Trial Title: Development of a Live Attenuated Vaccine against *Salmonella* Paratyphi A

Internal Reference Number / Short title: Development of a Vaccine against *Salmonella* Paratyphi A (VASP)

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Funder:	Medical Research Council

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AJP is Chair of UK Dept. Health and Social Care's (DHSC) Joint Committee on Vaccination & Immunisation (JCVI), and is a member of the WHO's SAGE. Oxford University has entered a joint COVID19 vaccine development partnership with Astra Zeneca

Confidentiality Statement

This document contains confidential information that must not be disclosed to anyone other than the Sponsor, the Investigator Team, HRA, host organisation, and members of the Research Ethics Committee and Regulatory Authorities unless authorised to do so.

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Protocol signature page

The undersigned has read and understood the trial protocol detailed above and agrees to conduct the trial in compliance with the protocol.

Andrew J Pollard		Oxford	13/04/2022
Chief Investigator	**Signature**	**Site name or ID number**	**Date**

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1. KEY TRIAL CONTACTS

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2. LAY SUMMARY

Typhoid and Paratyphoid fever are both forms of an illness called Enteric fever. Their names come from the bacteria that cause them: *Salmonella* Typhi (typhoid) and *Salmonella* Paratyphi A (paratyphoid). They both cause high fevers, headache, muscle and joint aches, abdominal pain, constipation and feeling generally unwell. If severe or left untreated, it can result in complications, long-term carriage of the bacteria or death.

There are approximately 14.3 million cases of Enteric fever every year, with 3.3 million of these due to paratyphoid. It is spread by the faeces of an infected person, typically via contaminated water or food. It is found in parts of the world where people have inadequate access to clean water and sanitation.

Effective vaccines against typhoid fever already exist but there are no licensed vaccines against paratyphoid fever yet. The University of Maryland have developed an oral paratyphoid vaccine. It has already been given to humans and was shown to be safe. It now needs testing to see if it might prevent disease. The Oxford Vaccine Group has developed a method of testing vaccines called controlled infection or "challenge" studies whereby participants are given the vaccine and later a dose of bacteria which can cause disease. All participants are monitored closely and are treated if they become unwell, or 14 days after drinking the bacteria, whichever is sooner. In this study we will give participants either the vaccine or a placebo, and then the "challenge", to see if the vaccine prevents the disease.

This model of studying vaccines has been undertaken by participants in previous Oxford Vaccine Group studies since 2011. Samples taken will not only test the effectiveness of this potential vaccine but will also help us better understand how the immune system protects against this disease.

3. SYNOPSIS

Trial Title	Development of a Live Attenuated Vaccine Against Salmonella Paratyphi A
Internal ref. no. (or short title)	Development of a Live Attenuated Vaccine Against Salmonella Paratyphi A (VASP) (OVG 2018/07)
Trial registration	EudraCT number: 2021-003259-41
Sponsor	University of Oxford
Funder	Medical Research Council
Clinical Phase	1/2
Trial Design	Observer-blind, participant-blind, controlled, outpatient, ambulatory design human infection study
Trial Participants	Healthy adults aged 18-55 years inclusive
Sample Size	66-76 participants will be randomised 1:1 to receive CVD 1902 **oral** vaccine or a placebo. A minimum of 30 participants will be required to be challenged per group, assuming a minimum attack rate in the control group of 65%, in order to demonstrate a protective effect of vaccination of 60% in the CVD 1902

	group. To account for a possible drop-out rate of 10%-20%, 33-38 participants per group will be randomised.	
Follow up duration	12 months post challenge	
Planned Trial Period	Clinical phase: September 2021-December 2024	
Planned Recruitment period	August 2021-December 2023	
	Objectives	Outcome Measures
Primary	To determine the relative protective effect of two doses of CVD 1902 given 14 days apart compared with placebo (sodium bicarbonate) in a healthy adult paratyphoid challenge model	The proportion of participants developing clinical or microbiologically proven paratyphoid infection following oral challenge with 1-5x10^3 *S*. Paratyphi A (strain NVGH308) delivered in a sodium bicarbonate solution in the group who have received two doses of CVD 1902 compared with those who have received two doses of placebo
Secondary	a. To compare the clinical and laboratory features of the host responses following challenge with *Salmonella* Paratyphi A (strain NVGH308) in participants vaccinated with CVD 1902 compared to placebo, including the time course of illness, development of bacteraemia and the inflammatory response.	Comparison of the clinical course of paratyphoid infection after challenge between placebo and CVD 1902 groups, in particular: • time to onset of symptoms • duration of illness • symptom severity • time to onset of bacteraemia • time to onset of stool shedding • inflammatory response Using clinical reporting, physical examination findings, microbiological assays to detect *S.* Paratyphi A in blood and stool, and laboratory assays to monitor inflammatory responses.
	b. To compare the host immune response following vaccination with CVD 1902, compared with placebo including innate, antibody and cell-mediated responses and	Immunological laboratory assays to assess innate, humoral, cell-mediated and mucosal responses to vaccination at baseline (Day -42) and post-vaccination time points, these may include:

	persistence of immunity and to relate these responses to the protective effect of vaccination.	• *S.* Paratyphi A antigen specific antibodies and serum bactericidal antibody titres • Cell-mediated responses (including antigen specific cell frequencies, description of lymphocyte populations, B and T cell repertoire) • Cytokine and acute phase reactant profile and kinetics
	c. To compare the host immune response following *S.* Paratyphi A challenge following vaccination with CVD 1902 or placebo.	Immunological laboratory assays to assess innate, humoral, cell-mediated and mucosal responses to challenge will be taken at various time points following challenge (for time points see section 7.6, Table 3b and section 7.7, Table 3c) in a variety of sample types, these may include: • Cell-mediated responses (including antigen specific cell frequencies, description of lymphocyte populations, B and T cell repertoire) • Cytokine and acute phase reactant profile and kinetics • *S.* Paratyphi A antigen-specific IgA, IgM and IgG antibodies
	d. To assess the safety and tolerability of CVD 1902 including faecal shedding	Clinical observation and participant recording of symptoms, both solicited and unsolicited plus safety laboratory data and microbiological data from blood and stool cultures following vaccination
	e. To investigate immunological correlates of protection for *S.* Paratyphi A infection	Immunological response data post-vaccination (including *S.* Paratyphi A specific antibody titres, cell-mediated responses) will be combined with vaccine efficacy data following *S.* Paratyphi A challenge to investigate if particular immunological markers could be used to predict protection from paratyphoid infection
Exploratory (Laboratory analyses relating to	To investigate recruitment methods and reasons for participant exclusions from	Analysis of recruitment numbers, including:

exploratory endpoints may be performed following adoption of samples into the OVC biobank)	paratyphoid challenge models.	• Number of positive and negative responses to different recruitment techniques; • Number of participants excluded prior to attending screening visits and reasons for exclusion; • Number of participants attending for screening visits and reasons for exclusion.
	To explore the variation in genomic response to vaccination with CVD 1902, or placebo and subsequent *Salmonella* Paratyphi A challenge in participants.	Laboratory and high-throughput assays to measure gene expression and protein translation at baseline, post-vaccination and post-challenge time points
	To explore changes in the gut microbiome following a course of antibiotics	Samples of stool to measure the constituent microbiological flora by assays such as pyrosequencing and related metagenomic studies.
	To explore molecular changes occurring after vaccination, challenge and during acute infection	Application of techniques such as proteomics, metabolomics and epigenetics to samples from baseline, post-vacination and post-challenge timepoints

Vaccines		
1) Investigational	CVD 1902 (a live attenuated *Salmonella* enterica serovar Paratyphi A, containing deletions of guaBA and clpX) Unlicensed. Manufactured to GMP by Bharat Biotech	
	Pre-treatment:	120ml sodium bicarbonate solution (to neutralize stomach acid)
	Dose/formulation:	Not less than 2 x 10^{10} CFU suspended in 30ml sodium bicarbonate
	Dosing schedule:	Two doses, 14 days apart
	Route of administration:	oral
2) Comparator (placebo)	Sodium bicarbonate	
	Pre-treatment:	120ml sodium bicarbonate solution (to neutralize stomach acid)

	Dose/formulation:	Sodium bicarbonate solution, volume to match vaccine
	Dosing schedule:	Two doses, 14 days apart
	Route of administration:	oral
Challenge Agent		
Challenge Agent	*Salmonella* Paratyphi A (strain NVGH308)	
	Pre-treatment:	120 ml sodium bicarbonate solution (to neutralize stomach acid)
	Dose/formulation:	1-5x10^3 CFU suspended in 30ml sodium bicarbonate prior to oral ingestion
	Schedule:	Single dose, 28 days after second dose of CVD 1902 or placebo
	Route of administration:	oral

4. ABBREVIATIONS

AE	Adverse event
AR	Adverse reaction
CCVTM	Centre for Clinical Vaccinology and Tropical Medicine, Churchill Hospital, Oxford
CI	Chief Investigator
CRA	Clinical Research Associate (Monitor)
CRF	Case Report Form
CRO	Contract Research Organisation
CSP	Clinical study plan
CT	Clinical Trials
CTA	Clinical Trials Authorisation
DMSC	Data Monitoring and Safety Committee
DSUR	Development Safety Update Report
eCRF	Electronic Case Report Form
ESBL	Extended spectrum beta-lactamase
GCP	Good Clinical Practice

GP	General Practitioner
HRA	Health Research Authority
IB	Investigators Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IMP	Investigational Medicinal Product
MHRA	Medicines and Healthcare products Regulatory Agency
NHS	National Health Service
OVGL	Oxford Vaccine Group Laboratories
RES	Research Ethics Service
PD	Paratyphoid Diagnosis
PI	Principal Investigator
PIL	Participant/ Patient Information Leaflet
R&D	NHS Trust R&D Department
REC	Research Ethics Committee
RGEA	Research Governance, Ethics and Assurance
RSI	Reference Safety Information
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SDV	Source Data Verification
SMPC	Summary of Medicinal Product Characteristics
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reactions
TMF	Trial Master File
UKHSA	United Kingdom Health Security Agency
URT	Upper Respiratory Tract

5. BACKGROUND AND RATIONALE

Enteric fever remains a cause of significant disease in resource limited settings. It is principally caused by *Salmonella enterica* serovars Typhi and Paratyphi A. Enteric fever is a non-specific febrile illness affecting 14.3 million individuals annually in 2017; the proportion attributable to *Salmonella* Paratyphi A varies widely by geography but may be in the region of 25%[1]. Estimating the true burden of each serovar is challenging because sensitive diagnostic tests, adequate laboratory facilities and surveillance structures do not exist as standard in endemic areas. Due to these factors global estimates are thought to be an underestimate but the region of highest *S.* Paratyphi A burden appears to be Asia[2], though the ages of those most affected are not well understood. The incidence of *S*. Paratyphi *A* appears to be increasing in some areas[3-5] though the reasons for this are unclear. Cases are also seen in travellers[6-10], particularly those visiting friends and family[10].

Provision of sanitation and access to clean water are paramount in preventing enteric fever but these take time and rely on political will and economic investment. *S*. Typhi vaccines exist and Vi-conjugate vaccines are recently been shown to be effective in Nepal[11]. In the medium-term vaccination against *S*. Paratyphi A is likely to be a cost-efficient way to minimise disease burden, if combined with *S.* Typhi vaccination. Vaccination against *S*. Typhi is highly likely to become more widespread in endemic areas. A study in China suggested that introduction of *S.* Typhi Vi-polysaccharide vaccination was associated with an increase in the incidence of *S.* Paratyphi A, causing concern about so called "serovar replacement" [12] following *S.* Typhi vaccination. The lack of other data and wide geospatial variation in vaccine coverage and bacterial surveillance make such reports difficult to interpret. However even if such an increase does not occur with *S*. Typhi conjugate vaccination coverage, development of a *S.* Paratyphi A vaccine remains the best hope of tackling this pathogen.

This study will use an established human infection model to test a live attenuated oral vaccine candidate for *S.* Paratyphi A. Building on the success of using this model to evaluate a conjugate *S.* Typhi vaccine[13] this study aims to further the development of *S.* Paratyphi A vaccines by assessing efficacy and characterisation of the immune response to vaccination within a host-relevant model.

5.1. *Salmonella* Paratyphi A

5.1.1. Epidemiology and Burden

S. Paratyphi A is one of two main serovars of *Salmonella enterica* that cause enteric fever, the other being *S.* Typhi. These two serovars cause enteric fever, a collection of symptoms including fever, headache, malaise, myalgia and arthralgia. Clinically the disease caused by each is indistinguishable which has resulted in a research field where the two are typically combined.

There is increasing recognition that adequate surveillance and more accurate estimation of the burden of enteric fever is essential for both researchers and policy makers. Whilst recent estimate data show a decline in enteric fever cases from 25.9 million cases to 14.3 million cases in 2017[1], critically this systematic analysis provided separate data on *S.* Paratyphi A and attributed 3.4 million of these cases to it in 2017. These data are comparable with previous estimates[14,15] but are also subject to the same caveats in terms of wide uncertainty due to lack of data and lack of a gold standard diagnostic test. Various large-scale surveys such as the Surveillance for Enteric Fever in Asia Project (SEAP), Severe Typhoid Fever Surveillance in Africa (SETA) and Surveillance for Enteric Fever in India (SEFI) are underway to try and fill these knowledge gaps.

There is very wide geospatial variation in terms of the relative contribution of *S*. Typhi and *S*. Paratyphi A to clinical cases of enteric fever[1]. The major burden of disease appears to be Asia however there is a massive difference between different countries; the proportion of cases that are caused by *S.* Paratyphi A varied from 13.6% in Bangladesh[16], 31% in Pakistan[16] to over 80% in the Guangxi region of China in one study[12]. There are almost no cases reported in Africa[17] and sparse data from other areas of the world.

5.1.2. Pathogenesis

The study of the pathogenesis of *S.* Paratyphi A is hindered by human host restriction thereby precluding a robust animal model. *S*. Paratyphi A is typically ingested in contaminated food or water. As for *S*. Typhi knowledge about pathogenesis is derived from animal models of other *Salmonella enterica* serovars. Inoculating bacteria travel through the gastrointestinal tract to the terminal ileum where invasion via the M cells on Peyer's patches is thought to occur[18]. Once in the bloodstream bacteria are disseminated throughout the body showing a predilection for bone marrow, liver and the gallbladder. There is an incubation period between ingestion and the development of disease, during which it is likely that the *Salmonella* Paratyphi A replicates in these extravascular sites. A sustained bacteraemia is then seen after approximately 7-10 days and in addition bacteria are shed into the bile and thus into the stool, allowing others to become infected.

Acute and chronic complications of disease such as intestinal perforation and carriage occur in *S*. Paratyphi A as in *S*. Typhi[19]. Multi-drug resistant and fluoroquinolone resistant strains appear more likely to result in complications and mortality[20].

5.1.3. Transmission

Transmission dynamics of typhoidal *Salmonella* species are incompletely understood. It is thought they are spread via food and water contaminated by the faeces of infected individuals[21]. It has been shown that cases of typhoidal *Salmonellas* can be seen to be clustered around urban water spouts in Kathmandu and water from these sources is faecally contaminated[22,23]. Notably cases appear to increase following

rainfall[22]. Environmental studies show that whilst traditional bacterial cultures are often negative, *S.* Paratyphi A can be detected by PCR in water samples[23,24].

Case control studies suggest that the risk factors for transmission of *S*. Typhi and *S*. Paratyphi A may differ and *S*. Paratyphi A may be more likely to be transmitted from certain food sources[25]. Other studies of food handlers and environmental studies suggest that there is overlap common to both serovars[22,26].

5.1.4. Treatment

Correct and prompt antimicrobial treatment typically results in complete cure of enteric fever. In endemic areas however access to adequate diagnostic or clinical infrastructure is often inadequate, resulting in empiric antimicrobial treatment. No national or international guidelines exist to help guide clinicians[27].

Antimicrobial non-susceptibility is becoming a significant issue in *S.* Typhi [28]. In common with the epidemiological differences, antimicrobial susceptibility differs widely according to geography which may reflect local spread and prescribing patterns[29,30]. Susceptibility of *S.* Paratyphi A isolates to first line agents (co-trimoxazole, chloramphenicol, amoxicillin) has been high (>90%) in many areas[31,32] but increasing fluoroquinolone resistance is observed[29,32-35]. Third generation cephalosporin susceptibility in many areas appears to be preserved[31,36,37] but there are reports of ESBL-producing strains[38] which are of significant concern. Azithromycin resistance has also been recently demonstrated[39].

Treatment trials have been undertaken in enteric fever[40-42] but are complicated by where cases present, delayed diagnostics and local epidemiology. Despite evidence of increasing resistance, fluoroquinolones remain the treatment of choice in susceptible strains and particularly for the treatment of carriers[43].

5.2. Vaccination against *S*. Paratyphi A infection

5.2.1. Vaccine development

Immunity to invasive *Salmonella* species is complex, reflecting the different stages of infection from mucosal invasion, intracellular occupation and bloodstream infection. It is likely that in the course of natural infection, both humoral and cell mediated immunity are elicited.

Efficacious vaccines for *S*. Typhi exist[11,13,44], suggesting that vaccination against *S*. Paratyphi A may be biologically feasible. Existing licensed vaccines for *S*. Typhi are the oral live-attenuated Ty21a (Vivotif®), the i.m. or s.c. subunit Vi-polysaccharide vaccine (Typherix®, Typhim VI®) and Vi-conjugate vaccine (Typbar TCV®). In January 2018 the WHO pre-qualified a conjugated Vi-polysaccharide vaccine Typbar-TCV following a SAGE recommendation for its use in children in high burden countries in October 2017. Field work is ongoing to assess the efficacy of this vaccine in endemic settings; preliminary reports though are promising[11].

There are no licensed vaccines for *S.* Paratyphi A though there are a number in development, employing a range of approaches[45]. Focus has been on two main avenues: an oral, live-attenuated vaccine (CVD 1902; University of Maryland and Bharat Biotech[46]) and injectable conjugate vaccines based on the lipopolysaccharide antigen (US NIH, Chengdu and Lanzhou Institutes of Biological Products in China; Biological E and SVGH; International Vaccine Institute)[45].

Both *S*. Typhi and *S.* Paratyphi A are human restricted pathogens. Research to develop accurate diagnostics and vaccination is hampered by the absence of an animal model. Currently no easily measured humoral correlate of protection to *S*. Paratyphi A has been identified. An *in vitro* serum bactericidal assay has been developed to assess complement-mediated antibody-dependent bacteria killing which may act as a surrogate marker for protection[47].

S. Paratyphi A does not express the Vi capsular polysaccharide which may explain why there does not appear to be significant cross-protection conferred by effective typhoid vaccines in clinical trials[48] but in vitro data suggests that Ty21a (which does not express Vi) may induce some antibody-secreting cells which do cross-react against *S*. Paratyphi A[49]. It does however express O antigens O-1,2,12 and flagellar antigens H type A, both of which are known to be highly immunogenic and the conjugate vaccines in development are employing O:2 conjugated to one of a number of familiar conjugate proteins (TT, DT, CRM_{197}).

5.2.2. Role of a *S*. Paratyphi A vaccine

Whilst there is marked geospatial variation in the epidemiology of *S*. Paratyphi A within Asia at least there is gross epidemiological overlap with *S*. Typhi on this continent. The role of a *S*. Paratyphi A vaccine from a policy making perspective would be as a bivalent vaccine in combination with a *S*. Typhi vaccine with a view to deployment broadly within an Asian context. Therefore, if CVD 1902 is shown to provide protective efficacy there is no intention to progress it to licensure as a monovalent vaccine and consequently this trial should be considered a proof of concept efficacy trial.

5.2.3. Rationale for an oral live attenuated organism

Developing vaccines for Salmonella species is particularly challenging. During an infection bacteria are found both extra- and intracellularly hence vaccines may be required to elicit both humoral and cell mediated immunity[50]. The premise for an oral live attenuated vaccine is, on this basis more intuitive than the conjugate vaccines, especially as we do not understand what constitutes immunological protection to *S*. Paratyphi A. An oral vaccine delivers many antigens to the mucosal surface and therefore should be capable of eliciting a multifaceted immune response. In addition, oral vaccine delivery often offers improved safety, easier administration and simpler manufacturing procedures.

There are limited data on the effect of infection on subsequent immunity to *S.* Paratyphi A. For *S*. Typhi early challenge studies showed prior infection conferred only modest protection, but a large challenge inoculum may be sufficient to overcome this[51,52]. It is unknown if the same is true for *S.* Paratyphi A.

A recent challenge/re-challenge study in a *S*. Paratyphi A human infection model showed that there was a 55% reduction in diagnosis in the re-challenge group compared to the naïve group[53] in *S*. Paratyphi A over a year after the initial challenge. This study sample size was small and the reduction was not statistically significant but showed that prior *S*. Paratyphi A infection may be protective. Indirect evidence from the burden of disease across age groups may be helpful in establishing the protective effect of prior infection but surveillance data, especially in the youngest age groups is insufficient[54].

5.2.4. CVD 1902

CVD 1902 *Salmonella* enterica serovar Paratyphi A live oral vaccine was constructed from wild type parent stain *S*. Paratyphi A strain ATCC 9150 by deleting the *gua*BA chromosomal operon (which encodes two enzymes employed in the distal *de novo* guanine nucleotide biosynthesis pathway). A second attenuating mutation deleted *clp*X. This gene encodes a chaperone ATPase that functions with the serine protease encoded by *clp*P to form a complex that participates in a variety of metabolic processes, including playing a role in controlling the availability of regulatory proteins and the breakdown of misfolded proteins)[55,56]. This *clpX* mutation results in hyperexpression of flagella. The second attenuating mutation minimises the risk of a reversion to full virulence. It is fully sensitive to ciprofloxacin, trimethoprim/sulfamethoxazole and ampicillin[56].

Studies in animals confirm the attenuation of virulence and suggest that CVD 1902 offers protection. Due to human host restriction animal models require different routes of delivery to act as surrogate models. When mice were inoculated with 10^9 CFU CVD 1902 intranasally, 0/19 vaccinated mice died by day 3 post intraperitoneal challenge with wild type *S.* Paratyphi A compared with 4/20 of the control mice [unpublished data].

This vaccine has been tested in adults in a phase 1 trial. A total of 30 healthy young adults have received a single oral dose of CVD 1902 in a single-site, randomised, double-blinded phase 1 study[46,56]. This study was performed in the USA and a 14-day course of antibiotics (ciprofloxacin or trimethoprim/sulfamethoxazole) were commenced 12 days after vaccination with six days of treatment prior to discharge from the inpatient containment facility where this study was performed. The phase 1 study showed no faecal shedding beyond day 3 (similar to other live attenuated vaccines[57]), and no positive blood cultures[56]. All participants who are challenged will receive antibiotics following challenge but will not receive antibiotics after the vaccine.

This vaccine is being studied in this trial as a proof of concept as to whether this attenuated strain can offer protection. There are no plans to take CVD 1902 to licensure as a monovalent vaccine. If this trial were to show promising efficacy within the challenge model the next step would be development into a bivalent vaccine against both *S*. Typhi and *S*. Paratyphi A.

5.2.5. Rationale for dose and dosing schedule

Live oral vaccines such as Ty21a and Vaxchora (CVD103 HgR) do not have specific doses in terms of CFU, instead, a dose minimum or range is given (not less than 2 x 10^9, and 4 x 10^8 to 2 x 10^9 CFU respectively). A dose range approach will be taken with CVD 1902: 2 x10^{10} to 1.7 x 10^{11} CFU per dose. The lower limit of the dose range chosen for this trial (2 x 10^{10} CFU) corresponds with the highest dose category in the Phase 1 trial performed at the Center for Vaccine Development, University of Maryland School of Medicine. This dose was shown to be safe and generate both ASC and serum antibody responses to LPS and H antigens in 4/6 (67%) of subjects in this dose group[56]. As higher doses (10^9 and 10^{10} CFU) were required in the Phase 1 study to elicit immune responses a two-dose schedule is thought necessary to optimise response across participants whilst also testing a schedule that ultimately may be feasible at a population level. The upper value for the dose range is derived from the stability data from September 2021 and represents the maximum cell count in vials tested at this timepoint. A reduction in cell count viability would be expected during the duration of the trial hence this is anticipated to be the maximum CFU to be delivered in one dose. All dose ranges were well tolerated in the Phase 1 study, and whilst there was no 10^{11} dosage group in that study, a dose of one log higher is not anticipated to be associated with safety concerns. Two doses would however be expected to be more immunogenic than those seen after a single dose in the Phase 1 study. Participants will be monitored closely throughout the trial, with the DSMB providing independent oversight, with regular safety reviews particularly during the early phase of this trial. A sentinel group of six participants (for approximately three participants randomised to vaccination) will undergo vaccination after which there will be a DSMB review of the safety data as this maximum dose has not been given before.

The rationale for using two doses of CVD 1902 14 days apart is to try and ensure an optimum immunological response to the vaccine. Previously, a single-dose live attenuated oral vaccine for *S*. Typhi, M01ZH09 was trialled within a human challenge model and whilst immunogenic, was not shown to provide protection.[58] Consultation with experts in the field has indicated that multiple doses are required to provide robust priming with enteric immunisation. The oral vaccine for *S*. Typhi, Ty21a requires administration three to four times with 48-hour intervals between doses, thought to mimic a natural infection with mucosal exposure to the vaccine for a prolonged period. [redacted]. Other oral vaccines in development have used a 14-day dosing regimen[59]. Vaccines reliant on a humoral response when administered as multiple doses are usually delivered with a 2-4 weeks interval. Therefore a two-dose

regimen with a 14-day interval was felt to be a pragmatic dosing regimen aiming to achieve both cell-mediated and humoral immunity.

5.2.6. Explanation for comparison to placebo

There are no other licensed oral vaccines for *S*. Paratyphi A, thus a head to head comparison is not possible. The use of a live attenuated vaccine against *S*. Typhi may produce misleading results due to the potential for cross-reactivity between S. Typhi and S. Paratyphi A.[60] An alternative approach would be to compare with another live attenuated oral vaccine such as cholera (eg Vaxchora, CVD103 HgR) which is licensed in the US but is not available in the UK. This option has not been chosen due to the potential for interaction between another live agent and the challenge agent. Sodium bicarbonate can produce mild adverse events as described in the SmPC which will allow the blind to be maintained.

Challenge studies are often performed without preceding vaccination.[61-64] Informed consent will be taken from all participants which will include explanation of comparison of the novel vaccine CVD 1902 against a placebo. Over 450 participants have been challenged with enteric fever-causing organisms (*S*. Typhi or *S*. Paratyphi A) at the Oxford Vaccine Group in the last decade, all have been successfully treated and there have been no safety concerns. All participants are closely monitored by clinicians throughout the challenge period, and are diagnosed and treated promptly after showing signs of illness. Field efficacy trials for a *S*. Paratyphi A vaccine are likely to be unfeasible due to the large sample size that would be required to evaluate efficacy coupled with the lack of sensitivity of diagnostic methods. Testing a vaccine within a challenge model provides a robust and reproducible means to assess efficacy in a relatively small number of subjects.

5.3. Human challenge model of S. Paratyphi A

5.3.1. Human challenge models of enteric fever

A human challenge model of enteric fever using *S*. Typhi was established in the 1960s at the University of Maryland. This program was terminated due to ethical concerns regarding the study population of incarcerated individuals. The Oxford Vaccine Group has established its own human challenge models for *S*. Typhi and *S*. Paratyphi A in healthy adult participants. To date the Oxford Vaccine Group has run six human challenge studies, involving over 450 participants.

Dose finding and safety studies for each pathogen determined the parameters by which the model could operate in healthy participants (OVG 2009/10 Oxford A REC ref. 10/H0604/53; OVG 2013/07 Oxford A REC ref. 14/SC/0004)[64,65]. Other studies have explored the pathogenesis of disease (OVG2016/03 Oxford A REC ref. 16/SC/0358) and the effect of re-challenge with the same and alternative serovar (OVG 2014/01 Oxford A REC ref. 14/SC/1204). The *S*. Typhi model has also been successfully employed to assess the

efficacy of vaccines, both oral live attenuated and conjugate (OVG2011/02 Oxford A REC ref: 11/SC/0302; OVG 2014/08 Oxford A REC ref. 14/SC/1427)[13,58].

The ability to test a vaccine within a challenge model in humans is particularly powerful. It provides both a cost and time-efficient way of assessing candidate vaccines prior to roll out in large and prohibitively expensive field studies which are particularly problematic in diseases such as enteric fever for reasons outlined above. Following the result of the *S*. Typhi conjugate vaccine trial (OVG 2014/08 Oxford A REC ref. 14/SC/1427)[13], the WHO pre-qualified this vaccine Typbar TCV® for use in children which has permitted large scale trials of this vaccine in endemic areas.

This study is the first time that the *S.* Paratyphi A human challenge model has been used to assess vaccine efficacy. Participants will be followed up for one year post challenge to continue safety evaluation and long term immunogenicity.

5.3.2. *S*. Paratyphi A challenge model

The Oxford Vaccine Group developed an ambulatory human infection model of *S*. Paratyphi A. The dose of 1-5 x 10^3 CFU has been shown to lead to a diagnosis (by pre-specified composite endpoint of fever $\geq$38^{o}C for greater than 12 hours or a positive blood culture $\geq$72hrs after challenge) in 60% of individuals in a previous dose-finding study [65]. The model involves careful selection of participants, intensive monitoring after challenge administration and follow-up to ensure clearance of pathogen.

5.3.3. *S*. Paratyphi A NVGH308 challenge strain

The original *S*. Paratyphi A strain NVGH308 isolate was from a clinical study performed by the Oxford University Clinical Research Unit at Patan Hospital, Kathmandu, Nepal. It has been manufactured into batches to GMP standard by GenIbet BioPharmaceuticals, Portugal, and is supplied to the Oxford Vaccine Group by Novartis Vaccines for Global Health.

5.3.4. Rationale for timing of antibiotic treatment

In the Phase 1 study carried out in the US participants were confined to an inpatient facility. To satisfy the FDA requirements for these participants to return to the community participants were treated with a course of antibiotics on day 12 after vaccination. Prior to treatment with antibiotics participants were closely monitored for evidence of invasive disease or prolonged shedding, no participants had a positive blood culture and no participants shed vaccine strain beyond 3 days. The participants in this trial will be ambulatory outpatients and given the evidence from the Phase 1 study there is no indication to treat with antibiotics as participants will already be in the community and will have received advice regarding strict hygiene measures to observe. Participants will be closely monitored and have access to a study clinician at all times should they have any concerns. In addition treatment with antibiotics for all participants will

occur following challenge with the wild-type *S*. Parayphi A strain (NVGH308). Additional courses post vaccination would also expose participants to unnecessary extra antimicrobial exposure.

5.4. Aims of the study

This study aims to assess the efficacy of the orally administered live-attenuated vaccine CVD 1902 and extend our knowledge of the immune response both to *S*. Paratyphi A infection and vaccination. We hope to identify correlates of protection and characterise the humoral and cell mediated immunity generated by this vaccine.

6. OBJECTIVES AND OUTCOME MEASURES

	Objectives	Outcome Measures
Primary	To determine the relative protective effect of two doses of CVD 1902 given 14 days apart compared with placebo (sodium bicarbonate) in a healthy adult paratyphoid challenge model	The proportion of participants developing clinical or microbiologically proven paratyphoid infection following oral challenge with 1-5x10^3 *S*. Paratyphi A (strain NVGH308) delivered in a sodium bicarbonate solution in the group who have received two doses of CVD 1902 compared with those who have received two doses of placebo
Secondary	a. To compare the clinical and laboratory features of the host responses following challenge with *Salmonella* Paratyphi A (strain NVGH308) in participants vaccinated with CVD 1902 compared to placebo, including the time course of illness, development of bacteraemia and the inflammatory response.	Comparison of the clinical course of paratyphoid infection after challenge between placebo and CVD 1902 groups, in particular: • time to onset of symptoms • duration of illness • symptom severity • time to onset of bacteraemia • time to onset of stool shedding • inflammatory response Using clinical reporting, physical examination findings, microbiological assays to detect *S.* Paratyphi A in blood and stool, and laboratory assays to monitor inflammatory responses
	b. To compare the host immune response following vaccination with CVD 1902, compared with placebo including innate, antibody and cell-mediated	Immunological laboratory assays to assess innate, humoral, cell-mediated and mucosal responses to vaccination at baseline (Day -42) and post-

	responses and persistence of immunity and to relate these responses to the protective effect of vaccination.	vaccination time points, these may include: • *S.* Paratyphi A antigen specific antibodies and serum bactericidal antibody titres • Cell-mediated responses (including antigen specific cell frequencies, description of lymphocyte populations, B and T cell repertoire) • Cytokine and acute phase reactant profile and kinetics
	c. To compare the host immune response following *S.* Paratyphi A challenge following vaccination with CVD 1902 or placebo.	Immunological laboratory assays to assess innate, humoral, cell-mediated and mucosal responses to challenge will be taken at various time points following challenge (for time points see section 7.6, Table 3b and section 7.7, Table 3c) in a variety of sample types, these may include: • Cell-mediated responses (including antigen specific cell frequencies, description of lymphocyte populations, B and T cell repertoire) • Cytokine and acute phase reactant profile and kinetics • *S.* Paratyphi A antigen-specific IgA, IgM and IgG antibodies
	d. To assess the safety and tolerability of CVD 1902 including faecal shedding.	Clinical observation and participant recording of symptoms, both solicited and unsolicited plus safety laboratory data and microbiological data from blood and stool cultures following vaccination
	e. To investigate immunological correlates of protection for *S*. Paratyphi A infection.	Immunological response data post-vaccination (including *S*. Paratyphi A specific antibody titres, cell-mediated responses) will be combined with vaccine efficacy data following *S.* Paratyphi A challenge to investigate if particular immunological markers

		could be used to predict protection from paratyphoid infection
Exploratory (Laboratory analyses relating to exploratory endpoints may be performed following adoption of samples into the OVC biobank)	To investigate recruitment methods and reasons for participant exclusions from paratyphoid challenge models.	Analysis of recruitment numbers, including: • Number of positive and negative responses to different recruitment techniques; • Number of participants excluded prior to attending screening visits and reasons for exclusion; • Number of participants attending for screening visits and reasons for exclusion.
	To explore the variation in genomic response to vaccination with CVD 1902, or placebo and subsequent *Salmonella* Paratyphi A challenge in participants.	Laboratory and high-throughput assays to measure gene expression and protein translation at baseline, post-vaccination and post-challenge time points
	To explore changes in the gut microbiome following a course of antibiotics	Samples of stool to measure the constituent microbiological flora by assays such as pyrosequencing and related metagenomic studies.
	To explore molecular changes occurring after vaccination, challenge and during acute infection	Application of techniques such as proteomics, metabolomics and epigenetics to samples from baseline, post-vaccination and post-challenge time points

7. TRIAL DESIGN

7.1. Overview

This is an observer-blind, participant-blind, randomised, placebo-controlled trial of the oral live-attenuated vaccine CVD 1902 using a healthy adult participant controlled human infection model of paratyphoid. This study is blinded to reduce the effect of bias on the adverse event reporting which forms one of the secondary endpoints (safety and tolerability of CVD 1902).

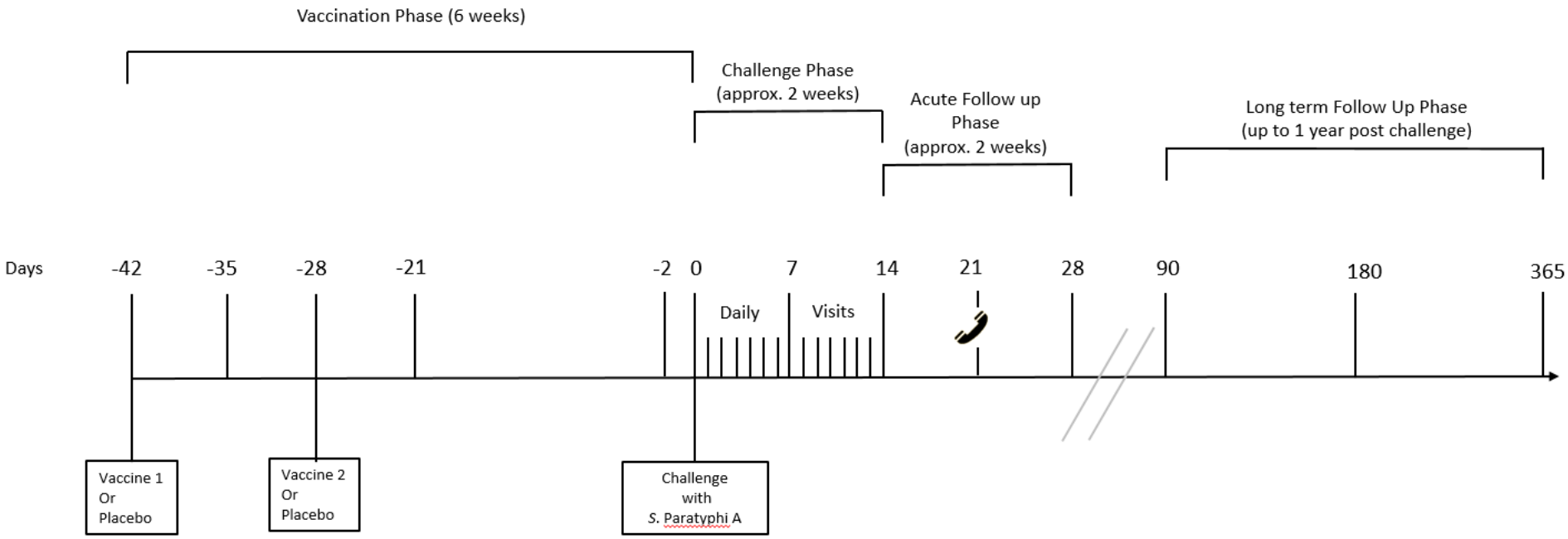
In total 66-76 participants will be randomised in a 1:1 ratio to receive a dose of not less than 2 x 10^{10} CFU of CVD 1902 or placebo (33-38 participants per group). They will receive two doses of vaccine or placebo 14 days apart.

Twenty eight days after their second vaccine or placebo dose participants will be challenged with *S*. Paratyphi A (strain NVGH308) at a dose of 1-5 x 10^3 CFU, the dose previously established to give a desired clinical/laboratory 'attack' rate of approximately 60% (as per the previous dose finding study (OVG 2013/07, REC Ref: 14/SC/0004)[65].

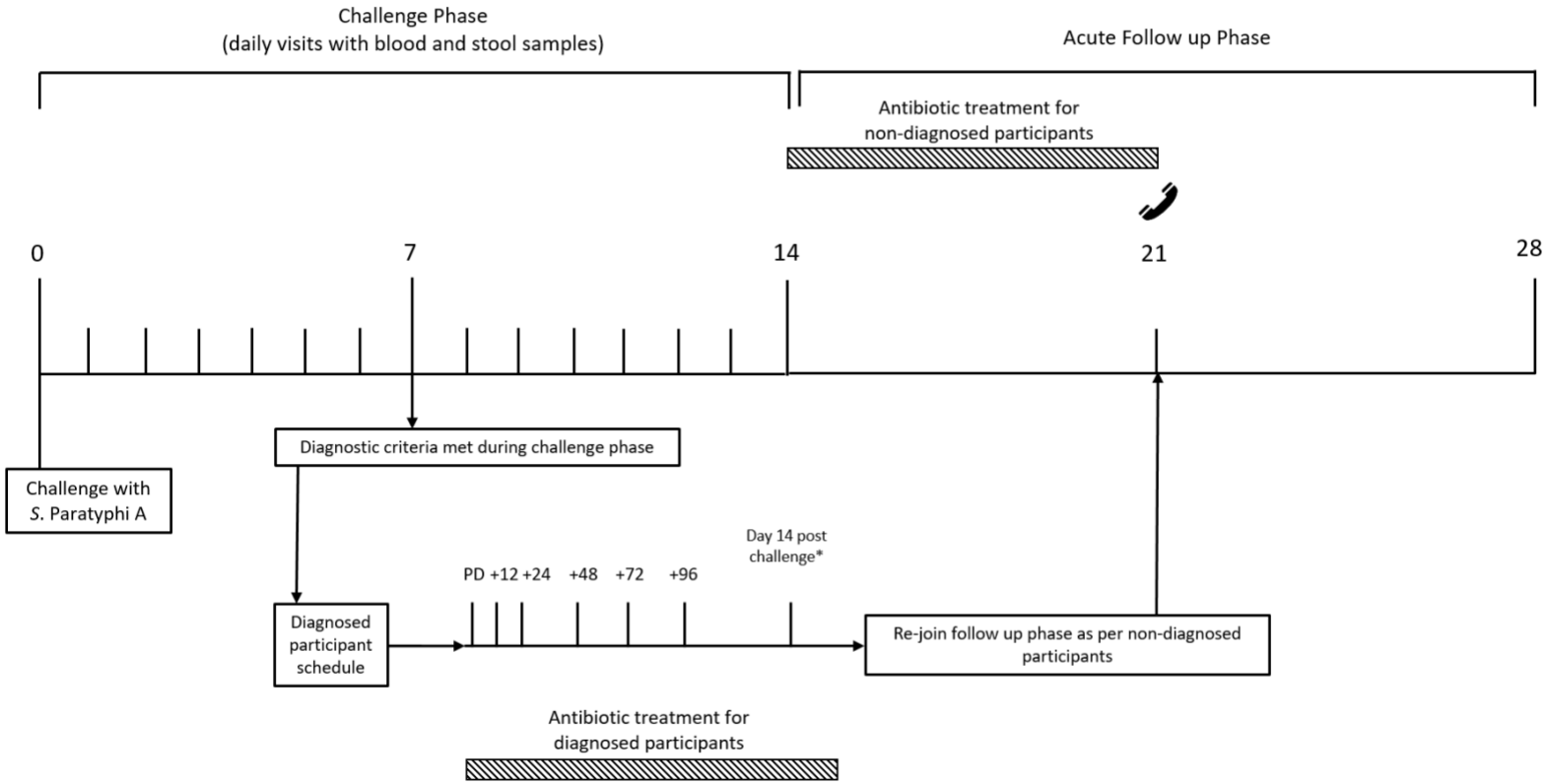
An overview of the study visits is found in Figures 1 and 2. An overview of the study procedures is shown in Tables 1a and 1b.

7.2. Figure 1: Visit Structure for whole study

Lines above timeline denote individual visits/study procedures



7.3. Figure 2: Structure of visits during challenge period



Notes: Diagnosis can occur on any day during the challenge phase, if this occurs diagnosed participants then follow the diagnosed participant schedule and then re-join the acute follow up phase visits as for non-diagnosed participants. *Day 14 post challenge visit in diagnosed participants occurs only if no other visit scheduled for this day, eg if the PD (paratyphoid diagnosis +48 visit fell on day 14 after challenge this would be the only visit on day 14 and visits would continue until PD+96 (ie day 16 after challenge)

7.4. Table 1a: Summary of screening procedures and tests

Screening procedures (within 120 days of enrolment)		
Informed consent		
Biobank consent		
Consent quiz		
Medical history		
Mood assessment		
Physical examination		
Vital signs		
Urine pregnancy test		
Urine sample		
Blood sample		
12 lead ECG		
Ultrasound scan		
Screening		
	Specimen	Container
FBC, ESR	Blood	EDTA vacutainer
Urea, Electrolytes & Creatinine	Blood	Heparin vacutainer
HIV, HBsAg, HCVA	Blood	Serum vacutainer
TTG and IgA	Blood	Serum vacutainer
HLA B27	Blood	EDTA vacutainer
Total blood volume		Maximum 17 mL
Blood glucose	Blood	NA
Urine dipstick	Urine	Standard urine pot
Urine Pregnancy test	Urine	Standard urine pot

7.5. Table 1b: Summary of study visits

	Screening	1st vaccine	Post-1st vaccination	2nd vaccine	Post-2nd vaccination	Pre-challenge	Intensive challenge period (first 14 days)					If Paratyphoid diagnosis made			Acute Follow Up	3 - 12 month follow-up	
		Va D-42	Vb D-35	Vc D-28	Vd D-21	D-2	D0	D1 to D6	D7	D8 to D13	D14	PD	PD+12hrs	PD +24, +48, +72, +96hrs, D14PD	D28	D90 and D180	D365
Enrolment		x															
Written Consent	x																
Biobank Consent	x																
Consent Quiz	x																
Written Continued consent							x										
Verbal continued consent		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
AE recorded and reviewed		x	x	x	x		x	x	x	x	x	x	x	x	x	x*	
SAE recorded and reviewed		x	x	x	x		x	x	x	x	x	x	x	x	x	x	x
Obtain 24 hr contact details		x															

* Medically attended AEs are recorded until day 90 (see section 11).

Medical history	x	x	x	x	x		x	x	x	x	x	x	x	x	x	x	x
Physical examination†	x											x					
Vital signs†	x	x	x	x	x		x	x	x	x	x	x	x	x	x	x	x
Urine pregnancy test†	x	x		x			x				x	x					
Urine sample†	x																
Stool sample‡†		x	x	x	x		x	x	x	x	x	x	x	x	x	x	x
Blood sample†	x	x	x	x	x		x	x	x	x	x	x	x	x	x	x	x
Saliva sample		x		x			x								x	x	x
12 lead ECG†	x																
Ultrasound	x																
Mood assessment†	x	x					x		x		x	x					
URT swabbing for SARS-CoV-2 PCR†						x					x	x					

† This procedure may be performed at any time in the study at the discretion of the study team eg if clinically indicated.

‡ Stool samples will also be collected 1 weeks after completion of a 7 day antibiotic course, until 3 successive stool samples are culture negative for *S.* Paratyphi A (please see section 9.11 for action if these are positive).

	1st vaccine	Post-1st vaccination	2nd vaccine	Post-2nd vaccination	Intensive challenge period (first 14 days)					If Paratyphoid diagnosis made			Acute Follow Up	3 - 12 month follow-up	
	Va D-42	Vb D-35	Vc D-28	Vd D-21	D0	D1 to D6	D7	D8 to D13	D14	PD	PD+12hrs	PD +24, +48, +72, +96hrs, D14PD	D28	D90 and D180	D365
Vaccine randomisation	x														
Vaccination with CVD 1902 or placebo	x		x												
Challenge with *S.* Paratyphi A					x										
e-Diary entries§	x	x	x	x	x	x	x	x	x	x	x	x	x		
Commence antibiotics									x	x					
Notification of GP**	x				x										
Notification of United Kingdom Health Securty Agency (UKHSA) **					x										
Letter informing close contacts and to offer screening	x		x		x				x	x			x		

§ Solicited symptoms after vaccination will be entered in the e-Diary for seven days post vaccination and for 21 days after challenge. The e-diary will remain open for unsolicited entries from Day -42 to Day 28 post challenge.

** UKHSA and participant GPs will also be notified at the time of stool shedding clearance.

7.6. Table 2: Window periods on visits

	1st vaccination	Post-1st vaccination	2nd vaccination	Post-2nd vaccination	Pre-challenge day	Challenge day	Intensive challenge period (first 14 days)	If Paratyphoid diagnosis made					Acute Follow Up	3 - 12 month follow-up	
Visit name	Va	Vb	Vc	Vd	Pre-challenge	0	D1-14	PD	PD+12	PD+24	PD+48 PD+72 PD+96	D14PD	D28	D90 D180	D365
Ideal	D-42	D-35	D-28	D-21	D-2	D0	D1 to D14	PD	PD+12hrs	PD+24hrs	PD +48, +72, +96hrs	D14	D28	D90 and D180	D365
Window period (days unless specified)	NA	+/- 2	-4	+/-4	+/-1	+28[††]	NA	NA	-6 hrs to +6 hrs	-0.5 to +1	-0.5 to +1	NA	+/-4	+/-14	+/-56
Acceptable range		D-37 to D-33	D-32 to D-28	D-25 to D-17	D-3 to D-1								D24 to D32	D76 to D104 D166 to D194	D309 to D421

[††] In exceptional circumstances where a participant was able and willing to be challenged having received two doses of the vaccine or placebo but was unable to be challenged within the specified visit window they may be challenged at a later date at the Investigator's discretion.

7.7. Table 3a: Sample collection – vaccination and post vaccination

Sampling time points, volumes and investigations may vary. Samples may be omitted as per the investigators' discretion for example where exploratory objectives are no longer being investigated.

Vaccine	Investigation	Pregnancy Test	Saliva	Stool sample	Blood culture	Full blood count	CRP, U+Es, LFTs	Blood for OVG laboratories	
	Sample tube	std urine pot	saliva coll-ection device	Stool Pot	Aerobic BACTEC bottle	EDTA Vacutainer	Heparin Vacutainer		
	Volume blood (mL)				10	1 → 3	2		**TOTAL***
	Day								
1	**D-42 (Va)**	x	x	x		2	3	74.5	79.5
	D-35 (Vb)			x	10	2	3	32.5	47.5
2	**D-28 (Vc)**	x	x	x	10	2	3	71	86
	D-21 (Vd)			x	10	2	3	32.5	47.5
								Total	260.5

Specific volumes and sample tubes for different assays for the OVG laboratories will be detailed in the Laboratory Analysis Plan and Clinical Study Plan.

7.8. Table 3b: Sample collection – challenge period

Challenge	Investigation	SARS-CoV-2 Test	Pregnancy Test	Saliva	Stool sample	Blood culture	Full blood count	CRP, U+Es, LFTs	Blood for OVG laboratories	
	Sample tube	NP swab	std urine pot	saliva coll-ection device	Stool Pot	Aerobic BACTEC bottle	EDTA Vacutainer	Heparin Vacutainer		
	D-2	x								
→	**D0**		x	x	x	10	2	3	81	96
	D1				x	10			20	30
	D2				x	10	2	3	up to 4	19
	D3				x	10			0	10
	D4				x	10	2	3	up to 4	19
	D5				x	10			0	10
	D6				x	10	2	3	0	15
	D7				x	10	2		58.5	70.5
	D8				x	10	2	3	0	15
	D9				x	10			0	10
	D10				x	10	2	3	0	15
	D11				x	10			0	10
	D12				x	10	2	3	0	15
	D13				x	10			0	10
	D14	x	x		x	10	2	3	58.5	73.5
									Total*	418

Specific volumes and sample tubes for different assays for the OVG laboratories will be detailed in the Laboratory Analysis Plan and Clinical Study Plan.

7.9. Table 3c: Sample collection – paratyphoid diagnosis, follow up and totals

Investigation	SARS-CoV-2 Test	Pregnancy Test	Saliva	Stool sample	Blood culture	Full Blood count	CRP, U+Es, LFTs	Blood for OVG laboratories	
Sample tube	NP swab	std urine pot	saliva coll-ection device	Stool Pot	Aerobic BACTEC bottle	EDTA Vacutainer	Heparin Vacutainer		
Day									**TOTAL***
PD	x	x		x	10	2	3	12.5	27.5
PD +12hrs**				x	10	2		50	62
PD +24hrs**				x	10	2	3	**	15
PD +48hrs				x	10	2	3	0	15
PD +72hrs				x	10	2	3	0	15
PD +96hrs				x	10	2	3	50	65
D14PD gp				x		2	3	0	5
D28			x	x		2	3	58.5	63.5
								Total	268
	Maximum total for first 3 months (excluding screening)			**If Paratyphoid NOT diagnosed**				**Total**	**738**
	Maximum total for first 3 months (excluding screening)			**If Paratyphoid Diagnosed (maximum blood volume)**				**Total**	**937.5**
D90			x	x		2	3	58.5	63.5
D180			x	x		2	3	48.5	53.5
D365			x	x		2	3	58.5	63.5
	Maximum total in 14 months (excluding screening)			**If Paratyphoid NOT diagnosed**				**Total**	**922.5**
	Maximum total in 14 months (excluding screening)			**If Paratyphoid Diagnosed (maximum blood volume)**				**Total**	**1122**

Specific volumes and sample tubes for different assays for the OVG laboratories will be detailed in the Laboratory Analysis Plan and Clinical Study Plan.

*The total volume of blood per visit represents the *maximum* amount of blood that would be taken if both "safety bloods" and "other bloods" are collected. The maximum amount of blood taken in diagnosed participants is calculated if participants are diagnosed on day 14 having already had their day 14 visit, they then do not have a day14PD visit.

** Blood samples for the OVG laboratory at PD+12 hrs may be alternatively taken at PD+24 hrs depending on sample processing availability at the time of the study visit.

Blood volumes in dark shaded columns (blood culture, full blood count, CRP, U+Es, LFTs) represent “safety bloods” and will be collected in all participants.

Blood volumes in the light shaded column (bacterial quantification, peripheral blood mononuclear cells, serum, plasma samples, functional genomics, DNA samples) represent “other bloods”, taken for exploratory laboratory assays, and may be collected in a subset of participants rather than all participants. These samples would be omitted if the participant developed anaemia which was felt to be clinically significant by the study team.

8. PARTICIPANT IDENTIFICATION

8.1. Study Eligibility

Male or female participants aged 18-55 years inclusive who are in good health (as determined by a study doctor, medical investigation and agreement by their general practitioner) and able to provide written informed consent will be eligible for inclusion in this study.

8.2. Inclusion Criteria

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

- Willing and able to give informed consent for participation in the study.
- Aged between 18 and 55 years inclusive at time of vaccination.
- In good health as determined by medical history, physical examination and clinical judgment of the study team.
- Willing to be available in Oxford for all required appointments
- Agree (in the study team's opinion) to comply with all study requirements, including capacity to adhere to good personal hygiene and infection control precautions.
- Agree to allow study staff to contact his or her GP to access the participant's vaccination records, medical history and have their opinion solicited as to the participant's appropriateness for inclusion.
- Agree to allow study staff to access NHS health records as required for study purposes.
- Agree to allow his or her GP (and/or Consultant if appropriate), to be notified of participation in the study.
- Agree to allow UKHSA to be informed of their participation in the study.
- Agree to give his or her close household contacts written information informing them of the participants' involvement in the study and offering them voluntary screening for *S.* Paratyphi A carriage.
- Agree to have 24-hour contact with study staff during the four weeks post challenge and are able to ensure that they are contactable by mobile phone for the duration of the vaccination and challenge period until antibiotic completion.

- Have internet access to allow completion of the e-diary and real-time safety monitoring.
- 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 provide their National Insurance/Passport number for the purposes of TOPS registration and for payment of reimbursement expenses.
- Participants must have received at least one dose of a SARS-CoV-2 vaccine that has been approved for use by the MHRA (or other national regulatory authority) ≥ four weeks prior to enrollment.
- Agree to not receive other vaccinations (eg Covid-19 vaccines) during the 7 days before and after study vaccination and during the 7 days before or 21 days post-challenge.

8.3. Exclusion Criteria

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

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

[‡‡] This includes anaemia. The acceptable lower limits for [haemoglobin] are 125 g/L for female participants and 135 g/L for male participants (Guidelines for the Blood Transfusion Services in the UK, 8th edition, 2018 < https://www.transfusionguidelines.org/red-book> Accessed 5th December 2018).

- Metabolic disease
- Autoimmune disease
- Psychiatric illness requiring hospitalisation
- Known or suspected drug misuse
- Known or suspected alcohol misuse (alcohol misuse defined as an intake exceeding 42 units per week)
- Infectious disease

- Have any known or suspected impairment of immune function, alteration of immune function, or prior immune exposure that may alter immune function to paratyphoid resulting from, for example:
  - Congenital or acquired immunodeficiency, including IgA deficiency
  - Human Immunodeficiency Virus infection or symptoms/signs suggestive of an HIV-associated condition
  - Receipt of immunosuppressive therapy such as anti-cancer chemotherapy or radiation therapy within the preceding 12 months or long-term systemic corticosteroid therapy.
  - Receipt of immunoglobulin or any blood product transfusion within 3 months of study start.
  - History of cancer (except squamous cell or basal cell carcinoma of the skin and cervical carcinoma in situ).
- 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.

[§§] If elevated scores are due to temporary significant life events, the questionnaire may be repeated after resolution of the event with a view to inclusion if normal.

- Anyone taking long-term medication (e.g. analgesia, anti-inflammatories or antibiotics) that may affect symptom reporting or interpretation of the study results.
- Contraindication to fluoroquinolones, macrolide antibiotics, co-trimoxazole or ceftriaxone.
- Family history of aneurysmal disease
- Female participants who are pregnant, lactating or who are unwilling to ensure that they or their partner use effective contraception[***] 30 days prior to vaccination and continue to do so until three negative stool samples have been obtained after completion of antibiotic treatment.
- Full-time, part-time or voluntary occupations involving:
    - Clinical or social work with direct contact with young children (defined as those attending pre-school groups or nursery or aged under 2 years), or
    - Clinical or social work with direct contact with highly susceptible patients or persons in whom typhoid infection would have particularly serious consequences
    - Commercial food handling (involving preparing or serving unwrapped foods not subjected to further heating)

  (unless willing to avoid work from vaccination until demonstrated not to be infected with *S*. Paratyphi A after challenge by clearance samples in accordance with guidance from UKHSA and willing to allow study staff to inform their employer).
- Close household contact with:
    - Young children (defined as those attending pre-school groups, nursery or those aged less than 2 years)
    - Individuals who are immunocompromised (including pregnancy).
- 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

[***] As defined by CTFG Recommendations related to contraception and pregnancy testing in clinical trials, current document: https://www.hma.eu/fileadmin/dateien/Human_Medicines/01-About_HMA/Working_Groups/CTFG/2014_09_HMA_CTFG_Contraception.pdf [accessed 23rd July 2019]

study within the 30 days prior to enrolment (e.g. significant volumes of blood already taken in previous study)[†††].

- Detection of any abnormal results from screening investigations (at the clinical discretion of the study team).
- 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 in the study,
    - adversely affect the interpretation of the primary endpoint data,
    - Impair the participant's ability to participate in the study.
- Have any history of allergy to vaccine/placebo components
- Having been resident in an enteric fever endemic country for 6 months or more.
- Have previously been diagnosed with laboratory-confirmed typhoid or paratyphoid infection or been given a diagnosis compatible with enteric fever.
- Have participated in previous typhoid or paratyphoid challenge studies (with ingestion of challenge agent).
- Have received any oral typhoid vaccination (e.g. Ty21a or M01ZH09) at any time.
- Have a prolonged corrected QT interval (>450 milliseconds) on ECG screening.
- Significant blood donation or planned blood donation prior to enrolment.

8.4. Temporary Exclusion at Vaccination

Participants will be temporarily excluded from receiving vaccination if presenting at a vaccination visit with the following:

- Acute gastrointestinal illness within 24-hours prior to vaccination.

[†††] As assessed by both participant questioning and registration with The Over Volunteering Prevention System (TOPS) database.

- Significant infection within the previous 7 days.
- Participant has experienced fever (>37.5°C) or subjective febrile symptoms within the previous 3 days (even with a negative COVID-19 test).
- Symptoms of COVID-19, or confirmed infection (according to government guidelines) within 14 days prior to vaccination visit; as per guidance in Section 9.11
- Validated positive SARS-CoV-2 test (NAAT or antigen) within 2 weeks prior to vaccination visit
- History of any antibiotic therapy during the previous 5 days.
- Any systemic corticosteroid (or equivalent) treatment in the previous 14 days, or for more than seven consecutive days within the past 3 months).
- Receipt of another enteric live vaccine within 4 weeks prior to vaccination or an injected live or killed vaccine within 7 days prior to vaccination.
- Plan to receive any vaccine other than the study vaccine within 7 days following vaccination.
- Therapy with antacids, proton pump inhibitors or H_2-receptor antagonists within 24 hours prior to vaccination.
- Unavailable for post-vaccination visits, second vaccination visit, and challenge visit as outlined in study procedures table (see section 7.4 table 1b).

If this is the first vaccine to be received and the temporary exclusion does not result in the participant becoming ineligible, then this vaccine visit can be rescheduled.

If this is the second vaccine to be received and the temporary exclusion does not result in the participant becoming ineligible, then this should be discussed with a study doctor as to whether they can be rescheduled for their second vaccination (see table 2, section 7.5 for visit windows) or withdrawn.

8.5. Temporary Exclusion at Challenge

Participants will be temporarily excluded from challenge if presenting at the challenge visit with the following. Participants can be challenged up to 28 days after their original challenge date (see table 2, section 7.5)

- Significant acute or acute-on-chronic infection within the previous 7 days or have experienced fever (>37.5°C) or subjective febrile symptoms within the previous 3 days (even with a negative COVID-19 test).
- Symptoms of COVID-19, or confirmed infection (according to government guidelines) within 14 days prior to challenge visit; as per guidance in Section 9.11
- Validated positive SARS-CoV-2 test (NAAT or antigen) within 2 weeks prior to challenge visit
- History of any antibiotic therapy during the previous 5 days.
- 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 H_2-receptor antagonists within 24 hours prior to challenge.
- Participant has not received two doses of the study vaccine/placebo.
- Anaemia felt to be clinically significant by the study team.
- Receipt of a COVID-19 vaccine in preceding 7 days
- Plan to receive any vaccine within 21 days following challenge.

8.6. Pregnancy and Contraception

The possible adverse effects of *S.* Paratyphi A infection or the effect of some antibiotics on the outcome of pregnancy are unknown; therefore, pregnant women will be excluded from the study. If relevant, female participants must have used an effective form of contraception in the four weeks prior to first vaccination. Vaccination with CVD 1902 and paratyphoid infection, with or without diarrhoea or vomiting, could reduce the efficacy of an oral hormonal contraceptive ('the pill') by altering absorption. For this reason, female participants who are taking oral contraception will be advised to use additional barrier contraception during the vaccination and challenge period until they have completed their course of antibiotics and shown, by the provision of stool samples to be clear of *S*. Paratyphi A infection.

Women of childbearing potential will be required to use an effective contraceptive measure. A woman is considered of childbearing potential, I.e fertile, following menarche and until becoming post-menopausal unless permanently sterile. Permanent sterilisation methods include hysterectomy, bilateral salpingectomy and bilateral oophrectomy. A post-menopausal state is defined as no menses for 12 months without an alternative medical cause. A high follicle-stimulating hormone (FSH) level

in the post-menopausal range may be used to confirm a post-menopausal state in women not using hormonal contraception or hormonal replacement therapy. However in the absence of 12 months of amenorrhoea, a single FSH measurement is insufficient.

Contraception should be maintained during the vaccination period and until the provision of negative clearance stool samples. Should a volunteer become pregnant during the trial this outcome will be recorded and the Sponsor and the DSMC will be notified if appropriate. She will be followed up for clinical safety assessment with her ongoing consent and in addition will be followed until pregnancy outcome is determined. No further non-essential trial procedures will be performed, however, procedures such as appropriate antibiotic treatment, screening for stool carriage and/or referral may be required for participant safety but no further study sampling except unless required for safety).

Female volunteers of childbearing potential are required to use an effective form of contraception until their last follow-up visit. The risk of teratogenicity or fetotoxcity with an attenuated strain of *S*. Paratyphi A is felt to be unlikely given limited evidence of adverse pregnancy outcomes with wild-type S.Paratyphi A. Therefore, the use of effective (rather than highly effective) measures of contraception throughout the use of the trial is advised. This approach has been safely followed with previous IMP and challenge studies over the past decades.

Acceptable effective forms of contraception for female volunteers include:

- Established use of oral, injected or implanted hormonal methods of contraception.
- Placement of an intrauterine device (IUD) or intrauterine system (IUS).
- Total abdominal hysterectomy.
- Bilateral tubal occlusion.
- Barrier methods of contraception (condom or occlusive cap with spermicide).
- Male sterilisation, if the vasectomised partner is the sole partner for the subject.
- Sexual abstinence defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatments (for this trial from vaccination to having been shown to be clear). The reliability of sexual abstinence needs to evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the subject.

Male participants with female partners are not required to use barrier methods for the purposes of contraception, given the unlikely risk of teratogenicity/fetotoxicty. There is no evidence that Salmonella Paratyphi A is transmitted by semen, it is therefore deemed that the risk of sexual

transmission after receiving an attenuated strainis negligible. Furthermore, the risks of vaccine excretion and faeco-oral transmission are negligible provided good hygiene measures are employed.

8.7. The Over-volunteering Prevention System

The Over-volunteering Prevention System (TOPS) is a database to guard against the potential for harm that can result from excessive volunteering in clinical trials involving IMPs or blood donations. Participants will have the TOPS database checked for conflicts at screening using their national insurance number or passport number if they do not have a national insurance number.

At screening, they will be registered on TOPS. The database will also be updated with vaccination doses and challenge dose as the study progresses.

The system will be updated in the event of the participant being withdrawn or excluded. Alternatively, TOPS will be updated on the participant's last visit.

8.8. Potential benefits to participants

There is no direct benefit to participants taking part in this study. Participants may benefit from being informed about their general health status.

9. TRIAL PROCEDURES

An overview of trial procedures is shown in Section 7.3.

9.1. Recruitment

All potential participants may be contacted by methods including but not limited to email, telephone, posters, leaflets, websites, advertisements in newspaper, radio and on social media and/or mail using a REC approved invitation letter or other advertising material using wording from REC approved study documents in the first instance to invite them to participate in the study.

Where mail-outs are used, participants may be identified via the electoral open register, or through National Health Service databases. These include the National Health Applications and Infrastructure Services (NHAIS) via a NHAIS data extract or equivalent. For the NHS databases initial contact to potential participants will not be made by the study team. Instead study invitation material will be sent out on our behalf by an external company, CFH Docmail Ltd (or equivalent company), in order to preserve the confidentiality of potential participants. CFH Docmail Ltd (or equivalent company) is

accredited as having exceeded standards under the NHS Digital Data Security and Protection Toolkit (ODS ID – 8HN70).

For mail-outs via the electoral register, we will have access to the names and addresses of individuals who are on the open electoral register (only contains the names of registered voters who have not opted out). In this instance, the study team will upload the mailing list to the CFH Docmail system (or equivalent company), and the study invitation pack will be sent out by CFH Docmail (or equivalent company).

Volunteers may also be recruited using direct SMS/text message, or emails to potential participants identified by GPs from their databases The study may be advertised on the electronic newsletter sent out to those potential participants signed up to the Oxford Vaccine Centre's Healthy Volunteers Database or by email distribution to a group or list only with the express agreement of the network administrator or with equivalent authorisation. Members of the public who have registered on this secure database have given their consent to be contacted when studies open for recruitment and understand that this is not a commitment to participate. Potential participants who are interested in study participation will be able to contact the Oxford Vaccine Group by telephone, email or online.

Once an expression of interest has been received by Oxford Vaccine Group staff, an information booklet will be sent via mail or email to the potential participants. Participants can also be directed to the appropriate website, where the information booklet will be available.

9.2. Initial screening

Once participants express an interest in joining the trial, they will be directed to a 2 stage online screening process. The first stage will assess for obvious exclusion criteria. If they pass this stage they will be asked to indicate their electronic consent to cover:

1) Reporting their medical history (stage 2)

2) Telephone screening visits to review their medical history.

Telephone screening visit(s)

Participants will be invited for telephone screening visit(s), which would then be completed by member(s) of the clinical team, based on the assessment of the part 2 responses. This will be recorded in a screening CRF. This will reduce the amount of time participants have with the clinical team during their screening procedures, should they progress to a face to face screening visit.

The face-to-face screening visit and enrolment interval may be up to a maximum of 120 days. Volunteers will be asked to contact the study team in the interim if there are significant changes to their health status during this time. The investigator will carefully assess whether any screening procedures (eg blood tests) need to be repeated since initial screening prior to enrolment. The consent to contact GP for medical and vaccination history is separate from informed consent for the study.

9.3. Informed Consent

The participant will personally sign and date the latest approved version of the informed consent form at the face-to-face screening visit before any study specific procedures are performed. Consent will be sought as described in relevant OVG and OVC SOPs. Written and verbal versions of the participant information and informed consent form will be presented to the participant, detailing no less than:

- the exact nature of and the rationale for performing the study
- implications and constraints of the protocol
- the risks and benefits involved in taking part
- The study team will be required to hold the name and 24-hour contact number of a close friend, relative or housemate who lives nearby and will be kept informed of the study participant's whereabouts for the duration of the study. This person is to be contacted if study staff are unable to contact the participant. The 24-hour contact will receive written information, and complete and sign a reply slip that the participant will give the study doctor/nurse before challenge.
- To inform close contacts of their involvement in the study by giving them letters provided by OVG. The letter details the study and offers screening for paratyphoid carriage. The low risk of spread will be emphasised to contacts to avoid undue anxiety.
- To contact their GP to confirm their medical and immunisation history and participation in the study, intended antibiotic treatment and clearance results.
- For UKHSA to be informed of study participation and clearance results.
- If the participant is involved in the provision of health or social care to vulnerable groups, then consent will be taken to inform his/her employer of their participation in the study.

It will be clearly stated that the participant is free to withdraw from the study at any time, for any reason and that they are under no obligation to give the reason for withdrawal. It will be explained to

participants that if they do withdraw, depending on the stage of the trial at which they withdraw they may still require follow up visits and samples to be taken to ensure their safety. The participant will be allowed adequate time to consider the information, and the opportunity to question the researcher, their GP or other independent parties to decide whether they will participate in the study. Written informed consent will be obtained by means of a dated signature of the participant and a signature of the study staff member who presented informed consent. A copy of the signed informed consent will be given to the participant and the original signed form will be retained at the study site. A doctor or nurse at OVG, who has been trained in the consent process, will conduct the informed consent discussion. Participants will be asked to complete a consent quiz as part of the informed consent process to ensure they have properly understood the study and provide an opportunity to review any areas that the participant may require further information before consent is taken.

9.4. Screening and Eligibility Assessment

Once informed written consent is obtained, the following baseline assessments and information will be collected as part of the assessment of inclusion/exclusion criteria:

- Participant demographics; age, sex and ethnicity,
- Medical history, including:
    - Details of any significant medical or surgical history based on participant recall and medical records. If medical clarification is required, additional medical notes may be obtained and/or discussion with other medical practitioners may be undertaken.
    - Blood donation history and planning.
    - Immunisation history (particularly receipt of previous typhoid vaccines)
- Females participants only:
    - Contraception use.
    - Highly-sensitive urinary pregnancy test.
- Use of concomitant medication (including over the counter medications, vitamins, illicit drug use and herbal supplements).
- Alcohol intake and smoking history.
- History of living in or visiting enteric fever endemic areas (age, place and duration).

- Physical examination; cardiovascular, respiratory, abdominal and gross neurological examination and calculation of Body Mass Index.
- All participants
    - Urine dipstick (and laboratory analysis if appropriate)
    - 12-lead ECG
    - Blood samples for: haemoglobin count, white cell indices, platelet count, erythrocyte sedimentation rate (ESR), serum sodium, serum potassium, serum urea, serum creatinine, liver function tests, C-reactive protein, serum amylase, TTG antibodies and IgA levels, HIV, Hepatitis B and C serology, HLA B27.
    - Random capillary or venous blood glucose.
    - Abdominal ultrasound (to screen for gallbladder disease).
    - Mood assessment by the Hospital Anxiety and Depression Score.
    - Responses regarding any personal or domestic reason that may lead to concern regarding a participant's ability to maintain good personal hygiene.

The medical history, vaccination history and prescribed medication lists are based primarily on participant recall. With participant approval, the GP will be contacted to confirm the history by providing a copy of the participant's medical summary. The GP of a potential participant will be contacted and asked to raise any concerns regardinga participant's suitability for inclusion into the trial but decisions regarding enrollment remain the responsibility of the Investigators.

Consent will be taken to register them onto TOPS (see section 8.7).

Participants will be informed that they would also be eligible for BioBank (OVC Biobank HRA South Central - Hampshire B Research Ethics Committee, 21/SC/0161). BioBank is a separate study and optional to all participants of studies conducted by OVC. Separate consent will also be sought for this at screening.

All laboratory results will be reviewed and collated by the study team who will record these in the CRF. Separate CRFs will be used for unblinded information during the vaccination period and regarding some samples. These CRFs will only be visible to members of the study team who are permitted to access this unblinded information (see section 11.4.4). Specific guidance is provided in existing OVG SOPs. If a test result is deemed clinically significant, it may be repeated, to ensure it is not a single occurrence. If a test remains clinically significant, the participant will be informed. Depending on the

nature of the result the participant may be asked to see their GP and be given the relevant information from any test results from the trial, alternatively with consent the participant's GP may be contacted to discuss a particular result or finding. Decisions to exclude potential participants from enrolling in the trial or to withdraw a participant from the trial will be at the discretion of the Chief and Co-Investigators.

9.5. Definition of enrolment

Enrolment will occur at prime vaccination. A maximum duration of 120 days will be accepted between initial screening and enrolment into the study.
GPs will be notified at the time of enrolment (vaccination) that the subject is taking part in the study.

9.6. Randomisation

Participants will be randomised to receive CVD 1902 or placebo on Day -42. A statistician at OVG will generate a randomisation list using varying block sizes (2 and 4). The randomisation list aims to ensure equal target sample size is reached per group. The final randomisation list will be sent to an IT manager at OVG to upload into a web-based randomisation system. The web-based randomisation system will ensure the allocation concealment is valid. The final randomisation list is only accessible by the unblinded team, the IT manager and the trial statistician. Randomisation of participants will be carried out by unblinded study staff (which may include laboratory staff), who are independent from the blinded team and do not perform any post vaccination procedures (such as ongoing eligibility, sample collection).

9.7. Blinding

This study will be conducted observer- and participant-blind from the time of randomisation until participant unblinding which will occur once the *last* participant has completed their Day 28 post-challenge visit. Observer and participant blinding is required to minimise the risk of bias on the reporting of adverse events following the administration of vaccine and challenge.

Unblinded individuals:

- Statisticians
- Data managers
- Laboratory staff involved in vaccine preparation
- Monitors

- Study internal QA/monitoring team
- Named clinician with responsibility for safety (including SUSAR) reporting
- Unblinded clinical team responsible for reviewing stool culture results in the vaccination phase (see below)
- Unblinded nursing team responsible for vaccine administration

Blinded individuals:

- Chief Investigator
- Co-Investigators
- Clinical staff

All vaccine and placebo vaccine doses will be administered in a blinded fashion such that the participant, nor the clinical staff, will be aware of which vaccine they have received. It is acknowledged that the practical steps to blind administration are not absolute and there may be a difference in the appearance of the vaccine and placebo. To minimise the risk of unblinding the clinical team vaccine and placebo doses will be administered by a separate unblinded nursing team. This team will have no other clinical involvement in the trial but may also administer challenge agent.

Vaccine and placebo vaccine will be reconstituted and checked by trained laboratory staff who will be unblinded. For assays requiring blinding in the laboratory (eg Elispots), spots will be counted by at least one individual blinded to vaccine or placebo vaccine allocation.

Participants will be notified by the study team of whether they received the vaccine or placebo vaccine at the time of unblinding.

As CVD 1902 is a live-attenuated vaccine and participants in this study will receive two doses for the first time, the safety data before challenge will be closely monitored and reviewed by the DSMC. The Phase 1 study at the University of Maryland did not show shedding of the vaccine strain beyond day 3 after dosing. We therefore do not anticipate shedding at the first stool sample collection point (7 days post vaccination), however it is possible that stool culture data during the vaccination period (day -42 to day 0) could unblind the study. To avoid inadvertent unblinding, stool samples will be labelled with an alternative number not related to the participant's main study number and these results will be reviewed and entered into the CRF by the unblinded team only. These results will be shared with and reviewed regularly by the DSMC. If there are any safety concerns regarding the stool shedding data between DSMC meetings, the unblinded team will communicate with the DSMC to arrange an emergency closed meeting to review the safety data from the vaccination period. The DSMC will make

a recommendation on whether any unblinding (either for an individual participant or for the whole study) is needed after reviewing the data.

9.8. Unblinding

Scheduled unblinding will occur once the *last* participant has completed their Day 28 post-challenge visit.

Unblinding may also occur at an earlier time point in the event of participant withdrawal, or the occurrence of SAEs, SARs or SUSARs (please see section 11). This will be conducted under the guidance of the DSMC. Due to the live nature of the vaccine it may be necessary to unblind for an unrelated SAE, for example if a participant was hospitalised soon after receiving the live vaccine unblinding may be required for infection prevention control purposes.

Circumstances may arise in which unblinding is required for one specific participant. Examples of this include when a participant has a Serious Adverse Reaction (SAR) or requires medical intervention which would be influenced by whether they have received the investigational vaccine or placebo (eg administration of antibiotics). Unblinding will be undertaken according to trial specific working instruction and group allocation will be sent to the attending clinician.

In emergency situations the investigator may need to break the treatment code immediately, or as quickly as possible. The investigator will therefore have access to the emergency unblinding system 24 hours a day. They will have the final decision and unilateral right to unblind in this situation. The sponsor will not be involved in the decision to unblind or be able to stall or delay unblinding in an emergency situation. The investigator will have responsibility for documenting and informing the Sponsor promptly of any unblinding.

At the time of scheduled unblinding, the participants, their GP will be informed of their vaccine/placebo allocation.

9.9. Vaccination procedures

9.9.1. Initial vaccination visit (day -42)

Pre-vaccination procedures (blinded team)

Revalidation of consent (oral)

Obtain 24-hour contact details
Interim medical history
Check temporary exclusion to vaccination
Schedule all study visits
Vital signs
Urine pregnancy test for female participants
Sample collection as per Table 3a (section 7.6)

Randomisation (unblinded study staff)

Randomise the participant (1st vaccination only)

Vaccination procedure (unblinded team)

These visits will require the following procedures:

Participants must be fasted for 90 minutes prior to vaccination
Administer pre-treatment bicarbonate solution
One minute later administer vaccination or placebo as per randomisation list

Post vaccination procedures (blinded team)

These visits will require the following procedures:

Participants will be directly observed for 15 minutes to ensure they do not vomit and monitored for a total of 60 minutes (+/- 30 minutes)..
Vital signs
Participant will then be asked to fast for 90 minutes in total post vaccination.
Issue participant with a study pack
Record all doses given on Study Vaccination Record Card
Notify GP with details regarding participant enrolment in the study (at 1st vaccination only)

Ensure participant has study centre contact details (including 24-hour telephone contact details for study doctor)
Instruct participant on notifying study centre of any serious adverse events/reactions
Instruct participants to use antipyretics only to treat fever or other adverse reactions, rather than pre-emptively
Provide participant with access to a vaccination e-diary and paper backup and instructions on how to use
Provision of information for close contacts (including invitation to screening)
Enteric precautions education
Check scheduling of future visits
Update TOPS database

9.9.2. E-diary for participants to record symptoms during vaccine period

Participants will be instructed to access an online vaccine e-diary to record oral temperatures twice-daily and to describe any symptoms or the use of any medications for 7 days after each vaccination. The foreseeable adverse reactions following vaccination include fever, nausea, vomiting, diarrhoea, anorexia, malaise, abdominal cramps, and headache. These adverse events will be listed as solicited adverse events provided they occur with 7 days of the day of vaccination. See section 11.3.1 on e-diary AEs.

9.9.3. Follow-up visit after initial vaccination (day -35)

Study procedures will be performed as per section 7.5 table 1b.

9.9.4. Second vaccination visit (day-28)

Procedures as for first vaccination following original randomisation allocation, see section 9.7.2. and section 7.5 table 1b.

9.9.5. Follow-up visits after second vaccination

Day-21 will include study procedures as per section 7.5 table 1b.

9.10. S. Paratyphi A challenge procedure

Assessment and challenge with *S.* Paratyphi A (strain NVGH308) will take place on Day 0. These procedures are described below, and in further detail in the relevant OVG SOPs, Clinical and Laboratory Study Plans.

9.10.1. Assessment

Pre-challenge

These visits will require the following procedures:

Revalidation of consent
Check 24-hour contact details
Interim medical history including AEs and any significant events
Check temporary exclusion criteria to challenge
Vital signs
Urine pregnancy test for female participants
Sample collection as per Table 3b (section 7.6) and replace stool collection pots
Mood assessment

9.10.2. Preparation of challenge agent

The solution for ingestion (containing *S.* Paratyphi A strain NVGH308) will be prepared in a Class II biological safety cabinet within a containment level 3 laboratory at the CCVTM that is solely used for the purpose of preparing the challenge solution. The strain will be prepared, checked and given to participants as outlined in relevant OVG SOPs and Laboratory and Clinical Study Plans. Challenge solution preparation is conducted by laboratory staff and dose, challenge agent identity, and date/time of preparation is checked by two laboratory staff members. Two clinical study team members check the challenge solution immediately prior to ingestion by the participant (participant identification, correct challenge agent, challenge prescription, dose, date and time on challenge agent label, volume). Administration is recorded in the challenge administration log. The water and bicarbonate used for the preparation will be commercially available food products. Following solution ingestion, the single-use containers will be returned to the laboratory for inspection, autoclaving and disposal.

9.10.3. Administration of S. Paratyphi A (strain NVGH308)

S. Paratyphi A challenge will be administered by the oral route with sodium bicarbonate at a dose of 1-5 x 10^3 CFU. Participants will fast for 90 minutes before and after challenge.

These visits will require the following procedures:

Check participants are fasted for 90 minutes prior to challenge
Administer pre-treatment bicarbonate solution
One minute later administer challenge agent

9.10.4. Procedure after challenge

These visits will require the following procedures:

Directly observe for 15 minutes post challenge‡‡‡
Vital signs
Participant will then be asked to fast for 90 minutes post challenge.
Ensure participant has study centre contact details (including 24-hour telephone contact details for study doctor)
Instruct participant to notify study centre of any serious adverse events/reactions that occur prior to next review.
Instruct participant on notifying study centre of any temperature readings ≥38°C
Instruct participants **not** to use antipyretics
Instruct participant to notify study team if they require the use of any medications.
Provide participant with access to a challenge e-diary and paper backup and instructions on how to use
Ensure participant still has Medic Alert-type card

‡‡‡ Participants who vomit for any reason within 60 minutes of the challenge will be withdrawn from the trial and treated with antibiotics as described in section 9.9.6.

Check details of mobile telephone number and explain procedures in the event that they are uncontactable
Provision of information for close contacts (including invitation to screening)
Enteric precaution information, including hand-washing demonstration and observation
Check scheduling of future visits
Update TOPS database
Notification of GP and UKHSA

9.10.5. E-diary for participants to record symptoms during the challenge period

Participants will be instructed to access an online challenge e-diary to record oral temperatures twice-daily and to describe any symptoms or the use of any medications for 21 days after challenge. The foreseeable adverse reactions following challenge include fever >37.5°C, diarrhoea, anorexia, malaise, abdominal cramps, headache, nausea, vomiting. See section 11.4.1 on e-diary AEs.

9.10.6. Post-challenge visits; non-paratyphoid diagnosis visits (D1 to D14)

These visits will require the following procedures (outlined in section 7.5, table 1b):

Revalidation of consent (oral)
Interim medical history
Check for occurrence of SAEs
Review E-diary entries including any adverse events/medications
Vital signs
Sample collection as per Table 3b and Table 3c (sections 7.7 and 7.8) and reissue stool collection pots (including pregnancy test for female participants at day 14)
Mood assessment (days 7 and 14)
Prescribe and issue concomitant medication for symptom control if required (see section 9.9.5.)

Re-iterate participant requirements such as completion of the e-diary, refraining from use of antipyretics, notification of any medication administration, requirement to be contactable at all times and notification of study staff of any fever ≥38.0°C (where appropriate).
Prescribe and issue antibiotic therapy (see section 9.11.6.) (day 14 only)
Provision of information for close contacts (including invitation to screening) (day 14 only)
Check scheduling of future visits

9.10.7. Day 21 phone call

Revalidation of consent (oral)
Interim medical history
Check for occurrence of SAEs
Review E-diary entries including any adverse events/medications
Confirm completion of full course of antibiotics
Remind re: clearance samples and dates/arrangements to deliver samples
Check scheduling of future visits

9.10.8. Follow up visits (D28 to D365)

These visits will require the following procedures:

Revalidation of consent (oral)
Interim medical history
Check for occurrence of adverse events/SAEs
Check e-diary and clarify any issues (day 28 only)
Vital signs
Sample collection as per Table 3c (section 7.9) and replace stool collection pots

Check clearance samples received and notification of GP/UKHSA regarding clearance completed
Check scheduling of future visits (if applicable)

9.10.9. Outside of scheduled visits and unscheduled visits

Unscheduled visits will be arranged, if required, to ensure participant safety as further history, examination and investigation may be needed. These visits will be at the discretion of the clinical study team. If participants are unwell and unable to attend CCVTM for a visit, they will be directed to the John Radcliffe Accident and Emergency department (or appropriate other medical facility) and relevant medical personnel (eg Consultant Physician providing clinical oversight, General Medical Registrar) will be made aware. This will be emphasised at screening and throughout the study.

9.11. Paratyphoid diagnosis and paratyphoid diagnosis visits (PD to PD+96 hours including D14PD)

These visits will require the following procedures:

Revalidation of consent (oral)
Interim medical history
Obtain and document paratyphoid diagnosis including physical examination findings (at PD and at PD+12 to PD+96 if applicable)
Assessment by a study doctor at the time of PD to assess severity and potential need for in-patient admission (section 9.9.3.), further visits can be conducted by a clinical study team member
Check for occurrence of SAEs
Review E-diary entries including any adverse events/medications
Vital signs
Sample collection as per Table 3c (see section 7.8) and reissue stool collection pots
Urine pregnancy test for female participants
Mood assessment

Prescribe and issue antibiotic therapy (see section 9.9.6.)
Prescribe and issue concomitant medication for symptom control if required (see section 9.9.5.)
Re-iterate participant requirements such as completion of the e-diary, adherence to antibiotic therapy and maintaining contact with the study team.
Provision of information for close contacts (including invitation to screening) (at PD visit)
Check scheduling of future visits

9.11.1. Diagnosis of paratyphoid infection

For the purposes of data analysis and reporting of paratyphoid cases to UKHSA, paratyphoid infection will be defined as specified in **Table 4**.

Paratyphoid fever is diagnosed if ANY of the following apply
A positive blood culture for *S.* Paratyphi A from 72 hours post-challenge
A positive blood culture for *S.* Paratyphi A within 72 hours post-challenge, with one or more signs/symptoms of paratyphoid infection (such as recorded temperature ≥38.0°C)
Persistent positive blood cultures (two or more blood cultures taken at least 4 hours apart) for *S.* Paratyphi A within 72 hours post-challenge.
Oral temperature ≥38.0°C persisting for 12 hours

Table 4. Criteria for the diagnosis of paratyphoid infection

S. Paratyphi A bacteraemia occurring before 72 hours may reflect a transient primary bacteraemia and not paratyphoid fever; however, participants who are bacteraemic before 72 hours AND have one or more symptoms/signs consistent with paratyphoid infection (such as a temperature ≥38.0°C) will also be deemed to have reached the definition for paratyphoid fever.

Microbiologically, the earliest indication of *S.* Paratyphi A bacteraemia will be the identification of Gram-negative bacilli by Gram staining of aerobic blood/broth culture specimens. Formal identification of the organism as *S.* Paratyphi A, will take a minimum of a further 24-hours. Participants in whom a Gram-negative bacillus is identified in the aerobic blood culture bottle will, therefore, be

defined as having paratyphoid fever for the purposes of clinical management (including antibiotic treatment) and collection/handling of blood, urine, and stool samples.

9.11.2. Severe paratyphoid fever

Severe paratyphoid fever will be defined as illness that includes any of the criteria satisfied in **Table 5**.

Severe paratyphoid fever is diagnosed if ANY of the following apply
Oral temperature ≥ 40.0°C
Systolic blood pressure ≤ 85 mmHg
Significant lethargy or confusion
Gastrointestinal bleeding
Gastrointestinal perforation
Any grade 4 or above laboratory abnormality, as defined in Appendix C: Grading the severity of laboratory Adverse Events

Table 5. Criteria for severe paratyphoid diagnosis

9.11.3. Admission to inpatient facility

Admission to the John Warin Ward (or other appropriate in-patient ward, Oxford University Hospitals NHS Foundation Trust) will be considered by a study doctor under the following circumstances:

Admission to in-patient facility to be considered if ANY of the following apply
Severe paratyphoid fever as defined in Table 5
Failure of symptoms to improve within 48 hours after starting antibiotic therapy
Inability to tolerate oral antibiotics
Dehydration requiring intravenous fluid therapy
Unanticipated concerns about the participant's home conditions

Table 6. Criteria for considering admission to inpatient facility.

In addition, any participant who deviates from the protocol, including taking antipyretics prior to a diagnosis of paratyphoid infection is made, will be considered for hospital admission at the discretion

of the clinical study team. At any stage, if clinically indicated, an additional review will be arranged by the clinical study team or if severely unwell they will be directed to the John Radcliffe Accident and Emergency department (or appropriate other medical facility) and relevant medical personnel (eg Consultant Physician providing clinical oversight, General Medical Registrar) will be made aware. This will be emphasised at screening and throughout the study.

Ultimately, all decisions regarding admission will be assessed by the clinical study team in conjunction with the Infectious Diseases Consultant on call. The consultant will be made aware of the study protocol and the suggested treatments outlined below, but in-patient management is at the discretion of the supervising consultant.

9.11.4. Blood sampling for participants with paratyphoid fever

For participants who develop paratyphoid fever, blood tests will be performed as per Table 3c (section 7.9) rows labelled Paratyphoid Diagnosis (PD). This schedule replaces other scheduled bloods during these days. Participants will be seen for approximately 5 further visits after diagnosis, additional visits may be required for safety purposes. If no other visit is scheduled for day 14 after challenge in diagnosed participants then a visit will be scheduled for this day (day 14PDgroup in Table 3c, section 7.9). This will only occur in participants diagnosed on or before day 9. The next blood sample will occur on the Day 28 visit, unless further blood samples are required for participant safety purposes.

9.11.5. Concomitant medication for symptoms of paratyphoid infection

Concomitant medication can be provided for symptomatic control of paratyphoid infection, before and after diagnosis. Concomitant medication after paratyphoid diagnosis includes antibiotics and antipyretics if required.

Drug	Indication	Dose	Route	Frequency
Paracetamol	Fever and discomfort (**after antibiotic therapy started**)	500mg - 1 Gram	Oral	PRN, max QDS
Codeine	Pain including headache	15-60mg	Oral	PRN (max. 240mg/24 hours)
Senna	Constipation	2-4 tablets	Oral	PRN
Cyclizine	Nausea and/or vomiting	50mg	Oral	PRN (max. 150mg/24 hours)
Chlorpheniramine	Allergy	4mg	Oral	PRN TDS-QDS (max. 24mg/24 hours)
Oral rehydration salts	Dehydration, vomiting or diarrhoea	1-2 sachets	Oral	PRN
Sando-K	Hypokalaemia	2-4 tablets	Oral	PRN, up to TDS dependent on potassium deficit

Table 7. Concomitant medication for symptom control.

9.11.6. Antibiotic treatment

Antibiotic therapy is commenced when one or more of the criteria in Table 8 are satisfied.

Antibiotics are commenced if ANY of the following apply
Any participant meeting the definition of paratyphoid infection (Table 4)
Any participant with 3 or more of the following symptoms ***severe enough to interfere with all normal activity;*** • **Headache** • **Malaise** • **Anorexia** • **Abdominal pain** • **Nausea/vomiting** • **Myalgia** • **Arthralgia** • **Cough** • **Rash** • **Diarrhoea** • **Constipation**
Any participant who has not received antibiotics by day 14 post-challenge
Any participant in whom antibiotic use is felt to be clinically necessary (as decided by a medically qualified study doctor)

Table 8. Criteria for commencing antibiotic treatment.

The first line antibiotic will be oral ciprofloxacin 500mg twice daily for 7 days.

Cautions and contraindications for the use of ciprofloxacin include:

- Pregnancy – a negative pregnancy test is required of all female participants of childbearing potential prior to treatment,
- Absorption of ciprofloxacin is decreased by antacids and iron supplements, and participants will be counselled not to take these during the antibiotic course.

For any participant in whom a contraindication to these first line antibiotics becomes apparent, the following regimens of licensed antibiotics will be used:

- 2nd line: Oral trimethoprim/sulfamethoxazole 160/800 mg twice daily for 7 days or azithromycin 1g stat dose followed by 500mg daily for 6 days.
- 3rd line: Oral amoxicillin 500mg TDS for 7 days.

The participant's GP will be notified in writing that a participant has been challenged (see section 9.10). In this communication they will be informed of the planned antibiotic course (ciprofloxacin 500mg bd for 7 days) that will be prescribed on diagnosis or at day 14 post challenge if no diagnosis made, at the time of writing to notify them of challenge.

9.12. Notification of UKHSA and GP

Reporting to the Health Protection Unit

The relevant local Health Protection Unit (UK Health Security Agency) will be informed of the name, address and date of birth of all participants who;

- Undergo challenge with *S.* Paratyphi A
- Have completed clearance stool sampling following challenge (with additional information and continued contact if persistent stool shedding occurs)

In addition, any breaches in enteric precautions that result in another individual coming into contact with the excreta of a participant will be reported to UKHSA.

Reporting to the GP

Participants give their consent that their GP be informed of their participation in the trial. A participant's GP will be informed at the following timepoints:

- Following first vaccination with CVD 1902/placebo
- Following challenge with *S*. Paratyphi A
- Once they have completed clearance stool sampling following challenge (with additional information and continued contact if persistent stool shedding occurs).

In addition, with participant consent, any other relevant medical issues, related to the trial or otherwise may be discussed as required with the participant's GP to ensure continuity of care.

9.13. Clearance samples

For routine clinical infections (ie not within an experimental infection setting) UKHSA guidelines require naturally infected individuals within risk groups (such as those involved in preparing or serving raw food/food not subjected to further heating and health or social or nursery care workers) to provide evidence of clearance of typhoidal Salmonellas[66]. Cases identified in these groups require

faecal sampling as described below. UKHSA guidelines suggest individuals not in these risk groups do not require clearance faecal samples in routine clinical infections.

In this study to detect chronic carriage of *S*. Paratyphi A and to confirm clearance, **all** participants are required to produce three stool samples obtained a minimum of 48 hours apart produced at least one week after completion of the antibiotic course.

At the day 14 visit or PD+96 visit participants will be given stool clearance packs and instructions about the first date at which they are able to deliver a stool clearance sample. Participants are reimbursed per clearance sample for the inconvenience of delivering the sample to OVG. Once a participant has reached the appropriate timepoint to provide a clearance sample (one week after completion of the antibiotic course), they will be contacted weekly (by phone/email/text message) to remind them to provide samples. These contact attempts will be documented. After 12 reminders and in-person reminders at day 90 and day 180 visits if participants have failed to provide three clearance samples no further action will be taken.

Participants with three successive negative stool samples will be considered to be fully treated for *S.* Paratyphi A infection and no longer an infection risk.

UKHSA will be informed of all participants in whom clearance has been demonstrated and of any participant who fails to demonstrate clearance after the initial 7-day course of antibiotics or after any other antibiotic treatment. The employer of any participant involved in the provision of health or social care to vulnerable groups will be notified in writing once three successive stool samples are negative.

9.14. Screening of close contacts for carriage of *S.* Paratyphi A

The participant will provide letters from the study team to close contacts including household contacts. Contacts will be offered the opportunity to be screened for *S*. Paratyphi A infection, which will involve obtaining two stool samples 48-hours apart a minimum of seven days after the participant has begun antibiotic treatment. Contacts may also be screened during the vaccination period in the same way if requested. If either stool culture of a household contact is positive, he/she will be referred to a Consultant in Infectious Diseases for appropriate antibiotic management and UKHSA will be informed.

9.15. Transport of samples

All samples from participants must be a labelled with a 'Danger of Infection' sticker if transported outside of the CCVTM. If a specimen sample bag is to be used, this should also be labelled 'Danger of Infection'. Samples should be transported in accordance with local OVG SOPs.

9.16. Sample Handling & Laboratory Testing

Samples will be taken as detailed in sections 7.4 to 7.9 and handled as set out in the Laboratory Analysis Plan. Samples will be analysed as described below, some may occur on fresh samples, other samples may be frozen to allow analyses to the batched.

Samples will be stored for the duration of the study and thereafter if consent is given be transferred to the OVC biobank (see section 9.3).

In addition to blood samples needed for the safe conduct of the trial and assessment of the primary endpoint, blood, stool and saliva samples from the participants will also be subjected to laboratory analyses in order to assess the objective defined in the secondary endpoint, and potentially for the exploratory objectives. These samples will be relabelled with a laboratory number upon processing in the OVG laboratory, which is linked to the original participant number.

Stool samples supplied by participants should be delivered to CCVTM within 24 hours of being taken. If possible, the samples should be kept cool until delivered to the CCVTM and then stored at 2^{o}C to 8^{o}C. The time of sampling will be noted on the sample form.

Following the completion of primary and secondary endpoints, and with appropriate consent, samples will be transferred to the OVC Biobank. Where OVC Biobank consent in not received, samples will be destroyed following the completion of exploratory endpoints. All samples will be either transferred to the OVC Biobank, or destroyed, within 12 months of the end of trial notification.

<u>Oxford University Hospitals NHS Foundation Trust Laboratories:</u>

Microbiology: Blood cultures, stool cultures, SARS-CoV-2 NAAT testing

Haematology and Biochemistry: FBC, WBC differential counts, C-reactive protein, urea, creatinine, electrolytes, aspartate transaminase (AST), alkaline phosphatase (ALP), alanine transaminase (ALT), bilirubin, amylase

Immunology: Coeliac screen, IgA

Transplant Immunology: HLA B27

Oxford Vaccine Group Laboratories:

Immunology

a. Antibody responses

Exploratory assays of particular scientific interest may include quantification of *S*. Paratyphi A antigen specific antibodies (IgG, IgA and IgM, as well subtypes), assessment of antibody functional properties including bactericidal antibody activity, and characterisation of cell-mediated responses and cytokine/acute phase reactant profiles.

Antibody responses will be assessed using in-house developed ELISAs and/or Luminex-based quantification. Functional antibody responses may also be determined using methods including but not limited to bactericidal or opsonophagocytic assays.

b. Inflammatory responses

The kinetics of the inflammatory response may be measured in stored plasma samples by multiplex bead-array or ELISA-based analysis of cytokines/chemokines. Plasma samples will be isolated from heparinised blood and stored at -80°C until assays are performed.

c. Cellular immune responses

Cellular immune responses will be analysed using PBMCs isolated using density gradient centrifugation and/or cells from fixed whole blood. Analysis may include but is not limited to characterisation of activation, proliferation, and cytokine and surface markers using intracellular staining and multi-chromatic flow cytometry of cells. Antibody-secreting cells that secrete antibodies against *S*. Paratyphi A specific antigens including but not limited to O and H will be measured by using ELISPOT/FluoroSpot. Memory B cell responses against *S*. Paratyphi A specific antigens including O and H will be measured by using ELISPOT/FluoroSpot following *ex vivo* stimulation of cells. Additional analysis may be performed using heavy metal ion tags (CyTOF) to increase the number of parameters being investigated. These investigations may be performed in collaboration with external research groups such as the Human Immunology Group at the Weatherall Institute of Molecular Medicine (WIMM), Oxford. Both innate and adaptive immune cell responses may be investigated.

These DNA samples may be used to analyse the genetic factors influencing vaccine responses (immunogenicity and reactogenicity) as well as response to challenge (susceptibility to infection and gene expression).

Other analyses

a. Additional Microbiology

Quantitative culture of whole blood will be performed to determine the number of organisms in the blood, using the Wampole™ Isostat® Isolator system (Oxoid Ltd, Basingstoke). Enumeration of *S.* Paratyphi A organisms in the blood will be performed by lysis centrifugation followed by direct plating onto selective media.

Isolates of *S*. Paratyphi A may be retained for phage typing or further investigation by the reference laboratory if challenge strain confirmation is required by PHE. Isolates derived from the challenge period will ultimately be analysed to confirm that these are challenge strain rather than vaccine strain. This will not occur in real time.

Additionally, quantitative stool cultures or PCR may be performed at the OVGL, to assess the burden of stool shedding. Isolates from stool samples will be stored frozen for future analysis, which may include phage typing or genetic sequencing.

b. Factors affecting susceptibility and response to infection

Analysis of gene expression changes in response to vaccination and challenge may be performed using peripheral blood. In addition, DNA samples obtained from peripheral blood will contribute to a Biobank of samples from multiple different Oxford Vaccine Group studies (separate consent will be sought for this).

Exploratory analysis may also include the application of techniques such as proteomics, metabolomics and epigenetics. These techniques can give insight into the molecular changes occurring after vaccination, challenge and during acute infection. Changes in the proteome, metabolome or epigenome may be of interest as markers of disease severity, and in combination with other data may highlight pathways or processes involved in vaccination responses and protection.

c. Microbiome analysis

Analysis of the relative composition of bacterial populations within the bowel, may be performed on collected stool samples. The faecal microbiome, bacterial dynamics and response to antibiotic treatment may be assayed using techniques including pyrosequencing. After collection, stool will be stabilised in RNA-later and stored at -80°C, prior to further assessment.

Other laboratories:

Samples collected as part of this study may also be used for other exploratory studies of scientific relevance by the OVG laboratory or any of the collaborating laboratories worldwide. These samples may include stool, serum, extracted DNA and RNA, and PBMCs. Frozen samples will be stored under the ethical approval for this study until the end of ethical approval. At this time, samples will be transferred to the Oxford Vaccine Centre Biobank subject to participant consent (see Section 9.3). Studies may include further investigation of the inflammatory and immunological response to vaccination and challenge.

9.17. Early Discontinuation/Withdrawal of Participants

Each participant can exercise his or her right to withdraw from the study at any time.

If, however, the participant decides to withdraw after they are challenged, they will be, for their safety and public health reasons, required to complete a course of antibiotics and may be required to attend additional hospital/non-study visits to ensure compliance. In addition, the investigator may terminate a participant's involvement in the study at any time if the investigator considers it necessary for any reason including, though not exclusive to, the following:

- Ineligibility (either arising during the study or in the form of new information not declared or detected at screening),
- Significant protocol deviation,
- Significant non-compliance with study requirements or risk to public health,
- Any adverse event which requires discontinuation of the study procedures or results in an inability to continue to comply with study procedures,
- Consent withdrawn,
- Lost to follow up
- Pregnancy of a female participant

Withdrawal from the study will not result in exclusion of the data generated by that participant from analysis. The reason for withdrawal, if given, will be recorded in the CRF.

All Participants (regardless of receipt of vaccine or placebo) who withdraw following vaccination prior to challenge will be asked to provide stool samples (3 samples taken 48 hours apart) to prove clearance. This is to protect the blind. If participants are unwilling or unable to do this, unblinding may be required to allow vaccine recipients to be treated with antibiotics. They can then be replaced in order to achieve 66-76 participants who have been both vaccinated and challenged. If participants

were found to have positive stool clearance samples they will be managed by a study clinician and may be treated with 7 days of ciprofloxacin 500mg bd.

Participants who are, for any reason, unable to be challenged having undergone vaccine or placebo administration can be replaced in order to achieve 66-76 participants who have been both vaccinated and challenged. Replacement participants will be randomised per standard processes and the sample size calculation has taken this into account.

9.18. Definition of End of Trial

The definition of the end of the study is when the last laboratory assay has been performed to determine the primary and secondary objectives of the study protocol.

9.19. Special circumstances (SARS-CoV-2 pandemic)

9.19.1 Conducting Controlled Human Infection Models of Paratyphoid in the context of the COVID-19 pandemic

The incidence of COVID-19 continues to change in the UK, with factors such as vaccination and new variants of concern making predictions about disease incidence difficult. It is likely that ongoing transmission within the UK will continue for some time including during the clinical delivery phase of this trial. To minimise the risk of infection we have stipulated that all participants must have received at least one dose of an approved COVID-19 vaccine prior to entry into the trial.

There are limited data on the risk of coincident COVID-19 infection and *Salmonella* Paratyphi A and it is unknown if co-infection would make the outcome of either infection more severe. As the risk of co-infection cannot be quantified, participants will be tested prior to the challenge section of the study and any participant who tests positive will not proceed with *Salmonella* Paratyphi A challenge.

Any participants who test positive at any stage of the study will be advised to follow current government guidelines in place. Positive COVID-19 PCR results will be reported as a notifiable disease as per UKHSA Guidance.

Fevers commonly occur following *Salmonella* Paratyphi A challenge and therefore may pose a diagnostic challenge as concurrent COVID-19 infection is likely to remain an ongoing possibility. Between days 3 and 14 development of fever would be an expected feature of the development of paratyphoid fever. Testing for COVID-19 during the challenge period has therefore been introduced if they develop a fever and at the point of paratyphoid diagnosis or commencement of treatment if they

have not had a swab in the preceding 24 hours. For participant safety, if a participant swabs positive for COVID-19 in the 14 days post challenge and prior to paratyphoid diagnosis they will receive treatment for paratyphoid regardless of whether they have reached the criteria for paratyphoid diagnosis and will be automatically withdrawn from the study. Visits following treatment in this scenario will be managed on a clinician managed risk basis to balance the need to minimise clinic visits and to ensure safety. These participants will continue to be monitored for safety. Participants who have COVID-19 symptoms during other stages of the study will be advised on COVID-19 testing and procedures as per current national and local guidance. Any participants who test positive for COVID-19 at stages of the study other than the 14 days post challenge will be discussed with a senior clinician and decision as to whether they will continue in the study or be withdrawn will be made on a case-by-case basis.

Social distancing requirements to control COVID-19 will likely remain in place to some extent in England. To abide with these, participants' attendances at study clinics will be carefully planned to adhere to social distancing guidelines. Participant flow will be arranged so that contact with other people is minimised and Personal Protective Equipment (PPE – compromising apron, gloves, surgical mask and eye protection) will be worn by members of clinical staff for all participant visits. This will follow an infection control SOP that covers all PPE requirements for clinical trials at the Oxford Vaccine Centre in the era of the COVID-19 pandemic. Any clinic visits that are carried out on a participant that is self-isolating will be conducted in a separate clinical area with side access to avoid any contact with other volunteers.

The national COVID-19 vaccination programme continues to roll out and it is likely that some of our participants may have their second or subsequent vaccine(s) during the trial. It is unknown what effect concomitant COVID-19 vaccine administration at the time of CVD 1902 vaccination or paratyphoid challenge may have on the immune response to the vaccine or to challenge following vaccination, both of which are being studied in this trial. To minimise the impact of COVID-19 vaccination affecting the results of the trial participants are asked not to schedule COVID-19 vaccinations for 7 days either side of their study vaccination and for 7 days before or 21 days after paratyphoid challenge.

9.19.2 Participants with fever during vaccination period

No fevers were seen with CVD 1902 vaccination in the Phase 1 study. For this reason if a participant did develop a fever during the vaccination period they would be reviewed by the study team and may be tested for COVID-19. If symptoms of COVID-19 other than fever develop a participant may be

advised to attend routine NHS COVID-19 testing. If the test is negative the participant can continue with study visits as per protocol.

9.19.3 Participants with fever during challenge period

Post challenge if a participant develops a fever ≥37.8°C or other symptoms consistent with possible COVID-19 disease, they will be advised to inform the study team before attending their clinic visit. As long as the participants' symptoms are judged by the Investigator as not significant enough to require referral to secondary care, they will be advised to attend the study clinic where their visit will be conducted in a separately-accessed isolated clinic room with study staff wearing appropriate PPE. If a participant fulfils COVID-19 testing criteria, a SARS-COV-2 PCR swab from the nasopharynx and throat will be taken by the study team in addition to the study specified study blood tests.

If a participant has a persistent fever or study clinicians are clinically concerned for COVID-19 disease, then a SARS-COV-2 PCR swab can be repeated at the discretion of the Investigator, if the first SARS-COV-2 PCR was negative.

If a participant is self-isolating because of COVID-19 disease and they are unable to travel to the clinic safely, then a home visit may be conducted by the study team in lieu of a clinic visit to allow collection of safety blood tests.

If following paratyphoid challenge and prior to paratyphoid diagnosis and treatment, a participant is found to have a positive SARS-COV-2 PCR swab from any source, they will be commenced on paratyphoid treatment, irrespective of the severity of COVID-19 disease and irrespective of paratyphoid symptoms or blood culture result at that time point.

All volunteers will undergo a combined nasopharyngeal and throat swab for SARS-COV-2 PCR on the day of paratyphoid diagnosis or when paratyphoid treatment is commenced, unless they have had a negative COVID-19 PCR swab within the preceding 24 hours. The reasoning for this is that participants may develop fever after commencement of paratyphoid treatment and if a participant develops a fever after starting treatment, a negative COVID-19 swab will allow exclusion of concurrent COVID-19 disease.

9.19.4 Risk assessment for the trial

For the purposes of this trial, the CI will risk assess with relevant parties where necessary (eg DSMB, MHRA) on the prevailing situation at a given time, for example to assess:

- The appropriateness of initiating vaccination
- Progression of vaccinees to wild-type challenge
- Whether the study should be paused

Given the rapidly changing nature of the pandemic modifications to the way in which the trial is conducted may be necessary and these will be detailed through updates to the Clinical Study Plan and/or amendments to the study protocol and other study documents as required.

10. TRIAL INTERVENTIONS

10.1. Investigational Medicinal Product(s) (IMP) Description

Vaccine: CVD 1902

CVD 1902 is a live attenuated strain of *Salmonella* Paratyphi A, an unlicensed, experimental oral vaccine for *Salmonella* Paratyphi A infection developed by the Center for Vaccine Development at the University of Maryland in Baltimore. A dose contains not less than 2 x 10^{10} CFU and vaccine recipients in this trial will receive 2 doses delivered in 30 mL carrier sodium bicarbonate solution (see section 10.2), 14 days apart.

QP certification will be performed by IKSA, B.V., Rotterdam.

The vaccine will be manufactured in glass vials. It will be transported to OVG frozen and kept within the appropriate range as per manufacturer's instructions/IMPD. The investigator (or delegate) will make an inventory and acknowledge receipt of all shipments of study vaccines. Vaccines will be stored as per manufacturer's instructions. The vaccine is a cloudy solution containing live attenuated *Salmonella* Paratyphi A bacteria suspended in glycerol.

The product is manufactured, tested and labelled according to current EMEA guidelines in keeping with Good Manufacturing Practice (GMP). See the IB and IMPD for detailed descriptions of the final drug product.

Placebo: The vaccine placebo is 30ml 1.3% wt/vol sodium bicarbonate solution made up using BP sodium bicarbonate powder with sterile water.

The vaccine was originally manufactured in February 2020 but due to the COVID-19 pandemic there has been a delay in starting the clinical trial. The expiry date was originally 21 May 2021. Stability

testing will be undertaking at an extra timepoint in July/August 2021 to provide data for extension of the expiry date and relabelling.

3.16.6. **Blinding of IMPs**

The reconstituted vaccine is a cloudy fluid which differs from the placebo which is clear. Blinding of the participant at vaccine or placebo administration will be achieved via the use of a concealed container. It is acknowledged that this blind may not be absolute for participant or blinded observer.

10.1.2. Storage of IMP

CVD 1902 will be stored in glass vials in secure freezers at -80°C at the Oxford Vaccine Group. These freezers are continuously monitored by automated telemetry systems to ensure frozen vaccine stocks are kept within the appropriate temperature range. It will be administered to participants within 4 hours of preparation. Once the vaccine doses are made up they will kept on wet ice prior to administration (as for challenge doses).

Sodium bicarbonate powder and sterile water for the reconstitution of IMP will be stored in a secure location within the Containment level 3 laboratory

10.1.3. Compliance with Trial Treatment

Participants will be observed drinking CVD 1902 or placebo and the container will be checked after administration by study staff to ensure the whole dose has been taken.

10.1.4. Accountability of the Trial Treatment

CVD 1902 is manufactured tested, packaged and labelled by Bharat Biotech®, according to current EMEA guidelines in keeping with Good Manufacturing Practice (GMP). All vaccines are labelled with a label specifying 'For clinical trial use only' and no less than the following:

- The clinical trial identifier (by reference code)
- The content of each vial
- Dose route
- The batch number
- The chief investigator
- Expiry date

The vaccine will be stored at the CCVTM pending authorised release for use in the clinical trial.

Vaccine and placebo doses will be accounted for within an accountability log stored in the CL3 laboratory and unblinded TMF. The unblinded laboratory team will keep a log of vaccine and placebo reconstitution. The blinded clinical team will keep a corresponding administration log. Unused vaccines at the end of the trial may be retained for laboratory use only (such as laboratory assay development). Any recall of study vaccines required for use in the study or reporting of defective vaccines will be performed according to relevant OVG and OVC SOPs.

10.1.5. Post-trial Treatment

Study medication will not be continued beyond the study period.

10.2. Other Treatments (non-IMPS)

10.2.1. Sodium bicarbonate solution for both vaccine and placebo arms (pre-treatment)

Both groups will receive 120ml of sodium bicarbonate solution prior to vaccine or placebo. Sodium bicarbonate solution will be reconstituted from pharmaceutical grade sodium bicarbonate powder and sterile water at a concentration of 1.3%wt/vol. Sodium bicarbonate powder and sterile water for the pre-treatment bicarbonate solution prior to vaccine and challenge administration will be stored in a secure drug cupboard within a temperature-controlled room.

10.2.2. *S.* Paratyphi A challenge strain

10.2.2.1. GMP manufacture

A parent seed lot, S888P5SP01, was established in March 2010 after serial colony selections on Luria Broth PTK agar plates and stored in the Novartis Vaccines and Diagnostics bacterial seed bank (Siena, Italy). This lot was used to establish the GMP Master Cell Bank, SA-13-002. Under GMP conditions in GenIbet BioPharmaceuticals, Portugal, 3 dose levels of the challenge agent were produced in chemically-defined media, using sucrose as the carbon source, to prepare a bulk bacterial suspension (Active Substance, 00513). Prepared vials containing the challenge agent were stored at -80°C ± 5°C and transferred to the Oxford Vaccine Group Laboratory in 2013.

Strain characterisation has included:

- Serotyping confirms *Salmonella enterica* serovar Paratyphi A.
- Antibiotic sensitivity profile of the challenge agent in June 2013 demonstrates a fully antibiotic sensitive strain.

- Analysis by the Novartis Vaccines Institute for Global Health confirms presence of the O:1 and O:2 polysaccharide antigen.
- Biochemical profiling.

10.2.2.2. Storage of the challenge strain

S. Paratyphi A (NVGH308) for inoculation of participants will be stored as a frozen suspension in soya tryptone medium containing 10% sucrose. Suspensions will be labelled with no less than the contents (*S*. Paratyphi A NVGH308 strain), 'working cell bank', date of manufacturer, storage conditions and vial number. Following GMP manufacture, NVGH will ship the *S*. Paratyphi A NVGH308 strain challenge agent via accredited courier to Oxford for storage.

10.2.2.3. Accountability for the challenge strain

The investigator will be responsible for adequate and accurate accounting of *S.* Paratyphi A vials prepared for administration to participants. The investigator or designee will administer the study *S.* Paratyphi A vials only to individuals included in this study following the procedures set out in this study protocol and the associated OVG SOPs and Study Plans. The date, dosage and time of administration will be recorded.

The study team will track all vials of *S.* Paratyphi A that have been used, administered to participants and wasted, within an accountability log.

10.2.3 Antibiotics

Antibiotics detailed in Section 9.11.6 are used to treat diagnosed *Salmonella* Paratyphi A infection or at day 14 post challenge for those who have not been diagnosed.

Antibiotics are stored in a secure drug cupboard within a temperature-controlled room. All non-IMPS will be received in accordance with local OVG SOPs and all doses will be accounted for within an accountability log.

Participants are observed taking their first dose of antibiotic and this is recorded in the eCRF. Participants are asked to record their subsequent doses in their e-diary which will be checked daily to make sure that the participant is entering doses correctly. Participants will be reminded by text message to take their antibiotic doses and contacted if a dose is not recorded in their electronic diary. Participants are called at day 21 to ensure that they have completed their course of antibiotics.

10.2.4 Concomitant medications

Concomitant medications detailed in Section 9.11.5 are used to treat symptoms that may occur during symptomatic *Salmonella* Paratyphi A infection. They will be stored in a secure drug cupboard within a temperature-controlled room. All non-IMPS will be received in accordance with local OVG SOPs and all doses will be accounted for within an accountability log.

Participants are asked to record any doses of medication taken in their e-diary. Regular medications (such as the contraceptive pill) that are pre-existing before entry into the trial are recorded in the eCRF and are not double entered in the e-diary. If medications are started after the e-diary has closed then these will be entered onto the eCRF.

All non-IMPS will be received in accordance with local OVG SOPs and all doses will be accounted for within an accountability log. Details of reconstitution will be included in the Clinical Study Plan.

11. SAFETY

11.1. Potential risks for Participants

The general risks to participants in this study are associated with the vaccine, placebo, study-fatigue, phlebotomy, symptomatic infection following challenge and the small risk of subsequent complications.

11.1.1. Complications of CVD 1902

Foreseeable vaccination reactions listed in the IB include fever, diarrhoea, anorexia, malaise, abdominal cramps, and headache. Nausea and vomiting are listed as possible adverse reactions. It is also noted that shedding of the vaccine strain may occur.

In the Phase 1 trial "CVD 1902 was well tolerated without clinically significant adverse reactions attributed to the vaccine."[56] Hypersensitivity to the vaccine is extremely unlikely given the constituents present in the vaccine preparation (water, sodium bicarbonate, *Salmonella* bacteria).

There may be unforeseeable side effects, including severe ones which cannot be predicted. Subjects will have the details of a 24-hour contact study doctor and can be seen for unscheduled visits as required. If participants develop diarrhoea or vomiting, they may be treated with oral rehydration salts. If the blinded clinical team feel that antibiotic treatment may be warranted this would be discussed with the blinded Co-I(s) (Professor Angus and/or Dr Ramasamy) and procedure for unblinding for a specific participant would be followed as per section 9.6.

11.1.2. Complications of Placebo

Hypersensitivity reactions to the placebo (sodium bicarbonate solution: concentration of 1.3% wt/vol) are exceedingly unlikely. The solution has an unpleasant taste. The SmPC states that stomach cramps and flatulence can be caused by oral administration.

11.1.3. Study-fatigue

This may occur due to the prolonged nature of the study, intense frequency of the visits, especially during the challenge period. Participants are compensated for their time and every effort will be made to make study investigations as swift and uncomplicated as possible. Interventions and visits will be limited in number and will be arranged to fit with individual schedules and other obligations, as far as is practical.

11.1.4. Phlebotomy

The volume of blood drawn over the study period should not compromise healthy adult participants. Potential participants will be bled 17 ml at a screening visit. If recruited to the study a maximum of 1122 ml of blood will be taken over the course of the study (1 year plus 6 weeks). As a comparison, women are able to donate a maximum of 1410 ml of blood per year, and men 1880 ml, to the National Transfusion Service. At screening, history of blood donations will be checked to ensure that the total volume of blood taken is safe to take. Participants will be closely monitored both clinically and by laboratory results for haemoglobin during the study. Should anaemia develop during the study, sample volumes will be minimised to include only essential safety bloods and blood cultures. Risks from venepuncture include mild tenderness, bruising, light-headedness and, rarely, syncope or arterial puncture.

11.1.5. Symptomatic infection

Some study participants will develop symptomatic paratyphoid infection following challenge. During the challenge phase (before treatment with antibiotics) participants will be reviewed at least daily by a clinical study team member. They are also sent reminder texts to ensure participant safety, and to reiterate the participants are to contact the study team if they have any concerns. Participants will be made aware of the potential symptoms of paratyphoid infection and will be monitored closely throughout the challenge for the development of these symptoms. Symptoms of fever, headache, malaise, anorexia (loss of appetite), abdominal pain, nausea/vomiting, myalgias and arthralgias, cough, rash, diarrhoea and constipation will be solicited each day in their e-diary after challenge. Participants will be instructed to record their oral temperature twice a day with a provided thermometer and should they feel feverish and will be instructed to contact study staff immediately should they have a fever $\geq$38°C or have any concerns.

The further management of paratyphoid fever is outlined in section 9.9.

Complications of paratyphoid fever, such as perforation or haemorrhage, occur almost exclusively in patients who do not receive appropriate antibiotic treatment for an extended period. Participants in this study will be treated within 24 hours after developing fever (typically much less depending on time of fever and availability to come in for diagnosis) or if *S.* Paratyphi A is recovered from a blood culture drawn 72 hours or more after challenge (see section 9.9.1). They will be closely monitored during the initial study phase until a 7-day course of antibiotics is completed to minimise the risk of complications. Participants who are not diagnosed with paratyphoid fever within 14 days post-challenge will also be treated with a 7-day course of antibiotics.

The risks associated with paratyphoid challenge will be greatly minimised by complying with study visits and maintaining close contact with the study team. A previous challenge study using the same strain of *S.* Paratyphi A at OVG has demonstrated a good safety profile[65].

11.1.6. Chronic carrier state

Approximately 2-5% of patients fail to clear typhoidal *Salmonella* infection following recovery from their acute illness and typically carry bacteria in their gallbladder[19]. These individuals can then asymptomatically shed bacteria and transmit infection to others[67]. The chronic carrier state of typhoidal *Salmonella* is often associated with gallbladder disease, typically calculi[68] and is more common in women[69]. Typhoidal Salmonellas are well adapted to live within the biofilm coating the surface of gallstones. Only participants with a normal ultrasound examination of the gallbladder will be included in this study.

The likelihood of developing chronic carriage is extremely low in the challenge setting. Participants are treated with ciprofloxacin, a fluoroquinolone antibiotic which is the preferred class of antibiotics for prevention of chronic carriage. A previous study demonstrated that, of more than 200 patients treated for typhoid fever with ciprofloxacin, none became carriers[43].

To ensure clearance of infection and to exclude chronic carriage, stool samples for culture will be obtained upon completion of the initial antibiotic course. If participants remain positive after a second course of antibiotic treatment (subsequent to the initial course) then participants will be referred to an Infectious Diseases Consultant at the Oxford University Hospitals NHS Foundation Trust for further management.

11.1.7. Antibiotics

Potential participants with known antibiotic hypersensitivity, allergy or contraindication to either of the first-line antibiotics (ciprofloxacin, co-trimoxazole, azithromycin or other macrolide antibiotics)

will be excluded. Participants who have a known specific allergy to ceftriaxone will also be excluded as this would be the intravenous treatment of choice if a participant required inpatient admission.

The antibiotics to be used in this study are generally well tolerated and are only occasionally associated with side effects however all participants will be counselled about antibiotic side effects during the consent process at screening. Common side effects of ciprofloxacin include gastrointestinal disturbance, rash, thrush, headache and deranged liver function tests; rarely side effects can include leukopenias, thrombocytopaenia, psychiatric disturbance, seizures and tendonitis (full details in SmPC). Counselling will include side effects of antibiotics in general and include specific details about the side effects of ciprofloxacin. For ciprofloxacin this will also include the provision of the MHRA leaflet "Fluoroquinolone antibiotics (-oxacins): what you need to know about side effects of tendons, muscles, joints, and nerves" [Dated March 2019, or subsequent version as appropriate] in keeping with routine clinical practice whereby all patients in the NHS who received ciprofloxacin are given this leaflet. The participant will also be given this leaflet when their antibiotics are dispensed and will be reminded to contact the study team if they develop any side effects.

Should an antibiotic cause allergy or intolerance this will be managed by a study doctor and a different antibiotic will be used for subsequent management.

There is evidence that a course of antibiotics has an effect on the diversity of the gastrointestinal microbiome which may exist beyond the antibiotic treatment course. Participants will be informed that this is one aspect of exploratory analysis in the study.

11.2. Potential risks to close contacts of participants

In view of the low infectivity of S. Paratyphi A without bicarbonate buffer and the high standard of hygiene and sanitation in the UK, secondary transmission of the challenge strain to household or other close contacts after discharge is highly unlikely. It is thought that typhoidal Salmonellas, unlike *Shigella sp.*, enterohaemorrhagic *Escherichia coli* or hepatitis A virus, are virtually never transmitted by direct faecal-oral contact. This is in part due to the higher oral inoculum of these bacteria required to cause clinical disease.

It is acknowledged, however, that transmission within households can occur if the individual excreting *S.* Paratyphi A fails to practice effective hand washing after defecation and is subsequently involved in uncooked food preparation. If food is kept at ambient temperatures, bacterial proliferation occurs

such that an infective dose level is reached, and the food then may act as a vehicle for paratyphoid transmission.

Throughout the period of possible excretion of the challenge strain, participants must practice stringent hand washing techniques after defecation. Participants will be given soap and paper towels for use at home and detailed advice on how to prevent transmission of *S.* Paratyphi A. Participants will be taught and observed practising good hygiene technique at their initial challenge visit. The importance of adhering to sanitation advice will be emphasised to participants. It is important to note that participants in this trial will be fully informed about the risks of transmission and how to prevent this prior to challenge. As such, participants will be in the position to implement this from the point of infection which will reduce the chance of secondary transmission. This is very different from the situation with travellers returning from abroad where paratyphoid diagnosis is usually delayed by several weeks allowing a prolonged period of exposure to contacts before precautions are put in place. Since most individuals living in upper middle income countries practice good personal hygiene and food hygiene, secondary transmission of *S*. Paratyphi A within households by returning travellers with paratyphoid fever is rare. Furthermore, the delay in diagnosis that occurs in travellers with paratyphoid fever leads to a prolonged period of time in which *S.* Paratyphi A has been excreted. We will treat all participants in this study very early in the course of disease, leading to rapid clearance of bacteria and a very limited period of excretion, reducing potential exposure to contacts.

When occasional transmission of paratyphoid infection occurs, it is usually related to unknowingly infected food handlers[70]. For this reason, food handlers will be excluded from this study. Potential participants employed in clinical or social work with direct contact with young children (those attending pre-school groups, nursery, or aged less than 2 years) or highly susceptible patients or persons in whom paratyphoid infection would have particularly serious consequences (such as the elderly) also represent an increased risk and will be excluded unless willing to not work until it has been demonstrated that they are not infected with *S.* Paratyphi A in accordance with UKHSA guidance[66].

Even in the absence of precautions to prevent secondary transmission (as is seen in returning travellers), the rate of transmission of enteric fever causing bacteria is exceptionally low within the UK. In a large recent study of 251 contacts of patients with typhoid fever in London, only one patient was identified as a suspected case of secondary transmission[71]. Similarly, a study in Scotland showed a very low secondary transmission rate in the absence of precautions[72].

11.3. Safety Reporting Definitions

Adverse Event (AE)	Any untoward medical occurrence in a participant to whom a medicinal product has been administered, including occurrences which are not necessarily caused by or related to that product.
Adverse Event of Special Interest	An adverse event of special interest is one of scientific and medical concern specific to a product or trial, for which ongoing monitoring and rapid communication by the investigator to the safety committee or Sponsor may be appropriate.
Adverse Reaction (AR)	An untoward and unintended response in a participant to an investigational medicinal product which is related to any dose administered to that participant. The phrase "response to an investigational medicinal product" means that a causal relationship between the IMP and an AE is at least a reasonable possibility, i.e. the relationship cannot be ruled out. All cases judged by either the reporting medically qualified professional or the Sponsor as having a reasonable suspected causal relationship to the trial medication qualify as adverse reactions.
Serious Adverse Event (SAE)	A serious adverse event (SAE) is any untoward medical occurrence that: • results in death • is life-threatening (i.e. the participant was, in the view of the investigator, at immediate risk of death from the event that occurred). This does not include an AE that, if it occurred in a more serious form, might have caused death. • requires inpatient hospitalisation or prolongation of existing hospitalisation. Hospitalisation (including inpatient or outpatient hospitalisation for an elective procedure) for a pre-existing condition that has not worsened unexpectedly does not constitute a serious AE. • results in persistent or significant disability/incapacity • congenital anomaly or birth defect • an 'important medical event' (that may not cause death, be life threatening or require hospitalisation) may also be considered a serious adverse event when, based upon appropriate medical judgement, the event may jeopardise the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.
Serious Adverse Reaction (SAR)	An adverse event that is both serious and, in the opinion of the reporting Investigator, believed to be possibly, probably or definitely

	due to an IMP or any of the trial procedures, based on the information provided.
Suspected Unexpected Serious Adverse Reaction (SUSAR)	A serious adverse reaction, the nature and severity of which is not consistent with the Reference Safety Information for the medicinal product in question set out: • in the case of a product with a marketing authorisation, in the approved summary of product characteristics (SmPC) for that product • in the case of any other investigational medicinal product, in the approved investigator's brochure (IB) relating to the trial in question.

NB: to avoid confusion or misunderstanding of the difference between the terms "serious" and "severe", the following note of clarification is provided: "Severe" is often used to describe intensity of a specific event, which may be of relatively minor medical significance. "Seriousness" is the regulatory definition supplied above. See Appendix A: GRADING THE SEVERITY OF SOLICITED AND UNSOLICITED SYSTEMIC ADVERSE EVENTS

Any pregnancy occurring during the clinical trial and the outcome of the pregnancy should be recorded and followed up for congenital abnormality or birth defect, at which point it would fall within the definition of "serious".

11.4. Reporting procedures for all Aes

We will record all Aes occurring from first vaccination until 28 days after challenge that are observed by the Investigator or reported by the participant. All SAEs will be recorded from time of consent.

Aes will be recorded in either:

- the e-diary
- the eCRF

At the day 90 visit participants will be asked if they have had any Aes that have required medical attention (contact with GP, visit to emergency department) since their last visit. These will be recorded in the eCRF. After the day 90 visit only SAEs will be collected. Serious adverse events will be collected from consent until Day 365.

All Aes that result in a participant's withdrawal from the study will, subject to participant consent, be followed up, where possible until a satisfactory resolution occurs, or until a non-study related causality is assigned.

3.16.6. E-diary Aes

Solicited adverse events

Solicited adverse events will be recorded by the participant in an electronic diary and graded by the participant alone (appendix A).

Participants will be asked to complete an electronic diary, during the vaccination period from the time of each vaccine administration for 7 days post-vaccination (i.e. day 0 to day 6).

Participants will be asked to complete an electronic diary, during the challenge period from the time of challenge administration for 21 days post challenge (i.e. day 0 to day 20).

Solicited adverse events will be reviewed daily during the periods of recording as detailed above by the clinical study team. If the clinical team have concerns about the severity or frequency of an event this will be followed up with the participant by phone or at a scheduled visit. All ≥ grade 3 solicited adverse events recorded in the vaccination diary will be followed up with the participant by the clinical team in order to monitor for possible stopping rules (see 11.14 and 11.15).

Unsolicited adverse events

These may be recorded by the participant in an electronic diary from the time of first vaccine administration until day 28 post challenge.

Unsolicited adverse events will be reviewed at clinic visits. If clarification of any event is required then the study nurse or doctor will seek this from the participant during a clinical visit or by telephone call. Unsolicited adverse events recorded in the e-diary will be severity graded by the participant as per appendix A. Causality will also be assigned as per section 11.5.

11.4.2. Vital sign related Aes

At all visits vital signs are taken. These will be recorded directly into the eCRF at the time of review and severity grading will be automatically assigned as per Appendix B. In the event of an abnormal reading, the measurement should be carried out again after a further 5 minutes and the second result will be recorded in the data field of the eCRF and an annotation discrepancy note added to the initial result, action taken and any external influence. Where a moderate or severe (grade 2 or 3) AE is identified a clinician should review the participant in clinic and document the clinical assessment carried out. Changes in vital signs that are deemed clinically significant by a CI-delegated clinician will be causality assessed.

For analysis purposes, an isolated raised systolic or diastolic blood pressure will not be considered an adverse event unless persistent on three or more consecutive occasions or equivalent to a grade 2 or grade 3 adverse event after an adequate period of rest.

11.4.3. Visit elicited Aes

Additionally, at visits occurring from day -42 to day 28 participants will be asked about the occurrence of Aes and if any are elicited that have not already been recorded they will be recorded. For the visit at day 90, any Aes have required medical attention (contact with GP, visit to emergency department) since their last visit will be recorded in the eCRF.

11.4.4. Laboratory Aes

All laboratory tests will be recorded onto a results eCRF and automatically graded (Appendix C). If a test is deemed clinically significant, it may be repeated, to ensure it is not a single occurrence. If a test remains clinically significant, the participant will be informed and advised with regards appropriate medical care. Laboratory results can be out of normal range for a number of reasons other than physiological disturbance (eg hot weather, delayed transit to processing laboratory). If judged to be clinically significant these will undergo causality assessment.

There will be separate CRFs which will be visible to different members of the team depending on their role, their need to access the data within a particular CRF and whether they are blinded or unblinded:

<u>Blinded safety CRFs:</u>

Both blinded and unblinded clinical study team will have access to the data contained therein which will include

- all trial safety blood results
- blood cultures results
- stool culture results taken from day 1 after challenge until the conclusion of the study

<u>Unblinded CRFs:</u>

This is to maintain the blind (any result showing shedding of vaccine strain in stool will unblind clinical team members as this would only occur in vaccinees). Only the unblinded study team will have access to the data contained therein which will be:

- Stool cultures results from samples taken during the vaccination period of the study until the day of challenge
- Randomisation and vaccination allocation

11.4.5. Notes on recording Aes

Pre-existing medical conditions (present prior to enrolment into the study) are considered "concurrent medical conditions" and should not be recorded as Aes. However, if the participant experiences a worsening or complication of the condition, the worsening or complication should be recorded as an AE. Study staff will ensure that the AE term recorded captures the change in the condition (e.g., "worsening of").

Non-serious Aes considered related to the trial vaccine or other study procedures as judged by a medically qualified investigator or the Sponsor will be followed up either until resolution, or the event is considered stable.

11.5. Causality assessment

All solicited Aes recorded and graded by the participant will automatically assumed to be related to the vaccine and therefore will not be formally causality assessed. Diaries will be reviewed daily by clinicians.

For every unsolicited AE during the vaccination period (day -42 to day 0), the CI-delegated clinician will make an assessment of the causal relationship of the intervention (vaccine administration). This assessment will be based on the type of AE, the temporal relationship of the AE to the intervention, and the known biology of the vaccine (Table 9). Alternative causes of the AE, such as the natural history of pre-existing medical conditions, concomitant therapy, other risk factors and the temporal relationship of the event to vaccination will be considered and investigated.

Causality assessment will take place ahead of planned safety reviews and interim analyses (e.g. if a holding or stopping rule is activated).

All unsolicited Aes during the challenge period will undergo causality assessment in relation to the IMPs, challenge agent and non-IMPs. It is acknowledged that causality assessment is confounded during the challenge period (day 0 to day 20) by the administration of challenge agent and antibiotics to all participants.

Medically attended non serious Aes occurring between day 28 and day 90 elicited at the day 90 visit will not receive a causality assessment.

SAEs will receive a causality assessment throughout the study, at the time of reporting.

Not related	• No temporal relationship to vaccine administration or *S*. Paratyphi A ingestion or other study procedure, ***and*** • Alternative aetiology (clinical, environmental or other intervention), ***and*** • Does not follow pattern of 91ecognized response to vaccine administration or paratyphoid infection or other study procedure.
Possible	• Reasonable temporal relationship to vaccine administration or *S*. Paratyphi A ingestion or other study procedure, ***or*** • Event not readily explained by alternative aetiology (clinical, environmental or other interventions), ***or*** • Similar pattern of response to that seen to vaccine administration or paratyphoid infection or other study procedure.
Probable	• Reasonable temporal relationship to vaccine administration or *S*. Paratyphi A ingestion or other study procedure, ***and*** • Event not readily produced by alternative aetiology (clinical, environment, or other interventions), ***or*** • Known pattern of response with vaccine administration or paratyphoid infection or other study procedure.
Definite	• Reasonable temporal relationship to vaccine administration or *S.* Paratyphi A ingestion or other study procedure, ***and*** • Event not readily produced by alternative aetiology (clinical, environment, or other interventions), ***and*** • Known pattern of response to vaccine administration or paratyphoid infection or other study procedure.

Table 9: Guidelines for assessing the relationship of study procedure to an AE

11.6. Reporting procedures for SAEs

SAEs will be collected throughout the entire trial period (from consent to D365 or withdrawal). These will be recorded in the SAE section of the eCRF which will include fields for causality and expectedness.

All serious adverse events (SAE) will be recorded on the OVG SAE reporting form and reported by email to the named clinician for safety reporting and DSMC Chair within 24 hours of discovery or notification of the event. Additional information received for a case (follow-up or corrections to the original case)

will be detailed on a new SAE form and emailed to the named clinician for safety reporting and DSMC Chair, as above.

The chair of the DSMC will perform an independent review of SAEs and request any further information required in a manner adherent to the procedures and timelines of the DSMC Charter. Documentation of this review will be kept in the blinded TMF. There are no serious events exempt from immediate reporting as SAEs.

11.7. Expectedness

For SAEs that require reporting, expectedness of SARs will be determined according to the relevant RSI section of the Investigators' Brochure or SmPC for licensed products by the delegated clinician for SUSAR reporting. The RSI used (within the IB or SmPC) will be the current Sponsor and MHRA approved version at the time of the event occurrence. All SAEs at least possibly related to CVD 1902 will be considered unexpected and be reported to the MHRA and REC as SUSARs within the regulatory timelines, as in section 11.9. For assessment of expectedness in the Development Safety Update Report, see section 11.12 below.

11.8. Foreseeable medical occurrences

- Adverse reactions to CVD 1902: fever >37.5°C, diarrhoea, anorexia, malaise, abdominal cramps, headache, nausea, vomiting.
- Clinical Paratyphoid infection (between days 4 to day 14 or PD+96):
    - Symptoms: fever >37.5°C, nausea, vomiting, diarrhoea, anorexia, malaise, abdominal pain, headache, constipation, rash, myalgia, arthralgia, cough.
    - Laboratory results: Grade 1-3: raised ALT, raised ALP, raised CRP, decreased platelets
- Adverse reactions to ciprofloxacin, azithromycin, co-trimoxazole, amoxicillin, paracetamol, cyclizine, chlorpheniramine, codeine phosphate, senna, Sando-K (as per relevant SmPC)

11.9. Reporting Procedures for SUSARs

All SUSARs (events considered possibly, probably or definitely related to the IMP) will be reported to the Sponsor, MHRA and to the REC and other parties as applicable. For fatal and life-threatening SUSARS, this will be done no later than 7 calendar days after the Sponsor or delegate is first aware of the reaction. Any additional relevant information will be reported within 8 calendar days of the initial

report. All other SUSARs will be reported within 15 calendar days. Principal Investigators will be informed of all SUSARs for the relevant IMP for all studies with the same Sponsor, whether or not the event occurred in the current trial.

11.10. Reporting procedure for serious unforeseen medical occurrences related to a trial procedure or non-IMP

Challenge agent

Unforeseen serious adverse reactions which are related not to an IMP but to a challenge agent cannot be reported as SUSARs. However they will be assessed to determine if any actions are needed (such as urgent safety measures or requests for substantial amendments etc). Depending on the nature of such actions taken, the competent authorities will be contacted in compliance with current legislation.

Non-IMPs

The study team will report any serious adverse reactions not in keeping with the SmPCs for the licenced non-IMPs used in the trial (ciprofloxacin, azithromycin, co-trimoxazole, paracetamol, cyclizine, chlorpheniramine, codeine phosphate, senna, Sando-K) to the MHRA using the electronic 'Yellow Card' System and this will be recorded in TMF.

11.11. Adverse Events of Special Interest (AESI)

An adverse event of special interest is one of scientific and medical concern specific to a product or trial, for which ongoing monitoring and rapid communication by the investigator to the safety committee or Sponsor may be appropriate.

Due to the additional study procedures the following events will be considered AESIs.

- Severe paratyphoid infection (as defined in section 9.9.2.).
- Failure to clinically or bacteriologically cure a participant of paratyphoid infection
- Progression to chronic carrier state
- Relapse of paratyphoid infection
- Transmission of *S*. Paratyphi A to a contact of a participant.
- Aes requiring a physician visit or Emergency Department visit which, in the opinion of study staff, are related to the challenge with *S*. Paratyphi A
- Pregnancy

The possible adverse effects of *S.* Paratyphi A infection or the effect of some antibiotics on the outcome of pregnancy are unknown.[73,74]. Therefore, pregnant women will be excluded by history and laboratory tests, and female participants will be specifically instructed to prevent conception during the vaccination and challenge periods of the study until completion of antibiotic therapy and clearance of paratyphoid infection is confirmed. Highly-sensitive pregnancy tests will be performed on female participants prior to each vaccination, challenge and antibiotic commencement. Should pregnancy occur, information about outcome of the pregnancy will be sought.

AESI will be reported to the CI and DSMC as soon as possible but within 7 days of discovery. If an AESI meets the criteria for SAE (eg hospitalisation with severe paratyphoid infection) then this will be reported as an SAE on the SAE reporting form as per section 11.4. and additionally as an AESI in the AE eCRF on REDCap.

11.12. Development safety update reports

A development safety update report (DSUR) for the IMP will be prepared annually, on the anniversary of the MHRA approval for the trial. This will be submitted by clinician with safety reporting responsibility to the Sponsor, competent authorities, and ethical committee(s).

For assessment of SARs in the DSUR, the RSI that was approved at **the start of the safety reporting period** will be used. When there has been approved changes to the RSI by substantial amendment during the reporting period, the RSI used for the DSUR will differ to the RSI used to assess expectedness at the time of SAR occurrence for SARs which require expedited reporting.

11.13. Trial committees

11.13.1.Trial Management committee

The trial investigators will form the trial management committee and will provide frequent management oversight of the trial.

11.13.2. Data and Safety Monitoring Committee

A Data and Safety Monitoring Committee (DSMC) will be appointed to provide real-time oversight of safety and trial conduct.

The DSMC is independent and will review all safety data throughout the study according to the DSMC Charter. The DSMC will have access to data and, if required, will monitor these data and make recommendations to the study investigators on whether there are any ethical or safety reasons why

the trial should not continue. They will particularly review the safety and stool shedding data in the vaccination group. They will also monitor the attack rate in the vaccine and placebo groups to confirm the challenge model is proceeding as expected.

The DSMC will provide guidance for any unblinding decision(s) required during the trial.

A summary of all AESIs and SAEs to date will be provided to the DSMC on request. The DSMC will also be notified if the study team have any concerns regarding the safety of a participant or the general public (e.g. if a participant is not contactable after *S*. Paratyphi A challenge and potentially infectious to others).

The outcome of each DSMC review will be communicated directly to the study investigators and documentation of all reviews will be kept in the relevant blinded or unblinded TMF. The Chair of the DSMC will also be contacted for advice when the CI feels independent advice or review is required. Reports for the DSMC will be prepared from these databases by the appropriate team (with respect to the blind).

11.14. Safety holding Rules

In the event of any of the following, vaccination of further individuals will be paused pending DSMC review:

- New scientific information is published to indicate that subjects in the trial are being exposed to undue risks as a result of administration of the IMP, or as a result of the trial procedures or follow-up schedule.
- Bacteraemia with *Salmonella* Paratyphi A of any participant during the vaccination period
- Serious concerns about the safety of the IMP arise as a result of one or more vaccine related SAE(s) occurring in the subjects enrolled
- If at least two subjects develop a 'severe' adverse event related to the study drug, as assessed by a clinician, independent of within or not within the same-organ-class.

The DSMC chair will then undertake a review of the data to decide whether a temporary halt is required as an urgent safety measure and what the scope of the halt will be. A full meeting of the DSMC can be called at the chair's discretion. If, following DSMC review, a halt is required, the Sponsor, REC and appropriate regulatory authorities will be notified within 3 days and a substantial amendment submitted within 15 days. If the DSMC decide that a halt is not required then the trial may continue.

Following a halt to the trial, if it is decided that the trial may re-start, then a request and substantial amendment will be made to the Sponsor, the REC and the MHRA in order to do so, otherwise the trial will be terminated.

11.15. Individual stopping Rules

Stopping rules for individual volunteers will apply (i.e., indications to withdraw individuals from further vaccinations). Study participants who present with at least one of the following stopping rules will trigger a clinical review as to whether the participant should be withdrawn from further vaccination in the study, would not undergo challenge and would be followed up only from a safety perspective if participants consent to this.

- Laboratory Aes: the participant develops a confirmed $\geq$ grade 3 laboratory AE considered possibly, probably or definitely related within 7 days after vaccination
- Solicited adverse events: the participant develops a $\geq$ grade 3 systemic solicited AE considered possibly, probably or definitely related within 2 days after vaccination (day of vaccination and one subsequent day) which is deemed severe by clinician assessment
- Unsolicited adverse events: the participant has a $\geq$ grade 3 adverse event, considered possibly, probably or definitely related to vaccination which is deemed severe by clinician assessment or has a SAE considered possibly, probably or definitely related to vaccination.
- The participant has an acute allergic reaction or anaphylactic shock following the administration of the vaccine investigational product.

11.16. Other safety reviews

As an additional safety measure, a sentinel group of six participants (for approximately three participants randomised to vaccination) will undergo vaccination. Following their first vaccination, relevant safety data will be reviewed by the DSMC. If there are no safety concerns these participants can proceed to their second vaccination. Recruitment will continue but no further participants will be vaccinated until this safety review has taken place. Further review of safety data will take place as outlined in the DSMC charter.

As part of the safety review, the DSMC will confirm that none of the 'Safety holding Rules' as detailed in section 11.14 have been fulfilled. If the stopping rules are not fulfilled, and there are no safety concerns, the trial will continue as planned, but if the stopping rules are triggered then dosing will be halted and the procedures detailed in section 11.14 will be followed.

In addition to formal DSMC review, there will also be local safety monitoring reviews. The unblinded team will regularly review the stool shedding and other safety data. They will liaise with the DSMC regarding these results.

12. STATISTICS

12.1. Statistical Analysis Plan (SAP)

A statistical analysis will be produced for this study. The statistical methods for the study are outlined in this section.

12.2. Description of Statistical Methods

The primary objective of this study is to determine the relative protective effect of CVD 1902 vaccine compared to a placebo group, using a healthy adult *S.* Paratyphi A challenge model. The null and alternate hypotheses are:

H_0 : Attack $Rate_{CVD\ 1902}$ = Attack $Rate_{placebo}$

H_1 : Attack $Rate_{CVD\ 1902}$ ≠ Attack $Rate_{placebo}$

Where Attack $Rate_{placebo/CVD\ 1902}$ the proportion of participants given a diagnosis of Paratyphoid infection (see section 9.11.1, Table 4 for definition) who have been vaccinated with placebo vaccine or CVD 1902, respectively.

Time-to-event endpoints 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 log-transformed prior to 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.

12.3. The Number of Participants

Based on findings from the previous challenge study performed at OVG[65], the assumption of attack rate of 65% was used in the control group. To demonstrate a protective effect of 60%, resulting in a reduction in attack rate from 65% to 26%, 30 participants would be needed per group to archive 80% power (1-β) at two-sided 5% significance level (α).

We observed less than a 10% of dropout rate from previous vaccine trial using typhoid challenge model[13]. As this study will use a two-dose vaccine schedule with a longer period between randomisation and challenge compared with previous studies, we would expect the dropout rate of 10%-20%. The sample size will be inflated to 33-38 participants per group to account for 10%-20% dropout. We expect to randomise 66-76 participants in total.

12.4. Populations for analysis

As the participants to be recruited are healthy adult volunteers and the primary objective is to establish the absolute protection afforded by the IMP vaccine (CVD 1902), the Per Protocol (PP) population will be used for evaluation of the primary endpoint. For the current study, the PP population is defined as,

All participants who:

- Have received two doses of the allocated study vaccine (or the actual vaccine received in case of randomisation error), and
- Have been successfully challenged with the challenge organism
- Have received no bias or interference that may interfere with potential vaccine effect or infection challenge, either according to the protocol or in the view of the study investigators.

Description of the final population to be analysed for the primary endpoint will be reported in accordance with the CONsolidated Standards of Reporting Trials (CONSORT) Statement.

If a participant later withdraws from the study, data up until that point will be included in the analysis. If participants are withdrawn before it is determined whether or not they develop paratyphoid within the specified 14-day period after being challenged, then sensitivity analyses will be undertaken to explore different assumptions for the missing data.

Secondary endpoints will be analysed in the following populations:

- **Post-challenge clinical and laboratory features** – vaccinated and challenged population (those having taken the *S.* Paratyphi A challenge agent in the required manner) providing post-challenge symptom data and at least one evaluable clinical specimen post-challenge according to protocol, without a major violation (a protocol violation considered by the investigators to have an impact (quantitative or qualitative)) which may have an effect on symptom reporting/laboratory testing.
- **Post-vaccination immune responses** – vaccinated population providing at least one evaluable post-vaccination clinical specimen, without a major violation (a protocol violation considered by the investigators to have an impact (quantitative or qualitative)) which may have an effect on the immunological response (per protocol population).
- **Safety and tolerability of CVD 1902** – vaccinated population (those having actually received at least one dose of the allocated vaccine or placebo) providing post-vaccination symptom data/at least one evaluable faecal sample, without a major violation (a protocol violation considered by the investigators to have an impact (quantitative or qualitative)) which may have an effect on symptom reporting and providing faecal sample.

- **Post-challenge immune correlation** – vaccinated (have received two doses of the allocated vaccine or placebo) and challenged population supplying at least one evaluable clinical specimen post-challenge, without a major violation (a protocol violation considered by the investigators to have an impact (quantitative or qualitative)) which may influence the immunological response (per protocol population).

The laboratory analysis of some parameters may be terminated before samples from all time points have been analysed if it is felt that further analysis is not of scientific value. This is likely to be if it has already been demonstrated that parameters for any endpoint have returned to baseline. The decision to not process latter time points will be at the discretion of the Chief Investigator. Further exploratory analysis may be conducted if findings of scientific interest become apparent during the study or processing of the data.

12.5. Analysis of demographic and baseline characteristics

Descriptive statistics relating to participant characteristics at baseline will be calculated overall and by group. No formal statistical comparisons of baseline characteristics between randomised groups will be conducted.

12.6. Analysis of the primary endpoint

The proportion of participants with a diagnosis of Paratyphoid fever (i.e. the attack rate) and the associated 95% confidence intervals will be presented by group at Day 28 after challenge. When calculating paratyphoid diagnosis proportions, the numerator will be the number of participants who meet the criteria for diagnosis and the denominator will be the per protocol population defined in 12.4**.** The difference in proportions between the CVD 1902 and placebo groups will be analysed using Pearson's chi-squared test (or Fisher's Exact test if expected counts in any group are less than 5). To fulfil the primary objective, the protective effect of CVD 1902 over placebo will be calculated by:

$$PE = 100 \times (AR_{Placebo} - AR_{CVD\ 1902})/AR_{Placebo} = 100 \times (1 - AR_{CVD\ 1902}/\ AR_{Placebo}),$$

Where PE is the protective effect and AR is the attack rate.

The 95%CI of the $AR_{CVD\ 1902}/\ AR_{Placebo}$ can be calculated using standard methods for calculation of 95%CI on a rate ratio. The corresponding 95%CI for PE will also be calculated.

A secondary analysis of the primary endpoint will be conducted using the Kaplan-Meier method which will include all participants. Participants who withdrew or had potential interference with vaccine effect or infection challenge, (e.g. treated prior to Day 14 with no diagnosis of paratyphoid) will be censored in the

analysis at the time of withdrawal or interference. Non-diagnosed participants will also be censored in the analysis at the time point of final monitoring of blood culture or temperature (Day 28).

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

The time variable in time-to-diagnosis analyses will be the time at which a blood culture positive blood sample was taken rather than the time at which it was recognised to be positive. Clinical diagnosis times will be the time at which a temperature first exceeded 38.0°C which subsequently lasted at least 12 hours.

12.7. The Level of Statistical Significance

P values lower than 0.05 will be considered statistically significant.

12.8. Criteria for the Termination of the Trial

The CI, with the DSMC will have the right to terminate the study at any time on grounds of participant safety. If the study is prematurely terminated the clinical study team will promptly inform the participants and will ensure appropriate therapy and follow-up.

If the study is terminated, the Sponsor, Oxford University Hospitals NHS Foundation Trust, MHRA and relevant Ethics Committee will be notified within 15 days of this occurring.

12.9. Procedure for Accounting for Missing, Unused, and Spurious Data.

Reasons for missing data (including withdrawal of consent, loss to follow-up, removal from study due to serious side effects, death, or inability to obtain any laboratory results) will be indicated, but missing data will not be imputed. The quantity of missing data for the vaccine and placebo groups and the appertaining demographic characteristics will be compared. There will be an intention to publish all collected data, or at least open clarification about which additional variables have been measured if reporting is ultimately selective, so that readers can self-determine the possible impact of "data dredging", i.e. selective reporting of seemingly interesting results.

12.10. Procedures for Reporting any Deviation(s) from the Original Statistical Plan

A final statistical analysis plan (SAP) will be signed off before the final database lock. Any additional analysis or deviations from the SAP will be documented in the final analysis report and updated according to the statistical standard operating procedure.

13. DATA MANAGEMENT

The data management aspects of the study are summarised here with details fully described in the Data Management Plan.

13.1. Source Data

Source documents are original documents, data, and records from which some participants' electronic data where the data is first recorded. These include, but are not limited to, hospital records (from which medical history and previous and concurrent medication may be summarised into the electronic data capture database), clinical and office charts, laboratory and pharmacy records, diaries, microfiches, radiographs, and correspondence. In this study, electronic data entries will be considered source data when it is the site of the original recording. All documents will be stored safely under strict confidentiality and with restricted access. The participant will be referred to by the study participant number/code on study-specific documents, other than the signed consent forms, participant contact sheet and information for GPs and UKHSA. Participant details populated from the electronic database are kept in the form of an electronic participant and screening log located on a password protected OVG network drive.

13.2. Access to Data

Direct access will be granted to authorised representatives from the Sponsor and host institution and the regulatory authorities to permit trial-related monitoring, audits and inspections.

13.3. Data Recording and Record Keeping

The investigators will populate the content of participants' CRFs and all the study data will be recorded directly into an Electronic Data Capture (EDC) system (e.g. OpenClinica, REDCap, or similar) or onto a paper source document for later entry into EDC if direct entry is not available. Any additional information that needs recording but is not relevant for the CRF (such as signed consent forms etc.) will be recorded on a separate paper source document. All documents will be stored safely and securely in confidential conditions.

The EDC system (CRF data) uses a relational database (MySQL/ PostgreSQL) via a secure web interface with data checks applied during data entry to ensure data quality. The database includes a complete suite of features which are compliant with GCP, EU and UK regulations and Sponsor security policies, including a full audit trail, user-based privileges, and integration with the institutional LDAP server. The MySQL and PostgreSQL database and the webserver will both be housed on secure servers maintained by Oxford Vaccine Group IT personal and local site IT personal. The servers are in a physically secure location in EU and data are backed up on secure servers operated by the University of Oxford IT Services physically located in EU zone. Backups will be stored in accordance with the IT department schedule of daily, weekly,

and monthly retained for one month, three months, and six months, respectively. The IT servers provide a stable, secure, well-maintained, and high capacity data storage environment. REDCap and OpenClinica are widely-used, powerful, reliable, well-supported systems. Access to the study's database will be restricted to the members of the study team by username and password.

Participant's personally identifiable information will be stored in a separate password protected Access databased saved on a secure University of Oxford server. Only Oxford staff have access to the Access database and are permitted for data entry.

Each study participant will have a unique participant number which will be allocated at the time a screening visit is booked and all names and/or identifying details are not included in any study data electronic file. After enrolment the participants will be identified by a study specific participants number and/or code. Samples sent to laboratories for processing will be identified by trial number and participant number only. To avoid the stool samples taken during the vaccination period unblinding the blinded clinical team stool samples during the vaccination period will have an additional participant number, separate to their main study number. This will allow stool culture results to be accessed by the unblinded team and not be inadvertently seen by the blinded team. The team statistician will retain the lists linking the participant numbers (participant number, stool participant number, laboratory number). Samples sent to laboratories for processing will be identified by, laboratory number and participant initials.

The study team will use names and contact details to contact participants about the research study, and make sure that relevant information about the study is recorded for their care, in relation to their health during the study and to oversee the quality of the study. At the completion of the study, unless participants consent otherwise (e.g. requesting to be informed of other trials), participant's personal details will not be used to contact them other than exceptional circumstances concerning their safety. If consent is provided by participants to take part in another study carried out by the study site, personal information and medical information including blood test results may be accessed to avoid unnecessary repetition. If participants provide specific consent, we will use personal identifiable data to invite participants for future research.

Bank details will be stored for 7 years in line with University financial policy.

13.3.1 Data integrity

Data collection and storage will be inspected throughout the study by performed by the Oxford Vaccine Group and monitoring will be carried out by the study Sponsor, University of Oxford Research Governance, Ethics and Assurance (RGEA).

13.3.2 Data archiving and storage

Study data may be stored electronically on a secure server, and paper notes will be kept in a key-locked

filing cabinet at the site. All essential documents will be retained for a minimum of 5 years after the study has finished. The need to store study data for longer in relation to licensing of the vaccine will be subject to ongoing review. For effective vaccines that may be licensed, we may store research data securely at the site at least 15 years after the end of the study, subject to adjustments in clinical trials regulations. Participants' bank details will be stored for 7 years in line with the site financial policy. De-identified research data maybe be stored indefinitely. General archiving procedures will be conducted in compliance to SOP OVC020 Archiving.

14. QUALITY ASSURANCE PROCEDURES

14.1. Risk assessment

The trial will be conducted in accordance with the current approved protocol, GCP, relevant regulations and standard operating procedures. Approved and relevant Standard Operating Procedures (SOPs) and Laboratory and Clinical Study Plans will be used at all clinical and laboratory sites. A risk assessment and monitoring plan will be prepared before the study opens and will be reviewed as necessary over the course of the trial to reflect significant changes to the protocol or outcomes of monitoring activities.

14.2. Monitoring

Monitoring will be performed according to Good Clinical Practice (GCP) by the RGEA or parties appointed by the Sponsor. Following written SOPs, the monitors will verify that the clinical trial is conducted, and data are generated, documented and reported in compliance with the protocol, GCP and the applicable regulatory requirements. The investigator site will provide direct access to all trial related source data/documents and reports for the purpose of monitoring and auditing by the Sponsor and inspection by local and regulatory authorities.

Monitoring by the DSMC is addressed in section 11.13.2 The unblinded study team will regularly review the stool shedding safety data during the vaccination phase and provide regular reports to the DSMC.

14.2.1. Direct access to source data/documents

Direct access will be granted to authorised representatives from the Sponsor, host institution and the regulatory authorities to permit trial-related monitoring, audits and inspections.

14.3. Audit and Inspection

The Quality Assurance manager operates an internal audit program to ensure that the systems used to conduct clinical research are present, functional, and enable research to be conducted in accordance with study protocols and regulatory requirements. Audits include laboratory activities covering sample receipt,

processing and storage and assay validation. The internal audits will supplement the external monitoring process and will review processes not covered by the external monitor.

The Sponsor may carry out audits to ensure compliance with the protocol, GCP and appropriate regulations. GCP inspections may also be undertaken by the MHRA to ensure compliance with the protocol and the Medicines for Human Use (Clinical Trials) Regulations 2004 and amendments.

14.4. Procedure to be followed in the event of an abnormal finding

Abnormal clinical findings from medical history, examination or blood tests, will be assessed as to their clinical significance using the severity grading criteria for Adverse Events tables (see Appendix A, B, C). If a test result is deemed clinically significant, it may be repeated, to ensure it is not a single occurrence. If a test remains clinically significant, the participant will be informed, and appropriate medical care will be arranged with the permission of the participant. Decisions to exclude potential participants from enrolling in the trial or to withdraw a participant from the trial will be at the discretion of the study team.

14.5. Staff and Investigator safety

All staff working on the project will be required to follow strict infection control techniques as outlined in local OVG SOPs. All staff members working at the CCVTM will be informed of the commencement of the challenge study.

14.6. Protocol deviations

A trial related deviation is a departure from the ethically approved trial protocol or other trial document or process (e.g. consent process or IMP administration) or from Good Clinical Practice (GCP) or any applicable regulatory requirements. Any deviations from the protocol will be documented in a protocol deviation form and filed in the trial master file.

15. SERIOUS BREACHES

The Medicines for Human Use (Clinical Trials) Regulations contain a requirement for the notification of "serious breaches" to the MHRA within 7 days of the Sponsor becoming aware of the breach.

A serious breach is defined as "A breach of GCP or the trial protocol which is likely to affect to a significant degree –

(a) the safety or physical or mental integrity of the subjects of the trial; or

(b) the scientific value of the trial".

If a serious breach is suspected the Sponsor must be contacted within 1 working day. In collaboration with the CI the serious breach will be reviewed by the Sponsor and, if appropriate, the Sponsor will report it to the REC committee, Regulatory authority and the relevant NHS host organisation within seven calendar days.

16. ETHICAL AND REGULATORY CONSIDERATIONS

16.1. Declaration of Helsinki

The Investigator will ensure that this trial is conducted in accordance with the principles of the Declaration of Helsinki.

16.2. Guidelines for Good Clinical Practice

The Investigator will ensure that this trial is conducted in accordance with relevant regulations and with Good Clinical Practice.

16.3. Approvals

Following Sponsor approval, the protocol, informed consent form, participant information sheet and any proposed advertising material will be submitted to an appropriate Research Ethics Committee (REC), HRA (where required), regulatory authorities (MHRA in the UK), and host institution(s) for written approval.

The Investigator will submit and, where necessary, obtain approval from the above parties for all substantial amendments to the original approved documents.

16.4. Reporting

The CI shall submit once a year throughout the clinical trial, or on request, an Annual Progress Report to the REC, host organisation, and Sponsor. In addition, an End of Trial notification and final report will be submitted to the MHRA, the REC, host organisation and Sponsor.

16.5. Transparency in Research

Prior to the recruitment of the first participant, the trial will have been registered on a publicly accessible database.

Results will be uploaded to the European Clinical Trial (EudraCT) Database within 12 months of the end of trial declaration by the CI or their delegate.

Where the trial has been registered on multiple public platforms, the trial information will be kept up to date during the trial, and the CI or their delegate will upload results to all those public registries within 12 months of the end of the trial declaration.

16.6. Participant Confidentiality

The study will comply with the UK General Data Protection Regulation (GDPR) and Data Protection Act 2018, which require data to be de-identified as soon as it is practical to do so. The trial staff will ensure that the participants' data is de-identifed other than for uses (e.g. notification to UKHSA and communication with the GP) about which the participants will be specifically consented for. Participants will be identified by initials and a participant ID number on the CRF. Any electronic databases and documents with participant identifying details will be stored securely and will only be accessible by study staff and authorised personnel.

16.7. Expenses and Benefits

All participants will be reimbursed for their time, travel and for inconvenience based on the following figures:

	Amount per visit	Max number of visits	Total
Travel expenses	£15	32	480
Inconvenience of blood test	£10	30	300
Time required for visit	£20	32	640
Travel to deliver stool sample (max 3)	£15	3	45
Time off work reimbursement (for challenged participants only)	£150 (per day)	£1500 for 10 days total (reimbursed to all challenged participants)	1500
Total			£2965

Participants will receive a total of £2965 if they remain in the study for the entire period (includes payment for screening). Payments will be made via bank transfer. Participants will be required to provide banking details including account name, sort code and account number. All personal banking details will be stored confidentially and retained by OVG while the participant is actively involved in the study and participants' bank details will be stored for 7 years by the University financial policy. Consent will be obtained prior to requesting and storing personal bank account details.

Participant payments will be requested at the following visits: Screening, Day 0, Day 14, Day 90, Day 180, and Day 365.

Due to the generous reimbursement for scheduled visits, participants will not be given extra reimbursement for unscheduled visits.

17. FINANCE AND INSURANCE

17.1. Funding

Funding for the study has been provided by the Medical Research Council.

17.2. Insurance

The University has a specialist insurance policy in place, which would operate in the event of any participant suffering harm as a result of their involvement in the research (Newline Underwriting Management Ltd, at Lloyd's of London).

17.3. Contractual arrangements

Appropriate contractual arrangements will be put in place with all third parties.

18. PUBLICATION POLICY

The Chief Investigator will co-ordinate dissemination of data from this study. All publications (e.g., manuscripts, abstracts, oral/slide presentations, book chapters) based on this study will be reviewed by each sub-investigator and by the Sponsor prior to submission. All communication or publications concerning the project, including at a conference or seminar, shall acknowledge the Parties and the Medical Research Council's contribution.

19. DEVELOPMENT OF A NEW PRODUCT/ PROCESS OR THE GENERATION OF INTELLECTUAL PROPERTY

Ownership of IP generated by employees of the University vests in the University. The University will ensure appropriate arrangements are in place as regards any new IP arising from the trial.

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21. APPENDIX A: Grading the severity of solicited and unsolicited systemic Adverse Events

Adverse event	Grade	Definition (in degrees Celsius)
Temperature	0	< 37.6
	1	37.6 – 38.0
	2	38.1 – 39.0
	3	> 39.0

Participant grading of severity (vaccine phase)

	0	1	2	3	4
Nausea	No symptoms	Present but no interference with activity	Some interference with activity	Significant; prevents daily activity	Emergency department visit or hospitalisation
Vomiting	No symptoms	Present but no interference with activity or 1 – 2 episodes in 24 hours	Some interference with activity or more than 2 episodes in 24 hours	Significant; prevents daily activity	Emergency department visit or hospitalisation
Diarrhoea	No symptoms	3-4 loose stools in 24 hrs	5-6 loose stools in 24 hrs	7 or more loose stools in 24 hrs	Emergency department visit or hospitalisation
Eating less than usual or loss of appetite	No symptoms	Eat less than normal for 1-2 meals	Miss 1-2 meals completely	Miss all meals	Emergency department or hospital visit required
Generally unwell	No symptoms	Present but no interference with activity	Some interference with activity	Significant; prevents daily activity	Emergency department visit or hospitalisation
Abdominal/ stomach pain	No symptoms	Present but no interference with activity	Some interference with activity	Significant; prevents daily activity	Emergency department visit or hospitalisation

Headache	No symptoms	Present but no interference with activity	Some interference with activity	Significant; prevents daily activity	Emergency department visit or hospitalisation

Participant grading of severity (challenge phase)

	0	1	2	3	4
Headache	No symptoms	Present but no interference with activity	Some interference with activity	Significant; any use of codeine phosphate or prevents daily activity	Emergency department visit or hospitalisation
Generally unwell	No symptoms	Present but no interference with activity	Some interference with activity	Significant; prevents daily activity	Emergency department visit or hospitalisation
Eating less than usual or loss of appetite	No symptoms	Eat less than normal for 1-2 meals	Miss 1-2 meals completely	Miss all meals	Emergency department or hospital visit required
Abdominal/ stomach pain	No symptoms	Present but no interference with activity	Some interference with activity	Significant; any use of codeine phosphate or prevents daily activity	Emergency department visit or hospitalisation
Nausea/vomiting	No symptoms	Present but no interference with activity or 1 – 2 episodes in 24 hours	Some interference with activity or more than 2 episodes in 24 hours	Significant; prevents daily activity	Emergency department visit or hospitalisation
Muscle pain	No symptoms	Present but no interference with activity	Some interference with activity	Significant; any use of codeine phosphate or prevents daily activity	Emergency department visit or hospitalisation
Joint pain	No symptoms	Present but no interference with activity	Some interference with activity	Significant; any use of codeine phosphate or prevents daily activity	Emergency department visit or hospitalisation
Cough	No symptoms	Present but no interference with activity	Some interference with activity	Significant; any use of codeine phosphate or prevents daily activity	Emergency department visit or hospitalisation

Diarrhoea	No symptoms	3-4 loose stools in 24 hrs	5-6 loose stools in 24 hrs	7 or more loose stools in 24 hrs	Emergency department visit or hospitalisation
Constipation	No symptoms	Present but no interference with activity or 1 – 2 episodes in 24 hours	Some interference with activity or more than 2 episodes in 24 hours	Significant; prevents daily activity	Emergency department visit or hospitalisation
Rash	No symptoms	Yes/No			

22. APPENDIX B: Grading the severity of visit observed Adverse Events

Observation	Grade 1	Grade 2	Grade 3	Grade 4
Oral temperature (°C)	37.6 – 38.0	38.1 – 39.0	> 39.0	A&E visit or hospitalisation for hyperpyrexia
Tachycardia (beats/min)	101-115	116-130	>130	A&E visit or hospitalisation for arrhythmia
Bradycardia (beats/min)	50-54	45-49	<45	A&E visit or hospitalisation for arrhythmia
Systolic hyper-tension (mmHg)	141-150	151-155	>155	A&E visit or hospitalization for malignant hypertension
Diastolic hyper-tension (mmHg)	91-95	96-100	>100	A&E visit or hospitalization for malignant hypertension
Systolic hypo-tension (mmHg)	85-89	80-84	<80	A&E visit or hospitalization for hypotensive shock

23. APPENDIX C: Grading the severity of laboratory Adverse Events

Parameter	Grade 1	Grade 2	Grade 3	Grade 4*
Haemoglobin: decrease from baseline value (g/l)	10 – 15	16-20	21-50	>50
White cell count: elevated (10^9/L)	11–15	16–20	21–25	>25
White cell count: depressed (10^9/L)	2.5-3.5	1.5-2.4	1.0-1.4	<1.0
Neutrophil count (10^9/L)	1.5-2.0	1.0-1.4	0.5-0.9	<0.5
Platelets (10^9/L)	125-140	100-124	25-99	<25
Sodium: hyponatraemia (mmol/L)	132–134	130–131	125–129	<125
Sodium: hypernatraemia (mmol/L)	146	147	148–150	>150
Potassium: hyperkalaemia (mmol/L)	5.1–5.2	5.3–5.4	5.5–5.6	>5.6
Potassium: hypokalaemia (mmol/L)	3.3–3.4	3.1–3.2	3.0	<3.0
Urea (mmol/L)	8.2–8.9	9.0–11	>11	RRT
Creatinine (µmol/L)	132-150	151-176	177-221	>221 or RRT
ALT and/or AST (IU/L)	1.1–2.5 x ULN	>2.6–5.0 x ULN	5.1-10 x ULN	>10 x ULN
Bilirubin, with increase in LFTs (µmol/L)	1.1–1.25 x ULN	1.26–1.5 x ULN	1.51–1.75 x ULN	>1.75 x ULN
Bilirubin, with normal LFTs (µmol/L)	1.1–1.5 x ULN	1.6–2.0 x ULN	2.1–3.0 x ULN	>3.0 x ULN
Alkaline phosphatase (IU/L)	1.1–2.0 x ULN	2.1–3.0 x ULN	3.1–10 x ULN	>10 x ULN
Albumin: hypoalbuminaemia (g/L)	28–31	25–27	<25	Not applicable
C-reactive protein	>10-30	31-100	101-200	>200

Grade 4* Potentially life threatening

25. APPENDIX D: AMENDMENT HISTORY

Amendment No.	Protocol Version No.	Date issued	Author(s) of changes	Details of Changes made
-	2.0	19th October 2021	Kate Emary Naina McCann	Section 5.2.5 Additional information regarding dosing added including maximum dose and dose range Section 5.2.6. Additional justification of comparison to placebo added Section 8.6 Definition of women of child-bearing potential added. Additional justification of use of effective methods of contraception added. Additional justification added for why barrier methods for male particiapnts are not required. Section 9.2 Wording clarified to explain participants may require repeated tests at enrollment. Setion 9.4 Clarified that highly-sensitive urine pregnancy test will be used. Section 9.8 Emergency unblinding procedure added. Section 9.17 Pregnancy listed as a discontinuation criterion. Section 11.4. Wording clarified. All Aes will be recorded. All Aes relating during vaccination period will be causality assessed. All SAEs will be collected from time of consent. Section 11.6. SAEs will be reported from time of consent. Section 11.14 Safety holding rule added: 'If at least two subjects develop a 'severe' adverse event related to the study drug, as assessed by a clinician, independent of within or not within the same-organ-class.' Section 11.15 Individual stopping rules amended to change > grade 3 to ¨> grade 3and 72 hour duration removed. Section 11.16 Other safety rules. Addition of a sentinel group of 6 for vaccination.

				Appendix A. Typographical error removed (loose stool volume definition). Appendix B, grade 4 events defined and added. Throughout document; vaccine interval changed to 14 days (rather than 10-14 days).
Minor amendment 1	2.1	30th November 2021	Naina McCann Nisha Singh	• Section 7.4 we have removed the table with specific volumes in Table 1a Summary of screening procdures and tests. There is no change in the volume of blood collected at the screening visit, but distribution may change between the different tubes, as required by the labs. Erythrocyte sedimentation rate (ESR) has also been added in the table. It was mentioned in Section 9.4 but mistakenly missed off the table. • Section 11.4 – The statement has been updated to clarify that SAEs will be collected from consent (and not enrolment) until day 365. This was missed at the time the protocol was updated to version 2.0. • Section 11.15 – Typo corrected: "Solicited adverse events: the participant develops a ≥ grade 3 systemic…." This was incorrectly written as ">" previously, and has been corrected to "≥". • Other typos have been corrected.
Substantial amendment 1	3.0	7th March 2022	Naina McCann Nisha Singh	• Section 11.14 Clarification of process following triggering of safety holding rules • Section 11.4.1 Clarification that all grade 3 and above solicited events will be followed up by clinical team to monitor for stopping rules • Appendix B error amended – grade 3 temperature defined as > 39 • Section 9.7 and 9.9.1 amended to reflect change that small unblinded nursing team will administer vaccine • Multiple updates regarding timepoint of notification of UKHSA is time of challenge not time of vaccine (Section 7.5, 9.8, 9.1, 9.9.1, 9.10.4, 9.12) • Section 9.19.1 sentence regarding trial only preceeding if COVID-19 infection rates low has been removed and clarifications that local and national guidance will be followed in regards to testing and isolation.

				• Section 11.16 sentence to state that the safety reviews will take place in accordance with the DSMC charter. • Section 11.4.1 amended to clarify e-diaries will be collected for 7 days post vaccine and 21 days post-challenge • Section 11.8 amended to add forseeable blood results following paratyphoid infection • Blood taken at timepoints D2 and D4 changed to 'up to 4 ml' (Section 7.8, Section 7.9, Section 11.1.4) • Section 7.6 – Window period added for D180 visit • D14 PD visit procedures and window period clarified (Section 7.5, 7.6, 9.11) • Section 9.1 – Clarification added regarding use of NHS vaccine registers of GP databases • Section 10.2 numbering errors amended • Section 11.1 AESI reporting form removed. AESIs will be reported in the AE eCRF • Appendix C – Hb grade 1 changed from < 15 to 10-15 • PHE changed to UKHSA throughout • CTRG changed to RGEA in the "Trial Key Contacts" table
Minor amendment 2	3.1	11th April 2022	Nisha Singh Hanna Robinson	• Amending the inclusion criteria (Section 8.2) to remove 'Thames Valley Area' and clarify that participants will be included if they are willing to be available in Oxford for appointments • Amending the UKHSA notification section (9.12) to say 'relevant local Helath Protection Unit' will be informed removing reference to Thames Valley