Effectiveness of a Low-cost Intervention: Changes to Cytokines and Microbiome Among Bioaerosol Exposed Dairy Workers

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Introduction and Objective

- Livestock workers experience a disproportionate burden of respiratory health effects.
- Livestock operations generate bioaerosols containing inflammagens (i.e., endotoxin).
- A large proportion of bioaerosol mass spans 10-100 µm in aerodynamic diameter (Figure 1a-1c)
- Bioaerosols are widely recognized to impact the respiratory health of dairy workers.
- Given the size range, a large fraction of bioaerosols deposit in the nasopharyngeal region.
- Understanding exposure health outcomes in the nose are important, especially those related to its microbiome.
- Interventions are needed to improve health of dairy workers but current engineering controls in this setting have not been economically feasible or practical.
- Hypertonic saline (HTS) has exhibited anti-inflammatory properties in the clinical setting.
- Here we report a novel, low-cost and non-invasive intervention using HTS as a nasal wash.

a. b. c.

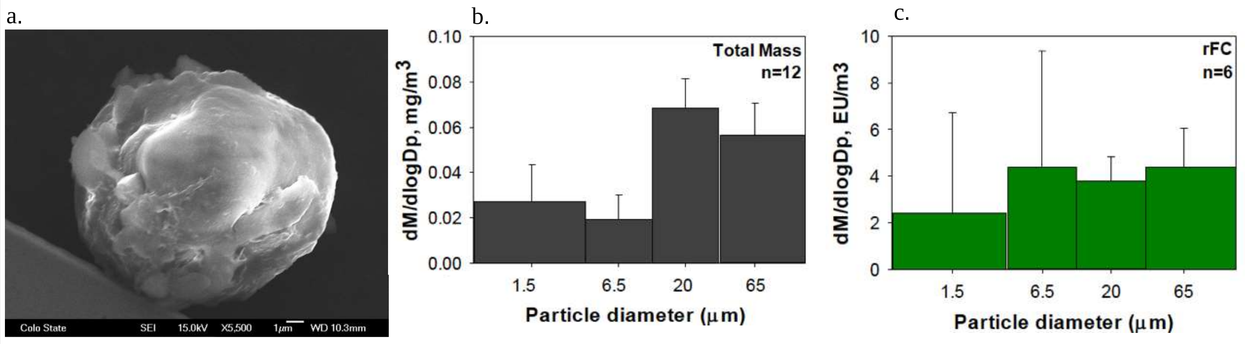

Figure 1: From left to right (a) SEM Image of dust sample (b) total mass size distribution (c) Endotoxin size distribution.

Our study aimed to: (1) pilot test the efficacy of an HTS nasal lavage as a low burden intervention to reduce the inflammatory response among bioaerosol-exposed dairy workers and (2) determine the impact of an HTS lavage on the nasal microbiome. We hypothesize that (1) bioaerosol exposure may influence nasal microbiome and cause perturbations associated with subclinical health impacts like increases in pro-inflammatory cyotkines (2) the hypertonic saline intervention will reduce the inflammatory response in dairy workers.

Methods

Exposure assessments and intervention applications were conducted over five consecutive days (8-hour shifts) at a dairy in the high plains and intermountain region. Dairy workers (n=10) were randomly assigned to an experimental group (n=5) that received hypertonic saline (400 mOsm) while the remaining five workers were administered normotonic saline (308mOsm) as part of the control group (Figure 2b). Each group was also fitted with a SKC Button sampler to measure particulate matter and endotoxin exposure (Figure 2a & 2c).

Figure 2: From left to right (a) Sampling pump set up. (b) Nasal lavage administration. (d) Particulate matter sample.

Table 1. Analysis technique and method across sample type

Compound	Analysis Technique	Analysis Method	End Point
Particulate	Gravimetric	Study Specific Method	PM_{100} Concentration (mg/m^3)
Pro-Inflammatory Cytokines	MSD V-PLEX Assay	MSD Specific Method	IL-6, IL-8, IL-10, and TNF-a Concentration (pg/ml)
Endotoxin	Fluorescent Assay	Lonza Pyrogene Kit Protocol	Endotoxin Concentration (EU/m^3)
Bacterial Communites	Illumina MiSeq	16S V4 rRNA Sequencing Protocol	Bacterial Taxa and Abundance

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Results (cont'd)

Dust concentrations were relatively high while endotoxins were low (as compared to previous dairy studies). Using a significance level of 0.05, we observed a significant promotion of the IL-10 anti-inflammatory cytokine among dairy workers in the treatment group when compared to workers in the control group (Figure 3). For IL-6 and IL-8, there was a significant difference by group. Expression of IL-6 and IL-8 in the treatment group was significantly higher than expression in the control group at the end of the study ($p < 0.05$). There was an overall significant treatment effect for IL-6 and IL-8 cytokines. Distinct nasal microbiome bacterial communities were observed in the AM and PM lavages (Figure 4). Similar bacterial communities were observed in the air and post-shift nasal lavage samples.

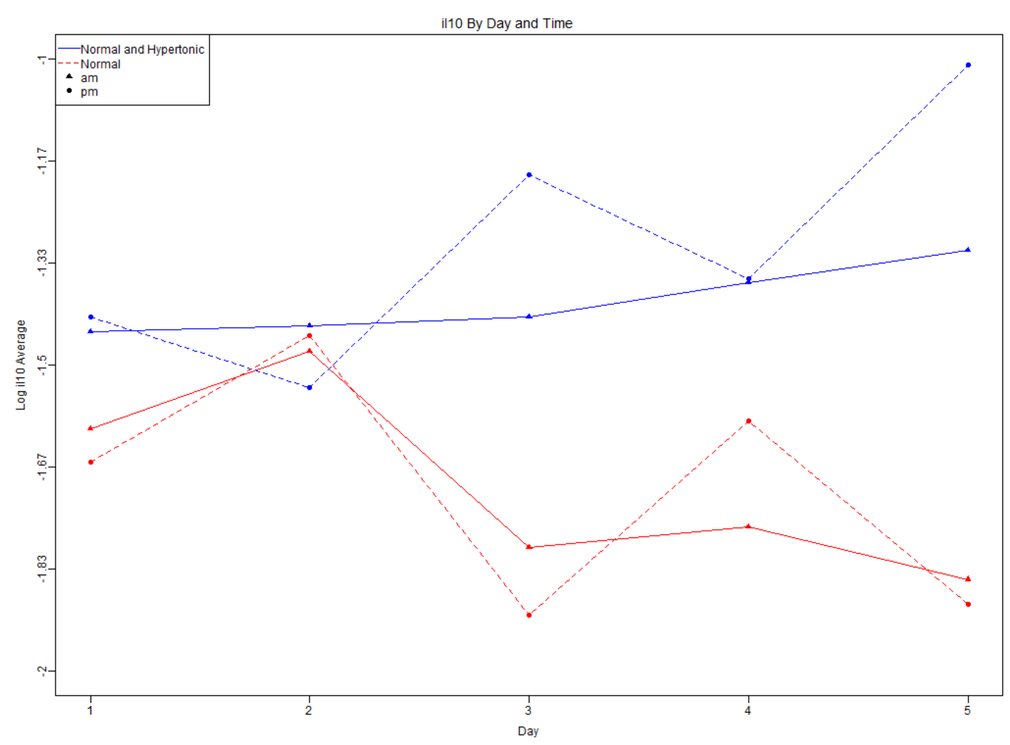

Figure 3: Normalized AM and PM sample plot of IL-10 anti-inflammatory cytokines by day. Each line represents a different participant with blue representing controls and red representing treatment.

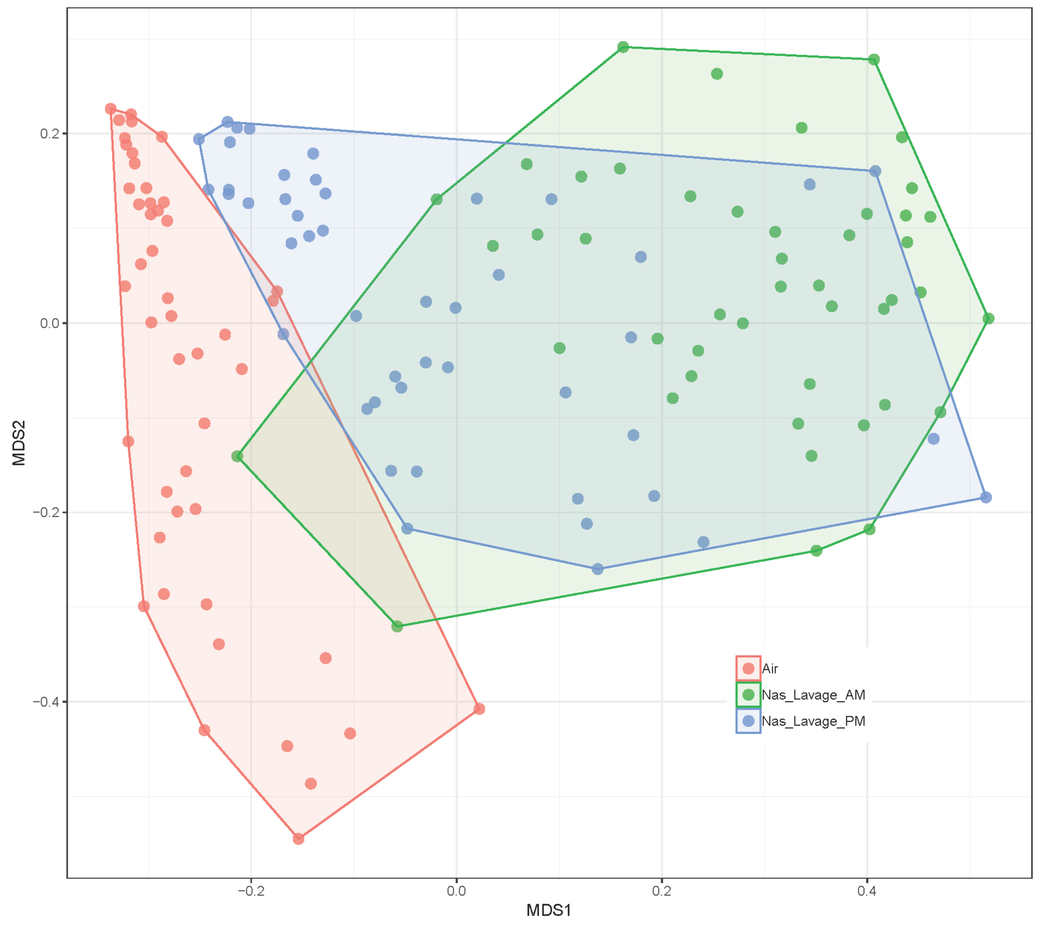

Figure 4: Bacterial community ordination plot of air (red), AM lavage (green), and PM lavage (blue).

Conclusions & Future Studies

- Hypertonic saline solution significantly upregulated anti-inflammatory cytokine levels when compared to those in the normotonic saline control group (p-value < 0.005).
- Treatment group levels of IL-6 and IL-8 pro-inflammatory cytokines levels deviated from the hypothesis with treatment group levels being significantly higher (p-value < 0.05).
- Distinct bacterial communities were observed in the pre and post shift lavage samples.
- Post shift sample communities closely resembled the air microbiome indicating bacterial deposition during work shifts.
- The HTS nasal lavage is a promising low-cost intervention to reduce upper airway inflammation in dairy workers.
- A subsequent large-scale study is underway testing the intervention at various dairies across Colorado and Texas that will:
  - Further investigate the effect of the intervention on the nasal microbiome and vice versa.
  - Characterize the nasal carriage of livestock-associated methicillin resistant *Staphylococcus aureus* among dairy workers

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