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ABSTRACT

Salmonella enterica serovars Typhi and Paratyphi (*S.* Paratyphi) A and B cause enteric fever in humans. Of the paratyphoid group, *S.* Paratyphi A is the most common serovar. In 2000, there were an estimated 5.4 million cases of *S.* Paratyphi A worldwide. More recently paratyphoid fever has accounted for an increasing fraction of all cases of enteric fever. Although vaccines for typhoid fever have been developed and in use for decades, vaccines for paratyphoid fever have not yet been licensed. Several *S.* Paratyphi A vaccines, however, are in development and based on either whole cell live-attenuated strains or repeating units of the lipopolysaccharide O-antigen (O:2) conjugated to different protein carriers. An O-specific polysaccharide (O:2) of *S.* Paratyphi A conjugated to tetanus toxoid (O:2-TT), for example, has been determined to be safe and immunogenic after one dose in Phase I and Phase II trials. Two other conjugated vaccine candidates linked to diphtheria toxin and a live-attenuated oral vaccine candidate are currently in preclinical development. As promising vaccine candidates are advanced along the development pipeline, an adequate supply of vaccines will need to be ensured to meet growing demand, particularly in the most affected countries.

Salmonella bacteria, of the Enterobacteriaceae family, comprise a group of Gram-negative, non-spore-forming, facultative anaerobic bacilli [1]. Collectively, these bacteria are responsible for a broad-spectrum of gastrointestinal and systemic illnesses that include but are not limited to enteric fever, food-borne diarrheal illness and invasive nontyphoidal *Salmonella* disease. *Salmonella enterica* serovar Typhi (*S.* Typhi) and *Salmonella enterica* serovar Paratyphi (*S.* Paratyphi) A and B cause enteric fever, a febrile illness exclusively in humans. Of the paratyphoid group, *S.* Paratyphi A is the most common serovar. The remaining *Salmonella* bacteria are mostly zoonotic nontyphoidal serotypes that tend to infect a variety of hosts [2]. In 2000, there were an estimated 5.4 million cases of *S.* Paratyphi A worldwide [3], with highest burdens on the Indian subcontinent and South East Asia. Unlike NTS and *S.* Typhi, there does not seem to be such high burden of paratyphoid in sub-Saharan Africa.

Studies from South Asia have reported an increased number of paratyphoid fever cases, but the same has not been true in other regions. For example, there is a high prevalence of *S.* Typhi in urban areas of Kenya, Malawi, Tanzania and the Democratic Republic of Congo but virtually no reports of *S.* Paratyphi [4,5]. Low prevalence, however, does not translate into low risk. Severe complications from *S.* Paratyphi infection mirror those of *S.* Typhi, including hypotensive shock, small bowel perforation, bradycardia, meningitis, osteomyelitis and multi-organ abscesses [6]. Chronic carriage and long-term bacterial shedding, well characterized for *S.* Typhi, has also been described for *S.* Paratyphi A [7]. Because patients with *S.* Paratyphi A generally present with non-specific febrile illness, diagnosis is dependent on laboratory confirmation. However, there is a relative 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 (80–95% sensitive). However, this is rarely used and is instead substituted with blood culture in areas where it is available. Stool culture can also be utilized as a means for case identification but may require a logistically challenging collection of multiple samples to increase sensitivity.

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The lack of a clinically relevant animal model has made it difficult to develop improved diagnostics. However, a number of new diagnostic technologies are being developed *de novo* or adapted from other fields. For example, researchers at the International Centre for Diarrheal Disease Research, Bangladesh recently developed the TPTest which detects *Salmonella*-specific IgA responses in lymphocyte culture supernatant [8]. Results of the TPTest can be used to distinguish enteric fever infection; it cannot, however, differentiate between *S.* Typhi and *S.* Paratyphi A. Furthermore, the time to identification is not significantly faster than blood culture. Molecular methodologies such as quantitative real-time PCR, DNA fingerprinting, pulse-field electrophoresis and, more recently, whole-genome sequencing are also being used to differentiate and quantify bacterial strains in clinical samples. The sensitivity and specificity of these diagnostics are influenced by multiple factors, including antimicrobial use, bacterial burden in the peripheral blood, the timing of blood collection and the volume of blood collected, particularly for children under the age of five years. Despite their potential, these leading-edge technologies may not be available in most resource-constrained laboratories for some time.

The strategies and challenges of preventing and treating paratyphoid are similar to those for typhoid. The treatment of *S.* Paratyphi has been complicated in recent years by the emergence of antimicrobial resistance, specifically to fluoroquinolones and naladixic acid [9,10]. There is evidence that *S.* Paratyphi A has a greater tendency toward resistance than *S.* Typhi. 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 uncontaminated food coupled with implementation of standard hygienic practices can significantly reduce transmission in endemic settings. As *S.* Paratyphi A is primarily transmitted outside of the home [11], case identification and treatment of travelers can be effective in preventing outbreaks [12]. While the most effective means of controlling *S.* Paratyphi is through the availability of clean water supplies and working sanitation services, these infrastructural changes tend to occur slowly. Given the complexities and limitations of other interventions, the development of a safe and effective vaccine remains a priority for controlling the spread of paratyphoid disease.

1. Biological feasibility and general approaches for paratyphoid vaccine development for low- and middle-income markets

Vaccines for *S.* Paratyphi are currently not available. However, microbiological similarities between serovar *S.* Typhi and *S.* Paratyphi A and the fact that there are licensed, available vaccines for *S.* Typhi support the biological feasibility for *S.* Paratyphi vaccine development. A killed, whole-cell parenteral TAB (Typhi/Paratyphi A/Paratyphi B) vaccine was used for several decades and consisted of killed strains of 1000 million *S.* Typhi, 750 million *S.* Paratyphi A, and 750 million *S.* Paratyphi B cells that provided some level of protective immunity against infection. Although this vaccine is no longer administered on account of its relatively severe side effects, the development of the vaccine attests to the possibility of vaccine-induced protection against *S.* Paratyphi [13]. *S.* Paratyphi A vaccines currently in development are primarily based on whole-cell live-attenuated strains and repeating units of the lipopolysaccharide O-antigen, (O:2) conjugated to a range of protein carriers. Recently, vaccination with the oral, live-attenuated *S.* Typhi vaccine, Ty21a strain, has been shown to elicit a humoral immune response with *in vitro* cross-reactivity against *S.* Paratyphi A and B [13]. Additional efforts are underway to develop subunit vaccines based on the lipopolysaccharide antigen that has been previously described as a virulence factor and a target of host immunity [14]. Regardless of the type of vaccine developed, the co-endemicity of paratyphoid A with typhoid fever in areas such as South and Southeast Asia, will likely require a bivalent vaccine with a focus on infants and young children.

2. Technical and regulatory assessment

In 2013, the World Health Organization published a guidance document on the regulation and prequalification of typhoid conjugate vaccines [15]. Although no such pathway has yet been developed for paratyphoid vaccines, the typhoid vaccine framework can serve as a surrogate until one is established. There is currently no immune correlate of protection identified for *S.* Paratyphi A in humans as exists for anti-Vi and protection from *S.* Typhi infection. There are, however, *in vitro* assays that quantify the positive correlation between serum antibody levels and *in vitro* bactericidal activity (SBA) induced by either natural infection or immunization. Still, there is no well-established animal model for *S.* Paratyphi A infection to evaluate pre-clinical efficacy. To address this deficiency in vaccine development, an experimental human challenge model using *S.* Paratyphi A is being developed [16] to evaluate clinical outcomes and immune response following natural infection and vaccination.

3. Status of vaccine R&D activities

Several research groups and vaccine manufacturers are in the process of developing monovalent *S.* Paratyphi A and bivalent *S.* Paratyphi A/*S.* Typhi glycoconjugate vaccines (Table 1). The US National Institutes of Health (NIH) has developed an O-specific polysaccharide (O:2) conjugated to tetanus toxoid (O:2-TT) that was found, in Phase 1 and 2 trials, to be both safe and immunogenic after one dose, although a booster antibody response was not evident after a second dose [17]. The NIH transferred the technology to the Chengdu and Lanzhou Institutes of Biological Products in China, the latter of which is currently conducting additional Phase 2 trials. The GSK Vaccines Institute for Global Health, with funds from the Wellcome Trust, developed an *S.* Paratyphi A conjugate using O:2 conjugated to CRM_{197}, a nontoxic mutant of diphtheria toxin (O:2-CRM_{197}), intended to be combined in a bivalent formulation with Vi-CRM197. This vaccine component has been shown to be immunogenic with strong SBA against *S.* Paratyphi A when delivered alone or in combination with Vi-CRM_{197}. SBVGH transferred this technology to Biological E, Ltd. in India, which intends to commercialize a bivalent vaccine—comprising Vi-CRM197 and O:2-CRM_{197}—that has activity against *S.* Typhi and *S.* Paratyphi A[18]. The International Vaccine Institute has also conjugated the O:2 of *S.* Paratyphi A to diphtheria toxoid (O:2-DT), though with an adipic acid dihydrazide linker [19]. Clinical testing of this product has not yet commenced. Finally, 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 [20]. A single dose of CVD 1902 was also well tolerated and immunogenic in Phase I trials. UMB has licensed the product to Bharat Biotech Ltd, Hyderabad, India, which will direct future vaccine production and clinical research with guidance from UMB. 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.

Table 1
Development status of current *Salmonella* Paratyphoid A vaccine candidates (POC = proof-of-concept trial).

Candidate name/identifier	Preclinical	Phase I	Phase II	POC	Phase III
O:2,12-TT + Vi-TT [NIH, Lanzhou]			X		
O:2,12-CRM_{197} + Vi-CRM_{197} [Biological E and SVGH]	X				
CVD 1902 + CVD 909 [UMB, Bharat Biotech]		X			
O:2,12-DT + Vi-DT [International Vaccine Institute]	X				

4. Likelihood for financing

Though paratyphoid fever has garnered some attention from global health funding agencies, commitment for vaccine development, licensure and deployment requires sustained efforts. The Strategic Advisory Group of Experts on Immunization, in 2011, highlighted the need for developing a combined typhoid and paratyphoid vaccine [21]. Despite these efforts, no concrete steps by the GAVI Alliance have been taken for designating a priority for vaccines against paratyphoid A, as was done for typhoid conjugate vaccines in 2008. The implementation of a Phase III clinical trial to establish efficacy the most promising *S.* Paratyphi A vaccine candidate will require funding from a variety of sources. With the increasing burden of disease from *S.* Paratyphi A, and growing attention to the identity of *Salmonella* enterica serovars responsible for invasive *Salmonella* disease, control through vaccines appears to become a significant global health priority. Whether vaccines for paratyphoid will be cost-effective as compared to other preventive interventions, will require rigorous studies once paratyphoid vaccines have shown to be efficacious.

Conflicts of interest

Dr. Martin is an employee of GlaxoSmithKline; Drs. Simon and Tennant are inventors on U.S. patents: "Broad spectrum vaccine against non-typhoidal *Salmonella*" (patent # 9,050,283) and "Broad spectrum vaccine against typhoidal and non-typhoidal Salmonella disease" (patent # 9,011,871); Dr. MacLennan is a former employee of the Novartis Vaccines Institute for Global Health and recipient of a Clinical Research Fellowship from GlaxoSmithKline; Drs. Khan and Sahastrabuddhe have no conflicts of interest.

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