

Occupational Dairy Exposure and IgE-mediated Allergic Disease in Yakima, WA

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Abstract

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Background: Many studies have linked childhood farm exposures to a protective effect against allergy and asthma. Although continued exposure in adulthood may maintain this effect, little work has been done looking specifically at adult occupational exposures. This study focuses on dairy farming exposures in the Yakima Valley in Washington state, a region whose trends toward intensification of dairy farming and shifts to a primarily Latino work force represent the recent changes in the larger U.S. industry. Objective: The objective of this study is to evaluate the prevalence of allergic sensitization in dairy farm workers as compared to community controls. Methods: Total and cow epithelium specific IgE levels were measured from the serum of 41 dairy farm workers and 36 community controls. Relevant information regarding occupation, demographics, and allergy symptoms was obtained via a questionnaire. Linear regression methods were used to compare IgE levels between exposure groups and adjust for potential confounders. Additionally, the relationship between IgE levels and exhaled nitric oxide (eNO) was evaluated using linear regression and counts for allergy symptoms and asthma diagnosis were evaluated with Fisher's Exact Test. Results: There was no significant evidence for the association between dairy farm exposure and total IgE, but sensitization to bovine allergen was higher in dairy workers as compared to controls. Additionally, sensitization to

bovine allergen was associated with higher levels of eNO. Conclusion: No evidence for a protective effect of farm exposure on total IgE was found, but dairy workers may be at higher risk for sensitization to bovine aeroallergens and subsequent airway inflammation.

INTRODUCTION

Working on a farm involves exposures to a variety of microbial species and components, which may be either pathogenic or beneficial in different contexts. Microbes well-known as biological hazards and animal pathogens capable of causing gastrointestinal problems in humans, such as *Campylobacter*, *Cryptosporidium*, and *E. coli*, are abundant in animal husbandry settings. In addition, microbial components in organic dust are known to have negative effects on lung health in livestock farmers because of their pro-inflammatory properties.^{1,2} Respiratory health of workers may also be impacted by the exposure to animal dander, a known allergen, which over time may lead to allergic sensitization.³ The trend towards large, confined animal operations increases the risk of these hazardous exposures by increasing the concentration of exposures on farms.⁴

At the same time, an increasing amount of work on the “farm effect” shows that farm exposure may actually protect against allergy, hay fever, and asthma.⁵ These studies suggest that there may be immune benefits to certain microbial exposures.⁶ Recent immunological studies show that both resident microbiota as well as microbes in the environment may be vital for immune maturation and regulation.⁷ Although specific immune mechanisms underlying this “biodiversity hypothesis” remain uncertain, epidemiological studies show that time spent on dairy farms remains one of the strongest protective environmental exposures for allergy and asthma in children. A number of these studies also report lower levels of blood Immunoglobulin E (IgE), a measure of allergic sensitization, in these children.⁸ Although some studies in adult populations exist, the effect is less clear and may be limited to exposure in childhood

maintaining a protective effect in later life.⁹ It is important to note that farming practices differ by geographical region, and most of the cohort studies have been conducted on European farms. A better understanding of the role of farm exposures in the development allergy and asthma is needed for effective prevention and appropriate occupational health interventions for farm workers. This study aims to contribute to understanding the associations between dairy farm exposures and immune status in dairy farm workers in Washington state by using data from the Healthy Dairy Worker Project, which follows a cohort of dairy farm workers and community controls in the Yakima Valley.

BACKGROUND

Serum IgE levels are associated with allergy and asthma.

Many types of allergy and asthma result from IgE-mediated hypersensitivity reactions centered on the development of a pro-inflammatory T-helper type 2 cell (Th2) reaction.¹⁰ Consequently, levels of IgE in serum are associated with these diseases and reflect the amount of IgE bound to immune cells in the tissues where symptoms occur. A genetic predisposition for elevated IgE and resulting allergic disease is known as atopy, but environmental and immunological factors also contribute to disease in susceptible individuals.⁹ In the past 50 years, allergy and asthma have been on the rise in industrialized nations. As described by the “hygiene hypothesis,” societal changes towards improved sanitation have correlated with a rise in hypersensitivity reactions and autoimmunity.¹¹ In a healthy immune system, the Th2 response targets parasitic infections, but when these are lacking, this response may be wrongly directed against common substances in the environment, in the case of allergy, or the body itself, in the case of autoimmunity.¹¹

Dairy farm workers are exposed to biological hazards.

Within the developed world, animal husbandry settings are environments where human and animals are still exposed to “less hygienic” environments that contain fecal matter, organic dust, and animal dander. Organic dust contains substantial amounts of endotoxin (lipopolysaccharide

or LPS), peptidoglycans, and (1-3)- β -D glucans from gram-negative bacteria, gram-positive bacteria, and cell wall components of molds, respectively.¹² These have been shown to induce chronic respiratory illness through their pro-inflammatory properties, although these seem largely dependent on dose, duration, and frequency of exposure.¹ The kind of exposure mainly depends on the farming practices and size of dairy farms, and can also be variable within a farm. Studies suggest that dairy farmers in Denmark are exposed to organic dust and endotoxin concentrations averaging between 0.8 and 2.4 mg/m³ and 220 and 1500 EU/m³ respectively.¹³ These exposures can cause organic dust syndrome and may worsen IgE-mediated diseases in susceptible individuals.

Biological exposures may have beneficial effects on training the immune system.

The microbial components described above are also implied in the “hygiene hypothesis” or “farm effect,” both of which suggest that microbes trigger a protective immune effect against allergic sensitization and allergic asthma in populations exposed to farming environments. The main studies supporting the immune benefits of growing up on a farm were done using large pediatric cohorts on small-scale, family-run European farms. These studies show lower prevalence of asthma and sensitization to a mix of common allergens (including dust mites, birch, timothy, cat, dog, horse, molds, and food allergens) in children growing up on farms. In addition, recent work in these cohorts show associations between farm exposures and lower total IgE as well as immune shifts towards production of Th1 and immunoregulatory cytokines.^{14,15} Previous work suggests that the protective effect may be timing-dependent, with the greatest effect occurring in utero or early life.⁵ It is not clear whether exposures in adulthood act alone or in combination with earlier exposures to contribute to an individual’s risk of allergic disease.⁹ One study, conducted in northern Germany, found that if adult farm exposure followed early life exposure, it did not further decrease risk of sensitization to common allergens. It also suggests that the lower prevalence of sensitization in adults was due to self-selection out of farming for allergic individuals and that starting exposure in adulthood may actually increase the risk of sensitization.¹⁶

The microbiome may contribute to the “farm effect” through immune training.

It is important to consider the biological plausibility of the “farm effect” described by epidemiological observations. Microbes and microbial components that influence the immune system can be found both within the human body (the microbiome) or in the environment. They modulate the immune system through interaction with pattern-recognition receptors on innate immune cells and the priming of T cells through the production of short-chain fatty acids.¹⁷ Recent advances in describing microbiota communities has enabled connecting immunological disorders, such as asthma and allergy, to the microbiome. Establishment of the microbiome and the training of the immune system both occur in early life but can be modified throughout the lifespan by alterations in diet, antibiotic administration, change of geographical location, as well as other environmental exposures.⁸ With increasing work in this area, the idea of the “hygiene hypothesis” has been replaced with the “biodiversity hypothesis” that states that it is not the lack of exposure to microbes, but rather the lack of microbial diversity that impairs immune training. Additionally, recent studies have shown that children living in rural environments also have a protective advantage over those living in urban environments with lower microbial diversity.^{8,17}

Long-term exposure to animal dander leads to allergen-specific sensitization.

In addition to microbes, dairy farm workers are exposed to high concentrations of animal proteins from animal urine, hair, saliva, blood, and amniotic fluids. These are high-molecular weight proteins with allergenic properties that induce IgE-mediated rhinitis, hives, asthma, and anaphylaxis. Increasingly, farm animals that produce large amounts of these allergens, such as cows and horses, are kept in larger herds and in more confined spaces, which increases the concentration of these proteins in the farm working environment. Although some studies have looked at the concentration of bovine allergen in dust samples collected on farms, results differed widely between farms with estimates of cow hair and epithelium in dusts ranging from 4,760 to 559,400 $\mu\text{g}/\text{m}^2$.^{3,18}

Atopy, a predisposition to allergic disease that manifests most commonly as childhood eczema, hay fever, food allergy, or allergic asthma,¹⁹ is an established risk factor for high sensitization to

cow epithelium.²⁰ Another main risk factor for sensitization to animal allergens is long-term, continuous exposure to that animals that shed that allergen. Over time and repeated exposures, effector immune cells are primed with IgE antibody, which mediate allergic responses and diseases. Although hair and dander are the primary bovine allergens, sensitization to proteins from urine, hair, saliva, and amniotic fluid can also occur.⁷ They can be found in air-borne dust, settled dust, farmer's clothes, and even in the homes of farm employees, which exposes other members of the family. Recent studies on dairy farms in Scandinavian countries suggest that anywhere from 5 to 20% of dairy farmers may be sensitized to bovine allergen.⁷

Quantifying allergen-specific IgE in serum is one measure of sensitization to an allergen. In a case report of two dairy farmers with debilitating labor-associated allergy symptoms, it was shown that both had elevated total serum IgE (383 and 477 U/ml) and elevated levels of bovine-specific IgE to cow hair and dander (20 and 36 U/ml).²¹

Bos d 2 is the most common bovine aeroallergen

In contrast to common allergens, such as dust mites, cat, dog, and rodent proteins, far less is known about the characteristics, such as composition and potency, of bovine allergens. Studies in the 1980s identified seventeen different bovine proteins as having allergenic potential, including: Bos d 1, Bos d 2 (a lipocalin), Bos d 3, Bos d 4 (alpha-lactalbumin), Bos d 5 (beta-lactoglobulin), Bos d 6 (serum albumin), Bos d 7 (immunoglobulin), and Bos d 8 (caseins).³ Of these, the lipocalin Bos d 2 (MW 20 kDa) is the major respiratory allergen.³ Lipocalins are produced in an animal's sweat glands and serve as a carrier of pheromones. They are a common class of allergen for other types of animal allergy, such as the more common cat, dog, and rodent allergies.

Risk of asthma for dairy workers

Sensitization to animal allergens is known as an important risk factor for the development of asthma, the most common occupation lung disease in high-income countries.²² In the U.S., 3 to 15% of asthma is due to occupation.²³ It is characterized by airflow obstruction, airway hyperresponsiveness, and airway inflammation, which manifests with symptoms including cough, wheeze, sputum production, and chest tightness. Dairy farmers are at high risk for lung inflammation because of exposure to aerosols that contain a mixture of many different things in

manure, animal dander, animal feed, pesticides, diesel exhaust that can either induce or exacerbate asthma.²⁴ They also work long shifts, often more than 5 days per week. Therefore, they are exposed often to high concentrations for prolonged periods of time.

Not all asthma is due to atopic disease, however one important pathophysiology for “atopic” or “allergic” asthma is chronic inflammation as a result of being exposed to allergens for which the individual is sensitized.²⁵ The inflammation can then cause acute allergen-mediated airway constriction. Therefore, elevated levels of specific IgE to common allergens or total IgE in blood is strongly associated with asthma. Recent studies suggest the importance of specific allergies, such as those to house mites or fungus, as having a distinct relationship with more severe asthma. Little work has been done looking at the role of bovine sensitization specifically. It is also important to note that not all sensitized individuals show symptoms or allergy or develop asthma.

Elevated exhaled nitric oxide (eNO) is associated with asthma

Nitric oxide is a gaseous molecule formed by various cell types in the lower airways, which can be used clinically as a marker of airway inflammation in the diagnosis of asthma as well as other airway diseases. Previous studies report that elevated levels of eNO can be a consequence of chronic exposure to inflammatory stimuli, such as animal allergens, and subsequent sensitization to those allergens.²⁶ Allergen exposure may be driving the inflammatory processes that contribute to both asthma and the production of eNO.

The association between animal allergy and eNO has been studied in laboratory animal workers, individuals who suffer from work-induced allergies and asthma to animals commonly used in animal research such as rodents, rabbits, dogs, and non-human primates. In one study, eNO levels were raised in laboratory animal workers who showed symptoms of allergy, with an increasing trend in eNO levels from asymptomatic, to mild allergy symptoms, to asthma.²³ That study concluded that raised eNO levels increased symptoms severity in laboratory animal workers with allergic disease and that eNO may become a useful tool for monitoring disease in animal workers.

The need for the Healthy Dairy Worker Study and attention to allergic disease

This study focuses on dairy farming exposures in the Yakima Valley in Washington state, a region whose trends toward intensification of dairy farming and shifts to a primarily Latino work force represent the recent changes in the larger U.S. industry.²⁷ Since many of the larger studies on the hygiene hypothesis were done using pediatric cohorts, there is much more debate on the continuation of such an effect into adulthood.

In addition, very few studies have looked at sensitization specifically to cow epithelium in U.S. dairy worker cohorts, even though allergic sensitization to occupational allergens is an important public health concern that can reduce quality of life, limit productivity at work, and increase the cost of health care.²⁸ Understanding the effects of the current dairy exposures on the immune status of workers is crucial to understanding ways to make their work environment safer and healthier.

SPECIFIC AIMS

Primary Aim: To investigate differences in allergic disease between dairy farm workers and community controls by: 1) (aim) determining if there is an association between dairy farm exposure and total serum IgE, and 2) (sub-aim) determining if there is a difference in the reporting of allergy symptoms or asthma diagnosis between exposed dairy workers and unexposed community controls.

Secondary Aim: To determine if there is a difference in the sensitization to cow epithelium between dairy farm workers and community controls.

Tertiary Aim: To further investigate the association between between IgE levels and exhaled nitric oxide by, 1) determining if the association between total IgE and eNO is modified by occupational exposure to dairy farming, and 2) if there is an association between elevated bovine IgE and eNO, especially in dairy farmers.

Hypotheses:

The hypothesis for the primary aim is based on the idea of the hygiene hypothesis that the increased microbial exposures on a dairy farm may have immune benefits that are protective against allergic disease. Therefore, I expect lower total IgE levels and lower reporting of allergy symptoms as compared to community controls to reflect lower rates of sensitization to common allergens.

I hypothesize that due to long, frequent, high-dose exposures to cows, dairy farm workers may have increased levels of bovine-specific IgE as compared to community controls.

Finally, because sensitization contributes to higher levels of eNO in people who are exposed to allergens to which they are sensitized, I expect that there is a positive association bovine-IgE levels and eNO. I also expect to confirm the previously described association between total IgE and eNO.²⁹

METHODS

Study Design

I performed a cross-sectional analysis of baseline data from an ongoing longitudinal cohort study, the Healthy Dairy Worker Study, of dairy farm workers and community controls that is designed to investigate health outcomes associated with occupational exposure to the dairy farm environment.

Research Setting and Participant Characteristics:

With the help of the Washington State Dairy Federation, Washington State University Outreach officers, and Pacific Northwest Agricultural Safety and Health Center researchers, large dairy farms, with over one thousand cows each, were identified in Yakima County, Washington. Three farms served as sites for recruitment of occupationally exposed dairy workers after their owners agreed to study involvement. Although farm owners and managers allowed the enrollment of workers, they were not involved in the recruitment. The choice to participate was made by

individual workers after being screened for eligibility, being informed of study protocols, and consenting to collection of samples and personal information.

Dairy workers were recruited pre/post work shift on the farms. Their eligibility for participation was based on 1) being currently employed and working at the farm and 2) a willingness to provide samples and participate in the collection of data through questionnaires, blood draws and eNO measurements, and follow-up visits.

Community controls were recruited using contacts from people in Yakima County communities that had previously participated in research studies and snowball sampling, a method through which dairy workers spread the word about the study and recruited people they knew in their community. Their eligibility for participation was based on 1) not currently working on a dairy farm and not having worked on a farm within the last 5 years, 2) not living on a dairy farm, and 3) not living with someone who works on a dairy farm. The community controls oversampled males as compared to females to provide adequate controls for the exposed dairy workers, who are mostly male.

At the time of this analysis, 77 participants had been recruited, including 41 farm workers and 36 community controls.

Participants were enrolled between May 2017 and January 2020 and interviewed by research staff to collect demographic and health information at baseline, 12 months, and 24 months. At each of these time points, they were also asked for a blood sample for detection of Immunoglobulin E (IgE) levels and a measurement of exhaled nitric oxide (eNO).

The Healthy Dairy Worker Study protocols were reviewed and approved by the University of Washington Human Subjects Review Committee (IRB) before the study began. Research staff were trained in the protection of human subjects and securing the privacy of their health information as stated in the Health Insurance Portability and Accountability Act (HIPAA) and participants received a stipend for each visit.

Questionnaire

Both dairy farm workers and community controls completed a questionnaire to collect information on demographics, smoking status, time working with animals, and current health status related to allergies. It was administered by trained research staff, community health outreach workers with a history of trusting relationships with the people of Yakima County. The questionnaire had both English and Spanish question options, and the research staff is bilingual to accommodate for the primarily Hispanic population.

The questionnaire data was recorded in via tablets and managed by Research Electronic Data Capture tools (REDCap) hosted by the University of Washington. All analyses are based on data extracted from REDCap from 77 participants on January 28, 2020.

Assessment of Immune Status:

Total IgE and bovine-specific IgE was measured in all serum samples collected from dairy farm workers and community controls within a few days of their completion of the questionnaire. Blood was drawn at local clinics (Yakima, Sunnyside, or Pasco) by trained phlebotomists. It was then centrifuged (20 min at 1300 g at room temperature) within 4 hours of collection, serum was collected, and serum aliquots were frozen and kept at -80 degrees until analysis at the UW clinical laboratory.

Both total IgE and bovine-specific IgE were measured at University of Washington clinical laboratories by radioallergosorbent assays (Phadia CAP System IgE FEIA for total IgE, ImmunoCAP System Specific IgE FEIA for IgE specific to cow epithelium) and reported in units of antibody per milliliter (U/ml). Bovine-specific IgE specifically measured IgE to cow epithelium, the most common bovine aeroallergen.

Data Cleaning:

Questionnaire data was downloaded from REDCap (Research Electronic Data Capture) managed by the Center for One Health Research. Immunoglobulin levels were measured by the UW clinical laboratory and stored in Excel spreadsheets, which were manually merged with the REDCap data. All further data cleaning was performed in R Software version 3.6.1 using

tidyverse packages. All missing entries from the REDCap download were treated as missing data and any repeated entry was deleted so that it didn't count twice in the analysis. Incorrect data entries in RedCap were corrected after careful review by research staff.

Analyses

All data analyses were performed in R Software version 3.6.1 using the following packages: basic R and uwIntroStats.

Descriptive Statistics:

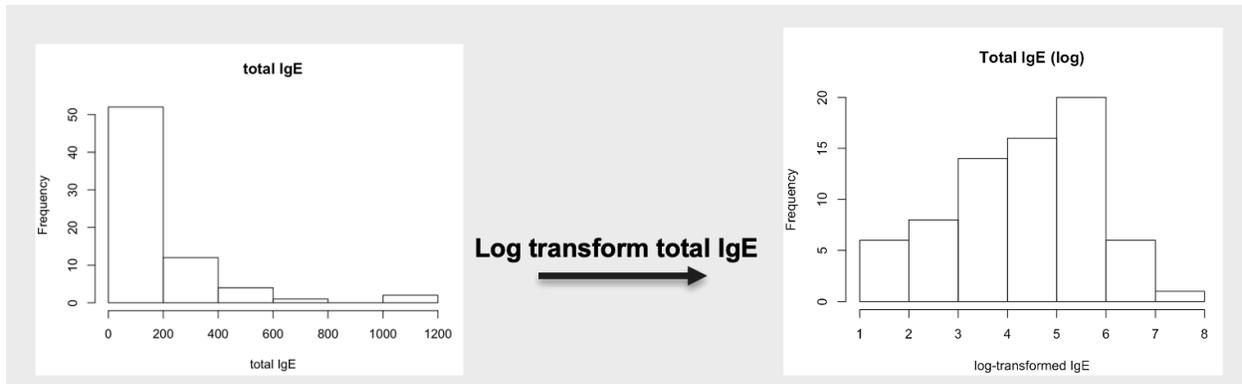
Descriptive analysis of questionnaire data was performed, including univariate analysis of continuous variables and frequencies of binary and categorical variables for key covariates. In addition, outcomes of total IgE and eNO are described before and after log (base e) transformation.

Analysis - Primary Aim – Part I

Determinants of Total IgE

The primary analysis investigated the association between occupational dairy farm exposure and total IgE, including as covariates other predictors of total IgE. This was done using cross-sectional data and linear regression methods using robust standard errors. Whereas classical standard errors require strong assumptions of homoscedasticity and normal distribution of the outcome of interest at any value of the predictor, robust standard errors rely solely on the assumption that the data are independent and from a random sample of the population and allows for more relaxed assumptions of the linear regression. As a result, robust standard errors tend to be larger than conventional standard errors. The distribution of the outcome of interest, total serum IgE levels, is right-skewed, and therefore was log-transformed prior to analysis to evaluate a linear relationship between predictor and outcome.

Figure 1: log transformation of continuous outcome, total serum IgE



Three linear regression models were used to investigate the association between dairy farm exposure and total IgE levels: 1) unadjusted model, 2) *a priori* adjusted model, and 3) extended adjusted model. The continuous outcome of interest in all models is the log-transformed total IgE levels. The unadjusted model includes only dairy farm exposure as the main predictor. The *a priori* model considers the additional covariates of age and smoking. Previous studies have shown a strong negative correlation between total IgE levels and age and a positive correlation between smoking and total IgE levels.³⁰

Potential confounders that will be explored in the extended model include growing up on a farm, owning a pet, and the season in which the blood was collected. Growing up on a farm has previously been shown to be associated with decreased total IgE levels.⁵ The directed acyclic graph (DAG) in Figure 2 displays the assumptions made about the relationships between variables.

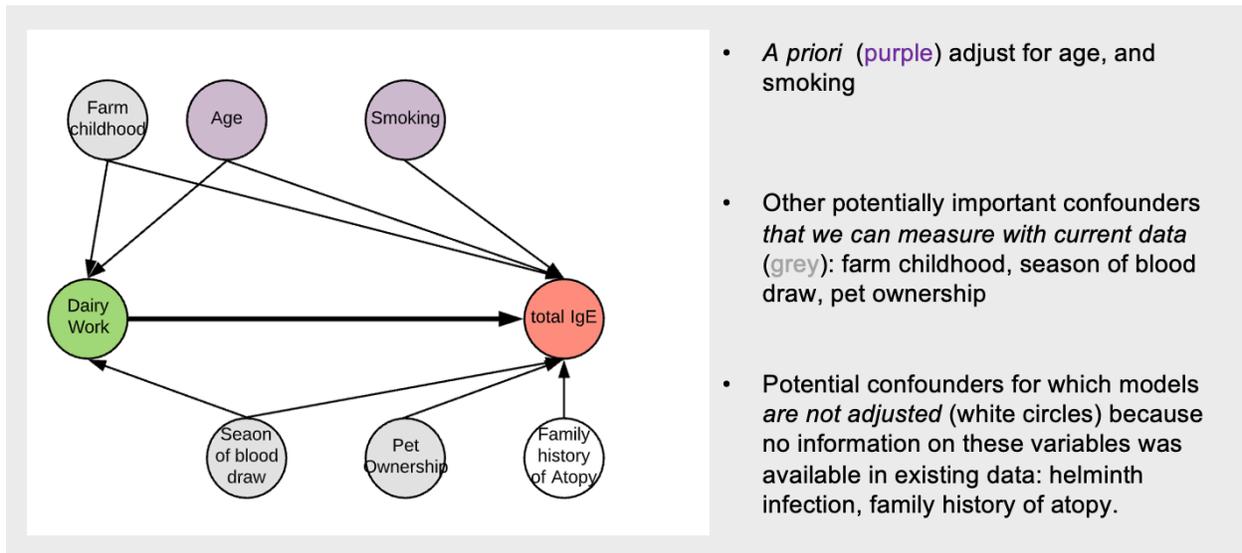
$$\text{Model: } E(\log \text{ total IgE} \mid \text{farm exposure}) = \beta_0 + \beta_1 X_A + \beta_2 X_B + \beta_3 X_C \dots \beta_K X_K$$

where β_1 is the regression coefficient for the **main effect** of interest, **dairy farm exposure** (yes/no), β_{2-K} are regression coefficients for other covariates in the model, X_A has a value of 0 for community controls and 1 for farm workers, and $X_{B,C,K}$ are values of the covariates in the model

Null hypothesis: $\beta_1 = 0$

Alternate hypothesis $\beta_1 \neq 0$

Figure 2: DAG displaying relationship between variables



Sensitivity analysis with binary total IgE outcome

Total IgE levels were dichotomized into those greater than and less than the threshold for clinically elevated levels of 300 U/ml. Logistic regression was then used to examine the odds of total IgE levels higher than 300 U/ml with dairy farm exposure, *a priori* adjusting for age and current smoking status. This was repeated for a 100 U/ml threshold, as there is some debate on the most appropriate threshold to use.

$$\text{Logit}(p) = \log(p/1 - p) = \log \text{odds} = \beta_0 + \beta_1 X_A + \beta_2 X_B + \beta_3 X_C \dots \beta_K X_K$$

$$\text{Odds of IgE} > 300 \text{ U/ml} = e^{\beta_0} \times e^{\beta_1 X_A} \times e^{\beta_2 X_B} \times e^{\beta_3 X_C}$$

where β_1 is the regression coefficient for the **main effect** of interest, **dairy farm exposure** (yes/no), β_{2-K} are regression coefficients for other covariates in the model, X_A has a value of 0 for community controls and 1 for dairy farm workers, and $X_{B,C,K}$ are values of the covariates in the model (age and current smoking status).

Analysis - Primary Aim – Part II

Difference in the reporting of allergy symptoms and asthma diagnosis

Two count tables were made to report the number of dairy farm workers and community controls who: 1) did and did not report allergy symptoms, 2) did and did not report having ever been diagnosed with asthma by a physician. Fisher’s Exact Test was used to test for a statistical

difference in the proportions of reporting between dairy workers and community controls for each count table. In addition, logistic regression analysis was used to adjust for age and current smoking status in the analysis of the odds of reporting allergy symptoms based on exposure to dairy farming.

Analysis - Secondary Aim

Dairy exposure predictor, bovine-specific IgE levels outcome

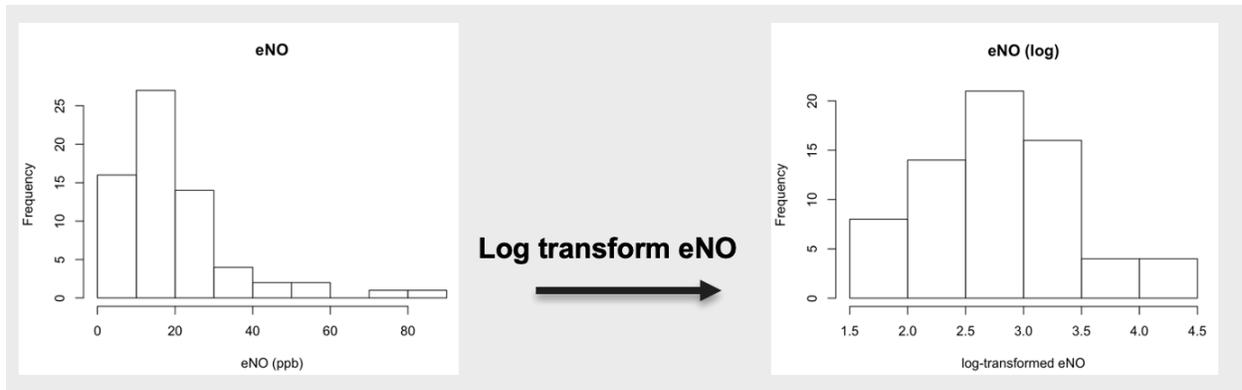
Bovine-specific IgE measurement values were dichotomized into greater than and less than the threshold of 0.35 U/ml, below which IgE measurements are clinically considered negative for sensitization to cow epithelium.³¹ To address the secondary aim regarding differences between dairy farm workers and community controls with respect to bovine IgE levels, the Fisher's Exact Test was used to find out if the proportion of dairy farm workers and community controls with elevated bovine-specific IgE was different.

Analysis - Tertiary Aim

IgE predictor, eNO outcome, and dairy exposure as potential effect modifier of their association

A previous DEOHS Master's thesis (Jose Carmona, 2019) showed that dairy farm workers had lower eNO as compared to community controls. In addition, previous studies show an association between eNO and total IgE.²⁹ With log-transformed (base e) eNO as the outcome of interest, cross-sectional baseline data was used in a linear regression with robust standard errors to investigate whether dairy farm exposure modified the effect of IgE levels on eNO levels. The predictor, total IgE levels was log (base 2) transformed so that the relationship between exposure and outcome could be considered as the fractional difference in eNO for a 2-fold (doubling) in total IgE.

Figure 3: log transformation of continuous outcome, eNO



Binary bovine IgE predictor, eNO outcome

The same binary bovine IgE predictor described above (above/below 0.35 U/ml) was used to determining if there was an association between elevated bovine-specific IgE levels (above 0.35 U/ml) and eNO levels using a linear regression with robust standard errors. As in the analysis with total IgE, eNO was log-transformed.

RESULTS

Provided in Table 1 are descriptive statistics of dairy farm workers and community control individuals at the time of enrollment. The average age of dairy workers was, on average, almost 6 years younger than community control subjects. Notably, dairy workers enrolled in the study were predominantly male, which is representative of the larger dairy industry workforce. Only one female dairy worker participated in the study. Most dairy workers grew up on a farm, indicating that they were exposed to dairy environments prior to the occupational exposure of interest in this study. Due to the timing of recruitment efforts, dairy workers and community controls were enrolled at different times of the year, which correspond to different allergy seasons. Whereas the majority of dairy workers were enrolled (and had their blood drawn) between April and September, months in which pollen counts are higher in Yakima County, the majority of community controls had their blood drawn during winter months. Season may also be relevant in terms of shedding for cows, wildfire season, and wet/dry season in Yakima. Dairy

farm workers had an average of 11.5 years working with animals, although the range included newly hired workers with less than 1 year of experience to a worker with 26 years of experience. The median time working with animals was 12.5 years. Longitudinal data was incomplete for both dairy workers and community controls (Table 2), but more community controls were lost to follow-up than dairy workers. Correlation between individual covariates and total IgE levels are reported (Table 4). None of the associations are statistically significant at an alpha level of 0.05, but the p-value for the association with age is 0.06.

The primary analysis did not find a significant association between dairy farm exposure and total IgE levels (Table 5), which agrees with our less formal, descriptive analysis in Table 3. Based on the confidence interval from the *a priori* linear model, the true difference in total IgE would likely be between 0.46 and 2.04 times higher for dairy farm workers as compared to community controls. A significant association between age and total IgE was found ($p = 0.03$), which gives additional evidence for the strong, previously reported relationship of lower levels of total serum IgE with increased age. Logistic regression analyses with total IgE thresholds of 300 U/mL and 100 U/mL (Table 6), serving as sensitivity analyses, contributed more evidence that dairy farm exposure and total IgE were not associated.

Although not part of the primary aim, the models in Table 5 suggest a significant association between age and total IgE levels, which adds evidence for lower levels of total serum IgE levels with increased age. In the extended model (including covariates for age, smoking, pet ownership, dairy exposure, and season), the association between farm childhood and total IgE was statistically significant, estimating that total IgE is 3.31 times higher for individuals who grew up on a farm as compared to those who did not. This does not agree with the correlation analysis on this covariate in Table 4.

Although no evidence was found for an association between dairy exposure and total IgE levels, there was a significant difference in the reporting of allergy symptoms ($p = 0.004$) between dairy workers and community controls. While only 10% (4/40) of dairy workers reported symptoms of allergy, 40% (13/32) community controls reported symptoms (Table 7a). This finding was still significant when investigated with a logistic regression to control for age and current smoking

(Table 7b). The difference in the proportion of dairy farmers and community controls who reported an asthma diagnosis was not statistically different with a Fisher's Exact test (p -value = 0.722) (Table 8). The four dairy farmer workers who reported having a diagnosis of asthma had eNO levels of 11, 28, 73, and 27 ppb. Of these, the individual with eNO levels of 73 ppb also had elevated bovine-specific IgE.

The difference in the proportion of dairy farmers and community controls with elevated bovine-specific IgE (above 0.35 U/mL) was not statistically significant with a Fisher's Exact test (p -value = 0.12), but the count table (Table 9a) indicates that 4 dairy farm workers had levels above 0.35 U/mL whereas no community controls had elevated levels above this clinically-significant threshold. Additionally, when repeated with a lower threshold (0.2 U/mL), it was significant and 7 dairy workers were positive for elevated bovine IgE (p -value = 0.016) (Table 9b).

The linear regression analysis of total log-transformed eNO levels and total IgE did not show a significant association between total IgE levels and exhaled nitric oxide levels. In evaluating the evidence that an association between total IgE and eNO levels would be different for those exposed to dairy farming, the interaction between dairy work and total IgE was not significant. (p -value = 0.43) (Table 10). However, the association between bovine-specific IgE and eNO was significant. The model estimates that those with elevated bovine IgE have eNO levels 2.49 times higher than those with bovine IgE levels below 0.35 U/ml (p = 0.006), although it would not be surprising if the true value of this difference was between 1.3 times and 4.7 times higher (Table 11a). When the same analysis was restricted only to dairy workers, the estimate was 2.13 times higher, but it did not achieve statistical significance (p -value = 0.07) (Table 11b). The eNO levels of the four dairy farm workers with elevated bovine IgE were 13, 23, 49, and 73 ppb, of which the worker with the highest eNO levels also had a diagnosis of asthma.

DISCUSSION

The study set out to investigate the hygiene and biodiversity hypotheses with respect to a potential protective effect of occupational dairy farming on total serum IgE levels. Although

there is no evidence from linear regression analyses for an association between dairy farm exposure and total serum IgE levels, there was a significant difference in the proportion of dairy farm workers and community controls that reported symptoms of allergies with a higher proportion of community controls reporting symptoms.

The secondary analysis suggests the potential impact of occupational dairy exposure on the prevalence of work-related sensitization to cow epithelium. Although the difference in the proportion of dairy farm workers and community controls with elevated bovine-specific IgE was not significant when using the commercial test's recommended threshold (0.35 U/ml), it is important to note that the four subjects with elevated bovine IgE were all dairy workers. Of 31 community controls included in this analysis, none had elevated bovine IgE. An elevated bovine-specific IgE level (above 0.35 U/ml) is one indication of immune response to the cow epithelium allergen and increases the likelihood development of allergic disease in the presence of cows.¹³ Additionally, one study proposes that commercially-available tests lower the cutoff level for sensitization to 0.2 U/ml as compared to the 0.35 U/ml.³¹ Based on this threshold, the prevalence of bovine sensitization for the participants in our study would be much higher and significant.

Additionally, sensitization to animal allergens is a major risk factor for asthma, and many individuals with asthma have elevated eNO levels. The data in this study suggest a significant association between bovine-specific IgE and eNO and estimates, on average, higher eNO levels with elevated bovine-specific IgE. According to the American Thoracic Society Clinical Practice Guideline, eNO levels less than 25 ppb in adults implies the absence of eosinophilic airway inflammation, values between 25 and 50 should be interpreted cautiously, and values above 50 ppb indicate airway inflammation. The four dairy farm workers with elevated bovine-specific IgE had values of 13, 23, 49, and 73 ppb. Two of those are close to or above the 50-ppb cut-off that indicate inflammation. Of note, the dairy worker with the eNO level of 73 ppb also reported a diagnosis of asthma.

It is especially important to note this relationship between sensitization to bovine IgE and elevated eNO as workers on a dairy farm are continuously exposed to bovine allergen. Previous

studies show that sensitization to an allergen followed by continued exposure to that allergen is associated with higher levels of eNO whereas sensitization without exposure is not. Therefore, there are two opportunities to protect workers: 1) to prevent sensitization to the allergen, 2) to reduce subsequent exposures, if they do get sensitized.

It is especially important for sensitized individuals to know that they are at risk so that they can take extra precautions to prevent the progression from sensitization to airway inflammation and asthma. Sensitization to animal allergens is known to be common in occupations other than dairy farming that involve working in close proximity with animals, such as veterinary medicine, zoo keeping, and laboratory animal research. Most of the current understanding of these allergies comes from studying laboratory animal workers. Because of the high prevalence of sensitization to animals in this group of worker, employers in research settings that use animals are required to establish and maintain occupational health and safety programs that educate employees on the risk of sensitization, screen employees for symptoms indicating sensitization, and prevent existing cases of sensitization from worsening.³² This is not common practice on U.S. dairy farms, but the prevalence of sensitization to cow epithelium among dairy workers in this study suggests that they may benefit from similar programs. Modifications to the work environment, job duties, and personal behavior as part of a program would potentially reduce a worker's exposure to cow epithelium at work.

The prevalence of sensitization to cow epithelium in this study, determined to be 10% (4 out of 40 dairy workers had elevated bovine IgE), may reflect the higher rates of sensitization reported for animal allergies over the last two decades. Between 1990 and 1999, studies in European countries suggested the prevalence of bovine allergen sensitization among dairy farmers was relatively low, between 2 and 5%.¹³ More recent studies in Scandinavian countries suggest a range of 5 to 20%.²² The prevalence of sensitization to laboratory animals in the U.S. is currently estimated to be between 10 and 55%.²⁸ The wide range of these estimates suggests that although animal allergens are ubiquitous in animal environments, the concentrations vary widely based on type of facility, geographical location, and species as well as breed of the animal.

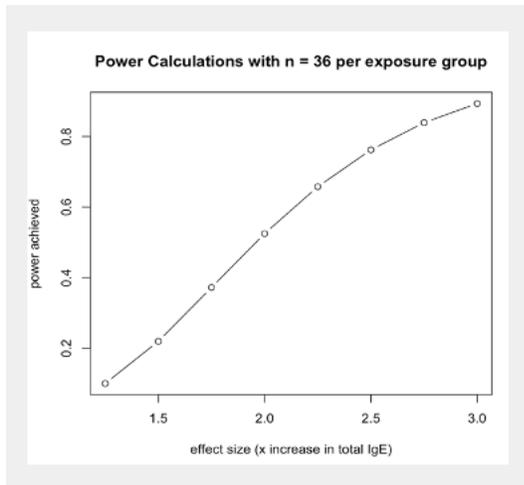
In the U.S. dairy industry, there is a lack of information regarding sensitization to cow allergens and this study was one of the first to contribute to the understanding of the current prevalence on industrial-scale dairy farms. Additionally, the association between elevated bovine IgE and eNO highlighted the need to address this issue of sensitization to cows in order to protect the health of dairy farmers.

LIMITATIONS

The biggest limitation in the primary analysis, the association between dairy farm exposure and total IgE, was the small sample size of the pilot study. The resulting confidence intervals around point estimates were quite large. Although a larger sample size may not give significant results, as there may be no difference between total IgE levels between exposure groups, it would provide a more precise estimate of the true association. It is important to consider that to achieve 80% power with $n=36$, the effect size would have required an effect size greater than 2.5-fold difference in total IgE between dairy workers and controls (Fig 4). Even if dairy workers do have lower total IgE than community controls, a difference this large is not what I would predict.

The significant finding of lower allergy reporting in dairy farm workers may be influenced by some limitations of the study arising from the potential for bias due to self-reported data and the healthy worker effect. Dairy workers may be healthier, both generally and in respect to allergies, than community controls in order to stay in their current job. Additionally, the controls recruited for the study were not the most appropriate controls. They were not necessarily workers, and those who were also worked in the agricultural sector. A more appropriate group of controls may have been an occupational cohort from an occupation known to have no exposure to livestock.

Figure 4: Power calculation showing power achieved for different effect sizes with a sample size of $n = 36$



Also, only two indicators of allergic sensitization were measured, total and cow epithelium IgE. Measures of sensitization to specific common allergens, such as grasses, house mites, cats, and dogs are often used as a measure of allergic disease in the context of the hygiene hypothesis when total IgE levels are inconclusive. Breaking down total IgE into specific-IgE components would have shed light on the drivers of IgE level and possible differences between dairy farm workers and community controls in respect to these. It is also possible that subjects were sensitized to an allergen not measured.

In addition, commercially-available tests for cow epithelium, like the one used in this study to measure bovine-specific IgE, may not be sensitive enough to always correctly identify individuals with sensitization to cow hair and dander allergens. A study in Germany showed that 32% of dairy farmers with cattle-related allergy symptoms and a clinical history indicative of animal allergy showed negative results with commercial RAST tests while showing reactivity with cow allergen in immunoblotting experiments.³¹ The same group proposes that commercially-available tests lower the cutoff level for sensitization to 0.2 U/ml as compared to the 0.35 U/ml used in this analysis, which was used in a sensitivity analysis (Table 9b).

Another key limitation of the study was the large amount of missing data, which may be due to turnover in research staff, changes in reporting protocols, and/or the length of the survey.

STRENGTHS

The pilot study provided a start to building a relationship between the research group and workers, dairy managers and owners, and local community members. Also, it is nested within a larger study, the Healthy Dairy Worker Study, which will allow results to be connected to studies of lung function and microbiome shifts for a better understanding of worker health. It is also unique in reporting the prevalence of sensitization of cow allergens in the dairy industry, a measure that can directly impact the creation of appropriate occupational health programs for dairy farms.

DIRECTION FOR FUTURE WORK

Because sensitization to animal allergen mostly happens within the first 3 years of occupational exposure, with the incidence of allergic rhinitis and asthma highest within the first year, longitudinal studies of newly hired workers would be valuable. It is hard for occupational studies to start early enough to really capture workers when they are still unexposed. Following newly hired dairy workers from unexposed to exposed would help to understand sensitization over time. Also, re-doing the primary analysis with more subjects would improve the ability to detect an association between dairy farm exposure and total IgE. At the same time, measuring sensitization to common allergens would increase the ability to look at a potential protective effect of the exposure. It would be interesting to look into the interaction between the different inflammatory exposures on a farm (endotoxin, allergens, and gases) and their contribution to allergic and airway diseases.

In addition to understanding the prevalence, incidence, and mechanism of allergic sensitization and disease in dairy workers, future studies should be done on the effectiveness of potential control measures that could be used to protect workers from sensitization.

CONCLUSION

Although there was no evidence for the association between dairy farm exposure and total IgE, the study found that more dairy workers were sensitized to cow epithelium than community controls. Additionally, sensitization to bovine allergen was associated with higher levels of eNO, suggesting that these workers may be at higher risk for asthma.

TABLES

Table 1: Descriptive characteristics of farm workers and community controls at baseline

	Dairy Farm Workers (N=41)	Community Control (N=36)	Total (N=77)
Age			
Mean (SD)	37.3 (7.04)	43.0 (13.4)	39.9 (10.8)
Median [Min, Max]	38.0 [19.0, 53.0]	42.0 [18.0, 75.0]	39.0 [18.0, 75.0]
Missing	0 (0%)	1 (2.8%)	1 (1.3%)
Gender			
Male	40 (97.6%)	22 (61.1%)	62 (80.5%)
Female	1 (2.4%)	5 (13.9%)	6 (7.8%)
Missing	0 (0%)	9 (25.0%)	9 (11.7%)
Farm Childhood			
Yes	37 (90.2%)	20 (55.6%)	57 (74.0%)
No	4 (9.8%)	10 (27.8%)	14 (18.2%)
Missing	0 (0%)	6 (16.7%)	6 (7.8%)
Pet Ownership			
Yes	23 (56.1%)	22 (61.1%)	45 (58.4%)
No	17 (41.5%)	10 (27.8%)	27 (35.1%)
Missing	1 (2.4%)	4 (11.1%)	5 (6.5%)
Season of blood draw			
April-September	31 (75.6%)	8 (22.2%)	39 (50.6%)
October-March	10 (24.4%)	28 (77.8%)	38 (49.4%)
Current Smoker			
Yes	13 (31.7%)	7 (19.4%)	20 (26.0%)
No	27 (65.9%)	25 (69.4%)	52 (67.5%)
Missing	1 (2.4%)	4 (11.1%)	5 (6.5%)
Time Working with Animals (Years)			
Mean (SD)	11.5 (7.33)	NA (NA)	11.5 (7.33)
Median [Min, Max]	12.5 [0.130, 26.0]	NA [NA, NA]	12.5 [0.130, 26.0]
Missing	2 (4.9%)	36 (100%)	38 (49.4%)
Allergies Symptoms (self-reported)			
Yes	4 (9.8%)	13 (36.1%)	17 (22.1%)
No	36 (87.8%)	19 (52.8%)	55 (71.4%)
Missing	1 (2.4%)	4 (11.1%)	5 (6.5%)
Asthma Diagnosis			
Yes	4 (9.8%)	4 (11.1%)	8 (10.4%)
No	36 (87.8%)	27 (75.0%)	63 (81.8%)
Missing	1 (2.4%)	5 (13.9%)	6 (7.8%)

Table 2: Descriptive characteristics of longitudinal data:

	Dairy Farm Workers	Community Control	Total
Baseline N	41	36	77
12 Months N (% of Baseline N)	28 (68%)	11 (31%)	39 (51%)
24 Months N (% of Baseline N)	10 (24%)	1 (2.7%)	11 (14%)

Table 3: Descriptive statistics of IgE measurements at baseline:

		Dairy Farm Workers (N=38)	Community Control (N=33)	Total (N= 71)
Total IgE	Above 100 U/mL	17 (45%)	14 (42%)	31 (44%)
	Below 100 U/mL	21 (55%)	19 (58%)	40 (56%)
Total IgE	Above 300 U/mL	8 (21%)	4 (12%)	12 (17%)
	Below 300 U/mL	30 (79%)	29 (88%)	59 (83%)
		Dairy Farm Workers (N=38)	Community Control (N=31)	Total (N = 69)
Bovine-specific IgE	Above 0.35 U/mL	4 (11%)	0 (0%)	4 (6%)
	Below 0.35 U/mL	34 (89%)	31 (100%)	65 (94%)

Figure 4: Distribution of total IgE at baseline; orange line represents 300 U/mL clinical threshold:

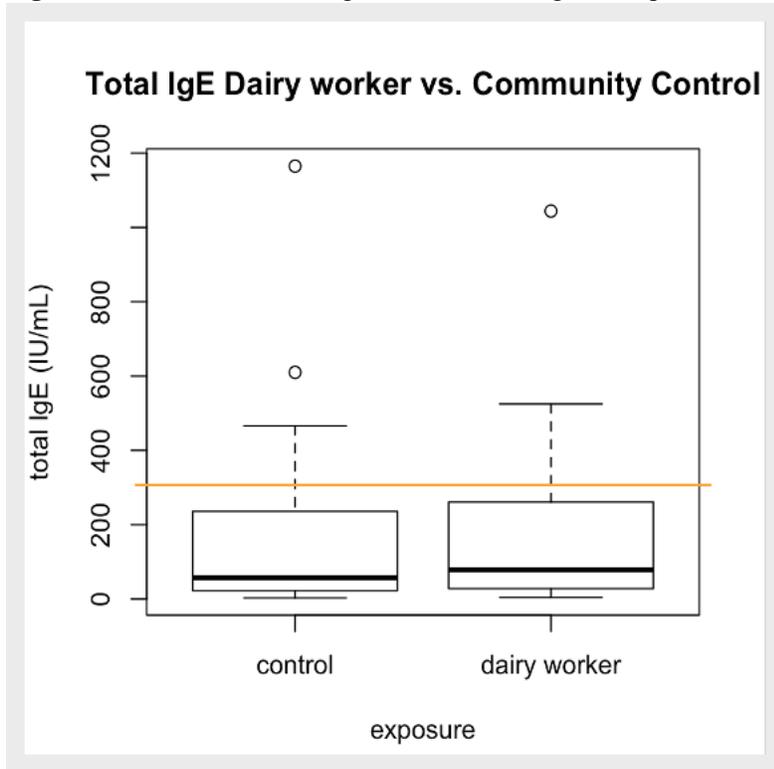


Table 4: Linear regression analysis of association between covariates and log-transformed total IgE (reporting exponentiated coefficients)

a) covariate: Age

	Estimate (CI)	p-value
Intercept	200.71 (62.75 , 641.96)	< 0.00005
Age	0.97 (0.95 , 1.00)	0.06395

b) covariate: Smoking

	Estimate (CI)	p-value
Intercept	77.55 (51.64 , 116.47)	< 0.00005
Current Smoker	0.65 (0.30 , 1.44)	0.28777

c) covariate: Farm childhood

	Estimate (CI)	p-value
Intercept	46.14 (19.14 , 111.24)	< 0.00005
Farm Childhood	1.65 (0.63 , 4.32)	0.30179

d) covariate: Farm childhood (analysis restricted to community controls only)

	Estimate (CI)	p-value
Intercept	54.93 (19.56 , 154.25)	< 0.00005
Farm Childhood	1.19 (0.33 , 4.20)	0.78354

e) covariate: Pet ownership

	Estimate (CI)	p-value
Intercept	82.44 (44.62 , 152.29)	< 0.00005
Farm Childhood	0.76 (0.36 , 1.61)	0.46840

f) covariate: Season (of blood draw)

	Estimate (CI)	p-value
Intercept	73.03 (45.03 , 118.46)	< 0.00005
Season	0.96 (0.49 , 1.89)	0.89789

Table 5: Linear regression analysis of the association between dairy farm exposure and log (total IgE) (reporting exponentiated coefficients)

Table 5a: Unadjusted

	Estimate (CI)	p-value
Intercept	67.59 (40.89 , 111.70)	< 0.00005
Dairy	1.11 (0.56 , 2.19)	0.76326

Table 5b: *A priori* adjusted for age and current smoking status

	Estimate (CI)	p-value
Intercept	298.10 (74.03 , 1200.42)	< 0.00005
Dairy	0.96 (0.46 , 2.04)	0.92376
Age	0.97 (0.94 , 1.00)	0.02994
Current Smoker	0.54 (0.24 , 1.25)	0.14735

Table 5c: Adjusted for age, current smoking status, pet ownership, farm childhood, and seasonality of blood draw

	Estimate (CI)	p-value
Intercept	250.09 (55.05 , 1136.25)	< 0.00005
Dairy	0.77 (0.30 , 2.01)	0.58977
Age	0.95 (0.93 , 0.98)	0.00347
Current Smoker	0.50 (0.22 , 1.17)	0.10832
Farm Childhood	3.31 (1.31 , 8.37)	0.01256
Pet Ownership	1.04 (0.49 , 2.24)	0.91095
Blood Draw Season	0.83 (0.36 , 1.92)	0.65358

Table 6: Logistic regression analysis: dairy farm exposure and binary IgE adjusted for age and current smoking

Table 6a: Total IgE (threshold 300 U/mL)

	Estimate (CI)	p-value
Intercept	1.43 (0.08, 25.35)	0.80391
Dairy	1.42 (0.35, 5.74)	0.61390
Age	0.95 (0.89, 1.01)	0.11120
Current Smoker	0.41 (0.07, 2.54)	0.33223

Table 6b: Total IgE (threshold 100 U/mL)

	Estimate (CI)	p-value
Intercept	8.93 (0.83, 96.16)	0.07035
Dairy	0.81 (0.28, 2.38)	0.69598
Age	0.95 (0.90, 1.00)	< 0.05
Current Smoker	0.58 (0.16, 2.05)	0.39216

Table 7: Self-reported allergy symptoms vs. dairy exposure

Table 7a: Counts table for self-reported allergy symptoms by exposure group for Fisher's Exact test

Allergy Symptoms	Dairy Farm Workers (N=40)	Community Control (N=32)
Yes	4	13
No	36	19

Fisher's Exact test p-value: **0.004345**

Reject null hypothesis that the true odds ratio is equal to 1.

Table 7b: Logistic regression analysis adjusted for age and current smoking

	Estimate (CI)	p-value
Intercept	2.87 (0.19, 42.53)	0.43678
Dairy Exposure	0.14 (0.04, 0.51)	< 0.05
Age	0.97 (0.91, 1.03)	0.33525
Current Smoker	0.88 (0.19, 4.20)	0.87401

Table 8: Counts table for self-reported asthma diagnosis

Asthma Diagnosis	Dairy Farm Workers (N=40)	Community Control (N=32)
Yes	4	4
No	36	27

Fisher's Exact test p-value: 0.722

Cannot reject null hypothesis that the true odds ratio is equal to 1.

Table 9a: Bovine-specific IgE (threshold 0.35 U/mL) – Counts table for Fisher's Exact test

Bovine-specific IgE	Dairy Farm Workers (N=38)	Community Control (N=31)
Above 0.35 U/mL	4	0
Below 0.35 U/mL	34	31

Fisher's Exact test p-value: 0.1197

Cannot reject null hypothesis that the true odds ratio is equal to 1.

Table 9b: Bovine-specific IgE (threshold 0.20 U/mL) – Counts table for Fisher's Exact test

Bovine-specific IgE	Dairy Farm Workers (N=40)	Community Control (N=31)
0.2 U/mL or above	7	0
below 0.2 U/mL	33	31

Fisher's Exact test p-value: 0.016

Figure 5: Distribution of eNO levels by dairy exposure at baseline:

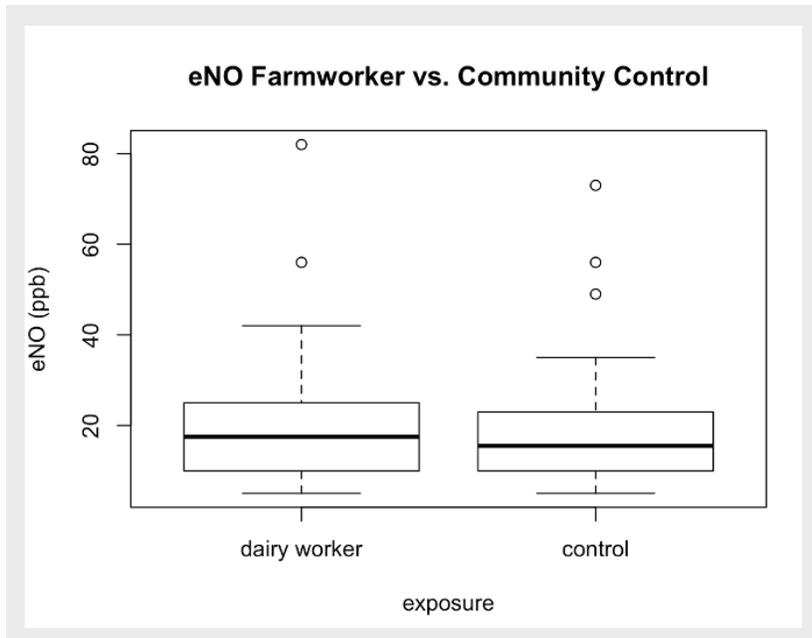


Table 10(a and c): Linear regression analysis of the association between total IgE levels and eNO (log)

Table 10a: *A priori* adjusted for age and current smoking status

	Estimate (CI)	p-value
Intercept	7.51 (2.97 , 18.99)	0.00005
Total IgE (2-fold change)	1.07 (0.99 , 1.17)	0.10418
Age	1.01 (0.99 , 1.02)	0.20629
Current Smoker	0.90 (0.62 , 1.30)	0.56282

Table 10b: Linear regression of association between eNO (log) and dairy exposure *a priori* adjusted for age and current smoking

	Estimate (CI)	p-value
Intercept	13.34 (6.78 , 26.24)	< 0.00005
Dairy Exposure	1.01 (0.71 , 1.44)	0.94548
Age	1.01 (0.99 , 1.02)	0.37955
Current Smoking	0.84 (0.56 , 1.24)	0.37292

Table 10c: *A priori* adjusted for age and current smoking with interaction between IgE and dairy farm exposure

	Estimate (CI)	p-value
Intercept	5.71 (1.74 , 18.81)	0.00486
Total IgE (2-fold change)	1.11 (0.97 , 1.27)	0.11856
Dairy Exposure	1.52 (0.54 , 4.30)	0.42034
Age	1.01 (0.99 , 1.03)	0.18543
Current Smoking	0.92 (0.61 , 1.38)	0.68543
Interaction IgE x Dairy Exposure	0.94 (0.79 , 1.11)	0.43407

Figure 6: Distribution of eNO levels by sensitization to cow epithelium at baseline:

6a – All participants (dairy worker and community control) **6b** – Dairy workers only

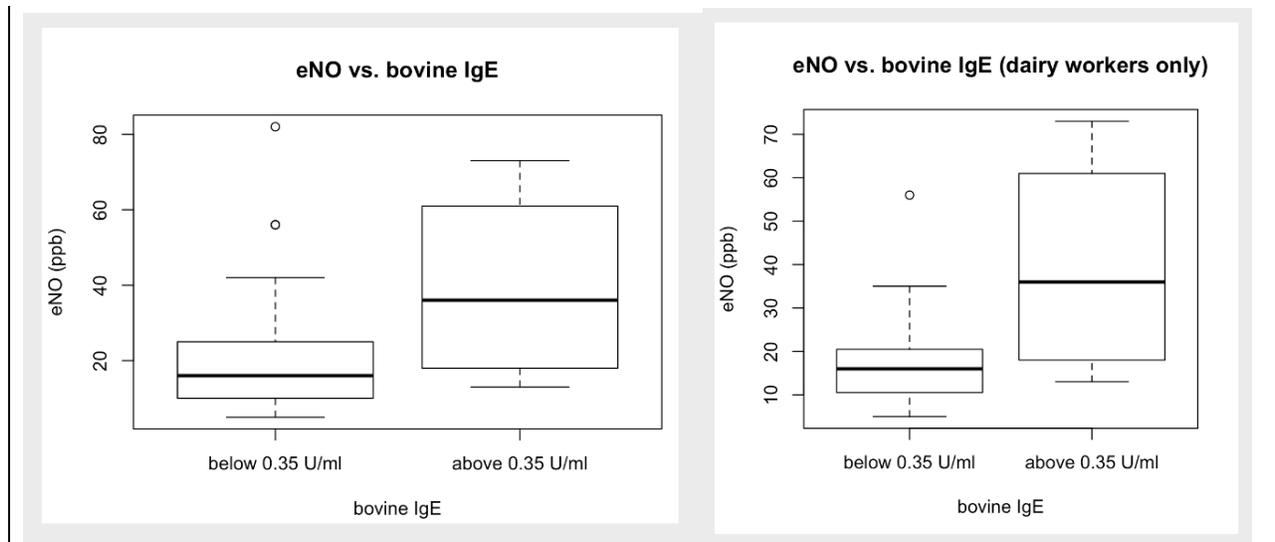


Table 11: Linear regression analysis of the association between bovine specific (binary) and eNO (log)

Table 11a: *A priori* adjusted for age and current smoking

	Estimate (CI)	p-value
Intercept	12.63 (6.98 , 22.85)	< 0.00005
Bovine IgE	2.49 (1.30 , 4.77)	0.00683
Age	1.01 (0.99 , 1.02)	0.32072
Current Smoker	0.74 (0.49 , 1.13)	0.16018

Table 11b: Same as 11a, but restricted to dairy farm workers

	Estimate (CI)	p-value
Intercept	11.84 (5.02 , 27.90)	< 0.00005
Bovine IgE	2.13 (0.92 , 4.92)	0.07496
Age	1.00 (0.98 , 1.03)	0.64463
Current Smoker	1.08 (0.65 , 1.81)	0.75512

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