A case-control investigation into the household distribution of invasive Salmonellae in Blantyre, Malawi

Malawi-Liverpool-Wellcome Trust
Clinical Research Programme

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Uncertain functional host adaptation of ST313 and other iNTS serovars and clades

127 index invasive disease iNTS
32/467 (family contacts carried Salmonella, 65% matched index by PFGE)
4 environmental isolates also similar, but very few animal isolates

Dione et al, Gambia PLOS NTD 2011
GEMS 14 NTS cases (8 diarrhoea, 6 healthy controls)
210 household animals (chicken, sheep goat)
21 animal NTS isolates (10%), MLST and serotyping
no sequence type overlap between human enteric and animal isolates

Nyirenda et al unpublished, Malawi 2014-15
60 healthy children aged 6-18 months sampled every month
~30% healthy children carried culture positive NTS short-term (higher by molecular)
Of these, 50% were Salmonella Typhimurium ST313
International consensus meeting on Invasive Salmonella Disease

November 2014, MLW, Malawi
Case control study of reservoirs of invasive Salmonella disease

Objectives

• Identify household shedding and household environmental reservoirs of invasive Salmonellae within Blantyre

• Compare invasive to human, animal and environmental strains using whole genome sequencing

• Information to inform control strategies for Salmonella infections in Africa
Recruitment and sampling

Index Case identification at QECH (Typhi + NTS)
60 cases
60 controls
URBAN

Visit case household within 2 weeks
Select Control household by bottle-spin and 100 yard walk
Obtain Consents

Conduct Sampling at Homes
Family Animals Environment

Culture and identification
WGS
<table>
<thead>
<tr>
<th>Household (HH)</th>
<th>iNTS Household</th>
<th>S.Typhi Household</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Case n=27</td>
<td>Control n=27</td>
</tr>
<tr>
<td>Median (IQR) or %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HH members</td>
<td>5 (5-7)</td>
<td>4 (3-6)</td>
</tr>
<tr>
<td>No. of rooms</td>
<td>4 (3-5)</td>
<td>3 (2-4)</td>
</tr>
<tr>
<td>HH keeps animals %</td>
<td>32</td>
<td>12</td>
</tr>
<tr>
<td>Has bathroom %</td>
<td>16</td>
<td>4</td>
</tr>
<tr>
<td>Walls unbaked mud %</td>
<td>4</td>
<td>20</td>
</tr>
<tr>
<td>Walls baked mud %</td>
<td>12</td>
<td>24</td>
</tr>
<tr>
<td>Walls plastered %</td>
<td>84</td>
<td>56</td>
</tr>
<tr>
<td>Tin roof %</td>
<td>100</td>
<td>96</td>
</tr>
<tr>
<td>Socioeconomic and sanitation</td>
<td>iNTS Household</td>
<td>S.Typhi Household</td>
</tr>
<tr>
<td>------------------------------</td>
<td>----------------</td>
<td>-------------------</td>
</tr>
<tr>
<td></td>
<td>Case n=25</td>
<td>Control n=25</td>
</tr>
<tr>
<td><strong>Socioeconomic:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Electricity supply %</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>HOH can read &amp; write %</td>
<td>100</td>
<td>80</td>
</tr>
<tr>
<td><strong>Sanitation:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pit latrine no slab %</td>
<td>40</td>
<td>48</td>
</tr>
<tr>
<td>Pit latrine with slab %</td>
<td>52</td>
<td>48</td>
</tr>
<tr>
<td>Flush toilet %</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>No. sharing toilet</td>
<td>9 (7-18)</td>
<td>9 (5-13)</td>
</tr>
<tr>
<td>Soap Available %</td>
<td>56</td>
<td>48</td>
</tr>
<tr>
<td>Drinking water</td>
<td>iNTS Household</td>
<td>S.Typhi Household</td>
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<tr>
<td>------------------------------------</td>
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<td>-------------------</td>
</tr>
<tr>
<td></td>
<td>Case n=25</td>
<td>Control n=25</td>
</tr>
<tr>
<td>Piped into house %</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Piped into plot %</td>
<td>28</td>
<td>24</td>
</tr>
<tr>
<td>Public tap or standpipe %</td>
<td>48</td>
<td>68</td>
</tr>
<tr>
<td>Public bore hole %</td>
<td>16</td>
<td>8</td>
</tr>
<tr>
<td>Other %</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Water Treatment used %</td>
<td>16</td>
<td>16</td>
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</tbody>
</table>
Sampling
- **Stool** (Human, adults and children)
- **Animal stool/rectal swabs** (chickens, pigs, cows, goats, sheep, cats, dogs, gecko)
- **Sterile boot sox** - perimeter, latrine, rubbish, bedroom, cooking areas
- **Water** (Stored, puddles, vessels)
- **Food** (left over)
Human Stool

Environmental Sample

- Enrichment BPW @ 37°C 18 – 24h
- Selective Selenite Broth @ 37°C 18 – 24h
- XLD @ 37°C 18 – 24h
- Colonies ID’d
  - Serology & API 20E
- Confirmed Salmonella for WGS

Water

- Filtered
- BPW @ 37°C 18 – 24h
- Selective RV Broth @ 37°C 18 – 24h
- Re-incubate Selective Selenite Broth @ 37°C 18 – 24h
<table>
<thead>
<tr>
<th>Total samples</th>
<th>Case Total Samples</th>
<th>No. of Salmonella Isolated</th>
<th>% Positive</th>
<th>Control Total Samples</th>
<th>No. of Salmonella Isolated</th>
<th>% Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human Stool</td>
<td>273</td>
<td>11</td>
<td>4</td>
<td>282</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Animal Rectal Swab</td>
<td>17</td>
<td>0</td>
<td>0</td>
<td>9</td>
<td>1</td>
<td>0</td>
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<tr>
<td>Animal Stool</td>
<td>32</td>
<td>2</td>
<td>6</td>
<td>34</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Bootsocks</td>
<td>305</td>
<td>6</td>
<td>2</td>
<td>297</td>
<td>24</td>
<td>8</td>
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<tr>
<td>Food</td>
<td>67</td>
<td>0</td>
<td>0</td>
<td>52</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Water</td>
<td>108</td>
<td>0</td>
<td>0</td>
<td>98</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>802</td>
<td>19</td>
<td>2.4</td>
<td>708</td>
<td>30</td>
<td>4.2</td>
</tr>
<tr>
<td>Sample Type</td>
<td>Case Total</td>
<td>Case no. Salmonella Isolated</td>
<td>Case % Positive</td>
<td>Control Total</td>
<td>Control no. Salmonella Isolated</td>
<td>Control % Positive</td>
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<td>0</td>
<td>0</td>
<td>9</td>
<td>1</td>
<td>0</td>
</tr>
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<td>6</td>
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<td>1</td>
<td>3</td>
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<td>-----------------------</td>
<td>----------------------------</td>
<td>------------</td>
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<tr>
<td>Human Stool</td>
<td>157</td>
<td>2</td>
<td>1.3</td>
<td>121</td>
<td>4</td>
<td>3.3</td>
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<tr>
<td>Animal Rectal Swab</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>1</td>
<td>16.7</td>
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<tr>
<td>Animal Stool</td>
<td>14</td>
<td>2</td>
<td>14</td>
<td>17</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Bootsocks</td>
<td>168</td>
<td>5</td>
<td>3</td>
<td>166</td>
<td>17</td>
<td>10</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>442</strong></td>
<td><strong>9</strong></td>
<td><strong>2</strong></td>
<td><strong>394</strong></td>
<td><strong>23</strong></td>
<td><strong>5.8</strong></td>
</tr>
</tbody>
</table>
### iNTS: households and controls (26 + 26)

<table>
<thead>
<tr>
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<th>Case Total Samples</th>
<th>No. of Salmonella Isolated</th>
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</thead>
<tbody>
<tr>
<td>Human Stool</td>
<td>116</td>
<td>9</td>
<td>8</td>
<td>97</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Animal Rectal Swab</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>31</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Animal Stool</td>
<td>18</td>
<td>0</td>
<td>0</td>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Bootsocks</td>
<td>137</td>
<td>1</td>
<td>1</td>
<td>131</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>Food</td>
<td>26</td>
<td>0</td>
<td>0</td>
<td>24</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Water</td>
<td>54</td>
<td>0</td>
<td>0</td>
<td>42</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>360</strong></td>
<td><strong>10</strong></td>
<td><strong>2.8</strong></td>
<td><strong>314</strong></td>
<td><strong>7</strong></td>
<td><strong>2.2</strong></td>
</tr>
</tbody>
</table>
Summary of isolation rates

- Multiple serovars isolated across the physical and living household environment

  3% overall isolation by culture
  Isolation 4% human, 6% animals, 5% soil

- No isolates from food or water – sampling / methodological
Predicted serovar

- Aberdeen
- Agoue|Cubana
- Amager
- Djama
- Gaminara
- Hadar
- Havana|ll 1,13,22:g.t:[1,5]
- II 40:b:-
- II 42:r:-|llb 42:r:-:[z50]
- Mguani
- Montevideo
- Ogbe|ll 43:z:1,5
- Senftenberg
- Typhimurium

Illumina sequencing
Enterobase phylogenetic trees
(S. Typhi blood stream isolates not included)
Predicted serovar

- Aberdeen
- Agouevecubana
- Amager
- Djama
- Gaminara
- Hadar
- HavanaII 1,13,22:g:t[1,5]
- II 40:b:-
- II 42:r:-IIlb 42:r:-[z50]
- Mgulani
- Montevideo
- OgbeteII 43:z:1,5
- Senftenberg
- Typhimurium

Bootsox house areas (controls)
- latrine, cooking area, bedroom, perimeter, rubbish tip

Bootsox house areas (cases & controls)
- latrine, cooking area, bedroom, perimeter, rubbish tip

Boot sox samples
Predicted serovar
- Aberdeen
- Agoueve/Cubana
- Amager
- Djama
- Gaminara
- Hadar
- Havana [II 1,13,22: g, t: [1,5]
- II 40:b:-
- II 42:r:- [IIIb 42:r:- [z50]
- Mgulani
- Montevideo
- Ogbete [II 43:z: 1,5
- Senftenberg
- Typhimurium

* Animal isolate
Predicted serovar

- Aberdeen
- Agoueve/Cubana
- Amager
- Djama
- Gaminara
- Hadar
- Havana/II 1,13,22:g,t:[1,5]
- II 40:b:-
- II 42:r:-/IIIb 42:r:-:[z50]
- Mgulani
- Montevideo
- Ogbele/II 43:z:1,5
- Sentfenberg
- Typhimurium

* Animal isolate
Predicted serovar

- Aberdeen
- Agoueve|Cubana
- Amager
- Djama
- Gaminara
- Hadar
- Havana||1,13,22:g,t:[1,5]
- II 40:b:-
- II 42:r-||IIIb 42:r-:z50
- Mguiani
- Montevideo
- Ogbele||43:z:1,5
- Senftenberg
- Typhimurium

Mixing of human and animal carried serovars
Predicted serovar

- Aberdeen
- Agoueve|Cubana
- Amager
- Djama
- Gaminara
- Hadar
- Havana|ll 1,13,22:g:t:[1,5]
- Il 40:b:-
- Il 42:r:-|ll|b 42:r:-:[z50]
- Mgulani
- Montevideo
- Ogbe|l|l 43:z:1,5
- Senftenberg
- Typhimurium

★ Adult stool
★ Child stool
★ Animal isolate
★ Boot sox isolates
Summary & conclusions

- iNTS and Typhoid case-control study – human, animal and environmental, urban slum setting

- Multiple serovars isolated across the physical and living household environment
  3% overall isolation by culture
  Isolation rate 4% human, 6% animals, 5% soil
  No isolates from food or water – sampling and methodological issues

- Clear overlap between asymptomatic human and animal serovars

- A phylogenetic ST313 match found only with family members (1 adult, one child)

- Supports (but does not prove) mainly human reservoir or iNTS
- Supports (but does not prove) human to human transmission of iNTS
Thank you

MALAWI
Reenesh Prakash
Leonard Koolman
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Rose Nyirenda
Brigitte Denis

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Chisomo Msefula
Nick Feasey
Paul Wigley
Jay Hinton
Sian Jones

Chisomo Msefula
Robert Heyderman