Associations Between Bioaerosol Exposures and Lung Function Changes Among Dairy Workers in Colorado

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Objective: Limited studies have examined effects of bioaerosols on the respiratory health of dairy workers; previous findings have been inconsistent across populations. **Methods:** Using a repeated measures design, exposures to dust, bioaerosols, and ozone were assessed and pre- and post-shift spirometry was performed for dairy workers ($n = 36$). Workers completed 1 to 8 visits. Linear mixed effect models estimated associations between air pollutant constituents and changes in spirometry. **Results:** There was an association between higher dust exposures and increased peak expiratory flow rate. However, for all other outcomes there was no association with the exposures considered. **Conclusions:** Relationships between bioaerosol exposures and respiratory health in dairy workers remain unclear. Future studies should increase sample sizes, include repeated measures designs, vary the timing of spirometry measurements, and include markers for Gram positive bacteria such as muramic acid or peptidoglycan.

Keywords: bioaerosol, dust, ozone, respiratory health, spirometry

The respiratory health of dairy workers has received increased research attention in the last decade due to the improved awareness of myriad exposures in agricultural environments. Respiratory diseases reported among dairy workers range from asthma and chronic obstructive pulmonary disease to cancer.[1] Livestock farm workers have higher than expected proportionate mortality ratios for respiratory diseases such as hypersensitivity pneumonitis, tuberculosis, asthma, and influenza.[2] Over the course of a work shift, dairy workers are exposed to several inhalation hazards with varying degrees of toxicity that are thought to be responsible for these respiratory health outcomes.[3] Documented exposures include dust, volatile organic compounds, and bioaerosols such as endotoxins, lactic acid bacteria, and fungal spores.[4–7] These exposures may induce immunological responses in exposed workers,[8–12] which are thought to underlie respiratory symptoms and sequelae.

Bioaerosol exposures are of particular interest on dairy farms due to their links to respiratory health outcomes and the high potential for exposure among workers. Factors related to bioaerosol exposures in dairy barns include the types of bedding used (eg, compost bedding compared to sawdust bedding)[13] handling feed or seeds,[5,14] the temperature and ventilation of dairy buildings,[15] and working in the milking parlor.[6] Endotoxin, a pro-inflammatory component of the cell walls of Gram-negative bacteria, has been linked to respiratory symptoms in both occupationally[16] and non-occupationally exposed populations.[17] Endotoxins that reach the lower part of the respiratory tract can trigger an innate immune response,[18] resulting in inflammation and airflow obstruction.[19,20] Interestingly, previous research has identified genetic mutations for endotoxin sensitivity such as variations in CD14 and Toll-like receptor 4 (TLR4).[12,21] Another common bioaerosol constituent found on dairy farms, β-glucan, is an indicator of fungal spores[22,23] and has also been linked to respiratory health effects in occupational settings where animals are present.[24] β-glucan exposures have been shown to induce inflammatory responses in human bronchial cells,[25] potentially impairing respiratory health. Increased inflammation may be associated with decreases in lung function over time,[26–28] though evidence of the direction of this association is mixed.[29] As both endotoxin and β-glucan have been linked to respiratory inflammation, their potential role in respiratory disease incidence among dairy workers warrants further research.[1]

In addition to dust and bioaerosol exposures arising from dairy activities, dairy workers may spend a large proportion of their work shifts outdoors, and thus be susceptible to health effects from ambient air pollution. Ozone is of particular concern in rural areas downwind of large metropolitan areas. Ozone concentrations are often higher in rural areas compared to urban centers due to regional effects such as higher elevation and deep atmospheric boundary layers and local effects such as ozone titration by nitrogen dioxide.[30] Similar to bioaerosol exposures, ozone exposures have been shown to induce inflammation and reduce pulmonary function in healthy adults.[31]

A limited number of studies have investigated the effects of bioaerosol exposures on dairy worker respiratory health measured using spirometry.[8,9,12] Spirometry has long been used in occupational settings as part of routine medical surveillance to detect changes in lung function over time.[32] Evidence from other occupational health studies suggests that small changes in cross-shift pulmonary function testing (PFT) can be cumulative and result in chronic lung function deficits.[33,34] These previous studies used cross-sectional designs measuring exposures and pulmonary function for a single shift and reported mixed results for associations between bioaerosols and lung function. Further, these studies have not accounted for other ambient air pollutant exposures such as ozone. Thus, research is needed to understand the combined effects of bioaerosol and ambient air pollutant exposures on the respiratory health of dairy workers. Such studies better characterize the physiologically relevant exposures experienced by this potentially vulnerable population.

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Funding: This research was funded by grant number 1U01 OH010840 (MPI: Reynolds, Schaeffer, Magzamen) from the National Institute of Occupational Safety and Health and the Centers for Disease Control and Prevention. The study was also supported by grants from National Institute of Environmental Health Sciences (R01 ES019325l PI: Poole) and the National Institute of Occupational Safety and Health (U54 OH010162; PI: Rautiainen).

The authors report no conflicts of interest

Clinical significance: Bioaerosol exposures are a recognized risk factor for respiratory health consequences, but the effects of dairy work exposures in workers without respiratory disease are unclear. In this study, there was no adverse effect in lung function measurements across several work shift days. Larger and longitudinal studies across time are needed.

Supplemental digital contents are available for this article. Direct URL citation appears in the printed text and is provided in the HTML and PDF versions of this article on the journal's Web site (www.joem.org).

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DOI: 10.1097/JOM.0000000000001856

The objective of this study was to assess whether exposure to dust, bioaerosols (endotoxin and β-glucan), or ozone were associated with changes in PFT measured via pre- and post-work shift spirometry. We collected pre- and post-shift spirometry data and assessed daily exposures to dust, bioaerosols, and ozone and used a repeated measures design to account for potential intra-subject variability in spirometry results. Our study hypothesis was that higher dust, bioaerosol, and ozone exposures were associated with greater decrements in lung function across a work shift.

METHODS

Study Area and Population

Four different dairy operations located in a High Plains state located in the western United States agreed to participate in this study. Each dairy was considered a large-herd operation with 1000 or more lactating cows. These study sites were identified by the High Plains Intermountain Center for Agricultural Health and Safety advisory board. Facility owners and operators were contacted, and operations were enrolled based on availability during sampling campaigns. The study population was recruited using a snowball sampling approach from each dairy based on group meetings at the facility. Participants were eligible to participate if they were at least 18 years old and spoke English or Spanish. Further, participants must have been engaged in tasks associated with high risk of exposure to bioaerosols. Key criteria for exclusion included current use of certain medications (ie, steroidal and nonsteroidal anti-inflammatory medications, as well as immune-suppressive, anti-autoimmune, or chemotherapy drugs) known to interfere with the health measurements performed in this study. All study participants provided informed consent in English or Spanish to participate in the study. All study protocols and materials were approved by the XXX Institutional Review Board.

Daily Work Shift and Health Questionnaires

Daily pre- and post-shift health questionnaires were administered to all participants in their native language (English or Spanish) by trained research staff. The questionnaires were based on the standard American Thoracic Society questionnaire, with modifications specific to work in dairy operations. Participants reported on relevant infections, use of antibiotics and personal protective equipment, hygiene, behaviors (eg, tobacco use, environmental smoke exposure, and alcohol consumption), and other key factors including acute and chronic respiratory symptoms. Additionally, workers were asked about the primary and secondary tasks performed on each day of participation. Farm characteristics including milking parlor design, stall design, herd size, and age of farm were collected through a combination of walkthrough surveys and self-administered questionnaires distributed to farm managers.

Exposure Assessment

Personal Monitoring

Exposures to inhalable dust and bioaerosols were based on personal monitoring data for each study participant. Personal breathing zone samples were collected using SKC Button samplers (SKC Inc., Eighty-Four, PA) fitted with 5 μm PVC filters as previously described.[35] Briefly, individual pumps were connected to each sampler and calibrated to a flow rate of 4 L/min using a primary standard. All sampling systems were calibrated before and after each sample; differences of ±5% were considered acceptable. Study participants wore the personal samplers for the duration of their work shifts (up to three days of sampling) and on one day off.

Gravimetric Analysis

Total mass of inhalable dust was measured using gravimetric analysis in a High Efficiency Particulate Air-filtered room. All filters were desiccated for 24 hours and static neutralized using a U-Electrode (Mettler-Toledo, Inc., Columbus, OH) before each weighing (pre- and post-sampling). All samples were weighed to the nearest 1.0 μg on an analytical balance (MT5; Mettler-Toledo, Inc., Columbus, OH) in duplicates and then averaged. A third measurement was collected if there was a >5 μg difference between replicates. Total mass was then calculated by subtracting the postsampling weight from the presampling weight. A calibration weight was used to assess and correct for potential drift in the balance. Time-weighted averages were determined by dividing the mass of dust on each filter by the volume of air sampled. Laboratory and field blanks were used to correct for measurement error and background signals.

Laboratory Analyses for Microbial Constituents

Endotoxin. Air samples were analyzed for biologically active endotoxin using the Pyrogene Recombinant Factor C Assay as previously described.[36,37] Briefly, sterile, pyrogen-free water with 0.05% Tween-20 was used to prepare serial dilutions of endotoxin standards, as well as extract inhalable dust from sample filters with continuous shaking at 22°C. The samples were added to a 96-well plate in triplicate and subsequently mixed with the buffer, enzyme, and fluorogenic substrate according to the manufacturer's protocol. Endotoxin concentrations of samples were calculated according to a standard curve; background fluorescence was subtracted, and log delta fluorescence was plotted against log endotoxin concentration. Assay reagent blanks served as reference and control to ensure pyrogen-free status of water, centrifuge, tubes, pipette tips and microplates. Quality assurance spiking assays were performed to assess matrix interference or enhancement.

(1-3)-β-D-Glucan (β-glucan). Airborne concentrations of β-glucan were determined using the Glucatell® assay (Associates of Cape Cod, Inc., East Falmouth, MA) according to the manufacturer's protocol. The assay was based on a limulus amebocyte lysate protease activation pathway that was specific for β-glucan. Serial dilutions of β-glucan standards were prepared using pyrogen-free water. Bioaerosol sample extracts (prepared previously for endotoxin measurement) were added, in triplicate, to a 96-well plate and mixed with a 100 μL of the provided reagent. Optical density was read using a spectrophotometric reader (HTX Synergy; Biotek, Winooski, VT); concentrations were calculated according to a standard curve with a limit of detection of 5 pg/mL. Assay reagent blanks and quality assurance spiking were included to control for background contamination and interference. The final concentration of β-glucan for each sample was reported in pg/m^3.

Ambient Ozone

For each day of the study, we assigned ozone exposures to all dairy workers using the concentration recorded at an Environmental Protection Agency monitor located within 50 km of the dairy. Consistent with the design value of the National Ambient Air Quality Standards,[38] we use the maximum daily 8-hour ozone concentration recorded each day as our exposure metric.

Lung Function Measurements

Using a KOKO® Legend II (nSpire Health, Inc., Longmont, CO), each participant completed spirometry assessments at the start and end of each work shift; morning and evening measurements were collected on days off. PFT data from up to four shifts were collected, and measurements were completed according to the American Thoracic Society guidelines.[39] To determine eligibility for spirometry, each participant filled out a brief survey on the day of the study visit that included questions on recent symptoms, respiratory infections, and surgeries. Participants were excluded if they

reported health conditions incompatible with spirometry, such as cardiovascular disease or recent stroke. We included five spirometry variables in our analysis: forced vital capacity (FVC), forced expiratory volume in the first second (FEV_1), the ratio of FEV_1 to FVC (FEV_1/FVC), forced expiratory flow at 25% to 75% (FEF_{25-75}), and peak expiratory flow rate (PEFR). FEV_1 and FVC are typical spirometry metrics used to assess whether there is an airway obstruction (indicated by lower FEV_1 and FEV_1/FVC) or an airway restriction (indicated by reduced FEV_1 and FVC).[40,41] FEF_{25-75} is a less commonly used metric and reflects the flow of air between the 25th and 75th percentile of FVC and serves as a marker for small airway disease. PEFR is a measure of maximum rate at which air is expelled out of the lungs after a full inspiration[42] and, like FEF_{25-75}, is less frequently reported in the respiratory health literature compared to the other spirometry measures.[43] PFT results were reviewed by an experienced physician (J.A.P.) to identify any abnormal test results. We interpreted a decrease in PFT measurements across the work shift as evidence of lung function impairment.

We assessed changes in pulmonary function in two ways. First, using the standard approach for spirometry,[43] we assessed change in pulmonary function as the difference between the percent predicted value at the post-shift measurement and the pre-shift measurement, where negative values indicate greater pre-shift lung function relative to post-shift. The percent predicted value is generated by the spirometer and incorporates data on the participant's ethnicity, height, weight, sex, and age.[44] However, the functions used to generate the percent predicted values were originally based on small populations that included few ethnic or racial minorities and may not fully reflect the pulmonary health measurements of non-white populations.[45,46] As an alternative to the percent predicted values, we also used the residuals from a linear mixed model that predicted pre- and post-shift lung function measures for our study population. These models included ethnicity and the aforementioned covariates that were also accounted for in the spirometer's percent-predicted values. PFT residuals have the same units as the original PFT results, which may aid in interpretation.

Statistical Analysis

All participant demographics and PFT variables were summarized using means and standard deviations (SD) or frequencies as appropriate. For the skewed exposure metrics, we summarized distributions using the geometric mean (GM) and geometric standard deviation (GSD).

We modeled the associations between each exposure (dust, endotoxin, β-glucan, or ozone) and each PFT outcome in separate linear mixed effect models. Exposures were log-transformed prior to model fitting due to high degrees of skewness. Because both the percent predicted values and the PFT residuals account for ethnicity, height, weight, sex, and age, we did not include these variables as covariates in our model. However, we did consider the effects of smoking and working outdoors as potential confounders in the final models. We also controlled for whether the study day was a work day or a nonwork day. We first considered single-pollutant models and then considered two-pollutant models with each combination of pollutants. For the two-pollutant models, we included an interaction term. Because exposures were log transformed, we reported effect estimates and 95% confidence intervals associated with a doubling in exposure.

All statistical analyses were performed in R version 3.6.1.[47]

RESULTS

Study Population Demographics

A total of 36 dairy workers participated in our study (Table 1). The mean (SD) age of the participants was 34 (12) years, and most (91%) identified as Latinx. Seven women (20%) participated in our study. A majority (66%) of the participants primarily worked outdoors.

TABLE 1. Participant Demographics

Variable	Mean (SD)	Min	Max
Age			
Years	34 (12)	19	64
Height			
In	66 (4)	56	75
Weight			
lbs.	168 (33)	110	270
	N		**%**
Sex			
Female	7		20
Male	28		80
Ethnicity			
Latinx	32		91
Not Latinx	3		9
Race			
Non-White	13		46
White	15		54
Smoking status			
Smoker	8		23
Nonsmoker	27		77
Primary work environment			
Indoors	12		34
Outdoors	23		66

Exposures to Dust, Bioaerosols, and Ozone

Exposure data were available for 137 work shifts and nonwork days. Some participants repeated the study and had up to eight measurements collected. The number of days monitored for each participant ranged from 1 to 8, with a mean (SD) number of exposure measurements per participant of 3.9 (2.0). The geometric mean (GSD) dust exposures were comparable between outdoor and indoor work environments [0.22 (3.30) mg/m^3 vs 0.22 (3.04) mg/m^3, respectively; $P = 0.83$] (Table 2). Endotoxin exposures tended to be higher for workers who worked primarily indoors compared to outdoor workers [60.50 (11.46) EU/m^3 vs 37.93 (6.31) EU/m^3, respectively; $P = 0.03$], whereas β-glucan exposures were higher for workers who worked primarily outdoors [1633.28 (2.70) pg/m^3] compared to workers who worked primarily indoors [1185.20 (2.83) pg/m^3, $P = 0.32$]. Ozone exposures were similar for indoor and outdoor workers because exposures were based on the measurements recorded at the nearby air quality network monitor.

Changes in Pulmonary Function

In general, changes in lung function measured across the work shift were greater for workers who primarily worked outside compared to those who worked inside. Table 3 summarizes the change in percent predicted values and change in PFT residuals for the full study population and the population stratified by primary work environment. The greatest decline in PFT measures (based on the difference in percent predicted values) were observed for FEV_1 (1.70 unit decrease for outdoor workers). On average, FEF_{25-75} increased over the course of the shift for indoor workers compared to outdoor workers (0.4 unit increase for indoor workers vs 1.34 unit decrease for outdoor workers, $P = 0.52$). Contrary to most of the other lung function results, PEFR increased over the course of the work shift (2.51 units on average) and increases in PEFR were higher for indoor workers (6.29 units) compared to outdoor workers (1.13 units, $P = 0.10$). Patterns for changes in PFT residuals were similar to those of the changes in percent predicted values (Table S1 in the Supplementary Digital content, http://links. lww.com/JOM/A724).

TABLE 2. Summary of Exposure Measurements for All Participants and Participants Stratified by Primary Work Location (Outdoor vs Indoor)

Exposure	All Measurements ($N = 137$)	Outdoor Workers ($N = 101$)	Indoor Workers ($N = 36$)	*P* Value*
Dust (mg/m^3)				
GM (GSD)	0.22 (3.23)	0.22 (3.30)	0.22 (3.04)	0.83
Min	0.01	0.01	0.01	
Max	3.81	3.81	1.29	
Endotoxin (EU/m^3)				
GM (GSD)	42.67 (7.53)	37.93 (6.31)	60.50 (11.46)	0.03
Min	0.04	0.17	0.04	
Max	1595.06	1595.06	1349.35	
β-glucan (pg/m^3)				
GM (GSD)	1,505.65 (2.76)	1,633.28 (2.70)	1,185.20 (2.83)	0.32
Min	57.88	189.13	57.88	
Max	22598.25	22598.25	6447.10	
Same-day ambient ozone (ppm)				
GM (GSD)	0.05 (1.21)	0.05 (1.20)	0.05 (1.22)	
Min	0.03	0.03	0.03	
Max	0.08	0.08	0.08	

EU, endotoxin units; GM, geometric mean; GSD, geometric standard deviation, ppm, parts per million.
*Wilcoxon test comparing the distribution of exposure between outdoor and indoor workers.

Associations Between Dust, Bioaerosols, and Ozone Exposure and Changes in Pulmonary Function

In unadjusted single pollutant models, increases in endotoxin and dust exposures were associated with increases in percent predicted PEFR. A doubling in endotoxin exposure was associated with a 1.32 (95% CI: 0.29, 2.35) unit increase in percent predicted PEFR and a doubling in dust exposure was associated with a 4.82 (−6.70, 16.33) unit increase in percent predicted PEFR.

Associations persisted for dust and PEFR after controlling for potential confounders (Table 4). Associations between endotoxin and PEFR remained suggestive after adjustment for potential confounding; a doubling in endotoxin exposure was associated with a 1.03 (−0.31, 2.36) unit increase in percent predicted PEFR. After adjusting for current smoking, working primarily indoors, and whether measurements were collected on days off, a doubling in dust exposure was associated with a 2.24 (0.18, 4.30) unit increase in percent predicted PEFR.

There was little evidence of associations between our exposures and any of the other changes in percent predicted lung function measurements in the single pollutant models. Similar to the percent predicted values, there was no evidence of associations between any of the pollutants and the PFT residuals (Table S2, http://links.lww.com/JOM/A724).

We also explored two-pollutant models using the percent predicted values as our outcomes of interest (Table S3, http://links.lww.com/JOM/A724). In a two-pollutant model with endotoxin and dust exposures and no interaction term, the relation

TABLE 3. Summary of Differences in PFT Measurements

PFT Result	All Measurements ($N = 137$)	Outdoor Workers ($N = 101$)	Indoor Workers ($N = 36$)	*P* Value*
FVC Percent Predicted (% points)				
Mean (SD)	−1.26 (4.01)	−1.55 (4.18)	−0.46 (3.44)	0.13
Min	−15	−15	−8	
Max	11	11	6	
FEV_1 Percent Predicted (% points)				
Mean (SD)	−1.60 (4.99)	−1.79 (5.43)	−1.06 (3.54)	0.37
Min	−26	−26	−7	
Max	10	10	9	
FEV_1/FVC Percent Predicted (% points)				
Mean (SD)	−0.37 (3.34)	−0.28 (3.70)	−0.63 (2.09)	0.50
Min	−15	−15	−5	
Max	6	6	4	
FEF_{25-75} Percent Predicted (% points)				
Mean (SD)	−0.69 (14.07)	−1.08 (15.30)	0.40 (10.02)	0.52
Min	−62	−62	−15	
Max	26	26	26	
PEFR Percent Predicted (% points)				
Mean (SD)	2.51 (17.70)	1.14 (18.59)	6.29 (14.59)	0.10
Min	−54	−54	−23	
Max	59	59	34	

As differences in percent predicted values across work shifts for all participants and participants stratified by primary work location (outdoor vs indoor).
FEV_1, forced expiratory volume in the first second; FEF_{25-75}, forced expiratory flow at 25% to 75%, FVC, forced vital capacity; PEFR, peak expiratory flow rate.
*t test comparing the mean difference in PFT percent predicted values for outdoor and indoor workers.

TABLE 4. Difference (95% confidence interval) in Percent Predicted Pulmonary Function Test Results (post-shift–pre-shift) for a Doubling in Exposure to Dust, Bioaerosols, or Ozone[*,†]

Outcome	Dust	Endotoxin	β-glucan	Ozone
FVC (% points)	0.15 (−0.33, 0.64)	0.08 (−0.24, 0.39)	0.03 (−0.48, 0.54)	0.44 (−2.27, 3.15)
FEV_1 (% points)	0.22 (−0.36, 0.81)	−0.10 (−0.49, 0.28)	0.04 (−0.57, 0.65)	0.80 (−2.43, 4.04)
FEV/FVC (% points)	0.22 (−0.18, 0.61)	−0.06 (−0.32, 0.20)	0.13 (−0.28, 0.54)	0.04 (−2.15, 2.23)
FEF_{25-75} (% points)	0.33 (−1.32, 1.99)	−0.40 (−1.49, 0.69)	0.21 (−1.52, 1.95)	4.43 (−4.76, 13.62)
PEFR (% points)	**2.24 (0.18, 4.30)**	**1.03 (−0.31, 2.36)**	1.21 (−1.00, 3.42)	4.86 (−6.80, 16.53)

Boldface indicates statistical significance.

FEV_1, forced expiratory volume in the first second; FEF_{25-75}, forced expiratory flow at 25–75%, FVC, forced vital capacity; PEFR, peak expiratory flow rate.

[*]Exposures were log transformed and analyzed as continuous variables.

[†]Linear mixed effect models were adjusted for smoking (binary: current vs nonsmoker); primary work environment (binary: indoor vs outdoor) and whether measurements were collected on nonwork days (binary: day off vs work day).

between dust and PEFR was attenuated but remained suggestive of an association: a doubling in endotoxin exposure was associated with a 0.21 (95%CI: −1.49, 1.91) unit increase in percent predicted PEFR and a doubling in dust exposure was associated with a 2.04 (95%CI: −0.60, 4.68) unit increase in percent predicted PEFR. After adding the interaction term, these associations were further attenuated (Table S3, http://links.lww.com/JOM/A724). No other associations between the cross-shift change in percent predicted PFT outcomes and exposures were observed in the two-pollutant models.

DISCUSSION

Counter to our original hypothesis, we found little evidence of associations between higher bioaerosol or ozone exposures and decreases in PFT measurements across work shifts for our study population of dairy workers in high plains dairies. We did, however, find an association between higher levels of dust exposure and increases in PEFR that was robust to adjustment for confounding by smoking status, primary work environment, and whether measurements were taken on work days or days off.

Our findings differed from other studies recently published on the respiratory health of dairy workers. A cross-sectional study in California observed decreases in FVC for workers exposed to endotoxin;[8] this study also observed decreases in FVC with increasing number of hours worked. In contrast, a study of dairy workers in the Midwestern United States (Iowa, Minnesota, South Dakota, and Wisconsin) found no associations between cross-shift pulmonary function and dust or endotoxin.[9] Differences across studies may be due to the low sample sizes used in our study ($n = 36$) and the Midwestern US study ($n = 62$)[9] relative to the California study ($n = 205$ dairy workers).[8] Another explanation may be potential genetic differences in the study populations. A study of agricultural workers in Colorado and Nebraska identified greater decreases in pulmonary function after exposure to endotoxin for workers with polymorphisms in the Toll-like receptor 4 (TLR4) gene,[12] a signaling pathway that recognizes and responds to lipopolysaccharides such as endotoxin.[48] In this previous study, 65% of participants identified as not Hispanic or Latino,[12] whereas only 9% of our study population identified as not Hispanic or Latino (Table 1). Potential genetic variability in populations of differing ethnicities may have resulted in different effect estimates across prior studies.

It is also possible that our lack of supportive results is in part due to lower exposures for our population relative to other concentrations reported in the literature. The GM (GSD) dust and endotoxin exposures for our dairy workers were 0.22 (3.23) mg/m^3 and 42.67 (7.53) EU/m^3, respectively. Our endotoxin exposures in particular were generally lower than previously reported values and are below suggested endotoxin exposure guidelines based on studies from other livestock environments.[49,50] For example, Nonnenmann et al[9] reported a GM (GSD) for endotoxin of 118 EU/m^3 in midwestern dairies. Even higher values have been reported in France (128 EU/m^3),[15] California (up to 370 EU/m^3 for some dairy workers)[6], and The Netherlands (360 EU/m^3).[14] In contrast, our measured β-glucan concentrations are in line or higher than previously reported concentrations measured in greenhouses.[22,51] One prior study of β-glucan exposure in greenhouses in the Midwest region of the United States observed more prevalent self-reported respiratory symptoms at lower concentrations than those observed in our study (10 – 100 ng/m^3).[51] Low bioaerosol exposures may have contributed to our lack of supportive evidence, particularly for dust and endotoxin exposures.

In two pollutant models, we observed an attenuation of the effect of endotoxin on PEFR when controlling for dust. We also did not observe an interaction between dust and endotoxin on PEFR or other lung function parameters. These results suggest that the effects of dust on lung parameters may be due to an unmeasured component of the particulate matter. For example, we did not include a measurement of Gram-positive bacteria. Previous studies of bioaerosol exposures have demonstrated that Gram-positive bacteria account for part of inflammatory response.[52]

Increases in percent predicted PEFR with higher dust exposures may also be the result of diurnal patterns in both dust and PEFR. PEFR has historically been used in occupational studies to assess short term respiratory health effects of inhaled exposures.[53] In general, we expected PEFR to decrease across work shifts as a result of exposures to respiratory hazards, as has been demonstrated in other occupational health studies. However, PEFR may not be as reliable a measure of respiratory health compared to other metrics (eg, FEV_1), in part due to its increased sensitivity to upper airway function as well as participant effort or attitudes about the testing.[54] Studies in healthy adult populations have demonstrated diurnal patterns in PEFR, with lower rates measured in the morning and higher rates measured in the afternoon or evenings.[55,56] Similarly, data from agricultural operations in the United States suggest there are diurnal patterns in particulate matter concentrations in cattle feedlots and dairies. For example, cattle feedlots in the high plains of Kansas experienced daily fluctuations in coarse particulate matter (PM_{10}) concentrations, with peaks occurring in the late afternoon and evening hours.[57] Similar patterns were observed for a dairy in Washington.[58] In our study, a majority (91%) of pre-shift measurements were taken in the morning (before 10:00 AM). Thus, our increase in PEFR may be in part due to these diurnal patterns in PEFR and exposure rather than a true increase in PEFR resulting from exposure.

Importantly, our study may have been subject to the healthy worker bias.[59] The population of dairy workers recruited to participate in this study were relatively young and without significant underlying respiratory disease. Few of our participants reported any respiratory or other health symptoms during the study (data not shown). Workers with underlying asthma are at increased risk of

developing respiratory symptoms in agricultural settings,[60] and to the best of our knowledge, none of our sample cohort had asthma. We did not have information on how long our participants had worked in agricultural settings prior to enrolling in our study. Workers with a longer history of agricultural work may have developed a chronic inflammatory adaptation response, in which a previously unexposed person develops respiratory symptoms and airway hyper-responsiveness upon first exposure that dampens with sequential exposures.[61] Although the acute PFT response after exposures may have dampened, workers who have developed chronic inflammatory adaptation response due to repeated exposures may remain at-risk of developing chronic lung disease over repeated, long-term exposures. A majority of our participants (66%) primarily worked outdoors where inflammatory bioaerosol exposures tended to be lower; working outdoors at the dairy may be a more ideal occupational exposure environment despite the potential risk for increased exposures to ambient air pollutants such as dust or ozone.

There are some important limitations to note when interpreting the results of this study. First, our sample size was relatively small compared with other studies in the literature; we enrolled a total of 36 dairy workers. Although we incorporated repeated measures of exposure and outcome, it is likely the study was underpowered to detect small effects of dust and bioaerosols on respiratory health. Second, as discussed above, exposures were generally lower than reported elsewhere. Moreover, this was the first study (to the author's knowledge) to measure dairy workers' exposure to airborne β-glucan; a few studies have been published related to exposures in greenhouses.[22,51] Differences in protocols for sampling and performing filter extractions were observed, which may bias recovery of β-glucan. Hence, it is important to consider filter material and extraction conditions for measuring airborne β-glucan in future studies. Optimization and standardization of such procedures will help advance the field of bioaerosol exposure science. Third, we did not include an indicator of exposure to Gram-positive bacteria such as muramic acid. Lastly, we used data from an Environmental Protection Agency monitor located within 50 km of the dairies in our study to assign exposures to ambient ozone. Due to limitations in our personal sampling approach, we were not able to measure individual-level ozone exposures, resulting in the same exposures being assigned to workers regardless of whether they primarily worked indoors or outdoors. Variability in ozone exposures was further reduced because study visits were on sequential days within the same season, and daily ozone variability tended to be low. The use of a single monitoring location to assign exposures may have resulted in exposure misclassification because gradients in exposure (both between the dairies and between indoor and outdoor workers) would not have been well represented.

Despite these limitations, our study has some key strengths. We had repeated measures for exposures and respiratory health for our worker population, with some measurements collected on days off. Repeated measures allowed us to account for intra-individual differences in pulmonary function across the study period. Measuring exposures and respiratory function on nonwork days also allowed us to account for continued exposure among some of our participants; 17 of our 36 participants (47%) of our participants lived in employee housing on the dairy. Interestingly, our study population comprised of a larger sampling of female participants (20%) as compared to previous investigations, which might reflect an emerging trend in the field of agriculture that has been previously dominated by men. However, we are not able to detect potential differences based on sex in this small sample size. In addition to measuring pulmonary function with spirometry, we collected cross-shift biomonitoring data on cytokine levels. Future studies will focus on changes in cytokine levels that may be more sensitive to bioaerosol exposures than the pulmonary function testing used here.

CONCLUSIONS

Overall, we found no cross-shift associations between exposure to dust, bioaerosols, and ozone and the adverse changes in pulmonary function parameters. However, we did find associations between endotoxin and dust exposures and PEFR that were robust to adjustment for confounding. These relationships were in the opposite direction of our original hypothesis and may be related to the timing of data collection in our study. There remains uncertainty about how bioaerosol exposures impact the respiratory health of dairy workers. Future studies should aim to increase both sample size and incorporate a repeated measures design to better account for intra-individual variability in both exposures and respiratory health outcomes. Additionally, future studies may benefit from the creation of an inception cohort where new, nonexposed agricultural workers are recruited and followed to avoid issues related to the healthy worker bias and the chronic inflammatory adaptation response.

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