Department for Environment Food and Rural Affairs

Application for consent to release a GMO – organisms other than higher plants

Part A1: Information required under schedule 2 of the Genetically Modified Organisms (Deliberate Release) Regulations 2002

Part I: General information

1. The name and address of the applicant and the name, qualifications and experience of the scientist and of every other person who will be responsible for planning and carrying out the release of the organisms and for the supervision, monitoring and safety of the release.

Oxford Vaccine Group, University of Oxford
Centre for Clinical Vaccinology and Tropical Medicine (CCVTM)
Churchill Hospital
Old Road, Headington
Oxford
OX3 7LE

Chief Investigator: Director of the Oxford Vaccine Group (Oxford, UK).

Principal Investigator: Director of the Oxford Centre for Clinical Tropical Medicine (Oxford, UK).

The Oxford Vaccine Group: Experience of conducting *Salmonella* Typhi controlled infections studies in healthy adult volunteers since 2010.

2. The title of the project.

Investigating the role of typhoid toxin in the pathogenesis of enteric fever: A double-blinded, randomised, outpatient human challenge study.

Part II: Information relating to the Organisms

Characteristics of the donor, parental and recipient organisms

3. Scientific name and taxonomy.

In this application the wild type (parent) and GMO are the same organism. *Salmonella* enterica ssp. *enterica* serovar Typhi. The wild type and GMO strain is *Salmonella* Typhi (*S*. Typhi) Quailes strain.

Name

(i)	order and/or higher taxon (for animals)	Enterobacteriales
(ii)	genus	*Salmonella*
(iii)	species	*S. enterica*
(iv)	subspecies	*enterica*
(v)	strain	Quailes
(vi)	pathovar (biotype, ecotype, race, etc.)	Typhi
(vii)	common name	*Salmonella* Typhi

The purpose of the genetic modification is to construct a typhoid toxin-deficient *S*. Typhi Quailes strain. The GMO will be used to investigate the role of typhoid toxin in the pathogenesis of enteric fever in a controlled human infection model. The Oxford Vaccine Group (University of Oxford) has been undertaking controlled human challenge studies using *Salmonella* Typhi and *Salmonella* Paratyphi since 2010. The primary objective of the proposed study is to compare the proportion of participants developing clinical or microbiologically proven typhoid infection following oral challenge with the wild type *S.* Typhi Quailes strain with participants challenged with a typhoid toxin-deficient isogenic mutant of *S.* Typhi Quailes strain (named SB6000).

The GMO has been constructed following the deletion of 3 genes from the wild type S. Typhi Quailes strain (deletion of *cdtB*, *pltA* and *pltB* genes). The GMO is a typhoid toxin-deficient *S*. Typhi Quailes strain (SB6000).

The typhoid toxin comprises enzymatic (A) and binding (B) components and is composed of three polypeptide subunits (CdtB, PltA and PltB). The enzymatic component of the toxin is Cytolethal Distending Toxin B (CdtB), a homologue of active A subunit of Cytolethal distending toxin. This toxin is present in several bacterial pathogens and possesses DNAse activity capable of inducing cell-cycle arrest and cellular distension *in vitro*. CdtB is covalently linked to Pertussis-like toxin A (PltA), a homologue of the enzymatic A subunit of pertussis toxin, which has ADP-ribosylase activity. PltA in turn is linked to Pertussis-like toxin B, a homologue of one of the binding (B) sub-units of pertussis toxin. The crystal structure of the typhoid toxin has been resolved and shows the sub-units to be arranged into a relatively unique A_2B_5 structure, comprising two enzymatic A components (CdtB, PltA) and a homopentameric binding B portion (PltB). CdtB is thought to account for the majority of the toxin's enzymatic activity, but all three components are required for toxin delivery and toxicity.

4. Usual strain, cultivar or other name.

Parent/Recipient strain: *Salmonella* Typhi (*S*. Typhi) strain Quailes.

GMO: The GMO is an isogenic mutant of a wild-type *S.* Typhi Quailes strain. The purpose of the genetic modification is to construct a modified *S*. Typhi Quailes strain carrying the deletion of the *cdtB*, *pltA*, and *pltB* genes, to generate a typhoid toxin-deficient *S*. Typhi Quailes strain (SB6000).

5. Phenotypic and genetic markers.

The wild type strain and GMO can be readily identified as strains of *S*. Typhi by serum agglutination with 09, Vi and H(d) antisera. Identification of organisms cultured will be by biochemical (API, Analytical Profile Index; BioMérieux), microbiological and serological methods.

6. The degree of relatedness between the donor and recipient or between parental organisms.

The GMO was created by targeted gene deletion therefore there is no donor organism.

7. The description of identification and detection techniques.

The wild type (parent) and GMO will be detected by microbiological culture. The wild type and GMO (S. Typhi) will be identified by selective microbiological culture; PCR; agglutination for specific *S*. Typhi antigens.

The GMO can be distinguished genetically from the wild type strain by using polymerase chain reaction (PCR) analysis and nucleotide sequencing to show the absence of the *cdtB*, *pltA*, and *pltB* genes.

8. The sensitivity, reliability (in quantitative terms) and specificity of detection and identification techniques.

The microbiological culture, agglutination and Biochemical profiling techniques described (in Sections 5 and 7) are routinely used to identify *S*. Typhi strains and together demonstrate 100% specificity.

9. The description of the geographic distribution and of the natural habitat of the organisms including information on natural predators, prey, parasites and competitors, symbionts and hosts.

Enteric fever is a systemic illness caused by infection with human-restricted pathogens *Salmonella enterica* subspecies *enterica* serovar Typhi (*S.* Typhi) and *Salmonella enterica* subspecies *enterica* serovar Paratyphi (*S.* Paratyphi). It continues to pose a significant burden of disease with an estimated 25 million cases and 200,000 fatalities occurring globally in the year 2000.

The highest burden of disease is found in low-income countries, where poor sanitation results in the contamination of water and food products and the spread of disease. Most parts of South Asia, South-East Asia, Central Asia, Africa, and South America are considered endemic, with an annual incidence of >100 per 100,000 population.

Although conventionally thought to be a disease of school-aged children and young adults, there is increasing evidence for high rates of infection in children fewer than 5 years of age in endemic areas. Enteric fever also remains an important health consideration in developed countries for travellers visiting endemic regions and laboratory workers.

Humans are the only reservoir of typhoid infection, with the gallbladder thought to be the main site for long-term carriage. Spread between individuals occurs through faecal-oral transmission, mainly via contaminated food or water. In areas with

adequate sanitation (i.e. flushing latrines), person-to-person transmission is rare. A retrospective case note review in 2013 studied 645 contacts of 329 cases of enteric fever in London. All had travelled to endemic regions, and only 10 additional cases were identified, all of whom had travelled abroad with their contact and were presumably infected by the same source rather than from person-to-person contact.

10. The organisms with which transfer of genetic material is known to occur under natural conditions.

Negligible risk that the GMO will acquire copies of deleted genes from a donor organism.

The potential for genetic exchange with any other organisms in the environment is extremely low as the GMO does not contain any plasmids or antibiotic resistance markers. *S.* Typhi is highly monomorphic, meaning there is very little genetic variation within the global *S.* Typhi population, thereby indicating that its propensity for genetic exchange is extremely low. This is supported by a study that analysed of the whole genomes of 19 *S.* Typhi strains and identified only 1,954 single nucleotide polymorphisms (SNPs) between all of them; approximately 1 every 2,300 bp. Further, very little evidence of recombination between *S.* Typhi isolates or with other bacteria was found. Genomic insertions were rare in the sequenced isolates and evolution in the *S.* Typhi population seems to be characterised by ongoing loss of gene function caused by nonsense SNPs. All data supports the hypothesis that evolution in *S.* Typhi is dominated by genetic drift and loss of gene function rather than by diversifying selection or gain of function through point mutation, recombination or acquisition of new sequences.

The risk of the GMO acquiring copies of the deleted *cdtB, pltA* and *pltB* genes to regain a typhoid toxin positive phenotype, via genetic exchange (conjugation, transduction) with a donor organism, is considered to be negligible. The GMO does not have a selective or survival advantage in the environment. The GMO is not likely to become more persistent or invasive when administered to healthy adults in the proposed clinical study and pre-clinical studies indicate that *Salmonella* strains lacking the typhoid toxin have reduced intestinal survival.

11. Verification of the genetic stability of the organisms and factors affecting that stability.

Genetic stability during the construction of the GMO was observed. Colonies of the GMO were screened by PCR and nucleotide sequencing to identify *S*. Typhi carrying the expected gene deletions. PCR evidence indicates that the three attenuating deletion mutations in the chromosome of the GMO are stable (SB6000 Δ *cdtb*, Δ*pltA* and Δ *pltB*).

Whole genome sequencing confirmed the absence of the typhoid-toxin pathogenicity islet in the SB6000 GMO strain. We observed no difference between the wild type Quailes and SB6000 strains in relation to key virulence factors, including genes required for Vi-capsule expression. Differences between strains were confined to

highly-variable regions encoding phage proteins, which are not predicted to impact on bacterial survival in the environment or persistence in the human host.

The phenotypic characteristics expected of a typhoid toxin deficient strain also remain stable as assayed by microbiological assays (growth and purity testing), nucleotide sequencing and typhoid toxin activity assays. The presence of the deletion mutations is confirmed in each batch of GMP manufactured GMO (in addition to microbiological limit and antibiotic sensitivity testing).

12. The following pathological, ecological and physiological traits:

a. the classification of hazard according to existing Community rules concerning the protection of human health and the environment;

S. Typhi is an Advisory Committee on Dangerous Pathogens (ACDP) is a hazard group 3 organism. ACDP: The Approved List of Biological Agents, Health and Safety Executive (HSE) 2013.

The Control of Substances Hazardous to Health Regulations (COSHH) 2002 definition of a human pathogen Hazard Group 3 organism: can cause severe human disease and may be a serious hazard to employees; it may spread to the community, but there is usually effective prophylaxis or treatment available.

A chronic carrier state, in which *S.* Typhi is excreted in the stools for many years without symptoms, can develop after *S.* Typhi ingestion. The chronic carrier state is usually seen in older women with pre-existing gallbladder disease (primarily gallstones).

In the absence of effective antibiotic treatment *S*. Typhi can reach the gall bladder and induce an active local infection or exist asymptomatically in a chronic carrier state. Chronic carriage is defined as excretion of *S*. Typhi in stool for more than one year after clinical or sub-clinical infection. The chronic carrier state is thought to be responsible for contributing to the 'steady-state' of S.Typhi infection rates seen in endemic settings. 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, particularly as both the wild type and GMO *S*. Typhi strains are fully susceptible to antibiotics such as azithromycin and fluoroquinolones.

b. the generation time in natural ecosystems, and the sexual and asexual reproductive cycle;

S. Typhi divides asexually by binary fission and is restricted to a human host. The GMO Quailes strain is a virulent strain of *S.* Typhi which is able to undergo replication *in vivo* without a requirement for specific nutrient supplements.

c. information on survivability, including seasonability and the ability to form survival structures, including seeds, spores and sclerotia;

Wild type *S.* Typhi is effectively contained and inactivated by the normal sewage treatment process. Survival time of wild type *S*. Typhi in sewage is usually less than one week. *S*. Typhi does not form endospores or any other survival structures. The

GMO will not have a selection or survival advantage compared to the wild type strain.

The GMO is an isogenic mutant of the wild type (parent) *S*. Typhi Quailes strain and has been designed and manufactured to GMP standard for use as an oral challenge agent within a controlled human infection model to be conducted at the Oxford Vaccine Group (University of Oxford, UK). The GMO will be administered orally to healthy volunteers recruited to the study who are likely to shed the organism in faeces/stool. This will constitute the release of the organism resulting in release into the sewage system. Normal basic hygiene precautions, namely the use of toilets and hand washing, are considered sufficient to prevent person to person transmission. The GMO is expected to be effectively contained and inactivated by usual sewage system processes.

Survival and Persistence Characteristics
To evaluate the survival and persistence characteristics of both the wild type parent strain (Quailes) and typhoid toxin-deficient isogenic mutant (SB6000) a series of experiments were conducted in river water, sea water and soil. In conclusion the data demonstrate that the GMO strain SB6000 did not persist for any longer than the wild type Quailes strain from which it is derived from. The data demonstrate that both strains survive only transiently in the environment. The wild type and SB6000 strains are phenotypically similar with respect to persistence.

River and sea water:
River water aliquots were inoculated with the *S*. Typhi wild type (WT) and SB6000 GMO strains. All aliquots were then incubated statically at 21 °C and analysed at various time points following inoculation. At each time point the viable cell count (VCC) of the samples was determined and the study proceeded for each sample until no viable cells were recovered in 1 ml of water. The rate of decline over a period of 68 days was similar for both the WT and GMO (SB6000) strains (Figure 1).

Sea water aliquots were then incubated statically at 21 °C and analysed at various time points following inoculation. At each time point the VCC of the samples was determined and the study proceeded for each sample until no viable cells were recovered in 1 ml of water. Neither the GMO (SB6000) nor the WT *S*. Typhi persisted beyond day 51 (Figure 1).

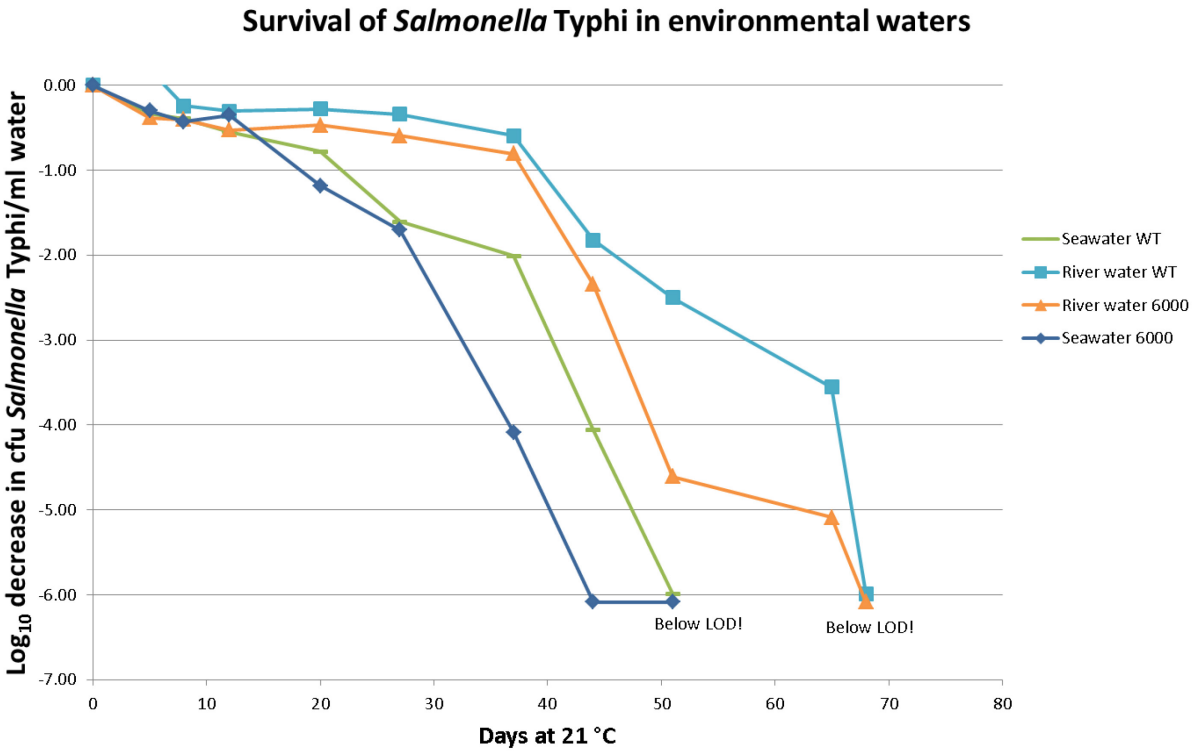

Figure 1. Survival of survival of *Salmonella* Typhi WT strain Quailes and its SB6000 GMO derivative in river and sea water.

Soil:
Aliquots of top soil were inoculated with the *S*. Typhi WT or SB6000 GMO strains. All aliquots were then incubated statically at 21 °C and analysed at various time points following inoculation. At each time point the viable cell count of the samples was determined and the study proceeded for each sample until no viable cells were recovered in 1 ml of soil suspension. It was concluded from this study that the GMO survived for only a limited time in soil. The GMO persisted for no longer than 44 days at the highest inoculum level tested and no longer than 37 days at the lower inoculum level (Figure 2).

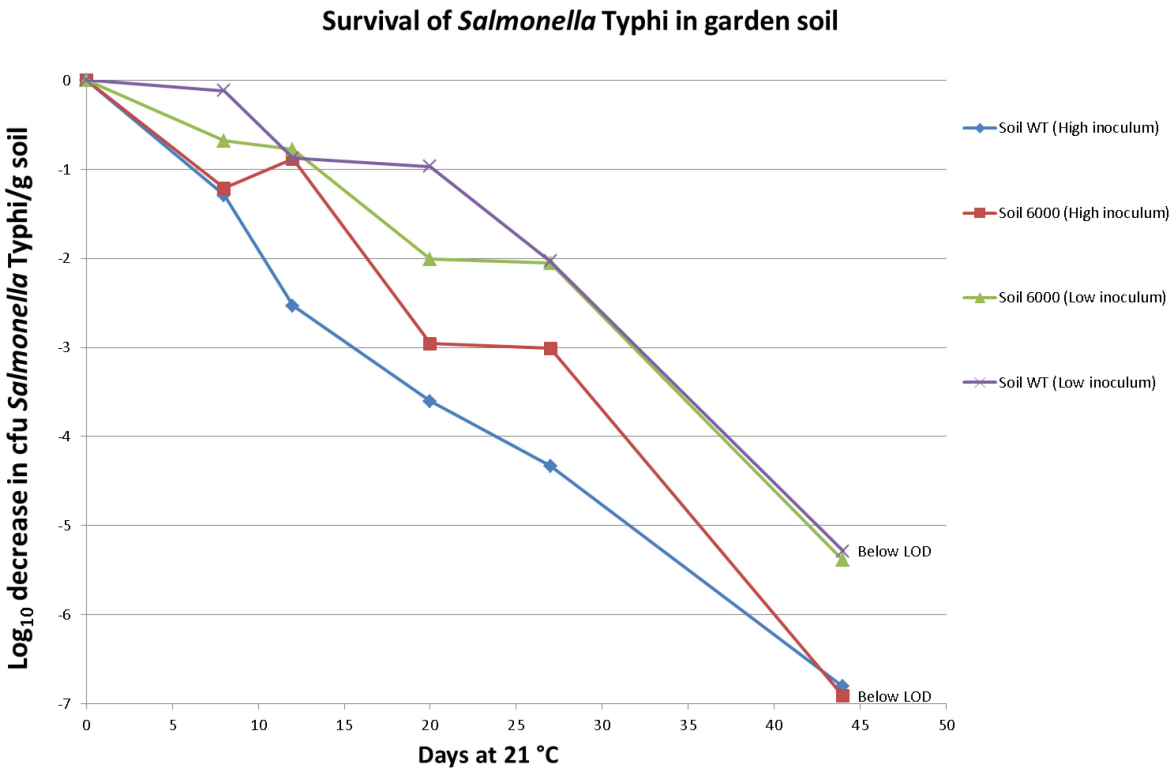

Figure 2. Survival of *Salmonella* Typhi WT strain Quailes and its SB6000 GMO derivative in garden soil.

d. pathogenicity, including infectivity, toxigenicity, virulence, allergenicity, carrier (vector) of pathogen, possible vectors, host range including non-target organisms and possible activation of latent viruses (proviruses) and ability to colonise other organisms;

Enteric fever is a systemic illness caused by infection with human-restricted pathogens *Salmonella enterica*chsubspecies *enterica* serovar Typhi (*S.* Typhi) and *Salmonella enterica* subspecies *enterica* serovar Paratyphi (*S.* Paratyphi).

Humans are the only reservoir of typhoid infection, with the gallbladder thought to be the main site for long-term carriage. Spread between individuals occurs through faecal-oral transmission, mainly via contaminated food or water. In areas with adequate sanitation (i.e. flushing latrines), person-to-person transmission is rare.

A retrospective case note review in 2013 studied 645 contacts of 329 cases of enteric fever in London. All had travelled to endemic regions, and only 10 additional cases were identified, all of whom had travelled abroad with their contact and were presumably infected by the same source rather than from person-to-person contact.

Infection in health adult volunteers can be achieved by the oral administration of greater than 1000 colony forming units (CFU) in a bicarbonate solution. Median incubation times and clinical attack rates have been shown to be dose dependent.

It is hypothesised that differences between typhoidal and non-typhoidal *Salmonella* may in part be explained by the acquisition of new virulence factors. One virulence factor that is limited to *S.* Typhi and *S*. Paratyphi is a recently described exotoxin termed the typhoid toxin. There is a growing body of evidence, which indicates that the typhoid toxin may play a key role in the pathogenesis of enteric fever. In this study we propose to utilise the human challenge model to further study host-pathogen interactions by investigating the role of a specific virulence factor, the typhoid toxin, in the pathogenesis of typhoid fever. This information will be used to inform vaccine design and development potentially influencing public health intervention strategies.

Following the natural ingestion of *S*. Typhi in contaminated food or water, the bacteria must first pass through the gastric acid environment of the stomach. The bacteria subsequently invade intestinal mucosa via attachment to the M (microfold) cells of Peyer's patches. It is thought that professional phagocytic cells ingest the bacteria within the lamina propria of the intestine. Subsequent dissemination of bacteria contained within macrophages is thought to cause a subclinical primary bacteraemia. This results in dissemination of *S.* Typhi to the liver, spleen, lymph nodes and bone marrow. After a latent incubation period of approximately one week, patients develop symptomatic secondary bacteraemia. Delayed or inadequate treatment can result in the development of complications including gastrointestinal perforation or haemorrhage due to hyperplasia, ulceration and necrosis of Peyer's patches.

Human Host Specificity

Wild type *S*. Typhi has no known animal reservoir (human restricted pathogen) and the GMO will not have a selective or survival advantage. The potential for genetic exchange with any other organism in the environment is extremely low given that the GMO does not contain any plasmids or antibiotic resistance markers. The GMO is not expected to persist in study participants following the completion of 14 days of antibiotic treatment.

Despite extensive studies, performed over a century in numerous animal species, it has not been possible to establish an infection with *Salmonella* Typhi that resembles typhoid or indeed any long term persistent infection other than in higher primates. *S*. Typhi can infect higher primates such as the chimpanzee but again, the disease does not fully replicate human typhoid, in part because the typhoid toxin receptor is not expressed in this species. Intensive efforts were made in the past several decades to genetically manipulate *S*. Typhi to become infective for other species, including the mouse, but these all failed.

S. Typhi remained avirulent in mice even if large regions of the genome of murine pathogenic *Salmonella* are mobilised into *S*. Typhi. By examining the genome of *S*. Typhi we now have a scientific explanation for these data in that (a) the Typhi genome harbours upwards of 300+ so called pseudogenes (inactivated genes), many of which are likely to be required for infectivity in other hosts (b) Typhi has acquired a number of different gene sets, including typhoid toxin, Typhi IV pili and Vi

antigen that facilitate infection in humans. Against this background it is hard to formulate a hypothesis that deletion of the typhoid toxin genes would enhance the ability of *S*. Typhi to infect other species, even if individuals were immunocompromised.

e. antibiotic resistance, and potential use of these antibiotics in humans and domestic organisms for prophylaxis and therapy;

The wild type and GMO strains do not possess any antibiotic resistance genes. Full antibiotic sensitivity of has been determined for the GMO as part of the GMP manufacture.

f. involvement in environmental processes including primary production, nutrient turnover, decomposition of organic matter and respiration.

Not applicable.

13. The sequence, frequency of mobilisation and specificity of indigenous vectors and the presence in those vectors of genes which confer resistance to environmental stresses.

Both the wild type and GMO do not contain indigenous vectors.

14. The history of previous genetic modifications.

Not applicable as the wild type *S*. Typhi strain Quailes has not been genetically modified. This is the first description of the GMO and its use in a clinical study with healthy adult volunteers. No release of this GMO has been made previously.

Characteristics of the vector

15. The nature and source of the vector.

The GMO does not contain any vectors. The GMO was generated by the deletion of genetic material. The GMO does not contain a functional insert as such. Chromosomal homologous recombination events during the construction of the GMO resulted in *S*. Typhi carrying either wild type copies or a complete deletion of the typhoid toxin genes (*cdtB*, *pltA* and *pltB*).

16. The sequence of transposons, vectors and other non-coding genetic segments used to construct the genetically modified organisms and to make the introduced vector and insert function in those organisms.

The GMO does not contain a functional insert as such. Chromosomal homologous recombination events during the construction of the GMO resulted in *S*. Typhi carrying either wild type copies or a complete deletion of the typhoid toxin genes (*cdtB*, *pltA* and *pltB*). Colonies were screened by PCR and nucleotide sequencing to identity *S*. Typhi mutants carrying the deletion of the *cdtB*, *pltA*, and *pltB* genes, generating the SB6000 typhoid toxin deficient S. Typhi Quailes strain.

17. The frequency of mobilisation, genetic transfer capabilities and/or methods of determination of the inserted vector.

The GMO does not contain any vector derived genetic material. *S.* Typhi mutants carrying the expected deletion of the *pltA* and *pltB* genes yielding the *S.* Typhi strain SB6000 carrying deletions in *cdtB*, *pltA*, and *pltB* genes were identified by nucleotide sequencing.

18. The degree to which the vector is limited to the DNA required to perform the intended function.

The GMO does not contain any vector derived genetic material.

Characteristics of the modified organisms

19. The methods used for the modification.

Standard genetic manipulation methods were used to construct the GMO (including DNA ligation, electroporation, DNA purification, polymerase chain reaction (PCR), bacterial transformation, colony selection and nucleotide sequencing).

20. The methods used -

a. to construct inserts and introduce them into the recipient organism;

Step 1: Construction of plasmid pSB5999 containing a complete deletion of the *cdtB* gene:

Two PCR reactions were performed using genomic DNA from *Salmonella* Typhi Quailes as a template using primers designed using the genome sequence of the S. Typhi strain CT18(Accession number AL513382). One PCR amplified immediately 5' of the *cdtB* gene, the other PCR amplified immediately 3' of the *cdtB* gene (1,000 nucleotides respectively). The resulting PCR products were mixed in equimolar concentrations with amplified pSB890 DNA and ligated using the Gibson assembly technique to yield the plasmid pSB5999 (carrying a deletion of the coding sequence of *cdtB*). The Gibson reaction was electroporated into *E. coli* CC118 λ pir with selection on LB agar. Isolated colonies were screened for the presence of the recombinant plasmid. Selected candidate plasmids were sequenced to verify the deletion of *cdtB*. The verified plasmid (pSB5999) was transformed into the *E. coli* strain ß2163 ∆*nic35* and selected on LB agar plates to allow the growth of the host bacteria. Plasmid DNA was isolated from the *E. coli* strain ß2163 ∆*nic35* and its nucleotide sequence confirmed.

Step 2: Construction of plasmid pSB6000 containing a complete deletion of the pltA and pltB genes

Nucleotide sequences consisting of 2,500 nucleotides immediately 5' and 3' from the coding sequences of the *pltA* and *pltB* genes, respectively, were amplified by PCR from the genome of *Salmonell*a Typhi Quailes strain using primers obtained from the genome sequence of the *S.* Typhi strain CT18 (Accession number AL513382). Two PCR reactions were perfumed. One PCR product amplified the region upstream of *pltB* whereas the second PCR product amplified the region downstream of *pltA*. The

PCR amplification of the suicide vector pSB890 was identical to the PCR described in the construction of the Δ*cdtB* suicide plasmid. The resulting PCR products were purified, mixed in equimolar concentration with amplified plasmid pSB890 and ligated using the Gibson assembly technique to yield the plasmid pSB6000 (carrying a deletion of the coding sequence of both *pltA* and *pltB*). The Gibson reaction was electroporated into *E. coli* CC118 λ Pir and isolated colonies selected on LB agar plates were screened for the desired recombinant plasmid. A selected candidate plasmid was sequenced to verify the deletion of *pltA* and *pltB*. This plasmid (pSB6000) was transformed into the *E. coli* strain ß2163 Δ*nic35* with selecting on LB agar. Plasmid DNA was isolated from the *E. coli* strain ß2163 Δ*nic35* and its nucleotide sequence confirmed.

Step 3: Construction of S. Typhi SB5999 strain containing a complete deletion of the cdtB gene

The *E. coli* strain ß2163 Δ*nic35* carrying the suicide plasmid pSB5999 was used to introduce the deletion of the *cdtB* gene into the chromosome of the *S*. Typhi Quailes strain by conjugation. A culture of both the donor (*E. coli* strain ß2163 Δ*nic35* carrying the suicide plasmid pSB5999) and recipient (*S.* Typhi Quailes) strains were mixed and filtered to facilitate mating. Growth selection for *S.* Typhi carrying plasmid pSB5999 and counter-selection for the integrated plasmid (which excised from the chromosome by homologous recombination) was performed. The recombination event resulted in *S.* Typhi carrying either a wild type copy or a complete deletion of *cdtB* with no plasmid derived sequences left remaining in the bacterial chromosome. Resulting colonies were screened by PCR and nucleotide sequencing to identify *S.* Typhi mutants carrying the expected deletion of the *cdtB* gene (leading to the identification of an *S.* Typhi strain SB5999 carrying a deletion of the entire coding sequence of the *cdtB* gene).

Step 4: Construction of an *S*. Typhi SB6000 containing a complete deletion of the cdtB pltA and pltB genes

The *E. coli* strain ß2163 Δ*nic35* carrying the suicide plasmid pSB6000 was used to introduce a complete deletion of the *pltA* and *pltB* genes in the chromosome of the *S.* Typhi Quailes derivative strain SB5999 (carrying a deletion of the *cdtB* gene). Introduction of the plasmid and selection of the transconjugants was performed as described in Step 2. Resulting colonies were screened by PCR. *S.* Typhi mutants carrying the expected deletion of the *pltA* and *pltB* genes yielding the *S.* Typhi strain SB6000 carrying deletions in *cdtB*, *pltA*, and *pltB* genes were identified by nucleotide sequencing.

b. to delete a sequence.

See Section 20a.

21. The description of any insert and/or vector construction.

Not applicable, no insert (targeted gene deletion).

22. The purity of the insert from any unknown sequence and information on the degree to which the inserted sequence is limited to the DNA required to perform the intended function.

Colonies were screened by PCR and nucleotide sequencing to identity *S*. Typhi mutants carrying the deletion of the *cdtB*, *pltA*, and *pltB* genes, generating the GMO (SB6000) typhoid toxin deficient S. Typhi Quailes strain.

PCR evidence indicates that the three attenuating deletion mutations in the chromosome of the GMO are stable (SB6000 Δ *cdtb*, Δ*pltA* and Δ *pltB*). The phenotypic characteristics expected of a typhoid toxin deficient strain also remain stable as assayed by microbiological assays (growth and purity testing), nucleotide sequencing and typhoid toxin activity assays. The presence of the deletion mutations is confirmed in each batch of GMP manufactured GMO (in addition to microbiological limit and antibiotic sensitivity testing).

23. The methods and criteria used for selection

As described in Section 20a.

PCR and nucleotide sequencing to identity *S*. Typhi mutants carrying the deletion of the *cdtB*, *pltA*, and *pltB* genes, generating the GMO (SB6000) typhoid toxin deficient *S*. Typhi Quailes strain.

24. The sequence, functional identity and location of the altered, inserted or deleted nucleic acid segments in question and, in particular, any known harmful sequence.

No harmful sequences have been introduced into the GMO. The GMO has been constructed following the deletion of 3 genes from the wild type S. Typhi Quailes strain (deletion of *cdtB*, *pltA* and *pltB* genes). The GMO is a typhoid toxin-deficient *S*. Typhi Quailes strain (SB6000).

Genetic manipulation of enteric microbes is known to occasionally introduce or select for off-target mutations in loci which are not the focus of gene deletion experiments. In order to assess for the presence of any off-target mutations, we performed whole genome sequencing (WGS) of the wild type *Salmonella* Typhi Quailes strain and the genetically modified organism (GMO) strain *Salmonella* Typhi SB6000, to be used in the planned human challenge study. Whole genome sequencing was also undertaken on the parent strains, from which the challenge lots were derived. Results of whole genome sequencing confirmed the expected absence of the typhoid-toxin pathogenicity islet in the SB6000 GMO strains compared with the wild type *S*. Typhi strains. Other than the expected difference in the typhoid-toxin islet, we observed three differences supported in the assemblies and by ambiguous base calls in the SNP analysis/differences in the mapped reads between the strains. The described differences between wild-type Quailes strain and the knock out SB6000 strain variants occur in genomic regions known to be highly unstable and have no known association with virulence ,persistence within the human host nor persistence

in the environment. In addition, no differences between strains were observed in SPI-7, a region encoding genes required for expression of the Vi-capsule.

Characteristics of the genetically modified organisms in their final form

25. The description of genetic traits or phenotypic characteristics and in particular any new traits and characteristics which may be expressed or no longer expressed.

The GMO has been constructed following the deletion of 3 genes from the wild type S. Typhi Quailes strain (deletion of *cdtB*, *pltA* and *pltB* genes). The GMO is a typhoid toxin-deficient *S*. Typhi Quailes strain (SB6000).

The typhoid toxin comprises enzymatic (A) and binding (B) components and is composed of three polypeptide subunits (CdtB, PltA and PltB). The enzymatic component of the toxin is Cytolethal Distending Toxin B (CdtB), a homologue of active A subunit of Cytolethal distending toxin. This toxin is present in several bacterial pathogens and possesses DNAse activity capable of inducing cell-cycle arrest and cellular distension *in vitro*. CdtB is covalently linked to Pertussis-like toxin A (PltA), a homologue of the enzymatic A subunit of pertussis toxin, which has ADP-ribosylase activity. PltA in turn is linked to Pertussis-like toxin B, a homologue of one of the binding (B) sub-units of pertussis toxin. The crystal structure of the typhoid toxin has been resolved and shows the sub-units to be arranged into a relatively unique A_2B_5 structure, comprising two enzymatic A components (CdtB, PltA) and a homopentameric binding B portion (PltB). CdtB is thought to account for the majority of the toxin's enzymatic activity, but all three components are required for toxin delivery and toxicity.
Much of what is known about the pathogenesis of typhoidal *Salmonella* has been inferred from studies using non-typhoidal *Salmonella enterica* serovars that can infect small animals. Administration of purified typhoid toxin in mice recapitulates several of the key features of enteric fever. Pre-clinical studies indicate that the typhoid toxin may play a key role in the pathogenesis of enteric fever and that the unique binding properties of typhoid toxin may, in part, account for the host restriction properties of typhoidal *Salmonella*. Thus, it is anticipated that the GMO lacking expression of the typhoid toxin will have reduced pathogenicity compared to wild type *Salmonella* Typhi.

26. The structure and amount of any vector or donor nucleic acid remaining in the final construction of the modified organisms.

There is no vector or donor nucleic acid remaining in the final GMO.

27. The stability of the organism in terms of genetic traits.

Genetic stability during the construction of the GMO was observed. Colonies of the GMO were screened by PCR and nucleotide sequencing to identify *S*. Typhi carrying the expected gene deletions. PCR evidence indicates that the three attenuating

deletion mutations in the chromosome of the GMO are stable (SB6000 Δ *cdtb*, Δ*pltA* and Δ *pltB*).

The phenotypic characteristics expected of a typhoid toxin deficient strain also remain stable as assayed by microbiological assays (growth and purity testing), nucleotide sequencing and typhoid toxin activity assays. The presence of the deletion mutations is confirmed in each batch of GMP manufactured GMO (in addition to microbiological limit and antibiotic sensitivity testing).

28. The rate and level of expression of the new genetic material in the organisms and the method and sensitivity of measurement of that rate and level.

Not applicable (gene deletion).

29. The activity of the gene product.

Not applicable (gene deletion).

30. The description of identification and detection techniques, including techniques for the identification and detection of the inserted sequence and vector.

Both the wild type and GMO strains can be cultured in the laboratory using standard selective media. GMO colonies have the morphological characteristics of wild type *S*. Typhi and can be identified by PCR, biochemical profiling and specific anti-sera against bacterial antigens Vi, 09 and H(d). PCR and nucleotide sequencing specifically identify GMO *S*. Typhi mutants carrying the deletion of the *cdtB*, *pltA*, and *pltB* genes (the SB6000 typhoid toxin deficient *S*. Typhi Quailes strain).

The phenotypic characteristics expected of a typhoid toxin deficient strain were confirmed in typhoid toxin activity assays. The presence of the deletion mutations is confirmed in each batch of GMP manufactured GMO in addition to microbiological limit and antibiotic sensitivity testing.

31. The sensitivity, reliability (in quantitative terms) and specificity of detection and identification techniques.

The microbiological, biochemical and genetic analyses described in Section 30 enable the GMO to be identified. PCR and nucleotide sequencing specifically identify *S*. Typhi mutants carrying the deletion of the *cdtB*, *pltA*, and *pltB* genes (the SB6000 typhoid toxin deficient *S*. Typhi Quailes strain).

32. The history of previous releases or uses of the organisms.

This is the first description of the GMO and its use in a clinical study with healthy adult volunteers. No release of this GMO has been made previously.

However, the wild type strain (parent) *S*. Typhi strain Quailes has been released previously in four clinical studies conducted within the UK at the Oxford Vaccine Group.

1) Understanding Typhoid Disease: developing a *Salmonella* Typhi challenge mdoel in healthy adults (active, recruitment completed).
2) Understanding Typhoid Disease after Vaccination: NCT01405521 (active, recruitment complete).
3) Investigating Enteric Fever – *Salmonella* Typhi and Paratyphi Challenge Study: NCT02192008 (active, recruiting).
4) Vaccines against *Salmonella* Typhi: NCT02324751 (active, recruiting).

All studies include a comprehensive monitoring of the safety of the wild type *S*. Typhi Quailes strain based on clinical, microbiological, hematology and biochemical measurements. In the proposed study described in this application, clinical study participants will be monitored for signs of infection with the GMO by identified of *S*. Typhi. The results of previous clinical studies using the wild type Quailes strain demonstrated no negative impact on the environment or on human health with the exception of causing enteric fever in the controlled human infection model.

33. In relation to human health, animal health and plant health

S. Typhi is a human restricted pathogen. There are no anticipated effects on animal or plant health.

a. the toxic or allergenic effects of the non-viable organisms and/or their metabolic products,

The GMO has not previously been administered to human subjects.

To date no toxic or allergenic effects were reported following administration of the wild type *S*. Typhi strain Quailes to adult human volunteers in the human challenge model conducted at the Oxford Vaccine Group (UK).

b. the comparison of the organisms to the donor, recipient or (where appropriate) parental organism regarding pathogenicity,

Much of what is known about the pathogenesis of typhoidal *Salmonella* has been inferred from studies using non-typhoidal *Salmonella enterica* serovars that can infect small animals. Administration of purified typhoid toxin in mice recapitulates several of the key features of enteric fever. Pre-clinical studies indicate that the typhoid toxin may play a key role in the pathogenesis of enteric fever and that the unique binding properties of typhoid toxin may, in part, account for the host restriction properties of typhoidal *Salmonella*. Thus, it is anticipated that the GMO lacking expression of the typhoid toxin will have reduced pathogenicity compared to wild type *Salmonella* Typhi.

Human Host Specificity

Wild type *S*. Typhi has no known animal reservoir (human restricted pathogen) and the GMO will not have a selective or survival advantage. The potential for genetic exchange with any other organism in the environment is extremely low given that the

GMO does not contain any plasmids or antibiotic resistance markers. The GMO is not expected to persist in study participants following the completion of 14 days of antibiotic treatment.

Despite extensive studies, performed over a century in numerous animal species, it has not been possible to establish an infection with *Salmonella* Typhi that resembles typhoid or indeed any long term persistent infection other than in higher primates. *S*. Typhi can infect higher primates such as the chimpanzee but again, the disease does not fully replicate human typhoid, in part because the typhoid toxin receptor is not expressed in this species. Intensive efforts were made in the past several decades to genetically manipulate *S*. Typhi to become infective for other species, including the mouse, but these all failed.

S. Typhi remained avirulent in mice even if large regions of the genome of murine pathogenic *Salmonella* are mobilised into *S*. Typhi. By examining the genome of *S*. Typhi we now have a scientific explanation for these data in that (a) the Typhi genome harbours upwards of 300+ so called pseudogenes (inactivated genes), many of which are likely to be required for infectivity in other hosts (b) Typhi has acquired a number of different gene sets, including typhoid toxin, Typhi IV pili and Vi antigen that facilitate infection in humans. Against this background it is hard to formulate a hypothesis that deletion of the typhoid toxin genes would enhance the ability of *S*. Typhi to infect other species, even if individuals were immunocompromised.

c. the capacity of the organisms for colonization, and

The wild type *S*. Typhi strain Quailes and the GMO are restricted to colonise humans.

d. if the organisms are pathogenic to humans who are immunocompetent –

The wild type and GMO are pathogenic to humans who are immunocompetent therefore strict exclusion criteria apply to the clinical study. Including any known or suspected impairment of immune function, alteration of immune function, or prior immune exposure that may alter immune function to typhoid 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), close household contact with young children (defined as those attending pre-school groups, nursery or those aged less than 2 years) and individuals who are immunocompromised.

A participant will not be enrolled into the study 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, 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, metabolic disease, autoimmune disease or infectious disease.

i. diseases caused and mechanisms of pathogenicity including invasiveness and virulence,

S. Typhi is the causative agent of typhoid fever in humans.

Following ingestion of bacteria in contaminated food or water, the bacteria must first pass through the gastric acid environment of the stomach. The bacteria subsequently invade intestinal mucosa via attachment to the M (microfold) cells of Peyer's patches. It is thought that professional phagocytic cells ingest the bacteria within the lamina propria of the intestine. Subsequent dissemination of bacteria contained within macrophages is thought to cause a subclinical primary bacteraemia. This results in dissemination of *S.* Typhi to the liver, spleen, lymph nodes and bone marrow.

After a latent incubation period of approximately one week, patients develop symptomatic secondary bacteraemia. Delayed or inadequate treatment can result in the development of complications including gastrointestinal perforation or haemorrhage due to hyperplasia, ulceration and necrosis of Peyer's patches.

It is hypothesised that differences between typhoidal and non-typhoidal *Salmonella* may in part be explained by the acquisition of new virulence factors. One virulence factor that is limited to *S.* Typhi and *S*. Paratyphi is the recently described exotoxin termed the typhoid toxin. There is a growing body of evidence, which indicates that the typhoid toxin may play a key role in the pathogenesis of enteric fever. This research project proposes to investigate the impact of typhoid toxin in the virulence of wild type *S.* Typhi by comparison with that of a toxin-defective isogenic mutant in a human volunteer model of *S.* Typhi infection.

ii. communicability,

Humans are the only reservoir of typhoid infection, with the gallbladder thought to be the main site for long-term carriage. Spread between individuals occurs through faecal-oral transmission, mainly via contaminated food or water. In areas with adequate sanitation (i.e. flushing toilets) person-to-person transmission is rare.

Throughout the period of possible excretion of the challenge strain, participants must practice stringent hand washing techniques, particularly after defecation. Participants will be given soap and paper towels for use at home and detailed advice on how to prevent transmission of *S.* Typhi. 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 at each visit. 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.

iii. infective dose,

Infection in health adult volunteers can be achieved by the oral administration of greater than 10,000 colony forming units (CFU) in a bicarbonate solution (1 – 5 x 10^4). The wild type Quailes strain has been used in challenge studies in over 200 healthy volunteers in studies conducted at the Oxford Vaccine Group (Section 32).

iv. host range and possibility of alteration,

The GMO is restricted to a human host.

The potential for genetic exchange with any other organisms in the environment is extremely low as the GMO does not contain any plasmids or antibiotic resistance markers. *S.* Typhi is highly monomorphic, meaning there is very little genetic variation within the global *S.* Typhi population, thereby indicating that its propensity for genetic exchange is extremely low.

The risk of the GMO acquiring copies of the deleted *cdtB, pltA* and *pltB* genes to regain a typhoid toxin positive phenotype, via genetic exchange (conjugation, transduction) with a donor organism, is considered to be negligible. The GMO does not have a selective or survival advantage in the environment.
The GMO is not likely to become more persistent or invasive when administered to healthy adults in the proposed clinical study and pre-clinical studies indicate that Salmonella strains lacking the typhoid toxin have reduced intestinal survival. For the safety of our study participants their health will be monitored very closely (up to daily for the first 14 days post challenge) by our clinical study team.

v. possibility of survival outside of human host,

The GMO does not have a selective or survival advantage in the environment (as described in section 12c). The wild type (parent strain) *S.* Typhi Quailes strain has no known animal reservoir and it does not persist in the environment in appreciable quantities sufficient to cause disease. Person to person transmission will be prevented by normal basic hygiene practice (primarily the use of toilets and hand washing).

vi. presence of vectors or means of dissemination,

The GMO does not contain a functional vector or insert as such. Chromosomal homologous recombination events during the construction of the GMO resulted in *S*. Typhi carrying either wild type copies or a complete deletion of the typhoid toxin genes (*cdtB*, *pltA* and *pltB*). Colonies were screened by PCR and nucleotide sequencing to identity *S*. Typhi mutants carrying the deletion of the *cdtB*, *pltA*, and *pltB* genes, generating the GMO SB6000 typhoid toxin deficient *S*. Typhi Quailes strain.

Humans are the only reservoir of typhoid infection, with the gallbladder thought to be the main site for long-term carriage. Spread between individuals occurs through faecal-oral transmission, mainly via contaminated food or water. In areas with adequate sanitation (i.e. flushing toilets), person-to-person transmission is rare. The GMO and the wild type strain are intended for use as an oral challenge agent in a controlled human infection model in healthy adult volunteers. The GMO and wild

type strain will be given orally to study participants who are likely to shed the organism in stool samples, which will constitute release of the organism into the sewage system. The GMO will disseminate into the sewage system which is designed to contain and clear bacteria such as *Salmonella*.

vii. biological stability,

Genetic stability during the construction of the GMO was observed. PCR evidence indicates that the three attenuating deletion mutations in the chromosome of the GMO are stable (SB6000 ∆ *cdtb*, ∆*pltA* and ∆ *pltB*). The phenotypic characteristics expected of a typhoid toxin deficient strain also remain stable as assayed by microbiological assays (growth and purity testing), nucleotide sequencing and typhoid toxin activity assays. The presence of the deletion mutations will be confirmed in each batch of GMP manufactured GMO (in addition to microbiological limit and antibiotic sensitivity testing).

viii. antibiotic-resistance patterns,

Full antibiotic sensitivity has been demonstrated for the GMO (GMP product).

The first line antibiotic will be oral azithromycin 500mg daily for 14 days. To ensure clearance of infection and to exclude carriage, stool samples for culture will be obtained upon completion of the initial antibiotic course. Should convalescent carriage occur (defined as stool cultures being positive for *S.* Typhi in at least 1 in 3 stool samples collected at least one week after completion of antibiotics) then participants will be referred to an Infectious Diseases Consultant at the Oxford University Hospitals NHS Foundation Trust for further management.

Potential participants with known antibiotic hypersensitivity or allergy to either of the first-line (azithromycin, ciprofloxacin, or other macrolide antibiotics) antibiotics will be excluded. The antibiotics to be used in this study are generally well tolerated and are only occasionally associated with 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. Public Health England will be informed of study participation, challenge outcome, commencement and completion of antibiotic course, and clearance results.

ix. allergenicity and x. availability of appropriate therapies.

Potential participants with known antibiotic hypersensitivity or allergy to either of the first-line antibiotics (azithromycin, ciprofloxacin, or other macrolide antibiotics) will be excluded from enrolment to the study. The antibiotics to be used in this study are generally well tolerated and are only occasionally associated with 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. The first line antibiotic will be oral azithromycin 500mg daily for 14 days. The most common side-effects include gastro-intestinal upset, headache, rash, dizziness or itch. Participants will be informed of possible side-effects of antibiotic treatment before enrolment into the trial.

To detect chronic carriage of *S*. Typhi, stool samples for culture will be obtained one week after completion of the antibiotic course until three samples (each taken at least 48 hours apart) are negative. Once these criteria are satisfied, the participant will be considered to be fully treated for *S.* Typhi infection and no longer an infection risk. If samples remain positive for *S.* Typhi four weeks after completion of antibiotics then the participant will be referred to a Consultant in Infectious Diseases (Oxford University Hospitals NHS Foundation Trust) for further management.

Public Health England will be informed of all participants in whom clearance has been demonstrated and of any participant who fails to demonstrate clearance after the initial 14 day course of antibiotics or after any other antibiotic treatment.

The possible adverse effects of *S.* Typhi infection or the effect of some antibiotics on the outcome of pregnancy are unknown. Therefore, pregnant women will be excluded by history and laboratory tests, and female participants will be specifically instructed to prevent conception during the challenge period of the study until completion of antibiotic therapy and clearance of typhoid infection is confirmed. Should pregnancy occur, information about outcome of the pregnancy will be sought.

e. Other product hazards

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

Complications of typhoid fever, such as perforation or haemorrhage, occur almost exclusively in patients who do not receive appropriate antibiotic treatment for an extended period of time. Participants in this study will be treated 12 hours after developing fever or if *S*. Typhi is recovered from a blood culture drawn 72 hours post-challenge. They will be closely monitored during the initial study phase, until a 14-day course of antibiotic is completed, minimising the risk of complications.

Humans are the only reservoir of typhoid infection, with the gallbladder thought to be the main site for long-term carriage. A chronic carrier state, in which *S.* Typhi is excreted in the stools for many years without symptoms, can develop after *S.* Typhi ingestion. The chronic carrier state is usually seen in older women with pre-existing gallbladder disease (primarily 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, particularly with newer antibiotics such as azithromycin and fluoroquinolones being available.

Part III: Information relating to the conditions of release

The release

34. The description of the proposed deliberate release, including the initial purpose or purposes of the release and any intention to use the genetically modified organisms as or in a product in the future.

Purpose of the deliberate release: In this clinical study we propose to utilise the human challenge model established at the Oxford Vaccine Group (UK) to further

study host-pathogen interactions by investigating the role of the typhoid toxin in the pathogenesis of typhoid fever. This information will be used to inform vaccine design and development potentially influencing public health intervention strategies. The title of the project 'Investigating the role of typhoid toxin in the pathogenesis of enteric fever; a double-blinded, randomized, outpatient human challenge study'.

The primary objective of the study: To investigate the contribution of typhoid toxin to *S.* Typhi infection and typhoid fever using a human challenge model of infection.

The primary endpoint of the study: The proportion of participants developing clinical or microbiologically proven typhoid infection following oral challenge with 1-5x10^4 CFU wild type *S.* Typhi Quailes strain (wild type), in comparison with 1-5x10^4 CFU of a typhoid toxin-deficient isogenic mutant of *S.* Typhi Quailes strain SB6000 (GMO).

The GMO and the wild type (parent) are intended for use as an oral challenge agent in a controlled human infection model in healthy adult volunteers, according to previously established protocols (Section 32). The GMO and wild type strain will be given orally to study participates who are likely to shed the organism in stool samples, which will constitute release of the organisms into the sewage system. Person to person transmission will be prevented by normal basic hygiene practice (primarily the use of toilets and hand washing).

35. The intended dates of the release and time planning of the experiment including frequency and duration of releases.

The study is expected to commence on the 1st of September 2016 (pending all necessary approvals) and will run for approximately 18 months (with an expected completion date of March 2018). All study participants will have follow-up visits up to 1 year post challenge. The study will have an enrolment target of 40 adult participants. A target of 20 participants will be administrated the GMO at a dose of 1 – 5 x 10^4 colony forming units (CFU).

The study is anticipated to commence on the 1st of September 2016 with the GMO administered to the first adult volunteer shortly after study recruitment has commenced. The GMO will be given orally and if likely to be shed in faeces at low levels until the completion of a 2 week course of antibiotics.
The duration of the release which includes both dosing (challenge period) and shedding phase of the study will be no longer than 18 months (with completion in March 2018). Monitoring will take place for the duration of the clinical study.

Following challenge with the GMO and wild type Quailes strain participants will be monitored daily for the first 14 days post-challenge. Continuous participant safety monitoring will occur throughout the challenge period through a combination of daily clinical review and monitoring of symptoms in an electronic diary. The protocol for visits will depend on whether the participant develops infection or not. Following diagnosis of enteric fever blood and stool sampling with be performed at 6, 12, 24, 48, 72 and 96 hours post diagnosis. Following completion of antibiotic treatment and confirmed clearance of the GMO in stool samples participants will be monitored a long term follow-up visits at Day 28, 90, 180 and 365. All study participants will

agree to have 24-hour contact with study staff during the four weeks post challenge and to be able to ensure that they are contactable by mobile phone for the duration of the challenge period until antibiotic completion.

36. The preparation of the site before the release.

All necessary study approvals must be place before the study can commence. The clinical study site at which the GMO will be administered to participants will be initiated according to Good Clinical Practice (GCP) and according to documented legal and local procedures and guidelines prior to study initiation. All site staff will be given study specific training and will be offered vaccination against typhoid through the University of Oxford Occupational Health Service.

37. The size of the site.

The proposed release will be conducted at the Oxford Vaccine Group, Centre for Clinical Vaccinology and Tropical Medicine (CCVTM), Churchill Hospital, Old Road, Headington, Oxford OX3 7LE at map reference SP543060. The size of the room at the site where the GMO will be administered to study participants is approximately 12.43 m^2 with wider release into the Oxfordshire area (via faecal shedding into the sewage system).

38. The method or methods to be used for the release.

The GMO will be administered orally in a sodium bicarbonate solution (to neutralise gastric acid) allowing a lower challenge dose to be used and resulting in a more consistent pattern of clinical infection. The outpatient model has been demonstrated to be safe and acceptable to participants (Section 32). The GMO will disseminate into the sewage system which is designed to contain and clear bacteria such as *Salmonella*.

39. The quantity of organisms to be released.

The study will have an enrollment target of 40 adult participants. A target of 20 participants will be administrated with the GMO at a dose of $1 – 5 \times 10^4$ colony forming units (CFU). The maximum release of the GMO in the study overall (assuming a 10% withdrawal rate) will be 1.1×10^6 CFU. A substantial proportion of the initial administered dose is unlikely to be shed as viable bacteria in faeces as the GMO and wild type strain will be taken up by host cells in areas of the gut or will die in transit in the gastrointestinal tract. For comparison, 244/274 (89.1%) of stool samples collected during earlier challenge studies were negative for *S.* Typhi following ingestion of the wild type Quailes strain.

40. The disturbance of the site, including the type and method of cultivation, mining, irrigation, or other activities.

Not applicable.

41. The worker protection measures taken during the release.

All clinical and laboratory staff will be appropriately trained according to GCP, GCLP, GMO risk assessments and local standard operating procedures (SOPs), including infection control, transportation of the GMO/associated clinical samples and GMO waste disposal policies. All study staff handling the GMO and who have direct contact with the study participants will use personal protective equipment (aprons and gloves) as appropriate. Existing SOPs govern the safe and accurate conduct of all study procedures in addition to a study-specific Clinical Study Plan.

All staff members working at the release site will be informed of the commencement of the challenge study and use of the GMO. All staff members associated with the study will be offered a typhoid vaccine through the University of Oxford Occupational Health Service. GMO exposure records for all staff members will be recorded in association with the University of Oxford Safety Office.

42. The post-release treatment of the site.

As a consequence of shedding through faecal material the GMO may be released into the sewage system in England and primarily within the Oxfordshire area. Post-release GMO shed in the stool samples of study participants will be eliminated and made safe in the sewage system.

Waste disposal and cleaning will be according to site standard operating procedures (SOPs) for handling both potentially infectious biological and GMO clinical waste. When required the dosing area will be cleaned and disinfected while wearing suitable personal protective equipment in accordance with documented local procedures including those for infection control. All clinical and laboratory waste will be treated by according to site standard operating procedures (SOPs) for handling GMO and potentially infectious GMO waste.

43. The techniques foreseen for elimination or inactivation of the organisms at the end of the experiment or other purposes of the release.

Local and site SOPs will be followed for inactivation of the GMO waste prior to disposal. Post-release GMO shed in the stool samples of study participants will be eliminated and made safe in the sewage system.

All participants diagnosed with typhoid infection, or after 14 days post-challenge will be treated with eradicative antibiotics. To ensure clearance of infection and to exclude carriage, stool samples for culture will be obtained upon completion of the initial antibiotic course. Should convalescent carriage occur (defined as stool cultures being positive for *S.* Typhi in at least 1/3 stool samples collected at least one week after completion of antibiotics) then participants will be referred to an Infectious Diseases Consultant at the Oxford University Hospitals NHS Foundation Trust for further management. All study participants will have follow-up visits up to 1 year post challenge.

44. Information on, and the results of, previous releases of the organisms and in particular, releases on a different scale or into different ecosystems.

This is the first description of the GMO and its use in a clinical study with healthy adult volunteers.

The environment (both on the site and in the wider environment)

45. The geographical location and national grid reference of the site or sites onto which the release

The proposed release will be conducted at the Oxford Vaccine Group, Centre for Clinical Vaccinology and Tropical Medicine (CCVTM), Churchill Hospital, Old Road, Headington, Oxford OX3 7LE at map reference SP543060.

46. The physical or biological proximity of the site to humans and other significant biota.

The site is in central Oxford, in an urban residential area known as Headington.

47. The proximity to significant biotopes, protected areas or drinking water supplies.

The research site is approximately 3 km from the River Isis (Thames) and approximately 2 km from the River Cherwell.

48. The climatic characteristics of the region or regions likely to be affected.

Not applicable.

49. The geographical, geological and pedological characteristics.

Not applicable.

50. The flora and fauna, including crops, livestock and migratory species.

Not applicable. The GMO (and wild type strain) are restricted to a human host.

51. The description of target and non-target ecosystems likely to be affected.

As a consequence of the release, the GMO will be released into the public sewage treatment system. The potential environmental impact of the release of the GMO is thought to be negligible as the bacterium will be inactivated by normal sewage and water treatment process.

The wild type (parent strain) *S.* Typhi Quailes has no known animal reservoir and it does not persist in the environment at appreciable levels.

52. The comparison of the natural habitat of the recipient organisms with the proposed site or sites of release.

The natural habitat of *S*. Typhi is humans. Study participants enrolled to the proposed clinical study will be primary recipient of the GMO. As a consequence of the release, the GMO will be released into the public sewage treatment system where it will be inactivated by normal sewage and water treatment processes.

All clinical and laboratory waste generated at the release site will be inactivated prior to disposal (according to local GMO standard operating procedures, legal and University policies). All site staff will be trained in infection control procedures.

The Thames Valley Health Protection Unit (Public Health England) will be informed of all participants who have been challenged with *S.* Typhi, satisfy the definition of typhoid infection, have commenced and completed antibiotics, and have completed clearance stool sampling (with additional information and continued contact if persistence stool shedding occurs). The participants GP will also be notified at the time of stool shedding clearance. 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 Public Health England.

53. Any known planned developments or changes in land use in the region which could influence the environmental impact of the release.

None known.

Part IV: Information relating to the interactions between the organisms and the environment

Characteristics affecting survival, multiplication and dissemination

54. The biological features which affect survival, multiplication and dispersal.

S. Typhi is a host-restricted and only infect humans. The GMO does not have a selective or survival advantage in the environment compare to the wild type strain. The GMO is not likely to become more persistent or invasive when administered to healthy adults in the proposed clinical study and pre-clinical studies indicate that *Salmonella* strains lacking the typhoid toxin have reduced intestinal survival.

The risk of the GMO acquiring copies of the deleted *cdtB, pltA* and *pltB* genes to regain a typhoid toxin positive phenotype, via genetic exchange (conjugation, transduction) with a donor organism, is considered to be negligible.

GMO will be excreted directly into the sewage system and it is expected that it will be contained there to be subject to normal sewage processing treatments. Survival time of wild type *S*. Typhi in sewage is usually less than one week. It is expected, based on evaluation of shedding in previous clinical trials, using the wild type S. Typhi Quailes strain, that the GMO will be shed by volunteers for approximately 17 days post-dosing.

In the absence of effective antibiotic treatment *S*. Typhi can reach the gall bladder and induce an active local infections or exist asymptomatically in a chronic carrier state. Chronic carriage is defined as excretion of *S*. Typhi in stool for more than one year after clinical or sub-clinical infection. The chronic carrier state is thought to be responsible for contributing to the ‘steady-state’ of S.Typhi infection rates seen in endemic settings. 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, particularly as both the wild type and GMO *S*. Typhi strains are fully susceptible to antibiotics such as azithromycin and fluoroquinolones.

55. The known or predicted environmental conditions which may affect survival, multiplication and dissemination, including wind, water, soil, temperature and pH.

GMO will be excreted directly into the sewage system and it is expected that it will be contained there to be subject to normal sewage processing treatments.

56. The sensitivity to specific agents.

Full antibiotic sensitivity of the *S*. Typhi wild type and GMO have been confirmed. All individuals challenged with the GMO will be treated with a two week course (14 days) of oral antibiotics (azithromycin 500mg daily), either at the time of acute infection or at Day 14 (whichever is sooner). The GMO has confirmed sensitivity against first, second, third and fourth line antimicrobials. Antibiotic treatment will ensure complete clearance of the GMO, which will be confirmed with collection of three serial stool specimens (at least 48 hours apart) upon completion of treatment.

Interactions with the environment

57. The predicted habitat of the organism.

The natural habitat of *S*. Typhi is humans. Study participants enrolled to the proposed clinical study will be primary recipient of the GMO. As a consequence of the release, the GMO will be released into the public sewage treatment system where it will be inactivated by normal sewage and water treatment processes.

58. The studies of the behaviour and characteristics of the organisms and their ecological impact carried out in simulated natural environments, such as microcosms, growth rooms and greenhouses.

Not applicable.

59. The capability of post-release transfer of genetic material-

a. from the genetically modified organisms into organisms in affected ecosystems,

The risk of genetic transfer with other organism is extremely low since the GMO does not contain functional insert or plasmid.

b. from indigenous organisms to the genetically modified organisms.

The risk of the GMO acquiring copies of the deleted *cdtB, pltA* and *pltB* genes to regain a typhoid toxin positive phenotype, via genetic exchange (conjugation, transduction) with a donor organism, is considered to be negligible.

60. The likelihood of post-release selection leading to the expression of unexpected or undesirable traits in the genetically modified organisms.

The GMO does not have a selective or survival advantage in the environment.

61. The measures employed to ensure and to verify genetic stability, the description of genetic traits which may prevent or minimise dispersal of genetic material and methods to verify genetic stability.

Genetic stability during the construction of the GMO was observed. Colonies of the GMO were screened by PCR and nucleotide sequencing to identify *S*. Typhi carrying the expected gene deletions.

PCR evidence indicates that the three attenuating deletion mutations in the chromosome of the GMO are stable (SB6000 Δ *cdtb*, Δ*pltA* and Δ *pltB*). The phenotypic characteristics expected of a typhoid toxin deficient strain also remain stable as assayed by microbiological assays (growth and purity testing), nucleotide sequencing and typhoid toxin activity assays. The presence of the deletion mutations is confirmed in each batch of GMP manufactured GMO (in addition to microbiological limit and antibiotic sensitivity testing). The risk of genetic transfer with other organism is extremely low since the GMO does not contain functional insert or plasmid.

Whole genome sequencing of SB6000 confirmed the expected absence of the typhoid-toxin pathogenicity islet and we observed no difference between the wild type and SB6000 strains in relation to key virulence factors. Differences between strains were confined to highly-variable regions encoding phage proteins which are not predicted to impact on bacterial survival in the environment or persistence in the human host.

62. The routes of biological dispersal, known or potential modes of interaction with the disseminating agent, including inhalation, ingestion, surface contact and burrowing.

S. Typhi has a very narrow host range and cannot colonise any organisms in the environment other than humans. Person to person transmission via the faecal-oral route will be minimised by issuing participants with information on enteric precautions, instructions for participants on obtaining stool specimens, provide the participant with stool sampling equipment. education of participants on correct hand washing techniques (including demonstration and observation), advise participants to inform the study team if any breaches of enteric precautions occur such that another individual comes into contact with excreta from the participant, issue participants with liquid hand soap and paper towels to aid with adherence to enteric precautions.

As a consequence of the release in faecal samples, the GMO will be released into the public sewage treatment system where it will be inactivated by normal sewage and water treatment processes.

63. The description of ecosystems to which the organisms could be disseminated.

The GMO will be released into the sewage system via faecal shedding from study participants who have received an oral dose of the organism. The GMO will be released into the public sewage treatment system where it will be inactivated by normal sewage and water treatment processes. Should a breach in the sewage system occur the GMO could enter other ecosystems such as soil and water bodies.

64. The potential for excessive population increase of the organisms in the environment.

Negligible, the GMO will be inactivated by normal sewage and water treatment processes.

65. The competitive advantage of the organisms in relation to the unmodified recipient or parental organism or organisms.

The GMO does not have a selective or survival advantage over wild type *S.* Typhi. The GMO will disseminate into the sewage system which is designed to contain and clear bacteria such as *Salmonella*.

66. The identification and description of the target organisms if applicable.

Healthy adult volunteers aged 18 -60 years will be the target organism in the proposed clinical study. Strict inclusion and exclusion criteria apply to the study protocol (as described in Section 33 and 77).

67. The anticipated mechanism and result of interaction between the released organisms and the target organisms if applicable.

In the proposed study, the human challenge model will be used to further our understanding of the pathogenesis of typhoid fever by exploring the contribution of a recently identified virulence factor, termed the typhoid-toxin, to the clinical syndrome of enteric fever. It is hoped that the insights obtained from this study will form the basis for future studies exploring the use of typhoid-toxin as a novel vaccine candidate as well as providing insights into disease pathogenesis that could facilitate the development of novel diagnostic strategies and therapeutics.

Converging lines of evidence suggest that typhoid toxin plays a central role in the pathogenesis of typhoid fever, which may account for the human-restriction properties of this pathogen as well as being a strongly immunogenic antigen following natural infection. These data raise the intriguing possibility that typhoid toxin could be a promising vaccine candidate for typhoidal *Salmonella.* The lack of a meaningful animal model has, however, hampered the ability to study the potential importance of typhoid toxin in the pathogenesis of typhoid fever.

Following ingestion of wild type *S*. Typhi bacteria in contaminated food or water, the bacteria must first pass through the gastric acid environment of the stomach. The bacteria subsequently invade intestinal mucosa via attachment to the M (microfold) cells of Peyer's patches. It is thought that professional phagocytic cells ingest the bacteria within the lamina propria of the intestine. Subsequent dissemination of bacteria contained within macrophages is thought to cause a subclinical primary bacteraemia. This results in dissemination of *S.* Typhi to the liver, spleen, lymph nodes and bone marrow. After a latent incubation period of approximately one week,

patients develop symptomatic secondary bacteraemia (identified by a positive blood culture).

68. The identification and description of non-target organisms which may be adversely affected by the release of the genetically modified organisms, and the anticipated mechanisms of any identified adverse reaction.

None expected as *S*. Typhi is a human restricted pathogen.

69. The likelihood of post-release shifts in biological interactions or in the host range.

The risk of the GMO acquiring copies of the deleted *cdtB, pltA* and *pltB* genes to regain a typhoid toxin positive phenotype, via genetic exchange (conjugation, transduction) with a donor organism, is considered to be negligible. The GMO does not have a selective or survival advantage in the environment. The GMO is not likely to become more persistent or invasive when administered to healthy adults in the proposed clinical study. For the safety of our study participants their health will be monitored very closely (up to daily for the first 14 days post challenge) by our clinical study team.

70. The known or predicted interactions with non-target organisms in the environment, including competitors, prey, hosts, symbionts, predators, parasites and pathogens.

None expected.

71. The known or predicted involvement in biogeochemical processes.

None expected.

72. Any other potentially significant interactions with the environment.

None expected.

Part V: Information on monitoring, control, waste treatment and emergency response plans

Monitoring techniques

73. Methods for tracing the organisms and for monitoring their effects.

This is the first description of the GMO and its use in a clinical study with healthy adult volunteers. No release of this GMO has been made previously. However, the wild type (parent) *S*. Typhi strain Quailes has been released previously in four clinical studies conducted within the UK at the Oxford Vaccine Group. All studies include a comprehensive monitoring of the safety of the wild type Quailes strain based on clinical, microbiological, hematology and biochemical measurements. In the proposed study described in this application, clinical study participants will be monitored for signs of infection with the GMO. The results of previous clinical studies using the wild type Quailes strain demonstrated no negative impact on the

environment or on human health (with the exception of causing enteric fever in the controlled human infection model). The potential environmental impact of the release of the GMO is thought to be zero.

Adult human volunteers will ingest the GMO orally where it will reach the intestine and the host will mount an immune response against the GMO. A proportion of individuals will develop acute typhoid fever following ingestion of the GMO. The attack rate following challenge with wild-type S. Typhi at a dose of 10^4 CFU is 65% and it is hypothesized that this will be lower in the individuals challenged with the GMO (attenuated strain). All individuals challenged with the GMO will be treated with a two week course of oral antibiotics (azithromycin 500mg), either at the time of acute infection or at Day 14 (whichever is sooner). The GMO has confirmed sensitivity against first, second, third and fourth line antimicrobials. Antibiotic treatment will ensure complete clearance of the GMO, which will be confirmed with collection of three serial stool specimens (at least 48 hours apart) upon completion of treatment.

Stool cultures will be taken at Day 0 (challenge), throughout the 14 day post-challenge period and at visits after typhoid diagnosis. Time to onset of stool shedding - time from challenge (Hours/Days) to the first positive stool culture, excluding the first 24 hours following ingestion of challenge agent will be documented. Participants will be required to supply further stool samples until proven not to be shedding *S.* Typhi. Stool samples will be collected at least one week after completion of a 14 day course of antibiotics, until 3 successive stool samples are negative for *S*. Typhi. If persistent stool shedding occurs after completion of antibiotics, participants will be referred to the Infectious Diseases Consultant at the Oxford University Hospitals NHS Foundation Trust. Additionally, quantitative stool cultures or PCR may be performed 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.

Blood samples will be monitored daily for the GMO using a combination of microbiological and molecular biology techniques. The Oxford Vaccine Group has developed a fast and highly sensitive novel TSB-bile blood culture-PCR assay which has been used to detect low levels of *S.* Typhi in the blood of participants after challenge. A BACTEC 9240 continuous monitoring system will be used to culture GMO organisms which will be identified as *S*. Typhi via biochemical and serological methods. Confirmed isolates will be tested for antibiotic susceptibility using standard methods.

74. Specificity (to identify the organisms and to distinguish them from the donor, recipient or, where appropriate, the parental organisms), sensitivity and reliability of the monitoring techniques.

No routine monitoring specifically for the GMO is planned. The *S*. Typhi GMO will be monitored as described in Section 73 (standard microbiological techniques for the detection of S. Typhi strains). However should this need arise the typhoid toxin gene deletions can readily be identified by specific methods such as PCR and nucleotide sequencing.

75. Techniques for detecting transfer of the donated genetic material to other organisms.

Not applicable (targeted gene deletion).

76. Duration and frequency of the monitoring.

Monitoring will take place for the duration of the clinical study. The study is expected to commence on the 1st of September 2016 (pending all necessary approvals) and will run for approximately 18 months (with an expected completion date of March 2018). All study participants will have follow-up visits up to 1 year post challenge.

Following challenge with the GMO and wild type Quailes strain participants will be monitored daily for the first 14 days post-challenge. Continuous participant safety monitoring will occur throughout the challenge period through a combination of daily clinical review and monitoring of symptoms in an electronic diary. The protocol for visits will depend on whether the participant develops infection or not. Following diagnosis of enteric fever blood and stool sampling with be performed at 6, 12, 24, 48, 72 and 96 hours post diagnosis. Following completion of antibiotic treatment and confirmed clearance of the GMO in stool samples participants will be monitored a long term follow-up visits at Day 28, 90, 180 and 365. All study participants will agree to have 24-hour contact with study staff during the four weeks post challenge and to be able to ensure that they are contactable by mobile phone for the duration of the challenge period until antibiotic completion.

An independent Data Safety Monitoring Committee (DSMC) will be established prior to the start of the study. The DSMC will be appointed to provide real-time oversight of safety and trial conduct. 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 and will particularly review the control group attack rate to confirm the challenge model is proceeding as expected. 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*. Typhi 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 trial master file. The Chair of the DSMC will also be contacted for advice when the Chief Investigator feels independent advice or review is required.

Stool Shedding

Following challenge with *Salmonella* Typhi, we collect daily specimens to detect shedding of the bacterium in the stool. In earlier challenge studies conducted by our group, stool shedding was detected in 63% of participants (Figure 3). Shedding typically preceded the development of clinical illness, although asymptomatic shedding was detected in 3/40 individuals. Convalescent shedding of *S*. Typhi in the stool is associated with antimicrobial resistance and shorter course antibiotic treatment. In the context of this study, challenge is performed with fully sensitive bacterial strains and all participants are treated with a prolonged course of oral antibiotics (ciprofloxacin 500mg PO BD for 14 days OR azithromycin 500mg PO OD for 14 days). No cases of convalescent stool shedding were detected after antibiotics treatment in earlier *S*. Typhi challenge studies performed by our group.

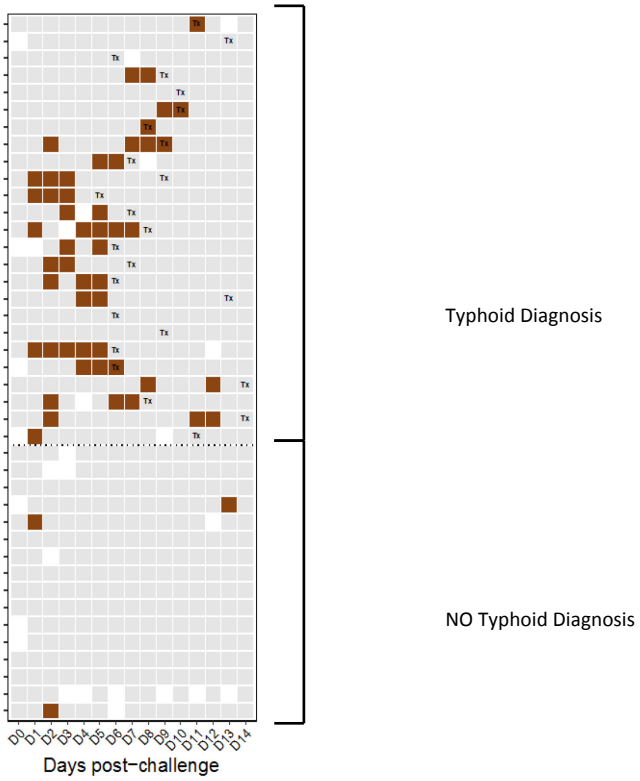

Figure 3 - Pattern of stool shedding following wild type *S.* Typhi challenge. Each row represents an individual challenged with *S*. Typhi. D0 = Day of challenge. D14 = End of follow up period. Coloured squares indicate stool culture results for *S*. Typhi (brown = positive; grey = negative; white = sample not collected). Tx = Day of treatment initiation. No cases of convalescent stool shedding were detected on follow up.

Upon completion of antibiotic therapy, three serial specimens are collected to ensure clearance of *S.* Typhi from the stool. Clearance specimens are collected in line with the latest guidelines from Public Health England, which state that three specimens should be collected at least 48 hours apart, at least one week after completion of antibiotic treatment. We contend that the clearance stool collection protocol undertaken in this study exceeds minimum requirements, as guidance from Public Health England states that 'low-risk' individuals (i.e. those not working in high risk occupations or with exposure to potentially vulnerable persons) do not require clearance stool collection following an episode of typhoid fever. Any individuals designated as high-risk are excluded from participation in the study. Clearance stool specimens are collected between Day 28 and Day 90, along with additional specimens at Day 90, Day 180 and Day 365.

Chronic Carriage

Chronic carriage of *Salmonella* Typhi is associated with gallbladder disease and the presence of chololithiasis/choledocholithiasis, around which the bacterium survives in a protected niche. To avoid the risk of chronic carriage, any individuals with a history of gall bladder disease or disease of the biliary tree are excluded from participation in the study. In addition, screening for evidence of gallbladder/biliary tree disease is undertaken via trans-abdominal ultrasound and screening blood tests (e.g. evidence of elevated Alakaline Phosphastase, hyperbilirubinaemia). In the unlikely event that shedding post antibiotics is detected, arrangements are in place for participants to be referred to a specialist Infectious Diseases department in the Oxford University Hospitals NHS foundation trust for further management (likely consisting of secondary antibiotic therapy and in exceptional cases cholecystectomy). No cases of chronic shedding have been detected in over 150 individuals challenged to date and no individuals have required referral for specialist management. The exclusion of individuals at risk of chronic carriage, combined with prolonged antibiotic treatment and confirmation of clearance is considered sufficient to ensure the risk of long term shedding is negligible.

Control of the release

77. Methods and procedures to avoid and/or minimise the spread of the organisms beyond the site of release or the designated area for use.

Wild type *S.* Typhi has a very narrow host range and cannot colonise any organisms in the environment other than humans. Person to person transmission via the faecal-oral route will be minimised by issuing participants with information on enteric precautions, instructions for participants on obtaining stool specimens, provide the participant with stool sampling equipment. education of participants on correct hand washing techniques (including demonstration and observation), advise participants to inform the study team if any breaches of enteric precautions occur such that another individual comes into contact with excreta from the participant, issue participants with liquid hand soap and paper towels to aid with adherence to enteric precautions.

Strict exclusion criteria have been set for the study including criteria to minimize the risk of transmission of the GMO. Full-time, part-time or voluntary occupations involving: clinical /social work with direct contact with young children (defined as those attending pre-school groups or nursery or aged under 2 years), or clinical/social work with direct contact with highly susceptible patients or persons in whom typhoid infection would have particularly serious consequences (unless willing to avoid work until demonstrated not to be infected with *S*. Typhi in accordance with guidance from Public Health England and willing to allow study staff to inform their employer). 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.

Female participants who are pregnant, lactating or who are unwilling to ensure that they or their partner use effective contraception 30 days prior to challenge and until three negative stool samples have been obtained after completion of antibiotic treatment. Full time, part time or voluntary occupations involving: commercial food handling (involving preparing or serving unwrapped foods not subjected to further heating), close household contact with: young children (defined as those attending pre-school groups, nursery or those aged less than 2 years) or individuals who are immunocompromised.

The health of our study participants is of the upmost importance and will be actively and closely monitored for the duration of the study. Any symptoms will be clinically managed by the site study physicians as appropriate. Person to person transmission will be prevented by normal basic hygiene practice (primarily the use of toilets and hand washing).

Participants will be screened for shedding of *S*. Typhi in the stool. Stool cultures will be taken at Day 0 (challenge), throughout the 14 day post-challenge period and at visits after typhoid diagnosis. Participants will be required to supply 3 further stool samples until proven not to be shedding *S.* Typhi in three consecutive samples. To detect chronic carriage of *S*. Typhi, stool samples for culture will be obtained one week after completion of the antibiotic course until three samples (each taken at least 48 hours apart) are negative. Once these criteria are satisfied, the participant will be considered to be fully treated for *S.* Typhi infection and no longer an infection risk. If samples remain positive for *S.* Typhi four weeks after completion of antibiotics then the participant will be referred to a Consultant in Infectious Diseases (Oxford University Hospitals NHS Foundation Trust) for further management. No evidence of stool shedding of the wild type *S*. Typhi Quailes strain after treatment or transmission to secondary contacts has been detected in previous challenge studies conducted at the Oxford Vaccine Group.

The Thames Valley Health Protection Unit (Public Health England) will be informed of all participants who have been challenged with *S.* Typhi, satisfy the definition of typhoid infection, have commenced and completed antibiotics, and have completed clearance stool sampling (with additional information and continued contact if persistence stool shedding occurs).

The participants GP will also be notified at the time of stool shedding clearance. 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 Public Health England.

78. Methods and procedures to protect the site from intrusion by unauthorised individuals.

Entry to the facility is restricted to authorised trained personnel only (swipe card access and where applicable swipe card in combination with unique personal access codes). The activities at site are regularly monitored with onsite security service provisions established.

79. Methods and procedures to prevent other organisms from entering the site.

Where applicable personal protective equipment will be used and all site staff will follow local operating procedures for handling the GMO and infection control.

All GMO (and wild type) bacterial stocks are assessed for purity as part of the GMP manufacturing process. Good laboratory practice and aseptic technique when handling of the GMO will minimise the risk of other organisms entering the facility. Single use vials will be used for challenge agent preparation to prevent other organisms entering the challenge agent bacterial stock.

Waste treatment

80. Type of waste generated.

Clinical waste (including but limited to: faecal/urine/blood/saliva/nasal swab samples, tissues, sharps, syringes, disposable clothing, gloves and aprons).

Laboratory waste (including but not limited to: plastic ware, microbiological waste (agar plates/blood culture bottles), gloves, disposable clothing, paper towels, and clinical samples as described above).

81. Expected amount of waste.

The amount of waste will be typical for the clinical site and laboratory operations amounting to a few clinical waste bags and bins per day waste. The amount of expected waste will be managed by standard operating procedures currently in place at the site.

82. Description of treatment envisaged.

All clinical and laboratory waste will be treated according to site standard operating procedures (SOPs) for handling GMO and potentially infectious GMO waste. All GMO activity at site will be inactivated by autoclaving prior to disposal and removal from the site. All associated procedures have been validated, site autoclaves are validated annually with contracts in place for regular equipment servicing and maintenance.

Post-release GMO shed in the stool samples of study participants will be eliminated and made safe in the sewage system.

Emergency response plans

83. Methods and procedures for controlling the organisms in case of unexpected spread.

Stringent precautions are in place to avoid the spread of the GMO from the study participant to others. Such spread has never been noted in previous studies conducted by the Oxford Vaccine Group using the wild type *S*. Typhi Quailes strain.

Participants who vomit for any reason within 90 minutes of the challenge will be withdrawn from the trial and treated with antibiotics. This will be treated as an emergency spill of the GMO and standard operating procedures will be followed by the research team. Suitable personal protective equipment and disinfectants will be used to inactive the GMO. All waste will be disposed according to legal, local GMO and University of Oxford standard operating procedures.

Participants will be instructed to notify the study team of any serious adverse events/reactions following administration of the GMO. All participants agree to have 24-hour contact with study staff during the four weeks post challenge and to be able to ensure that they are contactable by mobile phone for the duration of the challenge period until antibiotic completion.

A physician from the clinical team will be on-call 24 hours. In addition, participants agree to allow the study team to hold the name and 24-hour contact number of a close friend, relative or housemate who will be kept informed of the study participant's whereabouts for the duration of the challenge period (from the time of challenge until completion of antibiotic course). This person will be contacted if study staff are unable to contact the participant.

Participants will be issued with a Medic Alert-type card containing information including the antibiotic sensitivity of the *S.* Typhi strain (GMO and wild type Quailes strain), study doctor contact details and instruction for the research team to be contacted immediately in the event of illness/accident.

Potential participants with known antibiotic hypersensitivity or allergy to either of the first-line antibiotics (azithromycin, ciprofloxacin, or other macrolide antibiotics) will be excluded from the study. The antibiotics to be used in this study are generally well tolerated and are only occasionally associated with 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. The participant's GP will be notified in writing of the antibiotics received. Participants will receive telephone calls or by text messages to remind them to take their antibiotic dose.

There are provisions within the protocol and site facilities to allow for admissions of participants as inpatients to the John Warin Ward (Churchill Hospital, Oxford) in cases of severe typhoid fever and/or other circumstances.

84. Methods, such as eradication of the organisms, for decontamination of the areas affected.

Contaminated areas may be decontaminated using Virkon of Haztab (granules or solution).

85. Methods for disposal or sanitation of plants, animals, soils and any other thing exposed during or after the spread.

All clinical and laboratory GMO waste will be autoclaved prior to removal and disposal at the site.

86. Methods for the isolation of the areas affected by the spread.

All participants will receive antibiotic treatment at 14 days following challenge at the latest (if no diagnosis of typhoid fever has been made). All study participants will agree to have 24-hour contact with study staff during the four weeks post challenge and to be able to ensure that they are contactable by mobile phone for the duration of the challenge period until antibiotic completion.

Participants will be screened for shedding of *S*. Typhi in the stool. Stool cultures will be taken at Day 0 (challenge), daily throughout the 14 day post-challenge period and at visits after typhoid diagnosis. Participants will be required to supply 3 further stool samples until proven not to be shedding *S.* Typhi in three consecutive samples. To detect chronic carriage of *S*. Typhi, stool samples for culture will be obtained one week after completion of the antibiotic course until three samples (each taken at least 48 hours apart) are negative, according to Public Health England guidelines. Once these criteria are satisfied, the participant will be considered to be fully treated for *S.* Typhi infection and no longer an infection risk. If samples remain positive for *S.* Typhi after completion of antibiotics then the participant will be referred to a Consultant in Infectious Diseases (Oxford University Hospitals NHS Foundation Trust) for further management.

No instances of stool shedding of the *S*. Typhi Quailes strain after treatment or transmission to secondary contacts were detected in previous challenge studies conducted at the Oxford Vaccine Group.

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*. Typhi infection, which will involve obtaining two stool samples 48-hours apart. 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 Public Health England will be informed.

87. Plans for protecting human health and the environment in case of the occurrence of an undesirable effect.

The health of our study participants is of the upmost importance and will be actively and closely monitored for the duration of the study. Any symptoms will be clinically managed by the site study physicians as appropriate. Person to person transmission will be prevented by normal basic hygiene practice (primarily the use of toilets and hand washing).

Participants will be screened for shedding of *S*. Typhi in the stool. Stool cultures will be taken at Day 0 (challenge), throughout the 14 day post-challenge period and at visits after typhoid diagnosis. Participants will be required to supply 3 further stool samples until proven not to be shedding *S.* Typhi in three consecutive samples. To detect chronic carriage of *S*. Typhi, stool samples for culture will be obtained one week after completion of the antibiotic course until three samples (each taken at least 48 hours apart) are negative. Once these criteria are satisfied, the participant will be considered to be fully treated for *S.* Typhi infection and no longer an infection risk. If samples remain positive for *S.* Typhi four weeks after completion of antibiotics

then the participant will be referred to a Consultant in Infectious Diseases (Oxford University Hospitals NHS Foundation Trust) for further management. No evidence of stool shedding of the wild type *S*. Typhi Quailes strain after treatment or transmission to secondary contacts has been detected in previous challenge studies conducted at the Oxford Vaccine Group.

The Thames Valley Health Protection Unit (Public Health England) will be informed of all participants who have been challenged with *S.* Typhi, satisfy the definition of typhoid infection, have commenced and completed antibiotics, and have completed clearance stool sampling (with additional information and continued contact if persistence stool shedding occurs). The participants GP will also be notified at the time of stool shedding clearance. 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 Public Health England.

Part VI: A description of the methods used or a reference to standardised or internationally recognised methods used to compile the information required by this schedule, and the name of the body or bodies responsible for carrying out the studies.

The methods used in the application for Deliberate Release include the following:

- Construction of the GMO
- Stability and function of the GMO
- GMP manufacture of both the wild type and GMO *S*. Typhi Quailes strain.
- Experience of conducting controlled infection studies in healthy adult volunteers

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Application for consent to release a GMO

Part 2: Data or results from any previous releases of the GMO

Give information on data or results from any previous releases of this GMO by you either inside or outside the European Community [especially the results of monitoring and the effectiveness of any risk management procedures].

The *S*. Typhi Quailes GMO strain (SB6000) has been generated and manufactured to GMP specifically for the purpose of undertaking the proposed controlled human infection study at the University of Oxford according to previously established protocols. No data or results from any previous release of this GMO is applicable.

The GMO strain is derived from a wild type *S*. Typhi Quailes strain which has been used extensively in previous typhoid challenge studies. To date the Oxford Vaccine Group have conducted four typhoid challenge studies using the wild type *S*. Typhi Quailes strain.

1) Understanding Typhoid Disease: developing a *Salmonella* Typhi challenge mdoel in healthy adults (active, recruitment complete).
2) Understanding Typhoid Disease after Vaccination: NCT01405521 (active, recruitment complete).
3) Investigating Enteric Fever – *Salmonella* Typhi and Paratyphi Challenge Study: NCT02192008 (active, recruiting).
4) Vaccines against *Salmonella* Typhi: NCT02324751 (active, recruiting).

Part A3: Details of previous applications for release

Give details of any previous applications to release the GMO made to the Secretary of State under the 2002 Regulations or to another Member State under the Deliberate Release Directive 2001/18/EC.

The GMO *S*. Typhi Quailes strain (SB6000) has been generated and manufactured to GMP specifically for the purpose of undertaking the proposed controlled human infection study at the University of Oxford. No applications to release the GMO have previously been made to the Secretary of State under the 2002 Regulations or to another Member State under the Deliberate Release Directive 2001/18/EC.

Part A4: Risk assessment and a statement on risk evaluation

It is satisfied that the release of this GMO will not have an adverse effect on human health and the environment, providing the required precautionary measures are taken.

Risk Assessment: environmental impact of the release of the GMOs

This is the first description of the GMO and its use in a clinical study with healthy adult volunteers. The potential environmental impact of the release of the GMO is thought to be negligible as the bacterium will be inactivated by normal sewage and water treatment process. The wild type (parent strain) *S.* Typhi Quailes has no known animal reservoir and it does not persist in the environment at appreciable levels.

The GMO and the wild type strain are intended for use as an oral challenge agent in a controlled human infection model in healthy adult volunteers. The GMO and wild type strain will be given orally to study participants who are likely to shed the organism in stool samples, which will constitute release of the organism into the sewage system. Person to person transmission will be prevented by enhanced hygiene practice until clearance is confirmed (primarily hand washing, the use of toilets and avoidance of food handling). All microbial challenge studies are conducted according to guidelines from the Academy of Medical sciences and subject to approvals from a designated National Research Ethics committee.

Participants will be screened for shedding of *S*. Typhi in the stool. Stool cultures will be taken at Day 0 (challenge), daily throughout the 14 day post-challenge period and at visits after typhoid diagnosis. Participants will be required to supply 3 further stool samples until proven not to be shedding *S.* Typhi in three consecutive samples. To detect chronic carriage of *S*. Typhi, stool samples for culture will be obtained one week after completion of the antibiotic course until three samples (each taken at least 48 hours apart) are negative, according to Public Health England guidelines. Once these criteria are satisfied, the participant will be considered to be fully treated for *S.* Typhi infection and no longer an infection risk. If samples remain positive for *S.* Typhi after completion of antibiotics then the participant will be referred to a Consultant in Infectious Diseases (Oxford University Hospitals NHS Foundation Trust) for further management. No instances of stool shedding of the *S*. Typhi Quailes strain after treatment or transmission to secondary contacts were detected in previous challenge studies conducted at the Oxford Vaccine Group.

The Thames Valley Health Protection Unit (Public Health England) will be informed of the name, address and date of birth of all participants who have been challenged with *S.* Typhi, satisfy the definition of typhoid infection, have commenced and completed antibiotics, and have completed clearance stool sampling (with additional information and continued contact if persistence stool shedding occurs). The participants GP will also be notified at the time of stool shedding clearance. 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 Public Health England.

The potential for genetic exchange with any other organisms in the environment is extremely low as the GMO does not contain any plasmids or antibiotic resistance markers. *S.* Typhi is highly monomorphic, meaning there is very little genetic variation within the global *S.* Typhi population, thereby indicating that its propensity for genetic exchange is extremely low. This is supported by a study that analysed of the whole genomes of 19 *S.* Typhi strains and identified only 1,954 single nucleotide polymorphisms (SNPs) between all of them; approximately 1 every 2,300 bp. Further, very little evidence of recombination between *S.* Typhi isolates or with other bacteria was found.
Genomic insertions were rare in the sequenced isolates and evolution in the *S.* Typhi population seems to be characterised by ongoing loss of gene function caused by nonsense SNPs. All data in this study supports the hypothesis that evolution in *S.* Typhi is dominated by genetic drift and loss of gene function rather than by diversifying selection or gain of function through point mutation, recombination or acquisition of new sequences.

Whole genome sequencing confirmed the absence of the typhoid-toxin pathogenicity islet in the SB6000 GMO strain. We observed no difference between the wild type Quailes and SB6000 strains in relation to key virulence factors, including genes required for Vi-capsule expression. Differences between strains were confined to highly-variable regions encoding phage proteins, which are not predicted to impact on bacterial survival in the environment or persistence in the human host.

The risk of the GMO acquiring copies of the deleted *cdtB, pltA* and *pltB* genes to regain a typhoid toxin positive phenotype, via genetic exchange (conjugation, transduction) with a donor organism, is considered to be negligible. The GMO does not have a selective or survival advantage in the environment. The GMO is not likely to become more persistent or invasive when administered to healthy adults in the proposed clinical study and pre-clinical studies indicate that Salmonella strains lacking the typhoid toxin have reduced intestinal survival. For the safety of our study participants their health will be monitored very closely (up to daily for the first 14 days post challenge) by our clinical study team.

Risk assessment: factors affecting dissemination

Dispersal of wild type *S.* Typhi occurs via faeco-oral transmission (contamination of food or water with faeces of infected individuals). There is a short survival time for wild type *S.* Typhi in chlorinated drinking water. The GMO does not have a selective or survival advantage over wild-type *S.* Typhi and will not persist in the environment (as described in section 12c).

In this release, the GMO will be excreted directly into the sewage system and it is expected that it will be contained there to be subject to normal sewage processing treatments. It is expected, based on evaluation of shedding in previous clinical trials, that the GMO will be shed by volunteers for no longer than 17 days post-dosing.

Strict exclusion criteria have been set for the trial to minimise the risk of transmission of the GMO, and in particular to minimise transmission to potentially vulnerable

groups such as immuno-compromised individuals, pregnant women or the very young and elderly.
Volunteers will be instructed on how to maintain strict personal hygiene and proper hand washing will be taught and reinforced to minimise the risk of faecal-oral transmission.

Risk assessment: human health impact

This is the first description of the GMO and its use in a clinical study with healthy adult volunteers. No release of this GMO has been made previously. However, the wild type strain (parent) strain Quailes has been released previously in four clinical studies conducted within the UK at the Oxford Vaccine Group. All studies include a comprehensive monitoring of the safety of the wild type Quailes strain based on clinical, microbiological, hematology and biochemical measurements. In the proposed study described in this application, clinical study participants will be monitored for signs of infection with the GMO. The results of previous clinical studies using the wild type Quailes strain demonstrated no negative impact on the environment or on human health (with the exception of using enteric fever in the controlled human infection model). The potential environmental impact of the release of the GMO is thought to be zero. The GMO has confirmed sensitivity against first, second, third and fourth line antimicrobials.
Antibiotic treatment will ensure complete clearance of the GMO, which will be confirmed with collection of 3 serial stool specimens (at least 48 hours apart) upon completion of treatment.

The wild type *S.* Typhi Quailes has no known animal reservoir and it does not persist in the environment in appreciable quantities sufficient to cause disease. The GMO and the wild type strain are intended for use as an oral challenge agent in a controlled human infection model in healthy adult volunteers.

The GMO and wild type strain will be given orally to study participants who are likely to shed the organism in stool samples, which will constitute release of the organism into the sewage system. Person to person transmission will be prevented by normal basic hygiene practice (primarily the use of toilets and hand washing).

Participants will be screened for shedding of *S*. Typhi in the stool. Stool cultures will be taken at Day 0 (challenge), throughout the 14 day post-challenge period and at visits after typhoid diagnosis. Participants will be required to supply further stool samples until proven not to be shedding *S.* Typhi in three consecutive samples. To detect chronic carriage of *S*. Typhi, stool samples for culture will be obtained one week after completion of the antibiotic course until three samples (each taken at least 48 hours apart) are negative. Once these criteria are satisfied, the participant will be considered to be fully treated for *S.* Typhi infection and no longer an infection risk. If samples remain positive for *S.* Typhi four weeks after completion of antibiotics then the participant will be referred to a Consultant in Infectious Diseases (Oxford University Hospitals NHS Foundation Trust) for further management. No instances of stool shedding of the *S*. Typhi Quailes strain after treatment or transmission to secondary contacts were detected in previous challenge studies conducted at the Oxford Vaccine Group.

Risk assessment: environmental impact

S. Typhi has a very narrow host range and cannot colonise any organisms in the environment other than humans. Person to person transmission via the faecal-oral route will be minimised by issuing participants with information on enteric precautions, instructions for participants on obtaining stool specimens, provide the participant with stool sampling equipment. education of participants on correct hand washing techniques (including demonstration and observation), advise participants to inform the study team if any breaches of enteric precautions occur such that another individual comes into contact with excreta from the participant, issue participants with liquid hand soap and paper towels to aid with adherence to enteric precautions.

Strict exclusion criteria have been set for the study including criteria to minimize the risk of transmission of the GMO. Full-time, part-time or voluntary occupations involving: clinical /social work with direct contact with young children (defined as those attending pre-school groups or nursery or aged under 2 years), or clinical/social work with direct contact with highly susceptible patients or persons in whom typhoid infection would have particularly serious consequences (unless willing to avoid work until demonstrated not to be infected with *S*. Typhi in accordance with guidance from Public Health England and willing to allow study staff to inform their employer). 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. Female participants who are pregnant, lactating or who are unwilling to ensure that they or their partner use effective contraception 30 days prior to challenge and until three negative stool samples have been obtained after completion of antibiotic treatment. Full time, part time or voluntary occupations involving: commercial food handling (involving preparing or serving unwrapped foods not subjected to further heating), close household contact with: young children (defined as those attending pre-school groups, nursery or those aged less than 2 years) or individuals who are immunocompromised.

All site GMO waste will be disposed according to local GMO standard operating procedures.

Risk assessment: monitoring the GMO

Stool cultures will be taken at Day 0 (challenge), throughout the 14 day post-challenge period and at visits after typhoid diagnosis. Time to onset of stool shedding - time from challenge (Hours/Days) to the first positive stool culture, excluding the first 24 hours following ingestion of challenge agent will be documented. Participants will be required to supply further stool samples until proven not to be shedding *S.* Typhi.
Stool samples will be collected at least one week after completion of a 14 day course of antibiotics, until 3 successive stool samples are negative for *S*. Typhi. If persistent stool shedding occurs after completion of antibiotics, participants will be referred to the Infectious Diseases Consultant at the Oxford University Hospitals NHS Foundation Trust. Additionally, quantitative stool cultures or PCR may be performed 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.

Blood samples will be monitored daily for the GMO using a combination of microbiological and molecular biology techniques. The Oxford Vaccine Group has developed a fast and highly sensitive novel TSB-bile blood culture-PCR assay which has been used to detect low levels of *S.* Typhi in the blood of participants after challenge. A BACTEC 9240 continuous monitoring system will be used to culture GMO organisms which will be identified as *S*. Typhi via biochemical and serological methods. Confirmed isolates will be tested for antibiotic susceptibility using standard methods. Not routine monitoring for the GMO is planned. However should this need arise deletion of the typhoid toxin genes can be readily identified by specific methods such as PCR or nucleotide sequencing.

Following challenge with the GMO and wild type Quailes strain participants will be monitored daily for the first 14 days post-challenge. Continuous participant safety monitoring will occur throughout the challenge period through a combination of daily clinical review and monitoring of symptoms in an electronic diary. The protocol for visits will depend on whether the participant develops infection or not. Following diagnosis of enteric fever blood and stool sampling with be performed at 6, 12, 24, 48, 72 and 96 hours post diagnosis. Following completion of antibiotic treatment and confirmed clearance of the GMO in stool samples participants will be monitored a long term follow-up visits at Day 28, 90, 180 and 365.

All study participants will agree to have 24-hour contact with study staff during the four weeks post challenge and to be able to ensure that they are contactable by mobile phone for the duration of the challenge period until antibiotic completion.

An independent Data Safety Monitoring Committee (DSMC) will be established prior to the start of the study. The DSMC will be appointed to provide real-time oversight of safety and trial conduct. 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 and will particularly review the control group attack rate to confirm the challenge model is proceeding as expected. 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*. Typhi 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 TMF. The Chair of the DSMC will also be contacted for advice when the Chief Investigator feels independent advice or review is required.

Risk assessment: emergency response

The health of our study participants is of the upmost importance and will be actively and closely monitored for the duration of the study. Any symptoms will be clinically managed by the site study physicians as appropriate. Person to person transmission will be prevented by normal basic hygiene practice (primarily the use of toilets and hand washing).

Participants will be screened for shedding of *S*. Typhi in the stool. Stool cultures will be taken at Day 0 (challenge), throughout the 14 day post-challenge period and at visits after typhoid diagnosis. Participants will be required to supply 3 further stool samples until proven not to be shedding *S.* Typhi in three consecutive samples. To

detect chronic carriage of *S*. Typhi, stool samples for culture will be obtained one week after completion of the antibiotic course until three samples (each taken at least 48 hours apart) are negative.

Once these criteria are satisfied, the participant will be considered to be fully treated for *S.* Typhi infection and no longer an infection risk. If samples remain positive for *S.* Typhi four weeks after completion of antibiotics then the participant will be referred to a Consultant in Infectious Diseases (Oxford University Hospitals NHS Foundation Trust) for further management. No evidence of stool shedding of the wild type *S*. Typhi Quailes strain after treatment or transmission to secondary contacts has been detected in previous challenge studies conducted at the Oxford Vaccine Group.

The Thames Valley Health Protection Unit (Public Health England) will be informed of all participants who have been challenged with *S.* Typhi, satisfy the definition of typhoid infection, have commenced and completed antibiotics, and have completed clearance stool sampling (with additional information and continued contact if persistence stool shedding occurs). The participants GP will also be notified at the time of stool shedding clearance. 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 Public Health England.

Participants who vomit for any reason within 90 minutes of the challenge will be withdrawn from the trial and treated with antibiotics. This will be treated as an emergency spill of the GMO and standard operating procedures will be followed by the research team. Suitable personal protective equipment and disinfectants will be used to inactive the GMO. All waste will be disposed according to local GMO standard operating procedures.

Participants will be instructed to notify the study team of any serious adverse events/reactions following administration of the GMO.
All participants agree to have 24-hour contact with study staff during the four weeks post challenge and to be able to ensure that they are contactable by mobile phone for the duration of the challenge period until antibiotic completion. A physician from the clinical team will be on-call 24 hours. In addition, participants agree to allow the study team to hold the name and 24-hour contact number of a close friend, relative or housemate who will be kept informed of the study participant's whereabouts for the duration of the challenge period (from the time of challenge until completion of antibiotic course). This person will be contacted if study staff are unable to contact the participant.

Participants will be issued with a Medic Alert-type card containing information including the antibiotic sensitivity of the *S.* Typhi strain (GMO and wild type Quailes strain), study doctor contact details and instruction for the research team to be contacted immediately in the event of illness/accident.

Potential participants with known antibiotic hypersensitivity or allergy to either of the first-line antibiotics (ciprofloxacin, azithromycin or other macrolide antibiotics) will be excluded from the study. The antibiotics to be used in this study are generally well tolerated and are only occasionally associated with 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. The participant's GP will be notified in writing of the antibiotics received. Participants will

receive telephone calls or by text messages to remind them to take their antibiotic dose.

There are provisions within the protocol and site facilities to allow for admissions of participants as inpatients to the John Warin Ward (Churchill Hospital, Oxford) in cases of severe typhoid fever and/or other circumstances.

Part A5: Assessment of commercial or confidentiality of information contained in this application

Identify clearly any information that is considered to be commercially confidential. A clear justification for keeping information confidential must be given.

It is our assessment that there is no commercially sensitive information contained within this application. The information contained herein pertains to a novel strain of *Salmonella enterica* subsp. *enterica* serovar Typhi (Quailes strain) engineered to be deficient in the typhoid-toxin. The strain will be used in a controlled human infection study to be performed at the Oxford Vaccine Group (University of Oxford), Churchill Hospital, Oxford and is being delivered with other academic partners (Yale University School of Medicine). The trial protocols will be registered on publically available databases, including clinicaltrials.gov and the European Clinical Trials Database, following receipt of the necessary approvals. This study is supported by a grant from the Bill and Melinda Gates foundation.

Part A6: Statement on whether detailed information on the description of the GMO and the purpose of the release has been published

Make a clear statement on whether a detailed description of the GMO and the purpose of the release have been published, and the bibliographic reference for any information so published.
This is intended to assist with the protection of the applicant's intellectual property rights, which may be affected by the prior publication of certain detailed information, e.g. by its inclusion on the public register.

There are currently no publications providing a detailed description of the specific GMO (*S.* Typhi Quailes SB6000 strain). This is because the GMO has been generated and manufactured specifically for the purposes of undertaking the proposed controlled human infection study at the University of Oxford.

The GMO strain is derived from a wild type *S*. Typhi Quailes strain, which has been used extensively in previous typhoid challenge studies and has been modified so as to be deficient in a specific virulence factor, termed the typhoid-toxin. There is extensive literature on the typhoid toxin and converging lines of evidence suggest that typhoid toxin plays a central role in the pathogenesis of typhoid fever.

The components of the typhoid toxin (CdtB, PltA, PltB) are encoded within a pathogenicity island that is present in a limited number of *Salmonella enterica* serovars, including *S.* Typhi and *S.* Paratyphi A (the etiologic agents of typhoid and paratyphoid fever). Attenuated GM strains containing specific point mutations in typhoid toxin sub-units have been described in published pre-clinical reports. In contrast, the GMO strain contains a complete deletion of the typhoid-toxin pathogenicity islet and has not been previously reported prior to this proposed study.