The human challenge model for *Salmonella* Paratyphi A: The clinical response and importance for future vaccine development

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Coalition Against Typhoid, Bali 2015
Salmonella Paratyphi A Challenge model

- Human-restricted pathogen
- CL3 pathogen in UK
- Inclusion and exclusion criteria
- Non-specific symptoms with fever
  - diagnostics
- Monitoring
- Antibiotic treatment and clearance
Paratyphoid Challenge Model

Clinical features

Oomics

CMI

Serology & Cytokines

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Challenge

Timepoint

log₁₀ ELISA units/mL

M01ZH09 Placebo Ty21a anti-LPS IgG

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TNF-α (log₂ FC)
## Criteria for diagnosis

Paratyphoid fever is diagnosed if ANY of the following apply

<table>
<thead>
<tr>
<th>Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>A positive blood culture for <em>Salmonella Paratyphi</em> from 72 hours post-challenge</td>
</tr>
<tr>
<td>A positive blood culture for <em>Salmonella Paratyphi</em> within 72 hours post-challenge, with one or more signs/symptoms of paratyphoid infection (such as recorded temperature $\geq 38^\circ C$)</td>
</tr>
<tr>
<td>Persistent positive blood cultures (two or more blood cultures taken at least 4 hours apart) for <em>Salmonella Paratyphi</em> within 72 hours post-challenge.</td>
</tr>
<tr>
<td>Oral temperature $\geq 38^\circ C$ persisting for 12 hours</td>
</tr>
</tbody>
</table>

*Note: The criteria provided are for illustrative purposes and may not align with the current standards or guidelines. Always refer to the latest and specific guidelines for diagnostic criteria.*
Clinical outcomes

Primary outcome: ‘attack rate’ of 60-75%

‘High dose’ $10^3$ CFU:
  – 12/20
    • Clinical criteria (fever) 3/12
    • Bacteraemia 9/12 (additional 4 cases with fever $\geq 38^\circ$)

‘Low dose’ ~800 CFU:
  – 8/20
    • All diagnoses based on BC (2 cases with fever $\geq 38^\circ$)
All typhoid and paratyphoid diagnoses

Kaplan-Meier plots demonstrating time to diagnosis following challenge at two dose levels with *S. Paratyphi* (10^3 CFU and ~800 CFU) and *S. Typhi* (10^3 and 10^4 CFU).
Kaplan-Meier plot demonstrating time to first positive blood culture following challenge at two dose levels with S. Paratyphi ($10^3$ CFU and ~800 CFU) and S. Typhi ($10^3$ and $10^4$ CFU).
A mild headache, muscle and/or joint pain often preceded diagnosis by 3-4 days, resolved and then returned with increased intensity.

All participants who were diagnosed with paratyphoid fever (positive blood culture or fever ≥38°C for 12 hours).

Percentage of participants who recorded symptoms related to paratyphoid infection at any time during the intense challenge period.
Paratyphoid participants had fewer and less severe symptoms than those in typhoid studies.
Microbiological diagnosis

Diagnosed on day 8

Stool culture positive

Bacteremia

Temperature (°C)

CRP (mg/L)
Symptomatic diagnosis

Diagnosed on day 6

No growth of S. Paratyphi from stool

Bacteremia

Temperature (°C)

CRP (mg/L)
Duration of bacteraemia

CFU/ml at time of diagnosis

Figure 1 - Duration of S. Typhi and S. Paratyphi bacteraemia in participants with enteric fever diagnosis (Days - Median, IQR; * p=0.0078).

See M. Gibani et al. poster

Figure 2 - Quantitative blood culture at time of diagnosis (CFU/ml - median, IQR). There was no significant difference in bacteraemic burden at time of diagnosis following challenge at all doses tested (p=0.493).

www.ovg.ox.ac.uk
Paratyphoid vs. typhoid in non-diagnosed participants

Not Diagnosed with Paratyphoid Fever

Number of participants with symptom (%)

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Severe</th>
<th>Moderate</th>
<th>Mild</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constipation</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>20</td>
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<tr>
<td>Cough</td>
<td>30</td>
<td></td>
<td></td>
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<tr>
<td>Arthralgia</td>
<td>40</td>
<td></td>
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<tr>
<td>Myalgia/vomiting</td>
<td>50</td>
<td></td>
<td></td>
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<tr>
<td>Abdominal pain</td>
<td>60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Loss of appetite</td>
<td>70</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Generally unwell</td>
<td>80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Headache</td>
<td>90</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Not diagnosed with Typhoid Fever (n=10, placebo arm of vaccine study)
Paratyphoid Blood parameters

Hemoglobin

White cell count

Platelets

CRP

See G. Napolitani et al. poster
Paratyphi and stool

- Stool clearance
- Carriage question

<table>
<thead>
<tr>
<th>Organisms</th>
<th>BBL CHROMagar Salmonella</th>
<th>XLD Agar</th>
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<tbody>
<tr>
<td>E. coli, Citrobacter</td>
<td>Inhibited or blue-green colonies with or without mauve halos</td>
<td>Large, flat, yellow. Some strains may be inhibited.</td>
</tr>
<tr>
<td>Enterobacter/Klebsiella</td>
<td>Partially inhibited; blue-green to blue colonies with or without mauve halos</td>
<td>Mucoid, yellow</td>
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<tr>
<td>Proteus</td>
<td>Inhibition partial to complete</td>
<td>Red to yellow. Most strains have black centers.</td>
</tr>
<tr>
<td>Salmonella, H₂S-positive</td>
<td>Growth; mauve (=rose-violet) to violet colonies*</td>
<td>Black or red with black centers</td>
</tr>
<tr>
<td>Salmonella, H₂S-negative</td>
<td>Growth; mauve (=rose-violet) to violet colonies*</td>
<td>Red</td>
</tr>
<tr>
<td>Shigella</td>
<td>Partially to completely inhibited; colorless or (rarely) blue-green colonies</td>
<td>Red</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>Inhibition partial to complete</td>
<td>Red</td>
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<tr>
<td>Aeromonas hydrophila, Stenotrophomonas maltophilia</td>
<td>Inhibition partial to complete; may rarely produce rose to mauve colonies; oxidase positive (S. maltophilia may be weakly positive or negative)*</td>
<td>Yellow or pink</td>
</tr>
<tr>
<td>Gram-positive bacteria</td>
<td>Inhibition partial to complete</td>
<td>Inhibition partial to complete</td>
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Stool shedding after challenge

<table>
<thead>
<tr>
<th>DAY from challenge</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14 clearance</th>
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<tbody>
<tr>
<td>fever</td>
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<td>bacteraemia</td>
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<td>No diagnosis</td>
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Lactoferrin after challenge

See H. Thomaides-Brears et al. poster

biomarker of neutrophil degranulation from mucosal surfaces
Spontaneous clearance without antibiotics

No fever, minimal symptoms and unremarkable blood results.
New control measures urgently needed

Future enteric fever prevention strategies in Asia must focus on S. Typhi and S. Paratyphi

- Emergence of drug-resistant strains
- Clinically indistinguishable disease
- Diagnostics: Need for improved diagnostics

A bivalent vaccine is needed to address enteric fever
Further enteric studies at OVG

Paratyphoid And Typhoid Challenge and re-challenge (PATCH):

• The mechanisms and determinants of mucosal and systemic protection against typhoid and paratyphoid fever, following controlled human challenge and natural disease.

Paratyphoid model developed for vaccine evaluation and now looking for candidates to test!
The Paratyphoid team

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