Estimating the Burden of Typhoid: The Role of Novel Techniques

Overview of presentation

- Background
- Objectives
- Methods
- Results
- Conclusion
Global Estimates of Typhoid Burden

- Globally, 14.3 million (95% uncertainty interval [UI] 12.5–16.3) cases of typhoid and paratyphoid fevers occurred in 2017.

- The highest disease burden regions are sub-Saharan Africa and South Asia.

- In Asia, a recent study conducted in Bangladesh, Nepal, and Pakistan reported high adjusted incidence of disease exceeding 100 per 100,000 person-years of observation (PYO) in all three countries.
Global Estimates of Typhoid Burden

The global burden of typhoid and paratyphoid fevers: a systematic analysis for the Global Burden of Disease Study 2017

Lancet Infect Dis 2019; 19: 369–81

WHO THRESHOLD FOR TYPHOID

100 cases per 100,000 PYO

Bangladesh, Nepal, and Pakistan
Ghana
Burkina Faso
Estimates of typhoid cases in Ghana

Monthly cases

- 2016-05-26
- 2017-08-26
- 2018-11-26
- 2020-02-26
- 2021-05-26
- 2022-08-26

COVID-19
Trend of typhoid cases

Proportion of Salmonella Typhi Cases

Years

Standard methods for typhoid surveillance
The need for additional tools for S.Typhi detection

Fecal-oral route

- Contaminated food or water
- Acid-tolerance genes allow for safe passage through the stomach
- Invasion of the mucous membrane of intestinal cells
- Inflammatory response results in diarrhea

Lack of proper hand-washing

Can spread to liver, spleen, and bone marrow to cause systemic disease

Prof Davis, lecture series, 2020
Project objectives

- Implement typhoid ES with partners at sites conducting blood-culture surveillance over a 12-month period

- Measure ES site characteristics and investigation of their association with detection of S. Typhi and human-restricted or other control organisms that indicate faecal contamination

- Investigate whether S. Typhi load and genetic diversity correlate with disease incidence rates in the local population
Studies on malaria
Studies on Typhoid
Fever without source

Catchment population 140,000
## ES sites

<table>
<thead>
<tr>
<th>Number of Sites</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>AGOGO</td>
<td>37</td>
</tr>
<tr>
<td>ANANEKROM</td>
<td>2</td>
</tr>
<tr>
<td>DOMEABRA</td>
<td>2</td>
</tr>
<tr>
<td>HWIDIEM</td>
<td>1</td>
</tr>
<tr>
<td>JUANSA</td>
<td>3</td>
</tr>
<tr>
<td>MAGYEDA</td>
<td>1</td>
</tr>
</tbody>
</table>

**Sum** 46

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**Map of ES sites:**
- Agog (Ashanti)
- Ananekrom
- Amantenaman
- Juansa
Study procedures

- 43 sites mapped out at the study site
- Monthly sampling (12 months)

ES site selection, characterization & validation

Sample collection (grab or trap samples)

Sample processing & DNA extraction

qPCR for *Salmonella* and *S. Typhi* targets (ttr, staG, tviB)

Typhoid ES methods: [https://www.medrxiv.org/content/10.1101/2021.05.21.21257547v1](https://www.medrxiv.org/content/10.1101/2021.05.21.21257547v1)

Protocols and community at: [https://www.protocols.io/workspaces/typhoides](https://www.protocols.io/workspaces/typhoides)
**Target genes for *Salmonella* Typhi detection**

<table>
<thead>
<tr>
<th>Primer/Probe Name</th>
<th>Sequence (5' -3')</th>
</tr>
</thead>
<tbody>
<tr>
<td>staG_F</td>
<td>CGC GAA GTC AGA GTC GAC ATA G</td>
</tr>
<tr>
<td>staG_R</td>
<td>AAG ACC TCA ACG CCG ATC AC</td>
</tr>
<tr>
<td>staG_P</td>
<td>[TAMRA] - CA TTT GTT CTG GAG CAG GCT GAC GG - [BHQ2]</td>
</tr>
<tr>
<td>ttr_F</td>
<td>CTC ACC AGG AGA TTA CAA CAT GG</td>
</tr>
<tr>
<td>ttr_R</td>
<td>AGC TCA GAC CAA AAG TGA CCA TC</td>
</tr>
<tr>
<td>ttr_P</td>
<td>[FAM] - CA CCG ACG GCG AGA CCG ACT TT - [BHQ1]</td>
</tr>
<tr>
<td>tvIB_F</td>
<td>TGT GGT AAA GGA ACT CGG TAA A</td>
</tr>
<tr>
<td>tvIB_R</td>
<td>GAC TTC CGA TAC CG GAT AAT G</td>
</tr>
<tr>
<td>tvIB_P</td>
<td>[JOE] - TG GAT GCC GAA GAG GTA AGA CGA GA - [BHQ1]</td>
</tr>
<tr>
<td>HF183_F</td>
<td>ATC ATG AGT TCA CAT GTC CG</td>
</tr>
<tr>
<td>HF183_R</td>
<td>CTT CCT CTC AGA ACC CCT ATC C</td>
</tr>
<tr>
<td>HF183_P</td>
<td>[FAM] - CT AAT GGA ACG CAT CCC - [BHQ1]</td>
</tr>
</tbody>
</table>

Detections of all targets (staG, ttr, tvib) is considered positive.
Field Training and community engagements

Training in protocol development

Field engagements in study implementation

Field training

Moore swabs preparation

Training by ICL and others
Validation of sites
Deployment of Moore swabs/Grab samples
Cold chain for sample transport
Data collection

- **Questionnaire for environmental site sample collection**
  - Date & time
  - Site ID
  - GPSaddress
  - Flow speed
  - Depth of wastewater
  - Width of wastewater
  - MSID, deployment duration,
  - Water quality measurements (Temp, pH, DO, EC, TDS, ORP, SAL, SSG)

**Data capture**
- Paper and tablets were used in capturing data
- Data were entered in REDCap
- Field and lab data were cleaned merged
Laboratory Processing and Testing

- Filter (0.45uM membrane)
- Elute by massaging in 10m RL
- Centrifuge and store pellets at -20

Grab

- 1L No enrichment
- Incubate overnight at 37°C
- Pipette 2 aliquotes of 20ml
- Filter (0.45uM membrane)
- Cut filters and place in powerbead tube
- Centrifuge and store pellets at -20°C

Moore Swab

- 450 ml pre-enrich in UPE
### Detection of *Salmonella Typhi*

<table>
<thead>
<tr>
<th>Location</th>
<th>Typhoid Positives</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGOGO</td>
<td>201 (31.7%)</td>
<td>633</td>
</tr>
<tr>
<td>ANANEKROM</td>
<td>2 (11.1%)</td>
<td>18</td>
</tr>
<tr>
<td>DOMEABRA</td>
<td>3 (15.7%)</td>
<td>19</td>
</tr>
<tr>
<td>HWIDIEM</td>
<td>8 (44%)</td>
<td>18</td>
</tr>
<tr>
<td>JUANSA</td>
<td>16 (30%)</td>
<td>53</td>
</tr>
<tr>
<td>MAGYEDA</td>
<td>0 (0)</td>
<td>14</td>
</tr>
<tr>
<td><strong>Sum</strong></td>
<td><strong>230</strong></td>
<td><strong>755</strong></td>
</tr>
</tbody>
</table>

### S. Typhi detection at ES sites

![Map showing detection locations](image)

- Agogo
- Ananekrom
- Domeabra
- Hwidiem
- Juansa
- Magyeda

Typhi Positives: 1 to 12
### Detection of faecal contamination marker at ES sites

#### Distribution of Typhoid Cases

<table>
<thead>
<tr>
<th>Location</th>
<th>HF183 Positives</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGOGO</td>
<td>526 (83.1%)</td>
<td>633</td>
</tr>
<tr>
<td>ANANEKROM</td>
<td>13 (72.2%)</td>
<td>18</td>
</tr>
<tr>
<td>DOMEABRA</td>
<td>17 (89.4%)</td>
<td>19</td>
</tr>
<tr>
<td>HWIDIEM</td>
<td>11 (61.1%)</td>
<td>18</td>
</tr>
<tr>
<td>JUANSA</td>
<td>43 (81.1%)</td>
<td>53</td>
</tr>
<tr>
<td>MAGYEDA</td>
<td>12 (14.3%)</td>
<td>14</td>
</tr>
<tr>
<td><strong>Sum</strong></td>
<td><strong>622</strong></td>
<td><strong>755</strong></td>
</tr>
</tbody>
</table>

HF183 detection at ES sites

![Map showing HF183 detection sites](image)
Salmonella Typhi detections in Grab and Moore swabs

Proportion of samples positive (%)

Month of sample collection

Sample
- Grab
- Moore swab
Faecal contamination and S.Typhi detections

Proportion of H183 (%)

Month of sample collection

Moore Swabs
S_Typhi
- Negative
- Positive

Grab
Predictors of Salmonella Typhi detections in wastewater

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Odds Ratio</th>
<th>P-value</th>
<th>Lower 95% CI</th>
<th>Upper 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method [Moore Swab]</td>
<td>4.43</td>
<td>&lt;0.0001</td>
<td>2.59</td>
<td>7.58</td>
</tr>
<tr>
<td>HF183 [Positive]</td>
<td>3.94</td>
<td>0.007</td>
<td>1.47</td>
<td>10.60</td>
</tr>
<tr>
<td>pH [High]</td>
<td>1.97</td>
<td>0.006</td>
<td>1.22</td>
<td>3.19</td>
</tr>
<tr>
<td>Dissolved_oxygen</td>
<td>1.45</td>
<td>0.109</td>
<td>0.92</td>
<td>2.30</td>
</tr>
<tr>
<td>Electrical_conductivity</td>
<td>0.84</td>
<td>0.359</td>
<td>0.58</td>
<td>1.22</td>
</tr>
<tr>
<td>Salinity</td>
<td>1.41</td>
<td>0.048</td>
<td>1.00</td>
<td>1.97</td>
</tr>
<tr>
<td>Seawater_specific_gravity</td>
<td>1.01</td>
<td>0.905</td>
<td>0.83</td>
<td>1.23</td>
</tr>
</tbody>
</table>
Conclusion

- Moore swab samples have better yield for *Salmonella* Typhi detection compared to Grab samples.

- pH level and level of faecal contamination is associated with *S.*Typhi detection in environmental samples. However more analysis is needed to provide additional evidence.

- While we have observed a high number of *S.*Typhi in the environment, the incidence of blood culture *Salmonella* detections is low. We plan to sequence the environmental *S.*Typhi detected in order to further confirm this.
Thanks to the study team and collaborators
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