Determining the Impact of Sanitation via Detection of Pathogenic *E. coli* in Environmental Samples from Rural Bangladesh

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**Background:** Diarrhea is the second leading cause of death among under-five children. Specifically, approximately 801,000 under-five children die due to diarrhea in developing countries such as Bangladesh.¹ Infectious diarrhea is caused by human enteric pathogens via the fecal-oral route as fecal material can be transmitted via consumption of contaminated food or water, exposure to polluted soil, and exposure to fecal-contaminated hands.² Diarrhea is preventable via interventions including improved sanitation, access to safe drinking water, and access to vaccinations.

**WASH Benefits:** The WASH Benefits study is a multi-institutional public health study led by researchers from the University of California, Berkeley, Stanford University, and the International Centre for Diarrhoeal Disease Research, Bangladesh, among others. The study’s primary objective is to measure the effects of health interventions, including improved water quality, improved sanitation, access to hand-washing, and access to nutrition, on child health and diarrheal incidence among children. A cluster-randomized control trial started in Mymensingh, Bangladesh in 2012 as part of the WASH Benefits study. The majority of the Mymensingh population has access only to unimproved sanitation, which consists of either a pit latrine with no concrete slab or open defecation.³ The trial enrolled pregnant women in order to study children under two because during the first two years of life, children born in low-income countries are at high risk for enteric infections. Environmental samples collected include hand-rinse samples from mothers (N=720), hand-rinse samples from children (N=360), soil samples (N=720), and stored water samples (N=720).

**Pathogenic *E. coli* (PEC):** There are five pathotypes of *E. coli* that cause gastroenteritis and infectious diarrhea.

<table>
<thead>
<tr>
<th>Pathotype</th>
<th>Signs &amp; Symptoms</th>
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<tr>
<td>Enteropathogenic <em>E. coli</em> (EPEC)</td>
<td>Watery &amp; bloody diarrhea in children</td>
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<tr>
<td>Enterotoxigenic <em>E. coli</em> (ETEC)</td>
<td>Childhood diarrhea &amp; traveler’s diarrhea</td>
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<td>Enterohemorrhagic <em>E. coli</em> (EHEC)</td>
<td>Bloody diarrhea &amp; hemorrhagic colitis</td>
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<td>Enteroinvasive <em>E. coli</em> (EIEC)</td>
<td>Watery diarrhea &amp; dysentery</td>
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<tr>
<td>Enteroaggregative <em>E. coli</em> (EAEC)</td>
<td>Persistent diarrhea in children &amp; adults</td>
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**Objectives:**

1. To determine if pathogenic *E. coli* is present in various environmental samples from the control arm and sanitation intervention arm of the WASH Benefits study

2. To determine the lower-detection limit of the culture-PCR method using ETEC
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**PEC Detection Experimental Design:** Upon sample collection, the IDEXX-Colilert test was used to simultaneously quantify total coliforms and *E. coli*. To investigate the presence of pathogenic *E. coli* (PEC) in samples, DNA was extracted using the Qiagen DNeasy Blood and Tissue Kit. Multiplex polymerase chain reaction (PCR) assays were used to amplify extracted DNA. Gel electrophoresis was used to visualize results and detect PEC.

**Results:**

**Lower-Detection Limit Experimental Design:** Flow cytometry was used to determine the lower-detection limit of the culture-PCR method used to detect PEC in Bangladeshi environmental samples. Various concentrations (1,000 cells of ETEC/mL-1 cell of ETEC/mL) were prepared via flow cytometry. Then, the IDEXX-Colilert test was conducted, followed by DNA extraction, PCR, and gel electrophoresis.

**Results:** Results indicate that as few as 2.5 cells/mL ETEC can be detected using the culture-PCR method. However, each Bangladeshi environmental sample has numerous other bacteria present in, which may compete for growth media nutrients and possibly increase the detection threshold.

**Conclusions:** Hands, soil, and water could all be key sources of exposure to pathogenic *E. coli*. Further analysis is needed to determine which source of exposure contributes most to diarrheal disease transmission in children. The most important reservoir of pathogens are the ones that children come in contact with most often. Overall, the WASH Benefits sanitation intervention seems to have a limited effect on the presence of pathogenic *E. coli* present in hands, soil, and water.

**Future Work:** Future work includes continuing to analyze remaining environmental samples for pathogenic *E. coli*, determining if fecal contamination is from human or animal origin, and modeling which source of exposure children have most contact with.

**References:**