I. About the Disease and Pathogen

Basic information on pathogen, including transmission, estimated global disease burden for those at risk, for morbidity and for mortality, including uncertainties/data gaps, geographical distribution, economic burden if available, age groups affected and target groups for vaccination.

Existing preventive, diagnostic and treatment measures and their limitations

The genus *Salmonella* belongs to the family of Enterobacteriaceae and are Gram-negative, non-spore-forming, facultative anaerobic bacilli (1). Collectively, these bacteria are responsible for a broad-spectrum of gastrointestinal and systemic illness including enteric fever, food-borne diarrheal illness and invasive nontyphoidal *Salmonella* (iNTS) disease. *Salmonella enterica* serovar *Typhi* (*S. Typhi*) and *Salmonella enterica* serovar *Paratyphi* (*S. Paratyphi*) A and B cause enteric fever, a febrile illness in humans but not in other animal hosts. Of the paratyphoid group, *S. Paratyphi* A is the most common serovar while *S. Paratyphi* B is presently less common, though there have been periods where *S. Paratyphi* B was the more common paratyphoid serovar. The remaining serotypes of the *Salmonella* genus comprise the group of nontyphoidal *Salmonella*, which do infect a variety of hosts and are frequently zoonotic (2).

In 2000, there were an estimated 5.4 million cases of *S. Paratyphi* A worldwide (3). The regions with the highest disease burden for paratyphoid are the same as those for *S. Typhi*, the Indian subcontinent and South East Asia. Recent data have indicated high burden of *S. Typhi* occurring in urban areas of Kenya, but no *S. Paratyphi* was identified (4). There have been reports from other locations in Africa as well including Malawi, DRC and Tanzania. Severe complications from infection with *S. Paratyphi* can occur. They include: small bowel perforation, bradycardia, meningitis, psychosis, subdural empyema, abscess of the brain, liver, spleen, psoas and thyroid, as well as osteomyelitis (5). In addition, hypotensive shock from systemic infection can occur. Chronic carriage and long-term bacterial shedding is well depicted in *S. Typhi*, but has also been described for *S. Paratyphi* A.

As patients with *S. Paratyphi* generally present with non-specific febrile illness, diagnosis is reliant on laboratory confirmation. However, there is a lack of reliable diagnostics for enteric fever, so most cases are treated without isolating or serotyping the infecting organism. The gold standard for diagnosis remains bone-marrow culture (which is 80 to 95 percent specific) as other diagnostic methods have lower levels of sensitivity and specificity. However, bone-marrow culture is rarely used and instead blood culture is performed in areas where this methodology is available. Stool culture can also be utilized as a means of case identification, but requires specialized equipment that may not be available in all endemic settings. There is an *S. Paratyphi* A test available for use in the food industry, but it has never been adapted for use in humans (5).

*S. Paratyphi* most commonly occurs in low- and middle-income countries where blood serum testing can be unavailable, prohibitively costly, or take too long for results to impact clinical care. Antimicrobial use, the volume of blood collected, and the timing of blood collection can also impact the sensitivity and specificity of blood culture. In addition, it may be difficult to obtain adequate volumes (at least 10mL to ensure sufficient outcome) of blood for culture in children under the age of five years, particularly when the bacterial burden circulating in peripheral blood is low (as low as less than one colony-forming unit/mL). The lack of a high-quality animal model has made it difficult to develop improved diagnostic testing methodologies. Researchers at ICDDR, B in Bangladesh recently developed the TPTest, which detects *Salmonella*-specific IgA responses in lymphocyte culture supernatant (6). Results of the Test can be used to detect acute infection from enteric fever; it cannot, however, differentiate between infection with *S. Typhi* or *S. Paratyphi* A. Furthermore, time to detection is presently not significantly faster than
blood culture. Another method, “MAMEF” (microwave-accelerated metal-enhanced fluorescence), is now capable of rapid diagnosis (less than 30 seconds) of *Salmonella* at very low infective loads (1 CFU/mL) (7). Although presented as research tools, neither of these tests has been used extensively in field settings nor have they been validated for implementation. Quantitative real-time PCR (qPCR) is another technique that is used to both amplify and quantify the amount of a specific strand of bacteria-specific DNA in a sample. Several qPCR-based assays are currently in development. Lastly, advanced molecular methodologies can be used to differentiate genetic similarities and differences between samples, including polymerase chain reaction (PCR) fingerprinting, pulse-field electrophoresis, and, more recently, whole-genome sequencing (8). Utility for diagnostics has yet to be demonstrated.

Individuals infected with *S. Paratyphi* respond well to antimicrobials when the bacteria are susceptible. However, antimicrobial resistance has been well documented. Specifically, resistance to naladixic acid, ciprofloxacin, and fluoroquinolone in *S. Paratyphi* has been reported (9). Furthermore, there is evidence that *S. Paratyphi A* seems to have a greater tendency than *S. Typhi* to develop antimicrobial resistance. Furthermore, when treatment is given, it can often be delayed due to the non-specific nature of clinical symptoms and the lack of a reliable test for either infection or drug resistance.

Because paratyphoid is spread by the fecal-oral route, provision of safe drinking water and hygienic food coupled with clean hygienic practices may have a long-term impact on disease prevention and may significantly affect community transmission in endemic settings. Data suggest that *S. Paratyphi A* is primarily transmitted outside of the home by consuming contaminated food. Case identification and treatment among travelers is effective in low-endemic countries (10). While the most effective means of controlling *S. Paratyphi* is through the provision of improved water supply and sanitation, the cost to improve such infrastructure in all regions endemic for *S. Paratyphi* is prohibitive. As such, vaccine development is a viable means for disease control as an adjunct to existing interventions.

**II. Overview of Current Efforts**

**A. Biological feasibility for vaccine development**

Evidence that vaccine development is biologically feasible including from development of naturally acquired immunity, from vaccine development for related pathogens, from animal models or in vitro data

Vaccines for *S. Paratyphi* are currently not available. However, microbiological similarities between serovar *S. Typhi* and *S. Paratyphi* and the fact that there are licensed, available vaccines for *S. Typhi* clearly support the biological feasibility of vaccine development for *S. Paratyphi*. Killed, whole-cell parenteral vaccine was used for several decades which included killed strains of 1,000 million *S. Typhi*, 750 million *S. Paratyphi A*, and 750 million *S. Paratyphi B* cells which provided some level of protective immunity against infection from these organisms. Although this vaccine is no longer administered due to the severity of its side effects, it attests to the possibility of vaccine-induced protection (11). More recently, cross-protective humoral immune response *in vitro* against *S. Paratyphi A* and *B* from vaccination with an oral, live typhoid vaccine using the Ty21a attenuated *S. Typhi* strain (12) was reported, although the field relevance of this finding has yet to be established. Apart from evidence from whole-cell vaccine approaches, there are efforts to pursue subunit, antigen specific approaches, as has been done for vaccines against typhoidal and nontyphoidal *Salmonella*. The lack of the surface antigen Vi in Paratyphi *A* and *B* precludes similar approaches that have been fairly successful for *S. Typhi*. However, a cross-protective effect of the *S. Typhi* Vi polysaccharide vaccine was found against *S. Paratyphi C*, which does have the Vi epitope (in some strains). Although the clinical significance of this finding is somewhat limited given the low rates of occurrence of *S. Paratyphi C*, all of this early evidence suggests that a safe and effective vaccine for *S. Paratyphi* is feasible, if the relevant antigens are defined. Both *S. Paratyphi A* and *B* share the O:12 antigen with *S. Typhi*.
**B. General approaches to vaccine development for low- and middle-income markets**

What are the scientific approaches and indications and target/age/geographic groups being pursued? What public health needs will these vaccines meet if successfully developed? Where there are several different possible indications/target groups, how much consensus is there as to prioritization between these for vaccine development in LMIC.

S. Paratyphi vaccines in development are based on whole cell live attenuated strains as well as subunit approaches that focus on the external repeating units of the lipopolysaccharide, O-antigen, with conjugation of the O polysaccharide (O:2) to a range of protein carriers, as for S. Typhi vaccines. This polysaccharide antigen has been previously described as playing a role in protection and virulence (13).

The disease is endemic in south and south East Asia, where typhoid fever is common. Therefore to address the issues of enteric fever, it could be feasible to have a vaccine that prevents enteric fever. Based on the available studies the epidemiology of paratyphoid in Africa is not clear, therefore the use of bivalent vaccine developed for both typhoid and paratyphoid may be limited to Asia only. The target age of vaccination is infants, and younger children.

**III. Technical and Regulatory Assessment**

Highlight perceived positive/negative aspects in clinical/regulatory pathways e.g. well established product development and regulatory pathway to licensure, accepted immune correlates and/or functional assays, accepted surrogate efficacy endpoints, existence of well accepted animal or challenge models, agreed trial designs and endpoints. Possibilities to develop case for correlates/surrogates should be included.

In 2013, the World Health Organization produced guidance on the regulation and prequalification of typhoid conjugate vaccines (WHO/BS/2013.2215). Although no such pathway is available for paratyphoid vaccines, this framework will likely provide a strong model for how to efficiently develop such guidelines once they are available.

There is currently no correlate of protection for S. Paratyphi, but there is an in vitro assay that is able to quantify the serum bactericidal activity (SBA) induced by S. Typhi, S. Paratyphi A, S. Typhimurium, and S. Enteritidis antibodies induced by natural infection or immunization. A correlation between antibody SBA and protection in humans against S. Paratyphi A has yet to be demonstrated. Additionally, there is no serologic correlate of protection established for S. Paratyphi A, as exists for anti-Vi and S. Typhi. No animal model exists for S. Paratyphi A infection due to human tropism. To determine vaccine efficacy in preclinical studies, a hog gastric mucin challenge model that is used for S. Typhi vaccines, and which is accepted by the US Food and Drug Administration, has been shown to be effective for S. Paratyphi A.

**IV. Status of Vaccine R&D Activities**

Summarize status of vaccine design, pre-clinical and clinical trial activity, including platforms, vectors, and adjuvants. Note academic, government, biotech and industry entities engaged. Summarize antigenic targets (if subunit approaches). Section on major advances in last 3-5 years, including key opportunities highlighted by recent science developments in the area.

Several research groups and developing-country vaccine manufacturers are in the process of developing vaccines for S. Paratyphi A and bivalent glycoconjugate vaccines, which will offer protection against both S. Typhi and S. Paratyphi A. Table 1 outlines the vaccine candidates currently under development.

The US National Institutes of Health (NIH) developed an O-specific polysaccharide (O:2) of S. Paratyphi A conjugated to tetanus toxoid (O:2-TT). In Phase 1 and 2 trials, this vaccine candidate was found to be both safe and immunogenic, although it failed to elicit a booster immune response. NIH transferred the vaccine technology to the Chengdu and Lanzhou Institutes of Biological Products in China, and the Lanzhou Institute is currently conducting further Phase 2 trials (13).
The Novartis Vaccines Institute for Global Health (NVGH), with funds from the Wellcome Trust, developed an S. Paratyphi A conjugate vaccine candidate using an O:2 conjugated to CRM$_{197}$, a nontoxic mutant of diphtheria toxin (O:2-CRM$_{197}$). This vaccine candidate has shown immunogenicity with strong serum bactericidal activity against S. Paratyphi A when delivered alone or in combination with Vi-CRM$_{197}$. The developers intend to produce a bivalent vaccine against S. Typhi and S. Paratyphi A that contains O:2 as well as Vi antigens conjugated independently to CRM$_{197}$. NVGH transferred this technology to Biological E, Ltd. in India for production and clinical development (14). In addition, NVGH is investigating the Generalized Modules for Membrane Antigens (GMMA) methodology for vaccine production, which appears promising as a possible platform for a variety of vaccines for Gram-negative bacteria, including different serovars of *Salmonella*. GMMA technology induces increased membrane blebs of small (50 to 90nm) immunogenic particles; GMMA are more immunogenic than glycoconjugate vaccines. The reactogenicity profile of GMMA is currently being evaluated in humans using a *Shigella sonnei* GMMA-based vaccine.

The Center for Vaccine Development at the University of Maryland Baltimore (UMB) has developed a live attenuated, oral vaccine candidate for S. Paratyphi A (CVD 1902). The vaccine has two independently attenuating mutations in *guaBA* and *clpX* and has been shown to be safe and immunogenic in preclinical studies (15). A single dose of CVD 1902 was also well tolerated and immunogenic in Phase 1 trials. Further Phase 1 studies are ongoing and results remain unpublished. UMB has indicated that Bharat Biotech in India will direct future vaccine production and clinical research. CVD 1902 is intended to ultimately become a part of a bivalent vaccine, along with the live attenuated CVD 909 vaccine candidate that targets S. Typhi.

The International Vaccine Institute has conjugated the O:2 of S. Paratyphi A to diphtheria toxoid (O:2-DT), with an adipic acid dihydrazide linker (16). Clinical testing has not yet commenced on this product, nor have any other project partners been announced.

### Table 1: Development Status of Current Vaccine Candidates (POC = Proof-of-concept trial)

<table>
<thead>
<tr>
<th>Candidate Name/Identifier</th>
<th>Preclinical</th>
<th>Phase I</th>
<th>Phase II</th>
<th>POC</th>
<th>Phase III</th>
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*Technology is available but is not presently being developed.

### References


