





OPEN LETTER

REVISED **The Challenge Non-Typhoidal *Salmonella* (CHANTS) Consortium: Development of a non-typhoidal *Salmonella* controlled human infection model: Report from a consultation group workshop, 05 July 2022, London, UK**

[version 2; peer review: 2 approved]

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Abstract


Invasive non-typhoidal *Salmonella* disease (iNTS) is a major cause of morbidity and mortality globally, particularly as a cause of bloodstream infection in children and immunocompromised adults in sub-Saharan Africa. Vaccines to prevent non-typhoidal *Salmonella* (NTS) would represent a valuable public health tool in this setting to avert cases and prevent expansion of antimicrobial resistance. Several NTS and combination typhoidal-NTS vaccine candidates are in early-stage development, although the pathway to licensure is unclear due to challenges in conducting large phase III field trials.

Controlled human infection models (CHIM) present an opportunity to accelerate vaccine development for a range of enteric pathogens. Several recent typhoidal *Salmonella* CHIMs have been conducted safely and have played pivotal roles in progressing vaccine candidates to pre-qualification and licensure. The Challenge Non-Typhoidal *Salmonella* (CHANTS) consortium has been formed with funding from the Wellcome Trust, to deliver the first NTS CHIM, which can act as a

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platform for future vaccine evaluation.

This paper reports the conclusions of a consultation group workshop convened with key stakeholders. The aims of this meeting were to: (1) define the rationale for an NTS CHIM (2) map the NTS vaccine pipeline (3) refine study design and (4) establish potential future use cases.

Keywords

Salmonella; Salmonella Typhimurium; Non-Typhoidal Salmonella; Human Challenge Model; Controlled Human Infection Model; Vaccines

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Any reports and responses or comments on the article can be found at the end of the article.

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REVISED Amendments from Version 1

This manuscript has been updated in response to comments from peer review. The introductory section has been updated to define the nomenclature surrounding non typhoidal *Salmonella* (NTS) more clearly, including diarrhoeal NTS disease (dNTS) and invasive NTS disease (iNTS). The section on NTS vaccines has also been updated to clarify the serovars currently targeted by vaccines in early-stage development, and the rationale for prioritising protection against iNTS. Additional references have also been included to reflect contemporary literature. Full responses to peer review comments can be found online at [Wellcome Open Research](#).

Any further responses from the reviewers can be found at the end of the article

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Introduction

Controlled human infection models (CHIM) provide a valuable method to study infectious diseases¹. Studies involving the controlled exposure of healthy volunteers to pathogenic or attenuated pathogens have been used for several decades to better understand the pathophysiology of disease – complementing insights from pre-clinical and field studies. Historically, a major use of such models has been in the field of vaccine development, in which a CHIM can be used to generate an early indication of vaccine efficacy and to suggest immune correlates of protection². This is particularly true for enteric pathogens for which, to date, CHIM studies have had a pivotal role in accelerating vaccine development for pathogens such as *Vibrio cholerae* and *Salmonella* Typhi, and are predicted to have an important role in vaccine development for *Shigella* spp³⁻⁵.

There is growing interest and awareness by regulatory agencies of the utility of CHIM studies in vaccine development for diseases which occur sporadically, or for which field efficacy trials are difficult to conduct. One such disease is invasive non-typhoidal *Salmonella* (iNTS). Over 2600 non-typhoidal *Salmonella* (NTS) serovars exist, which affect a broad host-range and are associated with a spectrum of clinical phenotypes in human infection, exhibiting significant geographic variation⁶. In high-income settings, NTS infections primarily cause a self-limiting enterocolitis, termed diarrhoeal NTS (dNTS). In contrast, invasive non-typhoidal *Salmonella* disease (iNTS) typically presents as a non-specific febrile illness – often without gastrointestinal symptoms – following invasion into sterile sites causing bloodstream infection, meningitis, or sepsis⁷. iNTS disease is a major cause of morbidity and mortality in sub-Saharan Africa (SSA), primarily affecting immunocompromised hosts. The most recent GBD 2019 data estimate iNTS were responsible for 535,000 cases, 77,500 deaths, and 4.26 million lost disability-adjusted life years (DALYs) in 2017⁸. The precise burden of disease is, however, relatively poorly understood and estimates vary depending on case-definitions and methods of surveillance^{9,10}. The global burden of diarrhoeal disease

attributable to non-typhoidal *Salmonella* serovars (dNTS) is less well characterised, but likely is responsible for a larger case load – preliminary estimates from the Institute of Health Metrics and Evaluation (IHME) give estimates of 73.9 million cases and 61,600 deaths attributable to dNTS in 2019¹¹. The World Health Organisation (WHO) has recently listed *Salmonella enterica* as a priority pathogen, identified as one of 12 families of bacteria that pose the greatest risk to human health through rising antimicrobial resistance¹².

Vaccines for iNTS would represent a valuable public-health tool in sub-Saharan Africa, in part because other effective methods for disease control are – arguably – slow and cost prohibitive in many endemic countries in the short-term. Several candidate vaccines are in development, including conjugate vaccines, live-attenuated oral vaccines, and those using the generalised modules for membrane antigens (GMMA) platform, typically in multivalent formulations^{13,14}. Vaccine development for iNTS is hampered, in part, by an incomplete understanding of mechanisms and determinants of immunity during natural infection, as well as a lack of commercial incentive. In addition, the epidemiology of iNTS disease dictates that the large-scale phase III trials of vaccine candidates needed to establish efficacy will require a large financial and time commitment. The most efficient pathway to vaccine licensure and deployment is unclear.

Unlike other enteric pathogens, there is relatively limited experience of controlled human infection studies of non-typhoidal *Salmonella* serovars¹⁵⁻¹⁷. The use-case for an NTS CHIM in vaccine assessment is controversial^{18,19}. Recently, the Wellcome Trust has funded the establishment of the Challenge Non-Typhoidal *Salmonella* (CHANTS) consortium, to develop a first-in-human model of *Salmonella* Typhimurium infection using contemporary strains. This consortium brings together stakeholders with expertise in *Salmonella* biology and controlled human infection studies to design a safe clinical study that de-risks the development of an NTS CHIM. The vision of this program is to develop an iNTS CHIM that dovetails with future iNTS vaccine efficacy studies, whilst simultaneously addressing fundamental questions on the immunological basis of susceptibility to iNTS disease.

A new CHIM for NTS will only significantly advance vaccine development if careful consideration is given to study design at an early stage, particularly with regards to selection of clinically meaningful endpoints and setting appropriate efficacy thresholds. Additionally, understanding the needs of key stakeholders is necessary to map out a potential use case in a vaccine product development pathway.

To address these issues, a consultation group workshop meeting was convened on 5th July 2022, with the aim of gathering input into the design and use-case of the proposed NTS CHIM. The consultation group meeting aimed to link the CHANTS consortium with key stakeholders in the NTS vaccine field, including regulators, academic researchers, funders, and vaccine developers. This meeting report summarises the key discussion points and outcomes from the workshop. We begin by reviewing the rationale for the development of an NTS CHIM and identify key learning points from other human challenge models for enteric diseases. This report focuses

on priority topics discussed in the meeting, including challenge strain selection; choice of endpoints; clinical considerations and mapping out a future use-case.

Rationale for an iNTS controlled human infection model

Unlike human host-restricted *Salmonella* Typhi, NTS serovars typically have a broad host range^{20,21}. Several animal models have been developed to assess NTS candidate vaccines²². Many of these models have provided valuable pre-clinical insights into host-pathogen interactions but do not always replicate the manifestations of disease in a context relevant for vaccine development. Historically, human challenge with wild-type or attenuated NTS serovars has been performed in a small number of volunteers, but a large-scale programme of activities for vaccine assessment has not previously been established¹⁸.

In the absence of an established NTS CHIM, there are several unknowns that hamper vaccine development. An NTS CHIM provides opportunities to understand NTS disease pathogenesis (both diarrhoeal and invasive) and the mechanisms that drive these distinct clinical phenotypes. Investigation of immune responses to the challenge agent at time points before and after exposure will provide insights into mechanisms of protective immunity and identification of immune correlates of protection. Once established, an NTS CHIM would provide a platform to evaluate vaccine efficacy and support licensure of vaccine candidates currently in early phase evaluation. Furthermore, controlled challenge with NTS can elucidate novel target antigens for future vaccine development, identify diagnostic biomarkers, and facilitate assessment of the impact of infection on the host gastrointestinal microbiome.

Whilst the anticipated benefits of conducting an NTS CHIM are many, there are several unknowns in relation to clinical responses to challenge. In particular, an NTS CHIM in healthy, immunocompetent adults from high-income settings is unlikely to accurately replicate an invasive disease phenotype most frequently observed in immunocompromised children with risk factors, including prematurity, malnutrition, and malaria infection, in lower- and middle-income countries (LMICs).

These uncertainties have been discussed at several expert meetings in the preceding years and have been discussed elsewhere^{18,19}. Acknowledging the gaps which remain in this field, it was concluded that to address these questions, a CHIM could be established to characterise the response to wild type NTS challenge in order to understand if – and how – a human challenge model of NTS disease could be used for future vaccine assessment.

The CHANTS consortium has since been funded by Wellcome Trust, including key partners from Imperial College London, PATH, University of Oxford, Malawi-Liverpool-Wellcome Trust Clinical Research Programme, and the University of Liverpool. This consortium aims to establish an NTS CHIM that offers maximum insight from controlled human infection, whilst being underpinned by the central principle of volunteer safety.

NTS human challenge – safety and ethics

In the first session, the workshop discussed the overarching considerations pertaining to the safety and ethical acceptability of an NTS challenge model. The principal tension in the development of an NTS CHIM is developing a framework that prioritises safety and ethical considerations, whilst simultaneously providing a clinically meaningful way to test vaccines that protect against invasive disease. Specifically, the development of a dNTS challenge model would pose fewer safety and ethical challenges but would not necessarily help to accelerate vaccines designed to address iNTS disease.

A CHIM that reliably achieves bloodstream infection (BSI) would have the greatest utility when viewed solely from the perspective of vaccine efficacy assessments against invasive disease. Previous *Salmonella* Typhi and Paratyphi CHIMs are good examples of this, as BSI occurs following challenge with these serovars in healthy, immunocompetent adults and responds rapidly to effective antibiotic treatment^{23,24}. In contrast, BSI with *Salmonella enterica* subspecies *enterica* serovars Typhimurium and Enteritidis is almost exclusively observed in clinically vulnerable populations with impaired host immunity^{7,25}. This includes a high mortality rate (5–15%) and the potential to seed deep-seated infections. It is plausible that silent or pauci-symptomatic BSI (as observed in typhoidal *Salmonella* challenge models) could be seen after challenge of healthy volunteers with NTS, but this is unlikely to represent the same pathophysiological processes as those associated with iNTS disease in the target population for vaccination.

Well recognised host-risk factors associated with invasive disease includes extremes of age, recent malaria infection, sickle cell disease, malnutrition, advanced HIV infection in adults and other defects in the immune system. Volunteers with these characteristics could arguably never be safely or ethically challenged with virulent *Salmonella*^{7,25}. Most CHIMs typically start by challenging exceptionally healthy volunteers in a narrow age range (e.g., healthy UK volunteers aged 18–50). It was acknowledged outright this population are not representative of the population at greatest risk of iNTS and are unlikely to develop invasive disease.

The relative contribution of pathogen specific factors in achieving invasive disease within the context of the model was discussed by the consultation group and is summarised below (Study Design). Importantly, it was noted that *Salmonella* Typhimurium ST313 isolates have been found in both invasive and diarrhoeal infections.

The consortium had a high degree of confidence in the establishment of a colonisation and gastrointestinal NTS disease model in immunocompetent adults. It was acknowledged that the prospect of achieving an invasive disease model representative of iNTS is less secure. The session finished by highlighting the key safety considerations which would be adopted to de-risk the model for participants, namely: establishment of an independent data and safety monitoring committee; thresholds for initiation of antimicrobial therapy; quality assurance of strain manufacture and administration, and close liaison with public health authorities including the

local health protection team and the UK Health Security Agency (UKHSA) in view of potential secondary household or community transmission.

NTS vaccine landscape

As listed WHO priority pathogens, invasive NTS isolates are increasingly associated with multi-drug resistance, significantly limiting treatment options^{26–29}. Development of effective vaccines for NTS may contribute to reduced case numbers, thus reducing antibiotic use and the expansion of antimicrobial resistance³⁰. The next session aimed to put an NTS CHIM within the context of vaccines currently under assessment. Key questions to address included where a CHIM would sit within the vaccine product development pathway, and what additional data could be generated that would meaningfully accelerate vaccine development.

In this workshop, Professor Cal MacLennan outlined the NTS vaccine landscape as of July 2022. He defined several key considerations in NTS vaccine development. These included:

- Whether an NTS vaccine could be developed as a stand-alone product or formulated in combination with other serovars
- Whether such a vaccine could provide protection against both invasive and diarrhoeal disease
- What would be the most appropriate age of administration and alignment within the Expanded Programme on Immunisation (EPI) schedule?
- What is the expected duration and putative mechanisms of protection?
- What would be the efficacy of a vaccine in individuals with comorbidities at higher risk of developing NTS?

Several NTS vaccines are currently under development which aim to target *S. Typhimurium* and *S. Enteritidis*. The most advanced include a bivalent conjugate vaccine comprising core and O-polysaccharides conjugated to serovar specific phase I flagellin protein (FliC) (University of Maryland/Bharat Biotech), where Phase I studies are complete³¹. Additionally, a bivalent vaccine developed by GSK Vaccines for Global Health (GVGH), based on the generalised modules for membrane antigens (GMMA) platform, is approaching Phase I³². Several other vaccines are in pre-clinical testing including a bivalent NTS vaccine developed by Boston Children's Hospital based on the multiple antigen presenting system (MAPS) technology¹³.

Several expanded-valency formulations of NTS and typhoidal *Salmonella* vaccines candidates are also in pre-clinical testing. These include a trivalent NTS (O-antigen conjugated to flagellin for Typhimurium and Enteritidis combined with Vi-TT Typhar-TCV [Centre for Vaccine Development, University of Maryland/ Bharat Biotech]); a trivalent NTS + Vi-CRM197 (GVGH); and a trivalent NTS + Vi-DT (IVI/SK). In addition, hypothetical quadrivalent vaccines for enteric fever (*S. Typhi* and Paratyphi A) with *Salmonella* Typhimurium and Enteritidis have been proposed¹³.

Analyses indicate that iNTS vaccines would be cost-effective in resource-limited countries, particularly when combined with other *Salmonella* serovars, as the priority for protection is a time-limited period where the burden of disease is highest – specifically the first three years of life. Cost effectiveness would rely on having the capacity to mass produce and roll-out a vaccine at low-cost³³.

As phase I data from vaccine trials become available, it was acknowledged there is a need to engage with vaccine developers early, particularly with respect to where a CHIM would fit in an already well-defined development pipeline. Until readouts from a pilot NTS CHIM are known, the future utility of the model for vaccine assessment remain uncertain.

Study design

Having considered the rationale for an NTS CHIM and its potential utility for supporting vaccine development, the consultation group reviewed different proposed challenge study designs, based on a draft template protocol.

The originally proposed study was to perform oral challenge with two wild-type strains of *Salmonella* Typhimurium administered in sodium bicarbonate in a dose escalation model targeting an attack rate of 60–75%.

Challenge strain selection

This study proposes to use two challenge strains belonging to ST19 and ST313 sequence types. This approach would directly address the contribution of African ST313 lineages to systemic disease compared with non-invasive isolates.

Dr Blanca Perez Sepulveda (University of Liverpool) outlined the rationale for the choice of strains used in this model. All human challenge models are limited in the number of strains that can be used and are frequently criticised for failing to replicate the global diversity of strains responsible for infection in endemic settings. Where possible, bacterial strains used for challenge should: be representative of circulating strains; be sensitive to multiple antibiotics used for treatment; have a traceable history from isolation; have been well characterised phenotypically and genotypically *in vitro* and *in vivo*; be capable of causing the disease of interest; and possess a representative array of antigens and virulence factors.

The NTS serovars commonly isolated in BSI varies by location, but usually include *Salmonella* serovars Typhimurium and Enteritidis³⁴. *S. Typhimurium* accounts for approximately two-thirds of iNTS in Africa. Sequencing of isolates from across Africa has identified multilocus sequence type (MLST) 313 as the major cause of invasive disease, which is distinct from the sequence types 19 and 34, which are more frequently associated with gastrointestinal disease in high-income settings³⁵. ST313 appears to have undergone sequential evolutionary changes that converge towards a genotype associated with invasive disease, in the same way as reported for *Salmonella* Typhi and Paratyphi serovars^{26,36}. The key changes are characterized by stepwise genomic degradation typified by multiple gene deletions and pseudogene

formation, often in genes associated with gastrointestinal survival²⁶. The data suggests that ST313 has adapted to a human niche and can evade mucosal immunity^{36–38}. Similar findings have been observed in invasive *S. Enteritidis* ST11 isolates from Africa³⁵. Transmission of invasive isolates is complex and likely involves a combination of zoonotic reservoirs (particularly for *S. Enteritidis*) as well as human-to-human transmission^{20,21}. Invasive NTS isolates are increasingly associated with multi-drug resistance, significantly limiting treatment options^{26–29}.

This study proposes to manufacture challenge stocks from the ST313 lineage 2 reference strain D23580, which can be considered as an archetypal isolate representative of strains causing invasive disease in sub-Saharan Africa. D23580 was originally isolated in 2004 from a blood culture taken from a 24-month-old HIV-negative child at Queen Elizabeth Central Hospital, Blantyre, Malawi. D23580 harbours four plasmids, including pSLT-BT, which encodes drug resistance to chloramphenicol, ampicillin, streptomycin, sulphonamides, and trimethoprim. The presence of drug resistant mobile genetic elements within D23580 was felt not to represent a significant risk when balanced against the benefits of using a well-characterised strain that is reliably sensitive to fluoroquinolones and more representative of isolates detected from endemic settings. In parallel, it was proposed to manufacture a challenge stock of the ST19 4/74 strain, originally isolated from a calf in England in 1974. Genomic analysis indicates that it is an archetypal ST19 strain, representative of current clinical infections. Both strains have undergone detailed genotypic, transcriptomic, proteomic, and phenotypic characterisation³⁹. Consensus was reached that challenge with highly characterised pathogens is advantageous from a participant safety perspective.

These proposals are predicated on the hypothesis that challenge of healthy volunteers with ST313 will be associated with a distinct clinical phenotype, as compared to challenge with ST19. There is a substantial body of phenotypic, genomic, and epidemiological evidence to indicate that infection with specific African lineages of ST313 will be associated with less gastrointestinal disease as compared with ST19 pathovariants³⁵. Whilst challenge with *S. Enteritidis* would be valuable, resource limitations currently preclude stock development, and

there was agreement that the most valuable data, within resource constraints, would be generated from a *S. Typhimurium* CHIM.

The consultation group were invited to consider challenge dosing. It was raised that the dose needed to achieve 60–75% attack rates might vary between strains and depend on what clinical endpoints are selected. For example, a relatively low dose of ST19 may cause a higher proportion of volunteers developing diarrhoeal disease compared with ST313. In view of the novelty of this model, it was suggested that a starting dose of 10³ CFU was rational, based upon prior experience with *S. Typhi* and Paratyphi A challenge administered with sodium bicarbonate^{23,24}.

All strains will be manufactured to GMP standard at the pilot biomanufacturing facility of the Walter Reed Army Institute of Research (MD, USA), following protocols previously established for typhoidal *Salmonella* studies. It was recommended that stability testing should be performed at least annually, and more frequently at the start of the study to ensure the challenge agent remains stable. Characterisation tests should include antibiotic susceptibility testing, whole genome sequencing and growth characteristics in different media and survival tests as a minimum.

Study endpoints

The selection of appropriate endpoints is critical to ensure utility of the model in future vaccine efficacy studies. An NTS CHIM could be designed to result in colonisation, diarrhoeal disease, systemic illness and/or bacteraemia. It was emphasised that these endpoints aren't mutually exclusive and that a participant may progress from one state to another. Exposure to the gastrointestinal tract is a prerequisite for the development of invasive disease, but kinetics of invasion after exposure are poorly understood. The consultation group discussed the relative merits of developing a colonisation/early infection model, or a disease model. It was acknowledged that the primary goal of vaccine development is to protect against invasive disease, but that protection against diarrhoeagenic disease would also be beneficial.

The consultation group was invited to consider the following possible endpoint definitions of Salmonellosis, outlined in [Table 1](#).

Table 1. Possible study endpoints to be used as composite or co-primary endpoints.

Possible Primary Endpoints
<ul style="list-style-type: none"> Isolation of <i>Salmonella</i> Typhimurium from stool on ≥ 2 occasions ≥ 24 hours from challenge and/or
<ul style="list-style-type: none"> Severe diarrhoea* and/or
<ul style="list-style-type: none"> Moderate diarrhoea** plus: <ul style="list-style-type: none"> Fever $\geq 38^{\circ}\text{C}$ on ≥ 1 occasion and/or ≥ 1 Grade 2 systemic symptoms (abdominal pain, nausea, vomiting, tenesmus) and/or
<ul style="list-style-type: none"> <i>Salmonella</i> Typhimurium bloodstream infection and/or
<ul style="list-style-type: none"> Fever $\geq 38^{\circ}\text{C}$ on ≥ 2 occasions ≥ 12 hours apart

*Severe diarrhoea defined as any one of (i) ≥ 6 loose/liquid stools in 24h (ii) $>800\text{g}$ loose/liquid stool in 24h (iii) ≥ 2 stools containing gross blood in 24h

**Moderate diarrhoea defined as any one of (i) 4–5 loose/liquid stools in 24h (ii) 400–800g loose/liquid stool in 24h

The consultation group discussed the merits and demerits of defining a composite endpoint or co-primary endpoints for each definition, generating attack rates for each of the definitions. Secondary and exploratory endpoints would also be generated, including duration/magnitude of diarrhoea, shedding, seroconversion, symptom profile and blood culture clearance.

Colonisation was thought to be insufficient as a surrogate marker for invasive disease. The primary concern was that colonisation as a primary endpoint will likely be achieved at a low infecting dose, preventing further dose escalation. Bloodstream infection and/or fever would be favourable in terms of model utility; however, care would be needed to minimise risks, as outlined above. It was acknowledged that the dose required to achieve invasive infection in healthy participants may be many orders of magnitude higher than in natural exposure, setting an unachievable threshold to demonstrate vaccine efficacy. The consultation group agreed that endpoints should have some flexibility, as the outcome following challenge is unknown.

There was consensus that a composite endpoint comprising colonisation, diarrhoeal disease and bacteraemia would not be informative. Co-primary endpoints would be preferable, to provide attack rates for colonisation, gastroenteritis and systemic disease and highlight strain differences. The challenge doses used could be escalated until safety signals require the escalation to be halted. Diarrhoea without fever could be designated as a secondary/exploratory endpoint so this would not preclude further dose escalation. The group highlighted the risk of prematurely ending the study if the target attack rate is achieved using a primary endpoint that is not representative of iNTS disease. There was broad consensus in having the scope to dose escalate with appropriate oversight by a data safety monitoring committee (DSMC).

Dose ranges and ceilings were also discussed. A stage might be reached where the investigators and DSMC consider the challenge dose to be grossly unrepresentative of natural exposure – at which point, consideration should be given to terminating the study. The consortium agreed that double-blind randomisation of participants is of value to maintain integrity in assessment of key subjective symptomatic outcome measures.

Clinical considerations

In the final session related to study design, the consortium was invited to consider practical clinical aspects of study delivery. Key considerations included location, inpatient vs. outpatient model; inclusion and exclusion criteria (with safety as priority); pre-enrolment tests; infection control considerations; strategies to mitigate complicated disease and treatment protocols.

Key clinical risks and risk mitigation strategies that should inform study design are highlighted in [Table 2](#).

Antibiotic treatment

The consultation group discussed antibiotic treatment at length. Opinions differed on the value of offering antibiotic treatment to all participants regardless of symptoms. All members agreed that antibiotic treatment would be indicated in cases of invasive disease. It was acknowledged that *Salmonella* gastroenteritis is typically self-limiting in healthy immunocompetent adults and that antibiotic treatment is not always indicated in this patient group – in part due to proposed risk of prolonged faecal shedding coupled with antibiotic related adverse events^{40,41}. Prolonged faecal shedding is less likely when fluoroquinolones are used for treatment of sensitive strains, when compared with other classes of antimicrobials⁴⁰. To protect participant safety and to minimise risk of prematurely terminating infection, treatment criteria should be distinct from diagnostic criteria. Signs of bloodstream infection or persistent fever will result in immediate antibiotic treatment, as will a combination of gastrointestinal symptoms that exceed a pre-defined threshold. Asymptomatic individuals who establish colonisation (positive stool culture detected on >2 occasions) could also receive antibiotic therapy at day 14. Whilst the use of antimicrobial therapy for uncomplicated NTS infection is contentious, the consortium agreed that antibiotic treatment could be justified within the context of a volunteer CHIM study with close post-challenge monitoring and assessment of clearance cultures. The consultation group also agreed that the study information booklet and screening procedures should counsel participants of the risks and benefits of antibiotic treatment.

Study location

Whilst extensive safety data are available from prior *Salmonella* CHIMs conducted as out-patient studies, the balance of risks, in conjunction with the novelty of challenge with NTS, led the group to favour an in-patient model. This followed from in-depth discussion regarding quarantine, balancing participant safety/public health with over-burdensome restrictions and the risk to mental well-being of participants. Discharge criteria and infection control considerations were developed with current public health guidance in mind. The UKHSA considers people non-infectious 48 hours after diarrhoea has ceased and clearance stool samples are not routinely required for NTS⁴². In this study, participants will be quarantined for at least seven days, until resolution of diarrhoea for at least 48 hours, negative blood clearance cultures if bacteraemia develops, and be medically fit as assessed by a study clinician.

Screening tests

Screening and pre-enrolment testing will consider inclusion and exclusion criteria to mitigate risk of adverse outcomes. Only healthy participants will be considered for participation, with targeted investigations undertaken to exclude occult conditions which may predispose to complications (biliary disease, abdominal aortic aneurysm). We will also exclude individuals with existing mild/sub-clinical irritable bowel syndrome (IBS) to avoid long-term complications post-infection.

Table 2. Risk assessment for *Salmonella* Typhimurium oral challenge. Participants should be counselled for these risks in the participant information sheet and during screening. Biological safety and laboratory risk-assessments are not detailed. The following risks were highlighted as areas of focus during protocol development but are non-exhaustive.

Possible study risks	Comments	Risk mitigation strategies
Clinical Considerations		
Primary complications of <i>Salmonella</i> infection		
Severe gastroenteritis with dehydration	<ul style="list-style-type: none"> Typical symptoms include watery diarrhoea, bloody diarrhoea, abdominal pain, nausea, vomiting, headache, and fever Overall duration of symptoms typically ranges from 4 to 7 days Total duration of illness is estimated to range between 3 to 19 days⁴⁰. 	<ul style="list-style-type: none"> In-patient quarantine with continuous oversight from study physician and nurses (initial 7 days) Daily stool output monitoring and culture Daily assessment of symptoms and severity grading Severe diarrhoea will be trigger antibiotic treatment Oral rehydration for diarrhoea Intravenous fluid replacement for severe hypovolaemia
Severe sepsis	<ul style="list-style-type: none"> Complications of invasive <i>Salmonella</i> infection can include septicemia, hypotension, tachycardia Occur almost exclusively in clinically vulnerable and/or those who do not receive appropriate antibiotic treatment. Estimated case-fatality rate 15–20% of INTS in vulnerable patients^{8,43}. ~5% of enteric infections with non-typhoidal <i>Salmonella</i> are thought to result in bacteraemia. May increase to ~10% depending on underlying host risk factors and serotype^{6,44}. Progression from enteric to systemic infection may occur more frequently in the context of altered gastric acid pH^{4,5}, altered microbiota (including prior antibiotic treatment)⁴⁶ and concomitant rotavirus infection⁴⁷⁻⁵¹. 	<ul style="list-style-type: none"> Exclusion of participants at higher risk of developing severe sepsis/invasive disease In-patient quarantine with continuous oversight from study physician and nurses. Daily blood cultures to detect bloodstream infection Early antimicrobial therapy in event of bloodstream infection or clinical signs/symptoms of developing sepsis. Backup admission to medical inpatient unit and/or high-dependency unit and/or intensive care unit if required.
Deep-seated focal infection	<ul style="list-style-type: none"> Bloodstream infection can lead to extra-intestinal focal infections at any site, including aortitis, osteomyelitis, meningitis, or arthritis. Almost exclusively in clinically vulnerable and/or those who do not receive appropriate antibiotic treatment Sickle cell disease is a recognised risk factor for osteomyelitis Endovascular disease is associated with atherosclerotic disease, valvular disease, or endovascular prosthesis Meningitis is a rare, but can occur in neonates, infants <12years of and in the context of advanced HIV infection. 	<ul style="list-style-type: none"> Exclusion of participants at risk e.g. underlying valvular heart disease, bone/joint disease (including prosthesis, metalwork), aneurysmal arterial disease Clinical examination to exclude valvular heart disease. Abdominal ultrasound during screening to detect asymptomatic biliary disease and aneurysmal disease of the abdominal aorta

Possible study risks	Comments	Risk mitigation strategies
Post-infectious/Para-infectious complications		
Prolonged <i>Salmonella</i> shedding and/or microbiological relapse	<ul style="list-style-type: none"> • Convalescent shedding of <i>Salmonella</i> is common after symptomatic or asymptomatic NTS infection. • Microbiological relapse (with or without clinical symptoms) may develop post recovery and/or treatment • The precise duration of shedding is poorly understood. • In some instances, antibiotic treatment is associated with prolonged symptoms or higher rate of microbiological relapse. • Chronic carriage (shedding for >12 months) is more common with host immunosuppression; in young children <5 and/or in the context of biliary tract abnormalities. • Theoretical risk of secondary transmission if hygiene measures are not followed. 	<ul style="list-style-type: none"> • Where required, use antibiotic classes not associated with prolonged shedding (e.g. fluoroquinolones) • Describe pattern of shedding by longitudinal collection of stool samples for detection of <i>Salmonella</i> spp. by PCR and culture. <ul style="list-style-type: none"> ◦ Distinguish between PCR positive and culture positive samples. • Clearance stool cultures will be obtained upon completion of antibiotic therapy. <ul style="list-style-type: none"> ◦ Follow guidance for typhoidal <i>Salmonella</i> i.e. three negative clearance stools. • Reinforce hygiene precautions after challenge. • Participants with chronic carriage (stool culture positive >4 weeks after antibiotics) can be referred to an Infectious Diseases specialist.
Clinical relapse	<ul style="list-style-type: none"> • Clinical relapse may develop post recovery and/or treatment • More common in immunocompromised patients. <ul style="list-style-type: none"> ◦ Advanced HIV infection⁵² ◦ Chronic granulomatous disease⁵³ ◦ Defects in specific cytokine pathways⁵⁴ ◦ Haematological malignancies⁵⁵ 	<ul style="list-style-type: none"> • Exclusion of at-risk individuals • Follow up of participants for up to 1 year • Study team contactable 24/7 <ul style="list-style-type: none"> ◦ Participants advised to contact study team in event of new symptoms
Reactive arthritis	<ul style="list-style-type: none"> • Gastrointestinal and genitourinary infections are commonly recognised triggers, including with <i>Salmonella</i> spp. • Typical symptoms - mono-arthritis or oligo-arthritis, often affecting the lower limbs, axial symptoms, enthesitis and/or dactylitis. • Symptom duration is highly variable – <ul style="list-style-type: none"> ◦ most patients will have little-to-no symptoms at 6–12 months post onset. ◦ A small proportion of patients may develop symptoms lasting >12 months⁵⁶. • Prevalence of HLA-B27 increased in patients with reactive arthritis, although the estimates vary depending on illness definition and study design⁵⁶⁻⁵⁸. • Presence of HLA-B27 is not essential for the development of reactive arthritis. 	<ul style="list-style-type: none"> • HLA-B27 screening prior to enrolment • Participants who develop symptoms of reactive arthritis will be referred to a Rheumatology service
Post-infectious irritable bowel syndrome (IBS)	<ul style="list-style-type: none"> • Post-infectious irritable bowel syndrome is estimated to occur in 3–10% of patients following bacterial diarrhoea. • Symptoms generally resolve within 1 year⁵⁹⁻⁶¹. 	<ul style="list-style-type: none"> • Exclusion of participants with pre-existing IBS • IBS questionnaire at baseline and post-challenge
Psychological impact of challenge and quarantine	<ul style="list-style-type: none"> • Study fatigue may occur due to the intense nature of the study procedures, especially during the quarantine period. • Participants may become anxious, lonely, or depressed by being confined to the quarantine unit for the minimum of 7 days. 	<ul style="list-style-type: none"> • Anxiety and depression questionnaire during screening and after challenge. • Facilities to make phone and video calls with friends and family. • A small number of visitors may be allowed but would have to comply with a strict hygiene protocol.

Possible study risks	Comments	Risk mitigation strategies
Risks associated with antibiotics		
Direct adverse effect of antibiotics	<ul style="list-style-type: none"> Antibiotic intolerance: <ul style="list-style-type: none"> gastro-intestinal upset, nausea, vomiting or other unspecified symptoms. Antibiotic hypersensitivity <ul style="list-style-type: none"> Rash, angio-oedema, difficulty in breathing anaphylaxis Abnormal liver function tests or other biochemical abnormalities Risk of antibiotic associated diarrhoea and/or <i>Clostridioides difficile</i> infection. 	<ul style="list-style-type: none"> Baseline ECG (QTC) Regular measurements of liver function tests and electrolyte monitoring Keep antibiotic treatment duration as short as possible Exclude participants with known antibiotic hypersensitivity or allergy to either of the first-, second- and third-line antibiotics (ciprofloxacin, azithromycin, or other macrolide antibiotics and cephalosporins)
Carriage of multi-drug resistant organisms (MDRO)	<ul style="list-style-type: none"> Antibiotic treatment may in theory increase the risk of carriage of drug resistant bacteria. 	<ul style="list-style-type: none"> Stool culture for carriage of multidrug resistant organisms (Carbapenem resistant organisms and Extended spectrum beta lactamase producing <i>Enterobacteriales</i>)
Infection control considerations		
Nosocomial spread of <i>Salmonella</i> between volunteers	<ul style="list-style-type: none"> Theoretical risk of cross-transmission between volunteers impacting study endpoints 	<ul style="list-style-type: none"> Strict enteric precautions Isolation of participants in side-rooms
Nosocomial spread of other pathogens, including SARS-CoV-2 (in-patient model)	<ul style="list-style-type: none"> Theoretical risk of outbreak of SARS-CoV-2 (or other respiratory viruses) within quarantine facility. 	<ul style="list-style-type: none"> Use of personal protective equipment Participants and investigators to have completed at least a primary SARS-CoV-2 vaccination course Negative SARS-CoV-2 lateral flow test prior to admission to quarantine Hospital infection prevention and control guidelines to be maintained throughout the quarantine phase Investigators with relevant respiratory symptoms to isolate and avoid contact with volunteers.
Transmission to study investigators	<ul style="list-style-type: none"> Theoretical risk of cross-transmission from study volunteers to study investigators 	<ul style="list-style-type: none"> Investigators will adhere to strict hygiene measures Use of personal protective equipment per hospital guidelines for enteric infections
Public health considerations		
Onward community transmission (ranging from household secondary cases to outbreak)	<ul style="list-style-type: none"> Person-to-person spread has been described, especially when patients are symptomatic with diarrhoea. Transmission within households can occur if the individual excreting <i>S. Typhimurium</i> fails to practice effective hand washing after defecation and is subsequently involved in uncooked food preparation. Person-to-person spread has been described, especially when patients are symptomatic with diarrhoea. Clearance cultures following NTS infection are not typically indicated according to UKHSA guidance². <ul style="list-style-type: none"> This contrasts with UK public health guidance following infection with the typhoidal <i>Salmonella</i> serovars (<i>S. Typhi</i> and Paratyphi) 	<ul style="list-style-type: none"> In-patient quarantine during putative infectious period. Defined de-isolation criteria <ul style="list-style-type: none"> Complete resolution of diarrhoea (Bristol stool type 6-7) for 48 hours Participant counselling on stringent hygiene measures until microbiological clearance confirmed Exclusion of participation from high-risk occupational groups <ul style="list-style-type: none"> food handlers ongoing contact with highly susceptible persons or young children healthcare workers Access to screening for household and other close contacts Inform health protection services at time of challenge and time of confirmed clearance. Engage with national reference laboratories to sequence isolates from participants to compare with any future community outbreaks.

Serological pre-screening for prior *Salmonella* exposure was not viewed as being feasible, owing to lack of standardised assays at the time of writing. Instead, a post-challenge analysis could be stratified by baseline sero-status to inform future studies. Practically, a clinical history and/or microbiological evidence of documented *Salmonella* infection and/or prior Typhoid oral Ty21a vaccination, would exclude participation due to potential cross-reactivity.

Future use case

The final workshop session focussed on defining the future use case for an NTS CHIM. The consultation group agreed that any model would preferably have a clear use in NTS vaccine assessment whilst simultaneously allowing investigators a platform to better understand NTS infection biology. It was acknowledged that these are not mutually exclusive aims but that trade-offs may be required given feasibility and budgetary constraints.

A primary application of CHIMs is to define immune correlates of infection- or vaccine-derived immunity. Prior CHIMs for enteric pathogens have played a key role in elucidating serum anti-LPS IgG and vibriocidal antibodies as correlates of protection against *Shigella* spp. and *Vibrio cholerae*, respectively^{62,63}. Whilst this study offers the opportunity to investigate a wide range of immune responses to infection, the consortium agreed certain assays should be prioritised, and standardised, to align with those targeted by NTS vaccine candidates currently in development. The study proposes to characterise antigen-specific and functional antibody responses at baseline, during acute disease, and during convalescence. O-antigen ELISA and serum bactericidal assays will be utilised to characterise the humoral response to NTS challenge. Saliva and stool samples will be collected to characterise mucosal IgA responses at baseline and following challenge, whilst peripheral blood mononuclear cells will be used to measure antibody-secreting-cell and memory B-cell activity (via ELISpot assays). High throughput functional antibody assays, biophysical antibody profiling and Fc-glycosylation studies could also be undertaken as part of so-called systems serology platforms⁶⁴⁻⁶⁶.

It was acknowledged that correlates of protection may differ for colonisation, gastroenteritis, and invasive disease. Careful consideration is needed to balance de-risking phase 3 studies by providing early efficacy signals, and undermining confidence in vaccine candidates based on surrogate endpoints that might not be relevant to invasive disease. It was voiced that identification of correlates should be hypothesis driven and follow a well-constructed sample collection schedule. Exploratory measurements will include whole blood and/or single cell transcriptomics, which could also help to identify the establishment of invasive disease in study participants.

The target population for any future NTS vaccine is anticipated to be children <5 years of age, although other groups including adults living with HIV may also benefit. Invasive

disease is strongly linked to immune susceptibility; either in very young infants who lack protective antibody responses, or children with recent malaria infection, who may have macrophage and/or complement deficiencies. It was agreed that the main role of a CHIM for vaccine development would be increasing confidence that a candidate vaccine would work in these susceptible populations. Whilst a challenge study involving these groups could be considered ethically unjustifiable, it may be informative to have adult participants with recent malaria infection in an NTS challenge study and compare the data to immunogenicity studies from children administered a vaccine candidate as part of a field trial. From discussions held about the model described, there were no immediate concerns or additional issues perceived about future transfer of an NTS CHIM to endemic settings.

Conclusions

The CHANTS workshop has successfully generated key recommendations to inform the establishment of an NTS challenge model. The aim of this workshop was to provide an expert consultation platform at an early stage of trial design. It is hoped that these contributions increase the likelihood that an NTS CHIM can be appropriately used to accelerate, and not hinder, vaccine development. Future consultation groups will likely be convened to report on preliminary data and refine the future use case of the model. Inputs from the expert consultation group will be considered alongside the outcome of public engagement and involvement meetings to inform the final trial protocol, which will be published once all approvals are in place. Continuing engagement with stakeholders will be key to defining and prioritising appropriate immunological endpoints to measure, specifically to ensure these are appropriately aligned and calibrated with assays currently being applied in iNTS vaccine trials.

Our vision is to develop an iNTS CHIM programme that dovetails with future iNTS vaccine efficacy studies, whilst simultaneously addressing fundamental questions on the immunological basis of susceptibility to iNTS disease. Several iNTS candidate vaccines are in development, but the epidemiology of iNTS disease is such that phase-III trials require a large financial and time commitment before efficacy readout is obtained. There are several instances where CHIM studies have accelerated vaccine candidates through to licensure, in particular for enteric pathogens such as *Vibrio cholerae* and *Salmonella* Typhi. We contend that a safe, reproducible, and well-designed CHIM – with clinically meaningful endpoints – will allow the field to build upon insights from animal-models and could have an important role in accelerating vaccine development for iNTS.

Data availability

No data are associated with this article.

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Lisa E Emerson

Emory Vaccine Center, Emory University, Atlanta, Georgia, USA

No further comments. Authors have included requested revisions.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Vaccine development, Salmonella, extracellular vesicles

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 25 August 2023

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Lisa E Emerson

Emory Vaccine Center, Emory University, Atlanta, Georgia, USA

This article is an open letter detailing the conclusions of a group workshop regarding the development of a NTS vaccine. Several key challenges are made clear, including the host, ethical, and pathogenesis factors. The need for a NTS vaccine is made clear; however, what is not clear is the focus on NTS vs iNTS. In the safety and ethics section, it is suggested that the vaccine be tested

against the ability to protect against invasive disease; however, the reasons why this is preferred to any NTS clinical symptoms were not clear. Since the host and bacterial factors that cause an NTS infection to become invasive are not well known, a serious barrier exists to the development of this CHIM. Additionally, over 2600 serovars of NTS exists, but the article does not make that clear.

Of note, the selected challenge strain of ST313 is an excellent choice for creating a iNTS vaccine and the rationale written in the article is excellent.

I recommend the acceptance of the article given that the authors revise the letter to clearly state 1) why protection against iNTS is preferred vs all NTS infections 2) the serovars the developed vaccine would protect against 3) the distinction between NTS, iNTS, and dNTS

Is the rationale for the Open Letter provided in sufficient detail?

Yes

Does the article adequately reference differing views and opinions?

Yes

Are all factual statements correct, and are statements and arguments made adequately supported by citations?

Yes

Is the Open Letter written in accessible language?

Yes

Where applicable, are recommendations and next steps explained clearly for others to follow?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Vaccine development, Salmonella, extracellular vesicles

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 07 Sep 2023

Christopher Smith

Reviewer 2 Thank you for your comprehensive review and feedback.

"This article is an open letter detailing the conclusions of a group workshop regarding the development of a NTS vaccine. Several key challenges are made clear, including the host, ethical, and pathogenesis factors. The need for a NTS vaccine is made clear;

however, what is not clear is the focus on NTS vs iNTS. In the safety and ethics section, it is suggested that the vaccine be tested against the ability to protect against invasive disease; however, the reasons why this is preferred to any NTS clinical symptoms were not clear. Since the host and bacterial factors that cause an NTS infection to become invasive are not well known, a serious barrier exists to the development of this CHIM."

Thank you. The primary focus of this meeting was to discuss the potential merits of developing a NTS CHIM, and the possible applications such a model could have in supporting candidate vaccine evaluation and licensure. Development of these vaccine candidates is undertaken by other stakeholders and is not within the remit of the CHANTS consortium. Vaccines currently in early-stage development are reviewed in the 'NTS vaccine landscape' section. Candidates utilise differing vaccine platforms and technologies; however, all are targeted at providing protection against *S. Typhimurium* and/or *S. Enteritidis* – serovars which are known to account for >90% of iNTS disease causing isolates. The ideal vaccine candidate would offer effective protection against both iNTS and dNTS. However, in these early stages, the priority is to develop vaccines which offer effective protection against invasive *Salmonella* disease. The rationale for this is highlighted in the stark differences in morbidity and mortality observed between iNTS and dNTS (see epidemiology figures in Introduction, paragraph 2). Furthermore, increasing multi-drug resistance amongst iNTS disease causing serovars (e.g., ST313 D23580) highlights the urgent need for effective vaccination strategies in endemic settings to avert further expansion of antimicrobial resistance. It is anticipated that successful candidates could be in bivalent formulations, or trivalent, providing coverage for both NTS and typhoidal serovars in relevant settings. We wholly agree our understanding of the bacterial and host factors leading to differential clinical phenotypes (iNTS vs. dNTS) is incomplete. We aim to gain a deeper understanding of these factors through this first-in-human NTS CHIM, and this is a primary rationale for the study.

"Additionally, over 2600 serovars of NTS exists, but the article does not make that clear."

Thank you, this has been clarified in the introductory section.

"Of note, the selected challenge strain of ST313 is an excellent choice for creating a iNTS vaccine and the rationale written in the article is excellent. I recommend the acceptance of the article given that the authors revise the letter to clearly state 1) why protection against iNTS is preferred vs all NTS infections 2) the serovars the developed vaccine would protect against 3) the distinction between NTS, iNTS, and dNTS."

Thank you. These clarifications have been highlighted in response to comments above and in the main body of the manuscript.

Competing Interests: No competing interests were disclosed.

Reviewer Report 05 April 2023

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Joshua Osowicki 

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Thank you for the opportunity to review this Open Letter reporting on a 2022 CHANTS consortium workshop discussing a range of topics relevant to development of a non-typhoidal *Salmonella* (NTS) controlled human infection model (CHIM).

The authors and workshop participants are well-qualified to have pursued this initiative and their broad expertise adds weight to the discussion described in the report and its recommendations.

The report itself is a mature and thoughtful work, addressing contentious questions and areas of uncertainty with the even-handed seriousness that has become characteristic of modern investigator-initiated human challenge research (a standard set by a number of the authors and attendees at the workshop).

At the outset, there is a familiar tension here, as for many CHIM initiatives, in balancing the experimental aims to address unanswered questions related to NTS infection and disease in humans with the vision of the CHIM as a safe and robust clinical trial platform. Again, I think the authors have done a good job here, making the case that an NTS CHIM meets a “safe enough and useful enough” threshold to pursue further, looking forward to the use case for a successful CHIM without pre-judging the outcomes of the initial efforts to establish the CHIM.

The focus on study endpoints was strong, reminiscent of the 2018 publication by Chad Porter and colleagues regarding clinical endpoints in the Shigella CHIM (PMID: 29590182). The point that CHIM endpoints must be selected with specific consideration of vaccine indications and endpoints in later phase clinical trials was well made. Similar points are made in the Discussion section of the typhoid CHIM trial that evaluated the Vi-TT vaccine (Celina Jin et al, PMID: 28965718) and the linked editorial commentary (Feasey & Levine, PMID: 28965714).

As with most well-written meeting reports, there are few targets here for a specific critique. As the authors describe, there will be important decisions in developing the final protocol for the first NTS CHIM study. The devilish details of the final protocol may inspire more specific criticisms.

The meeting report outlines a cautious general approach, for an inpatient trial in extensively screened volunteers (including with abdominal ultrasound) with a relatively low threshold for antibiotic therapy and a relatively stringent requirement for microbiological eradication afterwards.

The risk assessment table is excellent and a model for other CHIM investigators.

I'm not certain that clinical examination can truly 'exclude valvular heart disease' given the significant subclinical fraction revealed by screening echocardiography studies (e.g. for rheumatic heart disease). I'm unconvinced that there's a specific need for further screening on this front though, if all other 'healthy adult' requirements are met. Somewhat similar, I wonder what is the prevalence of subclinical aortic and biliary tract pathology in otherwise healthy young adults? That said, so long as a standardised approach is taken to performing and reporting the abdominal ultrasounds, and members of the clinical trial team are trained to complete these targeted ultrasounds, they may not be too onerous, too expensive, and won't exclude too many otherwise eligible volunteers.

The term 'double-blind randomisation' will eventually need to be defined in more detail. Who exactly will be blinded? Presumably only the volunteers, the clinical trials facility staff, and those charged with assessing endpoints? Too much blinding too early in the development of a CHIM may be overly restrictive and prevent the almost inevitable flexible adjustments the protocol will require as early lessons emerge.

I understand the choice to exclude volunteers previously vaccinated against typhoid from the initial trial. Although resource constraints may preclude challenging some of these volunteers at a dose level that achieves a high attack rate in unvaccinated volunteers, I do wonder if samples from these participants could make a meaningful contribution to the immunological work to follow the clinical trial. Given the investments required to launch a CHIM study, this idea may not happen at all if it doesn't happen early and in parallel to another study.

The final reference regarding systems serology introduces these methods in relation to HIV research. The same others (Galit Alter et al) have since published systems serology investigations specifically related to CHIM studies (e.g. typhoid, shigella) that would seem to be even more appropriate references here?

I know that these authors have a strong record of work with clinicians and investigators in low- and middle-income countries. As they point out, the global burden of NTS disease is concentrated in LMICs and especially in Africa. However, there is limited representation from LMICs including African countries (besides Prof. Gordon) in the author group for this open letter and the workshop attendees. I do trust the consortium has plans to boost its engagement in this regard. Nonetheless, it does bear mentioning. I do note the authors have made a general comment in the conclusion regarding plans for stakeholder engagement.

The rationale for this Open Letter is clear: to introduce the CHANTS consortium and its mission to a broader audience, describing the rationale for developing an NTS CHIM and the seriousness with which the consortium is considering the safety of prospective participants and how to design the model in a way that will boost its contribution to accelerating NTS vaccine development. It is never possible to predict 100% of all impacts that will flow from the successful establishment of a new CHIM. The contributors to this meeting have made a strong case that a CHIM can be pursued with sensible safety guardrails (safe enough) and that it is a worthwhile objective that is more likely than not to meaningfully contribute to NTS science and vaccine development beyond what has been achieved to date with in vitro studies, animal models, and studies of naturally infected

humans (useful enough). I look forward to news of the study protocol and the early findings that emerge from a new NTS CHIM.

Is the rationale for the Open Letter provided in sufficient detail?

Yes

Does the article adequately reference differing views and opinions?

Yes

Are all factual statements correct, and are statements and arguments made adequately supported by citations?

Yes

Is the Open Letter written in accessible language?

Yes

Where applicable, are recommendations and next steps explained clearly for others to follow?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: I am a paediatric infectious diseases physician and human infectious challenge researcher. In my PhD I led the CHIVAS-M75 study to establish a *Streptococcus pyogenes* pharyngitis CHIM in healthy adult volunteers. I am now undertaking *S. pyogenes* challenge trials to evaluate vaccines and accelerate development of improved long-acting penicillin formulations (primarily for secondary prevention of rheumatic heart disease). I am also involved in efforts towards developing new human models of bacterial and mycobacterial skin infections and sexually-transmitted infections.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 07 Sep 2023

Christopher Smith

Reviewer 1 Thank you for your comprehensive review and feedback.

"I'm not certain that clinical examination can truly 'exclude valvular heart disease' given the significant subclinical fraction revealed by screening echocardiography studies (e.g. for rheumatic heart disease). I'm unconvinced that there's a specific need for further screening on this front though, if all other 'healthy adult' requirements are met. Somewhat similar, I wonder what is the prevalence of subclinical aortic and biliary tract pathology in otherwise healthy young adults? That said, so long as a standardised approach is taken to performing and reporting the abdominal

ultrasounds, and members of the clinical trial team are trained to complete these targeted ultrasounds, they may not be too onerous, too expensive, and won't exclude too many otherwise eligible volunteers."

Thank you. We wholly agree on points raised regarding potential subclinical aortic, valvular, and biliary tract disease. Our intended participant cohort will be comprised of healthy adults (aged 18-50) without chronic disease or risk factors for severe Salmonellosis. Screening protocols are designed to select such individuals through comprehensive medical history, physical examination, and review of primary care records. We recognise the pre-test probability of subclinical biliary or aortic disease in this cohort is very low. However, we have agreed on abdominal ultrasonography with standardised assessment and reporting protocols as an appropriate measure to exclude these specific risk factors for complicated infection. Abdominal USS is accessible, non-invasive, and can be performed and reported in a timely manner. Transthoracic echocardiography was carefully considered but felt unnecessary due to burden of imaging and reporting times – again, in the context of a low pre-test probability for subclinical valvular disease. Furthermore, *Salmonella* endocarditis is thought to represent a less likely complication even amongst the small proportion who may develop bacteraemia. Timely antibiotic therapy will be commenced immediately upon development of sustained fever and/or bacteraemia, significantly reducing the probability of such complications developing.

"The term 'double-blind randomisation' will eventually need to be defined in more detail. Who exactly will be blinded? Presumably only the volunteers, the clinical trials facility staff, and those charged with assessing endpoints? Too much blinding too early in the development of a CHIM may be overly restrictive and prevent the almost inevitable flexible adjustments the protocol will require as early lessons emerge."

Thank you. As suggested, blinding will be applied to participants, clinical study staff delivering direct patient care, and the same clinical team members responsible for assessment of clinical endpoints. Unblinding will occur at study conclusion, or as deemed necessary by the Data Safety and Monitoring Committee in response to safety thresholds.

"I understand the choice to exclude volunteers previously vaccinated against typhoid from the initial trial. Although resource constraints may preclude challenging some of these volunteers at a dose level that achieves a high attack rate in unvaccinated volunteers, I do wonder if samples from these participants could make a meaningful contribution to the immunological work to follow the clinical trial. Given the investments required to launch a CHIM study, this idea may not happen at all if it doesn't happen early and in parallel to another study."

Thank you. Participants will only be excluded if they have received prior vaccination with oral typhoid Ty21a vaccine (or confirmed prior *Salmonella* infection). As Ty21a is a live attenuated vaccine, this would be considered prior 'oral challenge' with a clinically relevant *Salmonella* serovar. No other prior formulation of typhoid vaccination would preclude participation. Immunological assays will be applied following the clinical study on samples obtained from all participants, irrespective of attack rate.

"The final reference regarding systems serology introduces these methods in relation to HIV research. The same others (Galit Alter et al) have since published systems serology investigations specifically related to CHIM studies (e.g. typhoid, shigella) that would seem to be even more appropriate references here?"

Thank you. The final references have been updated to reflect your appropriate suggestions.

"I know that these authors have a strong record of work with clinicians and investigators in low- and middle-income countries. As they point out, the global burden of NTS disease is concentrated in LMICs and especially in Africa. However, there is limited representation from LMICs including African countries (besides Prof. Gordon) in the author group for this open letter and the workshop attendees. I do trust the consortium has plans to boost its engagement in this regard. Nonetheless, it does bear mentioning. I do note the authors have made a general comment in the conclusion regarding plans for stakeholder engagement."

Thank you for this important comment. As suggested, we aim to maximise engagement with key stakeholders from LMICs, where the greatest burden of iNTS disease is concentrated. Members from academic and research institutions in sub-Saharan Africa have been consulted throughout the protocol development stages of this study, and also form central roles within our Data Safety and Monitoring Committee. Future applications and use case of the model will involve ongoing collaboration with representatives from SSA, including potential transfer of the model to an endemic setting, and in planning future vaccine efficacy studies.

Competing Interests: No competing interests were disclosed.