This is a postprint of an article published in
Guzman, C.A., Borsutzky, S., Griot-Wenk, M., Metcalfe, I.C., Pearman, J., Collioud, A., Favre, D., Dietrich, G.
Vaccines against typhoid fever
(2006) Vaccine, 24 (18), pp. 3804-3811.

Vaccines Against Typhoid Fever

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Abstract

Because of high infectivity and significant disease burden, typhoid fever constitutes a major global health problem. Implementation of adequate food handling practices and establishment of safe water supplies are the cornerstone for the development of an effective prevention program. However, vaccination against typhoid fever remains an essential tool for the effective management of this disease. Currently, there are two well tolerated and effective licensed vaccines. One is based on defined subunit Vi polysaccharide antigen and can be administered either intramuscularly or subcutaneously and the other is based on the use of live attenuated bacteria for oral administration. The advantages and disadvantages of the various approaches taken in the development of a vaccine against typhoid fever are discussed, along with the potential for future vaccine candidates.

Key words: typhoid fever, vaccine, oral administration, Ty21a, Vi antigen

1. Typhoid Fever

Typhoid fever, which is typically characterised by fever, headache, malaise, anorexia, splenomegaly and a relative bradycardia, is an acute and often life-threatening febrile illness. The causative organism is the bacterium *Salmonella enterica* serovar Typhi; *S. enterica* Paratyphi types A and B cause paratyphoid fever, a less common but clinically similar enteric fever [1]. Ingestion of these highly infectious organisms resulted in typhoid fever in up to 55% of study volunteers who ingested 10^5 organisms [2]. As contaminated food and water are the main sources of infection, the risk of disease is highest in developing countries with poor sanitation. For this reason, industrialized country travellers to, and military personnel stationed in endemic regions in developing countries major at-risk groups. It is estimated that ~ 16 million new typhoid fever cases are recorded each year, resulting in more than 600,000 deaths [3, 4].

After ingestion of contaminated food or water, the initial stages of infection involve bacterial survival within the acidic content of the stomach, subsequent passage into the small intestine and final penetration across the mucosal barrier (Fig. 1). Following penetration of the mucosal epithelium in the non-immune host, bacteria can reach mesenteric lymph nodes via the lymphatics, and microorganisms may translocate via the blood stream to the reticuloendothelial cells of liver and spleen, in which they replicate.

Local (particularly secretory antibodies) and systemic immune responses constitute the main host defence during initial infection. An effective immune response may prevent bacteria from reaching the lamina propria, thereby blocking the subsequent stages of the infection process. Immune responses against circulating extracellular bacteria encompass anti-lipopolysaccharide (O), anti-capsular (Vi), and anti-flagella (H) antibodies [5]. However, the

acquired immune response stimulated by typhoid infection may not result in protection from relapse and re-infections. In fact, a relapse can occur in 15-20% of individuals who recover from a clinical infection and some investigators report that a few percent may not exhibit any detectable immune response after typhoid infection [6]. This suggests that *S.* Typhi may exhibit immunosuppressive traits.

Although in the past several different antibiotic therapies have been successfully employed to treat typhoid fever, the clinical management of patients is becoming increasingly difficult due to a global emergence of multi-drug resistant strains of *S.* Typhi [7]. Of particular concern is an increment in the number of resistant strains against the usually effective fluoroquinolones [8]. Furthermore, the treatment of typhoid fever is associated with considerable direct and indirect costs that place a substantial burden on national health care facilities. For example, the average cost of medical treatment in the U.S. has been calculated at U.S.$4,500 per adult case of typhoid fever [9].

Thus, to reduce the impact of typhoid fever, we are increasingly depending on achieving effective control of the disease through prevention strategies, such as improved sanitation, hygiene and the use of efficacious vaccines.

2. Types of Vaccines Used to Prevent Typhoid Fever

Different mechanisms contribute to the overall protection against infections caused by *Salmonella*. The general clearance mechanisms of the mucosal barrier (e.g., mucus, ciliary activity, lytic enzymes, pH) together with the innate immune system constitute the first non-specific line of defence against infections. The adaptive immune response represents a more refined and pathogen-specific second line of defence, which in turn leads to memory responses able to protect against subsequent challenges. *Salmonella*-specific responses

stimulated through vaccination constitute a cost-effective prevention strategy. In this context, vaccines that are able to stimulate both systemic and mucosal immune responses present several advantages. In particular, the stimulation of local responses at the portal of entry an not only protect against disease but also against infection (i.e., colonisation) and may reduce the risk of pathogen transmission to other susceptible hosts.

Different approaches have been successfully pursued to develop vaccines against *Salmonella*. These include the use of either inactivated whole-cell vaccines, live attenuated vaccines or subunit vaccines (Table 1).

2.1 Inactivated Whole Cell Vaccines Against Typhoid Fever

Parenteral whole cell typhoid vaccines were obtained by inactivating virulent microorganisms with heat or chemicals. The destruction of some heat-labile antigens during preparation was believed to compromise the overall efficacy of heat-inactivated, phenol-preserved vaccine. This problem was in part been solved by the use of acetone for inactivation, followed by drying. The establishment of appropriate quality control tests (e.g., potency and killing) was very difficult for the inactivated whole cell vaccines.

The original concept of a vaccine to protect against typhoid fever was introduced simultaneously in England and Germany in 1896 and resulted in the production of the first parenteral whole-cell vaccines (9). During the 1960s and 1970s, randomized controlled field trials of inactivated parenteral whole cell vaccines were sponsored by the World Health Organisation (WHO) in Eastern Europe and Guyana. These studies demonstrated the efficacy of vaccines consisting of *S.* Typhi inactivated by heat and phenol or by acetone ranged from 51% to 88% in children and young adults and protection persisted for up to 7 years [10, 11, 12]. Although these early parenteral vaccines were clearly able to confer protection against

typhoid fever, their global use as public health tools for routine vaccination was undermined by their high reactogenicity: the parenteral whole cell vaccines caused fever (6-30% of the recipients), headache (10%) and severe local pain (up to 35%) [13, 14]. Furthermore, up to 10% of the vaccinees missed work or school owing to the vaccination [14]. Consequently, the whole cell vaccines have been replaced by the well-tolerated Vi-based parenteral subunit vaccine and the oral attenuated live bacterial vaccine Ty21a.

2.2 Vi-based Subunit Vaccines Against Typhoid Fever

Another approach is the use of subunit vaccines that are generated using purified Vi (virulence) capsular polysaccharide of *S.* Typhi. A subunit vaccine was developed from wild type *S.* Typhi strain Ty2 on the basis of non-denatured purification of the Vi polysaccharide. Vi consists of ([alpha]1-4),2-deoxy-2-N-acetyl galacturonic acid, which is partially O-acetylated at carbon 3 and forms a capsule that protects the bacteria against complement-mediated lysis and phagocytosis [15, 16]. For vaccine production, the Ty2 cells are cultured in large-scale bioreactors. The capsular polysaccharide is precipitated from the culture supernatant, purified and vacuum dried before the antigen is resuspended in buffer. The vaccine also contains phenol as a preservative. Initial Vi purification attempts failed as the polysaccharide required for its immunogenicity was denatured [17]. Only the establishment of more gentle antigen purification processes, that retain the original structure of the polysaccharide, resulted in the successful development of potent Vi-based vaccine [18, 19]. The vaccine is administered as a single intramuscular or subcutaneous dose containing 25 µg of non-denatured Vi-antigen; revaccination is recommended every 2 years in the US [20].

Immunisation with Vi-antigen results in the induction of anti-Vi antibody titres in vaccinees in endemic and non-endemic areas (a fourfold rise in anti-Vi antibodies is defined as

seroconversion). Previous exposure to *S.* Typhi does not seem to influence the immune response [21, 22, 23]. The efficacy of the Vi polysaccharide was assessed in the late 1980s in field trials in typhoid-endemic areas in Nepal and South Africa. In South Africa, the vaccine was given to more than 11,000 children 6-14 years of age and exhibited a protective efficacy of 64% during the first 21 months after vaccination and an efficacy of 55% over three years [22, 24]. In Nepal, the vaccine was tested in 6,900 individuals 5-44 years of age and resulted in 72% protective efficacy over 17 months [21, 25]. Two weeks after immunization, about 80% of vaccinees exhibit a fourfold rise in antibody titres [20]. Hence, it is recommended that an interval of at least two weeks exist between immunisation and expected exposure. Vi capsular polysaccharide is well tolerated and safe. The most common side effects are pain, redness and induration at the injection site, and fever. In very rare cases, allergic reactions and rashes have been observed [20]. Currently, a monovalent Vi-based vaccine is available, as well as two bivalent vaccines combining Vi-antigen with a hepatitis A vaccine.

An important drawback of the parenteral administration of Vi-polysaccharide vaccines lies in their inability to stimulate mucosal immunity and the fact that revaccination does not elicit any booster effect, as shown in clinical trials [26]. This absence of a booster effect is due to the fact that immune responses against polysaccharides do not involve T cells, therefore immunological memory cannot be established. Furthermore, these vaccines are not very effective in infants or toddlers. The linkage of the T-independent Vi polysaccharide antigen to a T-dependent protein carrier molecule results in a T-dependent conjugate vaccine that can overcome these limitations. This approach has been employed successfully for the development of vaccines against *Haemophilus influenzae* type b, meningococci and. For *S.* Typhi, covalent binding of Vi polysaccharide to recombinant *Pseudomonas aeruginosa* exotoxin A (rEPA) resulted in the induction of higher and more sustained IgG antibody

responses in comparison to pure Vi polysaccharide. Enhanced immunogenicity was not only observed in adults, but also in children 5 to14 years of age and in pre-school children age 2-4 years. This vaccine also stimulated a booster response in two to four year-old children. A recent field trial conducted in Vietnam showed a two-dose immunisation schedule resulted in 92% protection of children two to five years of age [27].

2.3 Live Attenuated Oral Vaccines Against Typhoid Fever

The use of attenuated live bacterial vaccines (LBV) constitutes another typhoid vaccine strategy. Attenuated strains administered orally mimic the mucosal and systemic immune responses elicited by natural infection [28, 29]. Oral vaccination is generally associated with lower rates of side effects and higher acceptance by vaccines and can be logistically simpler [30].

The safety of attenuated live vaccines must be evaluated carefully to demonstrate that the optimal balance between safety and immunogenicity has been found. The potential risk of reversion to a virulent state can largely be overcome by the use of multiple well-defined attenuating deletion mutations. Nevertheless, regulatory authorities require a careful and exhaustive evaluation of the potential impact of environmental release of a live vaccine. Particularly relevant is to evaluate the duration and rate of shedding, the survival rate, as well as the risk of horizontal gene transfer [31].

The first human trials using attenuated strains to prevent typhoid fever were made in the early 1970s with a streptomycin-dependent mutant of serovar Typhi [32]. Oral immunisations with freshly harvested bacteria conferred approximately 80% protection against experimental challenge with *S.* Typhi. However, lyophilised preparations more amenable to large-scale manufacture were found to be less immunogenic and did not exhibit any protective efficacy

[32].

2.3.1 *S.* Typhi Ty21a

Ty21a is an attenuated mutant strain of *S.* Typhi Ty2 that is safe and protective as a live oral vaccine. This mutant was isolated in the early 1970s by chemical mutagenesis and has a GalE- and Vi-negative phenotype [33]. The mutation of the *galE* gene results in a complete deficiency of the enzyme uridine diphosphate (UDP)-galactose-4-epimerase, which is responsible for the conversion of UDP-glucose to UDP-galactose and vice versa. Because of this enzyme deficiency, UDP-galactose cannot be metabolised and accumulates in the cytoplasm to cause cell lysis and attenuation, when galactose is present in the growth medium. However, *galE* deficiency alone was found to result in premature lysis when administered to mice, thereby preventing the elicitation of an adequate immune response. Therefore, strain Ty21a resulted from a further selection for reduced levels of enzymes involved in the synthesis of UDP-galactose from exogenous galactose, namely galactose permease, galactokinase, and galactose-1-phosphate-urydiltransferase [34]. Since galactose is incorporated into the lipopolysaccharide (LPS) core moiety via UDP-galactose, the absence of *galE* leads to the formation of rough LPS, i.e., LPS devoid of part of the core and the O-antigen. Since the O-antigen is the main antigenic determinant on the cell surface, Ty21a is supplied with a source of exogenous galactose during production of the vaccine. This enables bacteria to generate UDP-galactose by an alternative route, thereby expressing complete immunogenic LPS. Thus, although the immunogenic properties of wild type *S.* Typhi are maintained when Ty21a is grown under appropriate conditions, the *galE* phenotype contributes to strain attenuation *in vivo*.

As a result of the mutagenesis method used during the generation of the vaccine strain, further

spontaneous mutations were generated. These mutations included the *via* and *ilvD* genes, leading to the loss of the Vi capsular polysaccharide and an auxotrophic phenotype for isoleucine and valine, respectively, and a mutation precluding H_2S utilisation [35]. An additional mutation in the *rpoS* gene, which also contributes to the avirulence of the Ty21a strain [36], was inherited from wild type parental strain Ty2. It is likely that the poor capacity of Ty21a to survive starvation conditions and resist various environmental stresses results, at least in part, from the *rpoS* mutation [37]. This, combined with the low shedding rate, reduces the environmental risks posed by use of Ty21a.

The Ty21a strain is the active constituent of Vivotif® (Berna Biotech Ltd, Switzerland), currently the only licensed live oral vaccine against typhoid fever [38]. During the production process of the *S.* Typhi Ty21a vaccine, bacteria are grown in large-scale fermenters under controlled conditions in medium containing a digest of yeast extract, an acid digest of casein, dextrose and galactose. The bacteria are harvested by centrifugation, mixed with a stabiliser containing sucrose, ascorbic acid and amino acids, and lyophilised. The lyophilised bacteria are mixed with lactose and magnesium stearate as excipients and filled into gelatin capsules that are coated with an organic solution to render them resistant to dissolution in stomach acid. The enteric, coated capsules are then packaged into blisters for distribution. Each capsule contains 2-10 x 10^9 (2-6 x 10^9 in the US) lyophilised live bacteria [39]. The capsules are administered orally, one hour before meals, and in three doses (four doses in the US and Canada) within a time frame of one week. The full course is recommended to be completed one week prior to potential exposure.

Protection against *Salmonella* is mediated by mucosal and serum antibodies (mucosal IgA and serum IgG), as well as cell-mediated immunity (CMI) [40]. The induction of mucosal antibodies provides protection against both infection and disease [39]. Increased serum IgG

antibodies and gut-derived IgA antibody secreting cells (ASC) against the O-antigen are the best surrogate markers of protection. Vaccination with three doses of Ty21a induces strong serum IgG and IgA responses [41]. An increase in O-specific faecal IgA was observed 1 to 8 months post immunisation [42]. Ty21a also triggers CMI, which is crucial for protection against intracellular bacterial pathogens [43]. Ty21a induced strong systemic CD4+ T-helper type 1 responses in vaccinees, characterised by the production of IFN-γ in the absence of IL-4 [41]. Vaccination with Ty21a also elicited strong CD8+ cytotoxic T cells (CTL), which persisted for at least 2 years after immunisation. A strong correlation was found between the CTL activity and the frequency of IFN-γ secreting CD8+ T cells [40].

The efficacy of Ty21a was assessed in a large number of clinical trials (Table 2), with over 500,000 vaccinated adults and children. Excellent tolerability and an overall protective efficacy of 67-80% (applying 3 doses of enteric-coated capsules or a liquid formulation) were demonstrated for up to seven years [38, 44]. The field studies conducted in Santiago (Chile) confirmed the efficacy and tolerability of Ty21a, and provided evidence of indirect protection (herd immunity) [38]. The incidence of typhoid fever fell in the placebo control group in the first field trial during the years in which Ty21a field trials were performed in other parts of Santiago and started to rise again when vaccination with Ty21a was not carried out [45, 46]. Two possible mechanisms have been suggested for the herd immunity effect of Ty21a. Firstly, individuals vaccinated with Ty21a have significantly reduced excretion of virulent *Salmonella* in comparison with the non-vaccinated population, thereby resulting in reduced contamination of water supplies. Secondly, fewer temporary carriers (i.e., children with sub-clinical or incubating acute infections) may reduce the transmission of the disease.

The optimum booster schedule for Vivotif® Vaccine has not been determined. Efficacy has been shown to persist for at least seven years. Further, there is no experience with Vivotif®

Vaccine as a booster in persons previously immunised with parenteral typhoid vaccine. In the US, it is recommended that a re-immunisation dose consisting of four vaccine capsules taken on alternate days be given every five years under conditions of repeated or continued exposure to typhoid fever [47].

The excellent safety and tolerability profile of Ty21a was further confirmed in more than 200 million vaccinees during its over 20-years use worldwide. Recent post-marketing surveillance has identified only mild and infrequent adverse events associated with Ty21a [48]. In the 10 years from 1990 to 2000, more than 38 million people were vaccinated with Ty21a with only 743 spontaneous reports of adverse events, an incidence of 0.002%. The most common adverse events reported with Ty21a were mild and transient gastrointestinal disturbances, followed by general symptoms such as pyrexia. The multiple mutations of Ty21a collectively render it genetically stable. Reversion to virulence has not been observed *in vitro* or *in vivo* [49]. This excellent safety profile is astonishing, particularly in the light of clinical data generated with a similar mutant strain [50]. The *gale,via* mutant strain EX645, which was developed by site-directed mutagenesis of the parent strain Ty2, did not exhibit the same safety profile as Ty21a. Despite the attenuated phenotype shown in mice, EX645 promoted typhoid-like disease with fever and bacteremia in two out of four volunteers in a phase 1 clinical trial [50]. The additional mutations present in Ty21a are therefore instrumental in the added safety level of Ty21a. Clinical trials have also shown either a limited and transient level or a complete lack of shedding in the stools of volunteers depending on the administered dose of Ty21a [51]. With a 10^{10} colony-forming unit (CFU) dosage (higher than commercial formulations of Vivotif®) a low rate of excretion, mainly on day one post-vaccination, was observed [49]. Further studies showed a lack of faecal excretion of Ty21a upon administration of the commercial formulation. The inability to culture Ty21a from the small intestine

suggests that the strain has a limited ability to proliferate *in vivo* [49]. Neither person-to-person transmission [52] nor invasion of the bloodstream has been observed in vaccinees [53]. The very low excretion rate of Ty21a combined with its genetic attenuation significantly reduces its ability to survive in humans and the environment.

2.3.2 Novel attenuated *S.* Typhi strains

Although there have been no compliance issues [54], the need to administer multiple doses to elicit a protective response is viewed as a drawback of Ty21a. Hence, vaccine researchers are attempting to develop novel attenuated *S.* Typhi strains that may serve as single-dose, oral typhoid vaccines. In most cases, these candidate strains have been engineered by targeted mutagenesis of *S.* Typhi Ty2. Among the most extensively evaluated vaccine candidates are mutant strains carrying mutations in the aromatic amino acid synthesis pathways. In the strain CVD 908 (Ty2 *aroC aroD*), deletions in the *aro* genes lead to an auxotrophy for aromatic amino acids, as well as for *p*-aminobenzoic acid and 2,3-dihydroxybenzoate. The mutant bacteria become attenuated under *in vivo* conditions because they are unable to scavenge these compounds in the human. Following a single oral immunisation in humans, CVD 908 was strongly immunogenic [55]. However, although well tolerated, CVD 908 resulted in silent vaccinemia in a proportion of subjects vaccinated with high doses; consequently, development of CVD 908 was discontinued. Similarly, an *aroC aroD* derivative of the serovar Typhi isolate ISP1820 (strain CVD 906), and *aroA aroD* (strain PBCC211) or *aroA aroD htrA* (strain PBCC222) derivatives of the CDC10-80 strain were found to cause fever and other adverse reactions, including vaccinemia [56]. Therefore, a further attenuating mutation was introduced into strain CVD 908 by deletion of the *htrA* gene; *htrA* encodes a periplasmic serine protease that degrades aberrant proteins during extracytoplasmic stress conditions. The *htrA* deletion attenuates *Salmonella* strains by impairing their response to stress and ability to

survive inside macrophages [57]. In clinical trials, CVD 908-*htrA* (Ty2 *aroC aroD htrA*) does not cause vaccinemia yet is highly immunogenic and constitutes a leading candidate for a single dose oral typhoid vaccine [58, 59].

Another Ty2-derivative, Ty800, harbors deletions in the *phoP-phoQ* regulon, which controls bacterial survival in phagosomes. This strain was well tolerated and immunogenic in a phase I clinical trial, eliciting anti-O-antigen IgG and IgA in young adults [60]. Another candidate tested in a Phase I clinical trial, has deletions of *cya* (encodes adenylate cyclase) and *crp* (encodes the cyclic AMP receptor protein); these two factors constitute a global regulatory system that controls a large number of virulence genes [61]. The *cya crp* mutant of S. Typhi caused clinical adverse reactions and vaccinemia. Additional mutants were generated, such as strain *X*4073, which carries mutations in both the *cya crp* regulator genes and the virulence gene *cdt* involved in bacterial dissemination from the gut to deeper tissues. This strain was shown to be safe in phase I clinical trials and elicited modest IgG and IgA antibodies against the O-antigen [62]. Finally, the recently developed strain ZH9 lacks the *aroC* and *ssvA* genes; the latter encodes a protein involved in protein secretion via a type III secretion system. This strain was also shown to be safe and immunogenic in a phase I clinical study [63].

Interestingly, none of the live bacterial vaccines developed so far induces strong immune responses against the Vi antigen. This may be due to the fine-tuned regulation of Vi expression under *in vivo* conditions. In particular, the Vi-expression seems to be down regulated after the salmonellae have gained access to the phagosomal compartments of professional antigen-presenting cells. In order to achieve stronger Vi-based immunity after immunisation with live attenuated strains, the original promoter sequence of the Vi expression cassette in strain CVD 908-*htrA* was replaced by the strong constitutive P_{tac} promoter. The resulting strain CVD 909 was found to express high levels of the Vi antigen even under

conditions mimicking the *in vivo* situation [64]. In a phase 1 clinical trial, CVD 909 was found to be safe. However, while this vaccine strain elicited strong gut-derived IgA ASC responses against Vi-antigen, only 2 out of 32 vaccinees exhibited anti IgG anti-Vi in serum [65]. Interestingly, the cell-mediated and serum IgG responses against the O- and H-antigens were comparable to those observed previously after immunization with the parent strain CVD 908-*htrA* [65].

2.4 Cross-protection Against *S.* Paratyphi

Although serovar Typhi is by far the most common causative agent of enteric fever, *S.* Paratyphi A and B cause a minority of enteric fever cases and some experts argue that a paratyphoid vaccine would be useful for disease control in some developing regions of the world [66].

Currently, epidemiological studies are being conducted to elucidate the burden of *S.* Paratyphi. Like serovar Typhi, *S.* Paratyphi is a pathogen increasingly associated with multi-drug resistance. From an immunological perspective, *S.* Paratyphi A and B lack the Vi-antigen, rendering Vi polysaccharide-based *S.* Typhi vaccines ineffective. For example, cases of typhoid fever due to infection with *S.* Paratyphi A and B were reported for travellers to India and Morocco despite immunisation with the Vi-based subunit vaccine [67]. However, *S.* Paratyphi A and B share the somatic O12-antigen with serovar Typhi, thus providing a basis for cross-protection against *S.* Paratyphi from a live serovar Typhi-specific vaccine triggering immune responses against the O12-antigen (Figure 2). Accordingly, immunologic studies have shown that volunteers vaccinated with Ty21a displayed a significant increase in cross-reactive cell-mediated immunity against *S.* Paratyphi A and B [42]. These findings support results observed during large-scale clinical trials of Ty21a [45, 68]. In a large-scale

field trial conducted in Area Occidente in Santiago (Chile) (that included more than 120,000 children), fewer cases of enteric fever due to *S.* Paratyphi B (10 cases, 45.1 cases/10^5) were observed in vaccinated children than in the placebo group (17 cases, 77.6/10^5; 45% vaccine efficacy, $p > 0.05$) [68]. In a second study with more than 90,000 children, the incidence of enteric fever due to *S.* Paratyphi B in the vaccinated group was 29 per 10^5, whereas the rate in the placebo group was 55 per 10^5 ($p > 0.05$) [45]. Therefore *S.* Typhi-based LBV, unlike Vi based vaccines, may elicit some cross-protection against *S.* Paratyphi.

3. Concluding Remarks

Many different vaccine candidates have been developed to prevent infections caused by *Salmonella* Typhi and Paratyphi in humans. Two different general approaches exhibited success leading to licensed vaccines. The first approach is based on a parenteral subunit vaccine containing the Vi-polysaccharide; the second is based on the development of attenuated *S.* Typhi strains for use as live oral vaccines. Development of the next generation typhoid vaccines aims to improve on these approaches. Thus, a Vi-conjugate vaccine elicits stronger anti-Vi responses and immunological memory. Some new attenuated strains of *S.* Typhi elicit strong immune responses following ingestion of just a single oral dose.

Acknowledgements

We are in debt to M. Rohde and S. Saftic for providing us FESEM micrographs and graphics, respectively.

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Figure Legends

Fig. 1. Field Emission Scanning Electron Microscopy of *Salmonella*-infected Host Cells. Micrograph of Cos7 cells infected with *S. enterica* serovar Typhimurium, in which bacterial-induced membrane ruffling is shown (magnification 40,000x).

Fig. 2. Surface Structures of Serovars Typhi and Paratyphi. The most important surface antigens of *Salmonella enterica* serovar Typhi are the LPS (O-antigen), flagella (H-antigen) and polysaccharide capsule (Vi-antigen), which are shown schematically. While *S. typhi* expresses the Vi-polysaccharide capsule at its surface, the serovar Paratyphi does not express capsular polysaccharide. The most important surface antigen of *S. enterica* serovar Paratyphi is the O12-antigen, which is shared with serovar Typhi.

Table 1. Comparison of currently available typhoid vaccines

	Whole cell vaccine	Vi vaccine	Ty21a vaccine
Route of administration	parenteral	parenteral	oral
Adverse systemic reactions	10-20%	2%	< 1%
Adverse local reactions	10-50%	10-40%	NA
Protection rate	~ 60 - 80%	64 - 72%	~60 - 80%
Duration of protection	7 years	> 17 - 21 months	4-7 years
Booster doses / revaccination	3 years	2-3 years	1-7 years
Medical professional needed for vaccination	yes	yes	no

Table 2. Clinical Trials Performed with Ty21a

Clinical site	Number of Subjects	Protective Efficacy	Length of protection	Reference
Egypt	32,388	96%	3 years	52
Chile	> 420,000	Up to 79%	3 years	46, 2, 38, 44, 68
		62%	7 years	
Challenge trial (challenge performed two months post vaccination)	71	87%	2 months	49