PARACHIM –
Paratyphi A Controlled Human Infection Model

Title of the Study

Salmonella Paratyphi A Controlled Human Infection Model in an Endemic Setting: Determining Safety, Dose Escalation, and Correlates of Protection

Investigators

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Contents

Synopsis

Summary Information type	Summary details

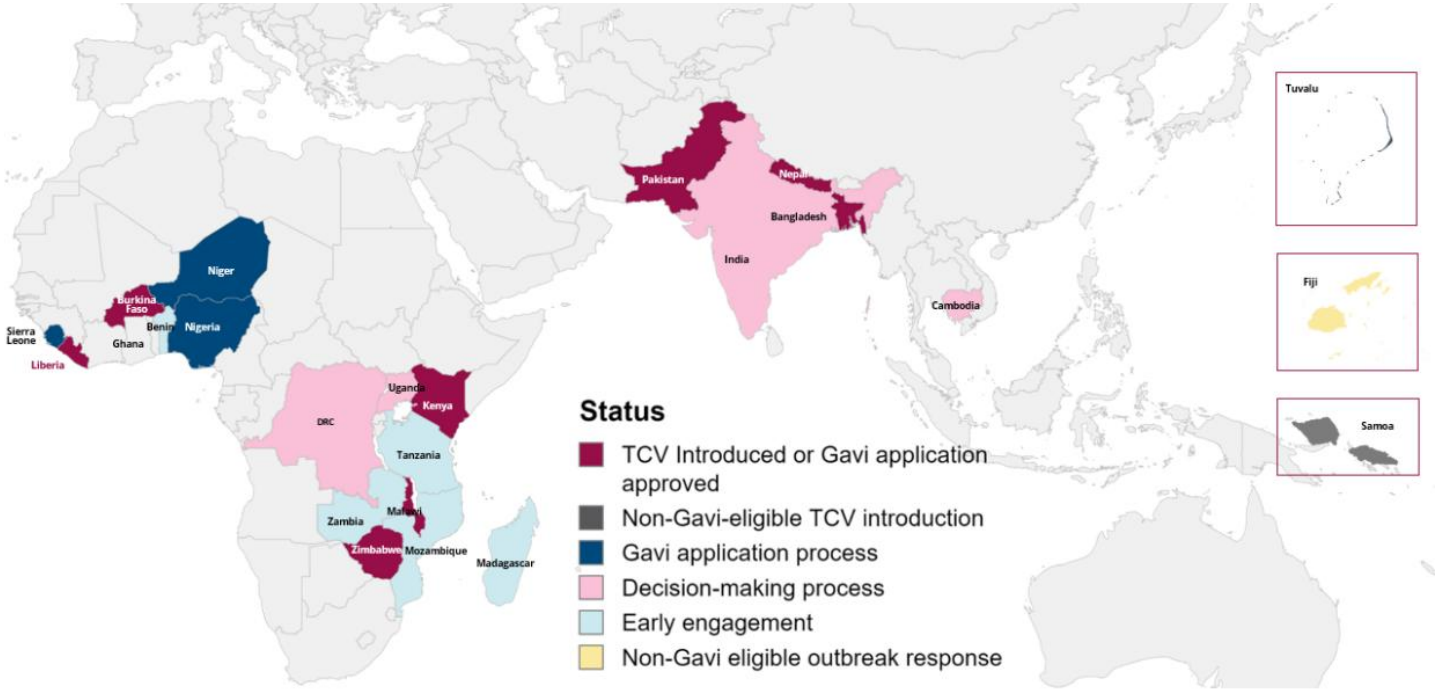
Study title	*Salmonella* Paratyphi A Controlled Human Infection Model in an Endemic Setting: Determining Safety, Dose Escalation, and Correlates of Protection
Acronym	**PARACHIM** – *Paratyphi A Controlled Human Infection Model*
Chief Investigator and Principal Investigator	Prof Farah Naz Qamar
Study design	Descriptive, dose level escalation, controlled human infection model (CHIM) study.
Study Participants	Healthy adults aged 18-55 years inclusive, living in an area endemic for *Salmonella* Paratyphi A
Sample size	The continuous reassessment method will be used for the dose-finding with groups of 3- 5 volunteers and a total sample size of up to 60 participants
Study Setting	Single centre: The Aga Khan University, Pakistan Challenge administration setting: The Aga Khan University Hospital, Pakistan Karachi, Pakistan
Challenge agent	Wild-type *S*. Paratyphi A strain NVGH308
Follow up duration	6 months post challenge
Planned Study Period	August 2025 – August 2027

Primary Objectives	1. 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 in Pakistan.
Secondary Objectives	2. To describe the human physiological response to *Salmonella* Paratyphi A challenge, and in those developing or not developing infection.
	3. To evaluate the sensitivity of the pre-defined criteria for Paratyphoid A infection in an endemic area, using subsequent clinical, microbiological and laboratory outcomes.
	4. To describe the characteristics of bacterial dynamics after challenge, including onset and duration of bacteraemia, bacterial burden at diagnosis and stool shedding.
	5. To describe the human immune response to challenge, including innate, humoral, cell-mediated and mucosal responses.
	6. To investigate immunological correlates of protection for *S*. Paratyphi A infection

Exploratory (Laboratory analyses relating to exploratory endpoints may be performed following adoption of samples into the biobank)	7. To compare the impact of antibiotics used to treat challenged participants on the gut microbial ecosystem and its reservoir of antimicrobial resistance (AMR) genes.
	8. To compare immunological results with other endemic and non-endemic populations to identify possible correlates of protection for *S*. Paratyphi A infection.
	9. To explore the variation in genomic response to *Salmonella* Paratyphi A challenge in participants
	10. To explore molecular changes occurring after challenge and during acute infection
	11. To discover, develop and evaluate novel diagnostic methods for *Salmonella* Paratyphi A infection

Background

Figure 1: Current status of Typhoid conjugate vaccine (TCV) roll out and plans in 2024 (courtesy of PATH)



Enteric fever, a life-threatening infection caused by *Salmonella* enterica serovar Typhi (*S*. Typhi) and *S.* Paratyphi A (SPA), imposes a substantial burden of disease in populations with limited access to clean water and inadequate sanitation (1) Management of enteric fever is increasingly complicated by the spread of antimicrobial resistance (2). Improving control of this disease through vaccination will have a substantial impact on the health and well-being of some of the most vulnerable populations in low-income settings including young children. Following World Health Organization (WHO) recommendations supported by the previous work of Oxford University vaccine on the controlled human Infection model (CHIM) for typhoid (3) and large-scale trials in Southeast Asia and Africa (4, 5), since 2021 over 60 million doses of *Salmonella* Typhi Vi-conjugate vaccines (TCVs) have been distributed to protect children in low-income countries against this disease (see Figure. 1). However, for comprehensive control of enteric fever, bivalent vaccines that also target *S.* Paratyphi A are needed because this organism causes approximately one-third of the global burden of enteric fever, mainly in South Asia (estimated 3 million cases)(1).

While new TCVs can be licensed for use based on non-inferiority immunogenicity trials against licensed typhoid polysaccharide and conjugate vaccines, this route is not available for paratyphoid vaccines as no licensed products are available as a comparator. Therefore, using the CHIM, pioneered and uniquely available at Oxford, provided an alternative route for vaccine development other than the traditional phase III field efficacy trial, which requires a huge sample size due to the low incidence of paratyphoid fever and associated diagnostic challenges. Indeed, WHO has advised (Product Development for Vaccines Advisory

Committee, PDVAC, December 2022/23) that licensure of vaccines for paratyphoid should be supported by evidence of protection in the CHIM (6), linked to field immunogenicity data (with appropriate safety data) because field efficacy trials (requiring randomization of 60,000-100,000 children) are not considered affordable. All the available SPA CHIMs studies were conducted in non-endemic settings and regulators, such as FDA and EMA, prefer efficacy data in the target population for licensure of vaccines, therefore, there is a need to establish CHIM models in endemic settings to test vaccine efficacy in endemic settings.

Rationale for Conducting a Controlled Human Infection Model (CHIM) Study in Paratyphoid endemic settings (Pakistan)

Controlled Human Infection Model (CHIM) studies are crucial for accelerating vaccine development by rapidly generating data on efficacy, safety, and immune responses. They reduce the time and cost of traditional trials while minimizing early-stage risks. However, over 99% of the >40,000 volunteers in human challenge studies (HCS) since World War II have been from high-income countries (HICs), limiting the applicability of findings to low- and middle-income countries (LMICs), where disease burden and immune responses may differ due to frequent natural exposure. (7) In recent years, CHIM studies have been successfully conducted in endemic settings, such as Malawi, where Africa's first pneumococcal challenge study provided key insights into vaccine protection and bacterial carriage reduction. (8) Similarly, ongoing Shigella CHIM studies in Kenya aimed at dose-finding and verification, paving the way for future vaccine trials in populations that stand to benefit the most. (9) Expanding CHIM studies in LMICs is essential for cost-effective vaccine evaluation and targeted public health interventions.

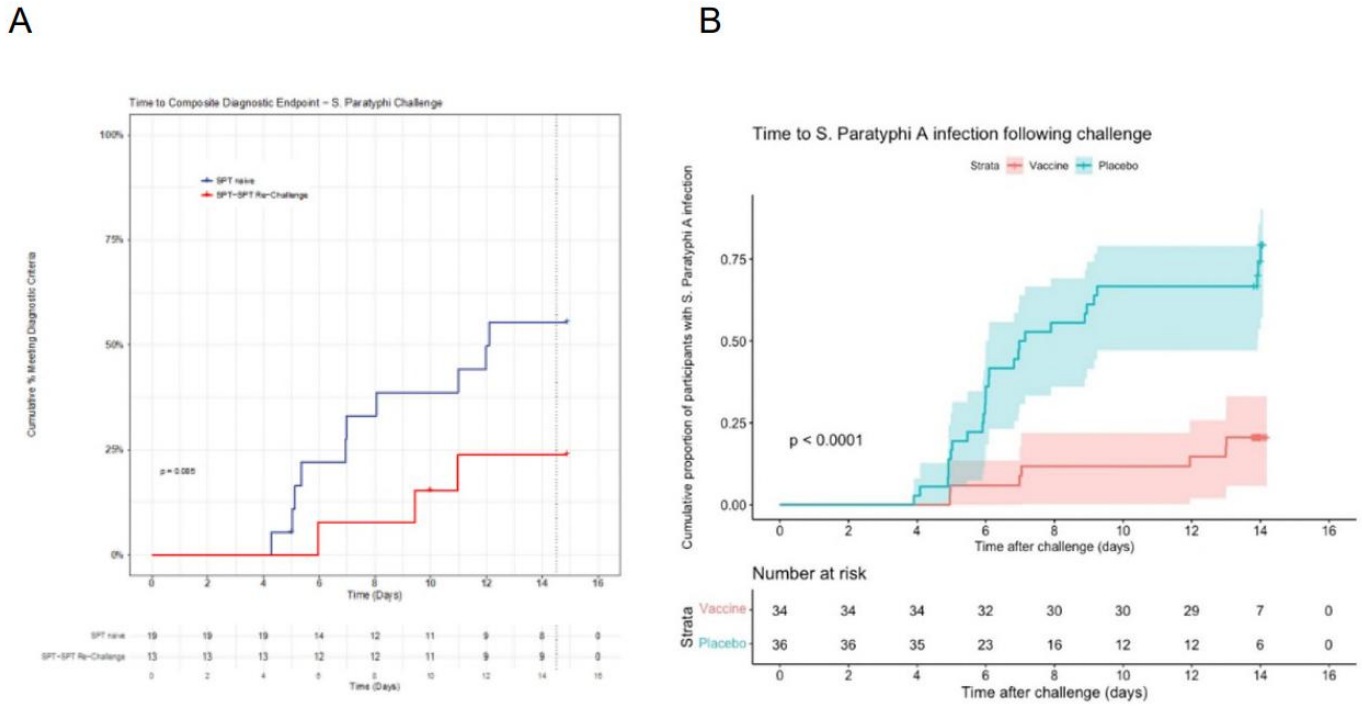
Pre-existing immunity in endemic populations can influence vaccine responses, sometimes enhancing protection but also potentially dampening immune responses due to prior exposure. Studies on vaccines for other infections, such as oral cholera, yellow fever, and influenza, have shown that immune responses can vary significantly between populations in endemic and non-endemic settings. (10-13) This variation underscores the critical need to test vaccines in the populations most affected by the disease to ensure they are effective.

Paratyphoid fever remains a major public health concern in LMICs, particularly in areas with poor sanitation and limited access to clean water. In Pakistan, where 80% of the population lacks access to safe drinking water, exposure to contaminated sources drives the spread of enteric infections, including *S.* Paratyphi A. (14) South Asia and sub-Saharan Africa have the highest incidence rates (77.4 cases per 100,000 people), compared to just 0.8 per 100,000 in North Africa and the Middle East. (15) *S*. Paratyphi A accounts for 8.5% if all blood culture proven enteric fever cases in across Pakistan (*unpublished data*). The economic burden is significant, with the median cost per case at $127.57 ($104.35 direct cost) which is an out-of-pocket expense for most patients due to weak healthcare infrastructure and lack of health insurance. (16)

This CHIM study will be the first for *Salmonella* Paratyphi A in an endemic region, contributing to the broader effort to establish Correlates of Protection (CoPs) for paratyphoid vaccine development. The Oxford Vaccine Group has developed the only existing paratyphoid CHIM, which has already been used to test vaccines showing early signs of protection. (17) (May 2024, See Fig 2) With TCV licensed in Pakistan since 2019, the introduction of a paratyphoid vaccine could enable the development of a bivalent vaccine targeting both S. Typhi and S. Paratyphi A.

This study will provide new insights into protection against SPA. Previous studies suggest that inactivated paratyphoid vaccines (now unavailable) and prior exposure to live bacteria (through rechallenge studies in the University of Oxford's paratyphoid CHIM and recent vaccine trials) offer some protection; however, key protective antigens, mechanisms of protection, and correlates of immunity remain unknown. (18, 19). This CHIM efficacy studies will provide an unparalleled opportunity to comprehensively evaluate CoP against paratyphoid, linking immunological readouts after vaccination to robust clinical and microbiological endpoints in the CHIM.

Figure 2: A) shows a lower attach rate in individuals with prior exposure to S. Paratyphi A (red line) than those on first exposure (blue line) in the CHIM (Gibani er al). B) Evidence of protection with live attenuated paratyphoid vaccine (CVD-1902) in the CHIM



This study builds on the Oxford Vaccine Group's successful track record of conducting paratyphoid vaccine CHIM trials in the UK. Since prior exposure to *Salmonella* Paratyphi A can confer some level of protection, we will use a dose-escalation approach. We will begin with the same challenge dose used in UK CHIM studies as a baseline in an **immune-naïve population**. However, given the continuous environmental exposure in Pakistan, this dose may not be effective in eliciting the desired infection rate.

Therefore, we will gradually increase the dose to determine the amount (in colony-forming units) of *Salmonella Paratyphi A* (challenge strain NVGH308) needed to achieve a 60% to 75% attack rate in healthy adult volunteers in Pakistan.

Objectives

The study objectives and their corresponding outcome measures are outlined in Table 1 below.

Table 1: Study objectives

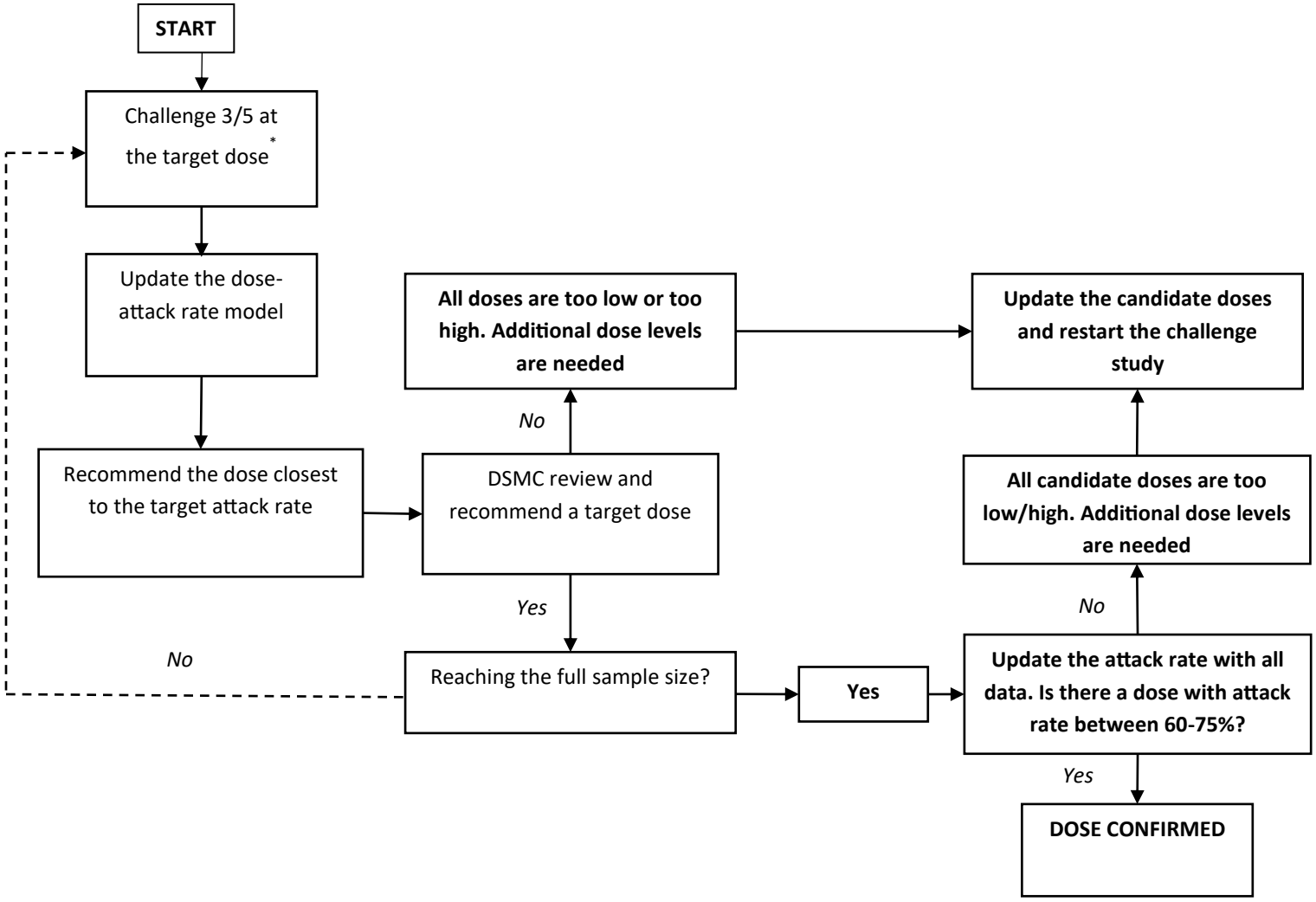
	Objectives	**Outcome Measures**
Primary	To determine the dose (in colony forming units) of *Salmonella* Paratyphi A, challenge strain NVGH308, needed to produce a 60% to 75% attack rate when ingested with sodium bicarbonate solution, in healthy adult volunteers in Pakistan.	a) Proportion of participants developing *Salmonella* Paratyphi A infection within 14 days following oral challenge with S. Paratyphi A strain NVGH308
Secondary	1. To describe the human physiological response to *Salmonella* Paratyphi A challenge, and in those developing or not developing infection.	Occurrence of: a. Solicited events in the 21 days after challenge b. Clinical and laboratory observations within 14 days after challenge Description of the clinical course after challenge using, for example, participant symptom profiles, temperature measurements and other recorded clinical and laboratory observations

2. To evaluate the sensitivity of the pre-defined criteria for Paratyphoid A infection in an endemic area, using subsequent clinical, microbiological and laboratory outcomes.	a) Determination of challenge dose/kg (dose/surface area) actually ingested by those developing and those not developing paratyphoid infection at each dose level. b) 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.
3. 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 A after challenge in blood, stool and urine, including assessment of quantitative level of bacteraemia in diagnosed participants
4. 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. a. Quantification of *S.* Paratyphi A antigen-specific IgG, IgA and IgM antibodies b. Antigen-specific cell-mediated responses (including antigen specific cell frequencies, description of lymphocyte and T cell repertoire, B cell responses and B-cell repertoire) to *S.* Paratyphi A antigens, such as Lipopolysaccharides (LPS) c. Functional responses to *S.* Paratyphi A antigens (Serum bactericidal assay titres, opsonophagocytosis assay) d. Mucosal responses (secretory IgA) e. Cytokine and acute phase reactant profile
5. To investigate immunological correlates of protection for *S*. Paratyphi A infection	To determine if particular immunological markers (including *S*. Paratyphi A specific antibody titres or serum bactericidal assay titres) at baseline, induced by natural exposure pre-challenge, correlate with protection against the clinical endpoint of *S.* Paratyphi A infection

Exploratory (Laboratory analyses relating to exploratory endpoints may be performed following adoption of samples into the biobank)	6. To compare the impact of antibiotics used to treat challenged participants on the gut microbial ecosystem and its reservoir of antimicrobial resistance (AMR) genes	To evaluate the effects of fluoroquinolones on the gut microbial ecosystem and its reservoir of antimicrobial resistance (AMR) genes, we will also compare the impact of antibiotics on the gut microbiota of individuals from regions with low and high antibiotic usage (e.g., the UK vs. Pakistan)
	7. To compare immunological results with other endemic and non-endemic populations to identify possible correlates of protection for *S*. Paratyphi A infection.	Exploratory analysis of immunogenicity data with previously collected samples from CHIM performed in the UK as well as endemic region samples from field surveys
	8. To explore the variation in genomic response to Salmonella Paratyphi A challenge in participants.	Laboratory and high-throughput assays to measure gene expression and protein translation at baseline and post-challenge time points
	9. To explore molecular changes occurring after challenge and during acute infection	Application of techniques such as proteomics, metabolomics, epigenetics and metagenomics to samples from baseline, post-challenge time points
	10. 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.

Study Design overview

Figure 3: Decision-making algorithm for dose escalation/de-escalation.



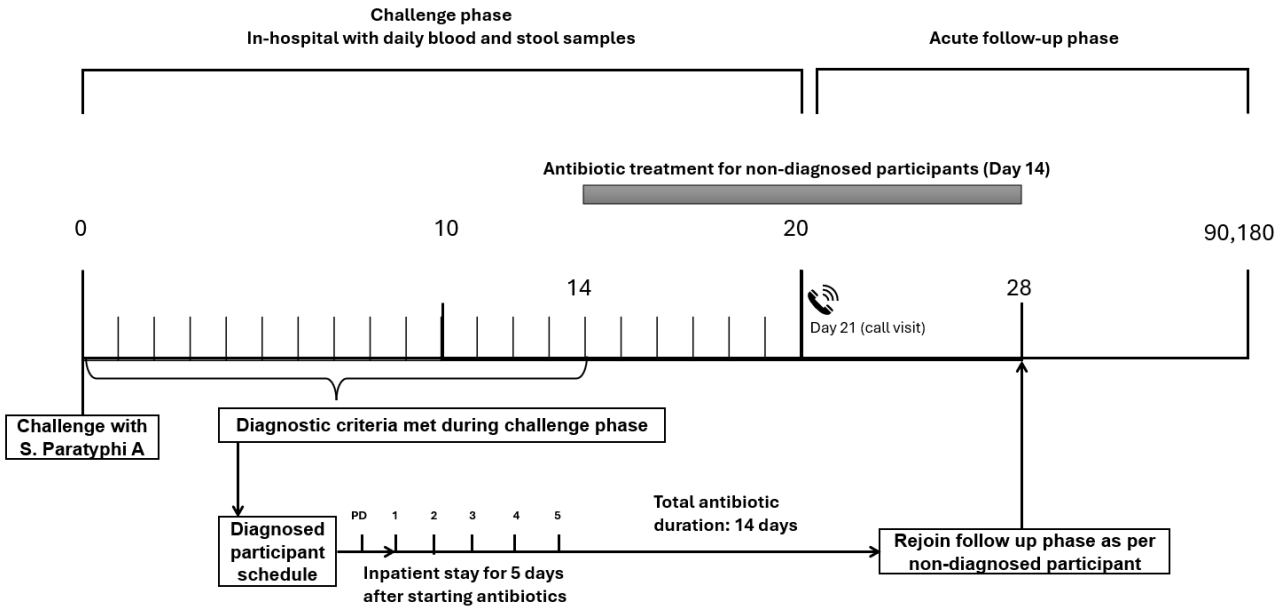
**The target dose for the first cohort will be 1-5 x 103 CFU, and the two cohorts will be 3 participants each*

This is an inpatient dose-finding controlled human infection model (CHIM) study of *Salmonella* Paratyphi A infection in healthy adult participants in Pakistan. In total, up to 60 participants will be recruited. A flowchart of the study design for dose escalation of each challenge strain is presented in Figure 3. Participants will be challenged with *S.* Paratyphi A (strain NVGH 308) beginning at a dose of 1-5 x 10^3 CFU/30 ml, the dose previously established to give a desired 'attack' rate of approximately 66% (as per prior Oxford Vaccine Group *S.* Paratyphi A studies: OVG 2013/07, REC Ref: 14/SC/0004; OVG 2014/01, REC Ref: 14/SC/1204, OVG 2018/07, REC Ref: 21/SC/0330). Because of pre-existing immunity in an endemic country, we anticipate that a higher dose of challenge may be required to successfully reach the target attack rate; the programme will therefore run as a dose-escalation study, increasing a log of the dose at a time for each cohort of 3-5 participants enrolled. Given this study is the first SPA CHIM in Pakistan, we will

start with a cohort of 3 participants. The first cohort will be further split into two groups (1+2 participants with an interval of at least 7 days to monitor the safety closely. The first dose review by the Data Safety Monitoring Board (DSMB) will be conducted after the first 3 participants have been challenged and followed up for 14 days. The second cohort will be another 3 participants. After we challenged the first 6 participants (cohorts 1 and 2), the DSMB will review the safety data and if there are no safety concerns, we will increase the cohort size to 5 participants. A dose review will be done after each of the cohorts reaching the 14 days of follow-up.

To ensure safety of the participants and also because of the lower reliability of closed-sanitation systems in Karachi, the study will be run on an inpatient basis at Aga Khan University (AKU) to ensure that volunteers are managed in a containment environment that avoids risk of onward transmission of paratyphoid A in the community (and conversely infection of the volunteers from contaminated water at home), and, as such, they will stay in an in-patient facility for the 10-20 days following oral challenge until at least 5 days of antibiotics have been received by the participants but not earlier then day 10 post-challenge.

Figure 4: study structure



Notes: Diagnosis can occur on any day during the challenge phase; if this occurs, diagnosed participants then follow the diagnosed participant (PD) schedule and then re-join the acute follow up phase visits as for non-diagnosed participants at D21. The D21 is a visit performed over the telephone.

Each PD assessment is scheduled 24 hours apart starting at PD-1 (+24hrs). Assessment at PD+12hrs will be optional if the study teams consider it is needed.

Minimum duration of admission will be 10 days post challenge in Diagnosed participants

Gut microbiome analysis

This part of the study will assess the impact of antibiotic (Ciprofloxacin) on the gut microbiome and its reservoir of antimicrobial resistance (AMR) genes in a setting with high antibiotic use. Stool samples will be collected at defined time points: before the challenge, after challenge before stating antibiotics, during antibiotic treatment, and at follow-up intervals. The project leverages metagenomic long-read sequencing to generate detailed microbial assemblies, enabling linkage of AMR genes to specific bacterial hosts and mobile genetic elements, providing insights into the effects of antibiotics on gut microbiota in a low-resource, endemic setting.

Operational definitions

Paratyphoid Diagnosis (PD)

A participant will be diagnosed with paratyphoid infection and start treatment with antibiotics under any one of the following conditions:

1. A positive blood culture for *Salmonella* Paratyphi A >72 hours after challenge administration.
2. A positive blood culture with *Salmonella* Paratyphi A <72 hours after challenge administration with fever of 38C (100.4F) or above.
3. Persistent positive blood culture <72 hours after challenge administration.
4. Persistent fever, defined as, oral temperature of 38C (100.4F) or above, two spikes at least 12 hours apart

Severe Paratyphoid fever:

Severe paratyphoid infection will be referred to cases of Salmonella enterica serovar Paratyphi A infection that exhibit complications such as:

- High, persistent fever (>39°C for more than 4–5 days)
- Severe gastrointestinal symptoms (profuse diarrhea or intractable vomiting)
- Sepsis or bacteremia
- Neurological symptoms (e.g., delirium, confusion)
- Complications such as:
    - Intestinal perforation
    - Gastrointestinal bleeding
    - Hepatitis
    - Myocarditis
    - Pneumonia
    - Septic shock

- Failure to respond to oral antibiotics, requiring IV therapy (criteria for starting IV therapy explained under heading “Antibiotics for the study”)

Paratyphoid infection clearance:

Clearance of *Salmonella* Paratyphi infection is confirmed when either of the following criteria is met:

1. A blood culture collected by the study phlebotomist, following an earlier confirmed positive blood culture, tests negative for *Salmonella* Paratyphi after starting antibiotics.
2. Absence of fever alone will not be enough to classify as paratyphoid clearance.

Paratyphoid Stool clearance

1. *Salmonella* Paratyphi can continue to be shed in the stool of infected individuals even after completing treatment, allowing them to transmit the pathogen to others.
2. Three stool clearance samples, collected at least 24 hours apart beginning one week after completing a 14-day course of antibiotics, must test negative to confirm infection clearance.
3. Until then, strict WASH (Water, Sanitation, and Hygiene) precautions are essential to prevent further transmission.

Antibiotics for the study

We will use oral Ciprofloxacin for the treatment of all participants enrolled in the study who received the Challenge agent. This study will utilize a strain of Salmonella Paratyphi A that is fully sensitive to ciprofloxacin. It has demonstrated no resistance in the Oxford study, where it was successfully treated in an outpatient setting using ciprofloxacin. To ensure complete resolution of infection, regular blood cultures and stool clearance tests will be performed.

If a participant does not show clinical improvement, defined as improvement in fever pattern and negative cultures, within 5 days of initiating ciprofloxacin, second-line treatment will be initiated. This will involve intravenous ceftriaxone for 10-14 days.

If there is inadequate response to both first- and second-line therapy, third-line treatment will be initiated. Depending on the participant's clinical condition, this will include either oral azithromycin or intravenous meropenem. The decision regarding the appropriate route and choice of antibiotic will be made by the study physician, an adult infectious diseases specialist, based on clinical evaluation.

Sample Size and Statistical Considerations

Sample Size

The sample size and study design (cohort size) were chosen based on operational characteristics from simulations using the continual reassessment method (CRM), along with practical constraints, such as the capacity of the inpatient facility. The CRM is a model-based design, which was first proposed in 1990. This approach is widely applied in cancer drug dose-finding early phase studies to replace the traditional 3+3 design (rule-based design). The most attractive characteristic of CRM compared with the traditional rule-based dose finding studies is that the CRM method borrows information across all dose levels, which means it will find the true dose with higher probability than the rule-based method in most situations, especially when the maximum sample size is small. The first dose-finding CHIM study using CRM was implemented in a Non-Typhoidal *Salmonella* dose-finding CHIM by our team, which demonstrated the efficiency of CRM over traditional rule-based design. (18) The simulation for this study will focus on the choice of sample size and cohort size, instead of repeating the comparison between model-based and rule-based designs. A flowchart of the study design for dose escalation is given in Figure 3.

Based on previous *S*. Paratyphi A challenge studies at 1-5x10^3 CFU at Oxford Vaccine Group in the UK, the attack rate in a non-endemic population is estimated to be around 66% (49/38=74%) (19, 20). Because of pre-existing immunity, higher doses of challenge may be required in Pakistan; a dose-escalation study, starting with the UK dose (1-5x10^3 CFU) and increasing a log at a time will be used. The parameters for the CRM are listed below:

- Four doses for each challenge agent: 1-5x10^3 CFU, 1-5x10^4 CFU, 1-5x10^5 CFU, 1-5x10^6 CFU;
- Target attack rate: 67.5%;
- Dose-attack rate model: empiric model;
- Dose-attack rate skeleton: 60%, 75%, 85%, 90%;
- Inference: Bayesian;
- Decision rule: the dose closest to the target attach rate (absolute difference);
- Sample size: 30; 40; 50 and 60;
- Cohort size=3 or 5 or a mixture of 3 and 5;
- Safety modifications: starting at the lowest dose and only one dose escalation at a time;
- Stopping rule: the attack rate at the highest dose deemed too low (<50%) after at least 15 participants were challenged at the highest dose;

We ran 1000 simulations to compare the operational characteristics under different sample sizes and cohort sizes, including:

- Sample size: maximum sample size of 30, 40, 50 or 60;
- Cohort size: 3 or 5 or a mixture of 3 and 5 (3 for the first two cohorts and 5 for the rest cohorts);

We chose three simulation scenarios:

- Scenario 1 (optimal dose within Dose 1-2): **60%, 75%**, 80%, 85%
- Scenario 2 (optimal dose within Dose 2-3): 40%, **60%, 75%**, 80%
- Scenario 3 (optimal dose outside the range, last dose too low): 20%, 30%, 40%, 50%

Table 2: Probabilities of correctly selecting the dose in different scenarios under different sample size and cohort size

Probabilities of choosing the correct dose												
Sample sizes	N=30			N=40			N=50			N=60		
Cohort sizes	n=3	n=5	Mixed	n=3	n=5	Mixed	n=3	n=5	Mixed	n=3	n=5	Mixed
Scenario 1												
First dose too high	4.5%	3.9%	4.4%	3.5%	3.2%	3.7%	1.3%	1.1%	1.2%	0.9%	0.9%	1.0%
Dose 1 (60%) / Dose 2 (75%)	**90.8%**	**92.8%**	**91.1%**	**93.3%**	**93.9%**	**93.2%**	**96.2%**	**96.6%**	**96.2%**	**97.5%**	**98.1%**	**96.7%**
Dose 3 (80%)	4.5%	3.3%	4.3%	3.2%	2.9%	3.1%	2.5%	2.2%	2.6%	1.6%	1.0%	2.3%
Dose 4 (85%)	0.2%	-	0.2%	-	-	-	-	0.1%	-	-		-
Last dose too low	-	-	-	-	-	-	-	-	-	-		-
Scenario 2												
First dose too high	-		-	-	-	-		-	-	-	-	-
Dose 1 (40%)	2.6%	3.8%	2.5%	1.2%	0.4%	0.9%	0.5%	0.7%	0.2%	0.5%	0.4%	0.2%
Dose 2 (60%) / Dose 3 (75%)	**93.8%**	**93.7%**	**93.9%**	**96.4%**	**97.3%**	**96.9%**	**97.1%**	**97.5%**	**97.7%**	**97.5%**	**98.9%**	**97.8%**
Dose 4 (80%)	3.6%	2.4%	3.6%	2.4%	2.3%	2.2%	2.4%	1.8%	2.1%	2.0%	0.7%	2.0%
Last dose too low	-	0.1%	-	-	-	-	-	-	-	-	-	-
Scenario 3												
First dose too high	-	-	-	-	-	-	-	-	-	-	-	-
Dose 1 (20%)	-	-	-	-	-	-	-	-	-	-	-	-
Dose 2 (30%)	-	-	0.1%	-	0.1%	0.1%	-	-	-	-	-	-
Dose 3 (40%)	5.0%	7.9%	5.0%	1.6%	3.6%	2.3%	1.2%	1.4%	1.1%	0.2%	0.5%	0.2%
Dose 4 (50%)	43.3%	53.0%	45.1%	37.4%	44.6%	38.2%	28.8%	37.5%	29.7%	24.0%	30.3%	24.8%
Last dose too low	**51.7%**	**39.1%**	**49.8%**	**61.0%**	**51.7%**	**59.4%**	**70.0%**	**61.1%**	**69.2%**	**75.8%**	**69.2%**	**75.0%**

Based on the simulation results, we will recruit up to a total of 60 participants as scenario 3, where the highest dose cannot achieve the target attack rate, could happen in an endemic setting. We do not want to recommend a dose with low attack rate as this will increase the risk of failure for future vaccine efficacy trials using this CHIM. A mixture cohort size of 3 and 5 (first two cohorts of 3) provides similar operational characteristics compared with cohort sizes of 3, while it can accelerate the study by challenging more participants at the same time.

Statistical Analysis

All analyses will be descriptive and will not include formal hypothesis testing. Binary endpoints for the primary endpoint and reactogenicity endpoints will be described using percentages and 95% binomial

exact (Clopper-Pearson) confidence intervals. Time-to-event endpoint analyses will be conducted using the Kaplan-Meier method and presented as Kaplan-Meier plots. Immunogenicity data are expected to be highly skewed and will be log10-transformed before analysis. Results will be presented as geometric means with 95% confidence intervals. Values below the limit of detection will be replaced by half the value of the lower limit.

Data Collection and Management

SAMPLE COLLECTION

Samples can be divided into safety samples and immunobiology samples. **All specific tube types and volumes will be specified in the Laboratory Analysis Plan.**

Safety samples are samples collected for safety reasons and related to the primary endpoint. They are represented in the dark shaded columns in tables 3 and 4. They may include samples for haematology and biochemistry analysis, blood culture, and stool culture. All visits will include safety samples collection for all participants; exceptions to this are the following:

a. Stool cultures, which are not always able to be provided by the participant at all time points;
b. Safety samples may not be required at PD when blood collection happened less than 3 hours prior.
c. The PD+12hrs samples are not required and will only be taken if the participant is clinically unwell. Other participants will proceed directly to daily samples identified as PD-1,2,3,4, and 5 at 24 hour intervals.

Immunobiology samples represent all other samples, related to primary, secondary and exploratory laboratory assays, and they will be collected for all participants unless clinically contraindicated, such as in cases of anemia**.** They are represented in the light shaded columns on tables 3 and 4. They represent blood for bacterial quantification, serum samples, peripheral blood mononuclear cells, plasma samples, functional genomics, DNA samples, saliva and stool for research purposes. Immunobiology samples may be omitted as per the investigators' discretion, for example when participant is anaemic, or when exploratory objectives are no longer being investigated, or when sample collection is impractical.

Sampling timepoints for safety blood tests may vary in case a participant requires extra (unscheduled) visits for safety reasons. Total blood volume taken will not exceed that of two blood donations (the limit in a year is three donations for both men and women, according to the Sindh Blood Transfusion Authority)

Stool for microbiome

This part of the study will utilise a unique existing gut microbiome sample set, taken before, during and after antibiotic use. Stool samples will be collected for analysis of microbiome before challenge (D0), after challenge

(D4), during antibiotic phase in both diagnosed and undiagnosed participants (D15-19 or PD+1-PD+5), and on follow-up visits (D28, D90 and D180) during the study period for microbiome analysis.

Table 3: Summary of sample collection (no diagnosis)

Investigation	Pregnancy Test	Stool sample	Blood culture	Complete blood count	CRP, U+Es, LFTs	Saliva	Immunobiology samples
D0/Pre-challenge	x	x		x	x	x	x
D1		x	x				x
D2		x	x	x	x		
D3		x	x				
D4		x	x	x	x		
D5		x	x				
D6		x	x	x	x		
D7		x	x				x
D8		x	x	x	x		
D9		x	x				
D10		x	x	x	x		x
D11		x	x				
D12		x	x	x	x		
D13		x	x				
D14	X*	x	x	x	x	x	x
D15		x	x				
D16		x	x	x	x		
D17		x	x				
D18		x	x	x	x		
D19		x	x				
D20		x	x	x	x		x
D28	x	x		x	x		x
D90		x		x	x	x	x
D180		x		x	x	x	x

***urine pregnancy test before starting antibiotics, even though participants will be admitted during the challenge period, it will reduce risk associated with ciprofloxacin (by increasing sensitivity of pregnancy test with longer abstinence period)**

Table 4: Summary of sample collection (PD pathway)

Investigation	Urine Pregnancy test	Stool sample	Blood culture	Complete Blood count	CRP, U+Es, LFTs	Immunobiology samples	Saliva samples (for a subset of participants)
PD	X**	x	x	x	x	x	
PD +12hrs*		x	x	x	x	x	x

PD +1 (24hrs)*	x	x	x	x	x	x
PD +2 (48hrs)	x	x	x	x		x
PD +3 (72hrs)	x	x	x	x		x
PD +4 (96hrs)	x	x	x	x		x
PD +5 (120hrs)	x	x	x	x	x	x
D14PD	x	x	x	x		

*** PD+12hrs will be collected only if needed i.e. participant is unwell. PD+ 1 (24hrs) samples will be collected for all participants along with Immunobiology samples.**

**** urine pregnancy test before starting antibiotics, even though participants will be admitted during the challenge period, it will reduce risk associated with ciprofloxacin (by increasing sensitivity of pregnancy test with longer abstinence period)**

Site protocol

Study site

The study will take place in-patient setting at Aga Khan University to ensure a contained environment. Due to the prevalence of SPA in the natural environment, any wild infection during the period of the study will alter the results of CHIM. Keeping study subjects in the hospital will allow continuous daily monitoring and proper disposal of waste to prevent transmission of the pathogen into the environment.

Because of the lower reliability of closed-sanitation systems in Karachi, the in-patient monitoring also ensures that volunteers are managed in a containment environment that avoids the risk of onward transmission of paratyphoid A in the community (and conversely infection of the volunteers from contaminated water at home), and, as such, they will stay in an in-patient facility for the 10-20 days following oral challenge till at least 5 days after antibiotics initiation or Day 10 post challenge, whichever is longer.

Study team

This Controlled Human Challenge Study will be the first of its kind conducted in Pakistan. To ensure the smooth execution of day-to-day procedures, we will recruit a qualified and experienced research team (table 5). Comprehensive training, along with periodic refresher sessions, will be provided throughout the study duration to ensure strict adherence to protocols and ethical guidelines.

Table 5: Summary of study team designation and description of work

Name of Position	Description of Work
Instructor	The instructor will serve as the team lead, overseeing study operations and ensuring seamless communication and coordination between principal investigators and the research team. Responsibilities include supervising all study activities, managing timelines, troubleshooting challenges, and ensuring that protocols and ethical guidelines are strictly followed.
Research Specialist	The Research Specialist will oversee the implementation of study protocols and ethical guidelines. This role includes training research staff, ensuring compliance

	with protocols, monitoring study procedures, and reporting any adverse events or protocol deviations. The Research Specialist will also be responsible for timely communication of updates, changes, or challenges to the team and leadership
Research Associate	The Research Associate will oversee daily study activities, including participant recruitment, screening, informed consent, and eligibility assessment. This role involves leading and coordinating the research team, ensuring smooth workflow, and addressing operational challenges. Additionally, the Research Associate will report all adverse events to the study leadership and ensure adherence to ethical and procedural protocols.
Study Physician	The Study Physician will be responsible for conducting participant screening, including medical history reviews, physical examinations, and consent procedures in collaboration with the Study Nurse. Physicians will be responsible for administering the challenge agent and monitor inpatient participants during 12-hour day and night shifts, ensuring regular monitoring of vitals, symptoms, and protocol adherence regarding sample collection and blood tests. They will document and report all adverse events to the study leadership.
Study Nurse	The Study Nurse will assist the Study Physician during participant screening, consent procedures, and inpatient monitoring. Responsibilities include administering care, recording vitals, monitoring symptoms, and ensuring adherence to study protocols.
Senior Research Assistant	The Senior Research Assistant will conduct pre-screening education and consent processes. They will approach potential participants at community field sites and through the online portal, introduce the study, provide educational materials, address initial questions, and facilitate participant enrollment for the screening process.
Lab Technicians	The lab technicians will be responsible for handling and preparing the challenge agent. Their duties include maintaining quality control and strictly adhering to safety protocols for the handling and storage of the challenge agent. They will work closely with the research specialist and study physician team to dispense the challenge agent and will oversee the processing and management of all participant samples collected during screening, the inpatient phase, and follow-up visits.
Phlebotomist	The Phlebotomist will be responsible for collecting blood samples from participants during screening, inpatient monitoring, and follow-up visits. They will ensure proper labeling, handling, and timely delivery of samples to the laboratory while maintaining participant comfort and safety.
Team Senior Manager	The Team Senior Manager will oversee the administrative and operational aspects of the study. Responsibilities include managing personnel, coordinating between various teams (research, clinical, and laboratory), monitoring progress, and ensuring that study timelines, goals, and budgets are met.
Data Analyst	The Data Analyst will be responsible for managing and analyzing all study data. Duties include developing data management plans, ensuring data quality and integrity, performing statistical analyses, generating reports, and presenting findings to the research team. The Data Analyst will also ensure compliance with data privacy and security guidelines.

Recruitment

Target Population:

Healthy adults aged 18–55 years, residing in Karachi or available to relocate for the duration of the study including all follow-up visits. Individuals in good health with no significant medical history and who meet the study's inclusion criteria are able to provide informed consent.

Recruitment Channels:

- **Community Outreach:** We will leverage local community centers, workplaces, and universities to raise awareness about the study. The Outreach and Research Program of the Department of Paediatrics and Child Health operates four community field sites across Karachi for research purposes. These sites offer access to a broad and diverse population, representing various ethnic backgrounds and socioeconomic statuses, ensuring a wide range of exposures for the study.
    1. Ibrahim Hyderi
    2. Ali Akber Shah (Korangi)
    3. Ali Akber Shah (Korangi) Extension
    4. Rehri Goth
    5. Bhains Colony
- **AKU Network:** We will promote the study through the Aga Khan University Hospital's outpatient clinics and affiliated healthcare networks by distributing pamphlets with information regarding the procedure and safety of the Challenge study and contact details for further clarification and providing details about the study, eligibility criteria, and benefits of participation. AKU staff and their families are also welcome to take part in the study.

Pre-screening

Participants will be recruited from field sites, outpatient clinics, and healthcare networks. A research associate will administer a pre-screening questionnaire, which the study instructor will review. Eligible participants may be invited to AKU for a detailed discussion about the study. After addressing their questions, they will provide written informed consent to take part in the study if found eligible.

Consent

Informed Consent Process:

The informed consent process is a crucial step in participant enrollment, ensuring that participants fully understand the study before agreeing to take part. A comprehensive verbal explanation of the study will be provided to participants in Urdu, their native language, to ensure clarity and accessibility. Additionally,

participants will be given physical copies of the participant information sheet, also translated into Urdu, allowing them to review the details at their own pace and make an informed decision.

Deliberation Period:

Participants will be given adequate time to carefully review the consent form and reflect on their decision. They will have the opportunity to ask additional questions to clarify any concerns.

- Official Written Consent:

Participants who decide to proceed with the study will sign the consent form in the presence of the study physician and a witness. At this stage, participants will:

1. Confirm their understanding and agreement to the study requirements, including hospital stay, infection control measures, contraceptive measures, and follow-up visits and ensure all information they have provided is accurate.
2. Provide the contact details of a designated 24-hour contact person, who will be available to assist if participants become unreachable during the study. The contact person will provide written agreement to this role.

Screening

Participants will be requested to come to AKU where they will undergo a comprehensive screening process under the supervision of a study physician. This process will include a review of medical history, physical examination, blood tests, ultrasound of the abdomen, and an ECG. A separate room will be assigned to maintain confidentiality.

Screening will ensure participants meet all inclusion and exclusion criteria and are fit for participation. A summary of screening procedures is provided in Table 6.

Study Eligibility

Participants of any gender or ethnicity, aged 18-55 years inclusive, who are in good health (as determined by a study doctor, medical investigation and are able to provide written informed consent will be eligible for inclusion in this study.

Inclusion Criteria

Participants must satisfy all the following criteria to be considered eligible for the study:

- Willing and able to give informed consent.
- Aged 18 -55yrs.

- In good health as determined by medical history, physical examination and clinical judgment of the study team.
- Available for all required appointments (agree to being residential in Karachi until confirmed clearance).
- Able to comply with all study requirements, including infection control precautions.
- Agree to medical record review.
- Agree to avoid antipyretic/anti-inflammatory treatment from challenge until advised by a study doctor or until 14 days after challenge.
- Agree to refrain from donating blood for the duration of the study.
- Agree to avoid vaccination during the study period.
- For participants of childbearing potential, they should be willing to ensure that they or their partner use effective contraception until confirmed clearance.
- Agree to remaining admitted in the hospital and take leave from work for a period of 10-20 days

Exclusion Criteria

The participant will not be enrolled if any of the following apply:

- History of significant organ/system disease that could interfere with study conduct or completion, in the opinion of the study team. Including, for example, but not restricted to:
  - Cardiovascular disease
  - Respiratory disease
  - Haematological disease
  - Endocrine disorders
  - Renal or bladder disease, including history of renal calculi
  - Biliary tract disease, including biliary colic, asymptomatic gallstones or polyps or previous cholecystectomy
  - Gastro-intestinal disease including chronic diarrhoea, inflammatory bowel disease, irritable bowel syndrome or diseases requiring use of regular antacids, H2-receptor antagonists, proton pump inhibitors, laxatives or prokinetic agents
  - Neurological disease
  - Metabolic disease
  - Psychiatric illness requiring hospitalisation, or other mental health condition
  - Known or suspected drug abuse
  - Known or suspected alcohol misuse
  - Infectious disease
  - Coagulation disorder
- 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

  - History of auto-immune disease
  - Human Immunodeficiency Virus 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)
- HLA-B27 positive
- 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 doctors.
- Weight less than 50 kg.
- Presence of implants or prosthetic material.
- Anyone taking long-term medication (e.g. analgesia, anti-inflammatories or antibiotics) that may affect symptom reporting or interpretation of the study results or that may interact with antibiotics used for treatment of paratyphoid A (in particular drugs that could prolong corrected QT interval).
- Contraindication to fluoroquinolones, macrolide antibiotics, co-trimoxazole, cefixime or ceftriaxone.
- Family history of aneurysmal disease.
- Participants who are pregnant, lactating or unwilling to ensure that they or their partner use effective contraception 30 days until three negative stool samples confirm clearance.
- 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) or plan to enroll in another research study during the follow-up study period.
- Significant blood donation or planned blood donation prior to enrolment.
- Detection of any abnormal results from screening investigations (at the clinical discretion of the study team).
- Have a prolonged corrected QT interval (>450 milliseconds) or significant clinical abnormality on ECG screening.
- Evidence of HIV or Hepatitis B or Hepatitis C infection.
- Presence of gallbladder abnormalities such as stones/calculi or polyps, as seen on ultrasound.
- Presence of any degree of haematuria.
- Inability to comply with any of the study requirements (at the discretion of the study staff and the participant's General Practitioner).
- Any other social, psychological or health issues which, in the opinion of the study staff, may

- put the participant or their contacts at risk because of participation on the study
- adversely affect the interpretation of the primary endpoint data,
- Impair the participant's ability to participate in the study.

Temporary Exclusion at Challenge

Participants will be temporarily excluded from challenge if presenting at the challenge visit with any of the following:

- Acute or acute-on-chronic infection within the previous 5 days, that is considered clinically significant by the Investigator
- History of any systemic antibiotic therapy during the previous 5 days for short-acting antibiotics and 15 days for long-acting antibiotics.
- Any systemic corticosteroid (or equivalent) treatment in the previous 14 days, or for more than seven consecutive days within the past 3 months.
- Therapy with antacids, proton pump inhibitors or H2-receptor antagonists or prokinetic agents within 24 hours prior to challenge.
- Receipt of any vaccine in preceding 7 days.
- Plan to receive any vaccine within 21 days following challenge.

Figure 5: Enrollment pathway

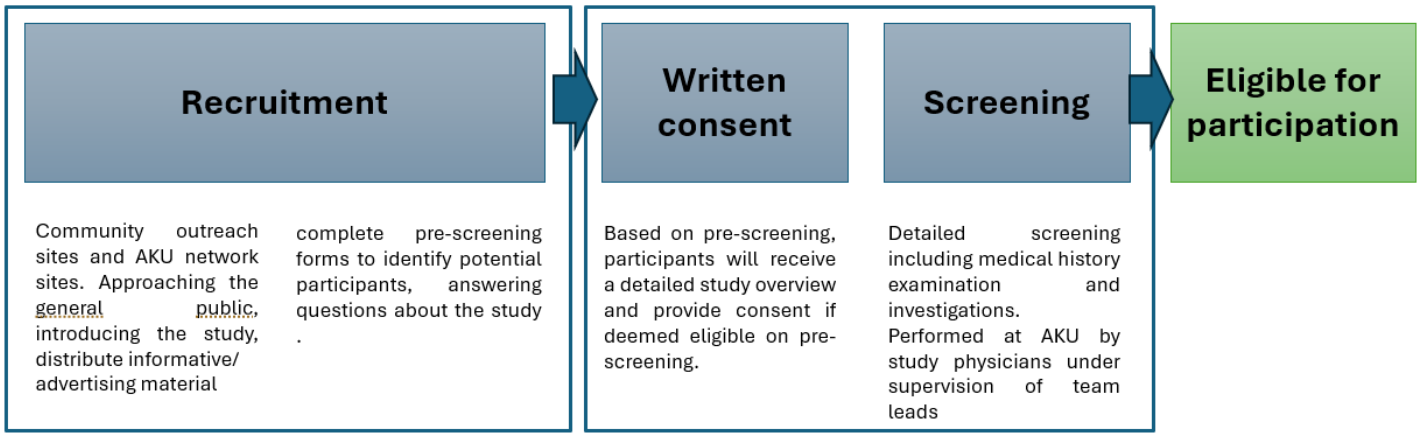


Table 6: Summary of screening procedures and tests

Screening procedures
Consent presentation, discussion
Written Informed consent (including optional biobank/ bio-repository consent)
Medical history (also collected during online eligibility assessment via specific questions, which may require clarification prior to in-person visit)
Mood assessment using HADS questionnaire
Physical examination including vital signs

Screening Tests	
Blood sample	CBC
Blood sample	Urea, electrolytes (Na and K) & creatinine, bilirubin, ALT, ALP, albumin, amylase, C-reactive protein
Blood sample	Serologies: HIV, HBsAg, HCV Antibodies
Blood sample	TTG and IgA level
Blood sample	HLA B27
Blood (point of care)	Blood glucose/HbA1c
Urine sample	Urine dipstick
Urine sample	Urine Pregnancy test
12 lead ECG	
Ultrasound scan: targeted gallbladder ultrasound	

In-Patient protocol

After a participant has been deemed eligible on pre-screening assessment and has shown interest in participation they will be called to AKU where they will undergo medical history, examination, and laboratory tests as part of screening under supervision of study physician and study nurse. Once they clear all inclusion and exclusion criteria, signed fully informed written consent with an optional clause for biobank (University of Oxford) and/or bio-repository (AKU) consent and provided all contact details (refer to recruitment section and figure 5) they will be admitted to the in-patient unit for challenge administration. If more than 120 days have elapsed from the screening visit to the challenge visit, some of the screening visit procedures may be repeated before admission.

Site:

Participants will be required to spend 10-20 days at the Aga Khan Hospital. The third floor of Princess Zahra Pavillion has been approved for use for this purpose. 3-5 participants will be admitted at a time to single rooms and administered a challenge dose (refer to section Procedures). Study physicians and nurses will monitor the volunteers 24 hours for the entire duration of the stay, record and review any adverse events (AE). 3 meals per day will be provided to each patient from the hospital, no food or drinks from outside will be allowed as to minimize chances of food or waterborne infections.

Monitoring

Close monitoring of all vital signs including blood pressure, heart rate, respiratory rate, temperature, and oxygen saturation will be done 6-8 hourly or more frequently if needed by the designated study nurse on duty and documented in eCRFs. Written instructions along with training will be provided to the team for reporting any AE and verbal continued consent will be sought daily. Mood assessment using Hospital Anxiety and Depression Scale (HADS) will be performed on D0 and D14 or at paratyphoid diagnosis (PD). Details of participant monitoring are summarized in table 3 and table 7.

Sampling

Blood samples will be collected by a trained study phlebotomist at defined time points throughout the study duration. Stool and urine samples will be taken by the participants themselves. Details of samples collected are summarized in table 3 (for all participants receiving the challenge) and table 4 (for participants diagnosed with Salmonella Paratyphoid after challenge).

Visitation Guidelines

To minimize the risk of infection, strict movement and exposure restrictions will be in place for participants challenged with *Salmonella* Paratyphi. To protect both the participant and vulnerable individuals—including children under 2 years old, the elderly, and immunocompromised individuals (e.g., those with uncontrolled diabetes, undergoing chemotherapy, or taking long-term steroids)—visits will not be permitted during the challenge phase and for five days after starting antibiotics.

Participants must adhere to strict isolation and infection control protocols and remain within designated premises.

Hospital Discharge Protocol

Participants will stay in the in-patient facility for 10–20 days and will be discharged based on the following criteria:

If Paratyphoid Infection is Diagnosed Before Day 14:

Once a Paratyphoid infection is confirmed according to the specified criteria, the participant will begin a 14-day course of antibiotics. They will remain in the facility for close monitoring, including daily blood and stool cultures to ensure treatment compliance and response. Participants will stay hospitalized for a minimum of 10 days from the start of the challenge phase and will only be discharged once they have completed at least 5 days of antibiotics and are symptom-free, whichever period is longer.

Discharge Timing:

Participants may be safely discharged after day 10 of the challenge phase after completing at least five days of antibiotic treatment, provided they meet specific criteria. Discharge will only occur if the participant is fever-free, a repeat blood culture taken 48 hours after starting antibiotics shows no growth.

For participants who are not diagnosed based on blood culture or symptom criteria, antibiotics will be initiated on Day 14. These participants will remain under observation for an additional five days before being discharged.

These measures ensure the participant's safety while minimizing the risk of infection to others.

Treatment Completion

After hospital discharge, participants will complete their remaining course of antibiotics at home.

Out-patient and follow-up visits

The first follow-up for all participants will occur on day 21 post-challenge through a phone call. The call will be made by a study nurse/physician who will document any symptoms experienced by the participant and ensure compliance of treatment. This will be followed by a long-term follow-up phase with in-person visits scheduled on days 28, 90, and 180 post-challenge. Details of the procedures and laboratory tests planned for these follow-up visits are provided in Table 7.

Table 7: Summary of study inpatient course and clinic visits

		Challenge phase (from challenge until D14 if not diagnosed)				If Paratyphoid diagnosis made (Diagnosed participant schedule)		Acute Follow Up phase		Long-term follow-up phase	
	Screening	Pre-challenge/ D0	D1 to D19	D14	D20	PD	PD+12hrs[1]	PD 1 (+24), 2 (+48), 3 (+72), 4 (+96)5 (+120hrs), D14PD[5]	D21 (phone visit)	D28	D90, D180
Enrolment		x									
Written Consent	x										
Biobank Consent	x										
Consent Questionnaire	x										
Verbal continued consent		x	x	x	x	x	x	x	x	x	x
AE recorded and reviewed[1]		x	x		x	x	x	x	x	x	
SAE recorded and reviewed		x	x		x	x	x	x	x	x	x
NOK contact details		x									
Medical history	x	x	x		x	x	x	x	x	x	x
Physical examination[2]	x					x					
Vital signs	x	x	x		x	x	x	x	x	x	
Urine pregnancy test	x	x								x	
Urine sample	x			x		x					
Stool sample[3]		x			x	x	x	x	x	x	x

[1]PD+12hrs optional

		Challenge phase (from challenge until D14 if not diagnosed)				If Paratyphoid diagnosis made (Diagnosed participant schedule)		Acute Follow Up phase		Long-term follow-up phase	
	Screening	Pre-challenge/ D0	D1 to D19	D14	D20	PD	PD+12hrs[1]	PD 1 (+24), 2 (+48), 3 (+72), 4 (+96)5 (+120hrs), D14PD[5]	D21 (phone visit)	D28	D90, D180
Blood sample	x	x			x	x	x	X	x	x	x
Saliva sample[4]		x		x			x	x			x
12 lead ECG	x										
Ultrasound	x										
Mood assessment	x	x		X		x					
Challenge with *S.* Paratyphi A		x									
Commence antibiotics				x		x					

[1] AE will be recorded from enrolment to D28, SAE will be recorded from enrolment until D180,

[2] This procedure may be performed at any time in the study at the discretion of the study team, eg if clinically indicated for participant

[3] Stool samples will also be collected 1 week after completion of antibiotic course, until 3 successive stool samples are culture negative for S. Paratyphi A (clearance samples)

[4] This will only be collected for a small subset of participants

5For D14PD samples the participant will either visit site or study team may visit participants residence for collection

Table 8: Window periods on visits

	Pre-challenge	Challenge day	Challenge phase (first 14 days)	If Paratyphoid diagnosis made (Diagnosed participant schedule)					Acute Follow Up phase		Long term Follow-up phase	
Visit name	D-2	0	D1-20	PD	PD+12 (if needed)	PD+1 (24hrs)	PD+2 (48hrs) PD+3 (72hrs) PD+4 (96hrs) PD+5 (120hrs)	D14PD	D28		D90	D180
Window period (days, unless specified)	NA	NA	NA	NA	-6 hrs to +6 hrs	-0.5 to +1	-0.5 to +1	NA	NA		+/-14	

Procedures

Challenge Material Preparation

A challenge dose of 1-5 x 10^3 CFU/30 ml *Salmonella* Paratyphi A (NVGH308 strain) will be used as the starting dose in controlled human infection models involving *Salmonella* Paratyphi A, with a logarithmic increase in dose until infection symptoms appear. The challenge doses are provided by Novartis Vaccines for Global Health (NVGH) and has been manufactured to Good Manufacturing Practice (GMP) standards by GenIbet (Portugal). The NVGH308 strain has been typed as *Salmonella* enterica serovar Paratyphi A and was originally isolated from a patient in Kathmandu (Nepal) who participated in a clinical study conducted by the Oxford University Clinical Research Unit. This is the same strain which has been successfully used in multiple CHIM studies at Oxford University. The challenge dose will be prepared with sodium bicarbonate solution following the SOP Preparation of *Salmonella* Paratyphi A Challenge Dose, V.08 or subsequent updated versions.

Dedicated lab staff will have access to the challenged material and its preparation, dose calculation, storage, and transportation. Inoculum stock will be handled in a designated Class II biological safety cabinet. Performance quality control (QC) will be checked and verified for all the equipment used in these procedures. Strict adherence to approved SOPs, including the provenance of reagents, cleaning protocols,

purity checks, and sequencing, will be monitored by the lab team supervisor. The study sites and laboratory activities may be audited by an independent or internal auditor during or after the study to ensure protocol compliance.

Laboratory assays

Different patients' samples collected at baseline and various time points will be subjected to blood & stool cultures, as well as hematological, serological and biochemical analysis. Hematological and biochemical assays include a complete blood count (CBC), serum electrolytes, urea, creatinine, C-reactive protein, and liver function (bilirubin, alanine transaminase, alkaline phosphatase, amylase, and albumin). Serological analysis including anti-LPS IgG & IgA, FcR binding, antibody-dependent neutrophil phagocytosis (ADNP), antibody-dependent monocyte phagocytosis (ADMP), antibody-dependent NK cell activity (ADNK), and ELISpots (for B memory cells and T cells), assays will be optimized and performed at Clinical Lab at AKU. Additionally, B-cell receptor (BCR) and T-cell receptor (TCR) sequencing will be employed to provide insights into immune repertoire changes during immune challenges. This will be done at both Oxford and AKU laboratories. Peripheral Blood Mononuclear Cells (PBMCs) from whole blood collected in Sodium-heparin tubes will be separated at AKU Karachi to measure cell mediated immune response. B and T cell ELISpot will be performed at AKU with the assistance of our collaborator, Oxford University, who will fully validate the protocol to conduct these tests. PBMCs will be stored in aliquots in liquid nitrogen till further use. The blood taken for all participants will not be the same and will depend on the day of Paratyphoid diagnosis. For undiagnosed participants, 7 mL of blood will be collected on alternate days for CBC and biochemistry analysis, and 10 mL of blood will be collected daily for blood culture. Additionally, 40 mL of blood will be collected for PBMCs at eight times-points over the 6-month study period.

For participants diagnosed with Paratyphoid infection, 17 mL of blood will be collected daily until discharge and again on day 14 of antibiotic treatment. An additional 40 mL of blood will be collected for PBMC at four time points. The quantity of blood taken for different samples is outlined in Tables 9 and 10.

We will send around 5-10% of all samples tested in Pakistan to our collaborative laboratory at University of Oxford for QC analysis. This would help us validate our results. System serology experiments will also be

performed at University of Oxford and patient serum and PBMC samples will be shipped there after executing an MTA.

Table 9 volume of blood taken in undiagnosed participants

Pre-Diagnosis

	Challenge	Challenge Phase																				Follow up		
Sample	D0	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14	D15	D16	D17	D18	D19	D20	D28	D90	D180
Urine - Pregnancy test	Yes																					Yes		
Stool sample for SAFETY	1 Pot	1 Pot	1 Pot	1 Pot	1 Pot	1 Pot	1 Pot	1 Pot	1 Pot	1 Pot	1 Pot	1 Pot	1 Pot	1 Pot	1 Pot	1 Pot	1 Pot	1 Pot	1 Pot	1 Pot	1 Pot	1 Pot	1 Pot	1 Pot
CBC	2 mL		2 mL		2 mL		2 mL		2 mL		2 mL		2 mL		2 mL		2 mL		2 mL		2 mL	2 mL	2 mL	2 mL
U&Es, LFTs & CRP	5 mL		5 mL		5 mL		5 mL		5 mL		5 mL		5 mL		5 mL		5 mL		5 mL		5 mL	5 mL	5 mL	5 mL
Blood culture		10 mL	10 mL	10 mL	10 mL	10 mL	10 mL	10 mL	10 mL	10 mL	10 mL	10 mL	10 mL	10 mL	10 mL	10 mL	10 mL	10 mL	10 mL	10 mL	10 mL			
PBMC, plasma, Cytof	40 mL	40 mL						40 mL			40 mL				40 mL						40 mL	40 mL	40 mL	40 mL
Only Plasma and Cytof		10 mL																						
Serum	12 mL																							
Functional Genomics / RNA (PAXgene)	2.5 mL	2.5 mL									2.5 mL											2.5 mL		
Saliva	4 mL														4 mL								4 mL	4 mL

Table 10 volume of blood taken once participants are diagnosed with Paratyphoid infection

Post-Diagnosis

Sample	PD	PD +12hrs	PD +1 (24hrs)	PD +2 (48hrs)	PD +3 (72hrs)	PD +4 (96hrs)	PD +5 (120hrs)	D14PD
Stool sample for SAFETY	1 Pot	1 Pot	1 Pot	1 Pot	1 Pot	1 Pot	1 Pot	1 Pot
CBC	2 mL	2 mL	2 mL	2 mL	2 mL	2 mL	2 mL	2 mL
U&Es, LFTs & CRP	5 mL	5 mL	5 mL	5 mL	5 mL	5 mL	5 mL	5 mL
Blood culture	10 mL	10 mL	10 mL	10 mL	10 mL	10 mL	10 mL	10 mL
PBMC, plasma, Cytof	40 mL	40 mL	40 mL				40 mL	
Functional Genomics / RNA (PAXgene)	2.5 mL							
Bacterial Quantification	20 mL							
Saliva		4 mL	4 mL	4 mL	4 mL	4 mL	4 mL	

Retention of Samples

Participants will be informed that they may opt in to the Oxford Vaccine Centre (OVC) Biobank (REC 21/SC/0161) and /or the AKU Bio-repository to allow long-term storage of biological samples collected under the PARACHIM protocol for use in possible future research once the study closes. Participants will be informed that declining to take part in the OVC Biobank study or declining long-term storage (at AKU)

after the end of the study will not affect their participation in this study. If a participant elects to decline to take part in the OVC Biobank, all of their remaining samples outside of Pakistan will be destroyed or returned to AKU after the required period of storage to meet Good Clinical Practice (GCP). If a participant elects to decline to take part in the AKU Bio-repository their samples will be destroyed at the end of the study.

In accordance with country guidelines, where consent for long-term storage of samples is provided, AKU permits sample storage for up to 7 years following the conclusion of the study. For storage beyond this period, additional approval must be obtained (from the appropriate ethics committee). The Oxford Vaccine Group (OVG) has an ethically approved Biobank. OVG has been collecting samples from trial participants for 30 years, and over this time a huge library of retained samples has been kept and used for future research. Since the introduction of the Human Tissue Act in 2006 explicit consent for this storage and use has been essential. The OVC Biobank was initiated in 2009. The samples from this study may be stored in the OVC Biobank indefinitely, provided the participant provides a voluntary consent. If the participant does not consent to biobank or biorepository storage, it will not affect their participation in the study.

Study Oversight

Joint Steering Committee

A Joint Steering Committee (JSC) and an independent Data Safety Monitoring Board (DSMB) will oversee the study and advise the study team on key issues including any changes or amendments in the methodology of the study, decision related to premature conclusion of the study due to safety concerns and/or any other technical, methodological or safety concerns.

The JSC will comprise of study PI, Co-Is, collaborators and study statistician. JSC will be referred for deliberation and expert guidance related to the technical issues regarding the study protocol, amendments, design etc. They will also be informed regularly about the progress of the study and challenges being faced. The JSC will be an internal body which will meet fortnightly or need-based to discuss the study progress, enrollment, challenges, and issues.

Data Safety Monitoring Board (DSMB)

A Data Safety Monitoring Board (DSMB) will be constituted to oversee the safety signals, ensuring participants' safety, assess study progress and make recommendations about the continuation of the

study to the study investigators. All collaborators will be non-voting observers in DSMB along with an independent statistician/trialist, an adult infectious disease consultant and an epidemiologist as voting members. The DSMB will evaluate frequency of adverse events, safety data as specified in the DSMB charter (to be developed later). The DSMB will make recommendations concerning the conduct, continuation, or modification of the study for safety reasons to the investigators. The DSMB will review serious adverse events (SAE) deemed possibly, probably, or definitively related to study interventions. The DSMB will be notified within 24 hours of the Investigators' being aware of their occurrence. The DSMB can recommend placing the study on hold if deemed necessary following a study intervention-related SAE. The first dose review by the DSMB will be conducted after the first 3 participants have been challenged and followed up for 14 days. The second cohort will be another 3 participants. After we challenged the first 6 participants (cohorts 1 and 2), the DSMB will review the safety data and if there are no safety concerns, we will increase the cohort size to 5 participants. A review will be done after each of the cohorts reaching the 14 days of follow-up. Further, the DSMB will meet quarterly to evaluate data for any safety signals, SAEs, and any other issues. Early discontinuation of the trial may be considered if a significant proportion of participants (>10% overall) experience a suspected unexpected serious adverse reaction (SUSAR), or SAE.

Adverse event (AE), SAE and SUSAR are defined in appendix 1. SUSARS will be reported to the Aga Khan University ethical committee, national bioethics committee and DSMB within 24 hours.

Names and qualifications of DSMB members:

Rashida Ferrand Internal medicine and clinical specialist, Epidemiology. London School of Hygiene & Tropical Medicine

Prof Tom Darton DTM&H MRCP(UK)(Infectious Diseases) FRCPath. University of Sheffield, School of Medicine and Population Health

Kawsar Talaat- Pediatrics, Internal Medicine and Infectious Diseases. Johns Hopkins- Bloomberg School of Public health, International Health, Global Disease Epidemiology and Control

Celina Jin Senior Clinical Research Officer, Infectious Diseases and Immune Defence, Oxford Vaccine group

Moreno Ursino PhD, Mathematics for Engineering Sciences, Research Engineer CHU Robert Debré University Hospital, Public Assistance Hospitals of Paris.

Quality assurance procedures

Risk assessment

A REDCap project will be built to securely capture and store study data, compliant with all requirements according to ICH/GCP E6 [R2] Section 5.5. A risk assessment and monitoring plan will be prepared before

the study opens and will be reviewed as necessary over the course of the study to reflect significant changes to the protocol or outcomes of monitoring activities.

Monitoring

Study oversight and site quality assurance monitoring will be undertaken by local, independent monitoring organizations in Pakistan as well as centralized oversight from Metrics Research (Pvt) (Ltd).

Monitoring will be performed according to Good Clinical Practice (GCP) guidelines. In Pakistan Metrics Research will be responsible for study quality assurance monitoring activities. Metrics Research will monitor and verify that the study is conducted as per the protocol and the SOPs, and that the generated data are documented, and reported in compliance with the protocol, GCP and the applicable regulatory requirements. The monitors will provide regulatory support throughout the project, site preparation and initiation activities, ongoing remote and regular on-site monitoring including the review of the study documents, consent forms, assisting the site with maintaining the site-investigator file and preparing study close-out reports. The site will provide direct access to all study related source data/documents and reports for the purpose of monitoring and auditing by the Sponsor institution and inspection by local and regulatory authorities.

ETHICAL AND REGULATORY CONSIDERATIONS

Research Ethics Approval & Local Governance Authorization

The Investigator will ensure that this study is conducted in accordance with the principles of the Declaration of Helsinki and in accordance with Good Clinical Practice. Ethical approval will be obtained from both the Oxford Tropical Research Ethics Committee (OxTREC), University of Oxford, UK and the Ethical review committee of Aga Khan University (AKU ERC), and from the National Bioethics committee of Pakistan (NBC).

Amendments to the protocol

This study will be conducted in compliance with the current version of the protocol. Any change to the protocol document, written Informed Consent Form or any other document that affects the scientific intent, study design, participant safety, or may affect the participant willingness to continue participation in the study is considered an amendment and therefore will be written and filed as an amendment to this

protocol and/or informed consent form. All such amendments will be submitted to the both, University of Oxford OxTREC and AKU-ERC for approval prior to being implemented.

Confidentiality

Participant confidentiality is strictly held in trust by the participating investigators, research staff, and the collaborating institution and their agents. This confidentiality is extended to cover testing of biological samples in addition to the clinical information relating to the participants.

The study data and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party.

All laboratory specimens, screening forms, and other records, such as reporting of adverse events that leave the site will be identified only by the participant study ID and will not have any kind of participants' identifier to maintain participant confidentiality. In addition, no data will be shared outside Pakistan without prior data transfer agreement (DTA).

Clinical information will not be released without written permission of the participant, except as necessary for monitoring by regulatory agencies.

Participant Incentive

A fixed incentive will be offered to all participants at certain time points of the study (end of challenge phase/hospital stay and at each follow-up) of the study. The amount will be disclosed to participants in advance, enabling them to make an informed decision about participation.

For the management of these monetary incentives, a log will be maintained to document cash distribution, ensuring transparency and enabling audits.

Risk Mitigation Plan

Participant Safety, Adverse Events, and Antimicrobial Resistance (AMR) Mitigation

The deliberate exposure of participants to *Salmonella* Paratyphi A carries inherent risks, including moderate to severe symptoms such as prolonged fever, diarrhea, and dehydration if left untreated. To mitigate these risks, the study will use a strain previously tested in UK trials, ensuring its safety profile. Comprehensive pre-screening and strict eligibility criteria will be implemented to enroll only healthy individuals. The study will be conducted in a controlled inpatient setting at AKU, where participants will

receive 24/7 medical supervision from trained physicians and nurses, enabling early detection and prompt management of adverse events. All participants—whether diagnosed or not—will receive antibiotic treatment and undergo structured follow-ups to confirm complete infection clearance.

Given the high prevalence of drug-resistance in Pakistan, the study will use a strain fully susceptible to the selected study antibiotics, ensuring both safety and efficacy. To prevent resistance development and bacterial persistence, all participants will complete a full 14-day antibiotic course. Discharge will only occur after confirming infection clearance with negative blood cultures**.**

Preventing Environmental Contamination and Community Transmission

The potential for *S*. Paratyphi A to spread within the community poses a significant risk. To prevent unintended transmission, participants will be housed in single rooms with dedicated restrooms within a controlled, biosafety-compliant facility. Strict infection control measures will be enforced, including proper waste disposal and restricted visitation during the challenge phase. Participants will also receive clear guidelines on Water, Sanitation, and Hygiene (WASH) protocols to minimize the risk of spreading the bacteria after discharge. Those who test positive for *S.* Paratyphi A in their stool will be required to provide three consecutive negative stool cultures before being considered free of the pathogen.

Inadequate response to first-line antibiotics:

In the event that a study participant does not respond adequately to the first-line antibiotic—ciprofloxacin—within 5 days of treatment initiation, defined as persistence of fever or lack of culture clearance, the participant will be evaluated by the adult infectious diseases specialist, who also serves as a co-investigator on the study team. Based on this clinical assessment, the decision will be made to escalate therapy to either second-line or third-line antibiotics. Each of the antibiotics under consideration has a well-established safety profile, but potential side effects must be carefully monitored.

To mitigate the risks associated with inadequate response and potential drug-related adverse effects, the study will implement the following safeguards:

1. **Continuous Clinical Supervision**: Participants will remain under close medical supervision until they are clinically stable—defined as fever-free and culture-negative.

2. **Specialist Oversight**: The presence of an adult infectious diseases specialist as part of the core study team ensures timely and expert decision-making in response to treatment failure or adverse effects.

3. **Independent clinical oversight: In addition to the infectious disease's expert on the study team, the volunteers will be monitored by an** independent clinical oversight by CRO to minimise risk to the participant.

4. **Frequent Safety Monitoring**: Routine blood safety tests (including CBC, liver and renal function tests) will be performed regularly to detect early signs of drug toxicity or treatment complications.

5. **Home-Based Care (Where Applicable):** For participants requiring prolonged IV therapy (e.g., ceftriaxone), home administration will be managed by trained study personnel to ensure adherence and immediate reporting of any concerns.

Through these interventions, the study aims to ensure both clinical safety and therapeutic efficacy, while minimizing risks related to antimicrobial treatment failure or adverse events.

Ethical and Regulatory Considerations

Conducting a Controlled Human Infection Model (CHIM) study in an endemic setting raises ethical concerns regarding the deliberate exposure of participants to infection. However, *S.* Paratyphi A is already endemic in Pakistan, meaning that individuals in the study are not at increased risk beyond their routine environmental exposure. Participants will receive detailed verbal and written explanations of the study, its risks, and their rights in local language before enrollment. A deliberation period will be provided to ensure informed decision-making. Daily verbal consent will be obtained throughout the study to confirm participants' ongoing willingness to continue. The study will also undergo rigorous ethical review by the **Aga Khan University Ethical Review Committee (AKU-ERC), the National Bioethics Committee of Pakistan (NBC), and Oxford Tropical Research Ethics Committee (OxTREC)**. A Data Safety Monitoring Board (DSMB) will independently oversee participant safety and review all adverse events in real-time.

Managing Psychosocial and Logistical Challenges

The extended hospital stay of 10-20 days may cause psychological distress or reluctance to participate. To enhance participant comfort, they will be accommodated in private rooms equipped with WiFi and

televisions. Mental health will be monitored using the **Hospital Anxiety and Depression Scale (HADS)**, with assessments conducted at baseline and multiple points during the study. Logistical challenges related to compliance with follow-up visits will be addressed through structured follow-ups. If a participant cannot be reached for follow-up, an alternate 24/7 contact person (designated before study enrollment) will be contacted.

Ensuring Participant Retention and Compliance

Before enrollment, all study details and procedures — including the length of hospital stay, investigations, follow-up visits, and required precautions — will be clearly explained to participants. This will allow them to make an informed decision about participating. Only participants who agree to stay enrolled until study completion and sign the informed consent form will be included in the study.

All investigations, including laboratory tests and treatment specified in the study, will be provided at no cost to the participants.

For the management of monetary incentives, a log will be maintained to document cash distribution and transfers, ensuring transparency and enabling audits.

Political instability:

Political instability in Pakistan may disrupt study operations, including participant recruitment, hospital accessibility, and timely follow-ups. To mitigate these risks, recruitment will be conducted through AKU community sites and the AKU network, where research teams have established long-standing relationships with the community. These trusted connections remain stable despite political unrest, ensuring minimal disruption to recruitment efforts. Additionally, the study team will implement contingency measures such as flexible scheduling for participant visits, alternative transportation arrangements, and remote follow-up options when necessary.

Community Engagement:

Effective community engagement is essential for the success of this study, ensuring awareness, transparency, and trust among potential participants. To reach a diverse population, the study team will actively engage with communities by approaching congregational places such as mosques, where large gatherings provide an opportunity to inform people about the study. Trained research staff will conduct informational sessions, addressing concerns and answering questions to foster understanding and

participation. Additionally, advertisement materials with key study details, will be distributed in local markets and community hubs to maximize outreach. These efforts will help ensure broad representation and informed decision-making among potential participants.

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