Introduction and Objective

- Livestock workers experience a disproportionate burden of respiratory health effects.
- Livestock operations generate bioaerosols containing in-mammagens (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.

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).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Analysis Technique</th>
<th>Analysis Method</th>
<th>End Point</th>
</tr>
</thead>
<tbody>
<tr>
<td>Particulate</td>
<td>Gravimetric</td>
<td>Study Specific Method</td>
<td>PM&lt;sub&gt;10&lt;/sub&gt; Concentration (mg/m³)</td>
</tr>
<tr>
<td>Pro-Inflammatory</td>
<td>MSD V-PLEX Assay</td>
<td>MSD Specific Method</td>
<td>IL-6, IL-8, IL-10, and TNF-α Concentration (pg/ml)</td>
</tr>
<tr>
<td>Cytokines</td>
<td>Fluorescent Assay</td>
<td>Lonza PyroGnome Kit Protocol</td>
<td>Endotoxin Concentration (EU/ml)</td>
</tr>
<tr>
<td>Endotoxin</td>
<td>illumina MiSeq</td>
<td>16S V4 rRNA Sequencing Protocol</td>
<td>Bacterial Taxa and Abundance</td>
</tr>
</tbody>
</table>

Table 1. Analysis technique and method across sample type

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.

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.

Acknowledgements: This research was funded by the National Institute for Occupational Safety and Health U01 grant #IU01OH010840 & The Mountain and Plains Education Research Center for Occupational and Environmental Health and Safety.