Journal of Agromedicine

ISSN: (Print) (Online) Journal homepage: www.tandfonline.com/journals/wagr20

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To cite this article: Grant Erlandson, Sheryl Magzamen, Julia L. Sharp, James Seidel, Jill A. Poole, Mary Bradford & Joshua W. Schaeffer (2025) Hypertonic Saline Nasal Rinse Intervention: Immunomodulatory Effects in Dairy Workers, Journal of Agromedicine, 30:1, 27-37, DOI: 10.1080/1059924X.2024.2416425

To link to this article: https://doi.org/10.1080/1059924X.2024.2416425

JOURNAL OF AGROMEDICINE
2025, VOL. 30, NO. 1, 27–37
https://doi.org/10.1080/1059924X.2024.2416425

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Hypertonic Saline Nasal Rinse Intervention: Immunomodulatory Effects in Dairy Workers

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ABSTRACT

Objective: Increased risk of occupational exposure to bioaerosols has long been recognized in livestock operations including dairy facilities. Spanning the inhalable fraction (0–100 µm), dairy bioaerosols comprise a wide variety of inflammatory components that deposit in the nasopharyngeal region. The resultant inflammatory response from bioaerosol exposure is likely driving the increased prevalence of respiratory disease observed in dairy workers. It is also thought the microbiome of the upper respiratory system may help mediate this inflammation. We investigated the viability of a low-cost hypertonic saline nasal rinse intervention in modulating inflammatory responses in bioaerosol exposed dairy workers and its impact on microbial diversity.

Methods: Pre- and post-shift nasal rinses were administered and collected alongside full shift inhalable personal breathing zone (PBZ) samples for each participant for up to 5 consecutive days. Treatment group participants ($n = 23$) received hypertonic saline rinses while control group participants ($n = 22$) received normotonic saline rinses. Particulate matter (PM) and endotoxin concentrations were quantified from PBZ samples using gravimetric and enzymatic analytical methods, respectively. Pre- and post-shift rinses were analyzed for pro- and anti-inflammatory markers and microbial diversity using a multiplex assay and 16S rRNA sequencing, respectively.

Results: PM and endotoxin concentrations were comparable between groups indicating similar exposures. Post-shift pro-inflammatory markers were significantly higher than pre-shift for IL-13 ($p = .047$), IL-1β ($p < .001$), IL-6 ($p < .001$), IL-8 ($p < .001$), and TNF-α ($p = .024$). There was no evidence of a difference in log concentrations between intervention group or day among any of the measured inflammatory markers. Anti-inflammatory IL-10 concentrations increased across the 5 sample days, independent of treatment group suggesting tonicity may not be driving the change. However, this result was not significant ($p = .217$). Nasal microbiome alpha (within sample) and beta (between sample) diversity metrics did not differ significantly between group or day demonstrating no adverse washout intervention effects.

Conclusion: This study provided encouraging results that warrant future research to further evaluate saline nasal rinses as a workplace intervention.

KEYWORDS
Agricultural health; bioaerosols; inflammation; intervention; nasal lavage

Introduction

Dairy workers are at an increased risk of exposure to airborne biological particulate matter (bioaerosols) commonly found in livestock operations.[1–6] Dairy-specific bioaerosols exist as complex mixtures of dust that contain biologically derived particles, including allergens (e.g., dander), microbial communities, and pro-inflammatory constituents (e.g., endotoxin, muramic acid, and β-glucans).[3,7–9] Spanning an aerodynamic diameter of 0 µm to 100 µm, bioaerosols encompass the inhalable fraction that impacts in the nasopharyngeal region (e.g., nose, mouth, and pharynx).[4] Once inhaled, these microbial constituents have the capacity to elicit inflammatory immune responses in the upper airways and are likely driving the increased prevalence of rhinosinusitis and contributing to chronic obstructive pulmonary disease (COPD), asthma, allergic rhinitis, and respiratory pneumonitis well-documented in dairy workers.[10–14] Furthermore, bioaerosol exposure seems to significantly shift nasal microbiome composition in workers' noses and may provide protection against pathogens.[6,15–18]

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Supplemental data for this article can be accessed online at https://doi.org/10.1080/1059924X.2024.2416425

Previous studies have demonstrated bioaerosol exposure concentrations in dairy operations can vary by several orders of magnitude, ranging from 0.22 to 9.8 mg/m^3.[3,8,9,19,20] Currently, no enforceable standards exist for occupational bioaerosol exposure or its inflammatory constituents, making the clinical relevance of measured exposures difficult to determine without robust outcome data. However, a recommended exposure limit for bioaerosols of (2.4 mg/m^3) was suggested in a publication by Reynolds et al.[21] Furthermore, the Dutch Expert Committee on Occupational Safety developed the former Dutch standard for endotoxin, an inflammatory component of gram-negative bacterial cell walls, of 90 endotoxin units per meter cubed (EU/m^3).[22] Previous research comparing worker endotoxin exposures across 30 dairies in the High Plains region of the U.S. found 89% of sampled workers exceeded the former Dutch standard.[3] Based on reported concentrations, dairy workers are exposed to bioaerosols and inflammagens exceeding recommended occupational limits resulting in a disproportionate burden of respiratory diseases and chronic allergies. Consequently, bioaerosol exposure and efforts to mitigate it continue to be a major industry concern.

Dairy industry practices including 24-hour production schedules, extended work shifts, contact with livestock, and use of heavy equipment create a unique exposure environment compared to other agricultural industries.[23,24] The size of dairies has also increased dramatically as median herd size has grown from just 80 in 1987 to 1300 in 2007.[25] A notable shift towards larger operations working with bigger herds (>500 head) has altered production scale and dramatically changed the workplace dynamics of dairy operations.[23,24,26,27] Worker demographics have also contributed to these challenges, as reliance on an under experienced, immunologically naïve immigrant workforce (>90% Hispanic/Latino) has led to a highly exposed and highly susceptible workforce.[23,27,28]

Interventions attempting to control bioaerosols exposure and resultant respiratory illnesses in dairy settings have been encouraging, but major challenges regarding long term efficacy and economic feasibility remain.[6,29,30] Through guidance from the High Plains Intermountain Center for Agricultural Health and Safety (HICAHS) Advisory Board, we identified a low-cost intervention with the potential to improve dairy worker respiratory outcomes. Previously, we pilot tested the effectiveness of a hypertonic saline (HTS) nasal lavage,[19] based on previous *in vitro* and *in vivo* studies, to modulate the pro- and anti-inflammatory response.[31–37] Hypertonic saline has been shown to inhibit release of pro-inflammatory cytokines that signal for an inflammatory immune response and stimulate release of anti-inflammatory cytokines that limit inflammatory immune responses.[31–37] Dairy workers receiving HTS nasal rinses across 5 consecutive days experienced a significant increase in anti-inflammatory cytokine interleukin (IL)-10 compared to those receiving a normotonic saline (NTS), but also produced conflicting increases in IL-6 and IL-8 pro-inflammatory cytokines.[19] We aimed to build off of this result with a larger cohort to better understand the immunomodulatory effects of HTS in dairy workers.

Potential side effects from regular nasal rinses on the resident microbial communities present in the nose were not evaluated in the pilot study. Diverse resident microbiomes help maintain the stability and health of their environment, in this case the upper airways.[38,39] Previous studies have demonstrated a role for microbes and pathogens in the development of mucosal inflammation.[40,41] Therefore, perturbations to microbial diversity from hypertonic rinses could compromise stability and have adverse inflammatory effects. Consequently, the viability of HTS rinses as an intervention for respiratory disease in dairy workers required further testing in a larger cohort. We hypothesized that administering pre-and post-shift HTS nasal rinse to dairy workers would reduce the pro-inflammatory cytokine response and increase anti-inflammatory production when compared to an NTS control rinse. We also hypothesized the effect would be cumulative and would not cause perturbations or washout resident protective microbiota.

Methods

Participant recruitment

We identified and recruited representative modern dairy operations across Colorado and Texas through the HICAHS Dairy Advisory board. Participation in the study was offered to all dairy facility employees with availability during

sampling campaigns. The Colorado State University Institutional Review Board approved all study protocols and language-specific informed consent was obtained in person before data collection (IRB approval #3015). Sampling took place between May 2019 and August 2022, with visits non-uniformly distributed across seasons. A total of 45 study participants with varying shift times and work weeks agreed to participate and were randomly assigned to the intervention group ($n = 23$) or the control group ($n = 22$). Participant job tasks involved milking, birthing, insemination, office work, re-bedding, animal medical care, and animal transport. Personal protective equipment among workers was limited (e.g., long sleeves, boots, thick material pants, and sometimes cloth masks). Following informed consent, participants completed a medical history questionnaire containing exclusionary criteria in English or Spanish. Example exclusionary criteria included use of steroidal nasal sprays, immunosuppressant and autoimmune medications, recent surgery, chest injuries, and history of heart disease or stroke. Daily pre- and post-shift health and job task questionnaires were administered to record changes in symptoms among participants and animal contact time respectively. All questionnaires were based on the American Thoracic Society (ATS) standard questionnaire with dairy-specific modifications.

Sample collection

Inhalable dust

Full work-shift personal breathing zone (PBZ) inhalable dust samples were collected from each participant for up to 5 consecutive sampling days using the SKC Button sampler (SKC Inc., Eighty-Four, PA). Button samplers were equipped with 25 mm, 5 µm pore size PVC filters and connected to SKC XR5000 sampling pumps (SKC Inc., Eighty-Four, PA) calibrated to 4 L/min using a primary calibration standard (DryCal Defender 510 Mesa Labs, Butler, NJ). A pump flow rate drift less ± 5% between pre- and post-calibration was considered acceptable. Filters were dried in a desiccator 24hrs prior to gravimetric analysis before and after sampling. Gravimetry was performed with a Mettler MT5 balance (Mettler-Toledo, Columbus, OH). Field blanks were averaged and used to blank subtract sample weights before concentration calculations. Post-gravimetry, samples were stored in −80°C for downstream endotoxin analysis.

Endotoxin

Inhalable dust was extracted from PBZ filters in pyrogene-free LAL water with 0.05% Tween-20. Filter extracts were processed through the Recombinant Factor C (rFC) assay (Lonza, Basel, Switzerland) on a Biotek reader (Biotek Instruments FLX800TBIE, Winooski, VT) as previously reported.[2] Endotoxin quantification was based on level of fluorescence and reported in endotoxin units per cubic meter of air (EU/m^3). Reagent blanks, filter blanks, and quality control spikes were used to ensure accuracy of results.

Nasal lavage and pro-inflammatory cytokines

Participants were administered nasal lavages pre- and post-shift for up to 5 consecutive workdays. Intervention group participants received hypertonic saline solution (400mOsm) while control group participants received a normotonic saline solution (308mOsm). After collection, a protease inhibitor was added (1% v/v) to lavage samples before being put on ice and subsequent storage in a −80°C freezer. Lavages were vortexed and aliquoted into 1 ml supernatants prior to cytokine and microbiome analysis.

Lavage aliquots were analyzed via Meso Scale Discovery technology (MSD) for 10 cytokines and chemokines, including pro-inflammatory interleukin (IL) −1β, IL-2, IL-4, IL-6, IL-8, IL-12p70, IL-13, interferon gamma (IFN-γ), tumor necrosis factor (TNF)-α, and the anti-inflammatory cytokine IL-10. Samples were analyzed and quantified with the MSD V-PLEX assay and MSD QuickPlex SQ 120 (MESO Scale Diagnostics, Rockville, MD).

Nasal lavage aliquots were analyzed using 16S rRNA sequencing technology to characterize the microbial composition of workers nasal microbiome at the University of Oklahoma Health Sciences Center. Samples were extracted and isolated using Zymo research Quick-DNA kits (Irvine, CA). Libraries were prepared with IDT DNA Technology primers and KAPA reagents according to the Illumina 16S Metagenomic

Sequencing Library Prep protocol. PCR amplification of the 16S rRNA V3 and V4 regions was conducted with 5 ng of genomic DNA to construct individual libraries. Libraries were indexed for multiplex sequencing and sequenced with a MiSeq 600 Cycle Reagent Kit v2 on the Ilumina MiSeq platform. Resulting 300 bp paired end reads were processed in qiime2-2022.2 (q2).[42] Sequence reads were denoised and organized into a feature table using the q2 DADA2 plugin.[43] Taxonomic classifications of amplicon sequence variants (ASV) were performed with a custom Naïve Bayes trained Silva classifier. Finally, a phylogenetic tree was created from the SILVA 128 SEPP reference database with the q2 sepp-fragment insertion plugin.[44]

Statistical analysis

To limit the effect of sample concentrations below the limit of detection on data analysis, an exclusion criterion was applied to each analyte. In cases where more than 70% of measurements were above the LOD (e.g., detectable concentrations), the analyte was evaluated statistically. Analytes with less than 70% of measurements above the LOD were excluded from further analysis. Among analytes where more than 70% of measurements were above the LOD, all non-detect measurements were assigned a value equal to the LOD divided by the square root of two.

Bioaerosol exposure concentrations exhibited a log normal distribution and were log transformed for statistical analysis. Geometric means (GM) and geometric standard deviations (GSD) were calculated and compared to previously suggested occupational exposure limits.[21,22,45] A statistical analysis was conducted to determine the effect of the intervention on the measured inflammatory markers.

A linear mixed effects model was used for both dust and endotoxin to compare exposures between study variables to determine if exposures were consistent between treatment groups and sample days. Model fixed effects included group (treatment and control) and day (sampling day 1–5) with a random effect for participant to account for repeated measures. Two-way interaction effects were included in both models. All dust and endotoxin measurements were Log (base 10) transformed to address heteroskedasticity of residuals. Dust and endotoxin models are expressed as:

$$y_{ijk} = \mu + group_i + day_j + group * day_{ij} + p_k + \varepsilon_{ijk} \quad (1)$$

where y_{ijk} represents the log exposure level of the k^{th} participant in the i^{th} group (treatment, control) on day j ($j = 1, \ldots, 5$). Random effect for participant k is denoted as p_k which exhibits a normal distribution with mean 0 and variance σ_p^2. ε_{ijk} represents the random error term that follows a normal distribution with mean 0 and variance σ_ε^2. Exposure model results were used to establish whether dust and endotoxin measures would be included as a variable in the outcome model below.

An outcome model was built for each cytokine and used the same fixed and random effects as in (1) but also includes the fixed effect term $time\_of\_day$ and subsequent interaction terms $time\_of\_day * day_{jm}$, $time\_of\_day * group_{im}$, and $time\_of\_day * group * day_{ijm}$ where time of day has two levels of am and pm. A variance component covariance structure was used in the estimation of the random effects.

Finally, using the cytokine models, we conducted sensitivity analyses to assess the impact of animal contact time on cytokine concentrations. Animal contact time was quantified by totaling the time workers spent performing tasks that require close contact with cows (e.g., milking, birthing, veterinary care, etc.). Workers were categorized into low (<2 hours/day), medium (2–4 hours/day), and high contact (>4 hours/day). Animal contact was then added to each individual cytokine model and its importance was evaluated by measuring model AIC before and after its addition. Decrease in AIC after addition was considered indicative of an improvement in model fit. Animal contact was not significantly associated with any cytokine outcome and increased the AIC of each model when added. Therefore, animal contact was omitted from final models. A table of model estimates with animal contact time can be found in Supplementary Table 1.

Each of the mixed models were fit according to the Kenward Rogers method using the R package

"afex".[46] Within model multiple comparison adjustments using Holm's procedure was conducted with the "emmeans" package.[47] Model estimated marginal means and associated confidence intervals were plotted to investigate and interpret interactions. All tests were conducted using a significance level of 0.05 in R (Version 4.3.0).[48]

Metagenomics analysis

Statistical testing of nasal microbiome alpha and diversity metrics to determine differences in the intervention group and the control group was conducted with the core metrics pipeline in QIIME2 software using a significance level of 0.05. Sample alpha diversity was estimated with the q2 breakaway package.[49] Differences in alpha diversity between treatment group, day, and time were tested for with pairwise Kruskal-Wallis tests. Beta diversity estimates were determined with the q2 DEICODE package[50] and compared with pairwise PERMANOVA testing to determine significant differences between group, day, and time.

Results

A total of 45 dairy workers participated in the study for up to 5 consecutive days at five dairies in the High Plains region. With participation ranging from one to 5 days, 344 nasal lavages and 178 PBZ samples were collected and analyzed. Participation duration and animal contact time by group, shown in Table 1 were evenly distributed between groups. Geometric mean dust and endotoxin concentrations for the treatment group were 0.36 mg/m^3 and 52.99 EU/m^3, respectively. Geometric mean dust and endotoxin concentrations for the control group were 0.36 mg/m^3 and 56.81 EU/m^3, respectively. PBZ dust and endotoxin concentrations stratified by day and treatment group are presented in Table 2.

From the ten pro- and anti-inflammatory biomarkers measured from each nasal lavage, IFN-γ, IL-12p70, and IL-4 were removed for not meeting our criteria requiring at least 70% of values to be above the limit of detection. Group level comparisons among the remaining seven biomarkers indicated concentrations of TNF-α were significantly higher in the treatment group compared to the control group ($p = .018$). Log adjusted biomarker concentrations averaged by treatment group, day, and time are presented in picograms per milliliter (pg/ml) in Figures 1–4. A summary of results from the linear mixed effects model fits is presented in Table 3.

The dust model did not reveal sufficient evidence of an effect on average log mg/m^3 between

Table 1. Participant Characteristics by Treatment Group.

	Treatment (n = 23)		Contol (n = 22)	
Participant Characterisic	n	%	n	%
Length of Participation				
1 day	23	100	22	100
2 days	23	100	22	100
3 days	19	82.6	20	90.9
4 days	13	56.5	14	63.6
5 days	9	39.1	9	40.9
Animal Contact Time				
Less than 1 hour	5	21.7	5	22.7
1 to 4 hours	3	13.0	2	9.1
4 to 8+ hours	15	65.2	15	68.2

Table 2. Geometric mean (geometric standard deviation) dust and endotoxin concentrations by treatment group and day.

	Dust (mg/m^3)		Endotoxin (EU/m^3)	
	Treatment	Control	Treatment	Control
Day 1 ($N = 45$)	0.316 (3.35)	0.369 (3.02)	69.82 (6.28)	39.27 (9.61)
Day 2 ($N = 43$)	0.299 (3.11)	0.336 (2.44)	36.18 (9.33)	51.29 (5.36)
Day 3 ($N = 39$)	0.468 (4.61)	0.324 (2.69)	60.07 (9.43)	54.55 (4.49)
Day 4 ($N = 27$)	0.412 (3.84)	0.356 (2.57)	101.92 (4.72)	84.18 (4.28)
Day 5 ($N = 18$)	0.347 (5.27)	0.472 (2.47)	23.65 (8.64)	75.83 (5.92)

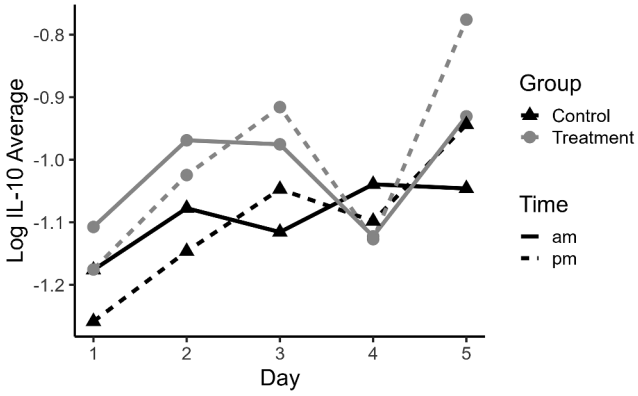

Figure 1. Average Log IL-10 across the 5 days for HTS treatment (green) and NTS control groups (orange) pre-shift (solid line) and post-shift (dotted line). Log IL-10 standard deviations ranged from 0.1035 to 0.6497.

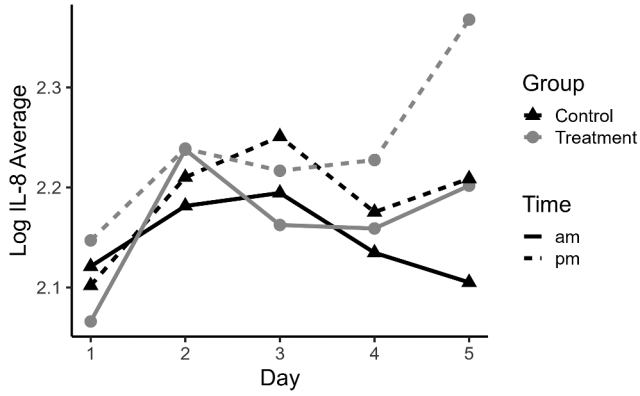

Figure 4. Average Log IL-8 across the 5 days for HTS treatment (green) and NTS control groups (orange) pre-shift (solid line) and post-shift (dotted line). Log IL-8 standard deviations ranged from 0.2217 to 0.6041.

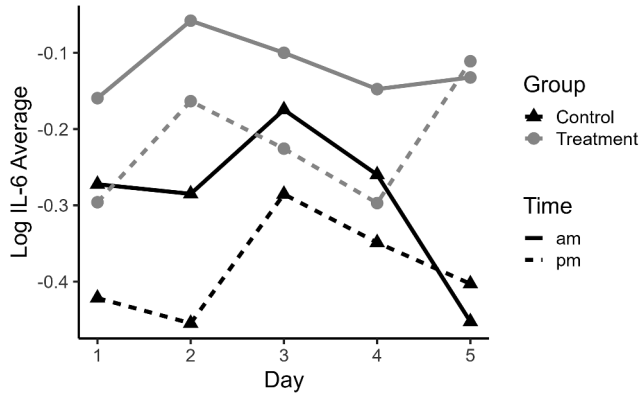

Figure 2. Average Log IL-6 across the 5 days for HTS treatment (green) and NTS control groups (orange) pre-shift (solid line) and post-shift (dotted line). Log IL-6 standard deviations ranged from 0.2811 to 0.9761.

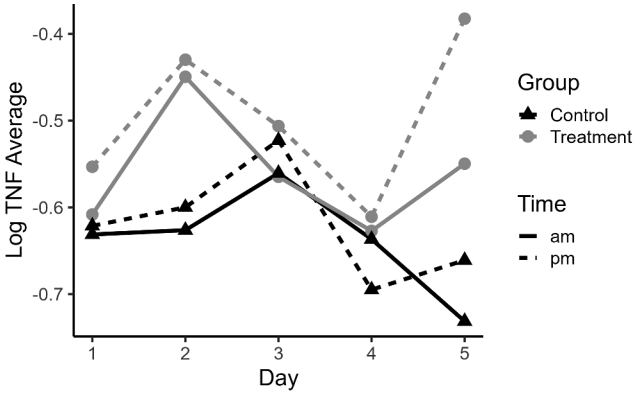

Figure 3. Average Log tnf-α across the 5 days for HTS treatment (green) and NTS control groups (orange) pre-shift (solid line) and post-shift (dotted line). Log TNF-α standard deviations ranged from 0.1233 to 0.6211.

treatment groups (HTS and control) ($p = .95$), day ($p = .826$), or an interaction effect between treatment and day ($p = .703$). Similarly, the endotoxin model did not indicate evidence of an effect on average log EU/m^3 between treatment groups ($p = .781$), among days ($p = .651$), or an interaction effect between treatment and day ($p = .458$). These results indicate similar dust and endotoxin exposure between treatment groups and sampling days. Therefore, exposure was not included as a variable in the biomarker model.

The biomarker model provided evidence of an interaction effect on average log concentrations between day and time of day for anti-inflammatory cytokine IL-10 ($p = .004$). However, neither day nor time of day were significant main effects. After adjusting for multiple comparisons using Holm's procedure, there was evidence that average log IL-10 concentrations were higher on day 5 than day 1 in AM samples ($p = .026$). To visualize the interaction in absence of main effects, we created a plot of the model estimated marginal means shown in Figure 5. The plot illustrates a crossover interaction for IL-10 production between pre-shift (AM) and post-shift (PM) samples with AM samples exhibiting a positive trend while PM samples exhibit a negative trend across the 5-day sampling. There was no evidence of an interaction effect on average log concentrations between treatment, day, and time of day among all biomarkers. Further, there was not sufficient evidence of an interaction effect on average log

Table 3. Linear mixed effect model results by Analyte.

	Group (df = 1)	Day (df = 4)	Time of Day (df = 1)	Group*Day (df = 4)	Group*Time of Day (df = 1)	Day*Time of Day (df = 4)	Group*Day*Time of Day (df = 4)
Dust	F = 0.01 (p = .950)	F = 0.38 (p = .826)		F = 0.54 (p = .703)			
Endotoxin	F = 0.08 (p = .781)	F = 0.62 (p = .651)		F = 0.91 (p = .458)			
IL-10	F = 0.55 (p = .463)	F = 1.45 (p = .217)	F = 0.06 (p = .807)	F = 0.45 (p = .774)	F = 0.44 (p = .510)	**F = 4.01 (p = .004)**	F = 0.10 (p = .982)
IL-13	F = 0.92 (p = .344)	F = 0.50 (p = .739)	**F = 4.00 (p = .047)**	F = 0.49 (p = .745)	F = 3.78 (p = .053)	F = 1.48 (p = .209)	F = 0.32 (p = .866)
IL-1b	F = 0.22 (p = .644)	F = 0.79 (p = .534)	**F = 0.71 (p = .001)**	F = 1.15 (p = .332)	F = 0.36 (p = .550)	F = 41 (p = .803)	F = 0.40 (p = .807)
IL-2	F = 1.11 (p = .298)	F = 1.99 (p = .096)	F = 2.82 (p = .094)	F = 0.91 (p = .457)	F = 0.69 (p = .407)	F = 0.83 (p = .507)	F = 0.92 (p = .451)
IL-6	F = 0.34 (p = .565)	F = 0.57 (p = .682)	**F = 14.36 (p = .001)**	F = 1.17 (p = .326)	F = 0.02 (p = .896)	F = 1.17 (p = .326)	F = 0.20 (p = .940)
IL-8	F = 0.16 (p = .691)	F = 1.28 (p = .279)	**F = 14.29 (p = .001)**	F = 0.68 (p = .610)	F = 1.05 (p = .307)	F = 1.39 (p = .236)	F = 0.76 (p = .550)
TNF-α	F = 0.26 (p = .612)	F = 1.11 (p = .352)	**F = 5.16 (p = .024)**	F = 1.57 (p = .183)	F = 1.68 (p = .197)	F = 1.17 (p = .323)	F = 0.26 (p = .902)

concentrations between treatment and day or treatment and time among all biomarkers. Analysis of main effects provided evidence of differences in average log concentrations between time of day (AM and PM) for IL-13 (p = .047), IL-1β (p < .001), IL-6 (p < .001), IL-8 (p < .001), and TNF-α (p = .024). Post-shift cytokine concentrations were higher than pre-shift cytokine concentrations for each significant result above. There was no evidence of differences in average log concentrations between treatment and control groups for any of the seven-modeled inflammatory biomarkers. There was no evidence of a difference in average log concentration between days among all biomarkers.

Microbiome diversity analyses revealed no significant differences in participant nasal microbiome alpha diversity, a measure of within sample diversity, between treatment and control groups. Beta diversity, a measure of between sample diversity, was not significantly different between group. There was evidence of differences in beta diversity between pre-shift and post-shift lavages (p = .002) indicating a work shift-related change in nasal microbiome. There was not sufficient evidence of differences in alpha diversity between pre and post shift. Additionally, there were not significant differences in alpha and beta diversity by day.

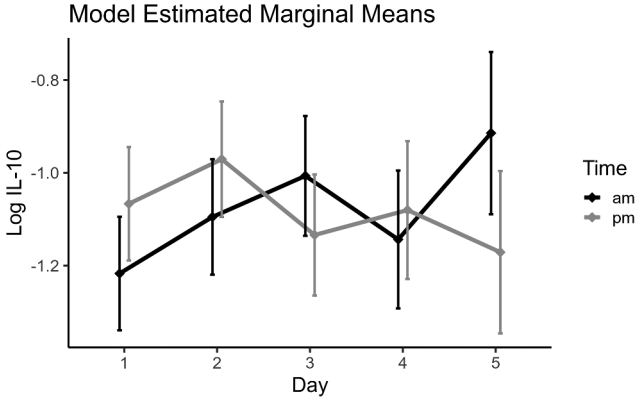

Figure 5. Model estimated marginal mean Log IL-10 concentrations across the 5 days for AM (orange) and PM (green) and associated confidence intervals.

Discussion

Exposure to bioaerosols and associated inflammagens (e.g., endotoxin) present in the dairy environment is associated with an increased risk of upper and lower respiratory disease. Past efforts to control dairy bioaerosol exposure have been mostly ineffective and cost prohibitive. In this study, pre- and post-shift hypertonic saline nasal lavages produced potentially advantageous albeit conflicting effects. Pre-shift pro-inflammatory cytokine concentrations were significantly lower than post-shift concentrations for IL-13, IL-1B, IL-6, IL-8, and TNF, confirming a work-related pro-inflammatory nasal response. Additionally, visual analysis of IL-10 anti-inflammatory concentrations shown in Figure 1 indicates an increasing anti-inflammatory response in both groups across the 5-day sample period. However, no significant differences were observed in the model between treatment groups or between day among all biomarkers. Evidence of a significant interaction effect was found for IL-10 between day and time of day (p = .004). Visual inspection of the model estimated marginal means (Figure 5) suggests a successful increase in log IL-10 across sample days among pre-shift samples while post-shift samples exhibit a downward trend. This crossover could be explained by IL-10s ability to auto regulate itself.[51] Essentially, upregulated pre-

shift IL-10 may be auto regulating itself and inhibiting a large post-shift IL-10 response.

Our results illustrate a less robust immunomodulatory effect of hypertonic saline on anti-inflammatory cytokine production in comparison to existing literature.[31–34] We demonstrated increases in IL-10 over time at a descriptive level in both groups, indicating HTS and NTS may be capable of upregulating the anti-inflammatory response. Still, model results revealed no significant differences by group or day. In contrast, *in vivo* testing of HTS was found to significantly increase IL-10 production in adult hemorrhagic trauma patients and produced a two-fold increase in peritoneal exudative macrophage IL-10 production compared to normotonic saline respectively.[33] The comparatively small effect in our study could be due to the much higher inflammatory state experienced by hemorrhagic patients or that cytokines were quantified from blood. Additionally, the muted anti-inflammatory effect we observed could be due to study design limitations surrounding the intervention. Previous studies investigating HTS's anti-inflammatory properties were able to test biomarkers at various intervals after administration allowing for quantitation of effects over time.[33,35] Our study was only capable of estimating transient changes in biomarkers due to samples being post-administration lavages.

For pro-inflammatory cytokines, there was no observed reduction in concentration attributable to the HTS treatment, contrary to our hypothesis. This result is a departure from our pilot study that measured significantly higher IL-6 concentrations in the HTS treatment group.[19] Earlier research found HTS treatment to be associated with significant decreases in TNF-a and IL-6 when compared to a normotonic saline treatment.[33] These studies were able to test lavage effects over time with multiple samples taken post-administration. Therefore, analysis of worker lavages as samples may be overlooking the true effect of the intervention on pro-inflammatory production. HTS can also illicit nasal mucosal irritation associated with transient increases in pro-inflammatory mediators. Analysis of post-administration lavages may be capturing this initial increase in inflammation while potentially missing subsequent decreases.[36,52]

Dust and endotoxin exposure concentrations provided by full-shift personal breathing zone samples were well below suggested agricultural exposure guidelines and were lower than most measures found in previous dairy environments.[6] Unavoidable wet and cool weather conditions observed during sampling campaigns (snowstorm and ice storm) likely altered the dry dusty conditions regularly present in high plains dairy environments. Independent of cause, reduced bioaerosol exposure results in a less inflammagenic, cleaner environment and may be concealing the immunomodulatory effects of the HTS rinse.

When evaluating a workplace intervention, it is essential to consider potential side effects, barriers of adoption, and worker reception. The primary side effect of concern regarding frequent nasal irrigation is potential washout of healthy diverse microbial communities present in the nose. Microbiome diversity analyses revealed that microbiome alpha and beta diversity did not significantly change after 5 consecutive days of the intervention. Moreover, the higher salinity of HTS lavages did not significantly shift nasal microbiome diversity compared to NTS lavages. Considering the low economic cost of hypertonic saline and the short amount of time to self-administer a nasal rinse (~10 sec), this intervention exhibits few adoption barriers in contrast to previous efforts.[29,30] Positive anecdotal comments made by participants also illustrate worker acceptability for this intervention.

It is important to interpret the results from this study in the context of its limitations and strengths. Foremost, our sample size ($n = 45$) may not have provided the adequate power needed to estimate the effects of HTS treatment over the full 5 days. Although we more than quadrupled the sample size from the pilot study, only 18 participants were able to complete all 5 days due to variable work schedules. Additionally, workers taking medication for existing respiratory conditions and workers unable to meet respiratory testing requirements were excluded due to participation requirements possibly introducing healthy worker bias. This study was also limited by only measuring inflammatory markers in post-administration lavages to estimate intervention efficacy.

Consequently, we were only able to measure transient changes in inflammatory markers. Additional measurement of inflammation at different intervals post-administration was not feasible in this study due to the nature of dairy operation schedules. The exclusion of other dairy bioaerosol inflammagens (i.e., peptidoglycans, bacterial pathogens, and viruses) and relevant pro-inflammatory mediators (i.e., histamine and leukotrienes) is another study limitation. Future investigations should expand exposure characterization and measure additional mediators to better estimate intervention efficacy. Finally, this study was limited by the absence of a true control group receiving no lavage. Future studies should include participants receiving no rinse to elucidate whether the rinse itself provides protective effects regardless of tonicity.

Conclusion

Regular bioaerosol exposure in the dairy environment and the resulting burden of respiratory disease in workers demonstrate the need for a low-cost, low-burden intervention. Pre- and post-shift administration of a hypertonic saline nasal lavage over 5 days was found to have limited but encouraging effect on the inflammatory response. When compared to controls receiving normotonic saline lavages, we observed no significant differences in inflammatory response in the HTS group. However, both groups experienced an increase in anti-inflammatory IL-10 concentration indicating the rinse itself may provide immunomodulatory benefits, independent of tonicity. Based on these results, it remains unclear if saline nasal rinses provide significant health benefits for bioaerosol exposed dairy workers. Therefore, we recommend future studies that measure inflammation incrementally post lavage and introduce a no-lavage control group to better estimate efficacy.

Acknowledgments

The authors would like to acknowledge and thank Dr. Stephen Reynolds and the HICAHS Dairy Advisory board for their help and expertise on this study. We would also like to acknowledge the dairy producers and workers who participated in data collection as well as Jessica Nunez and Lus Chavez for their valuable assistance with field sampling. We thank the Human Immune Monitoring Shared Resource at the CU Anschutz Medical Campus for their expert assistance in analysis of multiplex cytokine arrays. Analysis supported by the Cancer Center Support Grant (P30CA04634). JAP has received research regent unrelated to this project from AstraZeneca and is site investigator for clinical studies unrelated to this project with Takeda, GSK, and Regeneron.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This project was supported by HICAHS and NIOSH U01 grant #1U01OH010840. JAP is funded by NIOSH [1R01OH012-45] and the Department of Defense [PR200793]. National Institute for Occupational Safety and Health [1R01OH012-45]; U.S. Department of Defense [PR200793].

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