

Evaluation of the clinical and microbiological response to *Salmonella* Paratyphi A infection in the first paratyphoid human challenge model

Authors

Hazel C Dobinson^{1a}, Malick M Gibani^{1a}, Claire Jones^{1a}, Helena B Thomaides-Brears^{1*}, Merryn Voysey^{1,2}, Thomas C Darton¹, Claire S. Waddington¹, Danielle Campbell¹, Iain Milligan¹, Liqing Zhou¹, Sonu Shrestha¹, Simon A Kerridge¹, Anna Peters¹, Zoe Stevens¹, Audino Podda³, Laura B. Martin³, Flavia D'Alessio⁴, Duy Pham Thanh⁵, Buddha Basnyat⁶, Stephen Baker^{5,7,8}, Brian Angus⁹, Myron M Levine¹⁰, Christoph J Blohmke¹, Andrew J Pollard¹

¹Oxford Vaccine Group, Department of Paediatrics, University of Oxford and the NIHR Oxford Biomedical Research Centre, Oxford, United Kingdom; ²Nuffield Department of Primary Care Health Sciences University of Oxford, UK; ³ GSK Vaccines Institute for Global Health (GVGH), Via Fiorentina 1, 53100 Siena, Italy; ⁴ European Vaccine Initiative, Heidelberg, Germany; ⁵ The Hospital for Tropical Diseases, Wellcome Trust Major Overseas Programme, Oxford University Clinical Research Unit, Ho Chi Minh City, Vietnam; ⁶ Oxford University Clinical Research Unit, Patan Academy of Health Sciences, Kathmandu, Nepal; ⁷ Centre for Tropical Medicine and Global Health, University of Oxford, Oxford, United Kingdom; ⁸ The London School of Hygiene and Tropical Medicine, London, United Kingdom; ⁹ Nuffield Department of Medicine, University of Oxford, UK; ¹⁰ Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, MD USA

Corresponding author contact:

Helena Thomaides-Brears
Oxford Vaccine Group
Centre for Clinical Vaccinology and Tropical Medicine
Churchill Hospital
Old Road, Headington
Oxford, UK
OX3 7LE
Telephone 0044-1865-611364/Fax: 0044-1865 857420;
helena.thomaides-brears@paediatrics.ox.ac.uk

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^a H.C.D, M.M.G and C.J. contributed equally to this manuscript.

Running title: Human Challenge Model of *S. Paratyphi A*

Summary (40 words): The safe establishment of a protocol for a human challenge model for *S. Paratyphi A* can be used to expedite the evaluation of novel vaccine candidates and provides insight into the clinical and immune response to paratyphoid infection.

ABSTRACT:

Background: To expedite the evaluation of vaccines against paratyphoid fever, we aimed to develop the first human challenge model of *S. Paratyphi A* infection.

Methods: Two groups of 20 participants underwent oral challenge with *S. Paratyphi A* following sodium bicarbonate pre-treatment at one of two dose levels (Group 1: $1-5 \times 10^3$ CFU and Group 2: $0.5-1 \times 10^3$ CFU). Participants were monitored in an outpatient setting with daily clinical review and collection of blood and stool cultures. Antibiotic treatment was started when pre-specified diagnostic criteria were met (temperature $\geq 38^\circ\text{C}$ for ≥ 12 hours and/or bacteraemia) or at day 14 post challenge.

Results: The primary study objective was achieved following challenge with $1-5 \times 10^3$ CFU (Group 1), which resulted in an attack rate of 12/20 (60%). Compared with typhoid challenge, paratyphoid was notable for high rates of subclinical bacteraemia (at this dose 11/20; 55%). Despite limited symptoms, bacteraemia persisted for up to 96 hours after antibiotic treatment (median duration of bacteraemia 53hrs; IQR 24-85hrs). Shedding of *S. Paratyphi A* in stool typically preceded onset of bacteraemia.

Conclusions: Challenge with *S. Paratyphi A* at a dose of $1-5 \times 10^3$ CFU was well tolerated and associated with an acceptable safety profile. The frequency and persistence of bacteraemia in the absence of clinical symptoms was notable, and markedly different from that seen in previous typhoid challenge studies. We conclude that the paratyphoid challenge model is suitable for the assessment of vaccine efficacy using endpoints that include bacteraemia and/or symptomatology.

Clinicaltrials.gov Registration: NCT02100397

Keywords: Paratyphoid Infection, Enteric Fever, *Salmonella enterica* Paratyphi A, Human challenge study, Immune responses

Introduction

Efforts aimed at reducing the global burden of enteric fever are likely to require a co-ordinated strategy comprising improvements in water quality, sanitation and hygiene measures along with the development of effective vaccines. Whilst *Salmonella* Typhi remains the principal aetiological agent of enteric fever globally, *Salmonella enterica* serovar Paratyphi A (*S. Paratyphi A*) is responsible for an increasing proportion of enteric fever cases. In South and South-East Asia, annual incidence rates in some areas are estimated to be as high as 150 cases/100,000 person-years.[1]

Relatively little is known regarding the pathophysiology and host response to *S. Paratyphi A* infection. *Salmonella enterica* serovars Typhi and Paratyphi A possess similar genomic markers of host-restriction, but differ with regards to expression of certain virulence factors, such as the lack of Vi polysaccharide capsule expression by *S. Paratyphi A/B*. [2,3] Additionally, there are currently no licensed vaccines available for the prevention of paratyphoid fever. The licensed *S. Typhi* vaccine Ty21a can induce cross-reactive humoral immune responses to *S. Paratyphi A/B in vitro* and there is some evidence from field trials for cross protection against *S. Paratyphi B*. [4,5] Several promising vaccine candidates are in development, including live-attenuated strains and conjugate vaccines targeted against the O-specific polysaccharide (O:2) of *S. Paratyphi A*, although no candidate vaccines have undergone efficacy trials to date. [6] Development of vaccines to prevent paratyphoid infection is hampered by limited knowledge of immunological correlates of protection and the lack of a suitable small-animal model of infection.

Human challenge studies are increasingly used to identify promising vaccine candidates suitable for evaluation in large-scale field trials. [7] In this study, we sought to establish a *S. Paratyphi A* human challenge model in healthy adult volunteers by oral challenge to enable future assessment of *S. Paratyphi A* vaccines and to assess host-pathogen interactions under a strictly controlled setting.

Methods

Study Design

We performed an observational, dose-level modification human challenge study of *S. Paratyphi A* infection conducted in healthy community adult volunteers, at the Centre for Clinical Vaccinology and Tropical Medicine, University of Oxford, Oxford, United Kingdom. [8] Description of the study protocol and enrolment criteria are detailed elsewhere. [9][10]

The primary objective of the study was to determine the dose in CFU of *S. Paratyphi A* strain NVGH308 required to achieve an attack rate of 60–75%, when ingested with sodium bicarbonate solution to neutralise gastric acid.

Challenge strain

S. Paratyphi A strain NVGH308, was isolated in 2006 from a patient with paratyphoid fever in Kathmandu, Nepal, and is susceptible to most commonly used antibiotics, including ciprofloxacin (**Supplementary Table 1**).^[9] The NVGH308 strain was selected as it is a contemporary, circulating strain isolated from a patient in a highly endemic country.

Whole genome sequencing was undertaken to identify the phylogenetic relationship of NVGH308 to other circulating strains (**Supplementary Figure 1; Supplementary Methods**).

The challenge inoculum was freshly prepared on day of challenge (**Supplementary Methods**). An initial target dose of 1.5×10^3 CFU (Group 1) was selected based upon prior *S. Typhi* challenge studies in a similar study population.^[11] Following fulfilment of the primary objective at the initial target dose, we amended the original study protocol to include a dose de-escalation group ($0.5-1 \times 10^3$ CFU; Group 2) in order to affirm a relationship between dose and diagnostic endpoints.

Clinical Evaluation

Regular participant safety monitoring occurred through daily clinical review for a minimum of 14 days and included solicited symptoms (**Supplementary Table 2**) and twice daily temperature measurements.^[9]

Diagnostic Criteria

The primary outcome was the rate of paratyphoid infection, defined as a positive blood culture for *S. Paratyphi A* (taken ≥ 72 hours after challenge to avoid detection of primary bacteraemia) and/or oral temperature $\geq 38^\circ\text{C}$ persisting for ≥ 12 hours. ^[9] Treatment was initiated upon fulfilment of diagnostic criteria (or at day 14 for those without illness) and comprised oral ciprofloxacin 500mg twice daily for 14 days.

Serological Response

Blood was collected at baseline and day 10, 28 and 90 post challenge. Specific Immunoglobulin M (IgM), IgG and IgA to *S. Paratyphi A* lipopolysaccharide (LPS) and flagellin (H) were measured by an in-house enzyme-linked immunosorbent assay (ELISA) using goat anti-human IgM, IgG and IgA conjugated to horseradish peroxidase (see **Supplementary Methods**).

Statistical Analysis

All participants were included in the primary analysis and a post-hoc analysis was conducted comparing participants challenged with *S. Paratyphi A* in this study with those challenged with *S. Typhi* in our previous study.[11]

Clinical, laboratory and immunological data were collated using a clinical trials database (OpenClinica, version 3.1). Data analysis was performed as described in detail in the Supplementary Methods, using R version 3.2.2.[12]

Results

Forty healthy adult participants were enrolled into the study between 20th May and 20th November 2014. (**Table 1; Supplementary Figure 2**). Twenty participants were challenged with *S. Paratyphi A* at a target dose of $1-5 \times 10^3$ CFU (group 1). A second group of twenty participants was challenged at a target dose of $0.5-1.0 \times 10^3$ CFU (group 2).

Paratyphoid infection was diagnosed in 20/40 (50%) of all participants challenged. The primary study objective was achieved in challenge group 1, where 12/20 participants met the pre-specified diagnostic criteria for paratyphoid fever (Attack rate 60%; 95%CI 36–81%) (**Figure 1**). Eleven participants (11/12, 92%) in this group were diagnosed following a positive blood culture for *S. Paratyphi A* and one was diagnosed based on temperature criteria with subsequent blood culture confirmation. Median time from challenge to initiation of treatment was 6.4 days (range 5.9-8.3 days) (**Table 2**).

Paratyphoid infection was diagnosed less frequently in challenge group 2. In this group, 8/20 participants met the pre-specified diagnostic criteria following challenge (Attack rate 40%; 95%CI 19-64%), all of whom were diagnosed following a positive blood culture (**Table 2**). Median time from challenge to initiation of treatment was 8.3 days (range 7.1-12.0 days) (**Figure 1**).

Attack rates using alternative diagnostic criteria are outlined in **Table 2**. The hypothetical attack rate was lower in both study groups if clinical end-points alone (e.g. fever) were used to define paratyphoid disease.

All participants were managed with daily clinical review and no participants met the pre-specified criteria for severe enteric fever.[9] Two participants had positive stool cultures for *S. Paratyphi A* after completing a 14-day course of ciprofloxacin, neither of whom had evidence of gall-bladder disease at screening. Both were successfully re-treated with a 14-day course of oral azithromycin (500mg daily) and all subsequent clearance stools were negative. There were no episodes of relapse of clinical disease.[9] One serious adverse event was recorded (appendicitis six months after *S. Paratyphi A* challenge) and was assessed as being unrelated to study procedures. One participant developed mouth ulcers after treatment with ciprofloxacin, which resolved following a change to azithromycin. A second participant developed vaginal candidiasis following antibiotic treatment (ciprofloxacin), which resolved following treatment with topical clotrimazole.

The most common symptom reported by those with paratyphoid illness was headache (17/20; 85%), followed by malaise (15/20; 75%), abdominal pain (14/20; 70%) and myalgia (14/20; 70%). The majority of solicited symptoms were graded as mild and did not result in limitation of usual activity (**Figure 1, Supplementary Table 2**). In comparison with participants challenged with *S. Typhi* in earlier studies,[11] paratyphoid resulted in a milder symptom profile, characterised by fewer severe symptoms and lower cumulative symptom scores (**Figure 2, Supplementary Table 3**).

Laboratory parameters were in keeping with those expected for acute enteric fever (**Supplementary Figure 3**).[13] Amongst those meeting diagnostic endpoints, peripheral blood biochemistry demonstrated elevated C-reactive protein and alanine aminotransferase, whilst haematology revealed a modest fall in haemoglobin, neutrophil and lymphocyte counts.

We documented at least one positive blood culture in all participants diagnosed with paratyphoid (20/20; 100%; median number positive blood cultures 5.5, range 1-8). Median bacteraemia clearance times were 2.2 days (IQR 1-3.5 days) for *S. Paratyphi* (**Figure 2, Figure 3**). The median time from challenge to bacteraemia was 5.2 days (interquartile range [IQR], 5.0-6.4) in group 1 and 7 days (IQR 6.7-7.4) in group 2. Quantitative blood cultures performed prior to antibiotic treatment demonstrated median bacterial loads of 1.1 CFU/ml (range 0-4.1), in group 1 and 0.2 CFU/ml (range 0-6.9) in group 2 participants, which are comparable to those seen in cases of typhoid fever in earlier challenge studies and in endemic settings.[11,14] The rate of blood culture positive days was twice as high in *S. Paratyphi A* diagnosed participants than in *S. Typhi* diagnosed participants from our previous study (IRR 2.1; 95%CI 1.46 to 3.1, $p < 0.001$) (**Figure 2; Table 2**).

Sub-clinical bacteraemia was a common finding following challenge, with 11/20 (55%; 95%CI 32-77%) participants having demonstrable bacteraemia but remaining persistently afebrile following challenge, although other symptoms were observed as discussed above (**Supplementary Table 2**).

Afebrile bacteraemia was less common than in earlier typhoid challenge studies performed by our group (4/21; 19%; 95%CI 5-42%; $p=0.025$ Fisher's exact test).[11] Fever was recorded less frequently in group 2 (2/8; 25%) than in group 1 (7/12; 58%) (**Table 2**).

Shedding of *S. Paratyphi A* in stool occurred sporadically throughout the challenge period and at least one stool culture was positive in 17/40 participants (43%; 95%CI 27-59%) (**Figure 3**). Amongst participants with demonstrable bacteraemia 11/20 (55%; 95%CI 32-77%) had a positive stool culture for at least one time point and stool shedding frequently preceded the onset of bacteraemia (**Figure 3**). Asymptomatic shedding was identified in 6/20 participants (30%; 95%CI 12-54%) who did not reach the diagnostic criteria during the observation period. The rate of stool positivity was similar in *S. Paratyphi A* diagnosed participants as was observed previously in *S. Typhi* diagnosed participants (IRR 0.78; 95%CI 0.40 to 1.52, $p=0.457$).

Baseline anti-LPS IgG geometric mean concentrations were higher in undiagnosed participants prior to challenge ($p=0.034$) but did not relate to the incidence of bacteraemia (**Supplementary Figure 4**). Anti-LPS antibody responses peaked at day 28 post-challenge and were significantly higher in those diagnosed with paratyphoid compared with those without acute disease ($p<0.001$). In contrast, anti-H antibody responses were only marginally increased above baseline at D28 and only in those meeting diagnostic endpoints (**Supplementary Figure 4**).

Discussion

In 1909, Proescher reported a case of "two individuals who accidentally swallowed a small amount of a pure culture" of *Salmonella Paratyphi A* and who "became ill...five and seven days after imbibing" the culture.[15] Here, we describe the first deliberate and controlled human challenge model using a contemporary circulating strain of *S. Paratyphi A*. Whilst *S. Typhi* challenge studies have become well established over the past half-century, we have uniquely demonstrated that *S. Paratyphi A* challenge can also be undertaken safely in an outpatient setting. Challenge at a target dose of $1-5 \times 10^3$ CFU was sufficient to achieve an attack rate of 60% in a cohort of naïve volunteers, whilst challenge using a lower dose resulted in a reduced attack rate and increased time to bacteraemia.

Whilst Vi-conjugate typhoid vaccines are currently licenced in both India and China, the paratyphoid A vaccine pipeline is less well developed.[6] The effect of future Vi-based typhoid vaccine programmes on the burden of paratyphoid fever is unknown and there is conflicting evidence of serovar replacement following historical typhoid vaccination campaigns.[16,17] This presents a pressing need for accelerated paratyphoid A vaccine development, which could be facilitated by the establishment of a human paratyphoid A challenge model. In support of this, the WHO has stated that well-validated

challenge studies can provide “considerable supporting evidence of the efficacy” of typhoid Vi-conjugate vaccines, as well as offering insights into immunological correlates of protection.[18] We suggest that the model described herein could fulfil an equivalent role for paratyphoid by providing a platform to assess the efficacy of paratyphoid vaccines currently in development.

The choice of diagnostic endpoints is central to future applications of this model in the assessment of candidate paratyphoid vaccines. In this study, we used a composite diagnostic end-point of clinical and/or microbiologically defined paratyphoid infection and observed that 95% of individuals who met the diagnostic criteria did so based upon microbiological criteria (**Table 2**). The frequency of blood culture sampling in this study resulted in the identification of several cases of asymptomatic bacteraemia, which may or may not have progressed to symptomatic paratyphoid fever if left untreated. Whilst the consistent finding of bacteraemia represents an unambiguous diagnostic endpoint, this could set an overly stringent threshold in future vaccine efficacy studies and clinical endpoints might be preferable as this more accurately reflects the situation in the field (**Table 2**). Arguably, vaccines demonstrating at least moderate efficacy in this challenge model would perform better in field trials, where the endpoints would include a clinical syndrome of paratyphoid fever with culture confirmation. Vaccine efficacy studies using this model should include multiple alternative pre-specified secondary diagnostic endpoints, such as the proportion of cases diagnosed by clinical criteria alone, microbiological criteria alone or a composite of clinical symptoms with culture confirmation.

To our knowledge, no previous studies have explored the infectious dose required to induce paratyphoid disease, although it is frequently assumed that *S. Paratyphi A* requires a higher dose of bacteria to cause clinical disease than does *S. Typhi*. [19,20] In this study, an attack rate of 60% was achieved using a dose of $1-5 \times 10^3$ CFU *S. Paratyphi A*, which is comparable to the attack rate (55%) observed at an equivalent dose of *S. Typhi* in earlier studies.[11] Notably challenge with as few as 700 CFU *S. Paratyphi A* was able to induce acute disease, in the context of sodium bicarbonate pre-treatment. Our findings are broadly consistent with observations from *S. Typhi* challenge studies, where reducing the challenge inoculum results in a reduced attack rate and longer incubation period.[21] As with *S. Typhi*, it is possible that the infectious dose required to induce paratyphoid disease in a non-endemic setting may differ from that required in an endemic setting, where prior exposure could lead to partial immunity.

We performed a post-hoc comparison of symptom data collected during earlier typhoid challenge studies [11] and noted that paratyphoid challenge resulted in a milder disease profile than that previously observed following typhoid challenge. In our model, paratyphoid was characterised by high

rates of afebrile bacteraemia in groups 1 and 2 (11/20; 55%) with minimal symptoms, which may otherwise have escaped detection in the absence of intensive sampling. Case series from the pre-antibiotic era reported that *S. Paratyphi A* caused a spectrum of clinical manifestations that “closely resembles that of mild enteric fever” [22] and asymptomatic cases of paratyphoid infection are well described.[23,24] Although the largest published case series to date indicates that *S. Typhi* and *Paratyphi A* infections cause an indistinguishable clinical syndrome[19], this study was limited to patients who attended healthcare facilities and may not account for ambulatory or mild cases of paratyphoid in the community. In keeping with this, epidemiological data from endemic settings suggest that sub-clinical paratyphoid infection frequently occurs.[5] In light of our challenge model data, we interpret earlier data as being supportive of the hypothesis of an underappreciated and undiagnosed burden of paratyphoid disease in the community. In a proportion of individuals, exposure to the bacillus is followed by asymptomatic bacteraemia and asymptomatic stool shedding, potentially perpetuating both long- and short-cycle transmission. In keeping with this, asymptomatic shedding of *S. Paratyphi A* was also observed in our model, including in individuals who did not meet diagnostic criteria for paratyphoid disease.

Somewhat counter-intuitively, we observed prolonged bacteraemia and protracted time to blood culture clearance (≥ 96 hrs) in the paratyphoid challenge model, despite the low rate of fever. In addition, convalescent stool shedding was noted in two individuals despite a 14 day course of ciprofloxacin, both of whom were successfully treated with a further course of azithromycin. The relatively poor response to ciprofloxacin was unexpected, given that the NVGH308 strain is sensitive to ciprofloxacin according to current CLSI and EUCAST guidelines (MIC 0.06 $\mu\text{g/ml}$). [25,26] Studies in endemic settings have found fluoroquinolone MICs for *S. Paratyphi A* were typically higher than those for *S. Typhi*. [19] The mechanisms underlying the discrepancy between the clinical and microbiological profiles of paratyphoid infection seen in this study are unclear, but may represent a strategy of achieving infection-by-stealth that facilitates onward transmission and persistence of the bacterium within the environment.

Ethical and safety considerations in the design of this study, including early initiation of rescue therapy, limit the extent that our findings can be extrapolated to endemic settings. For example, a proportion of individuals with sub-clinical bacteraemia could have progressed to symptomatic disease had antibiotic treatment been delayed, as was observed in historical typhoid challenge studies.[27] As only a single strain was used in this study we cannot conclusively rule out a strain-specific effect for our observations. However, the NVGH308 strain is a recent clinical isolate from a symptomatic case with bacteraemia, and is closely related to currently circulating strains in South Asia. Additionally, *S. Paratyphi A* is a clonal monomorphic pathogen containing limited genomic variation, suggesting that

the pathogenicity and immune response to the NVGH308 strain will translate to other wild-type strains.[28] Future work could identify whether these observations apply to other strains of *S. Paratyphi A* and also investigate B and C strains in the human challenge model. The evaluation of only two challenge doses is one potential limitation of this study and challenge at of higher dose ranges and/or re-challenge studies could be undertaken to better define clinical outcomes.

In summary, we have established the first *S. Paratyphi A* human challenge model and have described the clinical and microbiological response to challenge in healthy adult volunteers, presenting marked differences from those previously seen with *S. Typhi*. Whilst *S. Typhi* challenge models have existed for several decades, the need for a *S. Paratyphi A* challenge model arguably exceeds that for *S. Typhi*, as far less is understood regarding the responses to infection and there are no specific control measures yet available. Development of a successful paratyphoid challenge model could offer distinctive insights into paratyphoid disease as well as providing a platform to expedite the development and implementation of much needed vaccines and diagnostics.

NOTES

Acknowledgements

The study team wish to acknowledge the contribution of study participants and the assistance of the following: Public Health England, Oxfordshire; Oxford University Hospital Laboratories; clinical and laboratory staff at Oxford Vaccine Group; University of Maryland provision of antigens and Emergent BioSolutions for provision of standard sera to complete the antibody work.

Funding

This work was supported by the European Vaccine Initiative (ref: PIM) and The Bill and Melinda Gates Foundation (ref: OPP1084259). The typhoid challenge study was supported by a Wellcome Trust Strategic Translational Award (grant number 092661). The study funders are independent of the study design, study management, analyses and interpretation of the study results. The Oxford Vaccine Group, University of Oxford, is supported by the National Institute for Health Research Clinical Research Network (CRN). LBM reports grants from the Wellcome Trust outside of the submitted work. AJP, TCD and CJB are supported by the NIHR Oxford Biomedical Research Centre (Translational Research Fellowship to TCD). CJB is supported by the European Union (FP7) as a Marie Curie fellow.

Conflict of Interest

LBM and AP are employees of GSK, which has partnered with Biological E (Hyderabad, India) for development of a bivalent typhoid and paratyphoid A vaccine. GSK Vaccines for Global Health have received a grant from the Wellcome Trust for development of a bivalent typhoid/paratyphoid A vaccine. MML reports a license agreement between the University of Maryland and Bharat Biotech for a bivalent typhoid/paratyphoid vaccine in early pre-clinical development, for which he is a co inventor. AJP has previously undertaken clinical studies on behalf of the University of Oxford, which were funded by vaccine manufacturers, but no longer does so. His department has received unrestricted educational grants from vaccine manufacturers to support delivery of a course on childhood infection. All other authors declare that they have no competing interests.

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Figure Legends

Figure 1

(a) Kaplan-Meier plots indicating time to diagnostic end points **(i)** Time to diagnosis **(ii)** Time to first temperature $\geq 38^{\circ}\text{C}$. **(b)** Clinical symptom profiles following *S. Paratyphi A* challenge. Percentage of participants reporting solicited systemic symptoms on one or more days following *S. Paratyphi A* challenge, recorded using an online diary for 21 days. Stacked columns display percentage of participants reporting maximum symptom severity as mild, moderate or severe. Individuals meeting pre-specified criteria for paratyphoid disease in group 1 **(i)** and group 2 **(ii)** Individuals who did not meet pre-specified criteria for paratyphoid disease in group 1 and group 2. None = No reported symptoms; Mild = Present but no limitation of usual activity; Moderate = some limitation of daily activity; Severe = unable to perform normal daily activity.

Figure 2

Comparison of the clinical and microbiological response to *S. Typhi* and *S. Paratyphi A* challenge. **(a)** Solicited systemic symptoms in individuals with acute typhoid (T, n=24) or paratyphoid (P, n=20) disease recorded using an online diary (Day 0 = Day of challenge; Day 14 = Final day of treatment initiation). Percentage of participants reporting one or more events. Stacked columns display percentage of participants reporting maximum symptom severity as mild (present but no limitation of usual activity), moderate (some limitation of daily activity) or severe (unable to perform normal daily activity); **(b)** Comparison of cumulative symptom severity scores between participants with acute typhoid (T, n=24) or paratyphoid (P, n=20) disease. Symptoms scores were calculated by summing numerical values assigned to the severity of individual symptoms between Day 0 to Day 14 (0=not present; 1=mild; 2=moderate; 3=severe). Box-and-whiskers display median, IQR and range; **(c)** Symptom severity scores for specific symptoms Day 0 to Day 14; **(d)** Results of quantitative blood

cultures (CFU/ml) collected immediately prior to initiation of antibiotic treatment for participants with typhoid or paratyphoid disease (See **Materials & Methods**).

Figure 3

Clinical and Microbiological dynamics for participants challenged with *S. Typhi* and *S. Paratyphi A*. PD1-20 = Confirmed paratyphoid disease; nPD1-20 = Paratyphoid disease not diagnosed. TD1-TD25 = Confirmed typhoid disease; nTD1-nTD16 = Typhoid disease not diagnosed. Day 0 = Day of challenge. **(a)** Pattern of blood culture results (red = positive; grey = negative; white = sample not collected). Numbers in parenthesis refer to quantitative blood culture colony count (CFU/ml) taken immediately prior to antibiotic treatment and correspond to day of treatment initiation; **(b)** Pattern of stool shedding following challenge. Coloured squares indicate stool culture results for *S. Paratyphi A* or *S. Typhi* (brown = positive; grey = negative; white = sample not collected). Tx = Day of treatment initiation. **(c)** Maximum temperature measurements by day. Tx= Day of treatment initiation

Table 1

Participant demographic characteristics

Characteristic	Group 1	Group 2	All
No. of participants	20	20	40
challenged			
Sex, Male, No (%)	10 (50)	11 (55)	21 (52.5)
Age, y, Median (Range)	27 (19-49)	23 (20-50)	25(19 -50)
Ethnicity, White British, No (%)	18 (90)	17 (85)	35 (88)
Country of Birth, UK, No (%)	15 (75)	17 (85)	32 (80)
Tobacco Smoking, Yes, No (%)	9 (45)	8 (40)	17 (43)
Alcohol Intake, Units/week, Median (IQR)	4 (0-10)	7 (4-11)	5 (2-10)
BMI, kg/m², Mean ± SD	23.5 ± 3.2	22.8 ± 4.2	24.1 ± 3.8

Table 2Attack rates and response to *S. Paratyphi A* challenge

Results	Group 1 (n=20)	Group 2 (n=20)	All (n=40)
Target challenge dose (CFU)	1-5x10 ³	0.5-1x10 ³	-
Attack Rate, n (% total) (95% confidence interval)	12 (60) (36-81)	8 (40) (19-64)	20 (50) (34-66)
Actual challenge dose, CFU x10³, median, range			
Paratyphoid A diagnosed	2.4 (2.2-2.8)	0.9(0.7-1.3)	-
Paratyphoid A NOT diagnosed	2.4 (2.2-2.7)	1.0 (0.7-1.3)	-
Paratyphoid A NOT diagnosed	2.5 (2.3-2.7)	0.8 (0.7-1.3)	-
Actual challenge dose/Body surface area^a, CFU/m² Mean ± SD			
Paratyphoid A diagnosed	1350 ± 166	519 ± 163	-
Paratyphoid A NOT diagnosed	1336 ± 196	533 ± 171	-
Paratyphoid A NOT diagnosed	1371 ± 119	509 ± 165	-
Mode of diagnosis, n (% total)			
Temperature ≥38°C for ≥12 hours with blood culture	1 (5)	0 (0)	1 (2.5)

confirmation			
Microbiological diagnosis^b	11 (55)	8 (40)	19 (47.5)
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Bacteraemia, n (% total)	12 (60)	8 (40)	20 (50)
Time to bacteraemia^c, days, median (interquartile range)	5.2 (5.0-6.4)	7 (6-11)	6.1 (5.1-7.1)
Time to antibiotics^d, days, median (interquartile range)	6.4 (6.2-7.4)	8.3 (8.2-9.2)	8.0 (6.4-8.3)
Overall duration of bacteraemia^e, days, median (interquartile range)	4.1 (3.4-5.0)	1.0 (1.0 -3.4)	4.0 (1.1-4.9)
Clearance time^f, days, median (interquartile range)	2.9 (2.1-3.6)	0.9 (0.7-1.9)	2.2 (1.0-3.5)
Quantitative blood culture, CFU/ml, Median (range)	1.1 (0-4.1)	0.2 (0-6.9)	1 (0-6.9)
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Temperature $\geq 38^{\circ}\text{C}$ any duration^g, n (% total)	7 (35)	2 (10)	9 (45)
in Paratyphoid A diagnosed	7 (35)	2 (10)	9 (45)
in Paratyphoid A NOT diagnosed	0 (0)	0 (0)	0 (0)
<hr/>			
Stool Shedding, n (% total)	12 (60)	4 (20)	16 (40)
in Paratyphoid A diagnosed	8 (40)	2 (10)	10 (25)
in Paratyphoid A NOT diagnosed	4 (20)	2 (10)	6 (15)

Attack rates using alternative**diagnostic criteria**

Temperature $\geq 37.5^{\circ}\text{C}$ (any duration), n (% total)	8 (40)	4 (20)	12 (30)
Temperature $\geq 38^{\circ}\text{C}$ (any duration), n (% total)	7 (35)	2 (10)	9 (45)
Temperature $\geq 37.5^{\circ}\text{C}$ (any duration) AND positive blood culture	7 (35)	4 (20)	11 (27.5)
Temperature $\geq 38^{\circ}\text{C}$ (any duration) AND positive blood culture	7 (35)	2 (10)	9 (22.5)

- a. Body surface area calculated according to Mostellar Method, $\text{BSA (m}^2\text{)} = (\text{height cm} \times \text{weight kg}/3600) \times 0.5$
- b. *Salmonella* Paratyphi A isolated from blood culture taken ≥ 72 hours from challenge
- c. Time from challenge to collection of first positive blood culture.
- d. Time from ingestion of the challenge agent to the fulfilment of diagnostic criteria.
- e. Time from collection of first positive blood culture to collection of first persistently negative blood culture.
- f. Time from initiation of antibiotics to time the collection of first persistently negative blood culture.
- g. Day 0 (Day of challenge) to Day 14.

Figure 1

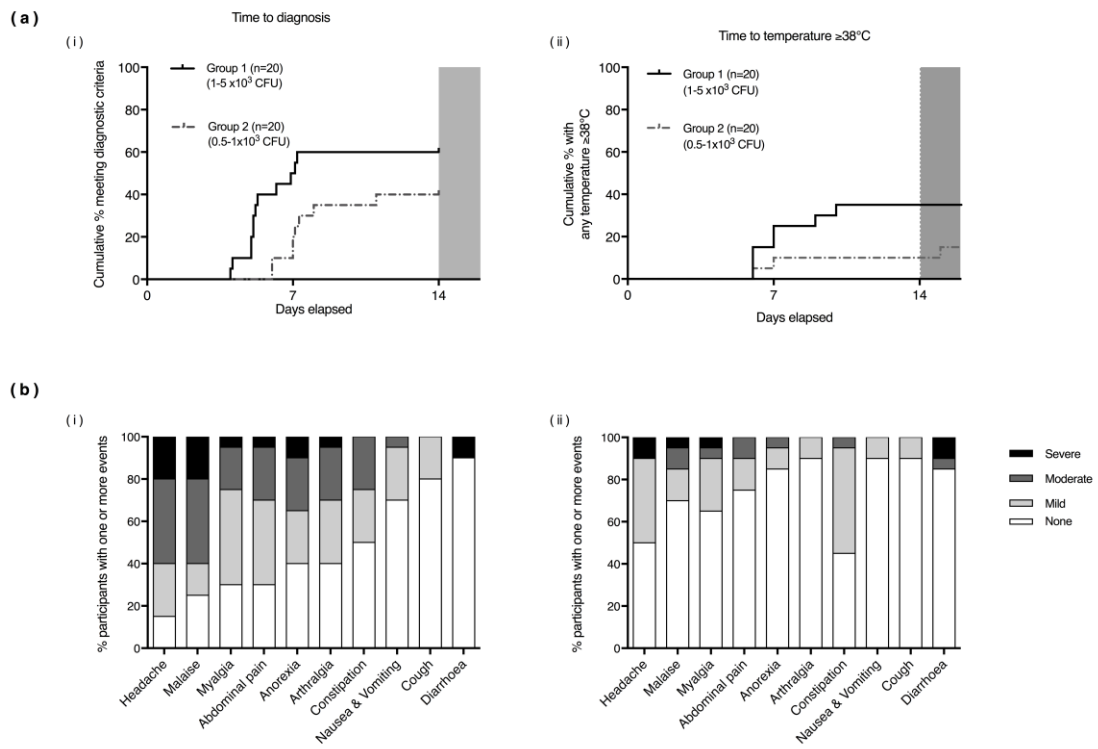


Figure 2

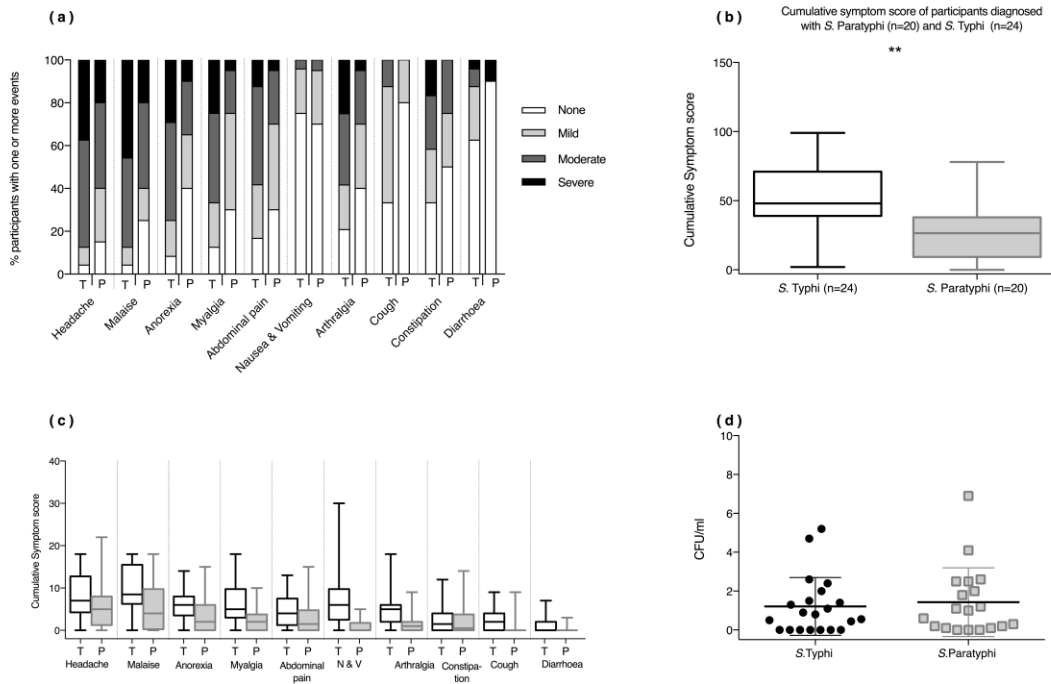


Figure 3

