Immunostimulation associated with environmental enteric dysfunction in children from a dual burden environment

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Faculty Disclosure

No conflicts to disclose.
Immunostimulation Associated with Environmental Enteric Dysfunction

**Exposures:**
- Pathogens
- Diet
- Genetics
- Stress
- Toxins/Drugs

**Balanced Gut Microbiota**

**Gut Dysbiosis**
- Chronic intestinal inflammation
- Poor intestinal barrier function (leaky gut)

**Endotoxemia**
- Lipopolysaccharides (LPS) or endotoxins initiate inflammation & humoral immune responses.
- Microbial translocation of gram-negative bacteria

**Immune Measures:**
- Inflammation: C-reactive Protein (CRP)
- Endotoxemia: Endotoxin Core IgG Antibodies (EndoCAb)
  - *E. coli*, *P. aeruginosa*, *K. aerogenes*, *S. typhimurium*
Immunostimulation in Galápagos & Comparisons

- Galápagos setting - Dual Burden Environment
- Are both pathogenic and dietary factors increasing immunostimulation?

Data sources:
Sample and Key Measures

- **164 children 2-10 years old**
- **Mother’s interviews:** Household information, children’s relevant birth and health information, hygiene practices, illness histories and diets, anthropometric assessments were conducted.
- **Diet:** Food frequency questionnaires were taken to determine very high, high, moderate, rare consumption patterns of local food items.
- **Fecal pathogen exposure:** A household water sample was collected and quantified for *E. coli* levels using Colilert\(^1\) reagents.
- **Inflammation:** Two dried blood spots were collected approximately 10 days apart and analyzed for high sensitivity C-reactive protein (CRP) using Quantikine ELISA kits\(^2\).
- **Endotoxemia:** One dried blood spot analyzed for Endotoxin Core IgG antibodies (EndoCAb IgG) using Hycult Biotech ELISA kits\(^3\).

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\(^1\) IDEXX Laboratories, Inc. Westbrook, MA.
\(^2\) R&D Systems, Inc. Minneapolis, MN.
\(^3\) Hycult Biotech, Inc. Plymouth Meeting, PA.
# Single Pathogen Exposures

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Percent of Sample</th>
<th>CRP Models</th>
<th>EndoCAb Models</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fecal contaminated household water</td>
<td>High <em>E. coli</em> levels (&gt;100 bacteria per 100mL) 12%</td>
<td>Low <em>E. coli</em> levels (&lt;100 bacteria per 100mL) 88%</td>
<td></td>
</tr>
<tr>
<td>Brushed teeth with tap water</td>
<td>Yes 87%</td>
<td>No 13%</td>
<td></td>
</tr>
<tr>
<td>Hand washing frequency</td>
<td>Sometimes-Rare 37%</td>
<td>Always 64%</td>
<td></td>
</tr>
<tr>
<td>Location of bath</td>
<td>Outside 15%</td>
<td>Inside 85%</td>
<td>+</td>
</tr>
<tr>
<td>Presence of pets or animals</td>
<td>Yes 55%</td>
<td>No 45%</td>
<td>+</td>
</tr>
<tr>
<td>Attended school</td>
<td>Yes 78%</td>
<td>No 22%</td>
<td>+</td>
</tr>
<tr>
<td>Swam in ocean</td>
<td>Yes 38%</td>
<td>No 62%</td>
<td>+</td>
</tr>
</tbody>
</table>

p<.05 in all reported relationships. Adjusted for field season, sex, and age for all models, and obesity and infectious symptoms for the CRP only.
### Single Dietary Consumption Factors

<table>
<thead>
<tr>
<th>Group</th>
<th>Percent in Sample</th>
<th>CRP Models</th>
<th>EndoCAb Models</th>
<th>Group</th>
<th>Percent in Sample</th>
<th>CRP Models</th>
<th>EndoCAb Models</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk</td>
<td><img src="chart1.png" alt="Pie Chart" /></td>
<td>Very High</td>
<td>High</td>
<td>Milk</td>
<td><img src="chart2.png" alt="Pie Chart" /></td>
<td>Very High</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Moderate</td>
<td>Fried Meats</td>
<td><img src="chart3.png" alt="Pie Chart" /></td>
<td>Very High</td>
<td></td>
</tr>
<tr>
<td>Cheese</td>
<td><img src="chart4.png" alt="Pie Chart" /></td>
<td>High</td>
<td>Moderate</td>
<td>Cheese</td>
<td><img src="chart5.png" alt="Pie Chart" /></td>
<td>Moderate</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Empanadas</td>
<td><img src="chart6.png" alt="Pie Chart" /></td>
<td>Moderate</td>
<td></td>
</tr>
<tr>
<td>Vegetables</td>
<td><img src="chart7.png" alt="Pie Chart" /></td>
<td>Moderate</td>
<td>Very High</td>
<td>Vegetables</td>
<td><img src="chart8.png" alt="Pie Chart" /></td>
<td>Very High</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ice Cream</td>
<td><img src="chart9.png" alt="Pie Chart" /></td>
<td>High</td>
<td></td>
</tr>
<tr>
<td>Beans</td>
<td><img src="chart10.png" alt="Pie Chart" /></td>
<td>High</td>
<td>Moderate</td>
<td>Beans</td>
<td><img src="chart11.png" alt="Pie Chart" /></td>
<td>Moderate</td>
<td></td>
</tr>
</tbody>
</table>

**Pie Chart Legend:**
- **Very High:** Every Meal
- **High:** Daily
- **Moderate:** Weekly
- **Low:** Monthly/Rare

*p*<.05 in all reported relationship from adjusted models, low consumption is the referent.
Predicted Mean Inflammation Levels from Aggregate Model

Mixed-effects model of log-transformed CRP with random effects on intra-individual CRP variability; adjusted for field season, sex, age, obesity, infectious symptoms and clustering at the household level.
Predicted Mean Endotoxemia Levels from Aggregate Model

OLS model of log-transformed EndoCAb; adjusted for field season, sex, age and clustering at the household level.

** p<.05
* p<.1

OLS model of log-transformed EndoCAb; adjusted for field season, sex, age and clustering at the household level.
Discussion

- Pathogenic and dietary factors can simultaneously impact immunostimulation associated with environmental enteric dysfunction in a dual burden environment.
- Some pathogenic factors increase immune activation: school attendance, swimming in contaminated oceans.
- Habitual exposure to fecal contaminated water not causing infection may help to prime humoral immunity & regulate anti-inflammatory networks - hygiene hypothesis.
- Dietary endotoxemia and inflammation in populations with overnutrition:
  - Fried foods & sweets ↑, vegetables and beans ↓ immunostimulation.
  - Elevated milk consumption is associated with both inflammation & endotoxemia.
Inflammation & Endotoxemia in Dual Burden Environment

- No structural diagnostic measures of environmental enteric dysfunction

Data sources:
Conclusion

- Dual Burden of the Intestinal Microbiome

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  - Genetics
  - Stress
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- Gut Dysbiosis
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- Balanced Gut Microbiota
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- Immune Measures:
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  - C-reactive Protein
  - Endotoxemia
  - Endotoxin Core IgG Antibodies
  - (E. coli, P. aeruginosa, K. aerogenes, S. typhimurium)

Future research: mediating factors of gut microbiome

Infographic source: http://www.who.int/nutrition/double-burden-malnutrition doubleburdenmalnutrition_infographic.png
Acknowledgments

Funding Support:
• NSF Doctoral Dissertation Research Improvement Grant
• Wenner-Gren Dissertation Fellowship
• Triangle Center for Evolutionary Medicine, Duke University
• UNC Institute for Global Health and Infectious Disease
• UNC Graduate School
• UNC Carolina Population Center
• Population Research Training Grant (5 T32 HD007168) from the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD)

• I thank Gyssell Zapata, the Galápagos Science Center, the Human Biology Lab at UNC, Universidad San Francisco de Quito, and the participants and residents of San Cristóbal, Ecuador.