Validation and Implementation of Methods in Assessing Job-Related Exposures in Dairy Farm Workers

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Abstract
Cattle are sources of bacteria; as a result, dairy farm workers are at a greater risk of exposure to infectious bacteria such as Campylobacter, E. coli O157:H7, and Salmonella. Yakima County is the leading dairy producing region within Washington State and has the largest inventory of cattle in any county in Washington. Yakima County has case rates for Campylobacter and Salmonella as high as 2 to 3 times the state of Washington and the case rate of E. coli O157:H7 has periodically exceeded Washington’s rates. The primary goal of this research project was to determine the environmental levels of these potential pathogens in the dairy setting to which workers may be exposed. Several routes of exposure on the Dairy Farm were considered significant including air surface contamination. Possible air borne bacterial pathogens were detected using the SKC Andersen 6-stage, the SKC Andersen single stage, and the SKC Biosampler. Swabs and contact plates with selective and non-selective media were used to sample different surfaces on the farm. Organisms grew on the swabs and both E. coli and Salmonella were detected in the SKC Andersen six-stage, SKC Andersen single stage, and contact plates. These organisms, however, have not been identified using the SKC Biosampler.

Introduction
• Despite relatively little information on the job-related routes of exposure to bacterial pathogens, there is considerable evidence demonstrating enteric illness resulting from direct or indirect farm animal contact.
• Enteric illness can cause severe diarrhea.
• The frequency in outbreaks of zoonotic diseases has been increasing, and can result in hospital visitations, missed days of work, loss of productivity, and financial burden.
• Zoonotic bacteria like Campylobacter spp., Salmonella spp., and E. coli O157:H7 are common causative agents leading to enteric illness in humans.
• There are numerous potential routes for exposure for farm workers on cattle farm to Campylobacter spp., Salmonella spp., and E. coli. including contaminated clothing or surfaces, vehicles, water, aerosols, fomites and direct contact with animals or animal wastes. [1]
• Campylobacter spp. has been recently described to be widely distributed on cattle farms in the State of Washington with a prevalence of 23% to 47%. [2]

Description of the Field Site
• Yakima County is primarily an agricultural area, with approximately half devoted to fruits and vegetables and the other half devoted to cattle and dairy farms.
• Yakima County is also the leading dairy producing region within the state and has the largest inventory of cattle and sheep in any county in Washington.
• As a result of the labor intensive agriculture in the county, there are large populations of minorities, primarily Hispanic, and migrant farm workers.
• Yakima County has a history of consistently elevated case rates for Campylobacter and Salmonella, 2 to 3 times Washington’s rates. [3]
• The case rate of E. coli O157:H7 in Yakima County has periodically exceeded the State case rates. [4]

Materials and Methods

Field Study Sampling:

Bioaerosols-
• The SKC Biosampler was run for 30 minutes in two different barns.
• Bacterial pathogens were detected using the SKC Andersen 6-stage and the SKC Andersen single stage. The stages were run for 30 seconds, 45 seconds, 1 minute, 1.5 minutes, and 2 minutes.

Surfaces-
• Swabs and contact plates with selective and non-selective media were used to sample different surfaces (barn gates, clothing, car tires, tractor handles, and water troughs) on the farm.
• The contact plates were pressed firmly on each surface for 30 seconds. Swabs were pre-moistened with Tween 80 and stored in PBS after sampling.

Laboratory Study:

Contact plates and SKC Andersen stages-
• The samples were incubated for 24 hours and then were processed in the laboratory for bacterial culture on selective and non-selective media.
• The resulting bacterial growth was enumerated, colony purified, morphologically characterized, and its identity confirmed by an API strip.

Swabs and SKC Biosampler-
• The samples were vortexed and then filtered. The filter plates were then incubated for 24 hours.
• The resulting bacterial growth was enumerated, the colony purified, its morphology characterized, and its identity confirmed by an API strip.

Media Used:
• Difco Nutrient Agar
• Difco MI Agar
• Difco MacConkey Agar
• Remel Brilliant Green Agar Modified
• Difco Actinomycete Isolation Agar

Results/Conclusions
• E. coli was isolated and confirmed using the SKC Andersen 6-stage, the SKC Andersen single stage, the swabs, and the contact plates.
• E. coli was confirmed at 99.6% using the API strips.
• Salmonella spp. was detected but not confirmed using the SKC Andersen 6-stage, the SKC Andersen single stage, and the contact plates.
• We have not identified Salmonella, Campylobacter, and E. coli using the SKC Biosampler.

Table 1: Average Number of Colonies Detected for Four Sampling Trips

<table>
<thead>
<tr>
<th></th>
<th>Brilliag Green Agar</th>
<th>Nutrient Agar</th>
<th>MacConkey Agar</th>
<th>MI Agar</th>
<th>AIA Agar</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 - Stage</td>
<td>52</td>
<td>4638</td>
<td>152</td>
<td>*</td>
<td>ND</td>
</tr>
<tr>
<td>Single Stage</td>
<td>18</td>
<td>7945</td>
<td>302</td>
<td>ND</td>
<td>7250</td>
</tr>
<tr>
<td>Contact Plate</td>
<td>ND</td>
<td>5327</td>
<td>544</td>
<td>*</td>
<td>3816</td>
</tr>
<tr>
<td>Swabs</td>
<td>*</td>
<td>6453</td>
<td>1154</td>
<td>116</td>
<td></td>
</tr>
<tr>
<td>Biosampler</td>
<td>*</td>
<td>758</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
</tbody>
</table>

* This agar was not used
ND = Not Detected

Table 2: Agent Containing Particles Per Unit of Area Sampled

<table>
<thead>
<tr>
<th></th>
<th>Brilliag Green Agar</th>
<th>Nutrient Agar</th>
<th>MacConkey Agar</th>
<th>MI Agar</th>
<th>AIA Agar</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 - Stage (ACPLA)</td>
<td>0.95</td>
<td>27.04</td>
<td>1.03</td>
<td>*</td>
<td>0.51</td>
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<tr>
<td>Single Stage (ACPLA)</td>
<td>0.37</td>
<td>24.72</td>
<td>1.03</td>
<td>ND</td>
<td>0.55</td>
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<tr>
<td>Contact Plate (Avg. CFU / sq cm)</td>
<td>ND</td>
<td>80.08</td>
<td>6.04</td>
<td>*</td>
<td>0.07</td>
</tr>
<tr>
<td>Swabs (Avg. CFU/cm²)</td>
<td>*</td>
<td>*</td>
<td>8.99</td>
<td>6.57</td>
<td></td>
</tr>
<tr>
<td>Biosampler (Avg. CFU)</td>
<td>0.27</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
</tbody>
</table>

Table 3: Agent Containing Particles Per Unit of Area Sampled

Future Study
• Assessing the para-occupational (take-home) exposure pathways for Campylobacter spp., Salmonella spp., and E. coli.
• Archiving the samples at -80°C and looking for antimicrobial resistant genes.

Acknowledgements
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References