Pulmonary Function and Airway Inflammation Among Dairy Parlor Workers After Exposure to Inhalable Aerosols

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Background *Inhalation exposure to organic dust causes lung inflammation among agricultural workers. Due to changes in production and work organization, task-based inhalation exposure data, including novel lung inflammation biomarkers, will inform exposure recommendations for dairy farm workers.*

Methods *Linear regression was used to estimate the associations of airborne exposure to dust concentration, endotoxin, and muramic acid with pulmonary outcomes (i.e., FEV_1, exhaled nitric oxide). Logistic regression was used to estimate associations with self-reported pulmonary symptoms.*

Results *Mean exposure concentration to inhalable dust, endotoxin, and muramic acid were $0.55\,mg/m^3$, $118\,EU/m^3$, and $3.6\,mg/m^3$, respectively. We found cross-shift differences for exhaled nitric oxide ($P = 0.005$) and self-reported pulmonary symptoms ($P = 0.008$) but no association of exposure with respiratory outcomes.*

Conclusions *Inhalation exposures during parlor tasks, which were lower than previously reported and were not associated with cross-shift measures of pulmonary health among dairy workers. Modern milking parlor designs may be contributing to lower inhalation exposure.* Am. J. Ind. Med. 60:255–263, 2017.   © 2017 Wiley Periodicals, Inc.

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INTRODUCTION

Work on dairy farms has been associated with adverse respiratory symptoms, primarily symptoms of bronchoconstriction, and decreased pulmonary function [May et al., 1986; Malmberg, 1990; Dalphin et al., 1993; Chaudemanche et al., 2003; Gainet et al., 2007; Eastman et al., 2013; Garcia et al., 2013; Reynolds et al., 2013]. Dairy workers may be at risk for lung inflammation based upon proximity to aerosol sources (e.g., cows) and exposure duration [Spaan et al., 2006]. Additionally, dairy farm workers often work 8–12 hr shifts for more than 5 days a week performing the same tasks (e.g., milking) [Donham, 1986, Douphrate et al., 2012, Garcia et al., 2013]. These aerosols may contain a mixture of manure, animal dander, hair, animal feed, gram-positive (i.e., muramic acid), and gram-negative (i.e., endotoxins) micro-biological components [Szponar and Larsson, 2001].

Agricultural workers inhalation exposure to dust measured over the work-shift has been reported from 0.8 to 20 mg per cubic meter (mg/m^3) [Donham et al., 1995; Kullman et al., 1998; Vogelzang et al., 1998; Spaan et al., 2006; Reynolds et al., 2012; Eastman et al., 2013; Garcia et al., 2013; Basinas et al., 2014; Mitchell et al., 2015]. Among dairy workers, Kullman et al. reported personal exposure geometric mean concentrations of inhalable dust and endotoxin at 1.78 mg/m^3 and 647 endotoxin units per cubic meter (EU/m^3). Additionally, Reynolds et al. [2012] reported geometric mean inhalable dust and endotoxin exposures of 2.37 mg/m^3 and 1,166 EU/m^3 among dairy farm workers, including parlor workers. To our knowledge, no studies have determined inhalable exposure concentrations of gram-positive bacteria (i.e., muramic acid) among dairy farm workers. This observation is surprising as gram-positive bacteria constitute the majority of bioaerosol in agricultural dust [Malmberg, 1990; Poole et al., 2010]. Therefore, future research examining associations of lung inflammation with inhalation exposures among agricultural workers should include exposure measurements of gram-positive bacteria (i.e., muramic acid) as this exposure metric remains relatively unexplored.

As dairy production has increased in size due to economies of scale, task-specialization has increased [Douphrate et al., 2009]. However, little information is available on the characterization of task-based exposures among dairy workers. Previous studies of inhalation exposure have combined exposure measurements across several tasks in dairy production (e.g., milking and feeding); consequently, limiting the application of the industrial hygiene hierarchy of exposure controls [DiNardi and Association, 1997; Kullman et al., 1998; Reynolds et al., 2012]. Recent studies have performed task-based exposure assessment among workers on dairy farms and found geometric mean exposures to inhalable dust ranging from 0.786 to 1.03 mg/m^3 and endotoxin 163–369 EU/m^3. The tasks of "feeding" and "bedding area maintenance" resulted in greater inhalation exposures compared to milking tasks [Garcia et al., 2013; Basinas et al., 2014]. However, these studies were based on farms with less than 500 head of dairy cows, and may not be representative of worker exposure during tasks on farms with larger herds (i.e., greater than 500 cows), which are common in the United States (US). Task-based inhalation exposure information is needed to develop recommendations for exposure controls specific to work on dairy farms.

Unlike the tasks of feeding and bedding, milking is a task that occurs on the farm for nearly 24 hr a day and has yet to be fully automated [Douphrate et al., 2009]. The milking task consists of the following activities: pre-dipping the teats in a sanitizing solution, wiping the teats, attaching the milking unit, detaching the milking unit (often automated), and finally applying a post-milking teat conditioner. An additional task associated with milking parlor work includes "pushing." Pushing involves the systematic movement of cows to and from the milking parlor. Pushing tasks may exclusively be assigned to one worker or may also be performed in conjunction with other milking tasks by the same worker during the work shift. Both milking and pushing tasks require work in close proximity to the milk cow [Douphrate et al., 2009]. Furthermore, milking tasks are performed in a semi-enclosed environment, which may result in exposure to greater concentrations of dust compared to other dairy farm tasks [Reynolds et al., 2013]. Other inhalation exposures such as gram-positive bacteria (i.e., muramic acid) among dairy workers during the milking tasks have yet to be characterized and may result in synergistic effects with other inhalation hazards (i.e., dust) [Poole et al., 2010]. Further, task-based exposure characterization will allow for the development of specific interventions (e.g., engineering controls) to reduce concentrations of inhalation hazards below recommended exposure guidelines (e.g., 100 EU/m^3 of endotoxin) [Donham et al., 1995; DECOS, 2010].

Dust inhalation exposure recommendations for work in other types of agricultural production exist. Specific recommendations for inhalation exposure to workers in poultry production are 2.4 mg/m^3 and 614 EU/m^3 and 2.5 mg/m^3 and 100 EU/m^3 for swine production [Donham et al., 1995, 2000; Reynolds et al., 1996]. However, studies of associations between inhalation exposure and lung inflammation are lacking among dairy workers and no inhalation exposure recommendations exist for workers in the dairy industry. Furthermore, inhalation exposure recommendations for Particles Not Otherwise Specified (PNOS) by the American Conference for Governmental Industrial Hygienists (ACGIH) for an 8-hr time-weighted average (TWA) may not apply to work on dairy farms as aerosols present in dairy production are complex mixtures which include inflammatory microbiological components (e.g., endotoxin) [ACGIH, 2015]. Therefore, associations of lung inflammation and dairy worker inhalation exposure need to be determined in order to establish inhalation exposure recommendations for dust, gram-positive and gram-negative bacteria.

Pulmonary function testing (e.g., spirometry) has been used extensively to assess lung volume changes (i.e., an indicator of lung inflammation) as a result of exposure to dust among agricultural workers [Heller et al., 1986; Castellan et al., 1987; Donham et al., 1995, 2000; Iversen and Dahl, 2000; Reynolds et al., 2012]. Cross-shift changes in lung volumes (e.g., Forced Expiratory Volume in 1 s, FEV_1) have been observed among workers exposed to aerosols containing endotoxin during agricultural work and have been used to establish exposure guidelines [Castellan et al., 1987; Donham et al., 1995; Reynolds et al., 2012]. However, some challenges persist when using spirometry. Specifically, spirometry requires a highly trained technician, can be

difficult to administer in remote farm locations and is invasive [Lemiere, 2007]. Other, easier to use tools should be evaluated for use on farms to assess lung inflammation associated with inhalation exposure [Lemiere, 2007; Quirce et al., 2010].

Specific biomarkers of lung inflammation (e.g., exhaled nitric oxide [eNO]) may be useful for detecting lung inflammation as a result of inhalation exposure among agricultural workers. Exhaled nitric oxide has been used successfully in a clinical setting for identifying individuals with lung inflammation and obstructive airway diseases such as chronic obstructive pulmonary disease and asthma [Clini et al., 1998; Maziak et al., 1998; Langley et al., 2003]. Recent advances in portable eNO instrumentation have allowed the use of these tools on farms to assess pulmonary inflammation among agricultural workers, however, eNO has not been extensively used to evaluate lung inflammation among agricultural workers [Kölbeck et al., 2000; Sundblad et al., 2002; Fortuna et al., 2007; Dressel et al., 2009]. Exhaled nitric oxide has also been used to evaluate the effectiveness of exposure interventions. For example, agricultural workers who used respiratory protection to reduce inhalation exposure to agricultural dust exhaled lower concentrations of NO compared to workers who did not use respiratory protection [Sundblad et al., 2002]. Exhaled nitric oxide has also been used to evaluate the effectiveness of an educational intervention to reduce inhalation exposures among asthmatic dairy farm workers [Dressel et al., 2009]. The instrumentation to measure eNO is easier to use compared to spirometry [Quirce et al., 2010].

To date, no study has measured the cross-shift associations between inhalation exposure to aerosols, markers of gram-positive (i.e., muramic acid) or gram negative bacteria (i.e., endotoxin) and eNO among dairy parlor workers. Furthermore, measuring both the inhalation exposure and markers of lung inflammation across the work shift will allow for determining the effect of inhalation exposures during milking parlor tasks on lung inflammation among dairy workers. If parlor tasks are associated with hazardous exposures, the work environment can be modified using the industrial hygiene hierarchy of controls. Therefore, the purpose of this study was to evaluate acute cross-shift changes in respiratory health among dairy workers performing milking parlor tasks using spirometry and eNO, and to determine associations between changes in respiratory health (i.e., spirometry and eNO) and measures of exposure (inhalable dust, endotoxin, and muramic acid).

MATERIALS AND METHODS

A non-randomized, cross-sectional study was conducted from May 2012 to January 2013 involving a total of 62 dairy parlor workers from nine dairy farms in Iowa, Minnesota, South Dakota, and Wisconsin. Farms were identified using state-based databases and internet searches for dairy farms among rural business databases (e.g., http://www.manta.com). Farm owners were contacted by the study staff and the study procedures were explained. If the farm owner agreed, study recruitment and data collection were scheduled. Study personnel arrived on the farm prior to the beginning of a work-shift and all potentially eligible workers were approached to participate in the study. Workers were eligible for participation if they were 18 years of age or older, current nonsmokers, worked in the milking parlor with the job title of "milker" or "pusher," able to speak English or Spanish, agreed to perform pulmonary function testing and wear exposure assessment equipment. The job title "milker" includes tasks associated with the milking of cows. The job title "pusher" includes tasks associated with moving the milk cows to and from the milking parlor and intermittent milking tasks. All study procedures were approved by the University of Iowa Institutional Review Board, which included a signed informed consent and the presence of Spanish speaking study staff.

Exposure Assessment

We measured inhalation exposure to inhalable dust, endotoxin, and muramic acid. Inhalable dust (50% cut-point at 100 µm) was sampled in the worker's breathing zone using an inhalable sampler (Button Aerosol Sampler, SKC Inc., Eighty Four, PA) and personal sampling pumps (AirChek XR 5000, SKC Inc.) for the duration of the work-shift and analyzed gravimetrically (Model XPE56—Microbalance, Mettler Toledo, Columbus, OH). Air was sampled at a rate of four liters per minute (LPM) on pre-weighed poly vinyl chloride filters (25 mm, 5.0 µm pore size, SKC Inc.). The aerosol samples were stored in $-20°C$ until analyzed for endotoxin. Air flow rates were both pre and post calibrated and only samples within $\pm5\%$ of target flow rate were used to estimate an air sample volume. Dust concentrations were calculated by using the filter mass change and the air sample volume after performing both field and laboratory blank correction. Dust exposure concentrations are reported in mg/m^3.

All filters were analyzed for endotoxin using a recombinant factor C (rFC) endotoxin assay (Cambrex, East Rutherford, NJ). Samples were extracted using sterile, pyrogen free water with 0.05% Tween-20 for 1-hr at 22°C while shaking the sample. Sample extract was added to a 96-well plate with 100 µl mixture of enzyme, buffer, and fluorogenic substrate. The plate was incubated for 1 hr at 37°C and analyzed using a fluorescence microtiter plate reader (Model FLX800TBIE, Biotek Instruments, Winooski, VT) with excitation/emission at 380/440 nm. Background fluorescence of extraction of zero EU/ml was subtracted and endotoxin was expressed in EU per mg of dust. Detailed methods have been previously described [Thorne et al., 2010].

Endotoxin inhalation exposure concentrations are reported as EU/m^3.

Sample extracts in Tween-20 were also analyzed for muramic acid using GC-MS/MS as previously described [Poole et al., 2010]. Briefly, samples and standards were lyophilized, and subsequently, digested in methanolic acid overnight at 100°C. Prior to mass spectrometry, muramic acid was isolated and derivatized using strong cation exchange solid phase extraction and a TMCS-pyridine cocktail, respectively. GC-MS/MS analysis was conducted using a Waters Quatro Micro system operated in the electron ionization positive and MRM modes (with collision energy of 6 eV). Results are presented in nanograms of muramic acid per milligram of dust (ng/mg).

Respiratory Health Outcomes

We measured pre- and post-shift pulmonary function with a symptom questionnaire, spirometry (i.e., FEV_1), and eNO. Pulmonary symptom questionnaires (pre- and post-shift) were administered to all participants in their preferred language, either English or Spanish. The questionnaires were based on an established American Thoracic Society questionnaire that has been previously used in dairy farm work and focused on acute and chronic respiratory symptoms in addition to work history [Rylander et al., 1990; Reynolds et al., 2009, 2012]. The self-reported pulmonary symptoms of interest were cough, phlegm, wheeze and shortness of breath. The post-shift questionnaire was administered to workers to assess changes in pulmonary symptoms after work.

Pulmonary function tests (PFTs) were administered by trained personnel both before and after the work-shift using a brass core Fleisch-type spirometer (KoKo PFT Spirometer, nSpire Health Inc., Longmont, CO). Trained technicians performed spirometry for all study participants both before and after the work-shift. The study procedure followed the American Thoracic Society (ATS)'s guidelines [Miller et al., 2005]. Spirometry measurements were repeated a minimum of three times and a maximum of six times to achieve a Forced Expiratory Volume in 1 s (FEV_1) within a range of 150 ml. of air for a set of three measurements. A physician blinded to the participant exposure and pulmonary symptom status reviewed all PFTs for evidence of poor effort. These efforts were excluded from the analysis (n = 9). Among the remaining participant spirometry measurements, the largest FEV_1 was selected from the pre-shift measurements and again from post-shift measurements and observed changes were reported in Liters (n = 53).

Finally, the NIOX MINO (Aerocrine, Morrisville, NC) was used to measure eNO of the participants. Participants inhaled air through a disposable filter attached to the NIOX MINO to remove endogenous nitric oxide from inhaled air. Participants exhaled through the mouthpiece of the instrument at a rate of 50 ml/s (±5 ml/s) for 10 s. The NIOX MINO instrument uses both visual and audio feedback to allow the participant to maintain the target exhalation rate and duration. Exhaled nitric oxide was measured both before and after the work-shift following guidelines published by the ATS and the manufacturer [Aerocrine, 2010]. Concentrations of eNO are reported in parts per billion (ppb).

Covariates

The following variables were selected from the self-reported questionnaire as potential confounders: gender (male vs. female), age (as a continuous variable), smoking (ever-smoked vs. non-smokers), months worked in agriculture, months in current job, and hours worked per week.

Data Analysis

Descriptive statistics and multivariable linear regression modeling were used to estimate associations between airborne particle exposure (i.e., inhalable dust concentration, inhalable endotoxin concentration, and inhalable muramic acid concentration) and pulmonary outcomes (i.e., post-shift FEV_1 and eNO). All exposure metrics and measures of pulmonary outcomes were tested for normality using the Kolmogorov–Smirnov test for normality. As a result, exposure variables were log-transformed. Given the small sample size, covariates were included in the multivariable regression model if P-value ≤ 0.2 (i.e., gender, ever smoking, months in current job, hours worked per week). Additionally, logistic regression models were used to estimate associations between dichotomized exposures (upper 50% of exposure vs. lower 50%) and the presence of any self-reported pulmonary symptom (i.e., cough, phlegm, wheeze, or shortness of breath). SPSS was used for the statistical analyses (IBM SPSS Version 20.0, IBM Corp. Armonk, NY).

RESULTS

The majority of study participants were Hispanic/Latino male workers (94%), with an average age of 32 years (Table I). Eighty-five percent of participants performed tasks associated with milking and 70% of the participants had ever smoked cigarettes. The majority of the milking parlors were of the "parallel" design where the cows are aligned parallel to one another with the rear of the cow perpendicular to the milking "pit." The mean age of the milking parlors was 6 years (SD = 4) with a mean milking herd size of 1,414 cows (SD = 598). The majority of the exposure samples were collected in the summer and fall months (n = 54), with an average temperature and relative humidity inside the parlor during the work shift of 24°C (SD = 5) and 45% (SD = 13).

TABLE I. Sample Characteristics Among Dairy Parlor Workers (n = 62)

Characteristics	Number (%) or mean (SD)
Sociodemographic	
Gender (male)	92%
Age (years)	32 (9.7)
Race (white)	92%
Hispanic/Latino ethnicity	94%
Family income (U.S. dollars)	10,000–30,000
Currently live on a farm	37%
Health-related	
Ever smoke	70%
Physician-diagnosed asthma	5%
Ever had fever or chills after exposure to dust	16%
Job-related	
Years working on any farm (years)	4 (4)
Years as a dairy parlor worker (years)	3 (4)
Months in current job	40 (44)
Hours per week	55 (9)
Seasonal migrant worker	74%
Occupation	
Milker	84%
Pusher	15%
Farm	
Age of parlor (years)	6 (4)
Parallel parlor design	67%
Herd size	1,417 (598)
Temperature (°C)	24 (5)
Relative humidity (%)	45 (13)
Inhalation exposure over the work-shift	
Dust (mg/m^3) (geometric mean [GM])	0.55 (2.6)
Endotoxin (EU/m^3) [GM, GSD]	118 (4)
Muramic acid (ng/m^3) [GM, GSD]	3.61 (4.2)

Physician diagnosed asthma was reported among three of the study participants (5%). Also, 10 of the participants (16%) reported experiencing fever or chills after their work-shift at least once.

The geometric mean exposure to inhalable dust was 0.55 mg/m^3 (GSD = 2.6), 118 EU/m^3 (GSD = 4.1), and muramic acid 3.6 mg/m^3 (GSD = 4.2). The mean pre-shift FEV_1 for all participants was 3.69 L compared with 3.66 L for the post-shift FEV_1 (Table II). The difference in the pre- and post-shift mean FEV_1 among workers was not statistically significant ($P = 0.149$). The proportion of workers reporting pulmonary symptoms (i.e., cough, phlegm, wheezing, or shortness of breath) decreased across the work-shift (Table II). Cough was reported by 19% of workers in the study prior to their work-shift, compared to 7% after their work-shift. The observed difference in the proportion of workers experiencing cough across the work-shift was statistically significant ($P = 0.004$). Approximately, 40% of the workers reported at least one pulmonary symptom (i.e., cough, phlegm, wheezing, or shortness of breath) at the beginning of the work-shift, compared to 23% after the shift (Table II). The observed decrease in any reported pulmonary symptoms across the work-shift was statistically significant ($P = 0.008$). Additionally, mean eNO concentrations in exhaled breath decreased across the work-shift, by 1 ppb. This decrease in eNO concentrations across the work-shift was statistically significant ($P = 0.005$).

All linear regression coefficients (i.e., β) of the association between the exposures of interest (i.e., concentrations of inhalable dust, endotoxin, and muramic acid) and cross-shift pulmonary health measures (i.e., FEV1 and eNO) are reported in Table III. Analyses of the relationship between dairy parlor worker inhalation exposure and pulmonary outcomes showed no statistically significant associations (all $P > 0.05$) after adjusting for potential confounders. Only the relationship between exposure to inhalable endotoxin and declines in FEV_1 (β = −0.057, $P = 0.081$) approached statistical significance (Table III). Logistic regression analyses resulted in no statistically significant relationships observed between the dependent variable of any reported pulmonary symptom (i.e., cough, phlegm, wheezing, or shortness of breath) and inhalation exposure.

DISCUSSION

Among a small sample of dairy parlor workers performing milking and pushing tasks, inhalation exposure to dust, gram-negative bacteria (i.e., endotoxin), and gram positive bacteria (i.e., muramic acid) were not associated with changes in mean FEV_1 or eNO. The geometric mean inhalation exposure to dust was 0.55 mg/m^3 (GSD = 2.6), 118 EU/m^3 (GSD = 4.1), and muramic acid 3.6 mg/m^3 (GSD = 4.2). The inhalable dust and endotoxin exposure concentrations measured in the study were lower than what has been previously reported [Kullman et al., 1998; Garcia et al., 2013; Mitchell et al., 2015]. It appears that engineering solutions to increase efficiency in dairy parlor technology has resulted in a work environment with lower concentrations of dust and endotoxin compared to older, enclosed milking technologies [Kullman et al., 1998]. This hypothesis is supported by the mean age of the milking parlors used on participant farms being 6 years. Other studies in slightly more arid environments reported geometric mean inhalable dust and endotoxin exposures of 0.786 mg/m^3 and 320 EU/m^3, respectively among milkers in California [Garcia et al., 2013]. Mitchell et al. [2015] reported geometric mean inhalable dust and endotoxin exposures at 0.812 mg/m^3 and 329 EU/m^3 using nearly identical methodology as this study; however, dairy worker tasks were not

TABLE II. Pulmonary Outcomes at Pre- and Post-Shift Among Dairy Parlor Workers (n = 62)

Outcomes	Number (%) or mean (SD) Pre-shift	Post-shift	P-value of the difference between pre and post-shift[*]
Pulmonary function measures			
FEV$_1$ (L) (n = 53)	3.69 (0.59)	3.66 (0.61)	0.149
eNO (ppb)	15 (7)	14 (8)	0.005
Self-reported pulmonary symptoms			
Cough	19%	7%	0.004
Phlegm	19%	8%	0.227
Wheeze	8%	3%	0.453
Shortness of breath	11%	5%	0.219
Any of the above	40%	23%	0.008

FEV$_1$, forced expiratory volume in 1 s (in L); eNO, exhaled nitric oxide.
[*]P-value from paired t-test for the difference of means; P-value from McNemar's chi-squared for paired proportions.

identified in this study. Poole et al. [2010] reported mean concentrations of muramic acid of 5 ng/mg among settled dust samples from dairy farms, which were approximately half the concentration of those observed in the present study (arithmetic mean 10.8 ng/mg, [SD = 11.1]). Additional studies are needed to determine the generalizability of the muramic acid exposure results observed in our study.

All the concentrations of eNO measured in this study were between 3.5 and 39 ppb, which represents the fifth to 95th percentile values of distribution for normal healthy individuals in the US population [See and Christiani, 2013]. Therefore, workers in this study failed to produce eNO concentrations above what is observed among members of the US population. The reason for this observation is unknown. A possible explanation is that the length of the work-shift varied between 6 and 12 hr and perhaps not enough time had elapsed from exposure to result in the formation of eNO in the lung. This is a possibility and we attempted to address this issue methodologically by performing a 24-hr follow-up eNO measurement among ten participants (data not shown). The 24-hr post-shift eNO measurements were nearly identical to the eNO measurements collected immediately after completion of the work-shift. Therefore, our conclusion is that the inhalation exposures experienced by the dairy farm workers in our study did not result in lung inflammation at a level in which the elevation of eNO would be observed.

Workers performing milking and pushing tasks seem to experience lower exposure concentrations of dust and endotoxin compared to other tasks on dairy farms. Other dairy farm tasks where inhalation exposures have been characterized by other researchers include: maintaining cow bedding areas (1.030 mg/m^3; 293 EU/m^3), moving cows (0.786 mg/m^3; 369 EU/m^3) and performing medical procedures (0.880 mg/m^3; 351 EU/m^3) [Garcia et al., 2013]. Additional research has reported the effect of task on dairy farm worker exposure. Specifically, Basinas et al. [2014] reported geometric mean inhalable dust and endotoxin exposures of 1.00 mg/m^3

TABLE III. Relationship (Beta Coefficient) Between Inhalation Exposures and Cross-Shift Pulmonary Health Measures

Inhalation exposures	n	Pulmonary health measures Adjusted only for pre-shift pulmonary measure β[b]	(SE)[c]	P-value	Adjusted for additional covariates[a] β[b]	(SE)[c]	P-value
		FEV$_1$					
Dust	53	0.006	(0.051)	0.620	0.006	(0.051)	0.880
Endotoxin	53	−0.057	(0.040)	0.805	−0.058	(0.039)	0.081
Muramic acid	53	−0.029	(0.038)	0.368	−0.029	(0.038)	0.446
		eNO					
Dust	61	0.006	(0.051)	0.880	0.045	(1.202)	0.501
Endotoxin	58	−0.057	(0.040)	0.100	0.075	(1.243)	0.358
Muramic acid	58	−0.029	(0.038)	0.446	0.099	(0.895)	0.172

FEV$_1$, forced expiratory volume in 1 s (in L); eNO, exhaled nitric oxide.
[a]Adjusted for covariates associated with the dependent variable at $P \leq 0.05$ in addition to pre-shift pulmonary measure.
[b]Linear regression coefficients. Given that inhalation exposures have been log transformed, the format for interpretation of the linear regression coefficients is that a one percent increase in the original untransformed scale among the inhalation exposure variable changes the pulmonary health variables by (β/100) units while all other variables in the model remain constant.
[c]Standard error.

and 360 EU/m^3 among dairy farm workers and emphasized the significance in accounting for the variability of task and farm equipment in exposure assessment. Interestingly, exposure reductions were observed among workers using simple engineering controls (i.e., rail feed dispensers, surface manure scrapers) [Basinas et al., 2014]. Kullman et al. [1998] reported personal exposure geometric mean concentrations of inhalable dust and endotoxin at 1.78 mg/m^3 and 647 EU/m^3 and noted elevated concentrations during feeding, bedding and lime application tasks while using a direct reading instrument to measure aerosols. Reynolds et al. reported geometric mean inhalable dust and endotoxin exposures of 2.37 mg/m^3 and 1,166 EU/m^3 among all dairy farm workers, not just workers who perform milking and pushing tasks.

Dairy farm size and parlor design may explain the observed exposures in this study. All farms recruited for this study milked over 500 head of cows resulting in multiple shifts of workers milking nearly 24 hr a day. Therefore, the task variability across workers was low, with the majority of workers only performing tasks associated with milking or pushing. None of the participants performed feeding or bedding tasks that have been identified by other researchers as resulting in higher exposures than what was observed in this study. The dairy farm parlor facilities that were observed in this study included modern milking parlor designs and technologies (rotary, parallel, and herringbone) all with automatic removal of milking equipment after task completion. Modern milking parlor designs include semi-enclosed parlors manufactured using concrete walkways, with steel barriers to manage cow movements [Douphrate et al., 2009]. Approximately, three to five workers were in the parlor (either milking or pushing) per farm. The relative humidity in semi-enclosed dairy parlor buildings where the study was conducted may have been higher, resulting in lower exposure concentrations of aerosols compared to other, more arid climates (e.g., Colorado and California) where previous studies have been conducted. Interestingly, Garcia et al. [2012] reported that increasing relative humidity was a variable associated with lower biologically active endotoxin concentrations. This analysis was unable to be performed in this study as relative humidity measurements were pooled across workers in the same parlor. However, this observation may lead to considerations for water-based engineering controls of aerosols on dairy farms in more arid climates which have been shown to reduce respirable dust exposures [Choudhry et al., 2012].

Study participants reported a lower prevalence of pulmonary symptoms after work compared to before work. The reason for the reduced prevalence of pulmonary symptoms after work is unclear. Several possible explanations exist; workers may be fatigued after work and want to report fewer symptoms after work so they can quickly complete the study procedures. This explanation seems unlikely as the post-shift questionnaire is much shorter than the pre-shift questionnaire. Workers could have misunderstood study procedures. This explanation is possible as 98% of the participants identified Spanish as their primary language. However, the study staff member that administered the questionnaire was fluent in Spanish language and explained the study procedures correctly. An additional possibility is the tendency toward social desirability [Grimm, 2010]. That is, for dairy workers in physically demanding jobs, reporting symptoms can be perceived as limiting in terms of social and professional relationships, and therefore, the workers may under report their pulmonary symptoms. An explanation as to why we observed a decrease in reported pulmonary symptoms after the work-shift is that social desirability may have biased symptom reporting after the shift to avoid embarrassment. Another possible explanation for this observation may be that the pulmonary symptom questionnaire that lacks validity, which seems unlikely.

Additional limitations were present in this study. The study design is cross-sectional, both the exposure and the pulmonary health outcomes were measured on the same day across a work-shift. However, the analyses of effect were conservative as pre-shift pulmonary health measurements were used as a baseline for pulmonary changes. No statistically significant increases in pulmonary symptoms were observed, however, an adaptation and selective survival phenomenon is likely occurring within this working population. For example, dairy workers who experience pulmonary symptoms that are problematic likely leave the working population, thereby maintaining a working population that has likely adapted to the inhalation exposures present during parlor work. Adaptation response to endotoxin is also likely occurring among dairy parlor workers. The original study design included efforts to collect study data during the first work-shift among workers returning to work after several days away from the dairy parlor. This approach proved to be logistically challenging and nearly impossible as the majority of dairy parlor workers work 6 days a week. Another limitation is that nine of the pulmonary function measures were identified as unusable due to poor participant effort. The reason for poor effort by the participants is unclear. Perhaps participant fatigue was a factor as parlor work has been reported as being physically demanding [Douphrate et al., 2012].

The study staff effort required for recruitment of workers was underestimated. Many of these farms visits were at distant locations (over 320 km) and required multiple visits to develop relationships with farm owners/managers and workers. Often, the initial contact with the farm owner was over the telephone and many farm owners were non-responsive or were not interested in participating. Future studies should carefully consider costs associated with the early establishment and maintenance of producer relationships. Developing a trusted relationship with producers requires considerable travel and personnel costs in addition to the costs associated with recruitment and data collection.

Future health and safety research in the dairy industry should be hierarchical and focus on tasks that expose workers to hazards for which acute health outcomes are anticipated. Based on the findings of this study, inhalation exposures associated with milking and pushing tasks were much lower than has been previously measured. Other more arid climates may result in higher inhalation hazards among workers in the dairy parlor. Also, the semi-enclosed design of dairy parlors may contribute to the lower exposures observed in this study.

AUTHORS' CONTRIBUTIONS

Each of the authors contributed in the following manner to the manuscript: conception or design of the work (MN, JL, VB, SR). The original grant proposal was developed by MN with guidance and mentorship from JL, VB, and SR. The acquisition, analysis, or interpretation of data for the work was completed by the entire project team (i.e., MN, DG, JL, DD, VB, JS, MG, MH, SR). The field data were collected by MN, MH, and MG. The endotoxin and muramic acid samples were analyzed by JS and SR. The remaining members of the team (DG, JL, DD, and VB) assisted MN with data analyses and interpretation. The initial drafting of the paper was completed by MN with the reminder of the team performing critical revisions and contributed intellectual content. The final approval of the version submitted and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved (MN, DG, JL, DD, VB, JS, MG, MH, SR).

ETHICS APPROVAL AND INFORMED CONSENT

This work was funded at UT Health Northeast, Department of Occupational Health Sciences, Tyler, Texas, USA' and performed at the University of Iowa, Department of Occupational and Environmental Health, Iowa City, Iowa. The University of Iowa institutional review board approved all aspects of the study and written informed consent was used (i.e., ID: 201109783).

DISCLOSURE (AUTHORS)

The authors report no conflicts of interest.

DISCLAIMER

None.

DISCLOSURE BY AJIM EDITOR OF RECORD

Steven Markowitz declares that he has no competing or conflicts of interest in the review and publication decision regarding this article.

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