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UNDERSTANDING PARATYPHOID INFECTION

Study Protocol for the Development of a Human Model of *Salmonella enterica* serovar Paratyphi A challenge in Healthy Adult Volunteers.

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ABSTRACT

Introduction: This study will develop the first human challenge model of paratyphoid infection which can then be taken forward to evaluate paratyphoid vaccine candidates. Salmonella Paratyphi A is believed to cause a quarter of the estimated 20 million cases of enteric fever annually. Epidemiological evidence suggests that an increasing proportion of the enteric fever burden is attributable to S. Paratyphi infection meriting further attention and interest in vaccine development. Assessment of paratyphoid vaccine efficacy in preclinical studies is complicated by the lack of a small animal model and the human-restricted nature of the infection. The use of experimental human infection in healthy volunteers provides an opportunity to address these problems in a cost-effective manner.

Methods and analysis: Volunteers will ingest virulent S. Paratyphi A bacteria (NVGH308 strain) with a bicarbonate buffer solution, to establish the infectious dose resulting in an "attack rate" of 60 – 75%. Using an a priori decision-making algorithm, the challenge dose will be escalated or de-escalated to achieve the target attack rate, with the aim of reaching the study endpoint while exposing as few individuals as possible to infection. The attack rate will be determined by the proportion of paratyphoid infection in groups of 20 healthy adult volunteers, with infection being defined by one or more positive blood cultures (microbiological endpoint), and/or fever, defined as an oral temperature exceeding 38°C sustained for at least 12 hours (clinical endpoint); 20 to 80 participants will be required. Challenge participants will commence a two week course of an oral antibiotic upon diagnosis of infection, or after 14 days follow-up.

Ethics and dissemination: The strict eligibility criteria aims to minimise risk to participants and their close contacts. Ethical approval has been obtained. The results will be disseminated in a peer-reviewed journal and presented at international congresses.

Trial Registration: Clinicaltrials.gov NCT02100397 **Trial Registration:** Clinicaltrials.gov NCT02100397

Strengths:

- This protocol describes the methodology for performing this unique, first in man challenge study with *S.* Paratyphi A.
- The development of a *S*. Paratyphi human challenge model should expedite the progress and evaluation of potential paratyphoid vaccine candidates, in particular by allowing the direct measurement of vaccine protective efficacy in a safe, reproducible host-relevant model.
- Studying the longitudinal physiological and immunological responses to infection will provide insight into this increasingly prevalent but poorly understood infection.

Limitations:

- The modest number of participants in challenge studies can make the model sensitive to individual variation.
- The response to challenge may not reflect the target population whom may have protection due to repeated natural exposure.
- The immuno-biological response to *S*. Paratyphi A exposure may differ between strains.

INTRODUCTION

Enteric fever is the term used to describe systemic illness caused by infection with *Salmonella enterica* serovars Typhi and Paratyphi A and C. Enteric fever is a leading cause of morbidity worldwide with at least 20 million cases annually, of which an estimated five million are caused by *S*. Paratyphi A. [1] Principally affected are young, school-aged children in resource-limited settings, and travellers to those areas.[1,2]

The proportion of disease caused by *S.* Paratyphi A is increasing, particularly in the highly endemic regions of South East Asia and the Indian sub-continent.[3] As a human restricted pathogen, targeted vaccination of those groups at high risk is likely to have a substantial impact on disease incidence.[4]

In view of the high burden of enteric fever and increasing antibiotic resistance, The World Health Organisation (WHO) has stated that countries with high-risk groups and populations 'should consider the programmatic use of typhoid vaccines for controlling endemic disease.[4]' Despite this recommendation, there has been a reluctance to introduce programmes with the available licenced typhoid vaccines, Vi polysaccharide (ViPS) and Ty21a, as both have limited efficacy, offer minimal cross-protection against paratyphoid infection, and cannot be given to children less than two years of age. This has focused development on firstly conjugate *S*. Typhi and Paratyphi A vaccines, which can be administered to infants and expected to provide long-lasting immune memory, as well as attenuated oral vaccines. A number of promising candidate vaccines are in early phase testing.

The aim of this study is to develop a safe, reliable human paratyphoid challenge model in which to validate vaccines and develop novel diagnostics. Human challenge studies with *S*. Typhi (Quailes strain) have been performed historically and more recently using an adapted model.[5–7] To our knowledge, human challenge with virulent *S*. Paratyphi has not been performed before so this study will provide a unique opportunity to study human paratyphoid infection. The study is designed to determine the dose of *S*. Paratyphi A

(challenge strain NVGH308) required to produce an infection attack rate of 60-75% in healthy adult volunteers who have not previously been exposed to typhoidal salmonella and therefore immunologically naïve to the challenge agent. With sufficient evidence of paratyphoid infection at a target dose, this model can then be taken forward to evaluate paratyphoid vaccine candidates.

METHODS/DESIGN

This is a descriptive, dose level escalation human infection study using *S.* Paratyphi A challenge of ambulatory, out-patient healthy community adult volunteers. For safety, the study will commence with the challenge of one individual at the initial dose of 1-5 x10^3 CFU. Consecutive groups of five or 10 participants will then be challenged a minimum of two weeks apart following a decision-making algorithm for dose escalation/de-escalation (see figure 1). This algorithm was used for the typhoid challenge model developed by the University of Oxford in 2009.[5]

Regulation and governance

In the UK investigational products, such as unlicensed medications and vaccines, are regulated by the Medicines for Human Use (Clinical Trials) Regulations 2004 which implement the European Clinical Trials Directive (2001/20/EC).[8] However most microbial challenge studies fall outside of the remit of the ECTD and are instead judged according to common law and best practice.[9] The typhoid and paratyphoid challenge models, using a fully characterised and non-genetically modified strain, falls under this category. To achieve best practice, challenge protocols undergo independent and rigorous peer review to assess the scientific quality and appropriateness of the study methodology to answer the key objectives. This includes conforming to Good Clinical Practice (GCP) guidelines with the support from a research ethics committee experienced in these studies.[10] As such, local or regional ethic committees, independent from the researchers and sponsors, are fundamental for research governance of microbial challenge studies.

Challenge strain

The original *S.* Paratyphi A strain NVGH308 was isolated in 2006 from a patient with acute paratyphoid fever as part of a study performed by the Oxford University Clinical Research Unit at Patan Hospital, Kathmandu, Nepal. It has been manufactured to Good Manufacturing Practice (GMP) standard and is supplied for study purposes by Novartis Vaccines for Global Health, Italy. Manufacturing to GMP standard, while not a regulatory requirement in the UK, fulfils best practice.[9]

A parent seed lot, S888P5SP01, was established in March 2010 after serial colony selections on Luria Broth PTK agar plates and stored in the Novartis Vaccines and Diagnostics bacterial seed bank (Siena, Italy). This seed lot underwent five sequential passages as part of the cell line cleaning process before being used to establish the GMP Master Cell Bank, SA-13-002, of the NVGH308 strain. GenIbet BioPharmaceuticals (Oeiras, Portugal) produced three dose levels of the challenge agent under GMP conditions. Prepared vials containing the challenge agent were stored at -80°C ± 5°C and transferred via an accredited courier to the Oxford Vaccine Group Laboratory in 2013.

Batch testing of the cell bank confirms O:1 and O:2 antigen positivity, batch purity and stable viable bacterial count. Ongoing stability testing of the seed lot has been performed by Novartis Vaccines and Diagnostics. The NVGH308 strain is fully characterised with known susceptibility to a number of antimicrobial options.

Study Objectives

The primary objective is to determine the dose (in colony forming units (CFU)) of *S.* Paratyphi A, challenge strain NVGH308, needed to produce a 60-75% attack rate when ingested with sodium bicarbonate solution by healthy adult volunteers.

The secondary objectives will describe the interaction between bacteria and human host at baseline, through inoculation and symptomatic infection (or asymptomatic immune protection) to recovery and long term follow up. These objectives are:

- To describe the human physiological response to *S.* Paratyphi A challenge, and in those developing or not developing infection.
- To evaluate the sensitivity of the pre-defined criteria for paratyphoid infection, using subsequent clinical, microbiological and laboratory outcomes.
- To describe the characteristics of bacterial dynamics after challenge, including onset and duration of bacteraemia, bacterial burden at diagnosis and stool shedding.
- To describe the human immune response to challenge, including the innate, humoral, cell-mediated and mucosal responses.
- To determine genetic features affecting host-pathogen responses, alteration of those responses through epigenetic changes, control of gene and protein expression.
- To discover, develop and evaluate novel diagnostic methods for *S.* Paratyphi A infection.
- To explore the factors, influences and motivation affecting volunteer's decision to participate in human challenge studies and their experiences of the study process.

Feedback and recommendations from Patient and Public Involvement (PPI) in addition to participant questionnaires from previous studies have been incorporated into this study. This beneficial process will continue with an anonymous questionnaire to be completed by participants 28 days after challenge.

Study setting

The study will be conducted at the Centre for Clinical Vaccinology and Tropical Medicine, Oxford, UK, which is a fully equipped vaccine research site with available clinical inpatient facilities and a Category III level laboratory on-site. The UK is non-endemic for enteric fever with the majority of cases travel-related; the rate of paratyphoid fever notification in the Thames Valley region, which includes Oxfordshire, is 0.5/100,000.[11]

Eligibility criteria

Male or female participants aged 18-60 years inclusive who are in good health (as determined by a study doctor, medical investigation and agreement by their general practitioner) and able to provide written informed consent will be eligible for inclusion in this study. Additional inclusion and exclusion criteria will be applied to ensure participants are appropriate for the study (supplementary 1). These strict criteria aim to minimise the risk of severe or complicated disease in participants and reduce potential risk of transmission to close contacts.

Interventions

A participant will be considered enrolled in the study on their day of challenge.

The method used for preparation of the challenge inocula is based on the one used for the recent typhoid challenge model.[5] Participants will fast for 90 minutes before ingesting 120ml of sodium bicarbonate (2.1g $NaHCO_3$) to neutralise stomach acid. This is followed 60 seconds later by the challenge inocula which will be freshly prepared prior to each challenge by defrosting and suspending the required bacterial dose in 30mls of sodium bicarbonate (0.53g $NaHCO_3$).

After *S*. Paratyphi A ingestion, participants will be seen daily for 14 days with blood, stool, saliva and urine samples taken at set time points (Supplementary 2). Monitoring for derangement of liver, renal, blood count parameters and inflammatory markers will be performed. Participants will also complete twice-daily temperature readings and record any symptoms experienced for 21 days; this data will be collected by electronic CRFs and diary cards. Subsequent follow-up appointments will be 28, 90, 180 and 365 days after challenge (Figure 2).

Paratyphoid infection will be diagnosed after challenge if one of the following applies:

- a positive blood culture for *S*. Paratyphi from 72 hours after challenge,
- a positive blood culture for *S*. Paratyphi within 72 hours, with one or more signs/symptoms of paratyphoid infection,

- persistent positive blood cultures for *S.* Paratyphi within 72 hours, or
- an oral temperature ≥38˚C persisting for 12 hours .

The earliest microbiological indication that a participant has a *S.* Paratyphi A bacteraemia will be the identification of a Gram-negative bacillus (GNB) from a positive blood culture. As formal identification of the organism may take a minimum of a further 24 hours, participants in whom a GNB is identified will be defined as having paratyphoid fever.

Ciprofloxacin 500mg twice daily for 14 days will commence on diagnosis of paratyphoid infection or at Day 14 after challenge. The rationale for using fluoroquinolones first-line is based on recommendations for treatment of enteric fever and prevention (and treatment) of chronic gallbladder carriage as therapeutic levels are reached in bile and gallbladder.[12,13] All positive blood culture isolates will have full susceptibility profiling performed using an antibiotic disc method and *E*-test to measure the ciprofloxacin MIC. Alternative antibiotics used as second-line therapy (in the event of adverse reactions or side-effects) include azithromycin and trimethoprim/sulfamethoxazole.

If the participant's symptoms fail to resolve after antibiotic administration, if they are unable to tolerate oral antibiotics, become dehydrated, or if unanticipated concerns regarding home circumstances emerge, inpatient admission to an infectious diseases unit will be considered. Patient care at this stage would be delegated to the hospital clinical team, which could include the provision of intravenous fluids, antibiotics and anti-emetics.

Safety

Participant safety during the study: Participants will be monitored closely with daily clinical review and completion of symptom diary cards. All adverse events will be recorded on case report forms (CRFs), with serious events notified to the Data Safety Monitoring Committee (DSMC) within 24 hours of the investigator becoming aware of the event. Adverse events of special interest (AESI) will also be reported to the DSMC in the same manner and include:

- complications of paratyphoid fever such as perforation or haemorrhage which occurs almost exclusively in patients who are untreated for an extended period,[14]
- failure to clinically or bacteriologically cure a participant of acute paratyphoid infection within 14 days of antibiotic therapy,
- relapse or progression to carrier state, the latter defined as a person who is still excreting *S*. Paratyphi A after two courses of appropriate antibiotic therapy,[15]
- transmission of S. Paratyphi to non-study participants.

Safety data collated from eCRFs and eDiary which include blood parameters, vital signs, and symptom recordings, will be reviewed by the DSMC after the first participant has been challenged and at each dose escalation. Approval from the DSMC will be required prior to any subsequent dose alterations.

Long term safety of participants: The risk of chronic carriage with *S*. Paratyphi A is minimised by treatment for two weeks with an effective antibiotic and excluding participants with gallbladder disease.[16] In addition, stool samples for culture will be obtained two weeks after completion of the antibiotic course and then weekly until two successive samples are negative. If samples remain positive for *S.* Paratyphi A four weeks after completion of antibiotics then the participant will be referred to a National Health Service infectious diseases consultant for further management.

Safety of non-study participants: The risk of secondary transmission to close contacts is unlikely in view of the low infectivity of *S*. Paratyphi A and the level of hygiene and sanitation in the UK.[17] Consent will not be obtained from close household contacts, but participants will be required to provide them with a written study summary detailing measures to reduce the risk of infection and offering screening for paratyphoid infection. Even in the absence of transmission precautions, the rate of secondary cases is exceptionally low within the UK.[18]

The participants will consent to the clinical study team informing the local Health Protection Unit of their involvement in the study. The Unit will be notified of their challenge date and

when stool clearance has been completed. Any breach in enteric hygiene precautions that result in another individual coming into contact with infectious material will be reported, with potential cases of transmission to be confirmed by sequence comparison to an isolate of the challenge strain stored at a Public Health England microbiology reference laboratory (Colindale, London).

Sample size

The selected sample size balances the need for a statistically reproducible attack rate while minimising the number of individuals exposed to paratyphoid infection. To meet the primary objective of a clinically reproducible attack rate of 60 to 75%, this careful, dose (de-) escalation protocol will be followed.[5] The maximum number of participants required will be 80, with the minimum 20 if the starting dose (1-5x10^3 CFU) satisfies our criteria. This is based on the probability that the criteria are satisfied according to true attack rate.

If the attack rate in the first group of 20 participants is greater than 75%, a lower dose will be decided based on the prior attack rate combined with laboratory and clinical findings. De-escalation to a dose lower than 1-5x10^3 CFU will also be considered if the target attack rate is reached and the chief investigator, with agreement from the DSMC, decides that a lower dose may achieve a similar attack rate.

Statistical analysis

The analysis of the primary endpoint will be descriptive only. The percentage of participants who meet the criteria for diagnosis of paratyphoid will be calculated with a 95% Clopper-Pearson Exact confidence interval. Those individuals who withdraw or are treated prior to Day 14 without prior diagnosis of paratyphoid would be excluded from this analysis. A secondary analysis of the primary endpoint will be conducted using the Kaplan-Meier method which will include all participants.

Time-to-event analyses of individual components of the primary outcome (e.g. positive blood culture for *S.* Paratyphi, oral temperature ≥ 38.0°C etc.) will be conducted using the

Kaplan-Meier method and will include all participants. Participants not meeting the criteria for an individual component of the primary endpoint will be censored in the analysis at the time of diagnosis or at Day 14 for those undiagnosed.

DISCUSSION

This will be the first *S*. Paratyphi A human challenge model developed. It is presumed that this study will be similar to the experience of recent typhoid challenge studies based on literature iterating the similar clinical presentation between typhoid and paratyphoid fever.[5,19,20] Clinical knowledge however of the NVGH308 paratyphoid A strain is limited to details from the original patient, in contrast to the *S*. Typhi Quailes strain where data from 762 challenged participants from the 1960s informed the re-establishment of a typhoid challenge model in 2009.[21] While the lowest infective inoculum of *S*. Paratyphi A is unknown, it is believed to be similar or higher than *S*. Typhi.[22,23] As such, we will use the same starting dose of 10^3 CFU that was used in the 2009 typhoid challenge model. This dose was the highest dose of *S*. Typhi that did not cause clinical infection in the historical typhoid challenge studies.[24] When co-administered with sodium bicarbonate solution, 10^3 CFU *S*. Typhi (Quailes strain) gave an attack rate of 55%, necessitating a dose escalation to reach the target attack rate of 60-75%.[5] We anticipate the same will occur with *S*. Paratyphi A challenge.

The outpatient management of participants challenged with typhoid is safe and achievable; this has been key in re-establishing the challenge model due to the prohibitive cost of in-patient care.[5] Participant satisfaction with this model, plus the monetary reimbursement for their time, travel expenses, blood draws, and potential days off work, is high.[25]

The modest number of participants in challenge studies can make the model sensitive to individual variation. Selecting an antigen-naïve cohort limits this variation, but the response may not reflect the target population whom may have protection due to repeated natural exposure, with consequent overestimation of the potential efficacy of a candidate vaccine.[26] Conversely, the challenge dose required to achieve a sufficiently high attack

rate within a manageable two week period is likely to be higher than encountered in the field. This may overwhelm candidate vaccines with erroneously discouraging protective efficacy, as was seen in the Maryland typhoid studies.[27]

The NVGH308 strain, while originally a clinical isolate, may not be representative of the current circulating strains in endemic settings. *S.* Paratyphi A however is a clonal monomorphic pathogen containing limited genomic variation,[28,29] making it likely that the pathogenicity and immune response to NVGH308 *S.* Paratyphi A challenge will translate to wild-type strains and that future vaccines will provide cross-strain protection.

Current promising candidate *S.* Paratyphi vaccines are based on whole cell live attenuated strains or subunit approaches that conjugate O polysaccharide (O:2) to a range of protein carriers. This O:2 polysaccharide antigen of *S.* Paratyphi A is known to play a role in protection and virulence.[30] A phase II trial is underway of O:2 conjugated to tetanus toxoid (O:2-TT), conducted after initial trials showed that it was safe and immunogenic.[31] A second conjugate vaccine moving into clinical testing is O:2 conjugated to CRM $_{197}$ (O:2-CRM_{197}) which demonstrated immunogenicity in pre-clinical studies with strong bactericidal activity against *S.* Paratyphi A when developed alone or in combination with Vi-CRM_{197}.[32] A live-attenuated oral vaccine candidate (CVD 1901, University of Maryland) has also been shown to be well tolerated and immunogenic in phase I trials and further phase I studies are ongoing.[33]

Though promising vaccines are in development, it is a long and costly process for any vaccine to get to licensure. The lack of a reliable correlate of protection and the poorly understood immunobiology of typhoid and paratyphoid infection adds to the difficulties in enteric fever vaccine development. Equally, highly sensitive and specific diagnostic tests for use in endemic settings are needed but their development and, particularly, validation has been hindered by the lack of a patient cohort immunologically naïve to typhoidal salmonella. Advancing knowledge on the microbiological and human-host response to exposure is necessary to inform transmission and impact modelling for vaccine roll-out, key for targeted vaccination programmes of high risk population groups.

The development of an *S*. Paratyphi A human challenge model could help overcome some of these limitations. As trials for paratyphoid and bivalent vaccine candidates are approaching Phase I/II stages, a paratyphoid challenge model could provide a crucial intermediate step in progressing efficacious vaccine candidates into more expansive field trials in endemic settings. The controlled and highly monitored nature of challenge studies provides a unique opportunity to further the understanding of disease pathogenesis in immunologically-naïve individuals and allows a comprehensive assessment of the human response to infection and evaluation of vaccine efficacy. Ideally, this will be translated into rapid, cost-effective diagnostics, contributing to the disease surveillance necessary for vaccination programs. Future measures to control enteric fever are expected to combine an effective bivalent vaccine against both serovars with public health measures that improve sanitation and access to clean water.

FOOTNOTES

Authors' contributions DM, HCD and TD drafted the manuscript. AP conceived the study design. All authors contributed to the development of the study protocol and have read and approved the final manuscript.

Competing interests LBM works for Novartis Vaccines Institute for Global Health which has partnered with Biological E (Hyderabad, India) for development of a bivalent typhoid and paratyphoid vaccine. All other authors declare that they have no competing interests.

Study status and ethics approval At the time of submission this study has been approved by the Oxford Research Ethics A Committee (Ref: 14/SC/0004) and patient recruitment is ongoing. Trust management Approval and Indemnity from the Oxford University Research and Development Office has been confirmed (Ref: 10602).

Patient consent Obtained.

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Sponsor This study is sponsored by the University of Oxford, UK.

Provenance and peer review Not commissioned; peer reviewed funding application and ethical approval.

Data sharing statement The results of this original research will be disseminated in a peer-reviewed journal and presented at international congresses.

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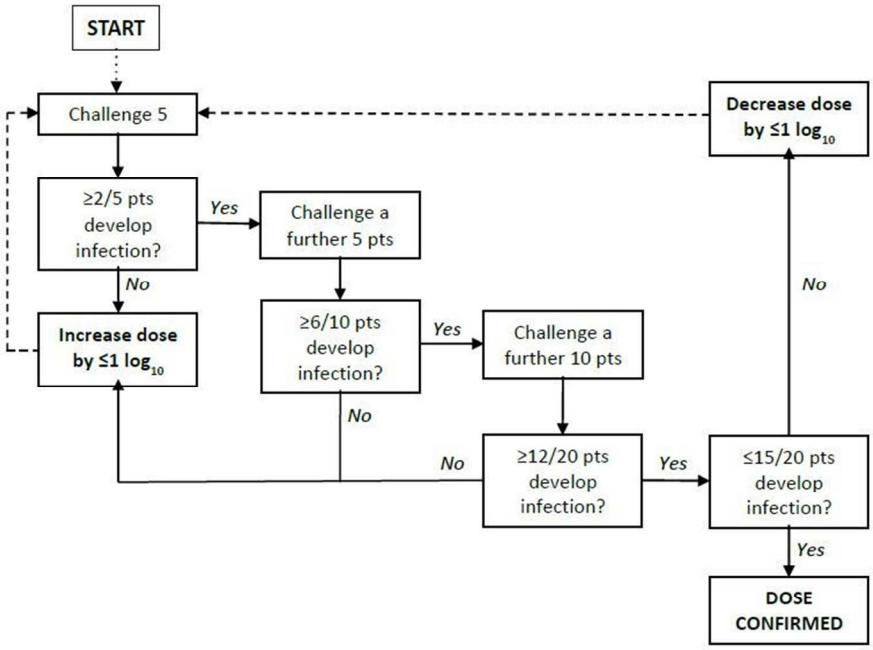
Figure 1. Decision making algorithm for *S*. Paratyphi dose escalation/de-escalation, starting at 1-5 x10^3 to reach the primary endpoint.

Figure 2. Participant journey through the study

Supplementary 1. Eligibility Criteria.

Supplementary 2. Summary of study procedures



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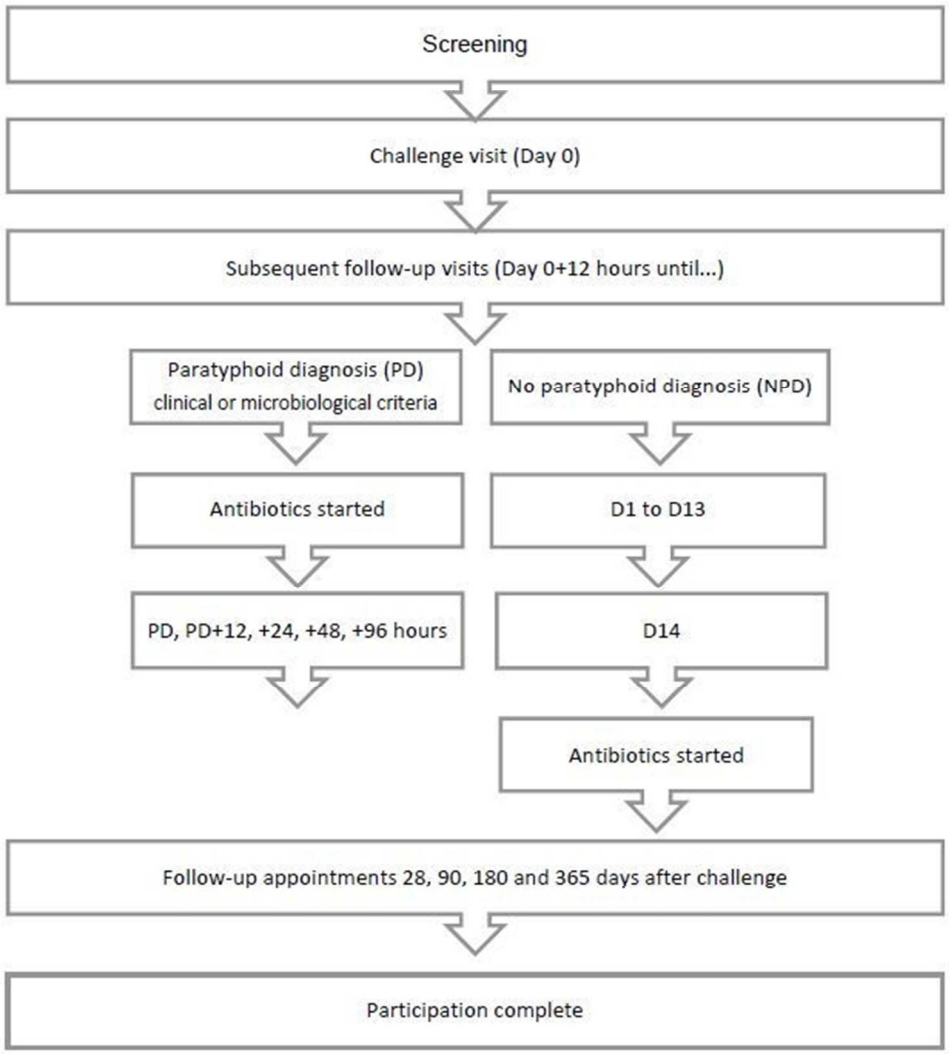

171x187mm (96 x 96 DPI)

Inclusion Criteria
Agree to give informed consent for participation in the study
Aged between 18-60 years inclusive at time of challenge
In good health as determined by medical history, physical examination and clinical judgment of the investigators
Agree (in the Investigator's opinion) to comply with all study requirements, including capacity to adhere to good personal hygiene and infection control precautions
Agree to allow his or her General Practitioner (and/or Consultant if appropriate), to be notified of participation in the study
Agree to allow Public Health England to be informed of their participation in the study
For those involved in provision of health or social care to vulnerable groups only – willing to allow his or her employer to be notified of participation in the study
Agree to give his or her close household contacts written information informing them of the participants' involvement in the study and offering them voluntary screening for *S.* Paratyphi carriage
Agree to have 24-hour contact with study staff during the four weeks post challenge and are able to ensure that they are contactable by mobile phone for the duration of the challenge period until antibiotic completion
Have internet access to allow completion of the eDiary and real-time safety monitoring
Agree to avoid antipyretic/anti-inflammatory treatment until advised by a study doctor or until 14 days after challenge
Agree to provide their National Insurance/Passport number for the purposes of TOPS registration and for payment of reimbursement expenses

Exclusion Criteria
History of significant organ/system disease that could interfere with study conduct or completion. Including, for example, but not restricted to:
Cardiovascular disease
Respiratory disease
Hematological disease
Endocrine disorders
Renal or bladder disease, including history of renal calculi
Biliary tract disease, including biliary colic, asymptomatic gallstones or previous cholecystectomy
Gastro-intestinal disease including requirement for antacids, H_2-receptor antagonists, proton pump inhibitors or laxatives
Neurological disease
Metabolic disease

Autoimmune disease Psychiatric illness requiring hospitalization or known or suspected drug and/or alcohol misuse (alcohol misuse defined as an intake exceeding 42 units per week) Infectious disease
Have any known or suspected impairment of immune function, alteration of immune function, or prior immune exposure that may alter immune function to paratyphoid resulting from, for example: Congenital or acquired immunodeficiency, including IgA deficiency Human Immunodeficiency Virus infection or symptoms/signs suggestive of an HIV-associated condition Receipt of immunosuppressive therapy such as anti-cancer chemotherapy or radiation therapy within the preceding 12 months or long-term systemic corticosteroid therapy Receipt of immunoglobulin or any blood product transfusion within 3 months of study start History of cancer (except squamous cell or basal cell carcinoma of the skin and cervical carcinoma in situ)
Moderate or severe depression or anxiety as classified by the Hospital Anxiety and Depression Score at screening or challenge that is deemed clinically significant by the study investigators
Weight less than 50kg
Presence of implants or prosthesis
Anyone taking long-term medication (e.g. analgesia, anti-inflammatories or antibiotics) that may affect symptom reporting or interpretation of the study results
Contra-indication to ciprofloxacin, azithromycin, trimethoprim/sulfamethoxazole and/or beta lactam antibiotics
Female participants who are pregnant, lactating or who are unwilling to ensure that they or their partner use effective contraception one month prior to challenge and continue to do so until two negative stool samples, a minimum of 2 weeks after completion of antibiotic treatment, has been obtained
Full-time, part-time or voluntary occupations involving: Clinical or social work with direct contact with young children (defined as those attending pre-school groups or nursery or aged under 2 years), or Clinical or social work with direct contact with highly susceptible patients or persons in whom typhoid infection would have particularly serious consequences (unless willing not to work until demonstrated to not be infected with *S.* Paratyphi in accordance with guidance from Public Health England)
Full-time, part-time or voluntary occupations involving commercial food handling (involving preparing or serving unwrapped foods not subjected to further heating)

Close household contact with: Young children (defined as those attending pre-school groups, nursery or those aged less than 2 years) Individual(s) who is (are) immunocompromised
Scheduled elective surgery or other procedures requiring general anaesthesia during the study period
Participants who have participated in another research study involving an investigational product that might affect risk of paratyphoid infection or compromise the integrity of the study within the 30 days prior to enrolment (e.g. significant volumes of blood already taken in previous study)
Detection of any abnormal results from screening investigations (at the clinical discretion of the study investigators)
Inability to comply with any of the study requirements (at the discretion of the study investigators and the participants General Practitioner)
Any other social, psychological or health issues which, in the opinion of the study investigator, may; put the participants or their contacts at risk because of participation in the study, adversely affect the interpretation of the primary endpoint data, or impair the participant's ability to participate in the study
Having previously received any live oral typhoid vaccine (those who have received Vi polysaccharide vaccine will not be excluded)
Having been resident in an enteric fever endemic country 6 months or more
Have previously been diagnosed with laboratory-confirmed typhoid or paratyphoid infection or been given a diagnosis compatible with enteric fever
Have participated in previous typhoid challenge studies (with ingestion of challenge agent)
Currently working for the Oxford Vaccine Group

		Intensive challenge period (14 days)						Diagnosed with paratyphoid			Follow-up visits	
	screening	D0	+12H	D1, D2, D3, D4, D5, D6	D7	D8, D9, D10, D11, D12,D13	D14	PD	+12H	+24,+48, +72,+96H	D28	D90, 180, 365
Informed consent	x											
Revalidation of consent		x										
Obtain 24 hr contact details		x										
Medical history	x											
Interim medical history		x	x	x	x	x	x	x	x	x	x	x
Physical examination	x	x						x				
Vital signs	x	x	x	x	x	x	x	x	x	x	x	
Urine pregnancy test	x	x					x	x				
Urine sample	x	x		x	x	x	x	x		x		x
Saliva sample		x		x	x	x	x	x		x		x
Stool sample		x		x	x	x	x	x		x		x
Blood sample	x	x	x	x	x	x	x	x	x	x	x	x
12 lead ECG	x											
Ultrasound scan	x											
Mood assessment	x	x			x		x	x				
Challenge with *S.* Paratyphi		x										
Issue study pack	x	x										
eDiary entries*				x	x	x	x	x	x	x	x	
Commence antibiotics							x	x				
Notification of public health and GP**		x										
Letter informing close contacts	x						x					
Letter to close contacts offering screening											x	

*eDiary will be open from day 0-21 for temperature and symptom recordings.
**The local Public Health England Unit and the participant's GP will be contacted on challenge and on completion of stool clearance samples. GPs will receive an additional letter when a participant has started antibiotic treatment.

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Understanding Paratyphoid Infection: Study Protocol for the Development of a Human Model of Salmonella enterica serovar Paratyphi A challenge in Healthy Adult Volunteers.

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Primary Subject Heading:	Infectious diseases
Secondary Subject Heading:	Immunology (including allergy), Infectious diseases, Public health, Research methods
Keywords:	MICROBIOLOGY, BACTERIOLOGY, IMMUNOLOGY, INFECTIOUS DISEASES, Tropical medicine < INFECTIOUS DISEASES

UNDERSTANDING PARATYPHOID INFECTION

Study Protocol for the Development of a Human Model of *Salmonella enterica* serovar Paratyphi A challenge in Healthy Adult Volunteers.

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Word count: 4368

ABSTRACT

Introduction: This study will develop the first human challenge model of paratyphoid infection which may then be taken forward to evaluate paratyphoid vaccine candidates. *Salmonella* Paratyphi A is believed to cause a quarter of the estimated 20 million cases of enteric fever annually. Epidemiological evidence also suggests that an increasing proportion of the enteric fever burden is attributable to *S.* Paratyphi infection meriting further attention and interest in vaccine development. Assessment of paratyphoid vaccine efficacy in preclinical studies is complicated by the lack of a small animal model and the human-restricted nature of the infection. The use of experimental human infection in healthy volunteers provides an opportunity to address these problems in a cost-effective manner.

Methods and analysis: Volunteers will ingest virulent *S.* Paratyphi A bacteria (NVGH308 strain) with a bicarbonate buffer solution, to establish the infectious dose resulting in an "attack rate" of 60 – 75%. Using an *a priori* decision-making algorithm, the challenge dose will be escalated or de-escalated to achieve the target attack rate, with the aim of reaching the study endpoint while exposing as few individuals as possible to infection. The attack rate will be determined by the proportion of paratyphoid infection in groups of 20 healthy adult volunteers, with infection being defined by one or more positive blood cultures (microbiological endpoint), and/or fever, defined as an oral temperature exceeding 38°C sustained for at least 12 hours (clinical endpoint); 20 to 80 participants will be required. Challenge participants will commence a two week course of an oral antibiotic upon diagnosis of infection, or after 14 days follow-up.

Ethics and dissemination: The strict eligibility criterion aims to minimise risk to participants and their close contacts. Ethical approval has been obtained. The results will be disseminated in a peer-reviewed journal and presented at international congresses.

Trial Registration: Clinicaltrials.gov NCT02100397

Strengths:

- The development of a *S*. Paratyphi human challenge model should expedite the development and evaluation of potential paratyphoid vaccine candidates, in particular by allowing the direct measurement of vaccine protective efficacy in a safe, reproducible host-relevant model.
- Studying the longitudinal physiological and immunological responses to infection will provide insight into this increasingly prevalent but poorly understood infection.

Limitations:

- The modest number of participants in challenge studies can make the model sensitive to individual variation.
- The response to challenge may not reflect the target population whom may have protection due to repeated natural exposure.
- The immuno-biological response to *S*. Paratyphi A exposure may differ between strains.

INTRODUCTION

Enteric fever is the term used to describe systemic illness caused by infection with *Salmonella enterica* serovars Typhi and Paratyphi A and C. Enteric fever is a leading cause of morbidity worldwide, particularly amongst young, school-aged children in resource-limited settings and increasingly among travellers to those areas.[1,2]

While infection with *S.* Typhi accounts for the majority of enteric fever cases, the proportion of disease caused by *S.* Paratyphi A is increasing, particularly in the highly endemic regions of South East Asia and the Indian sub-continent.[3] As a human restricted pathogen, targeted vaccination of those groups at high risk is likely to have a substantial impact on disease incidence.[4]

In view of the high burden of enteric fever and increasing antibiotic resistance, The World Health Organisation (WHO) has stated that countries with high-risk groups and populations 'should consider the programmatic use of typhoid vaccines for controlling endemic disease.[4]' Despite this recommendation, there has been a reluctance to introduce programmes with the available licenced typhoid vaccines, Vi polysaccharide (ViPS) and Ty21a, as both have limited efficacy, offer minimal cross-protection against paratyphoid infection, and cannot be given to children less than two years of age. This has focused development on firstly conjugate *S.* Typhi and Paratyphi A vaccines, which can be administered to infants and expected to provide long-lasting immune memory, as well as attenuated oral vaccines.[5,6] A number of promising candidate vaccines are in early phase testing.[5–8]

The aim of this study is to develop a safe, reliable human paratyphoid challenge model in which to validate vaccines and develop novel diagnostics. Human challenge studies with *S.* Typhi (Quailes strain) have been performed historically and more recently using an adapted model.[9–11] To our knowledge, human challenge with virulent *S.* Paratyphi has not been performed before so this study will provide a unique opportunity to study human paratyphoid infection. The study is designed to determine the dose of *S.* Paratyphi A

(challenge strain NVGH308) required to produce an infection attack rate of 60-75% in healthy adult volunteers who have not previously been exposed to typhoidal salmonella and therefore immunologically naïve to the challenge agent. With sufficient evidence of paratyphoid infection at a target dose, this model can then be taken forward to evaluate paratyphoid vaccine candidates.

METHODS/DESIGN

This is a descriptive, dose level escalation human infection study using *S.* Paratyphi A challenge of ambulatory, out-patient healthy community adult volunteers. For safety, the study will commence with the challenge of one individual at the initial dose of 1-5 x10^3 CFU. Consecutive groups of five or 10 participants will then be challenged a minimum of two weeks apart following a decision-making algorithm for dose escalation/de-escalation (see Figure 1). This algorithm was used for the typhoid challenge model developed by the University of Oxford in 2009.[9]

Regulation and governance

In the UK investigational products, such as unlicensed medications and vaccines, are regulated by the Medicines for Human Use (Clinical Trials) Regulations 2004 which implement the European Clinical Trials Directive (2001/20/EC).[12] However most microbial challenge studies fall outside of the remit of the ECTD and are instead judged according to common law and best practice.[13] The typhoid and paratyphoid challenge models, using a fully characterised and non-genetically modified strain, falls under this category. To achieve best practice, challenge protocols undergo independent and rigorous peer review to assess the scientific quality and appropriateness of the study methodology to answer the key objectives. This includes conforming to Good Clinical Practice (GCP) guidelines with the support from a research ethics committee experienced in these studies.[14] As such, local or regional ethic committees, independent from the researchers and sponsors, are fundamental for research governance of microbial challenge studies.

Challenge strain

The original *S.* Paratyphi A strain NVGH308 was isolated in 2006 from a patient with acute paratyphoid fever as part of a study performed by the Oxford University Clinical Research Unit at Patan Hospital, Kathmandu, Nepal. It has been manufactured to Good Manufacturing Practice (GMP) standard and is supplied for study purposes by Novartis Vaccines for Global Health, Italy. Manufacturing to GMP standard, while not a regulatory requirement in the UK, fulfils best practice.[13]

A parent seed lot, S888P5SP01, was established in March 2010 after serial colony selections on Luria Broth PTK agar plates and stored in the Novartis Vaccines and Diagnostics bacterial seed bank (Siena, Italy). This seed lot underwent five sequential passages as part of the cell line cleaning process before being used to establish the GMP Master Cell Bank, SA-13-002, of the NVGH308 strain. GenIbet BioPharmaceuticals (Oeiras, Portugal) produced three dose levels of the challenge agent under GMP conditions. Prepared vials containing the challenge agent were stored at -80°C ± 5°C and transferred via an accredited courier to the Oxford Vaccine Group Laboratory in 2013.

Batch testing of the cell bank confirms O:1 and O:2 antigen positivity, batch purity and stable viable bacterial count. Ongoing stability testing of the seed lot has been performed by Novartis Vaccines and Diagnostics. The NVGH308 strain is fully characterised with known susceptibility to a number of antimicrobial options.

Study Objectives

The primary objective is to determine the dose (in colony forming units (CFU)) of *S.* Paratyphi A, challenge strain NVGH308, needed to produce a 60-75% attack rate when ingested with sodium bicarbonate solution by healthy adult volunteers. The secondary objectives will describe the interaction between bacteria and human host at baseline, through inoculation and symptomatic infection (or asymptomatic immune protection) to recovery and long term follow-up (see Table 1).

	Objective(s)	Outcome/Endpoint(s)
Primary	To determine the dose (in colony forming units) of *Salmonella* Paratyphi A, challenge strain NVGH308, needed to produce a 60% to 75% attack rate when ingested with sodium bicarbonate solution, in healthy adult volunteers.	Clinical or microbiologically proven paratyphoid infection following oral challenge with *Salmonella* Paratyphi A, strain NVGH308, delivered with sodium bicarbonate solution.
Secondary	i. To describe the human physiological response to *Salmonella* Paratyphi A challenge, and in those developing or not developing infection.	Description of the clinical course after challenge using, for example, participant symptom profiles, temperature measurements and other recorded clinical and laboratory observations.
	ii. To evaluate the sensitivity of the pre-defined criteria for paratyphoid infection, using subsequent clinical, microbiological and laboratory outcomes.	Determination of challenge dose/kg (dose/surface area) actually ingested by those developing and those not developing paratyphoid infection at each dose level. Analysis of the attack rate using alternative criteria including, for example, passive field surveillance definitions, alternative temperature thresholds and adjunctive microbiological and laboratory diagnostic assays.
	iii. To describe the characteristics of bacterial dynamics after challenge, including onset and duration of bacteraemia, bacterial burden at diagnosis and stool shedding.	Microbiological assays to detect and characterise Salmonella Paratyphi after challenge in blood, stool and urine.
	iv. To describe the human immune response to challenge, including the innate, humoral, cell-mediated and mucosal responses.	Immunological laboratory assays to measure innate, humoral, cell-mediated and mucosal responses to challenge.

	v. To determine genetic features affecting host-pathogen responses, alteration of those responses through epigenetic changes, control of gene expression and post-translational modifications.	Laboratory and high-throughput assays to measure genetic factors affecting susceptibility, gene expression and protein translation.
	vi. To discover, develop and evaluate novel diagnostic methods for *Salmonella* Paratyphi A infection.	Exploratory analysis of blood, faeces, saliva and urine samples using experimental assays and diagnostics.
	vii. To explore the factors, influences and motivation affecting volunteer's decision to participate in human challenge studies and their experiences of the study process.	Participant responses using questionnaires during the course of the study.

Table 1. Study objectives and outcomes

Feedback and recommendations from Patient and Public Involvement (PPI) in addition to participant questionnaires from previous studies have been incorporated into this study. This beneficial process will continue with an anonymous questionnaire to be completed by participants 28 days after challenge.

The questionnaire will be closely based on one used by the Oxford Vaccine Group (OVG) in previous typhoid studies to explore participants' study experience. This includes motivations, attitudes and factors influencing participation in human challenge research, as well as their experience of study procedures such as ingesting the challenge agent.

Study setting

The study will be conducted at the Centre for Clinical Vaccinology and Tropical Medicine, Oxford, UK, which is a fully equipped vaccine research site with available clinical inpatient facilities and a Category III level laboratory on-site. The UK is non-endemic for enteric fever with the majority of cases travel-related; the rate of paratyphoid fever notification in the Thames Valley region, which includes Oxfordshire, is 0.5/100,000.[15]

Recruitment

Several strategies may be employed in order to recruit the required cohort of volunteers. These include:

- Study invitation letters with reply slip, sent out by the National Health Applications and Infrastructure Services who hold the central National Health Services (NHS) patient database (Open Exeter),
- Website and poster advertising,
- direct mail-out using the Electoral register,
- E-mail communication to local tertiary education facilities.

The Oxford Vaccine Centre also manages a secure database for healthy volunteers who have expressed an interest in being contacted about potential studies. Potential participants will be invited for a screening and consent visit, where a member of the clinical team will assess their eligibility.

Participants will be offered reimbursement for their time, travel and inconvenience. The amount, frequency and method of payment will be described in the study information booklet. The payment schedule will hopefully encourage attendance of follow-up appointments.

Eligibility criteria

Male or female participants aged 18-60 years inclusive who are in good health (as determined by a study doctor, medical investigation and agreement by their general practitioner) and able to provide written informed consent will be eligible for inclusion in this study. Additional inclusion and exclusion criteria will be applied to ensure participants are appropriate for the study (supplementary 1). This includes a participant's ability to attend daily appointments for two weeks after challenge with *S*. Paratyphi A. These strict criteria aim to minimise the risk of severe or complicated disease in participants and reduce potential risk of transmission to close contacts.

Interventions

A participant will be considered enrolled in the study on their day of challenge.

The method used for preparation of the challenge inocula is based on the one used for the recent typhoid challenge model.[9] Participants will fast for 90 minutes before ingesting 120ml of sodium bicarbonate (2.1g $NaHCO_3$) to neutralise stomach acid. This is followed 60 seconds later by the challenge inocula which will be freshly prepared prior to each challenge by defrosting and suspending the required bacterial dose in 30mls of sodium bicarbonate (0.53g $NaHCO_3$).

After *S*. Paratyphi A ingestion, participants will be seen daily for 14 days with blood, stool, saliva and urine samples taken at set time points (Supplementary 2). Monitoring for derangement of liver, renal, blood count parameters and inflammatory markers will be performed. Participants will also complete twice-daily temperature readings and record any symptoms experienced for 21 days; this data will be collected by electronic CRFs and diary cards. Subsequent follow-up appointments will be 28, 90, 180 and 365 days after challenge (Figure 2).

Paratyphoid infection will be diagnosed after challenge if one of the following applies:

- a positive blood culture for *S*. Paratyphi from 72 hours after challenge,
- a positive blood culture for *S*. Paratyphi within 72 hours, with one or more signs/symptoms of paratyphoid infection,
- persistent positive blood cultures for *S*. Paratyphi within 72 hours, or
- an oral temperature ≥38˚C persisting for 12 hours .

The earliest microbiological indication that a participant has a *S.* Paratyphi A bacteraemia will be the identification of a Gram-negative bacillus (GNB) from a positive blood culture. As formal identification of the organism may take a minimum of a further 24 hours, participants in whom a GNB is identified will be defined as having paratyphoid fever.

Ciprofloxacin 500mg twice daily for 14 days will commence on diagnosis of paratyphoid infection or at Day 14 after challenge. The rationale for using fluoroquinolones first-line is based on recommendations for treatment of enteric fever and prevention (and treatment) of chronic gallbladder carriage as therapeutic levels are reached in bile and gallbladder.[16,17] All positive blood culture isolates will have full susceptibility profiling performed using an antibiotic disc method and *E*-test to measure the ciprofloxacin MIC. Alternative antibiotics used as second-line therapy (in the event of adverse reactions or side-effects) include azithromycin and trimethoprim/sulfamethoxazole.

If the participant's symptoms fail to resolve after antibiotic administration, if they are unable to tolerate oral antibiotics, become dehydrated, or if unanticipated concerns regarding home circumstances emerge, inpatient admission to an infectious diseases unit will be considered. Patient care at this stage would be delegated to the hospital clinical team, which could include the provision of intravenous fluids, antibiotics and anti-emetics.

Safety

Participant safety during the study: Participants will be monitored closely with daily clinical review and completion of symptom diary cards. All adverse events will be recorded on case report forms (CRFs), with serious events notified to the Data Safety Monitoring Committee (DSMC) within 24 hours of the investigator becoming aware of the event. Adverse events of special interest (AESI) will also be reported to the DSMC in the same manner and include:

- complications of paratyphoid fever such as perforation or haemorrhage which occurs almost exclusively in patients who are untreated for an extended period,[18]
- failure to clinically or bacteriologically cure a participant of acute paratyphoid infection within 14 days of antibiotic therapy,
- relapse or progression to carrier state, the latter defined as a person who is still excreting *S*. Paratyphi A after two courses of appropriate antibiotic therapy,[19]
- transmission of S. Paratyphi to non-study participants.

The DSMC for this study will be composed of an independent and trial-experienced group of infectious disease and public health clinicians and a statistician. Safety data collated from eCRFs and eDiaries which include blood parameters, vital signs, and symptom recordings, will be reviewed by the DSMC after the first participant has been challenged and at each dose escalation. Approval from the DSMC will be required prior to any subsequent dose alterations. This role and function of the DSMC will be described in a Charter, agreed prior to the start of recruitment.

Long term safety of participants: The risk of chronic carriage with *S.* Paratyphi A is minimised by treatment for two weeks with an effective antibiotic and excluding participants with gallbladder disease.[20] In addition, stool samples for culture will be obtained two weeks after completion of the antibiotic course and then weekly until two successive samples are negative. If samples remain positive for *S.* Paratyphi A four weeks after completion of antibiotics then the participant will be referred to a National Health Service infectious diseases consultant for further management.

Safety of non-study participants: The risk of secondary transmission to close contacts is unlikely in view of the low infectivity of *S.* Paratyphi A and the level of hygiene and sanitation in the UK.[21] Consent will not be obtained from close household contacts, but participants will be required to provide them with a written study summary detailing measures to reduce the risk of infection and offering screening for paratyphoid infection. Even in the absence of transmission precautions, the rate of secondary cases is exceptionally low within the UK.[22]

The participants will consent to the clinical study team informing the local Health Protection Unit of their involvement in the study. The Unit will be notified of their challenge date and when stool clearance has been completed. Any breach in enteric hygiene precautions that result in another individual coming into contact with infectious material will be reported, with potential cases of transmission to be confirmed by sequence comparison to an isolate of the challenge strain stored at a Public Health England microbiology reference laboratory (Colindale, London).

Sample size

The selected sample size balances the need for a statistically reproducible attack rate while minimising the number of individuals exposed to paratyphoid infection. To meet the primary objective of a clinically reproducible attack rate of 60 to 75%, this careful, dose (de-) escalation protocol will be followed.[9] The maximum number of participants required will be 80, with the minimum 20 if the starting dose (1-5x10^3 CFU) satisfies our criteria. This is based on the probability that the criteria are satisfied according to true attack rate.

If the attack rate in the first group of 20 participants is greater than 75%, a lower dose will be decided based on the prior attack rate combined with laboratory and clinical findings. De-escalation to a dose lower than 1-5x10^3 CFU will also be considered if the target attack rate is reached and the chief investigator, with agreement from the DSMC, decides that a lower dose may achieve a similar attack rate.

Statistical analysis

The analysis of the primary endpoint will be descriptive only. The percentage of participants who meet the criteria for diagnosis of paratyphoid will be calculated with a 95% Clopper-Pearson Exact confidence interval. Those individuals who withdraw or are treated prior to Day 14 without prior diagnosis of paratyphoid would be excluded from this analysis. A secondary analysis of the primary endpoint will be conducted using the Kaplan-Meier method which will include all participants.

Time-to-event analyses of individual components of the primary outcome (e.g. positive blood culture for *S.* Paratyphi, oral temperature ≥ 38.0°C etc.) will be conducted using the Kaplan-Meier method and will include all participants. Participants not meeting the criteria for an individual component of the primary endpoint will be censored in the analysis at the time of diagnosis or at Day 14 for those undiagnosed.

DISCUSSION

This will be the first *S*. Paratyphi A human challenge model developed. It is presumed that this study will be similar to the experience of recent typhoid challenge studies based on literature iterating the similar clinical presentation between typhoid and paratyphoid fever.[9,23,24] Clinical knowledge however of the NVGH308 paratyphoid A strain is limited to details from the original patient, in contrast to the *S*. Typhi Quailes strain where data from 762 challenged participants from the 1960s informed the re-establishment of a typhoid challenge model in 2009.[25] While the lowest infective inoculum of *S*. Paratyphi A is unknown, it is believed to be similar or higher than *S*. Typhi.[26,27] As such, we will use the same starting dose of 10^3 CFU that was used in the 2009 typhoid challenge model. This dose was the highest dose of *S*. Typhi that did not cause clinical infection in the historical typhoid challenge studies.[28] When co-administered with sodium bicarbonate solution, 10^3 CFU *S*. Typhi (Quailes strain) gave an attack rate of 55%, necessitating a dose escalation to reach the target attack rate of 60-75%.[9] We anticipate the same will occur with *S*. Paratyphi A challenge.

The outpatient management of participants challenged with typhoid is safe and achievable; this has been key in re-establishing the challenge model due to the prohibitive cost of in-patient care.[9] Participant satisfaction with this model, plus the monetary reimbursement for their time, travel expenses, blood draws, and potential days off work, is high.[29]

The modest number of participants in challenge studies can make the model sensitive to individual variation. Selecting an antigen-naïve cohort limits this variation, but the response may not reflect the target population whom may have protection due to repeated natural exposure, with consequent overestimation of the potential efficacy of a candidate vaccine.[30] Conversely, the challenge dose required to achieve a sufficiently high attack rate within a manageable two week period is likely to be higher than encountered in the field. This may overwhelm candidate vaccines with erroneously discouraging protective efficacy, as was seen in the Maryland typhoid studies.[31]

The NVGH308 strain, while originally a clinical isolate, may not be representative of the current circulating strains in endemic settings. *S.* Paratyphi A however is a clonal monomorphic pathogen containing limited genomic variation,[32,33] making it likely that the pathogenicity and immune response to NVGH308 *S.* Paratyphi A challenge will translate to wild-type strains and that future vaccines will provide cross-strain protection.

Current promising candidate *S.* Paratyphi vaccines are based on whole cell live attenuated strains or subunit approaches that conjugate O polysaccharide (O:2) to a range of protein carriers. This O:2 polysaccharide antigen of *S.* Paratyphi A is known to play a role in protection and virulence.[34] A phase II trial is underway of O:2 conjugated to tetanus toxoid (O:2-TT), conducted after initial trials showed that it was safe and immunogenic.[5] A second conjugate vaccine moving into clinical testing is O:2 conjugated to CRM $_{197}$ (O:2-CRM_{197}) which demonstrated immunogenicity in pre-clinical studies with strong bactericidal activity against *S.* Paratyphi A when developed alone or in combination with Vi-CRM_{197}.[7] A live-attenuated oral vaccine candidate (CVD 1901, University of Maryland) has also been shown to be well tolerated and immunogenic in phase I trials and further phase I studies are ongoing.[8]

Though promising vaccines are in development, it is a long and costly process for any vaccine to get to licensure. The lack of a reliable correlate of protection and the poorly understood immunobiology of typhoid and paratyphoid infection adds to the difficulties in enteric fever vaccine development. Equally, highly sensitive and specific diagnostic tests for use in endemic settings are needed but their development and, particularly, validation has been hindered by the lack of a patient cohort immunologically naïve to typhoidal salmonella. Advancing knowledge on the microbiological and human-host response to exposure is necessary to inform transmission and impact modelling for vaccine roll-out, key for targeted vaccination programmes of high risk population groups.

The development of an *S.* Paratyphi A human challenge model could help overcome some of these limitations. As trials for paratyphoid and bivalent vaccine candidates are approaching Phase I/II stages, a paratyphoid challenge model could provide a crucial intermediate step in

progressing efficacious vaccine candidates into more expansive field trials in endemic settings. Ideally, this will be translated into rapid, cost-effective diagnostics, contributing to the disease surveillance necessary for vaccination programs. Future measures to control enteric fever are expected to combine an effective bivalent vaccine against both serovars with public health measures that improve sanitation and access to clean water.

FOOTNOTES

Authors' contributions DM, HCD and TD drafted the manuscript. AP conceived the study design. All authors contributed to the development of the study protocol and have read and approved the final manuscript.

Competing interests LBM works for Novartis Vaccines Institute for Global Health which has partnered with Biological E (Hyderabad, India) for development of a bivalent typhoid and paratyphoid vaccine. All other authors declare that they have no competing interests.

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Data sharing The results of this research will be disseminated in peer-reviewed, open access journals and presented at relevant international congresses. Published articles will also be distributed to study participants by mail. Access to data will be granted to authorised monitors to ensure compliance with regulations and anonymised data may be requested by the Bill and Melinda Gates Foundation. Within OVG, data may be accessed and used for future academic research.

Figure 1. Decision making algorithm for *S.* Paratyphi dose escalation/de-escalation, starting at 1-5 x10^3 to reach the primary endpoint.

Figure 2. Participant journey through the study

Supplementary 1. Eligibility Criteria.

Supplementary 2. Summary of study procedures

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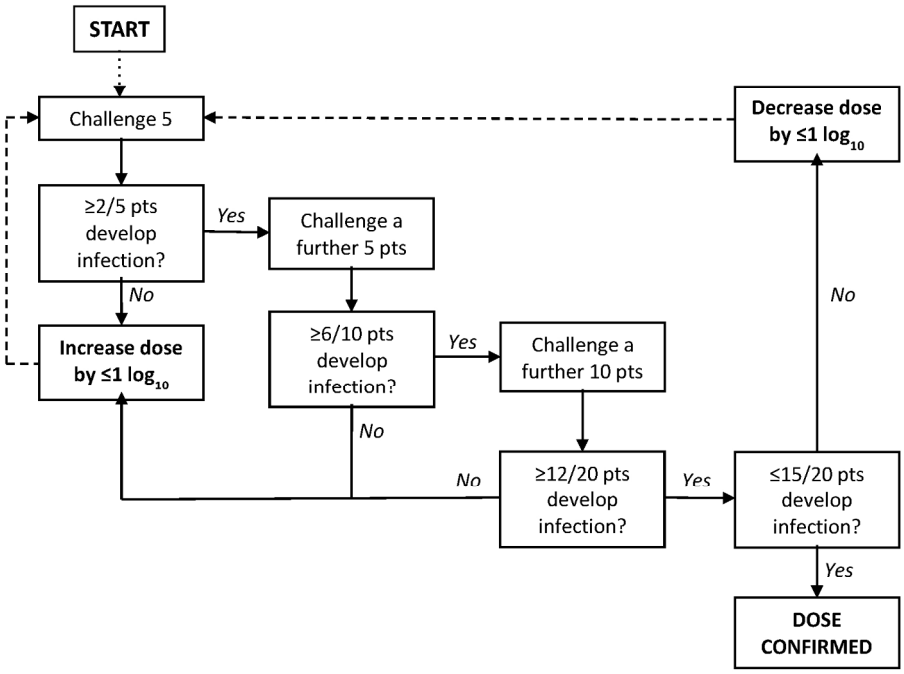

Figure 1. Decision making algorithm for S. Paratyphi dose escalation/de-escalation, starting at 1-5 x10e3 to reach the primary endpoint.
178x124mm (300 x 300 DPI)

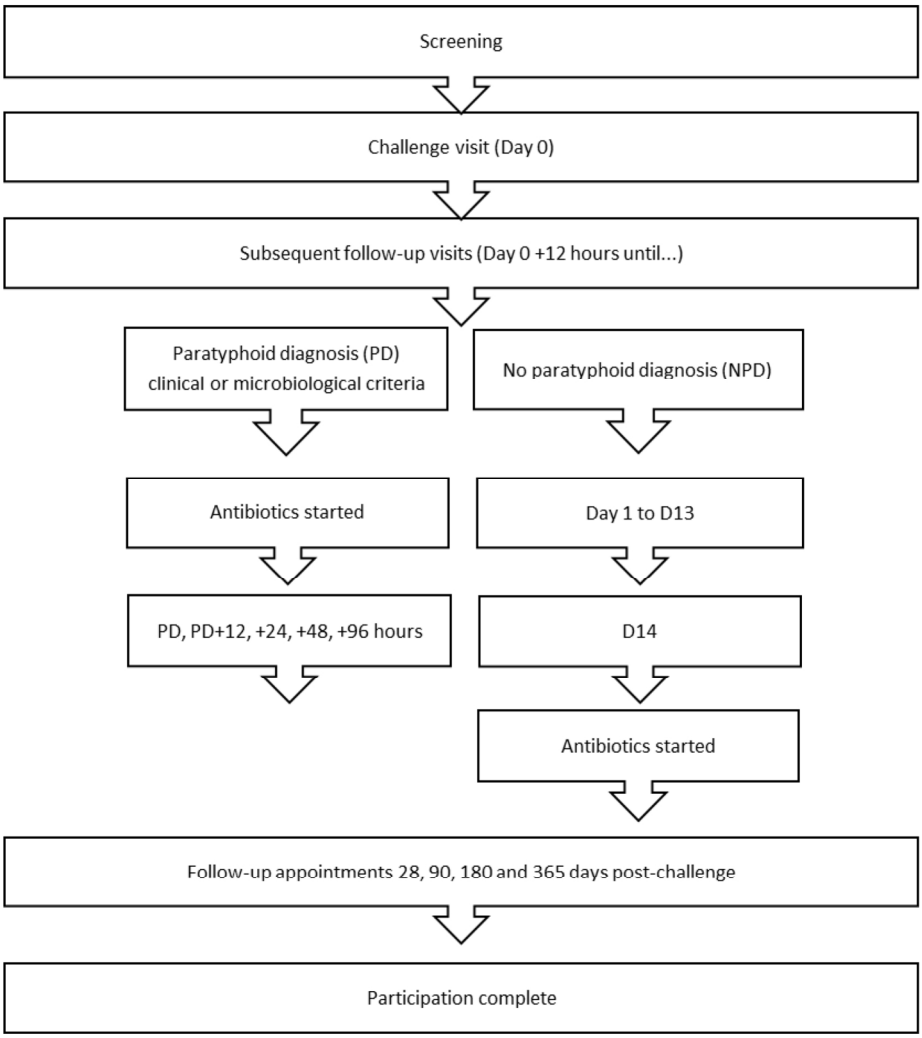

Figure 2. Participant journey through the study
153x167mm (300 x 300 DPI)

Supplementary 1. Eligibility Criteria.

Inclusion Criteria
Agree to give informed consent for participation in the study
Aged between 18-60 years inclusive at time of challenge
In good health as determined by medical history, physical examination and clinical judgment of the investigators
Agree (in the Investigator's opinion) to comply with all study requirements, including capacity to adhere to good personal hygiene and infection control precautions
Agree to allow his or her General Practitioner (and/or Consultant if appropriate), to be notified of participation in the study
Agree to allow Public Health England to be informed of their participation in the study
For those involved in provision of health or social care to vulnerable groups only – willing to allow his or her employer to be notified of participation in the study
Agree to give his or her close household contacts written information informing them of the participants' involvement in the study and offering them voluntary screening for *S.* Paratyphi carriage
Agree to have 24-hour contact with study staff during the four weeks post challenge and are able to ensure that they are contactable by mobile phone for the duration of the challenge period until antibiotic completion
Have internet access to allow completion of the eDiary and real-time safety monitoring
Agree to avoid antipyretic/anti-inflammatory treatment until advised by a study doctor or until 14 days after challenge
Agree to provide their National Insurance/Passport number for the purposes of TOPS registration and for payment of reimbursement expenses
Exclusion Criteria
History of significant organ/system disease that could interfere with study conduct or completion. Including, for example, but not restricted to: Cardiovascular disease Respiratory disease Hematological disease Endocrine disorders Renal or bladder disease, including history of renal calculi Biliary tract disease, including biliary colic, asymptomatic gallstones or previous cholecystectomy Gastro-intestinal disease including requirement for antacids, H_2-receptor antagonists, proton pump inhibitors or laxatives

Neurological disease Metabolic disease Autoimmune disease Psychiatric illness requiring hospitalization or known or suspected drug and/or alcohol misuse (alcohol misuse defined as an intake exceeding 42 units per week) Infectious disease
Have any known or suspected impairment of immune function, alteration of immune function, or prior immune exposure that may alter immune function to paratyphoid resulting from, for example: Congenital or acquired immunodeficiency, including IgA deficiency Human Immunodeficiency Virus infection or symptoms/signs suggestive of an HIV-associated condition Receipt of immunosuppressive therapy such as anti-cancer chemotherapy or radiation therapy within the preceding 12 months or long-term systemic corticosteroid therapy Receipt of immunoglobulin or any blood product transfusion within 3 months of study start History of cancer (except squamous cell or basal cell carcinoma of the skin and cervical carcinoma in situ)
Moderate or severe depression or anxiety as classified by the Hospital Anxiety and Depression Score at screening or challenge that is deemed clinically significant by the study investigators
Weight less than 50kg
Presence of implants or prosthesis
Anyone taking long-term medication (e.g. analgesia, anti-inflammatories or antibiotics) that may affect symptom reporting or interpretation of the study results
Contra-indication to ciprofloxacin, azithromycin, trimethoprim/sulfamethoxazole and/or beta lactam antibiotics
Female participants who are pregnant, lactating or who are unwilling to ensure that they or their partner use effective contraception one month prior to challenge and continue to do so until two negative stool samples, a minimum of 2 weeks after completion of antibiotic treatment, has been obtained
Full-time, part-time or voluntary occupations involving: Clinical or social work with direct contact with young children (defined as those attending pre-school groups or nursery or aged under 2 years), or Clinical or social work with direct contact with highly susceptible patients or persons in whom typhoid infection would have particularly serious consequences (unless willing not to work until demonstrated to not be infected with *S.* Paratyphi in accordance with guidance from Public Health England)
Full-time, part-time or voluntary occupations involving commercial food handling (involving

preparing or serving unwrapped foods not subjected to further heating)
Close household contact with: Young children (defined as those attending pre-school groups, nursery or those aged less than 2 years) Individual(s) who is (are) immunocompromised
Scheduled elective surgery or other procedures requiring general anaesthesia during the study period
Participants who have participated in another research study involving an investigational product that might affect risk of paratyphoid infection or compromise the integrity of the study within the 30 days prior to enrolment (e.g. significant volumes of blood already taken in previous study)
Detection of any abnormal results from screening investigations (at the clinical discretion of the study investigators)
Inability to comply with any of the study requirements (at the discretion of the study investigators and the participants General Practitioner)
Any other social, psychological or health issues which, in the opinion of the study investigator, may; put the participants or their contacts at risk because of participation in the study, adversely affect the interpretation of the primary endpoint data, or impair the participant's ability to participate in the study
Having previously received any live oral typhoid vaccine (those who have received Vi polysaccharide vaccine will not be excluded)
Having been resident in an enteric fever endemic country 6 months or more
Have previously been diagnosed with laboratory-confirmed typhoid or paratyphoid infection or been given a diagnosis compatible with enteric fever
Have participated in previous typhoid challenge studies (with ingestion of challenge agent)
Currently working for the Oxford Vaccine Group

Supplementary 2. Summary of study procedures

		Intensive challenge period (14 days)						Diagnosed with paratyphoid		Follow-up visits	
	screening	D0	+12H	D1, D2, D3, D4, D5, D6	D7	D8, D9, D10, D11, D12,D13	D14	PD	+12H	+24,+48, +72,+96H	D28	D90, 180, 365
Informed consent	x											
Revalidation of consent		x										
Obtain 24 hr contact details		x										
Medical history	x											
Interim medical history		x	x	x	x	x	x	x		x	x	x
Physical examination	x	x						x				
Vital signs	x	x	x	x	x	x	x	x		x	x	
Urine pregnancy test	x	x					x	x				
Urine sample	x	x		x	x	x	x	x		x		x
Saliva sample		x		x	x	x	x	x		x		x
Stool sample		x		x	x	x	x	x		x		x
Blood sample	x	x	x	x	x	x	x	x		x	x	x
12 lead ECG	x											
Ultrasound scan	x											
Mood assessment	x	x			x		x	x				
Challenge with *S.* Paratyphi		x										
Issue study pack	x	x										
eDiary entries*				x	x	x	x	x		x	x	
Commence antibiotics							x	x				
Notification of public health and GP**		x										
Letter informing close contacts	x						x					
Letter to close contacts offering screening											x	

*eDiary will be open from day 0-21 for temperature and symptom recordings.

**The local Public Health England Unit and the participant's GP will be contacted on challenge and on completion of stool clearance samples. GPs will receive an additional letter when a participant has started antibiotic treatment.