive - Cannot show the exact allergen(s) to which an individual is sensitized to, but demonstrates a specific reaction to at least one of the allergens of the mixture, whichever the reaction(s) may be. - The patient may be mono-sensitized to food or other allergens not covered by Phadiatop.

Besides the objective tests, questionnaire-based surveys are commonly used in epidemiologic studies.[15-17] Atopic status can be, therefore, estimated by questionnaire in large

population-based studies.[18] If questionnaire report is to be used in place of objective measures of SPT, we must ensure that questionnaire report of allergy accurately reflects the objective measure. A quick, simple and accurate questionnaire can be preferred to a more costly and involved objective method. [15-17] Due to those reasons, questionnaire report is now used in many studies.

2.5.2. Statistical methods to evaluate diagnostic tests

Measurement issues such as validity and reliability are of concern in epidemiological studies.[19-21] Current knowledge have shown that there is no perfect diagnostic test. In order to evaluate diagnostic tests, measurement issues including validity, reliability of the test or diagnosis have a place of importance to play.

Common indices of validity include sensitivity, specificity, Positive Predictive Value (PPV), and Negative Predictive Value (NPV). Sensitivity is the ability of a test to correctly classify an individual as ′diseased′, and the ability of a test to correctly classify an individual as “not having disease” is called the test′s specificity.[22] PPV is the percentage of patients with a positive test who actually have the disease, and NPV is the percentage of patients with a negative test who do not have the disease.[22] Predictive values of PPV and NPV are more useful in clinical application.[23] The characteristics of population as well as the prevalence of atopy in the population can influence the PPV and NPV. For example, the low PPV can be seen from screening results for a rare disease such as systemic lupus erythematosus (SLE) in the population because of the high number of false positives.[24] However, the high PPV can be seen from a test of a specific population of patients with signs of SLE, because the prevalence of SLE is much higher in patients’ population.

Second, information about the quality of measurements is reflected through the measurement of reliability and agreement. Traditionally, percent agreement is one of the methods to measure interrater reliability. Also, the kappa statistic is frequently used to test interrater reliability.[25] The calculation of the kappa statistic is based on the difference between

the “observed” and “expected” agreement.[26,27]Kappa statistic accounts for both percentage agreement and the percentage of agreement expected by chance. Cohen’s Kappa coefficient is used in many studies to measure levels of agreement.[26,28] The values of agreement are often interpreted as: >0.80 (very good agreement), 0.61-0.80 (good), 0.41-0.60 (moderate), 0.21-0.40 (fair), and <0.02 (poor).[29] It is understood that Kappa value depends on the prevalence of diseases within certain populations which makes it difficult to compare results from different studies.[30]

2.5.3. Studies measuring agreement between questionnaire report and objective measures

The reliability of the objective test as well as the validation of questionnaires is of concern.[31] There have been a number of studies assessing the relationship between clinical measures of atopy (e.g., skin-prick test positivity, total IgE) and questionnaire report to evaluate the discrepancy or concordance of information shown on questionnaire and clinical diagnosis.[15,16,31-33] **Table 2** describes studies where results from objective tests were compared with each other and with questionnaire report. The discordance between questionnaire report and SPT results as well as between SPT results and other objective tests were shown in recent studies.[10,15,16,34-36] When comparing the agreement between questionnaire report and skin prick test results, questions on rhinitis were highly specific and high PPV to detect atopy among children with symptoms, but not helpful for detecting atopy in a general population of children.[16] Also, SPT has the best positive predictive value and the best efficiency to diagnose respiratory atopic diseases in a study to compare the results from 3 different objective tests including IgE, skin prick tests, and Phadiatop.[10]

Table 2: Comparison of different objective tests and those tests with questionnaire report

Lead author (Year published)	Country	Study population (age, N, Sub-N)	Comparison	Definition of atopy (cut-off)	Findings
Braun-Fahrlander et al [16] (1997)	Switzerland	- 7, 10 and 14-years old - 2,954 - 2,120	SPT and Questionnaire	SPT (≥3 mm). At least one positive response to at least one of six common aeroallergens (grass mixture, birch, mugwort, D. pteronyssinus, cat and dog dander)	- ISAAC core questions on rhinitis were highly specific (77.5% to 97.6%), high positive predictive value (63%-70%) in detecting atopy among children with symptoms, but not helpful for detecting atopy in a general population of children because of the low sensitivity (2.6% to 42.7%).
Tschopp et al [10] (1998)	Switzerland	- Adults - 8,329	IgE, skin prick tests, and Phadiatop	SPT (≥3 mm). At least one positive response to at least one of eight common aeroallergens - Positive total IgE (IgE ≥ 100 kU/l)	- Diagnostic efficiency of SPT was significantly higher than that of Phadiatop (83.1% vs 79.9% and 77.6 vs 71.9%, respectively; both P < 0.001) to diagnose current allergic rhinitis (CAR) and current allergic asthma (CAA). - IgE and SPT had equal efficacy (77.6%), which was significantly higher than that of Phadiatop, to diagnose current allergic asthma (71.9%; both P < 0.001). - SPT had significantly the best positive predictive value for CAA (5.2% for SPT vs 4.6% for

					both IgE and Phadiatop; both P < 0.001) and CAR (48.7% for SPT vs 43.5% for Phadiatop and 31.6% for IgE; both P < 0.001).
Garcia-Marcos et al[35] (2007)	Spain	- 9–12 years old - 1,471 - 621	SPT and PhadiatopTM test	- A positive SPT was that with at least a wheal having a maximum diameter of 3 mm, allergens: *Dermatophagoides pteronysinus, Dermatophagoides farinae,* cat, *Alternaria,* mixed trees *(Betula, Alnus and Corylus)* and mixed grasses *(Dactylis, Lolium, Festuca, Poa, Phelum and Avena),* olive and Parietaria. - PhadiatopTM included the following allergens: Mites (*D. pteronyssinus and D. fariane),* pets (cat and dog), mixed moulds (*Penicillium, Cladosporium, Aspergillus and Alternaria*), mixed grasses (*Parietaria, Lolium, Phleum and Cynodon*), *Artemisia* and mixed trees (*Acer, Betula, Ulmus,* Quercus, Olea, Salix, Pinus, Eucalyptus, Acaciaand Malaleuca). Specific IgE cut-off: ≥0.35 kU/l.	Using SPT as the gold standard, sensitivity of Phadiatop = 85% (95% CI = 82.2–87.8%), specificity 85.5% (95%CI 82.7–88.3%), positive predictive value 72.7% (95%CI = 69.0–76.1%), negative predictive value 92.7% (95%CI 90.6–94.7%) and accuracy 85.3% (95%CI 82.3–88.0%).
Weinmayr et al[36]	Countries participated in	- 8 to 12 years old	SPTs, total and specific IgE	- SPT (3mm). Six extracts of common aeroallergens	- In non-affluent countries, a higher proportion of children with positive

(2010)	International Study of Asthma and Allergies in Childhood (ISAAC)- Phase II	children - 7,461		(*Dermatophagoides pteronyssinus*, *D. farinae*, cat dander, *Alternaria tenuis*, mixed tree pollen and mixed grass pollen) - Allergen-specific IgE antibodies to a mix of common inhalant allergens (*Dermatophagoides pteronyssinus*, *D. farinae*, birch, timothy, mugwort, cat, dog, horse, *Cladosporium*, olive pollen and Parietaria). A positive allergen-specific IgE test was defined as ≥0.35 kU^A/l. - The lower detection limit was 2 kU/l for total IgE.	SPT had no detectable specific IgE (sIgE) (range 37–61%) than in affluent countries (0–37%). - Total IgE was more strongly associated with sIgE than with SPT positivity. The geometric means ratio for the association of total IgE with sIgE (adjusted for presence/absence of positive SPT) was 3.66 [(95%-CI): 3.12;4.30], whereas the geometric means ratio for the association of total IgE with SPT results (adjusted for sIgE, cutpoint 0.35 kUA/l) was 1.60 (95%CI: 1.38-1.84)
Hoppin et al[15] (2011)	USA	- 1 year old and more - 12,862 - 8,334	IgE & Questionnaire	1 or more positive specific IgE ≥ 0.35 kU/L	- 53% reported at least 1 allergic condition. Discordance between atopy and allergic conditions: 37% of persons with atopy reported no allergic condition, and 48% of persons who reported an allergic condition were not atopic.

Among the aforementioned objective tests, the allergy skin prick test (SPT) is commonly used due to its simplicity, safety[37] and accuracy in determining the presence of atopy. According to the World Allergy Organization, the skin prick test is advantageous compared to other testing methods.[38] SPT is proved to possess relatively high sensitivity and specificity (77% and 65.3%, respectively) with the 3 mm cut-off of diameter wheal[39], low cost, and identification of patients'allergy status.[40]

However, use of a different cut-off of wheal size in different studies hampers the effort of comparing the results of studies to one another. Some studies used the cut-off of wheal diameter: 0 mm,[41] 2 mm[42] and recently most studies used 3 mm as standard. The cut-off of 3 mm wheal diameter of the skin prick test was recommended by the Global Allergy and Asthma European Network (GA(2) LEN), Allergic Rhinitis and its Impact on Asthma in 2012[43] and European standards[11] and American Academy of Allergy, Asthma and Immunology (AAAAI).[44]

In summary, not unique to questionnaire measures, a lack of agreement is seen in objective measures with 2 most common measures, specific IgE positivity and skin-prick test positivity. There is no gold standard measurement for allergy, since there are inconsistent results among the measures of atopy and allergy. Questionnaires remain the best and sometimes the only way to collect medical information in a large study sample. Increasing the precision of questionnaires will improve their utility; however, not all aspects of the allergy-atopy spectrum are well described using questionnaires. Researchers need to be aware of the limitations of all potential atopy measures, given the lack of concordance in many large population-based studies. It is also important to test the consistency between questionnaire report and objective measures in specific locations.

2.6. Epidemiology of atopy

2.6.1. Natural history of atopy

The natural history of atopic manifestations including eczema, asthma and rhinitis are called the "atopic march" or "allergic march". The "allergic march" is a progression of atopic disease from eczema to asthma, and then to allergic rhinoconjunctivitis.[45] Those clinical symptoms appear very early in life and persist over years, characterized by typical IgE antibody responses.

2.6.2. Prevalence of atopy

The prevalence of atopy varies internationally among children (20-60 fold difference). A study in Brescia, an industrialized town in Northern Italy, among 13-14-year-old schoolchildren between October 2002 and June 2003 found that 49% (308 out of 680) of children were positive for at least one of the 12 common allergens on SPT.[46] According to the National Health Interview Survey of the United States of America in 2010, 13 percent of US children aged 17 years and under suffered from skin allergies in the past 12 months.[47] Another study conducted by Batlles-Garrido et al[48] (2010) in 1143 children aged 10-11 years from Almeria (Spain) found the prevalence of atopy – defined by any SPT positivity of common allergens – was 42.5%. Among these, 34.9% could be regarded as subclinical sensitization because no symptoms were found in these patients. Among 342 children (0-14 years old) in Spain, 20% of them were found to have skin test positivity in a retrospective study (2000-2007).[49] Among these children, there was an increase in allergen sensitisation with age from 42.3 % in the 0-3 years age group to 93.3 % in the 7-14 years age group ($p < 0.0001$).

In Canada, there have been few studies examining atopic sensitization. More commonly, the prevalence of atopy among adults has been studied. A cross-sectional study was conducted in 2003 in 2,081 adults (18-79 years old) in the town of Humboldt, Saskatchewan.[50] The results showed that adults farmers were less likely to have atopic sensitization – defined by any positive reaction to 4 allergens used - compared to non-farmers

[OR=0.79; 95%CI: 0.65 - 0.97]. Another cross-sectional study was carried out in Quebec among 1,199 rural secondary school students aged 12 to 19 years in 2000.[51] Findings showed that adolescents raised on the farm were less likely to have atopic sensitization to any one of 24 common inhaled allergens [OR= 0.58; 95%CI: 0.46 - 0.75]. Furthermore, Chan-Yeung et al[41] conducted a cross-sectional study among adults aged 20-44 years in six study sites across Canada. The authors found that the overall prevalence of atopy – defined as at least one reaction (skin test over 0 mm to any allergen) among 14 allergens used- was 62.7%. The geographical variation in the prevalence of atopy in the six study sites was also determined. It was lowest [55.6%; 95% CI: 51.3-59.9] on Prince Edward Island and highest [66.0%; 95%CI: 61.7-70.3] in Montreal.

2.6.3. Temporal trends in atopy/atopic sensitization

Temporal trends in atopy have been studied and trends seem to be different among countries. Ronmark et al[52] found a significant increase from 21% in 1996 to 30% in 2006 ($P < 0.001$) in the prevalence of allergic sensitization which was defined by at least one positive reaction to 10 common allergens in school children in northern Sweden. The increasing pattern was also found in another study in Greenland.[53] In that study, 859 Greenlanders aged 15-80 years participated in population-based screening campaigns in 1987 and in 1998 and underwent blood tests for IgE. The frequency of atopy – which was defined as a positive result against 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].

Besides the increase of atopy prevalence in some countries, other studies also demonstrated a stable or decreasing pattern. Cross-sectional studies conducted in 1982, 1992, 2002 among school children aged 8-11 years in 1982 in Australia[54] showed that the prevalence of atopy – detected by skin prick testing with cut-off wheal size at 3mm- decreased 3.1% (from 39.3% in 1992 to 36.2% in 2002). Zollner and colleagues conducted 6 cross-sectional studies over 9 years (1992-2001) among 6762 school children (9-11 years old)

in Germany.[55] In those studies, the prevalence of atopic sensitization which was defined as the 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)].

2.6.4. Burden of atopy and allergic diseases

Atopic diseases such as asthma, allergic rhino-conjunctivitis and atopic eczema/dermatitis have a significant impact on children's health (physical, social and economic)[56] as well as their parent's burden and health care costs.[56,57]

Economic burden on health care systems in Europe and North America attributable to allergic diseases is also considered.[33,58] In the United Kingdom in 2004, a secondary analysis of health care burden posed by allergic disorders using national databases about healthcare burden and the costs of allergic disorders[59] showed that 0.8% of hospital admissions were for allergic diseases. Another analysis of national databases in Scotland in 2009[60] found that allergic disorders affect about one in three of the population at some time in their lives which accounts for 1.5% of hospital admissions and an estimated 130 million pound per year in direct healthcare costs. A national multicenter study to determine the extent and burden of allergic diseases in elementary schoolchildren in Turkey in 2010[33] showed that 34.2% of children with asthma and allergic diseases were absent from school for at least 1 day in that school year and some degrees of disturbance, and hospitalization.

In Canada, one study examining the burden of atopic dermatitis (AD) in 2006 showed that the total cost of AD was estimated to be 1.4 billion Canadian dollars annually and the annual per patient expenditure was estimated to be $82,454 Canadian dollars and $1,242 Canadian dollars for patients with mild, moderate and severe atopic dermatitis, respectively.[61]

2.6.5. Relation between atopy and asthma and allergic diseases

Atopy is strongly associated with asthma in children, but it is unknown to what extent atopy accounts for this disease.[62] While most children with conditions such as rhinitis,

asthma, and atopic dermatitis are atopic, some are not, and, conversely, some children who are atopic may not have atopic disease manifestation (rhinitis, eczema, etc.)[63]

At a population level, about 34% of asthma is suspected to be attributable to atopy defined by the occurrence of at least one positive allergen-specific test.[64] An estimation of prevalence of atopy and atopic disease among the population (children and adults) was established.[65] It was estimated that approximately 40% were atopic as defined by skin test positivity.[65] The prevalence of allergic rhinitis was 20%.[65] There is a considerable overlap between asthma and rhinitis; however, not all those with asthma are atopic. Atopic eczema has also an overlap with asthma and allergic rhinitis.[65]

From the Third National Health and Nutrition Examination Survey in the United States in 2007 with the subjects from 6 to 59 years old, it was concluded that 56.3% of the asthma cases were attributable to atopy which was defined as at least 1 positive allergen-specific test based on 10 common allergens.[66] In a population-based cross-sectional study (GABRIEL Advanced Studies- Phase II), 2,586 schoolchildren in rural Poland were chosen to participate in a study with in-depth questionnaire about specific farm exposures and objective measures of atopy.[67] It was found that only 22% of those with childhood asthma were sensitized to indoor allergens and 17% were sensitized to outdoor allergens. As for hay fever, only 21% of those with hay fever were sensitized to outdoor allergens.

In conclusion, the strong association found between atopy and clinical asthma suggests that atopy may play a role in causing asthma in genetically predisposed children only. Thus, understanding the factors that truly account for the development of atopy and its manifestations could be essential for the prevention of these subsequent outcomes.

2.7. General risk and protective factors of atopy in children

The etiology of pediatric allergic diseases and atopy is known to be complicated. Much of the epidemiological and clinical literature has divided those risk factors into personal and environmental factors.

2.7.1. Personal factors

Typically, personal factors are most consistently identified as important risk factors for atopy. Familial pre-disposition has been known to be an important predictor of atopic diseases.[68] The probability of being atopic in the child if both parents are atopic is 50% and 15% if one of the parents is atopic.[69] In childhood, boys are more likely to be allergic and atopic rather than girls but the pattern is reversed when it comes to adolescence and early adulthood.[70]

A number of factors at birth appear to influence the risk in the development of allergic sensitization and atopic diseases.[71] Gestational age equal to, or over 40 weeks compared with less than 36 weeks was associated with an increased risk of atopy [OR= 1.65, 95%CI: 1.16-2.34).[72] The relationship between prematurity at birth (<36 weeks' gestational age) and atopy is inconsistent among studies. Some studies linked the prematurity at birth to an increased risk of atopy;[73-75] whereas others showed the opposite[76,77] or no association[78,79] or a decreased long-term risk of atopic sensitization.[80]

It is suggested that first-born children appear to be at increased risk of allergic disease and atopic sensitization compared to those who are not first-born children.[81] It is also suggested that children who attended daycare before age 2 years had a reduced risk of atopy at 3-5 years.[82] The effect of breast feeding has been inconsistent.[83-87] Common childhood immunization in the first year is not associated with an increased risk of allergic sensitization based on the current literature.[88] Recent studies also suggested that Body Mass Index (BMI) is also associated with children's atopic condition, but the findings are controversial.[89-92] There has been debate on the association between sibship size/birth order and childhood allergic diseases.[93] Having older siblings appears to protect against the development of atopic diseases after the age of 2, but increase the incidence of early asthma.[94]

2.7.2. Environmental factors

The role of environmental factors in the development of atopy should also be considered since 23% of children without any familial pre-disposition appear to develop atopy.[95] Associations between environmental factors and atopy/allergic sensitization have been inconsistent. Studies have provided conflicting evidence of the effect of environmental tobacco smoke (ETS) on the development of allergic diseases in children. The effect of ETS on childhood allergic sensitization depended upon maternal history of asthma/allergy.[96] Children whose mothers had positive history of asthma/allergies have a reduced risk of atopic sensitization.[96] Parental smoking can trigger the risk of allergic sensitization in their children;[97,98] whereas others showed the protective effect[99,100] and even no association was found[101].

A more controversial environmental factor is pet exposures. Exposure to pets indoors appears to have a protective effect on atopic sensitization in children, especially for the exposure in the first two years of life.[102] Among farm children, early and current exposure to cats has a reduced risk of grass pollen sensitization and current contact with dogs confer protection against cat sensitization and grass pollen sensitization.[103] However, the effect of pet ownership on atopic sensitization depends upon which kinds of pets: dogs or cats. The results for cat ownership showed more inconsistency, but for dog ownership, results are more consistent suggesting no effect or may be protective against the development of specific sensitization to dog and allergic sensitization in general.[104]

2.8. Rural-urban difference in atopy prevalence in children

There have been inconsistent findings also found in some studies comparing the prevalence of allergic sensitization between rural and urban settings. Even among studies showing an association, the strength of association varies among studies.[105-110] **Table 3** describes studies examining the difference in atopy prevalence in children in urban-rural residence.

In general, the reported prevalence of atopy among children is lower in rural areas than in urban areas. A study among 404 school children aged 12-16 years old in Poland in 2005 showed that 63.7% (128/201) urban school children had an atopic sensitization- defined as at least one reaction to 15 allergens- compared to only 24% (46/203) of rural children [OR = 6.0; 95%CI: 3.9–9.3; p < 0001)].[106] Another cross-sectional study in 738 children aged 6-18 years old in 2007 in Konya, Turkey showed that that there were no statistically significant differences in the prevalence of atopy in rural and urban areas.[110]

Table 3: Difference between urban-rural residences in atopy prevalence in children

Lead author (Year published)	**Country**	**Design**	**Study population (Age, N, Sub-N)**	**Definition of atopy (Objective tests/cut-off wheal size chosen)**	**Prevalence & Association (OR; 95% CI)**
Barnes et al [105] (2001)	Greece	CS	- Age: 11-19 years - 1,392 - 997 returned parental questionnaire, 929 underwent SPT.	- SPT (3mm). - Allergens: mixed grass pollen (Mediterranean), house dust mite, cat, *Parietaria*, olive blossom, *Altenaria* and goat allergens	Urban vs rural: 19.6% vs 9.6 % (p< 0.001)
Majkowska–Wojciechowska et al [106] (2007)	Poland	CS	- Age: 12-19 years - 404	- SPT (3mm). - 15 allergens: *Dermatophagoides pteronyssinus* (Der P), *D. farinae*, cat, dog, rabbit, hamster, guinea pig, rat, swine, birch, grass mix, mugwort, plantain, *Alternaria tenius* and *Cladosporium herbarum.*	Urban vs Rural: 63.7% vs 22.7% (p< 0.001). [OR= 5.98;95%CI: 3.23–12.72]
Priftis et al [111] (2007)	Greece	Longitudinal cohort study	Phase 1: 801 Phase 2: 679	- SPT (3mm). - Allergens: mixed grass pollen, *Parietaria officinalis*, olive tree pollen,	- Sensitization was more prevalent in the urban areas at both phases. Phase 1: Urban vs Rural:

				Dermatophagoides pteronyssinus, *Dermatophagoides farinae*, *Alternaria alternata*, *Cladosporium herbarum*,cat and dog dander	19.0% vs. 12.1% (p= 0.009) Phase 2: Urban vs Rural: 20.0% vs. 14.1% (p= 0.048) - Residential area contributed independently to sensitization to ≥1 aeroallergens [OR=0.29; 95% CI: 0.13–0.66; p=0.003] and to polysensitization [OR=0.28; 95% CI: 0.10–0.82; p=0.02] in phase 1.
Guner et al [112] (2011)	Turkey	CS	- Age: 6-18 - 738 - 607 (some students were excluded from the study because they were not countersigned by the parents).	Atopy was defined as at least one manifestation of asthma, allergic rhinitis or eczema.	Urban vs Rural: 32.2% vs 30.0% (p=0.57)

Abbreviations: CI, Confidence Interval; CS, cross-sectional ; OR, Odds ratio

2.9. Atopy prevalence of atopy among children in farming and non-farming settings

In addition to studies of the urban and rural differences in atopy among children, there have been several studies with the focus on the relationship between farming exposure and atopic sensitization in children solely in rural settings. 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. **Table 4** summarizes studies examining the prevalence of atopy among children in farming compared to non-farming counterparts. Most of the studies were cross-sectional in design. Skin prick testing was used most often.

Epidemiological studies have been conducted in several countries such as Canada, Austria, Sweden, New Zealand, Eastern Finland, and the USA (as shown in **Table 4**). In Canada, Ernst et al[51] conducted a cross-sectional study in 1199 children and adolescents aged 12-19 years with skin prick test (SPT) positivity defined as a positive reaction to any one of 24 common inhaled allergens. The results showed that children living on a farm were significantly less likely to develop atopy compared to those not living on the farm [OR= 0.58; 95% CI: 0.46 to 0.75]. Some other following studies confirmed these inverse associations between atopy and farm children. Remes et al[113] conducted a cross-sectional study in 2000 among 366 farmers' and 344 non-farmers' children in eastern Finland and found that atopy – defined as children had positive reactions against one or more allergens among 6 chosen allergens - was less frequent among the farmers' children than the non-farmers' children [aOR=0.56 (95% CI 0.40-0.78)]. Alfven et al[114] also conducted a cross-sectional study in Austria, Germany, The Netherlands, Sweden and Switzerland with the sample size of 14,893 children aged 5-13 years. With the definition of atopy as the Serum IgE $\geq$ 0.35 kU/l , the authors found that farm children had less atopy than non-farm children [OR=0.53 (0.42-0.67)].

Table 4: Atopy prevalence among farmers' children and 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 (OR; 95% CI)**
Ernst et al[51] (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[115] (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).
Klintberg et al[116] (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[117] (2001)	Australia (two rural towns: Wagga Wagga in a mixed farming region, and Moree in a crop farming region)	CS	- Age: 7-12 years - 1,500 - 1,436 subjects underwent SPT	SPT (3mm). Any positive reactions to 8 allergens (*D.pteronyssunus*, rye grass *pollen Lolium perenne,* cat dander, the fungus *Alternaria alternat*, the fugus *Clasdosporium cladosporoides*, wheat wholegrain, grain mill dust and cotton lint)	Lower risk of atopy in Wagga Wagga between farm vs non-farm living (adjusted odds ratio [aOR=0.47; 95%CI: 0.32-0.72] but not in Moree [aOR= 0.97; 95%CI: 0.62-1.53].
Horak et al[118] (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[119] (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]
Remes et al[113] (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]
Remes et al[120] (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,	Little difference was observed in sensitization against the other allergens

				cat, dog, cow, and horse epithelial danders, cockroach (*Blatella germanica* , house dust mite (*Dermatophagoides pteronyssimus*), and storage mite (*Lepidoglyphus destructor*)].	between the farmers' (17.2%) and non-farmers (14.5%) children [aOR=1.11; 95%CI: 0.71-1.72]
Merchant et al[121] (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 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	- 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 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]
Alfven et al[114] (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[122] (2006)	Shropshire, England	2-stage cross-sectional study	- Rural primary schools (exact age not provided) - Stage 1: 4,767	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*,	Compared with rural nonfarming children: adjusted [OR= 0.68;95%CI: 0.40-1.16; p = 0.15]

			Stage 2: 879	*Tyrophagus putrescentiae*	
Holbreich et al[123] (2012)	Switzerland	CS	- Age: 6-12 years - (Amish:157, Swiss farmer: 3006 and Swiss non-farmer: 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% (10/157), Swiss farmer: 25.2% (223/3,006) and Swiss non-farmer: 44.2% (281/10,912)
Illi et al[124] (2012)	Austria, Germany, and Switzerland	CS (population-based)	- Age: 6-12 years - 7,682	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)	Children living on a farm were at significantly reduced risk of atopic sensitization [aOR=0.54;95%CI: 0.48-0.61; p< 0.001) compared with nonfarm children.
Macneill et al[67] (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;95%CI: 0.57- 0.91] and skin prick test [aOR = 0.65;95CI: 0.50- 0.86]

Abbreviations: CI, Confidence Interval; CS, cross-sectional

There have been some studies in which the difference in prevalence of atopy was similar or was not statistically significant (may not differ between farm and non-farm status). A birth cohort among 707 children aged 7-8 years in the island of Gotland, in the Baltic Sea, Sweden showed the prevalence of atopy among farming children and non-farming were 30.7% and 32.7%, respectively. Also, the study of Remes et al in 2005[120] showed little difference in atopic sensitization among farm and non-farm children.

Taken together, the available epidemiological literature suggests that a protective effect of farm residence on the development of atopy were consistent. However, there is considerable heterogeneity between studies with respect to the health outcomes (how to define atopic condition by skin prick testing or IgE blood test) that are inversely associated with atopy and with respect to the different type of farm between different regions which entail specific exposures.

2.10. The "farm effect" and farming activities

Several characteristics specific to the farm environment have been investigated to explain the farm effect on atopy. These include: livestock contact, animal feed contact and unprocessed milk consumption and the timing of those exposures.[125-129]

2.10.1. Contact with livestock

Livestock exposure was suggested as an important component of the protective effect in the farm environment in some European countries[113,122,130,131] where they mostly practice dairy-based farming. Together with farm residence, livestock contact confers protection against atopy in children. Regular contact with farm animals has been suggested to be the strongest protective predictor for atopy in rural subjects.[132] However, some studies in other countries or regions where farming practices vary showed less consistency in livestock effect, suggesting that protection may depend on the type of farming.[117,133,134] No such protective effect was found among children 7-12 years of age living in crop-related farms in Australia, but the combination of livestock contact and crop farming surprisingly demonstrated the inverse association between atopy and farm children against non-farm peers in the same region.[117] Moreover, a cross-sectional study of 293 children in New Zealand showed that current farm residence appeared to increase the risk of SPT positivity and concluded the effect of early life contact with pigs with SPT positivity [OR = 0.2, 95%CI: 0.1-0.9].[119]

Another cross-sectional study among 7981 children aged 13-14 years old in the Finnish ISAAC study (2002) demonstrated that living on a farm with livestock conferred protection from allergic rhinoconjunctivitis (aOR=0.69).[135] In another cross-sectional study among 366 farmers' and 344 non-farmers' children in eastern Finland in 2000, the authors found an inverse, dose-dependent association between the frequency of current livestock contacts and atopy- defined as having one or more positive skin prick test reactions [aOR=0.46, 95% CI: 0.22-0.97] for daily contact vs. no contact.[113] This protective effect was also found in a cross-

sectional study of 1,137 school age children (8-10 years old) in 1997 in a rural area of Austria, but the inverse association was not statistically significant [OR=0.75, 95%CI: 0.37-1.52].[115]

In Canada, a cross-sectional study was conducted among 8-20 year old members of the 4-H club in British Columbia in 2001.[136] These members had opportunities to have contact with livestock through club activities. Results showed that living in a farm residence with livestock was protective compared to living in a rural non-livestock area [diagnosed asthma aOR= 0.49;95CI: 0.27–0.89]; allergic rhinitis [aOR=0.51; 95%CI: 0.30–0.85] and atopic dermatitis [aOR=0.45; 95%CI: 0.24–0.84]. Although the SPT was conducted on a sub-population, the results of atopy were not reported in this paper. The authors concluded that contact with livestock was one of farm environment aspects which were protective for allergic conditions.

2.10.2. Contact with animal feed

In farming environments, animal sheds contain allergens, bacteria, viruses and fungi, few of these exposures have been assessed.

Phase I of the GARBRIEL study (A Multidisciplinary *Study* to Identify the Genetic and Environmental Causes of Asthma in the European Community) in rural regions of Austria, Germany, and Switzerland among 79,888 school-aged children in 2006 was conducted to identify specific exposures accounting for the protective effect of asthma and allergies. Of these, 7,682 had blood sample tests to identify specific IgE levels. The results showed that exposure to fodder storage and manure provided protection against atopic dermatitis.[124] In phase II of the GABRIEL project based in Poland and Alpine regions of Germany, Austria and Switzerland, 2586 schoolchildren were recruited and completed a more detailed questionnaire on specific farm exposures with objective measures of atopy. Atopy was defined by specific IgE $\geq$ 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 with histamine and saline controls and atopic sensitization was defined as a mean wheal size 3 mm greater than

the mean negative control for any of cat, *D. pteronyssinus*, grass or birch. Results showed that grain or related farming activities played a role in protecting children from atopy or allergic diseases.[67]

Another cross-sectional survey with school-age children aged 5-7 years in 1999 in two Bavarian districts with extensive farming activities showed that increased exposure to bacterial compounds in stables where livestock is kept confers protection against the development of allergic disorders in children.[131] In another cross-sectional study among 2283 children aged 8-10 years in 1997 from a mostly rural area in Australia found the low prevalence of hay fever, asthma and allergic sensitization in children living on a farm. The possible explanation was the increased exposure of farm children to microbial antigens in the stables or farmhouses.[115]

2.10.3. Unprocessed milk consumption

The consumption of unprocessed milk has been suggested as an independent protective factor for the development of atopic sensitization. Studies have shown that children living in rural areas consumed unpasteurized milk during their first year of life reduced the risk of atopic sensitization at school age.[130] The effect of farm milk consumption has also been shown to be independent regardless of farm residential status or farm-related exposures. This protective effect was not restricted to children living on a farm, but was also seen among non-farm populations consuming unpasteurized milk.[122] The findings of the inverse association between farm milk consumption and atopic sensitization have been fairly consistent between studies in different populations.[126] In a cross-sectional survey in rural areas of Austria, Germany, and Switzerland among 2,618 children aged 6-13-years in 1999, a strong protective effect was demonstrated in the development of atopic sensitization – defined as at least one positive specific IgE test result - in long-term and early-life exposure to farm milk.[130] Early life exposure was associated with a reduced prevalence of atopy (at least one positive specific IgE test result) [OR=0.43; 95%CI: 0.24-0.77]. Wickens et al[119] conducted a study among 293

7-10 year old New Zealand farm children to determine the pattern of reduced prevalence of allergy in farmers' children. The authors found the independent inverse associations between any unpasteurized milk consumption with atopic eczema/dermatitis syndrome (AEDS) [OR = 0.2; 95%CI: 0.1-0.8].

The protective effect of farm milk consumption on childhood asthma and atopy was also supported by a large population-based cross-sectional study in rural regions of Germany, Austria, and Switzerland in 8,334 school-aged children.[127] That study found that raw milk consumption was inversely associated with atopy - at least one positive specific IgE test result - [aOR=0.74; 95% CI: 0.61-0.90] regardless of farm exposures and no concrete explanation could be generated to explain the association between farm milk and atopy, except the association between whey protein fraction in milk and asthma.

However, not all studies reached statistical significance when examining the relationship of farm milk consumption and atopic sensitization. In a recent study (GABRIEL project) assessing whether a farming environment in childhood is protective against allergic diseases in Poland, it was found that even the protective effects against sensitization were not statistically significant [aOR = 0.88; 95%CI: 0.69-1.12].[67] A study conducted by Remes et al among 710 rural farm and non-farm children aged 6-15 years in Finland found that farm milk consumption was not associated with atopy.[113]

According to recent findings, it is suggested that fatty acid composition of unprocessed cow's milk may plays a role in protecting farm children against atopy and asthma.[137] However, current evidence of protective "farm milk effect" is still weak and needs better explanation.[128]

2.10.4. Timing exposure at the farms

The farming effect appeared to be the strongest with *in utero* exposure and during the first years of life,[125,138] although these findings are not entirely consistent.

The risk of atopic sensitization was determined strongly by maternal exposure to animal sheds during pregnancy. The strongest protective effect on atopic sensitization - defined as at least one positive specific IgE test result - was found children with prenatal exposure to animal sheds [aOR=0.36; 95%CI: 0.25-0.51]; first year of life exposure [aOR=0.54; 95%CI: 0.32-0.92]; and at school age [aOR=0.77; 95%CI: 0.49-1.22].[139] Also, in another cross-sectional survey in rural areas of Austria, Germany, and Switzerland among 2,618 6-13-year-old children from farming and non-farming families in 2001,[140] it was found that exposures to stables during the first year of life provided protection against atopic sensitization - defined as at least one positive specific IgE test result- compared to those had exposures to stables at age 1-5 years (12% versus 29%).

A prospective birth cohort study of 5509 subjects born in northern Finland in 1966 and followed up to the age of 31 years was conducted to examine the farming environment, especially farm animal contact, during infancy, with atopic sensitization and allergic diseases.[141] The result was in agreement with the conclusion that those with mothers who worked on farms during pregnancy and had contact to farm animals had a lower risk of being SPT positivity compared with those who were not born in a family with farm animals exposure [OR=0.67; 95%CI: 0.56-0.80].

However, the findings from a study of 293 children aged 7-10 years in small town and surrounding rural area of New Zealand did not show the protective effect of early life exposures to animals and atopic sensitization which contrasted with other recent studies.[119]

2.11. Mechanisms

The underlying mechanisms are still ill-defined, but are likely to involve a number of steps in innate and adaptive immunity and gen-environment interactions.[142]

An explanation for the protective effect of farming on atopy is thought to be the amount of exposure to microbial antigens in the stables leading to development of immunotolerance or the stimulation of TH1 cells and suppression of the TH2 cells.[115] Recently, endotoxin has

been suggested to be a candidate for the presence of atopy in children by activating TH1-type immune responses and reducing the development of TH2-type immune responses.[143] However, the actual exposure explaining the association remains unclear. Alternatively, the diversity of environmental microbial exposures has also been suggested to account for the protective effects.[125]

The mechanism of association of farming exposure, particularly farming activities and atopy is still obscure. In a review of farm effects on asthma and allergic disease, Wlasiuk and Vercelli (2012)[138] suggested that the great diversity of environmental microbial exposure was associated with asthma, but not atopy and also suggested that distinct mechanisms have a protective effect of atopy and asthma. Dust from animal sheds is rich in endotoxin[134,144] which possibly plays a role in atopic conditions and asthma.

2.12. Summary

Over the decades, the world has witnessed the increase in atopy prevalence in developed countries. However, there have been certain populations that have been experiencing a lower prevalence of atopic sensitization, especially those in school ages. Some studies have shown the protective effect of farming on atopic sensitization among children, but the mechanisms underlying that effect remain obscure. Possible explanations have been thought to be the diversity of farm exposure (livestock contact, farm milk consumption, and animal feed contact) and the presence of endotoxin and other microbial exposures.

There are limited Canadian studies examining the association of farm exposure and atopy among children. Although there have been several studies in European countries determining the importance of farm-related exposures and atopy among children, given the different farm practices in rural Canada compared with that in European countries, there is still a need to identify risk and protective factors in the context of rural Canadian children. However, there have been few studies examining the relationship between the specific farming exposures

(livestock contact, farm milk consumption, and animal feed contact and timing of exposure) and atopy which is measured by skin prick test in children in a rural Canadian context.

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Chapter 3

Prevalence and determinants of atopy and allergic diseases among school-age children in rural Saskatchewan, Canada

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Abstract

Background: There has been few investigations of the farming related activities or specific characteristics resulting in the associations between those exposures and atopic disease.

Objective: We sought to study the associations between farm-associated exposures and atopic disease.

Methods: As part of a longitudinal study of lung health in rural residents, we conducted a cross-sectional baseline study in rural Saskatchewan, Canada. This included an initial survey phase followed by a clinical testing phase. A sub-sample of 584 children (grades 1-8) completed skin prick testing to assess atopic status. Atopy was defined as a positive reaction to any of 6 allergens (local grasses, wheat dust, cat dander, house dust mite, Alternaria, Clasdosporium) ≥3mm compared to the negative control.

Results: Among those who completed clinical testing, the prevalence of atopy was 19.4%, hay fever was 8.8% and eczema was 27.4%. Based on SPT, sensitization was highest for cat dander (8.6%) followed by local grasses (8.2%) and house dust mite (5.1%). After adjustment for potential confounders, home location (farm vs. non-farm) was not associated with atopic status. However, livestock farming was protective for atopy (aOR=0.38, 95%CI: 0.17-0.88). In contrast, current residence on a farm was associated with an increase in the likelihood of hay fever in these children (aOR= 3.68, 95%CI: 1.29-10.45). Also, regular farming activities in the past year were associated with an increased risk of hay fever.

Conclusions: In children, livestock exposure has a protective effect on SPT positivity while farm living and activities increase the risk of hay fever.

Keywords: atopy; children; farming exposures; allergic diseases; hay fever

3.1. Introduction

Recently, studies have shown a lower prevalence of atopy and allergic diseases among rural and farming children compared to urban and non-farming children,[1-8] although there are some discrepancies.[7,9-18] Several characteristics specific to the farm environment have been investigated to explain the farm effect on atopy including contact with livestock or animal feed and unprocessed milk consumption.[19-23] It is also suggested that the timing of exposures may play a role in determining the presence of atopy and atopic disease.[24,25] These include prenatal exposures to animal sheds as well as exposures to the farming environment in the first year of life.

The results from Phase III in 2003 of the International Study of Asthma and Allergies in Childhood (ISAAC) conducted in 6-7 years old Canadian children showed that 10.8% of Canadian children suffered from allergic rhino-conjunctivitis symptoms, 18.2 % with asthma symptoms, and 12% with eczema symptoms.[26] Wang et al[18] conducted a cross-sectional study of 8334 adolescents aged 13 to 14 under the ISAAC Phase III program in 5 provinces in Canada. The authors found that the prevalence rates of allergic rhinoconjunctivitis ranged from 14.6-22.6% and 8.2-10.4% for atopic eczema and showed the disparity in regional variations for the development of allergic rhinoconjunctivitis and atopic eczema. However, few studies have been conducted in Canada examining the prevalence and risk factors for atopy and related conditions such as allergic rhinitis or atopic dermatitis in rural children. Results from a cross-sectional survey in British Columbia which included 4-H members 8–20 years of age[27] found the inverse association of farm residence or a rural livestock area and allergic rhinitis as well as atopic dermatitis and no report for atopy. Furthermore, there has been less investigation of the association between the farming related activities or specific characteristics and atopic disease in rural Canadian children. We conducted this study in order to: first, identify the prevalence of atopy, hay fever, hay fever symptoms, and eczema among children in a rural region of Canada; and second, identify the protective and risk factors of

atopy, hay fever, current allergic rhinitis symptoms, and eczema specific to a rural environment.

3.2. Methods

Data source and study design

The data in this paper were based on the baseline survey and clinical visit from the children's component of the Saskatchewan Rural Health Study. This is a longitudinal study (2010-2015) examining the health outcomes of rural adults and children in the Canadian province of Saskatchewan with a focus on lung disease and related conditions. The methodology for this study has been previously described.[28]

Study population and data collection

Four rural quadrants of Saskatchewan (Northwest, Northeast, Southwest and Southeast) were included in the Saskatchewan Rural Health Study. Quadrants were selected by a multistage stratified random sampling strategy.[28] With the definition of rural area being located at least 60 kilometers from an urban center,[29] a sample of 9 rural municipalities (RMs) was randomly selected for each quadrant from a purposeful sample of 48 RMs (12 from each quadrant). Overall, 32/36 (89%) RMs and 15/16 (94%) small towns participated.

Based on the adult study quadrant selection, schools located within the four quadrants were considered the target schools for the child study. Ten school divisions in the 4 quadrants agreed to participate and 43 schools within these divisions were chosen accordingly. Of the 43 selected schools, 39 agreed to take part in this study.

The study team prepared packages that included an information letter, questionnaire, consent, and assent forms. Following approval from the school district boards, all schoolchildren in Grades 1-12 were sent a study package containing a questionnaire for parents to complete. Classroom teachers distributed the packages to students. Completed questionnaires were sent back to the school. For the survey portion, 5667 children were

approached with 2383 children taking part for a response rate of 42%. A subset of students in Grades 1-8 attending from 16 pre-selected schools received a study package containing the questionnaire and a request to participate in clinical assessments including spirometry and skin testing for allergens. The schools where clinical assessments were conducted were selected based on school participation numbers in the survey in order to maximize efficiencies and reduce costs. We also excluded schools with high First Nations enrollments. One school division refused to allow clinical assessments in their schools. A total of 1768 students from 16 schools were approached for clinical testing. Of these, 584 took part.

Approval for the study protocol (for the children's survey and clinical portion of this study) was obtained from the Biomedical Research Ethics Board at the University of Saskatchewan (Study approval #Bio-10-177). Parental written consent and child written assent were in place prior to clinical assessment.

Study instrument

The parent-completed cross-sectional questionnaire was based on questions from standardized questionnaires including: the American Thoracic Society's 1979 Children's Respiratory Disease Questionnaire,[30] questionnaires used in a study in Estevan, Saskatchewan in 2000 and 2003,[31,32] the International Study of Asthma and Allergies in Childhood Study (ISAAC) questionnaire,[33] and a questionnaire used in a study conducted in Humboldt, Saskatchewan[34]. The questionnaire includes information on socio-demographics, the respiratory and general health of the child, allergic disease, life style, home environment, and early life exposures.

Skin prick testing (SPT)

A panel of allergens that are most common in Saskatchewan was used including *Alternaria* (mold), *Cladosporium* (mold), cat dander, local grasses, wheat dust and house dust mite (ALK – Abello Pharmaceuticals, Inc., Ontario, Canada). Histamine (10mg/ml) and

saline solution (0.9%) were used as positive and negative controls, respectively. Skin prick testing for atopy was conducted according to international standards for testing.[35,36]

3.3. Variables under study

Outcomes

Atopy was defined as a ≥3mm wheal formed for any of the allergens compared to the negative control on SPT as recommended by the Global Allergy and Asthma European Network (GA^{2}LEN) and Allergic Rhinitis and its Impact on Asthma in 2012[35] and European standards.[36] **Hay fever** was defined as a positive response to the question "Has this child ever had hay fever?" **Eczema** was defined as a positive response to the question "Has this child ever had eczema?" **Current allergic rhinitis** was defined as a positive response to the question "In the past 12 months, has you child ever had a problem with sneezing, or a runny, or a blocked nose when he/she did not have a cold or the flu?"

Exposures

The primary exposure of interest was the child's location of their home and was determined by the question: "Where is your home located?" (Farm, acreage or in town). This variable was recoded as farm (farm or acreage) and as non-farm.[37,38] **The secondary exposures** were "farm type and farm activities". For those children who answered yes to farm as location of home, farm type was determined by livestock (beef cattle, dairy cattle, pigs, and poultry) or grain (grain crops). Farming activities were determined by the question "In the past 12 months, on average, how often has this child spent 1 hour near or in the following activities?" Six farming activities were included: 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".[38] The responses of "less than once a month" and "never" were coded as "less regular activity".[38] In addition to this, early

farm living, unpasteurized milk consumption, farming exposure of the mother during pregnancy were assessed.

Covariates of interest included

Parental history of asthma or any allergy, history of breastfeeding, daycare attendance, number of siblings, pet exposure at the first year of life and currently, environmental factors (dampness in home, mildew in home, mice in home, etc.) were identified by the questionnaire. A child was considered to be born underweight if their reported birth weight was less than 2500g. Premature birth was determined as being born more than 2 weeks before the expected birth date. Exposure to environmental tobacco smoke (ETS) was determined by parental smoking habits in the home and was categorized as: "nonsmoker", "previous smoker" and" current smoker". Parents' educational attainment was determined by the question "What is the highest level of education completed by the child's father/mother?" and was categorized as high school or less vs. any postsecondary education. Body mass index (BMI) was calculated [weight (kg)/the square of height (m)]. Weight status was classified based on age and sex values using the cut-off value of the predicted adult equivalent of 25 for overweight and 30 for obese determined by standardized methods by Cole et al.[39] The height and weight of children were objectively measured as part of the clinical measurement component.

Statistical analysis

All analyses were conducted using the SPSS® version 19 statistical package (the Statistical Package for the Social Sciences). Statistical significance was set at $p < 0.05$ based on two-sided calculations. The overall prevalence of atopy and other allergic conditions was determined followed by independent sample chi-squared test (χ^2) to compare the prevalence of atopy between farm and non-farm dwelling children. Throughout the analyses all children

with valid response were included. In the multiple logistic regression modeling, only those with data available on all potential confounders were included (n=529).

We conducted multiple logistic regression analysis to identify determinants for each outcome. A purposeful modeling strategy was applied based on Hosmer et al.[40] The strength of association were assessed by the odds ratio and corresponding 95% CIs. Variables with p-value < 0.25 in the univariate analysis were considered candidates for further analysis in full multiple logistic regression models.[40] Variables in the full multiple logistic regression models that were statistically significant were retained in the final model. In this study, we were unable to control for clustering by family units because of the lack of available data. We applied the inflation factor of 4.9%[41] which was used by Barry et al using the same dataset to serve as a proxy analysis for clustering by family units. Potential confounding and biologically important variables were included in the final model as appropriate. Effect modification between sex and the primary exposure of interest as well as parental history of allergy and the primary exposure of interest were considered. This was based on previously reported in associations by sex[42,43] and the potential for a gene-environment interaction.[44-49] Parental history of allergy served as a proxy for the genetic component.[46,48,50]

3.4. Results

Characteristics of study population

The mean age of the participating children was 9.57 ± 2.21(SD) years. There was no difference for age between farm [9.67 ± 2.24 (SD) years] and non-farm children [9.48 ± 2.20 (SD) years]; (p=0.32). Farm children were more likely to have more than 2 siblings, have less parental smoking, were mostly Caucasian and were less likely to attend daycare at young age **(Table 1**). Farm children were more likely to live in a house using burning fuel, less natural gas usage, more mice or rats in the past 12 months, more signs of mold or mildew, more

wood fireplace use, less likely to have a dog or a cat in the home currently, and less use of an air conditioner **(Table 2)**.

Characteristics of early life and the last 12 month farming exposures are described in **Table 3**. As expected, farm children were more likely to live on a farm during the first year of life, were more likely to have their mother live or work on a farm while pregnancy compared to non-farm children. In the past 12 months, farm children were more likely to visit a farm (more than 3 times), were more likely to regularly performed farm-related activities.

Atopy and allergic disease prevalence

The prevalence of various symptoms of atopy as well as allergy and allergic disease is presented in **Table 4**. The overall prevalence of atopy measured by SPT was 19.4%. Findings were similar for farm and non-farm children (18.7% vs. 20%; p=0.71). Of the 6 allergens used in SPT, sensitization to cat dander showed the highest prevalence (8.6%). Another common sensitization was in local grasses (overall 8.2%). No statistically significant differences between farm and non-farm children to specific allergens.

No statistically significant difference was found in symptoms of current allergic rhinitis between farm and non-farm children (28.5% vs. 22.8%; p=0.13) or eczema ever (29.8% vs. 25.5%; p=0.27). Children living on a farm were more likely to have a report of hay fever (ever) than non-farm children (12.3% vs. 5.9%; p=0.009).

Risk and protective factors

As seen in **Table 5**, there were no statistically significant associations between atopy and allergic disease outcomes with farm and non-farm status, except for the report of hay fever (ever) (aOR=3.68, 95%CI=1.29-10.45). While there was an inverse association with atopy (aOR=0.81, 95%CI=0.09-7.07), it was not statistically significant.

Table 5 summarizes the final results of the multiple logistic regression model. There was an increased risk for hay fever (ever) with the parental history of allergy or atopy, a dog

currently in the home, mice in the home in the last 12 months and an air filter in the home. Frequent mildew odor or musty smell in the home was inversely associated with hay fever. There was an increased risk for eczema (ever) with parental history of any allergies or asthma, and dampness in the home. We did not find any effect modification between sex and the primary exposure of interest (home location) or between parental history of allergy and this primary exposure.

Farm type, farm-related activities and outcomes

Table 6 presents an analysis of farm-specific environmental risk factors for atopy and allergic disease including early life and current exposures to farm-related activities. Living on a livestock farm conferred protection against atopy (aOR=0.38, 95%CI= 0.17-0.88; $p<0.05$). Performing any regular farm-related activities increased the risk of hay fever among this population (aOR=2.83, 95%CI=1.14-6.99; $p<0.05$). Performing some farm-related activities also increased the risk of hay fever such as feeding livestock, cleaning or playing in barns, cleaning or playing in pens or corrals, and riding horses **(Table 6)**. Also, living on a farm during the first year of life and mother living or working on a farm while pregnant were positively associated with hay fever in the child.

3.5. Discussion

We found that the overall prevalence of atopy (measured by skin prick testing) was 19.4%, current allergic rhinitis prevalence was 25.3%; hay fever (ever) prevalence was 8.8% and eczema (ever) was 27.4%. We also found that the prevalence of atopy as well as allergen-specific sensitizations was similar between farm and non-farm children all living in a rural area_but support the notion that livestock farming is protective of atopy. Farm dwelling and farm-related exposures increased the risk for hay fever.

The prevalence of atopy in this study population was low (19.4%) compared to findings from studies in Europe including the ALEX study,[51] PARSIFAL study[1] and GARBIEL phase II

study.[14] It is noted that there is heterogeneity between studies given the different definition of atopic condition based on skin prick testing or IgE blood test and the number of allergens used, etc. Thus, it is challenging to compare the results of this prevalence with other large studies of atopy. The prevalence of hay fever in our study was within the range of 2.8%[1] to 10.9%[51] shown in the above three studies in Europe. The prevalence of eczema in our study was higher (2-fold) compared to the findings of these studies ranging from 8.5% [1] to 15.3 %.[14]

The prevalence of atopy for our study population was similar between children living and not living on a farm. This finding supported the results from a study in Sweden,[9] but was not in agreement with most other findings in the literature.[1,14,51] It is possible that there is a lack of variability in exposure between farm and non-farm populations of these children. The towns (i.e. non-farm children) were small and adjacent to farming areas. Similar studies have also compared rural farming to rural non-farming.[1,5,52] However, these other studies were European and New Zealand where farm practices may differ from Canadian practices. In the future, it would be advantageous to conduct studies to compare rural-urban environments in the presence of atopy and allergic diseases.

A higher prevalence of hay fever among farm children compared to non-farm children did not support typical findings of previous studies.[1,3,12,14,53-55] However, this result is consistent with a finding from a study of New Zealand children.[10] The explanation of higher prevalence of hay fever in farm compared to non-farm children was that the allergen load and irritants on farms trigger upper airway inflammation, resulting in the increased risk of hay fever.[10] In New Zealand, animals were kept outdoors in large holdings leading to the effect of hay fever.[10] Similar practices may result in our observed associations. Also, other research has suggested that fungi have a role to play because mold (and bacteria) can prosper in hay, grain, or straw in high-humidity storage condition[56] which could help explain the association observed in our study.

Living on a livestock farm protects children from atopy and this effect was not seen for living on grain farms. This result confirms previously identified inverse associations between livestock exposure and atopy.[17,55,57] In the 2001 cross-sectional study conducted with 1158 8 to 20 year old members of a Canadian agriculturally-based education club[27] who had opportunities to have contact with livestock through club activities, living on a farm residence with livestock was protective for allergic rhinitis [aOR=0.51 (0.30–0.85)] and atopic dermatitis [aOR=0.45 (0.24–0.84)]. Although skin prick testing for atopy was conducted in a subset of the population, the results were not reported.

The underlying mechanisms of farm protection against atopy are still ill-defined, but are likely to involve a number of steps in the innate and adaptive immunity and gene-environment interactions.[24] Explanations for the protective effect of farming on atopy is thought to be related to the amount of exposure to microbial antigens in the stables leading to development of immunotolerance or the stimulation of TH1 cells and suppression of the TH2 cells.[7] Dust from animal sheds is rich in endotoxin[16,58] which possibly plays a role in the observed associations. Endotoxin has been suggested to be a candidate for the inverse association with atopy in children by activating TH1-type immune responses and reducing the development of TH2-type immune responses. Endotoxin concentrations are higher in homes of children living on farms versus non-farms.[59]

We found in this study that performing any regular farm-related activity statistically increased the risk of hay fever in this population. Cleaning or playing in the barns, and riding horses are two particular activities that can trigger an allergic response airway inflammation and then possibly lead to hay fever and current allergic rhinitis, even eczema. This finding contrasts with the report from the literature.[27]

There are limitations of this study to be considered. The response rate was modest, which limits generalizing the results. There is potential for selection bias. However, when comparing those who were included in analysis and those who were not, they have similar characteristics

in demography as well as allergic disease patterns. Moreover, the modest response rate may have also affected statistical power by the inclusion of fewer participants than expected. However, we did find statistically significant relationships in some sub-analyses. Also, the Odds Ratio of 0.81 in our primary analysis was not overly strong. As such, power was not the major issue here but clinical importance was. Also, this was a cross-sectional study that simultaneously assessed exposures and outcomes. Temporality and along with it, causality, are difficult to establish. An example of this would be the association with hay fever and having a home air filter. It may be that those with hay fever bought an air filter in response to the condition being present. However, this study will allow us to generate hypotheses about the role of the farm environment in childhood atopy and allergy. In our study, the overall reported prevalence of atopy may be underestimated because we limited our allergy testing to six common allergens found in Saskatchewan. Furthermore, we did not include allergens that are closely related to a farming environment such as those associated livestock activity (e.g. horse or cow). However, we used the most common allergens in our region and included more allergen types than has been completed in previous studies using standard epidemiologic definitions,[60,61] hopefully minimizing the problems of prevalence underestimation. Moreover, we did not include any skin prick tests for livestock allergy. However, we did have a question about children having allergies to specific exposures where farm animals were one of the exposures and found that among children living on a farm, those who live on a livestock farm were less likely to have farm animal allergy compared to those children not living on a livestock (data not shown) supporting our findings. In the future, farm-related environment measurements such as livestock (pig, cattle, etc.) should be included in the allergens panel. The study was conducted in winter months when pollen levels and subsequent reported allergic responses for some students would be lower. Some parents could have refused to have their children tested for allergies because of previously identified allergic responses. As well, the research team excluded highly atopic children, those children who had a severe systemic

reaction to an allergen previously or had taken any antihistamines on the day of the test. The number of children who did not complete SPT even though they attended the clinical visit (n=55) had similar characteristics (data not shown) compared to those who completed SPT. Thus, the results were not affected by the exclusion of these children. Information bias resulting in misclassification due to recall bias and response bias from parents completing a self-report questionnaire should also be considered. The assessment of farm activities was self-reported and required parent recall of children's behaviors. However, given that the questionnaire was completed prior to atopy assessment and that atopy was assessed objectively, any misclassification should be non-differential and would tend to bias the results towards the null. It is also possible that the farming parents with allergic children would be more interested in our study and such participation could inflate the prevalence of atopic outcomes. However, we conducted an analysis (data not shown) to determine if there were differences in characteristics and outcomes of interest, and found that there were no differences between those who participated in SPT and those who only filled out the questionnaire but did not participate in SPT including the proportion of those who lived on a farm and the proportion of those reporting various allergic outcomes. The results shown in the SPT group can also then be generalized to other populations with similar characteristics (age, current home location, etc.) Moreover, the high prevalence of eczema derived from the questionnaire report may be due to the misclassification of eczema status, possibly mistaking it for dry winter skin given that the study was conducted in winter time. However, the question about eczema was adopted from an internationally recognized study (ISAAC) allowing for consistent comparison with other studies worldwide.[26] What is more, we found the prevalence of atopy in our study was similar in farming and non-farming areas. This may be due to the predominately rural nature of the study areas which would result in less variability in exposures. A more informative study could include an urban area for comparison.

There are substantial strengths of this study which also need to be considered. This study further identifies important specific agriculturally related activities that may influence the occurrence of or protection for common atopic conditions in childhood. This study used an objective measurement of atopy reducing the likelihood of biased results.

In conclusion, we found in this study that the prevalence of atopy, current allergic rhinitis, and eczema were similar between farm and non-farm children, but we observed the difference in hay fever prevalence which is higher in farm children compared to their counterparts who did not live on a farm. We also found the protective effect of living in livestock farm on atopy in this population and current residence on a farm was associated with an increase in the likelihood of hay fever in these children. Further studies are needed to look at the effects of farming and farming activities on atopy and allergic diseases.

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Table 1:Descriptive statistics of demographic, behavioral, and personal characteristics by farming status

	Overall (n=584) N (%)	Farm (n= 262) N (%)	Non-farm (n= 318) N (%)	P-value *
Sex				
Male	307 (52.6)	140 (53.4)	165 (51.9)	0.71
Female	277 (47.4)	122 (46.6)	153 (48.1)	
Missing	0			
Number of siblings				
0	228 (39.1)	91 (35.1)	135 (43.4)	0.03
1	215 (36.8)	97 (37.5)	117 (37.6)	
≥2	131 (22.4)	71 (27.4)	59 (19.0)	
Missing	10 (1.7)			
Obesity status (objective)				
Not overweight or obese	429 (73.5)	202 (77.1)	225 (70.8)	0.15
Overweight	115 (19.7)	47 (17.9)	67 (21.1)	
Obese	40 (6.8)	13 (5)	26 (8.2)	
Missing	0			
Maternal education				
High school or less	210 (36.0)	92 (35.4)	115 (37.3)	0.63
Any postsecondary education	362 (62.0)	168 (64.6)	193 (62.7)	
Missing	12 (2.2)			
Paternal education				
High school or less	294 (50.3)	145 (55.8)	145 (48.3)	0.08
Any postsecondary education	270 (46.2)	115 (44.2)	155 (51.7)	
Missing	20 (3.5)			
Mother smoking				
Never smoke	355 (60.8)	191 (73.2)	164 (51.9)	<0.001
Ever smoke	108 (18.5)	34 (13.0)	71 (22.5)	
Currently smokes	118 (20.2)	36 (13.8)	81 (25.6)	
Missing	3 (0.5)			
Father smoking				
Never smoke	321 (55.0)	172 (66.2)	146 (46.5)	<0.001
Ever smoke	98 (16.8)	35 (13.5)	63 (20.1)	
Currently smokes	159 (27.2)	53 (20.3)	105 (33.4)	
Missing	6 (1.0)			
Ethnicity				
Non-Caucasian	46 (7.9)	12 (4.6)	33 (10.6)	0.008
Caucasian	528 (90.4)	248 (95.4)	277 (89.4)	
Missing	10 (1.7)			
Children breastfeed				
No	103 (17.6)	37 (14.2)	62 (19.6)	0.08
Yes	477 (81.7)	223 (85.8)	254 (80.4)	
Missing	4 (0.7)			
Daycare attendance				
No	244 (41.8)	132 (50.6)	111 (35.5)	<0.001
Yes	334 (57.2)	129 (49.4)	202 (64.5)	
Missing	6			
Mother smoking during pregnancy				

No	451 (77.3)	214 (82.9)	234 (76.5)	0.05
Yes	117 (20)	44 (17.1)	72 (23.5)	
Missing	16 (2.7)			
The child was born before mother's due date				
No	386 (66.1)	170 (65.1)	213 (69.8)	0.23
Yes	184 (31.5)	91 (34.9)	92 (30.2)	
Missing	14 (2.4)			
Low birth weight (Less than 2,500g)				
No	527 (90.2)	238 (92.6)	286 (93.8)	0.58
Yes	39 (6.7)	19 (7.4)	19 (6.2)	
Missing	18 (3.1)			
Family history of asthma/allergic diseases				
No	237 (40.6)	100 (39.5)	134 (44.1)	0.27
Yes	324 (55.5)	153 (60.5)	170 (55.9)	
Missing	23 (3.9)			

*Based on valid percentage (no missing included)

Table 2: Descriptive statistics of environmental characteristics by farming status

	Overall (n=584) N (%)	Farm (n= 262) N (%)	Non-farm (n= 318) N (%)	P-value *
Type of housing unit				
One family house	551 (94.3)	245 (93.9)	302 (95.0)	0.56
Others	32 (5.5)	16 (6.1)	16 (5.0)	
Missing	1 (0.2)			
Older home				
Built before 1980	362 (62.0)	154 (61.8)	205 (67.9)	0.14
Built after 1980	193 (33.0)	95 (38.2)	97 (32.1)	
Missing	29 (5.0)			
Renovations in the past 12 months				
No	425 (72.8)	181 (70.2)	241 (76.8)	0.07
Yes	151 (25.9)	77 (29.8)	73 (23.2)	
Missing	8 (1.4)			
Burning fuel use in the home				
No	393 (67.0)	114 (43.5)	276 (86.8)	0.001
Yes	191 (32.7)	148 (56.5)	42 (13.2)	
Missing	0 (0.0)			
Natural gas use in the home				
No	151 (25.9)	133 (50.8)	18 (5.7)	0.001
Yes	433 (74.1)	129 (49.2)	300 (94.3)	
Mice or rats in the home in the past 12 months				
No	491 (84.1)	205 (81.0)	282 (89.0)	0.008
Yes	83 (14.2)	48 (19.0)	35 (11.0)	
Missing	10 (1.7)			
Dampness in the home				
No	401 (68.7)	121 (46.7)	169 (54.2)	0.07
Yes	179 (30.7)	138 (53.3)	143 (45.8)	
Missing	4 (0.7)			
Mildew odor or musty smell in the home				
No	434 (74.3)	193 (75.4)	237 (77.2)	0.61
Yes	133 (22.8)	63 (24.6)	70 (22.8)	
Missing	17 (2.9)			
Signs of mold or mildew				
No	459 (78.6)	191 (73.5)	265 (84.4)	0.001
Yes	119 (20.4)	69 (26.5)	49 (15.6)	
Missing	6 (1.0)			
Pesticides in home				
No	526 (90.1)	231 (88.8)	291 (92.1)	0.18
Yes	54 (9.2)	29 (11.2)	25 (7.9)	
Missing	4 (0.7)			
Air conditioners use in the home				
No	300 (51.4)	165 (63.0)	134 (42.1)	0.001
Yes	284 (48.6)	97 (37.0)	184 (57.9)	
Air filter				
No	360 (61.6)	163 (62.2)	195 (61.3)	0.82

Yes	224 (38.4)	99 (37.8)	123 (38.7)	
Humidifier				
No	439 (75.2)	192 (73.3)	244 (76.7)	0.34
Yes	145 (24.8)	70 (26.7)	74 (23.3)	
Dehumidifier				
No	332 (56.8)	146 (55.7)	183 (57.5)	0.66
Yes	252 (43.2)	116 (44.3)	135 (42.5)	
Wood fireplace				
No	500 (85.6)	200 (76.3)	296 (93.1)	0.001
Yes	84 (14.4)	62 (23.7)	22 (6.9)	
Dog in the home in the 1st year of life				
No	418 (71.6)	193 (73.7)	222 (69.8)	0.30
Yes	166 (28.4)	69 (26.3)	96 (30.2)	
Dog in the home currently				
No	340 (58.2)	170 (64.9)	168 (52.8)	0.003
Yes	244 (41.8)	92 (35.1)	150 (47.2)	
Cat in the home in the 1st year of life				
No	424 (72.6)	191 (72.9)	230 (72.3)	0.87
Yes	260 (27.4)	71 (27.1)	88 (27.7)	
Cat in the home currently				
No	372 (63.7)	181 (69.1)	188 (59.1)	0.01
Yes	212 (36.3)	81 (30.9)	130 (40.9)	

*Based on valid percentage (no missing included)

Table 3: Descriptive statistics of farming exposures in early life and the last 12 months by dwelling location

	Overall (n=584) N (%)	Farm (n= 262) N (%)	Non-farm (n= 318) N (%)	P value*
Early life exposure				
Farm milk consumption 1st year of life				
No	559 (95.8)	254 (97.7)	301 (96.8)	0.51
Yes	16 (2.7)	6 (2.3)	10 (3.2)	
Missing	9 (1.5)			
Living on a farm during the 1st year of life				
No	403 (69.0)	107 (40.8)	294 (93.3)	< 0.001
Yes	178 (30.5)	155 (59.2)	21 (6.7)	
Missing	3 (0.5)			
If yes, type of farm				
Grain	36 (6.2)	31 (21.4)	5 (27.8)	0.51
Livestock	35 (6.0)	29 (20.0)	5 (27.8)	
Both	94 (16.1)	85 (58.6)	8 (44.4)	
Missing	419 (71.7)			
Mother living or working on a farm while pregnancy				
No	395 (67.6)	103 (39.8)	291 (92.4)	< 0.001
Yes	183 (31.3)	156 (60.2)	24 (7.6)	
Missing	6 (1.0)			
If yes, type of farm				
Grain	38 (6.5)	32 (21.3)	6 (28.6)	0.71
Livestock	40 (6.8)	34 (22.7)	5 (23.8)	
Both	96 (16.5)	84 (56.0)	10 (47.6)	
Missing	410 (70.2)			
Current exposure				
Times the child visited a farm in the past 12 months				
Never	55 (9.4)	12 (5.6)	43 (14.1)	< 0.001
3 or fewer times	122 (20.9)	23 (10.8)	99 (32.5)	
More than 3 times	341 (58.4)	178 (83.6)	163 (53.4)	
Missing	66 (11.3)			
Current unpasteurized milk consumption				
No	547 (93.8)	245 (94.6)	298 (96.1)	0.38
Yes	26 (4.5)	14 (5.4)	12 (3.9)	
Missing	11 (1.9)			
Performed any regular farm-related activities				
No	361 (61.8)	94 (35.9)	266 (86.1)	<0.001
Yes	214 (36.6)	168 (64.1)	43 (13.9)	
Performed haying in the past year	9 (1.5)			
Never or irregularly	436 (74.7)	144 (55.0)	291 (94.5)	<0.001

Regularly	138 (23.6)	118 (45.0)	17 (5.5)	
Missing	10 (1.7)			
Performed feeding livestock in the past year				
Never or irregularly	419 (71.7)	131 (50.2)	287 (93.2)	<0.001
Regularly	154 (26.4)	130 (49.8)	21 (6.8)	
Missing	11 (1.9)			
Performed cleaning or playing in barns in the past year				
Never or irregularly	440 (75.3)	151 (57.6)	287 (93.2)	<0.001
Regularly	134 (22.9)	111 (42.4)	21 (6.8)	
Missing	10 (1.7)			
Performed emptying or filling grain bins in the past year				
Never or irregularly	528 (90.4)	227 (86.6)	298 (96.8)	<0.001
Regularly	46 (7.9)	35 (13.4)	10 (3.2)	
Missing	10 (1.7)			
Performed cleaning or playing in pens or corrals in the past year				
Never or irregularly	448 (76.7)	156 (59.8)	289 (93.8)	<0.001
Regularly	125 (21.4)	105 (40.2)	19 (6.2)	
Missing	11 (1.9)			
Performed riding horses in the past year				
Never or irregularly	523 (89.6)	222 (85.1)	297 (96.1)	<0.001
Regularly	51 (8.7)	39 (14.9)	12 (3.9)	
Missing	10 (1.7)			

*Based on valid percentage (no missing included)

Table 4: Prevalence of atopy in children living on a farm compared with children living in a non-farming environment based on skin prick testing

	Overall (N= 525) N (%)	Living on a farm (N=235) N (%)	Not living on a farm (N=290) N (%)	p-value
Skin prick testing				
Any atopy*	102 (19.4)	44 (18.7)	58 (20.0)	0.71
Local grasses	43 (8.2)	19 (8.1)	24 (8.3)	0.93
Wheat dust	21 (4.0)	11 (4.7)	10 (3.4)	0.47
Cat dander	45 (8.6)	20 (8.5)	25 (8.6)	0.96
House dust mite	27 (5.1)	14 (6.0)	13 (4.5)	0.44
Alternaria	17 (3.2)	7 (3.0)	10 (3.4)	0.76
Clasdosporium	17 (3.2)	8 (3.4)	9 (3.1)	0.84
Questionnaire report				
The child has had a problem with sneezing, or a runny, or a blocked nose in the past 12 months (current allergic rhinitis)	133 (25.3)	67 (28.5)	66 (22.8)	0.13
Hay fever (ever)	46 (8.8)	29 (12.3)	17 (5.9)	0.009
Eczema (ever)	144 (27.4)	70 (29.8)	74 (25.5)	0.27

Table 5: Adjusted associations with atopy based on skin prick testing, hay fever, hay fever symptoms, eczema

	Atopy OR (95% CI)	Hay fever ever OR (95% CI)	Current allergic rhinitis OR (95% CI)	Eczema ever OR (95% CI)
Home location (ref: non-farm)	0.81 (0.09-7.07)	3.68 (1.29-10.45)*	1.22 (0.68-2.17)	1.23 (0.71-2.11)
Sex (ref: female)	1.65(0.94-2.92)	0.97 (0.41-2.28)	1.46 (0.87-2.44)	0.81 (0.50-1.31)
Age	1.13 (0.99-1.29)	1.18 (0.96-1.45)	1.10 (0.98-1.23)	0.93 (0.83-1.04)
Parental history of any allergies or asthma (ref: No)	1.34 (0.73-2.44)	4.28 (1.48-12.35)*	1.69 (0.99-2.90)	2.23 (1.32-3.74)*
Maternal education (ref: high school or less)	0.99 (0.52-1.86)	1.32 (0.51-3.42)	1.17 (0.65-2.08)	0.88 (0.50-1.53)
Paternal education (ref: high school or less)	0.78 (0.42-1.44)	0.48 (0.18-1.23)	0.76 (0.43-1.32)	1.05 (0.61-1.79)
Dog in the home currently (ref: No)	0.52 (0.27-1.02)	2.23 (0.85-5.83)‡	0.85 (0.47-1.52)	0.84 (0.48-1.47)
Dog in the home in the 1st year of life (ref: No)	0.88 (0.42-1.84)	0.94 (0.32-2.74)	1.49 (0.80-2.79)	1.56 (0.85-2.83)
Cat in the home currently (ref: No)	0.85 (0.41-1.74)	0.79 (0.25-2.45)	0.62 (0.32-1.21)	1.06 (0.57-1.96)
Cat in the home in the 1st year of life (ref: No)	0.60 (0.27-1.32)	1.00 (0.29-3.34)	1.10 (0.55-2.19)	0.70 (0.36-1.36)
Dampness in the home (ref: No)	1.21 (0.65-2.27)	1.88 (0.71-4.97)	1.12 (0.63-1.98)	1.57 (0.91-2.71)‡
Dehumidifier use in home (ref: No)	0.57 (0.31-1.05)	0.67 (0.25-1.76)	1.11 (0.64-1.91)	0.79 (0.47-1.34)
Mildew odor or musty smell in home frequently (ref: No)	0.94 (0.44-2.01)	0.14 (0.03-0.60)*	1.21 (0.62-2.34)	0.92 (0.48-1.76)
Mice in home in the last 12 months (ref: No)	0.57 (0.22-1.41)	2.55 (0.77-8.37)	1.12 (0.53-2.35)	0.75 (0.35-1.57)
House type (ref: Not single family home)	1.76 (0.31-9.85)	0.55 (0.08-3.84)	0.33 (0.09-1.15)	0.42 (0.12-1.44)
Air filter in home (ref: No)	1.01 (0.56-1.83)	3.68 (1.36-8.37)*	1.40 (0.82-2.36)	1.17 (0.70-1.93)

Adjusted for sex, age, parental history of asthma/allergies, paternal and maternal education, weight at birth, maternal smoking, paternal smoking, maternal smoking during pregnancy, number of siblings, BMI, dampness in home, mildew in home, mice in home, house type, old house, major renovation past 12 months, air filter in home and air conditioners in home, pesticide in home, breast-feeding, dehumidifier in home, cat in home 1st year of life and currently, using wood fireplace, dog in home 1st year of life and currently

*p<=0.01

†p<=0.05

‡p<=0.1

Table 6: Adjusted association of farm exposures and atopy, hayfever, rhinitis symptoms and eczema

	Atopy OR (95% CI)	Hay fever ever OR (95% CI)	Current allergic rhinitis OR (95% CI)	Eczema ever OR (95% CI)
Farm type				
Livestock (ref: no)	0.38 (0.17-0.88)†	2.27 (0.79-6.49)	1.07 (0.55-2.10)	1.37 (0.73-2.57)
Grain (ref: no)	0.99 (0.49-2.01)	1.04 (0.35-3.08)	1.45 (0.75-2.77)	1.33 (0.72-2.45)
Farming activities				
Performed any regular farm-related activities (ref.: No)	0.79 (0.43-1.45)	2.83 (1.14-6.99)†	1.19 (0.69-2.03)	1.43 (0.85-2.37)
Performed haying in the past year (ref.: Never or irregularly)	1.08 (0.54-2.16)	1.68 (0.63-4.47)	1.06 (0.57-1.96)	1.66 (0.92-3.01)
Performed feeding livestock in the past year (ref.: Never or irregularly)	0.80 (0.40-1.59)	2.28 (0.89-5.84)‡	1.16 (0.64-2.10)	1.42 (0.80-2.51)
Performed cleaning or playing in barns in the past year (ref.: Never or irregularly)	1.03 (0.53-2.00)	3.58 (1.44-8.90)*	1.44 (0.80-2.59)	1.35 (0.77-2.36)
Performed emptying or filling grain bins in the past year (ref.: Never or irregularly)	0.91 (0.32-2.56)	1.60 (0.39-6.47)	2.04 (0.85-4.92)	1.70 (0.71-4.04)
Performed cleaning or playing in pens or corrals in the past year (ref.: Never or irregularly)	0.77 (0.37-1.58)	2.73 (1.06-6.99)†	1.33 (0.73-2.44)	1.22 (0.67-2.19)
Performed riding horses in the past year (ref.: Never or irregularly)	0.65 (0.21-1.98)	9.40 (2.80-31.49)*	1.10 (0.45-2.68)	1.83 (0.84-4.00)
Unpasteurized milk consumption in the first year of life (ref.: No)	0.84 (0,12-5.74)	-	-	1.99 (0.39-10.19)
Unpasteurized milk consumption currently (ref.: No)	-	-	0.14 (0.01-1.31)	0.54 (0.13-2.31)
Living on a farm during the 1st year of life (ref.: No)	0.93 (0.49-1.74)	2.74 (1.10-6.82)†	1.26 (0.72-2.19)	1.22 (0.71-2.07)

Mother living or working on a farm while pregnancy (ref.: No)	0.83 (0.44-1.55)	2.66 (1.09-6.49)†	1.31 (0.75-2.26)	1.34 (0.79-2.27)

Adjusted for sex, age, parental history of asthma/allergies, paternal and maternal education, weight at birth, maternal smoking, paternal smoking, maternal smoking during pregnancy, number of siblings, BMI, dampness in home, mildew in home, mice in home, house type, old house, major renovation past 12 months, air filter in home and air conditioners in home, pesticide in home, breast-feeding, dehumidifier in home, cat in home 1st year of life and currently, using wood fireplace, dog in home 1st year of life and currently

*p<=0.01

†p<=0.05

Chapter 4

Validation of Questionnaire Report of Atopic Outcomes in School-age Children: The Saskatchewan Rural Health Study

(The manuscript was submitted to *Clinical & Experimental Allergy)*

Abstract

Background and Objectives: Self-report of allergic status by questionnaire may not be as reliable as objective measures, but in population-based epidemiological studies, questionnaires remain the most efficient tool to assess the presence of allergy. In order to assure the quality of information, questionnaire validation is required. We sought to evaluate the predictive value of a questionnaire report of allergy to assess atopy in rural children, an understudied population with regard to atopy and allergic disease.

Methods: A total of 480 schoolchildren (Grades 1-8) from rural Saskatchewan completed a questionnaire report of allergy and atopic outcomes and participated in skin prick testing (SPT). SPT for 6 common allergens (local grasses, wheat dust, cat dander, house dust mite mixed, *Alternaria and Clasdosporium*) was completed. Subjects with at least one positive SPT (≥3mm) compared to the negative control were considered to be atopic. Agreement between questionnaire report and objective measures of atopy was considered overall and between the specific allergens tested on SPT and those assessed on questionnaire. We considered percent concordance, Kappa, sensitivity, specificity, and the positive and negative predictive values of reported allergies or allergic conditions in comparison to SPT as the gold standard.

Results: We found that 25.0% of children reported by questionnaire a history of any allergy and 19.4% were atopic based on skin pick testing. The agreement between questionnaire report of allergic triggers and atopy measured by SPT was high (83.0%-89.5%). The agreement between atopy and report of allergic conditions (hay fever ever, eczema ever, current rhinitis symptoms, and eczema symptoms) ranged from 67.1% to 79.6%. Individual allergic conditions demonstrated high specificity but low sensitivity. The questionnaire report of any allergy had a low positive predictive value in detecting atopy (47.3%) and high negative predictive value (86.3%). The positive predictive value of reported allergic

conditions was low (24.8%- 43.9%), but the negative predictive value was again high (82.0% - 82.9%).

Conclusions: We found that the agreement between questionnaire report of allergic symptoms and atopy measured by SPT was high and the agreement between atopy and report of allergic conditions was moderate. This questionnaire may be an efficient tool for studies involving the differential inclusion of subjects with and without atopy.

Key words: agreement, children, rural, allergy, atopy, spin prick testing, sensitivity and specificity, predictive value

4.1. Background

Questionnaires are an efficient tool for assessing outcomes of interest in epidemiological studies. These are usually conducted in large population-based studies.[1-3] A quick, simple and accurate questionnaire can be preferred to a more costly and involved objective measure of the same variable. If questionnaire report of allergy is to be used in place of objective measures such as skin prick testing (SPT), researchers must ensure that the questionnaire report of allergy accurately reflects the objective measure.

In the case of atopic diseases and allergy, in large-scale epidemiologic studies, researchers have commonly relied on reported allergic conditions or triggers reported by questionnaire[4-8] in order to assess the presence of these conditions. In order to clinically facilitate the diagnosis of allergic status or atopic conditions, objective measures such as skin-prick test positivity, elevated total IgE, and specific IgE are often used. The accuracy and agreement of questionnaire report compared to the results from objective measures are, therefore, of interest[1,2,9,10] to maximize validity and practicality. Accordingly, there have been a number of studies assessing the relationship between clinical measures of atopy (e.g., skin-prick test positivity, specific IgE, total IgE) and questionnaire reports to evaluate the discrepancy or concordance of information shown on questionnaire report and results from clinical diagnosis.[1,2,11-13]

In Canada, to our knowledge, few studies have been carried out to examine the agreement between questionnaire report of allergy and results from SPT in population. There was one hospital-based study in children aged 1-17 years in British Columbia, Canada.[14] Recently, in Saskatchewan, Canada, a group of researchers conducted the Saskatchewan Rural Health Study (SRHS). It is a large, population-based study that retrieved detailed information on allergy by questionnaire as well as clinical measures of atopy (skin prick testing) both in adults and children.[15] The objectives of the present analysis were: first, to

examine the agreement between atopy measured by SPT and allergy measured by the questionnaire report; second, to examine the agreement between atopy measured by SPT and atopic outcomes measured by the questionnaire; and third, to evaluate the predictive values of the questionnaire to assess atopic outcomes.

4.2. Methods

Study design and population

We compared the agreement between questionnaire report of allergy and allergic conditions to atopy measured from SPT in children. We used data from the baseline cross-sectional study of children from the SRHS.[15] The SRHS is a longitudinal study (2010-2015) examining the health outcomes of rural adults and children in the Canadian province of Saskatchewan with a focus on lung disease and related conditions. The methods of this longitudinal study were described elsewhere.[15] Two thousand three hundred eighty three children (participation rate: 42%) participated in the baseline survey. A subset of students in grades 1-8 attending 16 selected schools were invited to participate in the clinical assessments which included skin prick testing for atopy. The schools where clinical assessments were conducted were selected based on school participation numbers in the survey in order to maximize efficiencies and reduce costs. A total of 1768 students from 16 schools were approached for clinical testing and 584 of these schoolchildren agreed to participate in the clinical study. We did not use the data of those who did not complete SPT or those who identified themselves as non-Caucasian (8%) in order to reduce potential confounding as there are differences in atopic outcomes prevalence by ethnic background.[16,17] This resulted in 480 children with both adequate information on the questionnaire and SPT. Information from these children was used in the current analysis. Children who completed both the questionnaire report and SPT were defined as a "Questionnaire and SPT group" (N=480). Those who completed only the questionnaire report, not participating in SPT were defined as a "Questionnaire Group Only" (N=1154).

Ethical approval was obtained from the University of Saskatchewan Biomedical Research Ethics Board prior to data collection. Written consents from parents and written assents from children were completed prior to data collection of the clinical studies. Schools and school divisions approved the study.

Skin prick testing (SPT) and atopy definition

We used a panel of allergens that were most common in Saskatchewan including *Alternaria* (mold), *Cladosporium* (mold), cat dander, local grasses, wheat dust and mixed house dust mite (ALK–Abello Pharmaceuticals, Inc., Ontario, Canada). Histamine (10mg/ml) and saline solution (0.9%) were used as positive and negative controls, respectively. Skin prick testing for atopy was conducted according to international standards for testing.[18,19] The allergy skin prick testing was not completed if the child had a severe systemic reaction to an allergen previously or if the child had taken any cold preparations or antihistamine on the day of testing. Atopy was defined as a ≥3mm wheal formed for any of the allergens compared to the negative control on SPT as recommended by the Global Allergy and Asthma European Network (GA^2LEN) [18] and European standards.[19]

Questionnaire report of allergy

Parents completed a self-administered health questionnaire about their socio-demographics, the respiratory and general health of the child, allergic disease, lifestyle, home environment, and early life exposures. The cross-sectional questionnaire was based on questions from standardized questionnaires including: the American Thoracic Society's 1979 Children's Respiratory Disease Questionnaire;[20] the International Study of Asthma and Allergies in Childhood Study (ISAAC) questionnaire;[21] and questionnaires used in recent studies in Saskatchewan[22-24].

History of allergy was defined by the question "Has the child ever had an allergy (hives, runny nose, swelling, itchiness and/or wheezing) to any of the following: house dust,

grain dust/pollen, trees, grasses, mold or mildew, dog, cat, birds or feathers, farm animals, chemicals, foods). A positive answer to cat, grasses, wheat dust, house dust mite, and mold was included in the definition of **any allergy** due to the link to specific SPT allergens included in our study. Each of these specific allergens was also examined separately. The status of **hay fever** was defined according to the question: "Has this child ever had hay fever?" and the status of **eczema** was defined according to the question: "Has this child ever had eczema?" **Current allergic rhinitis** was defined as a positive response to the question "In the past 12 months, has your child ever had a problem with sneezing, or a runny, or a blocked nose when he/she did not have a cold or the flu?" **Eczema symptoms** were defined as a positive response to the question "Has your child ever had an itchy rash which was coming and going for at least 6 months?"

Statistical analysis

Agreement between questionnaire report of allergy and objective measures of atopy were considered overall and between the specific allergens tested on SPT and those assessed on questionnaire (house dust mite, cat, grasses, mold, and wheat dust). The percent concordance and Kappa statistic were used to quantify the agreement between atopy assessed by SPT and allergic disease based on questionnaire reports. We calculated the sensitivity, specificity, and positive and negative predictive values of each questionnaire variable and variable for atopy based on SPT. We assumed that SPT was the gold standard. SPSS® (the Statistical Package for the Social Sciences) version 21 statistical package was used for data analysis.

4.3. Results

Table 1 shows the characteristics of Grades 1-8 children in the "Questionnaire and SPT group" or "Validation Study Group" in comparison with those in the "Questionnaire Group Only". These two groups were similar in the current residence, paternal educational attainment, paternal smoking status, and family history of allergies or asthma. However, children in the "Validation Study Group" were more likely to be male compared to those the

"Questionnaire Group Only" (54.4% vs. 48.4%, p=0.03, respectively). Moreover, those in "Validation Study Group" were younger than those in the "Questionnaire Group Only" [9.60 years (±2.17) vs 10.27 years (±2.50); (p<0.001)]. **Table 1** also shows that they were similar in the prevalence of allergy and atopic conditions reported from the questionnaire.

Prevalence of atopic outcomes

The prevalence for each outcome is given in **Figure 1.** The overall prevalence of atopy was 19.4% (95%CI: 18.3%-20.5%). The prevalence of any allergy based on the questionnaire was 25.0% (95%CI: 21.0%-28.0%). The prevalence of hay fever (ever) was 8.5% (95%CI: 6.1%-10.8%). The prevalence of eczema (ever) was 26.9 % (95%CI: 25.6%-28.1%). The prevalence of current rhinitis symptoms was 25.1% (95%CI: 23.8%-26.3%). The prevalence of eczema symptoms was 15.4% (95%CI: 12.2%-18.5%).

Levels of agreement and diagnostic characteristics between results from SPT and questionnaire report of allergy

The percentage of agreement between results from skin prick testing and results from the questionnaire is shown in **Table 2**. The agreement of reported any atopy/allergy and the results from SPT was fair based on Kappa (Kappa=0.25, p<0.001) and the percentage of concordance was 74%. The concordance between report of specific triggers and overall results based on SPT ranged from 77.1% to 80.2%. For instance, the agreement of reported allergy to grain dust and any SPT positivity was 77.3% (Kappa=0.14, p<0.001). When comparing between report of specific triggers and specific results from SPT, the concordance ranged from 83.0% to 89.5%. For example, according to **Table 2**, the agreement of reported allergy to cat and the result from SPT (cat dander) was fair based on Kappa (Kappa=0.34, p<0.001) and the percentage of concordance was high (89.5%). The sensitivity of any allergy from questionnaire for atopy was 47.3% and specificity was 80.3% while the positive predictive value was 36.6% and the negative predictive value was 86.3% (**Table 4**).

Levels of agreement and diagnostic characteristics between results from SPT and questionnaire report of allergic conditions

Table 3 shows the agreement between atopy and reported allergic conditions. There was agreement beyond chance between reported hay fever ever and atopy based on Kappa (Kappa=0.17, p<0.001) as well as current rhinitis symptoms (Kappa=0.09; p=0.049). The percentage of concordance ranged from 67.1% to 79.6%. The discordance between atopy and allergic conditions was considerable (**Table 3**). **Table 4** summarizes the sensitivity, specificity and predictive values of the questionnaire variables for atopic outcomes. The sensitivity of the questionnaire variables of allergic conditions for atopy was generally low (19.3%-34.4%), while the specificity was higher (74.9%-94.0%). In general, the positive predictive value of allergic conditions was low (24.8% - 43.9%), but the negative predictive value was high (82.0% - 82.9%).

4.4. Discussion

We found that 25.0% of children reported a history of allergic conditions by questionnaire and 19.4% were atopic detected by skin pick test. In our study, the agreement between questionnaire report of specific allergic triggers and atopy measured by SPT was high (83.0% - 89.5%). This result showed that the information from the questionnaire report was reliable to predict the actual manifestations shown by SPT.

The question of hay fever was very specific but not sensitive given the low percentage of sensitivity and high percentage of specificity. This finding was in agreement with one study among 2,120 Swiss school children visiting the school health service in the International Study of Asthma and Allergies in Childhood (ISAAC).[2] Furthermore, the PPV of the questionnaire of hay fever in the earlier study was 70% [2] which was higher than in our study (45.7%). A consideration is that prevalence affects predictive values with increasing disease prevalence, the PPV increases, and with decreasing prevalence, the NPV increases.[25] This lower PPV in our study might be due to the higher number of those who reported no hay

fever on the questionnaire, but in fact were SPT positive (15.6%). Furthermore, it is a possibility that using SPT as criteria of hay fever or allergic rhinitis may underestimate the sensitivity of the questions, because not all those sensitized have symptoms.[26] The high negative predictive value found for all allergic conditions in this study suggests that the questionnaire could be used as a diagnostic tool for primary screening that can rule out subjects with asymptomatic allergy. The low prevalence of hay fever (8.5%), will influence the negative predictive value by increasing it.

We also found that the current rhinitis symptoms yielded a positive predictive value of 25.6%, which was lower than that of the ISAAC study in a population of Swiss school children (aged 5-15 years) (52.0%).[2] The prevalence of these symptoms in our study (25.1%) was quite similar to that of these Swiss school children (23.7%). The reason for our low PPV would be the high percentage of false positives at 13.0%. Moreover, the results of our study are in line with a study among Canadian children.[14] In this study, children aged 1-17 years currently having allergic symptoms were referred to the Children's Hospital in Vancouver. Of these, 48% were found to have allergies based on SPT to inhalant allergens. The parents were then asked about their children's symptoms using a standardized questionnaire containing detailed questions about allergic status by trained interviewers. The results showed that the standardized questionnaires accurately predicted the history of allergies among these children. In general, the specificity was above 80% and sensitivity ranged from 11% to 56%.[14]

It is challenging to compare results of our study to findings from other studies in which different methods of measuring and defining the presence of atopy were used (serum and specific IgE) and the age of participants varied. One study among 2067 adults aged 20-60 years examining the predictive value of the questionnaire relative to atopy, defined by the presence of serum specific IgE found that the positive predictive value of the questionnaire on hay fever was 41.8%, which was quite similar to our findings (43.9%).[27] The authors suggested that this low positive predictive value may be due to the overall low prevalence of

hay fever in this population (18.5%).[27] This result was similar to our findings where the overall low prevalence of hay fever was 8.7% which could lead to our low positive predictive value. We therefore extend our findings from adults in the previous study to children in our study.

There are limitations that should be considered. The main weakness was the modest participation rate (42%). However, we found that there were few differences between the "Validation Study Group" and the "Questionnaire Group Only" and no statistically significant differences in the prevalence of reported allergy between those two mentioned groups. Recall bias should be considered. It is possible that over time, when the children were no longer bothered by allergic conditions, their parents tended to forget a previous diagnosis that can lead to lack of predictive value of the questions.

There were strengths that should also be taken into account. In our study, we assumed that SPT was a gold standard. It is an objective measure with established methodology [19]. Moreover, prior to the allergy skin testing, we excluded children if they had taken any cold preparations or antihistamine on the day of testing as this could prevent a reaction from occurring and biasing the study results. Almost all participants experienced a histamine response in the SPT (97.8%) confirming that if a participant was susceptible to a reaction, it would occur. Also, the questionnaire was completed prior to atopy assessment, thereby limiting reporting bias that may occur with knowledge of the child's atopic status from the SPT.

In summary, questionnaires remain important, efficient, and sometimes the only way to collect medical information in a large study sample. Increasing the precision of questionnaires will improve their utility; however, not all aspects of the allergy-atopy spectrum are well described using questionnaires. In our study, the standardized questionnaire report of allergy and atopic conditions was shown not to efficiently and reliably predict atopy as a screening tool based on the low positive predictive value. However, given the good

specificity and the negative predictive value, the questionnaire may be an efficient tool for risk-factor epidemiological studies that involve the differential inclusion of subjects with and without atopy.[28] Although use of a questionnaire inevitably leads to some misclassification, it is a more rapid and cost-effective method than SPT or the use of other objective means of measuring allergic status in population-based studies. Researchers need to be aware of the limitations of all potential atopy measures, given the lack of concordance in many large population-based studies in order to verify the accuracy of the data.

4.5. Acknowledgements

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4.6. References

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Table 1: Characteristics of children who completed the skin-prick testing (SPT) and Questionnaire Report

	Questionnaire and SPT group* N= 480 (%)	Questionnaire Group Only† N=1154 (%)	p-value
Sex			
Male	261 (54.4)	559 (48.4)	0.03
Female	219 (45.6)	595 (51.6)	
Mean age in years (SD)	9.60 (2.17)	10.27 (2.50)	<0.001
Home location on farm			
Farming	222 (46.5)	511 (44.7)	0.49
Non-farming	255 (53.5)	633 (55.3)	
Maternal education			
High school or less	173 (36.3)	415 (36.4)	0.97
Any postsecondary education	303 (63.7)	724 (63.6)	
Paternal education			
High school or less	246 (52.1)	599 (52.8)	0.79
Any postsecondary education	226 (47.9)	535 (47.2)	
Mother smoking			
Never smoke	291 (61.0)	651 (57.0)	0.13
Ever smoke	93 (19.5)	275 (24.1)	
Currently smokes	93 (19.5)	217 (19.0)	
Father smoking			
Never smoke	262 (55.2)	625 (54.9)	0.34
Ever smoke	87 (18.3)	240 (21.1)	
Currently smokes	126 (26.5)	273 (24.0)	
Family history of allergies/asthma			
Yes	265 (57.1)	626 (56.5)	0.83
No	199 (42.9)	481 (43.5)	
Hay fever (ever)			
Yes	41 (8.5)	1051 (91.1)	0.80
No	439 (91.5)	103 (8.9)	
Eczema (ever)			
Yes	129 (26.9)	324 (28.1)	0.62
No	351 (73.1)	830 (71.9)	
Current allergic rhinitis			
Yes	117 (24.4)	269 (23.3)	0.64
No	363 (75.6)	885 (76.7)	
Eczema symptoms			
Yes	74 (15.4)	161 (14.0)	0.44
No	406 (84.6)	993 (86.0)	
Any allergy (questionnaire report)			
Allergic to any allergy			
Yes	120 (25.0)	296 (25.6)	0.78
No	360 (75.0)	858 (74.4)	
Allergic to grasses			
Yes	58 (12.1)	121 (10.5)	0.34

No	422 (87.9)	1033 (89.5)	
Allergic to mold/mildew			
Yes	57 (11.9)	126 (10.9)	0.57
No	423 (88.1)	1028 (89.1)	
Allergic to cat			
Yes	42 (8.8)	116 (10.1)	0.41
No	438 (91.3)	1038 (89.9)	
Allergic to house dust			
Yes	40 (8.3)	90 (7.8)	0.71
No	440 (91.7)	1064 (92.2)	
Allergic to grain dust			
Yes	56 (11.7)	142 (12.3)	0.71
No	424 (88.3)	1012 (87.7)	

* Grades 1-8 with SPT
† Grades 1-8 without SPT

Table 2: Measures of agreement between results from skin prick test and results from questionnaire (any allergy/atopy and specific allergens)

		Results based on SPT N=480		Kappa	% Concordance
		Positive (+) N=93 (%)	Negative (-) N=387 (%)		
Compared to any SPT positivity					
Any allergy	+	44 (47.3)	76 (19.6)	0.25*	74.0
	-	49 (52.7)	311 (80.4)		
House dust	+	13 (14.0)	27 (7.0)	0.09†	77.7
	-	80 (86.0)	360 (93.0)		
Grasses	+	26 (28.0)	32 (8.3)	0.23*	79.4
	-	67 (72.0)	355 (91.7)		
Mold	+	20 (21.5)	37 (9.6)	0.14*	77.1
	-	73 (78.5)	350 (90.4)		
Cat	+	20 (21.5)	22 (5.7)	0.20*	80.2
	-	73 (78.5)	365 (94.3)		
Grain dust	+	20 (21.5)	36 (9.3)	0.14*	77.3
	-	73 (78.5)	351 (90.7)		
Compared to specific allergen from SPT					
House dust	+	3 (12.0)	37 (8.1)	0.03	87.7
	-	22 (88.0)	418 (91.9)		
Grasses	+	8 (20.0)	50 (11.4)	0.07	83.0
	-	32 (80.0)	390 (88.6)		
Mold	+	11 (36.7)	46 (10.2)	0.18*	86.5
	-	19 (83.3)	404 (89.8)		
Cat	+	17 (40.5)	25 (5.7)	0.34*	89.5
	-	25 (59.5)	413 (94.3)		
Grain dust	+	5 (25.0)	51 (11.1)	0.07	86.2
	-	15 (75.0)	409 (88.9)		

*$p<0.001$
†$p<0.05$

Table 3: Measures of agreement between SPT and questionnaire report of atopic diseases (N=480)

		Results based on SPT N=480		Kappa	% Concordance	% False negative	% False positive
		Positive (+) N=93 (%)	Negative (-) N=387 (%)				
Hay fever ever	+	18 (19.4)	23 (5.9)	0.17*	79.6	4.8	15.6
	-	75 (80.6)	364 (94.1)				
Current rhinitis	+	30 (32.3)	87 (22.5)	0.09†	68.8	18.1	13.1
symptoms	-	63 (67.7)	300 (77.5)				
Eczema ever	+	32 (34.4)	97 (25.1)	0.08	67.1	20.2	12.7
	-	61 (65.6)	290 (74.9)				
Eczema	+	20 (21.5)	54 (14.0)	0.08	73.6	11.3	15.2
symptoms	-	73 (78.5)	333 (86.0)				

*p<0.001
†p<0.05

Table 4: Sensitivity, Specificity and Predictive values of questionnaire variables for atopic outcomes (N=480)

Questionnaire variables	% population (95%CI)	Sensitivity, %	Specificity, %	Predictive value, %	
				Positive	Negative
Any allergy from questionnaire	25.0 (21.0-28.0)	47.3	80.3	36.6	86.3
Hay fever ever	8.5 (6.1-10.8)	19.3	94.0	43.9	82.9
Eczema ever	26.9 (25.6-28.1)	34.4	74.9	24.8	82.6
Current rhinitis symptoms	25.1 (23.8-26.3)	32.2	77.5	25.6	82.6
Eczema symptoms	15.4 (12.2-18.5)	21.5	86.0	27.0	82.0

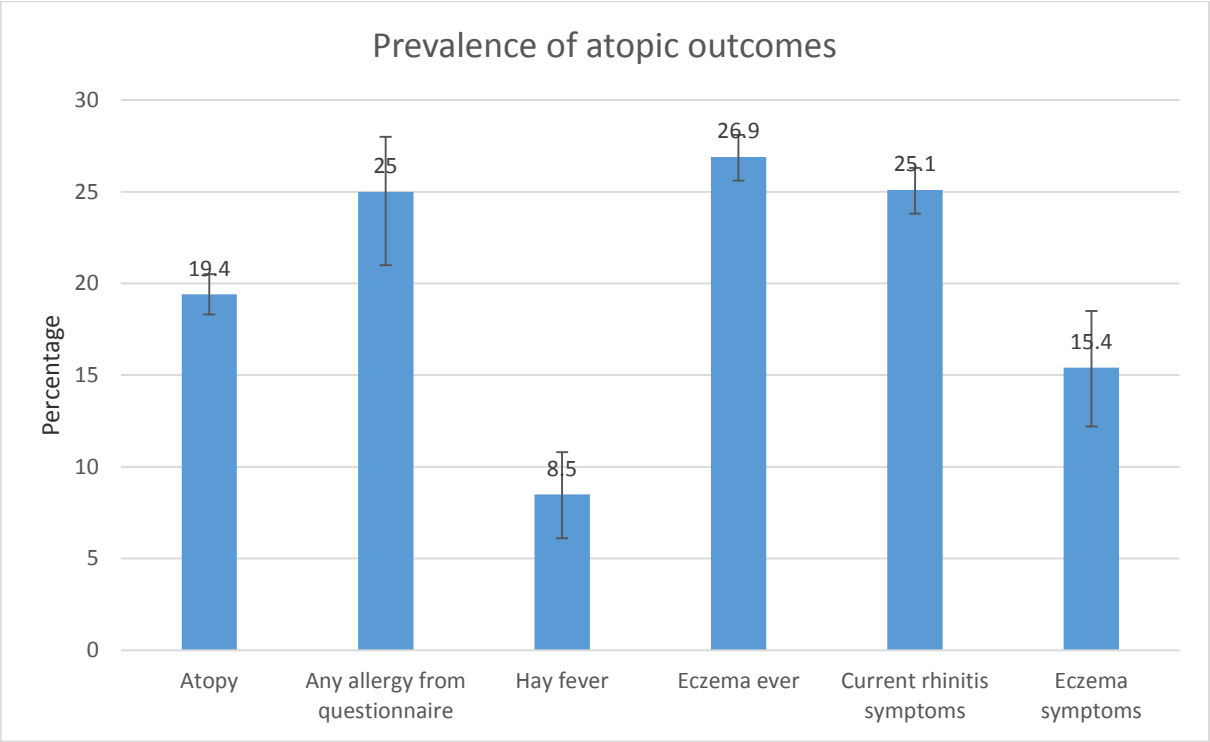

Figure 1: Prevalence of atopic outcomes

Chapter 5

Discussion

5.1. Summary and Bridge of the Two Manuscripts

The overall purpose of this thesis was to first identify the prevalence and determinants of atopy among school-age children in rural Saskatchewan, Canada, which was presented in Manuscript 1 (Chapter 3); and second, to examine the agreement between a questionnaire report of allergy and allergic diseases with an objective measure (SPT) of atopy, which was presented in Manuscript 2 (Chapter 4). Both manuscripts used data from the Saskatchewan Rural Health Study (SRHS) – Child's Cohort.

The findings of this thesis are summarized below by research question:

Research question 1: What is the prevalence of atopy in rural Saskatchewan children and is there a difference in atopy prevalence between farming and non-farming residential status?

- The overall prevalence of atopy measured by SPT in rural Saskatchewan children in our study was 19.4%.
- The atopy prevalence was similar for farm and non-farm children (18.7% vs. 20%; p=0.71).

Research question 2: What are the individual and environmental factors associated with atopy in Grade 1-8 rural Saskatchewan children with a focus on farming exposures?

- There were no statistically significant associations between atopy with farm and non-farm status (aOR=0.81, 95%CI=0.09-7.07).
- Living on a livestock farm conferred protection against atopy (aOR=0.38, 95%CI= 0.17-0.88; p<0.05).

Research question 3: What is the agreement between atopy assessed by skin prick testing and allergic disease based on questionnaire report?

- We found that 25.0% of children reported a history of allergic conditions by questionnaire and 19.4% were atopic detected by skin pick testing.

- The agreement of reported any atopy/allergy and the results from SPT was fair based on Kappa (Kappa=0.25, p<0.001) and the percentage of concordance was 74%. The concordance between report of specific triggers and overall results based on SPT ranged from 77.1% to 80.2%.

- The agreement between questionnaire report of specific allergic triggers and specific allergens measured by SPT was high (83.0% - 89.5%).

- The sensitivity of any allergy from questionnaire for atopy was 47.3% and specificity was 80.3% while the positive predictive value was 36.6% and the negative predictive value was 86.3%.

- There was slight agreement beyond chance between reported hay fever ever and atopy based on Kappa (Kappa=0.17, p<0.001) as well as current rhinitis symptoms (Kappa=0.09; p=0.049). The percentage of concordance ranged from 67.1% to 79.6%.

- The sensitivity of the questionnaire variables of allergic conditions for atopy was generally low (19.3%-34.4%), while the specificity was higher (74.9%-94.0%). In general, the positive predictive value of allergic conditions was low (24.8% - 43.9%), but the negative predictive value was high (82.0% - 82.9%).

5.2. Internal and External Validity of the findings

5.2.1. Internal Validity

5.2.1.1. Selection bias

Selection bias should be considered in our study. There were 584 children in participating schools who agreed to undergo the clinical testing including SPT. We excluded those children with a history of severe allergic reactions to avoid any potentially dangerous reaction to allergens used which could potentially affect their health. Also, we excluded those were on antihistamine in the day of testing to minimize bias resulting from inclusion of these subjects. Fifty-five children who attended clinical testing did not complete the skin prick testing (47 refused; 4 with other reasons and 4 missing). The number of children not

completing SPT (n=55) had similar characteristics (data not shown) compared to those who completed SPT. Thus, the results were not affected by these excluded children.

In Manuscript 2, we ran a descriptive analysis to compare the characteristics of two groups. Children who completed both the questionnaire report and SPT were defined as a "Questionnaire and SPT group". Those who completed only the questionnaire report, not participating in SPT were defined as a "Questionnaire Group Only". We observed that the participation in clinical testing did not result in the parents of children with asthma or allergies being more likely to complete the questionnaire than those parents of children without these conditions.

Another source of potential selection bias was the "healthy student effect" in a sense that children with atopic symptoms might move out from their current location on farm to settle in different places with less farm-related exposures or the activities that children with atopy are taking part in are modified because of their atopic conditions. Therefore, the lower prevalence of atopy may have been observed in children living on a farm compared to those who not living on a farm. However, in our study, the findings showed that there were no difference in atopy prevalence between farm and non-farm status, showing that it is unlikely that this effect differs by farm and non-farm residential status.

5.2.1.2. Information bias

Information bias should be considered in our study. Information was collected from self-administered parental questionnaire report, which is subject to errors in recall. In Manuscript 2, we found that 25.0% of children reported a history of allergic conditions by questionnaire and only 19.4% were atopic detected by skin pick test. Information bias can be derived from the methods or credibility of the exposure and outcomes measurement.

Regarding the exposure measures, we did not have a standard definition of a "farm" or which types of farm (part-time or full-time farming) as well as commencement and duration

of exposure (lifetime exposures and current exposures). Also, measuring other specific farm exposures was difficult from the questionnaire. It is possible that parents could underestimate the amount of time that their children spent on a farm doing their farm work. In our study, we used commonly used questions on the questionnaire, so such systematic error would be minimized.

Regarding the outcome measures, we used skin prick testing which is a widely used standardized technique to detect atopic status. Despite this, such a clinical measure of atopy can be difficult to compare across studies because of varying methodologies (specific allergens used, number of allergens, etc.). Furthermore, information of allergic conditions to specific triggers were collected from the questionnaire report. We found that the questionnaire report was fairly accurate to detect these allergic conditions. The agreement between questionnaire report of allergic triggers and atopy measured by SPT was high (83.0%-89.5%). Also, the agreement between atopy and report of allergic conditions (hay fever, eczema, current rhinitis symptoms, and eczema symptoms) ranged from 67.1% to 79.6%.

5.2.1.3.Confounding

In our study, we attempted to adjust for a number of potential confounders by using multivariable analyses. Due to the complexity of asthma and allergies etiology, there are other potential confounders that might have been adjusted for, but not available for inclusion in the analysis. For example, research has shown that prenatal maternal stress may influence the present of atopy of the child.[1] Also, active and chronic helminthic infections were reported to be protective from atopy.[2] While we did not include all potential confounders, our survey was focused on respiratory disease and related conditions and the common and important known risk factors and confounders were considered. We also fitted the model based on confounding in the data as well as confounding in the literature.

5.2.1.4. Cross-sectional design

Due to the characteristics of a cross-sectional design used in this study, we could not draw causal associations between exposures and outcomes. It is possible that parents practiced some prevention methods to avoid potentially harmful exposures or practices because of the condition (atopic symptoms) they observed in their children.

5.2.2. External Validity

Despite small number of Canadian children population (n=584) participating in the clinical phase, our findings can be generalized to our non-clinical study population since the characteristics between these clinical and non-clinical populations were similar (presented in Manuscript 2). The results of our study could be generalized to school-age children in Saskatchewan specifically and other Canadian children living in similar rural environments. Also, the applicability of these findings to non-Caucasian children may be limited due to a large number of Caucasian children who participated in our study (90.4%). Furthermore, due to the farm practices varying from country to country, or even in different regions within countries, the global generalization of the findings should also be carefully considered.

5.2.3. Other strengths and Limitations

The major advance in the two manuscripts are the objective assessment of atopy using standard methods for diagnosis (SPT), as opposed to self-report as well as the assessment of agreement between the objective and reported measures. Objective measures were used in determining atopic status (SPT) and BMI measurement, which can reduce the potential for measurement error. Given different approaches to answer the research questions in Manuscript 1 and 2, the limitations and strengths of each manuscript would be considered separately in addition to the information provided in the discussion section of each manuscript.

Additional limitations and strengths in Manuscript 1 should be considered. It is undeniable that the objective measure of atopy brings credibility for the interpretation of results. However, it is important to consider limitations in our questionnaire. Our questionnaire did not include wide range of information of allergic symptoms rather than "yes-no" answers in the response. Thus, the allergy-atopy spectrum were not collected adequately. For example, our questionnaire should have included wide range of hay fever/ allergic rhinitis symptoms to examine the accurate of allergic status of subjects. Besides SPT, physician-diagnosis information should also be included in the questionnaire. Moreover, when we re-calculated the actual statistical power, we observed that the power of our analysis was 15%, which can lead us to Type II error.

There are additional strengths in the thesis. The results from Manuscript 1 added evidence to the Bradford Hill's criteria on causation such as strength of the association between exposures and outcomes as well as consistency of results**.** We observed a statistically significant decreased risk of atopy in children living on a livestock farm compared to those not living on a livestock farm (aOR=0.38, 95%CI: 0.17-0.88, $p<0.05$). In contrast, current residence on a farm was associated with an increase in the likelihood of hay fever in these children (aOR= 3.68, 95%CI: 1.29-10.45, $p < 0.01$). According to Hill's criteria, inadequacy could derive from systematic errors such as reverse causation. There are possibilities that over- or underreporting of exposures. Moreover, our findings did not support most studies in the notion that lower prevalence of atopy in children was associated with living on a farm compared to those who do not live on a farm. The explanations could lay in different people, places, circumstances or time. However, we did find that living on a livestock farm protects children against atopy, which is in agreement with many studies in Europe and other places.[3-7] On the other hand, our findings of increased risk of hay fever among farm children did not entirely agree with recent findings from European studies, but did with one study in New Zealand.[8] What is more, multi-causation is very common in epidemiological as well as

clinical studies, given the complexity of disease etiology. Although we observed strong associations between atopy among children living on a livestock farm or the increased risk of hay fever among farm children, we could not pinpoint which factors on that farm account for the presence of atopy and hay fever among those children. Such factors on a livestock farm are suggested to include: Endotoxin,[9,10] muramic acid,[10] fungus, etc. Only studies with clearly defined exposures, well-defined health outcomes and well-established modeling strategies will probably account for specificity. Furthermore, in cross-sectional studies, exposures are determined retrospectively. In our study, questionnaire report was used to collect information on environmental exposures (farm-related exposures), and some diseases of interest (allergic diseases, allergies to specific allergens) at the same time. Furthermore, a dose-response relationship between exposures and health outcomes assessment is important for understanding the etiology of diseases as well as for possible interventions. There have been studies linking incidence of disease (allergic diseases) with timing and magnitude of the exposures (duration of farming exposures).[11,12] In our study, we tried to collect information of the "dose" of farm-related activities involvement, but due to the sample size and the frequency of the responses, we could not derive such dose-response relationships in our study. Beyond any doubt, this study's findings will add to the literature given the relative paucity of atopy and allergic diseases research in rural areas, especially in Canadian rural context.

Besides these mentioned strengths, there are several additional limitations of the thesis should be taken into account. First, the use of atopy definition in our study was not similar to that of some other studies, thereby limiting comparisons among studies. However, this limitation was addressed in many studies and comparisons among studies were warranted. What is more, the relative low levels of sensitization found could be due to the limited skin tests used in this study. We used a panel of 6 common allergens found in Saskatchewan, which possibly differs from that of different studies in different places. This would somehow under-estimate the atopy prevalence in our study. However, as mentioned in Manuscript 1 that

the common epidemiologic definition of atopy included only three common allergens including house dust mite, grass and cat (Birth cohort studies in Britain in 1997 and Kent, UK in 2007, a cohort study in Kent, UK in 2003, and a case-control study in Scotland). It is well recognized that such an epidemiologic definition of atopy will underestimate the true prevalence of atopy.

5.3. Recommendations and Applications

Based on the findings in this thesis, recommendations include (1) considering the benefits of exposures to livestock in children and (2) the application of questionnaire report to collect data of childhood allergies.

First, in our study, we found that living on a livestock farm conferred protection against atopy among school-age children. Even though this association has not been proven to be causal, the findings of protective effects of living on a livestock farm or livestock exposures were consistent in many countries given the difference in types of farm, timing of exposures, farming practices, etc. As such, identifying the possible protective effects on a livestock farm suggests possible future prevention consideration.

Second, in our study, given the agreement between the questionnaire report and the objective measure of allergy (SPT), the questionnaire we used could be applied in similar populations in rural Canada to detect allergic conditions in children. In population-based epidemiological studies with large sample sizes, it is known that SPT would be costly and time-consuming to conduct. Therefore, an effective questionnaire would reduce the cost and bring credible results similar to the findings from an objective measure such as SPT.

5.4. Future Research Directions

There are a number of suggestions/recommendations for future research on allergy epidemiology. First, in order to disentangle the direction of exposures leading to diseases, there is a need of more sophisticated and appropriate research designs used such as

longitudinal studies (e.g. Birth cohort) looking at the timing of farm exposures in relation to the respiratory diseases development. Second, due to the controversial results derived from different studies in different populations, a meta-analysis is suggested to improve power as well as conclusion evidence to draw any causal relationships between farming exposures and respiratory diseases. Third, it would also be valuable to perform similar studies in other rural and urban communities, and with more ethnically diverse study populations in order to assess the generalizability of the current study findings. Fourth, it is recommended that more comprehensive exposure measurement methods be used such as objective measures of endotoxin level, microbial compounds, grain dust, etc. to pinpoint what factors on a farm responsible for the development of childhood atopy and respiratory diseases. Also, the timing, frequency and duration of agricultural exposures should be collected. Fifth, standard definitions of asthma and atopy are needed to facilitate comparisons of data across studies. Sixth, population-based studies are required to assess the etiologic fraction, which is an important measure for the proportion of allergy attributable to exposures of interests (e.g. farming exposures, etc.). This etiologic fraction will be an efficient tool for setting priorities in prevention.

5.5. Conclusions from this thesis

This thesis examines the agreement between questionnaire report of allergy and allergic diseases with an objective measures of atopy, and identifies the prevalence and determinants of atopy among a large population of school-age children in rural Saskatchewan, Canada. Findings suggest that the questionnaire may be an efficient tool for risk-factor epidemiological studies that involve the differential inclusion of subjects with and without atopy. Moreover, findings also suggest that environmental exposures such as livestock farm may protect school-age children against atopy among school-age children in rural Saskatchewan. Further studies (e.g. cohort designs) are needed to look at the effects of farming and farming activities on atopy and allergic diseases.

5.6. References

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Appendix A

The Saskatchewan Rural Health Study Questionnaire

SASKATCHEWAN RURAL HEALTH PROJECT

Children Study

UNIVERSITY OF SASKATCHEWAN

TO PARENTS OR GUARDIANS:

The University of Saskatchewan is conducting this project to learn more about the lung health of rural dwellers in Saskatchewan.

PLEASE COMPLETE ONE QUESTIONNAIRE PER CHILD.

THE PERSON MOST FAMILIAR WITH THIS CHILD'S HEALTH SHOULD COMPLETE THE QUESTIONNAIRE.

This questionnaire has four parts. Please answer all parts as they concern your child and his or her surroundings. Please try to answer all of the questions, but remember you don't have to answer any questions if you choose not to.

Part One asks about contact information.

Part Two asks some general questions about your child and about your child's past and present health.

Part Three asks about your child's lifestyle and surroundings.

Part Four asks questions about child's personal and family history.

PLEASE NOTE:
ALL INFORMATION WILL BE KEPT CONFIDENTIAL AND USED ONLY TO GROUP YOUR RESPONSES WITH THE QUESTIONNAIRE RESPONSES OF OTHER PARENTS.

WHEN YOU HAVE FINISHED, PLACE THE QUESTIONNAIRE IN THE ENVELOPE, SEAL THE ENVELOPE AND RETURN IT TO THE SCHOOL.

The University of Saskatchewan

Sponsored by the Canadian Institutes of Health Research
(Canada's main funder of medical research)

PART TWO – HEALTH OF THIS CHILD

COUGH

1. Has your child ever had a dry cough at night or first thing in the morning **NOT** associated with a cold or chest infection?
Tick all that apply

- ☐ Yes, past 12 months
- ☐ Yes, before last 12 months
- ☐ No

2. Does this child usually cough at all during the rest of the day?

- ☐ Yes
- ☐ No
- ☐ Don't know

3. **In the past 12 months**, has this child woken up because of a cough?

- ☐ Yes
- ☐ No
- ☐ Don't know

CONGESTION AND PHLEGM

4. Does this child usually have congestion in the chest or bring up phlegm or mucus **apart from colds?**

- ☐ Yes
- ☐ No
- ☐ Don't know

If **YES**, has this congestion or phlegm been present for as much as 3 months in a row out of the year?
Tick all that apply

- ☐ Yes, past 12 months
- ☐ Yes, before last 12 months
- ☐ No

WHEEZING

5. Has this child ever had a wheeze or whistling noise that comes from the chest?

- ☐ Yes
- ☐ No
- ☐ Don't know

If **NO**, SKIP TO **QUESTION 10**

If **YES**, at what age did this child first start to wheeze?
_____ years

6. **In the past 12 months**, has this child had a wheeze or whistling noise that comes from the chest?

- ☐ Yes
- ☐ No
- ☐ Don't know

*IF **NO,** at what age did this child stop wheezing?
_____ years GO TO **QUESTION 10.**

*IF **YES,** CONTINUE ON TO **QUESTION 7**

7. Does the wheezing or whistling in the chest occur:

- ☐ apart from colds?
- ☐ with colds?
- ☐ both apart from colds and with colds?

8. How many attacks of wheezing or whistling in the chest has this child had **in the past 12 months**?

- ☐ none
- ☐ 1-3
- ☐ 4-12
- ☐ more than 12

9. Does wheezing or whistling in the chest occur most nights or days?

- ☐ Nights only
- ☐ Days only
- ☐ Both nights and days

PART 2

10. Has this child ever been short of breath with wheezing? (speech limited to one or two words between breaths) *Tick all that apply*

- ☐ Yes, past 12 months
- ☐ Yes, before last 12 months
- ☐ No

11. Has your child's chest ever sounded wheezy during or after exercise/sports? *Tick all that apply*

- ☐ Yes, past 12 months
- ☐ Yes, before last 12 months
- ☐ No

ASTHMA

12. Has this child ever been diagnosed as having asthma by a doctor?

- ☐ Yes
- ☐ No
- ☐ Don't know

IF NO or DON'T KNOW, Please go to question 20

IF YES, continue at question 13

13. At what age was the asthma diagnosed?

_____ year of age

14. **In the past 12 months,** has this child required services for asthma from the following places:

Hospital inpatient	☐ Yes	☐ No
Emergency room outpatient	☐ Yes	☐ No
Doctor's office	☐ Yes	☐ No

15. **In the past 12 months**, how many asthma episodes has your child had?

_____ (number of episodes)

16. Which of the following statements best describes this child's asthma medication use **in the past 12 months:**

- ☐ Never in the past 12 months
- ☐ At least once in the past 12 months
- ☐ At least once per month
- ☐ At least once per week
- ☐ Every day or nearly every day

17. Do you have a written action plan to manage your child's asthma?

- ☐ Yes
- ☐ No
- ☐ Don't know

18. **In the past 12 months** how many days of school has your child missed because of asthma?

_____ days

19. How often has your child's sleep been disturbed by asthma **in the past 12 months:**

- ☐ Never in the past 12 months
- ☐ At least once in the past 12 months
- ☐ At least once per month
- ☐ At least once per week
- ☐ Every day or nearly every day

ALLEGIC DISEASE

20. Has this child ever had an allergy (hives, runny nose, swelling, itchiness and/or wheezing) to any of the following:

House dust	No ☐	Yes ☐
Grain dust	No ☐	Yes ☐
Pollen	No ☐	Yes ☐
Trees	No ☐	Yes ☐
Grasses	No ☐	Yes ☐
Mold or mildew	No ☐	Yes ☐
Dog	No ☐	Yes ☐
Cat	No ☐	Yes ☐
Birds/feathers	No ☐	Yes ☐
Farm animals	No ☐	Yes ☐

If **YES**, what kind? _____

Chemicals No ☐ Yes ☐

If **YES**, what kind? _____

Foods No ☐ Yes ☐

If **YES**, what kind? _____

Other (please name) _____

21. Has your child **ever** had a problem with sneezing, or a runny, or a blocked nose when he/she did **NOT** have a cold or the flu?

Yes ☐ No ☐

22. **In the past 12 months**, has your child ever had a problem with sneezing, or a runny, or a blocked nose when he/she did **NOT** have a cold or the flu?

Yes ☐ No ☐

23. Has this child ever had hayfever?

Yes ☐ No ☐

24. Has your child ever had an itchy rash which was coming and going for at **least 6 months?**

Yes ☐ No ☐

If YES, Has your child had this itchy rash at anytime **in the last 12 months**?

Yes ☐ No ☐

25. Has your child ever had eczema?

Yes ☐ No ☐

OTHER ILLNESS AND PAST ILLNESS

26. Is this child **regularly** taking medicine that your doctor prescribed for a breathing problem?

☐ Yes ☐ No ☐ Don't know

If **YES**, please name the medicine(s) below:

_____

_____

_____

27. **In the past 12 months** has this child been kept at home from school for 3 or more days with a chest illness?

☐ Yes ☐ No ☐ Don't know

28. **In the past 12 months** have you or another family member missed work because of your child's chest illness?

☐ Yes ☐ No ☐ Don't know

29. Has a doctor ever said this child had any of the following illnesses when **younger than 3 years:**

Bronchitis	No ☐	Yes ☐
Pneumonia	No ☐	Yes ☐
Sinus trouble	No ☐	Yes ☐
Croup	No ☐	Yes ☐

PART 2-3

30. Which statement best describes this child's immunization status:

- ☐ Never immunized
- ☐ Partial immunization
- ☐ Fully up to date

31. Has this child have an operation to remove the tonsils or adenoids?

Yes ☐ No ☐

32. Has a doctor ever said this child had:

a. Diabetes	No ☐	Yes ☐
b. Arthritis	No ☐	Yes ☐
c. Tonsilitis	No ☐	Yes ☐
d. Ear infections	No ☐	Yes ☐
e. Stomach reflux	No ☐	Yes ☐

33. **During the past 12 months**, was this child seen by a doctor or other primary caregiver for:

a. Tonsilitis	No ☐	Yes ☐
b. Ear infections	No ☐	Yes ☐
c. Stomach reflux	No ☐	Yes ☐

34. **During the past 12 months**, how many times was this child injured and had to be treated by a doctor or nurse? (Injuries can include being hurt from activities, poisons, burns, etc.)

_____ Number of times

35. **In the past 12 months**, did you ever experience any difficulties getting the routine or on-going healthcare for this child?

☐ Yes ☐ No ☐ Don't know

36. How far do you travel (one dirction) to receive routine and ongoing medical care for this child?

_____ km

37. How far do you travel (one direction) to receive 24 hour emergency health care services for this child?

_____ km

PART THREE: LIFESTYLE & ENVIRONMENT

38. How many times per week does this child usually eat or drink ...?

Fruits	_____
Vegetables	_____
Fish	_____
Whole fat milk (homogenized)	_____
French fries	_____
Hamburgers	_____
Fast food	_____
Deep fried food	_____
Chicken nuggets	_____
Soft drinks or pops	_____
Potato chips	_____

39. **Over the past 7 days**, on how many days was this child physically active for a total of at least 60 minutes per day?

_____ days

40. Over a **typical or usual** week, on how many days was this child physically active for a total of at least 60 minutes per day?

_____ days

41. How many times a week does your child engage in vigorous physical activity long enough to make him/her breathe hard?

- ☐ Never or occasionally
- ☐ Once or twice per week
- ☐ Three or more times per week
- ☐ Don't know

42. How long does it usually take you to travel to school from your home? (Please tick one box only)

- ☐ Less than 5 minutes
- ☐ 5-15 minutes
- ☐ 15-30 minutes
- ☐ 30 minutes to 1 hour
- ☐ More than 1 hour

43. On a typical day, is the MAIN part of your journey TO school made by…? (Please tick one box only)

- ☐ Walking
- ☐ School bus
- ☐ Bicycle
- ☐ Car
- ☐ Other means

Please specify _____

44. Does your child participate in physical education at school?

- ☐ Never
- ☐ Some of the time
- ☐ All of the time

45. Do you consider your child to be

- ☐ Underweight?
- ☐ Just about right weight?
- ☐ Overweight?

HOME ENVIRONMENT

47. Where is your home located?

- ☐ Farm
- ☐ Acreage
- ☐ In town

If you answered "FARM", from the list above, please check each commodity that is **produced for sale** on your farm or ranch (*Check all that apply*).

- ☐ Grain crops
- ☐ Cattle (beef)
- ☐ Cattle (dairy)
- ☐ Pigs
- ☐ Poultry
- ☐ Vegetable/fruit
- ☐ Other: Please specify: _____

48. **In the past 12 months**, how many times did this child visit a farm?

- ☐ Never
- ☐ 3 or fewer times
- ☐ More than 3 times

49. How long has your child lived in your current home?

____ Years

PART 3

46. **In the past 12 months**, on average, how often has this child spent 1 hour near or in the following activities (*Please check the box that best applies*):

	Everyday	At Least Once a Week	At Least Once a Month	Less Than Once a Month	Never
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	☐	☐	☐	☐	☐
Riding horses	☐	☐	☐	☐	☐

50. Which best describes the type of housing unit in which you live?

- ☐ one family house
- ☐ a duplex
- ☐ a building for 3 or more families
- ☐ a mobile home or trailer

51. Which category of years do you think most closely matches when this building was built?

- ☐ 1980-present
- ☐ 1979 or before
- ☐ Don't know

52. **In the past 12 months**, have major renovations been done to the home?

☐ Yes ☐ No ☐ Don't know

53. How many bedrooms do you have in your home?

Number _____

54. How many people live in your home?

Number _____

55. In your house, what fuel is usually used for heating?

- ☐ Natural gas
- ☐ Propane
- ☐ Electricity
- ☐ Fuel oil
- ☐ Coal
- ☐ Geo-thermal
- ☐ Solar energy
- ☐ Wood
- ☐ Other, specify _____
- ☐ Don't know

56. **In the past 12 months**, have you had any problems with mice or rats in your home?

☐ Yes ☐ No ☐ Don't know

57. Do you have any of the following in your home?

Air conditioners	No ☐	Yes ☐
Air filter	No ☐	Yes ☐
Humidifier	No ☐	Yes ☐
Dehumidifier	No ☐	Yes ☐
Wood fireplace	No ☐	Yes ☐

58. Does your house have any damage caused by dampness (e.g., wet spots on walls, floors)?

Yes ☐ No ☐

59. **During the past 12 months**, has there been water or dampness in your home from broken pipes, leaks, heavy rain, or floods?

☐ Yes ☐ No ☐ Don't know

60. Does your home (including basement) frequently have a mildew odor or musty smell?

☐ Yes ☐ No ☐ Don't know

61. Are there signs of mold or mildew in any living areas in your home?

Yes ☐ No ☐

62. **In the past 12 months**, have you had any of the following pets living in your home? *(Please check yes or no for each type of pet).*

Cat	No ☐	Yes ☐
Dog	No ☐	Yes ☐
Bird	No ☐	Yes ☐
Any other pet	No ☐	Yes ☐

If yes, please specify

_____

PART 3

63. **Within the past 12 months**, were pesticides (including herbicides, insecticides, fungicides, rodenticides, fumigants) applied inside your residence?

☐ Yes Please specify _____
☐ No

64. Does any person smoke **inside** the house?

Yes ☐ No ☐

65. Has this **child's father** ever smoked?

Yes ☐ No ☐

If YES, Year started smoking? _____

66. Does this child's father currently smoke?

Yes ☐ No ☐

If NO but he has smoked, Year quit smoking? _____

67. Has this **child's mother** ever smoked?

Yes ☐ No ☐

If YES, Year started smoking? _____

68. Does this child's mother currently smoke?

Yes ☐ No ☐

If NO but she has smoked, Year quit smoking? _____

69. Other than the mother, father, and this child, does any other person living in the house smoke?

Yes ☐ No ☐

70. On average, how many cigarettes are smoked in your home a day? (Please take into account everyone who smokes in your home)

_____ Cigarettes/day

71. Is this child exposed to smoke in a car?

☐ Yes ☐ No ☐ Don't know

72. Do any of this child's friends smoke?

☐ Yes ☐ No ☐ Don't know

73. Has this child ever smoked tobacco? (At least one cigarette, cigar or pipe)

☐ Yes ☐ No ☐ Don't know

74. Is this child exposed to tobacco smoke from alternate caregivers on a regular basis?

☐ Yes ☐ No ☐ Don't know

75. Does this child take part in chores regularly that require the use of cleaning liquids/fluids?

☐ Yes ☐ No ☐ Don't know

76. Does this child have a paid job?

☐ Yes ☐ No

If YES, please specify: _____

PART FOUR: THIS CHILD, THE FAMILY, AND EARLY LIFE EXPOSURES

77. Child's sex: Male ☐ Female ☐

78. Date of Birth: ____ ____ ____
Mo Day Yr

79. Child's age: _____

80. How tall is this child? (*For best results please use a tape measure against a wall*)

_____ feet _____ inches

81. How much does this child weigh?

_____ pounds

82. What was the child's weight at birth?

___ pounds ___ ounces or ____ kg

83. Was this child born before mother's due date?

☐ Yes ☐ No ☐ Don't know

If **YES,** how many weeks early? _____weeks

84. Within this family, what is the birth order of this child? (*Please circle*)

1st 2nd 3rd 4th 5th 6th 7th

85. Was this child breastfed?

☐ Yes ☐ No ☐ Don't know

If **YES,** for how long? _____

86. Did this child consume unpasteurized milk (raw milk, farm milk) regularly **in the first year of life**?

☐ Yes ☐ No ☐ Don't know

87. Does your child **currently** drink unpasteurized milk regularly?

☐ Yes ☐ No

88. Did this child live on a farm during the first year of life?

☐ Yes, **If YES**, what type of farm? (Check all that apply)
- ☐ Grain
- ☐ Livestock

☐ No
☐ Don't know

89. While this child's mother was pregnant with this child, did she live on a farm or did she work on a farm?

☐ Yes, **If YES**, what type of farm? (Check all that apply)
- ☐ Grain
- ☐ Livestock

☐ No
☐ Don't know

90. In the first 12 months of this child's life, did you have any of the following pets living in your home? *Please check yes or no for each type of pet.*

Cat	No ☐	Yes ☐
Dog	No ☐	Yes ☐
Bird	No ☐	Yes ☐
Any other pet	No ☐	Yes ☐

If yes, please specify _____

91. Did this child's mother smoke during the pregnancy? *(Please check all that apply)*

☐ No
☐ 1st trimester
☐ 2nd trimester
☐ 3rd trimester
☐ Don't know

92. Did this child ever go to daycare?

☐ Yes ☐ No ☐ Don't know

If YES, At what age did this child begin attending? _____

93. Does the child's natural mother have any of the following conditions? *Tick any that apply*

☐ Asthma
☐ Hayfever
☐ Allergies
☐ Eczema
☐ Don't know

94. Does the child's natural father have any of the following conditions? *Tick any that apply*

☐ Asthma
☐ Hayfever
☐ Allergies
☐ Eczema
☐ Don't know

PART 4

95. How many of the child's natural brothers or sisters have had the following conditions?

- ☐ Asthma
- ☐ Hayfever
- ☐ Allergies
- ☐ Eczema
- ☐ Don't know
- ☐ No brothers or sisters

96. What is the highest level of education completed by the child's mother?

- ☐ Public school
- ☐ Grade 12
- ☐ Technical school
- ☐ University degree

97. What is the highest level of education completed by the child's father?

- ☐ Public school
- ☐ Grade 12
- ☐ Technical school
- ☐ University degree

98. Type of household

- ☐ Single parent home
- ☐ Two parent/partner home

99. What is this child's ethnic background?

- ☐ Caucasian
- ☐ First Nation/Metis
- ☐ African, African-American
- ☐ Other Please specify _____

THE END

Thank you for completing the questionnaire.

Please return in envelope to the child's classroom.

Comments:

PART 4

Appendix B

Additional tables for Manuscript 1

Table 1: Descriptive statistics of demographic, behavioral, personal characteristics by atopic status

	Overall (N=529) n (%)	Atopic (N= 105) n (%)	Non-atopic (N= 424) n (%)	P value
Home location				
Non-farm	290 (55.2)	58 (56.9)	232 (54.8)	0.71
Farm	235 (44.8)	44 (43.1)	191 (45.2)	
Livestock farm				0.14
Yes	121 (22.8)	19 (20.4)	102 (27.9)	
No	337 (63.7)	74 (79.6)	263 (72.1)	
Missing	71 (13.5)			
Grains farm				0.47
Yes	129 (24.3)	29 (31.2)	100 (27.4)	
No	329 (62.2)	64 (68.8)	265 (72.6)	
Missing	71 (13.5)			
Sex				
Female	249 (47.1)	41 (39.0)	208 (49.1)	0.06
Male	280 (52.9)	64 (61.0)	216 (50.9)	
Number of siblings				
0	204 (39.3)	38 (37.6)	166 (39.7)	0.87
1	191 (36.8)	37 (36.6)	154 (36.8)	
≥2	124 (23.9)	26 (25.7)	98 (23.4)	
Obesity status (objective)				
Not overweight or obese	429 (73.5)	79 (75.2)	308 (72.6)	0.49
Overweight	115 (19.7)	17 (16.2)	88 (20.8)	
Obese	40 (6.8)	9 (8.6)	28 (6.6)	
Maternal education				
High school or less	193 (37.2)	36 (35.0)	157 (37.7)	0.60
Any postsecondary education	326 (62.8)	67 (65.0)	259 (62.3)	
Paternal education				
High school or less	272 (53.0)	55 (53.9)	217 (52.8)	0.84

Any postsecondary education	241 (47.0)	47 (46.1)	194 (47.2)	
Mother smoking				
Never smoke	321 (61.1)	59 (56.2)	262 (62.2)	0.41
Ever smoke	98 (18.6)	24 (22.8)	74 (17.6)	
Currently smokes	107 (20.3)	22 (21.0)	85 (20.2)	
Father smoking				
Never smoke	284 (54.2)	60 (58.3)	224 (53.2)	0.52
Ever smoke	95 (18.1)	19 (18.4)	76 (18.1)	
Currently smokes	145 (27.7)	24 (23.3)	121 (28.7)	
Children breastfeed				
No	93 (17.7)	21 (20)	72 (17.1)	0.48
Yes	433 (82.3)	84 (80)	349 (82.9)	
Daycare attendance				
No	222 (42.4)	40 (38.5)	182 (43.4)	0.35
Yes	301 (57.6)	64 (61.5)	237 (56.6)	
Paternal history of hay fever				
No	442 (91.9)	84 (89.4)	358 (92.5)	0.31
Yes	39 (8.1)	10 (10.6)	29 (7.5)	
Paternal history of eczema				
No	443 (92.1)	86 (91.5)	357 (92.2)	0.80
Yes	38 (7.9)	8 (8.5)	30 (7.8)	
Paternal history of asthma				
No	432 (89.8)	85 (90.4)	347 (89.7)	0.82
Yes	49 (10.2)	9 (9.6)	40 (10.3)	
Paternal history of allergies				
No	360 (74.8)	66 (70.2)	294 (76)	0.25
Yes	121 (25.2)	28 (29.8)	93 (24)	
Paternal history of any allergy				
No	333 (69.2)	61 (64.9)	272 (70.3)	0.31
Yes	148 (30.8)	33 (35.1)	115 (29.7)	
Maternal history of allergies				
No	359 (70.4)	66 (64.7)	293 (71.8)	0.16
Yes	151 (29.6)	36 (35.3)	115 (28.2)	
Maternal history of asthma				

No	469 (92.1)	88 (87.1)	381 (93.4)	0.04
Yes	40 (7.9)	13 (12.9)	27 (6.6)	
Maternal history of hay fever				
No	455 (89.2)	86 (84.3)	369 (90.4)	0.07
Yes	55 (10.8)	16 (15.7)	39 (9.6)	
Maternal history of eczema				
No	436 (85.5)	88 (86.3)	348 (85.3)	0.80
Yes	74 (14.5)	14 (13.7)	60 (14.7)	
Maternal history of any allergy				
No	310 (60.8)	57 (55.9)	253 (62)	0.25
Yes	200 (39.2)	45 (44.1)	155 (38)	
How long has the child living in the house				
Less than 3 years	139 (26.3)	30 (28.6)	109 (25.7)	0.25
3-6 years	123 (23.3)	18 (17.1)	105 (24.8)	
More than 6 years	267 (50.5)	57 (54.3)	210 (49.5)	
Cat in the home last 12 months				
No	332 (62.8)	72 (68.6)	260 (61.3)	0.17
Yes	197 (37.2)	33 (31.4)	164 (38.7)	
Dog in the home last 12 months				
No	306 (57.8)	72 (68.6)	234 (55.2)	0.01
Yes	223 (42.2)	33 (31.4)	190 (44.8)	
Cat in the home 1st year of life				
No	368 (72.0)	79 (78.2)	289 (70.5)	0.12
Yes	143 (28.0)	22 (21.8)	121 (29.5)	
Dog in the home 1st year of life				
No	360 (70.2)	79 (76.7)	281 (68.5)	0.10
Yes	153 (29.8)	24 (23.3)	129 (31.5)	
Maternal smoking while pregnancy				
No	406 (79.0)	77 (75.5)	329 (79.9)	0.33
Yes	108 (21.0)	25 (24.5)	83 (20.1)	

Table 2: Risk of atopy between the children from farming and non-farming residential status, stratified by sex

		Atopic	Non-atopic	P-value	OR	95% CI
Males	Non-farm	32 (51.6)	116 (53.7)			
	Farm	30 (48.4)	100 (46.3)	0.77	1.087	0.618, 1.914
Females	Non-farm	26 (65)	116 (56)			
	Farm	14 (35)	91 (44)	0.29	0.686	0.339, 1.390

Table 3: Risk of atopy between the children from farming and non-farming residential status, stratified by maternal, paternal history of any allergy/asthma

	Overall N (%)	Atopic N (%)	Non-atopic N (%)	P value
No maternal history of any allergy/asthma				
Non-farm	166 (55.3)	32 (59.3)	134 (54.5)	0.52
Farm	134 (44.7)	22 (40.7)	112 (45.5)	
Maternal history of any allergy/asthma				0.48
Non-farm	112 (54.6)	22 (50)	90 (55.9)	
Farm	93 (45.4)	22 (50)	71 (44.1)	
No paternal history of any allergy/asthma				0.69
Non-farm	173 (54.7)	32 (57.1)	141 (54.2)	
Farm	143 (45.3)	24 (42.9)	119 (45.8)	
Paternal history of any allergy/asthma				0.20
Non-farm	86 (53.4)	22 (62.9)	64 (50.8)	
Farm	75 (46.6)	13 (37.1)	62 (49.2)	
No parental history of asthma				
Non-farm	222 (54.8)	46 (60.5)	176 (53.5)	0.26
Farm	183 (45.2)	30 (39.5)	153 (46.5)	
Parental history of asthma				
Non-farm	40 (51.3)	8 (44.4)	32 (53.3)	0.50
Farm	38 (48.7)	10 (55.6)	28 (46.7)	
No parental history of any allergy				
Non-farm	129 (57.6)	24 (64.9)	105 (56.1)	0.32
Farm	95 (42.4)	13 (35.1)	82 (43.9)	
Parental history of any allergy				
Non-farm	145 (52.5)	32 (51.6)	113 (52.8)	0.87
Farm	131 (47.5)	30 (48.4)	101 (47.2)	
No parental history of any allergy/asthma				
Non-farm	125 (58.1)	22 (62.9)	103 (57.2)	0.53
Farm	90 (41.9)	13 (37.1)	77 (42.8)	
Parental history of any allergy/asthma				
Non-farm	152 (52.8)	34 (53.1)	118 (52.7)	0.95
Farm	136 (47.2)	30 (46.9)	106 (47.3)	

Appendix C

Acceptance to Annals of Allergy, Asthma and Immunology

Ref.: Ms. No. 14-04-0146R2

Prevalence and determinants of atopy and allergic diseases among school-age children in rural Saskatchewan, Canada

Annals of Allergy, Asthma & Immunology

Dear Dr. Lawson,

The Editor is pleased to inform you that your manuscript entitled "Prevalence and determinants of atopy and allergic diseases among school-age children in rural Saskatchewan, Canada has been accepted for publication in the Annals of Allergy, Asthma & Immunology. Please send completed authorship forms, from all authors, to the editorial office (if this has been done, we thank you. Duplicate submission is not necessary but we will inform you if we determine that we do not have a copy of your signed authorship form.) If needed, the authorship form can be found on the EES Log In page.

Authorship forms should be either faxed OR mailed to:

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Sincerely,

Gailen D Marshall, MD, PhD

Editor-in-Chief

Annals of Allergy, Asthma & Immunology