Environmental Enteric Dysfunction

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for the
SHINE Trial Research Group

Sanitation Hygiene Infant Nutrition Efficacy
Stunting is only partially responsive to current diet and health interventions

• Environmental Enteric Dysfunction is now hypothesized to be a key underlying cause of stunting

• This hypothesis is being tested by several research groups
3 names for the **same** thing!

- Tropical Enteropathy (TE)
- Environmental Enteropathy (EE)
- Environmental Enteric Dysfunction (EED)
What is EED?

- Condition of the gut caused by prolonged and persistent exposure to enteric pathogens
  
  caused by ingestion of feces
  
  among people living in conditions of poor WASH

- Is virtually ubiquitous among people living in poverty

- Asymptomatic (Subclinical)

- Is fully reversible when fecal exposure is removed
During EED there are abnormal changes in Structure and Function of the small intestine.

<table>
<thead>
<tr>
<th>Structural Change</th>
<th>Functional Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Villous atrophy (flat)</td>
<td>Reduced nutrient absorption</td>
</tr>
<tr>
<td>Inflamed and permeable</td>
<td>Microbial translocation</td>
</tr>
</tbody>
</table>
The normal gut wall is only one cell thick.

The EED gut is permeable, gaps open up between cells.

Microbial products cross into bloodstream.

Chronic immune activation.
Chronic immune activation: IL-6 switches off Insulin-like Growth Factor-1 a hormone that regulates linear growth

↑ pro-inflammatory cytokines (IL6)

↓ Growth Factor (IGF-1)

Stunting
Cohort of Zimbabwean infants, who we had measured and collected blood every 3 months birth to 18 months in 1999

- Identified 100 children who were stunted at 18 months of age
- And 100 children NOT stunted at 18 months
- Pulled out their 3-monthly blood samples collected from birth to 18 months
- What was different in the blood samples of children who did and did not become stunted?
Stunted Children had higher markers of inflammation from 6 weeks to 12 months than Non-stunted children

Prendergast et al., PLOS One 2014
Stunted Children had lower levels of IGF-1 hormone from 6 weeks to 12 months than Non-stunted children.
Inflammation was correlated with IGF-1 concentrations

AGP
R = -0.51
P < 0.0001

CRP
R = -0.53
P < 0.0001
Stunting is an **inflammatory** condition

Children who were stunted at 18 months of age had

- Higher inflammation markers from **6 weeks*** – 12 months
- Lower concentrations of IGF-1 from **birth*** to 12 months
- AND – their mothers had lower concentrations of IGF-1 at **delivery***

*Note how early in life this inflammation and reduced IGF-1 occurs!
Chronic immune activation:
Increases **hepcidin** – principle iron regulation hormone: high hepcidin reduces iron absorption/mobilization

- ↑ pro-inflammatory cytokines
  - ↑ Hepcidin
  - ↓ Growth Factor (IGF-1)

- Anemia
- Stunting
Chronic immune activation

- ↑ pro-inflammatory cytokines
- ↑ Hepcidin
  - Anemia
- ↓ Growth Factor (IGF-1)
  - Stunting
- Immunosenescence (premature aging) of adaptive cell-mediated immune system
  - Impaired response to vaccines and infections
Could maternal EE reduce fetal growth?

Mother

LPS + other microbial products

Synthesis of proinflammatory cytokines
Inhibition of IGF-1

Enteric inflammation
Increased permeability

Gl tract

Reduced fetal growth

Local IL-6, TNF-α, IL-1β

Fetus

Increased IL-6, TNF-α, IL-1β
Reduced circulating IGF-1

Reduced IGF-1
Could maternal EE cause other adverse birth outcomes?

Other adverse obstetric outcomes associated with inflammation:

- Preterm birth
- Miscarriage or Stillbirth
Recent and on-going studies

**GEMS** – Global Enteric Multicenter Study

*Case-control study in 7 countries, collected fecal samples from:*

- 9439 children with moderate-severe diarrhea
- 13,129 children without diarrhea

*Which pathogens are the main causes of diarrhea?*

**MAL-ED** – Etiology, Risk Factors, and Interactions of Enteric Infections and Malnutrition and the Consequences for Child Health and Development

*Cohorts of 200 children in 8 countries followed from birth to 2 years*

3 Randomized Controlled Trials testing the independent and combined effects of improved WASH and improved IYCF on stunting and anemia:

- **WASH Benefits-Bangladesh**
- **WASH Benefits – Kenya**
- **SHINE** – Sanitation, Hygiene, Infant Nutrition Efficacy study - Zimbabwe
For many years it was assumed that diarrhea alone mediated the link between poor WASH and malnutrition.
Change in HAZ in a among children with and without mod-severe diarrhea over a 50-90 day interval (GEMS STUDY (7 countries))
Less diarrhoea but no change in growth: 15 years’ data from three Gambian villages

E M E Poskitt, T J Cole, R G Whitehead

<table>
<thead>
<tr>
<th>Year</th>
<th>x(SD) LAZ 12 months</th>
<th>x(SD) LAZ 24 months</th>
</tr>
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<tbody>
<tr>
<td>1979</td>
<td>-1.3 (1.0)</td>
<td>-2.0 (0.9)</td>
</tr>
<tr>
<td>1993</td>
<td>-1.7 (1.0)</td>
<td>-2.1 (0.9)</td>
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</tbody>
</table>
Diarrhea: the tip of the enteric disease iceberg

Diarrhea is episodic ~25 days per year but results in 800,000 deaths per year

Enteropathy is nearly constant ~250 days per year – not dramatic,

Reflects the fact that children living in conditions of poor WASH are infected with multiple enteric pathogens even when they do NOT have diarrhea: GEMS: 83% of cases had at least one pathogen in their stool and 72% of controls!
MAL-ED Study:
A major objective:

Is carriage of particular enteropathogens or enteropathogen profiles associated with stunting independent of diarrhea?

EED and diarrhea may be caused by different pathogens.
• Largely because of the chronicity of EED, **SHINE** hypothesizes that EED is the major mediator of improved WASH on improved linear growth.

• In a substudy, SHINE will measure the relative contribution of these two pathways:

  ![Diagram](diagram.png)
How can we measure EED?
Fecal samples, assess infant microbiome

Kosek’s 3 fecal markers
- Fecal calprotectin
- Lactulose-mannitol
- Sugar-absorption test, urine measurement

LPS, EndoCAb, bacterial DNA PCR in plasma

Immune stress indicators: IL-1, TNF, IFN-α, IL-6, CRP and T-cell activation

Assess behaviors

Fecal contamination
Altered bacterial load, composition and/or timing of colonization
Intestinal inflammation
Increased intestinal permeability
Microbial translocation
Systemic immune activation and T cell activation
Fecal samples, assess infant microbiome

Kosek's 3 fecal markers
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Systemic immune activation and T cell activation
Composite of 3 stool tests: a promising measurement of EED from the Mal-Ed Study

1. **Alpha-1 antitrypsin**: Serum protein that seeps into gut during inflammation, permeability, or ulceration

2. **Neopterin**: Marker of activated T-cells from the gut lining

3. **Myeloperoxidase**: Marker of activated neutrophils

• Each test measures a different aspect of gut inflammation
• All 3 tested independently predicted decline in length-for-age in subsequent 6 months
• Little correlation between them: As a composite, more strongly predicted growth faltering
Can improved WASH reduce EED and Stunting?
Clean and dirty houses in Bangladesh

• 119 Bangladeshi children <4 years old
• Houses categorized as ‘clean’ or ‘dirty’ based on water quality, latrines, etc
• Children in clean houses had higher HAZ
• Children in clean houses had more severe EED by several indicators

3 on-going trials

- WASH Benefits – Bangladesh
- WASH Benefits – Kenya
- SHINE – Zimbabwe

Results available 2016-2017
Comparing multiple combinations of WASH interventions:

- Water quality
- Sanitation
- Hand-washing
- All 3 WASH interventions
- Nutrition
- Everything

<table>
<thead>
<tr>
<th>ARM</th>
<th>Bangladesh N clusters (N children)</th>
<th>Kenya N clusters (N children)</th>
<th>Growth and Diarrhea Measurements (time since enrollment)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>180 (1,440)</td>
<td>200 (2,000)</td>
<td>Enroll</td>
</tr>
<tr>
<td></td>
<td>90 (720)</td>
<td>100 (1,000)</td>
<td></td>
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<tr>
<td></td>
<td>90 (720)</td>
<td>100 (1,000)</td>
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<tr>
<td></td>
<td>90 (720)</td>
<td>100 (1,000)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total 720 (5,760)</td>
<td>800 (8,000)</td>
<td>Child age range:</td>
</tr>
</tbody>
</table>

Child age range:
- 6 to 15 months
- 18 to 27 months
<table>
<thead>
<tr>
<th>Sanitation/Hygiene</th>
<th>Nutrition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. VIP latrine</td>
<td>• 20 g Nutributter daily provided for infants (6-18 mo)</td>
</tr>
<tr>
<td>2. 2 Tippy Taps</td>
<td>• Behavior Change! optimal use of local foods for complementary feeding</td>
</tr>
<tr>
<td>3. POU Water treatment</td>
<td></td>
</tr>
<tr>
<td>4. Clean area for eating and play</td>
<td></td>
</tr>
<tr>
<td>5. Behavior Change!</td>
<td></td>
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**Nutrition & Sanitation/Hygiene**

**Standard Care**
Strengthened early antenatal care and Exclusive Breastfeeding
Routes of fecal disease transmission and protective barriers *for babies!*

- **Sanitation**
  - Fluids
  - Fingers
  - Flies
  - Fields/floors

- **Clean water supply**
  - Food

- **Hygiene**
  - Laundry Water
  - Nappy Handling, Potties
Causes of intestinal damage in rural Zimbabwean infants

- Structured observation of 23 households
- 6 hour periods
- 130 hours total
- Recorded every object mouthed by the infant

Causes of intestinal damage in rural Zimbabwean infants

- Infant’s own fingers most frequently mouthed
- 38 times in 6 hours
- 75% of the time visibly dirty

- Frequent ingestion of soil, chicken feces and stones

Causes of intestinal damage in rural Zimbabwean infants

<table>
<thead>
<tr>
<th></th>
<th>% houses with E coli + sample</th>
<th>Mean E Coli cfu/g</th>
<th>Mean E Coli “per serving size” cfu/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infant food</td>
<td>0%</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Drinking water</td>
<td>54%</td>
<td>2</td>
<td>800</td>
</tr>
<tr>
<td>Wet shaded soil</td>
<td>60-80%</td>
<td>69</td>
<td>2,100</td>
</tr>
<tr>
<td>Chicken feces</td>
<td>100%</td>
<td>10,000,000</td>
<td>10,000,000</td>
</tr>
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Routes of fecal disease transmission and protective barriers *for babies!*

- **Sanitation**
  - Fluids
  - Fingers
  - Flies
  - Fields/floors

- **Clean water supply**
  - Food

- **Hygiene**
  - Protective Play Space

- **Laundry Water**

- **Nappy Handling, Potties**

- **Geophagia, dirty hands**
Perhaps MOST importantly

- Both WASH Benefits and SHINE are focusing huge attention and resources on behavior change
- Fidelity of implementation
- Uptake of promoted behaviors
Building toilets is important! But...
Toilets are necessary but not sufficient!
For SHINE

- 4 years of formative research
- 2 years of intervention design, piloting, re-design, re-pilot
- Village Health Workers trained for 8 weeks to deliver 5 WASH modules
- Village Health Workers mentored by Intervention Nurses (1:10) in groups, one-on-one
- More than 55% WASH budget goes to behavior change, 45% to latrine building
Interventions

• Each intervention has 5 key modules delivered by Village Health Workers

• Interactive tools and activities
Porridge Additive Flipbook
(leafy greens example)
Acknowledgements

Zvitambo Institute for Maternal Child Health Research, Zimbabwe
Mdu Mbuya, Robert Ntozini, Franne VanderKeilen, Kuda Mutasa, Naume Tavengwa, Florence Majo, Phillipa Rambanepasi

Zimbabwe Ministry of Health and Child Care
Goldberg Mangwadu, Acikaria Chigumira, Cynthia Chasokela

Johns Hopkins Bloomberg School of Public Health, USA
Jean Humphrey, Jim Tielsch, Larry Moulton

Queen Mary, University of London
Andrew Prendergast

Cornell University, USA
Rebecca Stoltzfus

University of Michigan
Andy Jones

University of British Columbia, Canada
Amee Manges

London School of Hygiene and Tropical Medicine
Val Curtis, Sandy Cairncross