

# Article

## Safety and Immunogenicity of a Shigella Bivalent Conjugate Vaccine (ZF0901) in 3 months to 5 years old Children in China

Yi Mo <sup>1,†</sup>, Wenjian Fang <sup>2,†</sup>, Hong Li <sup>3,†</sup>, Junji Chen <sup>1</sup>, Xiaohua Hu <sup>2</sup>, Bin Wang <sup>3</sup>, Zhengli Feng <sup>1</sup>, Honghua Shi <sup>4</sup>, Ying He <sup>4</sup>, Dong Huang <sup>5</sup>, Zhaojun Mo <sup>1</sup>, Qiang Ye <sup>3</sup>, and Lin Du <sup>2,6,\*</sup>

<sup>1</sup> Guangxi Center for Disease Prevention and Control, Guangxi 530027, China; [moyi0415@hotmail.com](mailto:moyi0415@hotmail.com) (Y.M.); [chenjunji2013@163.com](mailto:chenjunji2013@163.com) (J.C.); [fengfir@163.com](mailto:fengfir@163.com) (Z.F.); [mozhi@126.com](mailto:mozhi@126.com) (Z.M.)

<sup>2</sup> Beijing Zhifei Lvzhu Biopharmaceutical Co., Ltd, Beijing 100176, China; [fangwenjian@zhifeishengwu.com](mailto:fangwenjian@zhifeishengwu.com) (W.F.); [huxiaohua@zhifeishengwu.com](mailto:huxiaohua@zhifeishengwu.com) (X.H.)

<sup>3</sup> National Institutes for Food and Drug Control, Beijing 102629, China; [lihong@nifdc.org.cn](mailto:lihong@nifdc.org.cn) (H.L.); [binwang@nifdc.org.cn](mailto:binwang@nifdc.org.cn) (B.W.); [qiangyee@nifdc.org.cn](mailto:qiangyee@nifdc.org.cn) (Q.Y.)

<sup>4</sup> Yongfu Center for Disease Prevention and Control, Guangxi 541800, China; [yfshh2006@126.com](mailto:yfshh2006@126.com) (H.S.); [heyong0217@126.com](mailto:heyong0217@126.com) (Y.H.)

<sup>5</sup> Yizhou Center for Disease Prevention and Control, Guangxi 546399, China; [15878860518@163.com](mailto:15878860518@163.com) (D.H.)

<sup>6</sup> Beijing Bacterial Vaccine Engineering Research Centre, Beijing 100176, China

\* Correspondence: [dulin@zhifeishengwu.com](mailto:dulin@zhifeishengwu.com);

† These authors contributed equally to manuscript.

**Abstract:** No licensed shigella vaccine is presently available globally. A double blinded, randomized, placebo controlled, age descending phase II clinical trial of a bivalent conjugate vaccine was studied in China. The vaccine ZF0901 consisted of O-specific polysaccharides purified and detoxified from lipopolysaccharide (LPS) of *S. flexneri* 2a and *S. sonnei* and covalently bond to tetanus toxoid. A total of 224, 310, and 434 children, consented by parents or guardians, age 3 to 6, 6 to 12 months and 1 to 5 years old respectively were injected with half or full doses, with or without adjuvant or control Hib vaccine. There were no serious adverse reactions in all recipients of ZF0901 vaccine independently of age, dosage, number of injections or the adjuvant status. Thirty days after the last injection, ZF0901 induced robust immune response with significantly higher levels of type specific serum antibodies (geometric mean concentrations (GMCs) of IgG anti-LPS) against both serotypes in all age groups compared with pre-immune or the Hib control ( $p < 0.0001$ ). Here we demonstrated that ZF0901 bivalent shigella conjugate vaccine is safe and immunogenic in infants and young children and suitable for routine immunization.

**Keywords:** shigella conjugate vaccine; bivalent; safety and immunogenicity; clinical trial; infants and young children

**Citation:** Mo, Y.; Fang, W.; Li, H.; Chen, J.; Hu, X.; Wang, B.; Feng, Z.; Shi, H.; He, Y.; Huang, D.; Mo, Z.; Ye, Q.; Du, L. Safety and Immunogenicity of a Shigella Bivalent Conjugate Vaccine (ZF0901) in 3 months to 5 years old Children in China. *Vaccines* **2021**, *9*, x.

<https://doi.org/10.3390/xxxxx>

Academic Editor: Firstname Last-name

Received: date

Accepted: date

Published: date

**Publisher's Note:** MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



**Copyright:** © 2021 by the author. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

## 1. Introduction

Shigellosis remains to be a common, serious, and increasingly difficult to treat disease, especially because of the emergence of antibiotic resistant strains [1–4]. For example, a survey in 2016 showed that there were estimated more than 269 million cases of shigellosis with over 212,000 deaths, of which about 74 million cases of shigellosis were in children younger than 5 years old and caused more than 63,000 deaths [5]. In mainland China, the annual morbidity of bacillary dysentery ranked in the top five of 39 notifiable infectious diseases according to the Chinese Center for Disease Control and Prevention (CDC) [6–8]. Although the incidence rate of bacillary dysentery has declined markedly due to the improvement of sanitation and hygiene, there were over 80,000 dysentery cases in China in the year 2020 [9]. Similar to the global trend, in China the highest incidence of Shigellosis were among children under 5 years old and the prevalent serotypes were *S. flexneri* and *S. sonnei*. Vaccine research against shigella has been

under development for several decades; unfortunately, there is so far no licensed vaccine available [10–13].

Shigella is a rod-shaped, Gram-negative pathogen. There are four species of Shigella commonly isolated from patients with shigellosis; among them *S. flexneri* and *S. sonnei* are predominant [14–16]. In Africa and Asia, *S. flexneri* and *S. sonnei* are responsible for around 90% of shigellosis according [17]. *S. flexneri* 2a is the most prominent serotype among *S. flexneri* and its conjugate induces type 2a and cross-reactive other *S. flexneri* serotypes including *S. flexneri* 6 [17,18]. We reason that a novel bivalent vaccine containing both *S. flexneri* 2a and *S. sonnei* could offer broad protection against most common pathogenic shigella serotypes.

The LPS of *Shigella* spp. is a virulence factor and its O-polysaccharide component (named O-antigen) serves as one of the promising protective antigen. Several shigella conjugate vaccines based on O-antigen have been evaluated in clinical trials [13]. For instance, an investigational vaccine against *S. sonnei* showed 74% protection in young adults [19], similarly, the vaccines against *S. flexneri* 2a serotype showed safety and immunogenicity in different age groups [20,21]. Up till present, most shigella conjugate vaccine candidates consist of a single serotype.

There are advantages of using O-antigens extracted from bacteria. The purification involved minimum physical-chemical processing and was likely to retain the native carbohydrate structures, in particular, the non-reducing end sugars. In Gram negative organisms, the immunodominant epitopes are located at the non-reducing end, this fact is best demonstrated in *Vibrio Cholerae* where the methylation of the non-reducing end is the serotyping marker that distinguishes Ogawa from Inaba serotypes [22].

The polysaccharide vaccines are known for their non-interference character between serotypes when combined as a multivalent vaccine; as demonstrated in the recently licensed pneumococcal 20-valent PCV20 [23]. Here, for the first time, an O-SP based bivalent conjugate vaccine, *S. flexneri* 2a-TT and *S. sonnei*-TT, also showed to elicit robust antibody levels to both serotypes. However, there are pro and cons of using TT as the carrier protein. TT is known to be safe in infants and shown to be an excellent carrier. However, with the increasing number of conjugate vaccines using it as carrier, there is a concern that children could be inadvertently over dosed with TT. To address this problem, currently there are other potential carrier candidates; such as the newly licensed Meningococcal B outer membrane protein FHbp, or the under developed capsid protein VP8 from another major childhood diarrhea disease [24,25].

Previously we conducted a preliminary Phase I study of a bivalent *S. flexneri* 2a and *S. sonnei* vaccine (ZF0901), consisted of O-antigens covalently bound to tetanus toxoid (TT), in an age descending order from adults to infants 3 months old (Clinicaltrials.gov identifier: NCT03561181) (unpublished). This safety screening was a prerequisite for subsequent clinical trials following the Chinese National Medical Product Administration (NMPA) guidelines. The results demonstrated that ZF0901 was safe in all studied age groups and further investigation was subsequently granted.

Here we present a phase II safety and immunogenicity study of a bivalent Shigella conjugate vaccine ZF0910 in children 3 months to 5 years old. The vaccine was administered more than 7 days apart from any routine vaccination in infants and toddlers. The effect of adjuvant and dosage (full or half dose) were also investigated.

## 2. Materials and Methods

The Investigational New Drug and One-time Certification for Clinical Investigation were approved by NMPA (No. 2015L00057 and No.2018Y36); the study protocol and informed consent were approved by the Medical Ethics Committee of Guangxi (No. GXIRB2018-0017). Written informed consent was obtained from parents or guardians of vaccinees before screening. The trial was registered at Clinicaltrials.gov (Identifier: NCT04865497). This study was undertaken in accordance with the standards of the In-

ternational Conference on Harmonization guidelines on Good Clinical Practices (ICHGCP), and followed the ethical principles established in the Declaration of Helsinki.

### 2.1. Study design and participants Clinical Protocol

The present phase II clinical trial is double-blinded, randomized and placebo-controlled study in children age 3 months to 5 years old in a single geographic region. The randomization was generated with SAS (SAS 9.4) on blind bases. The site of the study was at Yongfu County, Guangxi Zhuang Autonomous Region in the Southwestern part of China, approximately 330 Km from the regional capital Nanning. It is an agriculture-based community, population 280,000 with birthrate approximately 1.3% per year.

All potential participants must meet all of the following inclusion criteria to be eligible for enrollment into the study. Healthy children 3 months to 5 years of age whose parents or legal guardians were willing and competent to provide signed and dated written informed consent after been informed of all pertinent aspects of the study. Key exclusion criteria were children with known allergic reaction to tetanus toxoid or had a history of severe adverse reaction associated with a vaccine and/or severe allergic reaction (e.g., anaphylaxis) to any component of the study vaccine. Participating in other studies during investigation period was also excluded. Children with immunodeficiency disease, or administered with immunosuppressive agents, with serious chronic disorder, had history of craniocerebral trauma, encephalopathy and psychosis, or any other disorder that, in the investigator's opinion, were excluded from participating in the study.

Criteria for temporary delay of vaccine administration including febrile illness (axillary temperature  $\geq 38^{\circ}\text{C}$  within three days) or other acute illness within 48 hours before investigational product administration. Children with diarrhea or bloody stool within three days were also temporarily disqualified.

Children recruited were divided into three age groups: 3 to 6 months, 6 to 12 months, and 1 to 5 years old and further randomly divided into 12 subgroups of different vaccine dosage, formulation or number of injections. In cohort 1, 250 young infants, aged 3 to 6 months old, were randomly assigned (2:2:1) to receive half or full dose of the trial vaccine or Hib vaccine as placebo. Each child received three intramuscular (IM) injections on day 0, 30 and 60. In cohort 2, 350 infants, age 6 to 12 months old, were randomly assigned (2:2:2:1) to receive half or full dose vaccine, full dose vaccine without adjuvant or Hib vaccine as placebo. Each child received two injections on day 0 and 30. In cohort 3, 350 toddlers, age 1 to 5 years old, were randomly assigned (2:2:2:1) to receive **one injection** of half or full dose, full dose vaccine without adjuvant, or Hib vaccine as placebo. In order to evaluate the effect of additional injection in toddlers, a separate cohort with 100 children in this age group were also recruited to receive two injections of full dose on days 0 and 30.

Administration of vaccines and blood sample collection were performed at the investigation site by appropriately qualified, Good Clinical Practice (GCP)-trained, and vaccine-experienced member of designated study staffs (e.g., physician, nurse, physician's assistant, nurse practitioner, pharmacist, or medical assistant) as allowed by local, state, and institutional guideline. Investigational product administration details were recorded on the **case report form (CRF)**.

Before vaccination, children were examined by the health staff and their axillary temperatures measured. Those without signs of infection and who had a normal temperature received a single dose (0.5 mL) of the study vaccine administered intramuscularly into the deltoid muscle. The vaccinees were under observation in site for at least 30 minutes after injection and body temperature taken prior to returning home.

Blood samples were collected before the first and 30 days after the last injection via venipuncture.

### 2.2. Vaccine

The investigational vaccine ZF0901 was developed by Beijing Zhifei Lvzhu Biopharmaceutical Co., Ltd (Beijing, China) and manufactured in accordance with the current Good Manufacture Practices. ZF0901 consisted of the O-SP, purified from LPS of *S. flexneri* 2a or *S. sonnei*, which was covalently bound to TT through a linker adipic acid dihydrazide to form conjugates. The residual endotoxin content in the final product was lower than 10EU/ $\mu$ g polysaccharide, which met the requirements of the product approved by NMPA. The injection volume was 0.5 mL and each full dose contained 10  $\mu$ g of *S. Sonnei* O-SP, 10  $\mu$ g of *S. Flexneri* 2a O-SP and 50  $\mu$ g of TT. Those adsorbed on aluminum phosphate as adjuvant contained 0.4 mg Al<sup>3+</sup>/mL. For half dose study, each vial contained 5  $\mu$ g of *S. Sonnei* O-SP, 5  $\mu$ g of *S. Flexneri* 2a O-SP and 25  $\mu$ g of TT.

Control vaccine injected in the placebo group, Hib-TT, was a licensed commercial product (Beijing Zhifei Lvzhu Biopharmaceutical; lot 201806001) containing 10  $\mu$ g of Hib capsular polysaccharide (CP) conjugated to 24  $\mu$ g of TT. Both the investigational vaccine and the placebo were packed in single-dose prefilled syringes, labeled according to study requirement with individual tracking bar codes for blinding. The vaccines were stored at 2–8°C with temperature continuous-monitoring systems.

### 2.3. Randomization and Blinding

For randomization statistician from an independent company used SAS software (SAS 9.4) to generate randomized blind base for three age groups. Among them, 3 to 6 months old subjects were randomly assigned to three groups according to the ratio of 2:2:1; 6 to 12 months old subjects were randomly assigned to four groups according to the ratio of 2:2:2:1; 1 to 5 years old subjects were assigned to five groups according to the ratio of 2:2:2:2:1. Investigators at trial site assigned study numbers of each age group strictly according to the order of screening sequence of eligible subjects.

Parents, guardians, participants, study coordinators, study related personnel, trial investigators and the sponsor were masked to the trial-group assignments. The statisticians responsible for randomization were not allowed to participate in other related work of these clinical trials or to disclose the blind code to any personnel participating in this clinical trial. Each prefilled syringes contained a unique code that ensured appropriate masking.

The unblinding was conducted after completion of serological assays by primary investigator at the presence of National Institutes for Food and Drug Control and Center of Disease Control, Guangxi.

### 2.4. Outcomes

#### 2.4.1. Assessment of Safety

We assessed the safety endpoints in the study population, which included all participants who received at least one dose of vaccination.

Participants were observed at the site for at least 30 minutes after each injection. Guardians of participants, using a paper based memory aid, kept daily record of local and systemic adverse events 30 days following each injection, and up to 6 months for any serious adverse events. Any serious adverse reaction occurred during the 6 months after the last injection should all be reported to the local drug administration, the ethics committee, the sponsor, and the clinical trial responsible party. Laboratory personnel directly conducting the safety compilation will remain blinded until the last subject completed the 6-month visit.

#### 2.4.2. Assessment of Immunogenicity

We assessed immunogenic endpoints in the per-protocol population, which included all participants who completed their assigned vaccination schedules and with available antibody results.

Blood samples were collected before and 30 days post last vaccination. Sera were separated immediately and stored at -20°C until assayed. The specific concentrations of IgG antibody against *S. sonnei* and *S. flexneri* 2a LPS were measured by ELISA at the National Institutes for Food and Drug Control, Beijing, China. Reference sera were pooled from adult donors with high levels of anti-LPS IgG and assigned to contain 100 ELISA units (EU) anti-LPS IgG per milliliter. Antibody concentrations were expressed in EU compared with the reference sera. We calculated GMCs and corresponding 95% CIs on the basis of standard normal distribution of the antibody concentrations. The minimum detectable level is 0.01 EU for *S. flexneri* 2a, 0.01 EU for *S. sonnei*. If the comparison among groups showed significant difference, we then did pairwise comparisons. Hypothesis testing was two-sided and we considered *P* values of less than 0.05 to be significant. Seroconversion rate was defined as greater than 4-fold rises. Laboratory personnel directly conducting the immunogenicity assays remain blinded throughout sample assay and data analysis. Unblinded immunogenicity data were analyzed at completion of the last blood sample. The analyses were descriptive and informed to all participating parties for internal program development decisions and potentially support regulatory interactions.

#### 2.4.3. Statistical Analysis

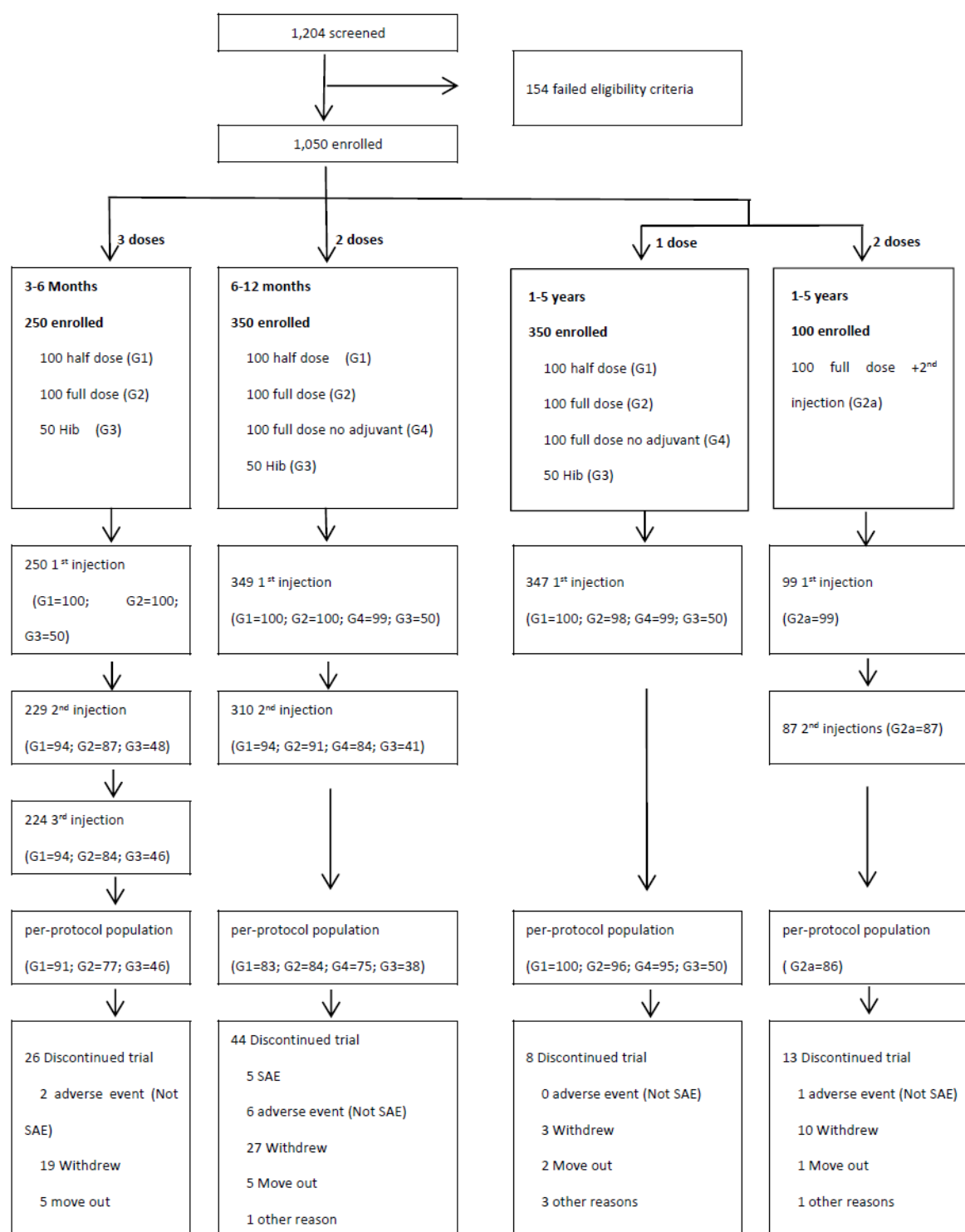
The immunogenicity against *S. sonnei* and *S. flexneri* 2a was evaluated respectively according to the anti-LPS IgG levels. In each study group, the serological conversion rate ( $\geq 4$  fold increase) was calculated individually. The two-sided 95% confidence interval was calculated by using **Clopper-Pearson method**. The chi-square test or Fisher's exact probability tests was used to test differences between the groups. If there were statistical differences between the groups, further statistical analysis would be done. Geometric mean and two-sided 95% confidence interval were used to describe the GMC of antigen specific IgG antibody after immunization and its growth multiple compared with that before immunization. Analysis of variance after logarithmic transformation was used to test the difference between the two groups.

**Medical Dictionary for Regulatory Activities (MedDRA) as a multi-axial, five-tiered hierarchical terminology was used for medical coding of adverse events and serious adverse events.** The number and incidence of all adverse events, adverse events related or not related to the vaccine in each group were calculated respectively. Fisher exact probability test was used to compare the differences between the groups. The severity, time distribution and dose distribution of adverse events and the relationship with the study vaccine were described statistically. The adverse events after each dose were statistically analyzed.

### 3. Results

#### 3.1. Study Participants

A total of 1,204 children were screened in this study, of which 154 excluded for failing the criteria, 1050 were enrolled, and 959 subjects completed the trial. The diagram depicts screening, enrollment, allocation of vaccine, and dose completion (Figure 1).



SAE: serious adverse event

Fig. 1 Study profile: subject screening, enrollment, and progress through the study

Demographic data of study participants by age groups are given in Table 1. In 3 to 6 months old group, the mean age of infants was 4.12 months (range, 3.0 to 5.9 months), there were a total of 141 (56.4%) male and 109 (43.6%) female. There were no significant differences in mean age ( $p=0.61$ ) or gender ( $p=0.67$ ) among each sub-groups. In 6 to 12 months old group, the mean age of children was 8.36 months (range, 6.0 to 11.9 months), 177 (50.7%) male and 172 (49.3%) female. There were no significant differences in gender

( $p=0.35$ ) but showed differences in mean age ( $p=0.0005$ ) among each sub-groups. In 1 to 5 years old group, the mean age of children was 2.49 years (range, 1 to 5 years), with 204 (45.7%) male and 242 (54.2%) female. There were no significant differences in mean age ( $p=0.66$ ) or gender ( $p=0.22$ ) among different vaccine cohorts.

Table 1. Demographic Characteristics of the Participants

| Table 1. Demographic Characteristics of the Participants |                      |                      |                                      |               |                   |
|----------------------------------------------------------|----------------------|----------------------|--------------------------------------|---------------|-------------------|
| 3-6 months old                                           | half dose<br>(N=100) | full dose<br>(N=100) | Hib<br>(N=50)                        |               |                   |
| Age at first vaccination — mo                            |                      |                      |                                      |               |                   |
| Mean(SD)                                                 | 4.25 (0.92)          | 4.16 (0.91)          | 4.30 (0.86)                          |               |                   |
| Median                                                   | 4.21                 | 4.09                 | 4.34                                 |               |                   |
| Min, Max                                                 | 3.0, 5.8             | 3.0, 5.9             | 3.1, 5.8                             |               |                   |
| Sex —no. (%)                                             |                      |                      |                                      |               |                   |
| male                                                     | 53 (53.00)           | 59 (59.00)           | 29 (58.00)                           |               |                   |
| female                                                   | 47 (47.00)           | 41 (41.00)           | 21 (42.00)                           |               |                   |
| 6-12 months old                                          | half dose<br>(N=100) | full dose<br>(N=100) | full dose without adjuvant<br>(N=99) | Hib<br>(N=50) |                   |
| Age at first vaccination — mo                            |                      |                      |                                      |               |                   |
| Mean(SD)                                                 | 8.59 (1.83)          | 7.71 (1.66)          | 8.66 (1.92)                          | 8.61 (1.90)   |                   |
| Median                                                   | 8.38                 | 7.11                 | 8.77                                 | 8.51          |                   |
| Min, Max                                                 | 6.0, 11.9            | 6.0, 11.8            | 6.0, 11.9                            | 6.0, 11.9     |                   |
| Sex —no. (%)                                             |                      |                      |                                      |               |                   |
| male                                                     | 44 (44.00)           | 50 (50.00)           | 55 (55.56)                           | 28 (56.00)    |                   |
| famale                                                   | 56 (56.00)           | 50 (50.00)           | 44 (44.44)                           | 22 (44.00)    |                   |
| 1-5 years old                                            | half dose<br>(N=100) | full dose<br>(N=98)  | full dose without adjuvant<br>(N=99) | Hib<br>(N=50) | 2 doses<br>(N=99) |
| Age at first vaccination — yr                            |                      |                      |                                      |               |                   |
| Mean(SD)                                                 | 2.97 (1.3)           | 3.01 (1.3)           | 3.12 (1.2)                           | 2.83 (1.1)    | 2.82 (1.5)        |
| Median                                                   | 2.91                 | 2.83                 | 3.21                                 | 2.78          | 2.84              |
| Min, Max                                                 | 1.0, 5.6             | 1.0, 5.6             | 1.1, 5.9                             | 1.1, 5.8      | 1.1, 5.6          |
| Sex —no. (%)                                             |                      |                      |                                      |               |                   |
| male                                                     | 44 (44.00)           | 37 (37.76)           | 54 (54.55)                           | 23 (46.00)    | 46 (46.46)        |
| female                                                   | 56 (56.00)           | 61 (62.24)           | 45 (45.45)                           | 27 (54.00)    | 53 (53.54)        |

SD: standard deviation

### 3.2. Adverse Reactions

No serious adverse reactions related to vaccines were noted. Fever accounted for the majority of adverse reactions (Fig. 2A).

For children age 3 to 6 months, a total of 123 (49.2%) of 250 recipients had at least one adverse reaction in all injections, among them, 48 (48%) in half dose group, 50 (50%) in full dose group and 25 (50%) in Hib group. There was no statistical difference in the incidence rate between groups by Fisher exact test ( $p=0.96$ ).

In the children age 6-12 months, 171 (49.0%) of 349 recipients had at least one adverse reaction of all injections, among them, 56 (55.5%) in half dose group, 47 (47.0%) in full dose group, 42 (42.4%) in full dose without adjuvant group and 26 (52.0%) in Hib

group. There was no statistical difference in the incidence rate between groups by Fisher exact test ( $p=0.29$ ).

In the children age 1-5 years, 118 (26.5%) of 446 recipients had at least one adverse reaction of all injections, among them, 29 (29.0%) in half dose group, 19 (19.4%) in full dose, 23 (23.2%) in full dose without adjuvant, and 17 (34.0%) in Hib group and 30 (30.3%) in full dose 2 injection group. There was no statistical difference in the incidence rate between groups by Fisher exact test ( $p=0.22$ ).

The adverse reactions also followed and compared after each injection (Fig. 2B). Similar adverse reaction rates were observed at each injection in all 3 age groups, the majority of adverse reactions were fever.

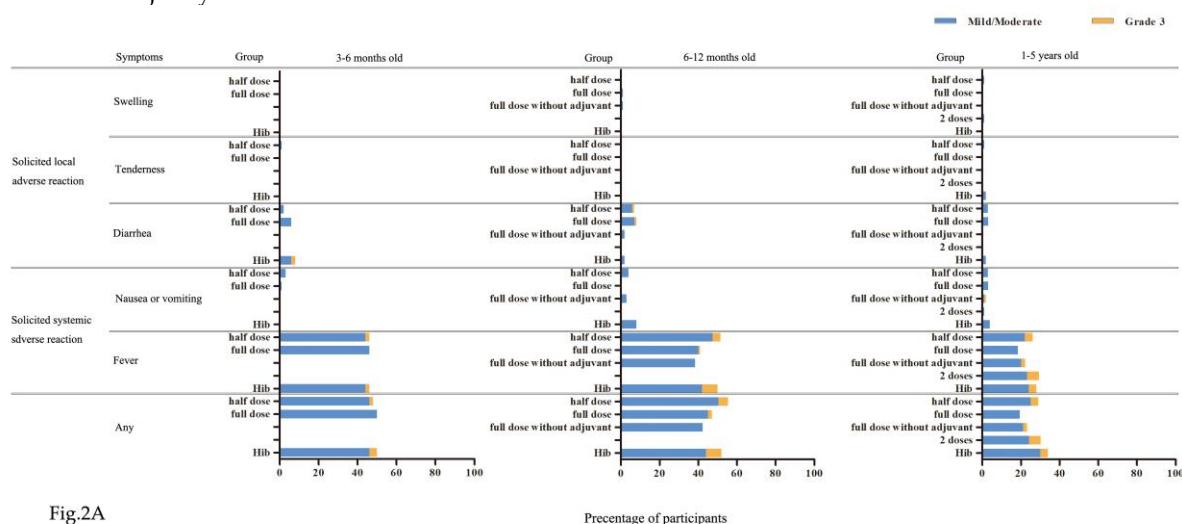


Fig.2A

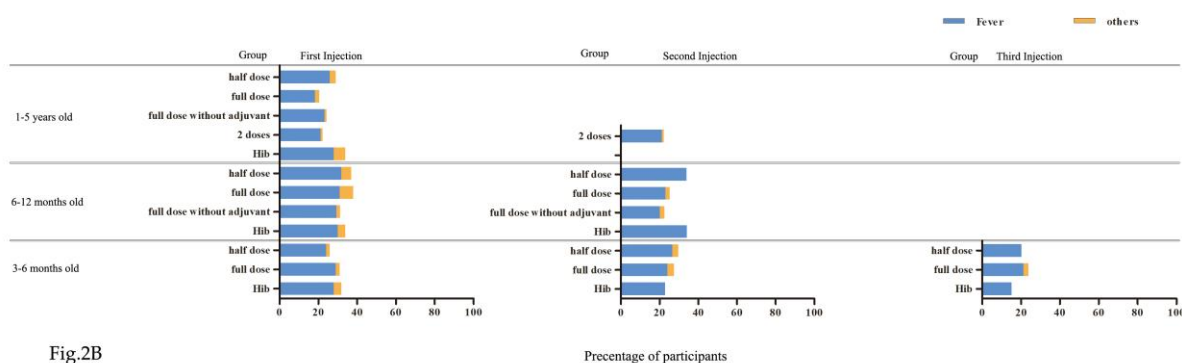


Fig.2B

Fig. 2. Incidence rate (%) of adverse reactions related to vaccination reported within 30 days after any injections, (A) Combined incidence rate according to ages. Blue bars indicate mild/moderate reactions and orange for grade 3 reactions. There is no statistical significance within the same age group receiving any type or number of vaccine injections, or between any age groups ( $p>0.5$ ). (B) Comparison of the rate (%) of adverse reactions according to the number of injections and vaccine types. Blue bars indicate fever incidences and oranges for other adverse reactions. There is no statistical significance in the incidence rate between: number of injections within the same age group, with or without adjuvant, full or half dosages ( $p>0.1$ )

### 3.3. Immunogenicity

There were detectable pre-vaccination antibodies to both *S. flexneri* 2a and *S. sonnei* LPS across the age groups (Fig. 3). The antibody titers decreased as the age increases and the difference between age groups were statistically significant (age 3 to 6 months vs 6 to 12 months or 1 to 5 years;  $p<0.001$ ; 6 to 12 months vs 1 to 5 years;  $p<0.002$ ). This pre-existing LPS antibodies declined faster with age for *S. sonnei* (all  $p<0.0001$ ).

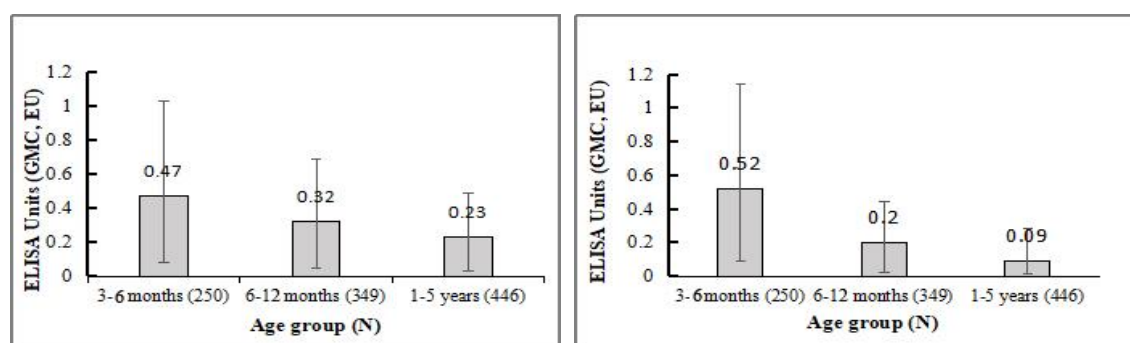


Fig. 3 Pre-vaccination GMC anti-LPS IgG in all participants

A. *S. flexneri* 2a antibody levels at day 0; B. *S. sonnei* antibody levels at day 0

### 3.3.1. 3-6 months old (Table 2)

Thirty days after the 3<sup>rd</sup> injection, more than half of the infants received ZF0901 showed a 4-fold seroconversion in both serotypes (57.7% for *S. flexneri* 2a and 60.7% for *S. sonnei*), and the conversion rates were significantly higher than the Hib control group ( $p < 0.0001$ ). There was no difference in seroconversion rate between groups receiving full or half doses of ZF0901 ( $p = 0.89$  for *S. flexneri* 2a;  $p = 0.07$  for *S. sonnei*).

GMC of serum anti-LPS IgG after 3 injections showed ZG0901 induced significantly higher response to both serotypes compared with their respective pre-immune levels (for *S. flexneri* 2a half dose, 3.68 vs 0.45, or full dose 2.85 vs 0.48,  $p < 0.0001$ ; for *S. sonnei* half dose, 5.67 vs 0.43, or full dose 3.23 vs 0.60,  $p < 0.0001$ ) or group received Hib ( $p < 0.0001$ ). When comparing the dosage effect, there was no statistically significant difference between groups received full or half dose for *S. flexneri* 2a (3.68 vs 2.85,  $p = 0.24$ ). However, for *S. sonnei* the group received half dose responded with higher GMC anti-bodies than those received the full dose (5.67 vs 3.23,  $p = 0.01$ ).

Table 2 Serum anti-LPS IgG levels in children age 3-6 months old vaccinated with half or full dose of the trial vaccine ZF0901

| 3-6 months old        |                     | half dose (n=91)     | full dose (n=77)     | Hib (n=46)         |
|-----------------------|---------------------|----------------------|----------------------|--------------------|
| <i>S. flexneri</i> 2a |                     |                      |                      |                    |
| Con (EU/ml)           | Conversion rate (%) | 58.24 (47.43, 68.50) | 57.14 (45.35, 68.37) | 4.35 (0.53, 14.84) |
|                       | Pre-                | 0.45 (0.34, 0.59)    | 0.48 (0.35, 0.65)    | 0.52 (0.36, 0.74)  |
|                       | Post-               | 3.68 (2.65, 5.10)    | 2.85 (2.19, 3.70)    | 0.23 (0.16, 0.33)  |
|                       | Fold rise           | 8.26 (4.96, 13.73)   | 5.99 (3.67, 9.76)    | 0.45 (0.33, 0.62)  |
| <i>S. Sonnei</i>      |                     |                      |                      |                    |
| Con (EU/ml)           | Conversion rate (%) | 67.03 (56.39, 76.53) | 53.25 (41.52, 64.71) | 4.35 (0.53, 14.84) |
|                       | Pre-                | 0.43 (0.33, 0.57)    | 0.60 (0.43, 0.83)    | 0.58 (0.40, 0.84)  |
|                       | Post-               | 5.67 (4.17, 7.73)    | 3.23 (2.37, 4.41)    | 0.25 (0.18, 0.34)  |
|                       | Fold rise           | 13.18 (7.78, 22.34)  | 5.38 (3.02, 9.61)    | 0.42 (0.31, 0.59)  |

### 3.3.2. 6-12 months old (Table 3)

More than half of the children in this age group received ZF0901 responded with 4-fold seroconversion to *S. flexneri* 2a (64.9%) and *S. sonnei* (86.4%) after 2 injections, and the seroconversion rates were significantly higher than those in the Hib control group ( $p < 0.0001$ ). The comparison between groups vaccinated with ZF0901 (half or full dose, or vaccine without adjuvant) showed no statistically significant difference in seroconversion rate across groups (all  $p > 0.4$ ).

The levels of serum GMC anti-LPS IgG in children injected with ZF0901 were significantly higher than those in pre-vaccination or the Hib group ( $p < 0.001$ ). There were no significant differences in GMC between the groups receiving full or half dose with the

half dose group responded slightly higher: for *S. flexneri* 2a, half vs full, 4.26 vs 3.08,  $p=0.17$ ; for *S. sonnei*, 6.85 vs 5.63,  $p=0.32$ . When comparing the antibody response in group injected with ZF0901 containing no adjuvant, it is noticeable that this group had lower antibody levels than groups with adjuvant. Comparing the adjuvant effect in serotype *S. flexneri* 2a: half & full without adjuvant, 4.26 vs 2.59,  $p=0.06$ ; full & full without adjuvant, 3.08 vs 2.59,  $p=0.52$ . Similar trend was found for *S. sonnei* (half & full without adjuvant, 6.85 vs 6.01,  $p=0.49$ ; full & full without adjuvant 5.63 vs 6.01,  $p=0.75$ ).

Table 3. Immunological response for children aged 6–12 months old with different vaccines

| 6–12 months old       |                     | half dose (n=83)     | full dose (n=84)     | full dose without adjuvant (n=75) | Hib (n=38)          |
|-----------------------|---------------------|----------------------|----------------------|-----------------------------------|---------------------|
| <i>S. flexneri</i> 2a |                     |                      |                      |                                   |                     |
|                       | Conversion rate (%) | 66.27 (55.05, 76.28) | 64.29 (53.08, 74.45) | 64.00 (52.09, 74.77)              | 15.79 (6.02, 31.25) |
| Con (EU/ml)           | Pre-                | 0.36 (0.27, 0.48)    | 0.30 (0.24, 0.39)    | 0.30 (0.23, 0.40)                 | 0.29 (0.18, 0.46)   |
|                       | Post-               | 4.26 (3.07, 5.91)    | 3.08 (2.22, 4.28)    | 2.59 (1.69, 3.97)                 | 0.42 (0.28, 0.65)   |
|                       | Fold rise           | 11.83 (7.99, 17.52)  | 10.15 (6.97, 14.77)  | 8.52 (5.61, 12.94)                | 1.48 (1.09, 2.00)   |
| <i>S. Sonnei</i>      |                     |                      |                      |                                   |                     |
|                       | Conversion rate (%) | 89.16 (80.41, 94.92) | 84.52 (74.99, 91.49) | 85.33 (75.27, 92.44)              | 10.53 (2.94, 24.80) |
| Con (EU/ml)           | Pre-                | 0.19 (0.14, 0.24)    | 0.23 (0.17, 0.30)    | 0.22 (0.17, 0.30)                 | 0.16 (0.11, 0.24)   |
|                       | Post-               | 6.85 (5.28, 8.89)    | 5.63 (4.22, 7.50)    | 6.01 (4.57, 7.90)                 | 0.20 (0.15, 0.27)   |
|                       | Fold rise           | 36.47 (24.13, 55.11) | 24.64 (15.96, 38.04) | 27.02 (17.63, 41.42)              | 1.25 (0.94, 1.66)   |

### 3.3.3. 1 to 5 years old (Table 4)

Most of the ZF0901 recipients showed 4-fold seroconversion after vaccination (89.4% for *S. flexneri* 2a, 95.5% for *S. sonnei*) and the conversion rates were significantly higher than the control Hib group ( $p<0.0001$ ). The rates were similar among vaccine groups: in difference dosages, with or without adjuvant (*S. flexneri* 2a: half & full  $p=0.09$ ; half & full without adjuvant  $p=0.96$ ; full & full without adjuvant  $p=0.10$ , *S. sonnei*: half & full,  $p=1.00$ ; half & full without adjuvant,  $p=1.00$ ; full & full without adjuvant,  $p=1.00$ ).

For both *S. flexneri* 2a and *S. sonnei*, the GMC anti-LPS IgG levels in all ZF0901 groups were significantly higher than the levels in pre-vaccination or Hib group ( $p<0.0001$ ). There was a dosage effect in antibody response for *S. flexneri* 2a, the group receiving full dose elicited significantly higher antibodies than the half dose group (8.06 vs 4.64,  $p=0.03$ ). In contrast, the dosage effect was not obvious for *S. sonnei* (full vs half, 9.06 vs 7.59,  $p=0.47$ ). The effect of alum adjuvant was also distinctive between the two serotypes. The *S. flexneri* 2a antibody concentration was much higher in the group injected with vaccine containing adjuvant compared with the group without (8.06 vs 5.10,  $p=0.04$ ), however the effect was reversed for *S. sonnei* (with adjuvant vs without, 9.06 vs 16.64,  $p=0.02$ ).

We included a separate cohort to evaluate the effect of additional injection given 30 days after the first. The GMC anti-LPS IgG were higher in recipients after the second injection (for *S. flexneri* 2a, 17.03 vs 8.06,  $p=0.0006$ ; *S. Sonnei*, 13.5 vs 9.06,  $p=0.10$ ), but the 4-fold conversion rates compared with single injection group were not statistically significant (for *S. flexneri* 2a,  $p=0.46$ ; *S. Sonnei*,  $p=0.72$ ).

Table 4. Immunological response for children aged 1–5 years old with different vaccines &amp; regimens

| 1–5 years old         | half dose<br>(n=100) | full dose (n=96) | full dose without<br>adjuvant (n=95) | Hib (n=50) | 2 doses (n=86) |
|-----------------------|----------------------|------------------|--------------------------------------|------------|----------------|
| <i>S. flexneri</i> 2a |                      |                  |                                      |            |                |

|                  |           |                     |                       |                        |                         |                    |                         |
|------------------|-----------|---------------------|-----------------------|------------------------|-------------------------|--------------------|-------------------------|
|                  |           | Conversion rate (%) | 85.00 (76.47, 91.35)  | 92.71 (85.55, 97.02)   | 85.26 (76.51, 91.70)    | 4.00 (0.49, 13.71) | 95.35 (88.52, 98.72)    |
| Con (EU/ml)      | Pre-      |                     | 0.18 (0.13, 0.25)     | 0.23 (0.17, 0.32)      | 0.24 (0.17, 0.33)       | 0.28 (0.18, 0.42)  | 0.25 (0.18, 0.34)       |
|                  | Post-     |                     | 4.64 (3.17, 6.82)     | 8.06 (5.84, 11.13)     | 5.10 (3.81, 6.83)       | 0.33 (0.20, 0.54)  | 17.03 (13.04, 22.24)    |
|                  | Fold rise |                     | 25.75 (18.11, 36.63)  | 35.04 (25.83, 47.53)   | 21.50 (15.58, 29.67)    | 1.20 (0.88, 1.64)  | 68.15 (47.87, 97.02)    |
| <i>S. Sonnei</i> |           |                     |                       |                        |                         |                    |                         |
|                  |           | Conversion rate (%) | 95.00 (88.72, 98.36)  | 94.79 (88.26, 98.29)   | 95.79 (89.57, 98.84)    | 6.00 (1.25, 16.55) | 96.51 (90.14, 99.27)    |
| Con (EU/ml)      | Pre-      |                     | 0.09 (0.07, 0.11)     | 0.08 (0.06, 0.10)      | 0.11 (0.08, 0.14)       | 0.09 (0.07, 0.13)  | 0.09 (0.07, 0.12)       |
|                  | Post-     |                     | 7.59 (5.49, 10.50)    | 9.06 (6.28, 13.09)     | 16.64 (11.34, 24.42)    | 0.15 (0.10, 0.22)  | 13.52 (10.01, 18.26)    |
|                  | Fold rise |                     | 85.99 (59.79, 123.68) | 112.06 (76.16, 164.87) | 158.03 (102.69, 243.22) | 1.55 (1.22, 1.96)  | 146.01 (103.11, 206.74) |

### 3.3.4. Antibody response in all 3 age groups

We compared the immune response across all 3 age groups following the designated immunization dosage and schedule (Fig. 4). The GMC anti-LPS IgG levels for both *S. flexneri* 2a and *S. sonnei* showed an age-dependent response. The specific IgG level was greater in children 1 to 5 years comparing with other age groups, especially in children immunized with full dose. In groups received full dose with adjuvant, statistical differences could be seen between 1 to 5 years group and the younger groups (for *S. flexneri* 2a, 3-6 mo vs 1-5 yr,  $p<0.0001$ , 6-12 mo vs 1-5 yr,  $p=0.03$ ; *S. Sonnei*, 3-6 mo vs 1-5 yr,  $p=0.001$ , 6-12 mo vs 1-5 yr,  $p=0.005$ ). Comparing groups received full dose without adjuvant, the GMC anti-LPS IgG were also higher in 1 to 5 years children than younger groups, but was only significant for *S. Sonnei* ( $p<0.0001$ ).

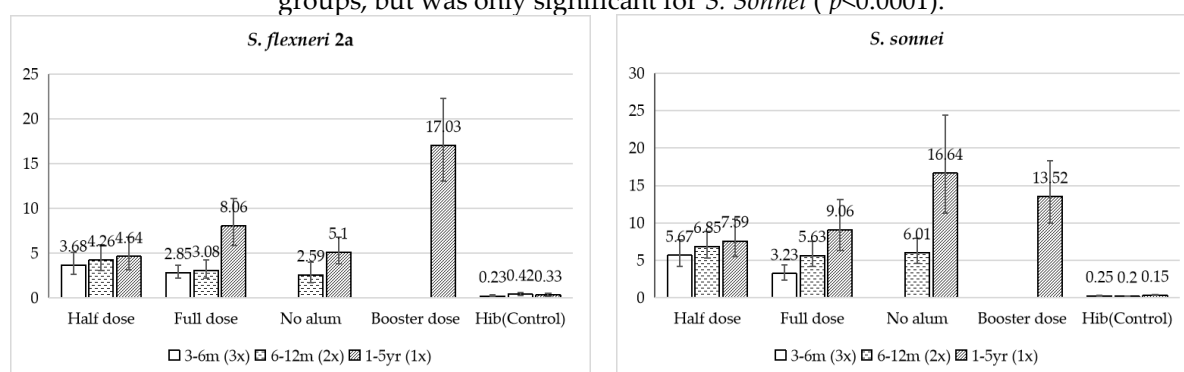


Fig. 4. Composite chart of GMC anti-LPS IgG. (A) *S. flexneri* 2a (B) *S. sonnei*

## 4. Discussion

There appears to be a strong correlation between the level of serum lipopolysaccharide (LPS)-specific IgG antibodies and serotype-specific immunity against shigella [12]. In the current pipeline of vaccine development, a number of O-antigen based conjugate vaccines were in clinical trials and showed promising immunity against the disease, including: bioconjugate vaccine [26,27], synthetic vaccine [28] and detoxified LPS conjugate

vaccines [29–31]. We designed a bivalent conjugate vaccine ZF0901 to elicit high levels of long-lived serum IgG O-SP antibodies to both *S. flexneri* 2a and *S. sonnei* that is suitable for routine immunization.

This study showed that ZF0901 was safe and immunogenic against both serotypes in neonatal and young children. There were no serious adverse reactions in all recipients of conjugate vaccine ZF0901 independent of age, number of injections or the adjuvant status. Fever was the most common side reaction in all age groups, over all 35.8% vs 41.3% ( $p>0.2$ ) in vaccinated participants and the Hib controls. The rate of fever was similar in all groups independent of the adjuvant status or number of injections. In all 3 age groups the overall incidence rate of diarrhoea, nausea/vomiting and dysphoria/irritability were 3.7%, 1.9%, and 1.8% in vaccine groups and were not different from the Hib control. The unsolicited ADRs with incidence rates of 0.4% in ZF0901 vaccinees and were not statistically different from Hib control.

ZF0901 vaccine elicited statistically significant rise of type specific antibodies against *S. flexneri* 2a and *S. sonnei* 30 days post-immunization in all vaccine groups. More than 50% of the recipients had >4-fold seroconversion in all age groups regardless of dosage or number of injections. There was no significant dosage effect observed. There seems to be an indication that the effect of alum adjuvant on immune response is opposite in 6–12 months old group and in 1–5 years old group, but the differences were not statistically significant.

We have found that the pre-immune sera contained pre-existing *S. flexneri* 2a and *S. sonnei* IgG antibodies in all ages and the titers declined as the age increased, as observed commonly in many other infectious diseases [32–34]. This observation indicated that mothers located in this endemic region probably had been exposed to shigella and in turn passively transferred shigella IgG antibodies to neonates and thus most subjects in the study were not immunologically naive. **Of course, this exposure is random, resulting in a much diverse in antibody level and a relatively high SD value.** If following the theory that serum *Shigella* antibody levels may constitute a surrogate for an as yet uncharacterized mechanistic intestinal protective response, then it could also explain the epidemiology finding that the highest incidence rate of shigellosis in GuangXi is between 1 to 5 years old children as the maternal antibodies waned [35,36]. We also noticed that there seems to be a “reversed” dosage effect in the younger groups, namely the half-dose infants responded with slightly higher GMT than those received the full dose. We have no satisfactory explanation except that it could possibly be due to the interference of seroconversion by maternal antibodies or immune tolerance in young infants [37].

There are several limitations of this study. The evaluation of ZF0901 immune response was based on the analysis of serum anti-LPS IgG and we did not perform other immunological analysis such as IgA isotype gut-homing anti-LPS antibody-secreting cells or other cellular immunities. The rationale of focusing on serum IgG was adopted from the well-established evidence of licensed polysaccharide vaccines (e.g., Hib, pneumococcal and typhoid conjugate vaccines) that the critical correlation of disease protection is the level of serum IgG elicited by the vaccine. We only compared the antibody levels between pre-immune and 30 days after the final injection. We did not follow longitudinal immune response which may be of interests, especially for children under 1 year old received multiple injections. The reason we focused on these two time points was again coming from experience of licensed polysaccharide vaccines that polysaccharide antibodies approached a decent level only after the final booster shots (three times for younger and 2 times for older infants). We plan to collect and analyze serum samples in a later date to gather additional information on antibody persistency. Lastly, since the purpose of this study was on *Shigella* immunity, we therefore did not analyze TT antibody levels.

## Conclusion

Bivalent *Shigella* conjugate vaccine ZF0901 showed to be safe and immunogenic in infants and young children. This locally manufactured vaccine candidate, following the precedent example of Vi typhoid vaccine, could potentially reduce the burden of diarrhea diseases in China and other high endemic regions [38]. The acceptable safety profile supports the vaccine candidate moving forward to vaccine efficacy trial, the immunological results for evaluating vaccine dosing and regimens provide guidance for next step clinical design.

**Author Contributions:** Conceptualization, Wenjian Fang and Lin Du; Investigation, Yi Mo, Hong Li, Junji Chen, Bin Wang, Zhengli Feng, Honghua Shi, Ying He, Dong Huang, Zhaojun Mo and Qiang Ye; Methodology, Yi Mo, Zhaojun Mo and Lin Du; Project administration, Yi Mo, Wenjian Fang, Zhaojun Mo and Lin Du; Resources, Lin Du; Supervision, Wenjian Fang, Xiaohua Hu and Lin Du; Writing – original draft, Lin Du; Writing – review & editing, Yi Mo, Wenjian Fang, Hong Li, Junji Chen, Xiaohua Hu, Bin Wang, Zhengli Feng, Honghua Shi, Ying He, Dong Huang, Zhaojun Mo, Qiang Ye and Lin Du.

### Acknowledgement

We would like to thank Dr. Rachel Schneerson and the late Dr. John B. Robbins for their initiation and guidance of this project.

### References

- Kotloff KL, Riddle MS, Platts-Mills JA, Pavlinac P, Zaidi AKM. Shigellosis. Lancet. 2018 Feb 24;391(10122):801-812. doi: 10.1016/S0140-6736(17)33296-8. Epub 2017 Dec 16.
- Baker S, The HC. Recent insights into Shigella. Curr Opin Infect Dis. 2018 Oct;31(5):449-454. doi: 10.1097/QCO.0000000000000475.
- Zachariah OH, Lizzy MA, Rose K, Angela MM. Multiple drug resistance of *Campylobacter jejuni* and *Shigella* isolated from diarrhoeic children at Kapsabet County referral hospital, Kenya. BMC Infect Dis. 2021 Jan 23;21(1):109. doi: 10.1186/s12879-021-05788-3.
- SalimiyanRizi K, Farsiani H, SasanMS. High rate of resistance to ceftriaxone and azithromycin among *Shigella* spp. isolates at three children's referral hospitals in Northeast Iran. J Infect Chemother. 2020 Sep;26(9):955-958. doi: 10.1016/j.jiac.2020.04.022. Epub 2020 May 20.
- Khalil IA, Troeger C, Blacker BF, Rao PC, Brown A, Atherly DE, Brewer TG, Engmann CM, Houpt ER, Kang G, Kotloff KL, Levine MM, Luby SP, MacLennan CA, Pan WK, Pavlinac PB, Platts-Mills JA, Qadri F, Riddle MS, Ryan ET, Shoultz DA, Steele AD, Walson JL, Sanders JW, Mokdad AH, Murray CJL, Hay SI, Reiner RC Jr. Morbidity and mortality due to shigella and enterotoxigenic *Escherichia coli* diarrhoea: the Global Burden of Disease Study 1990–2016. Lancet Infect Dis 2018 Nov;18(11):1229-1240. doi: 10.1016/S1473-3099(18)30475-4. Epub 2018 Sep 25.
- Liu Z, Tong MX, Xiang J, Dear K, Wang C, Ma W, Lu L, Liu Q, Jiang B, Bi P. Daily Temperature and bacillary dysentery: estimated effects, attributable risks, and future disease burden in 316 Chinese cities. Environ Health Perspect. 2020 May;128(5):57008. doi: 10.1289/EHP5779. Epub 2020 May 26.
- Zhang H, Si Y, Wang X, Gong P. Patterns of bacillary dysentery in China, 2005-2010. Int J Environ Res Public Health. 2016 Jan 27;13(2):164. doi: 10.3390/ijerph13020164.
- Chang Z, Zhang J, Ran L, Sun J, Liu F, Luo L, Zeng L, Wang L, Li Z, Yu H, Liao Q. The changing epidemiology of bacillary dysentery and characteristics of antimicrobial resistance of *Shigella* isolated in China from 2004-2014. BMC Infect Dis. 2016 Nov 18;16(1):685. doi: 10.1186/s12879-016-1977-1.
- China CDC. National data of class A, B and C communicable diseases in December 2020. Ji Bing Jian Ce. 2021, 36(1): 1 <https://doi.org/10.3784/j.issn.1003-9961.2021.01.001>.
- Mani S, Wierzba T, Walker RI. Status of vaccine research and development for *Shigella*. Vaccine. 2016 Jun 3;34(26):2887-2894. doi: 10.1016/j.vaccine.2016.02.075. Epub 2016 Mar 12.
- BöhlesN, BöhlesN, Busch K, Busch K, Hensel M, Hensel M. Vaccines against human diarrheal pathogens: current status and perspectives. Hum Vaccin Immunother. 2014;10(6):1522-35. doi: 10.4161/hv.29241. Epub 2014 May 26.
- Barry E, Cassels F, Riddle M, Walker R, Wierzba T. Vaccines Against *Shigella* and *Enterotoxigenic Escherichia coli*: A summary of the 2018 VASE Conference. Vaccine. 2019 Aug 7;37(34):4768-4774. doi: 10.1016/j.vaccine.2019.02.070.
- Barel LA, Mulard LA. Classical and novel strategies to develop a *Shigella* glycoconjugate vaccine: from concept to efficacy in human. Hum Vaccin Immunother. 2019;15(6):1338-1356. doi: 10.1080/21645515.2019.1606972.
- Anderson M, Sansonetti PJ, Marteyn BS. *Shigella* Diversity and Changing Landscape: Insights for the Twenty-First Century. Front Cell Infect Microbiol. 2016 Apr 19;6:45. doi: 10.3389/fcimb.2016.00045. eCollection 2016.
- Nisa I, Qasim M, Yasin N, Ullah R, Ali A. *Shigella flexneri*: an emerging pathogen. Folia Microbiol (Praha). 2020 Apr;65(2):275-291. doi: 10.1007/s12223-020-00773-w. Epub 2020 Feb 5.

16. Shad AA, Shad WA. *Shigella sonnei*: virulence and antibiotic resistance. Arch Microbiol. 2021 Jan;203(1):45-58. doi: 10.1007/s00203-020-02034-3. Epub 2020 Sep 14.
17. Livio S, Strockbine NA, Panchalingam S, Tennant SM, Barry EM, Marohn ME, Antonio M, Hossain A, Mandomando I, Ochieng JB, Oundo JO, Qureshi S, Ramamurthy T, Tamboura B, Adegbola RA, Hossain MJ, Saha D, Sen S, Faruque AS, Alonso PL, Breiman RF, Zaidi AK, Sur D, Sow SO, Berkeley LY, O'Reilly CE, Mintz ED, Biswas K, Cohen D, Farag TH, Nasrin D, Wu Y, Blackwelder WC, Kotloff KL, Nataro JP, Levine MM. *Shigella* Isolates from the Global Enteric Multicenter Study inform vaccine development. Clin Infect Dis. 2014 Oct 1; 59(7): 933–941.doi: 10.1093/cid/ciu468. Epub 2014 Jun 23.
18. Farzam N, Ramon-Saraf R, Banet-Levi Y, Lerner-Geva L, Ashkenazi S, Kubler-Kielb J, Vinogradov E, Robbins JB, Schneerson R. Vaccination with *Shigella flexneri* 2a conjugate induces type 2a and cross-reactive type 6 antibodies in humans but not in mice. Vaccine. 2017 Sep 5;35(37):4990-4996. doi: 10.1016/j.vaccine.2017.07.070. Epub 2017 Aug 7.
19. Cohen D, Ashkenazi S, Green MS, Gdalevich M, Robin G, Slepon R, Yavzori M, Orr N, Block C, Ashkenazi I, Shemer J, Taylor DN, Hale TL, Sadoff JC, Pavliakova D, Schneerson R, Robbins JB. Double-blind vaccine-controlled randomised efficacy trial of an investigational *Shigella sonnei* conjugate vaccine in young adults. Lancet. 1997;349:155–59.doi:10.1016/S0140-6736(96)06255-1.
20. Passwell JH, Harlev E, Ashkenazi S, Chu C, Miron D, Ramon R, Farzan N, Shiloach J, Bryla DA, Majadly F, Roberson R, Robbins JB, Schneerson R. Safety and immunogenicity of improved *Shigella* O-specific Safety and immunogenicity of improved *Shigella* O-specific polysaccharide-protein conjugate vaccines in adults in Israel. Infect Immun.2001;69:1351–57. doi:10.1128/IAI.69.3.1351-1357.2001.
21. Passwell JH, Ashkenazi S, Harlev E, Miron D, Ramon R, Farzam N, Lerner-Geva L, Levi Y, Chu C, Shiloach J, Robbins JB, Schneerson R; Israel *Shigella* Study Group. Safety and immunogenicity of *Shigella sonnei*-CRM9 and *Shigella flexneri* type 2a-rEPAsucc conjugate vaccines in one- to four-year-old children. Pediatr Infect Dis J. 2003;22:701–06.doi:10.1097/01.inf.0000078156.03697.a5.
22. Lei PS, Ogawa Y, Flippen-Anderson JL, Kovác P. Synthesis and crystal structure of methyl 4-6-dideoxy-4-(3-deoxy-L- glyco-ero-tetronamido)-2-O-methyl-alpha-D-mannopyranoside, the methy alpha-glycoside of the terminal unit, and presumed antigenic determinant, of the O-specific polysaccharide of *Vibrio cholera* O:1, serotype Ogawa. Carbohydr Res. 1995 Sep 15;275(1):117-29.doi: 10.1016/0008-6215(95)00147-1.
23. Klein NP, Peyrani P, Yacisin K, Caldwell N, Xu X, Scully IL, Scott DA, Jansen KU, Gruber WC, Watson W. A phase 3, randomized, double-blind study to evaluate the immunogenicity and safety of 3 lots of 20-valent pneumococcal conjugate vaccine in pneumococcal vaccine-naïve adults 18 through 49 years of age. Vaccine. 2021 Sep 7;39(38):5428-5435. doi: 10.1016/j.vaccine.2021.07.004. Epub 2021 Jul 24.
24. Perez JL, Absalon J, Beeslaar J, Balmer P, Jansen KU, Jones TR, Harris S, York LJ, Jiang Q, Radley D, Anderson AS, Crowther G, Eiden JJ. From research to licensure and beyond: clinical development of MenB-FHbp, a broadly protective meningococcal B vaccine. Expert Rev Vaccines. 2018 Jun;17(6):461-477. doi: 10.1080/14760584.2018.1483726. Epub 2018 Jun 22.
25. Wen X, Cao D, Jones RW, Li J, Szu S, Hoshino Y. Construction and characterization of human rotavirus recombinant VP8\* subunit parenteral vaccine candidates. Vaccine. 2012 Sep 21;30(43):6121-6. doi: 10.1016/j.vaccine.2012.07.078. Epub 2012 Aug 9.
26. Clarkson KA, Talaat KR, Alaimo C, Martin P, Bourgeois AL, Dreyer A, Porter CK, Chakraborty S, Brubaker J, Elwood D, Frölich R, DeNearing B, Weerts HP, Feijoo B, Halpern J, Sack D, Riddle MS, Fonck VG, Kaminski RW. Immune response characterization in a human challenge study with a *Shigella flexneri* 2a bioconjugate vaccine. EBioMedicine. 2021 Apr;66:103308. doi: 10.1016/j.ebiom.2021.103308. Epub 2021 Apr 1.
27. Talaat KR, Alaimo C, Martin P, Bourgeois AL, Dreyer AM, Kaminski RW, Porter CK, Chakraborty S, Clarkson KA, Brubaker J, Elwood D, Frölich R, DeNearing B, Weerts H, Feijoo BL, Halpern J, Sack D, Riddle MS, Fonck VG. Human challenge study with a *Shigella* bioconjugate vaccine: Analyses of clinical efficacy and correlate of protection. EBioMedicine. 2021 Apr;66:103310. doi: 10.1016/j.ebiom.2021.103310. Epub 2021 Apr 13.
28. Cohen D, Atsmon J, Artaud C, Meron-Sudai S, Gougeon ML, Bialik A, Goren S, Asato V, Ariel-Cohen O, Reizis A, Dorman A, Hoitink CWG, Westdijk J, Ashkenazi S, Sansonetti P, Mulard LA, Phalipon A. Safety and immunogenicity of a synthetic carbohydrate conjugate vaccine against *Shigella flexneri* 2a in healthy adult volunteers: a phase 1, dose-escalating, single-blind, randomised, placebo-controlled study. Lancet Infect Dis. 2021 Apr;21(4):546-558. doi: 10.1016/S1473-3099(20)30488-6. Epub 2020 Nov 10.
29. Passwell JH, Ashkenazi S, Banet-Levi Y, et al. Age-related efficacy of *Shigella* O-specific polysaccharide conjugates in 1-4-year-old Israeli children. Vaccine. 2010 Mar 2;28(10):2231-2235. doi: 10.1016/j.vaccine.2009.12.050.
30. Passwell JH, Harlev E, Ashkenazi S, Chu C, Miron D, Ramon R, Farzan N, Shiloach J, Bryla DA, Majadly F, Roberson R, Robbins JB, Schneerson R. Safety and immunogenicity of improved *Shigella* O-specific polysaccharide-protein conjugate vaccines in adults in Israel. Infect Immun. 2001 Mar;69(3):1351-7. doi: 10.1128/IAI.69.3.1351-1357.2001.
31. Cohen D, Ashkenazi S, Green M, Lerman Y, Slepon R, Robin G, Orr N, Taylor DN, Sadoff JC, Chu C, Shiloach J, Schneerson R, Robbins JB. Safety and immunogenicity of investigational *Shigella* conjugate vaccines in Israeli volunteers. Infect Immun. 1996 Oct;64(10):4074-7. doi: 10.1128/iai.64.10.4074-4077.
32. Palmeira P, Quinello C, Silveira-Lessa AL, Zago CA, Carneiro-Sampaio M. IgG placental transfer in healthy and pathological pregnancies. Clin Dev Immunol. 2012;2012:985646. doi: 10.1155/2012/985646.

- 
33. Guerra FM, Crowcroft NS, Friedman L, Deeks SL, Halperin SA, Severini A, Hatchette TF, Bolotin S; Immunity of Canadians and Risk of Epidemics (iCARE) Network. Waning of measles maternal antibody in infants in measles elimination settings - A systematic literature review. *Vaccine*. 2018 Feb 28;36(10):1248-1255. doi: 10.1016.
34. Healy CM, Rench MA, Swaim LS, Timmins A, Vyas A, Sangi-Haghpeykar H, Ng N, Rajam G; MPIR Laboratory Work Group, Havers F, Schiffer J, Baker CJ. Kinetics of maternal pertussis-specific antibodies in infants of mothers vaccinated with tetanus, diphtheria and acellular pertussis (Tdap) during pregnancy. *Vaccine*. 2020 Aug 18;38(37):5955-5961. doi: 10.1016.
35. Robbins JB, Chu C, Schneerson R. Hypothesis for vaccine development: protective immunity to enteric diseases caused by nontyphoidal salmonellae and shigellae may be conferred by serum IgG antibodies to the O-specific polysaccharide of their lipopolysaccharides. *Clin Infect Dis*. 1992 Aug;15(2):346-61. doi: 10.1093/clinids/15.2.346.
36. Li JB, Li Z, Pan B, Gong J, Li CY, Shi LW, Huang Y, Du L. Epidemiological surveillance of bacillary dysentery in Zhongshan County, Guangxi. *Ying Yong Yu Fang Yi Xue*. 2018 May;24(3):220-222. (Chinese)
37. Jones C, Pollock L, Barnett SM, Battersby A, Kampmann B. The relationship between concentration of specific antibody at birth and subsequent response to primary immunization. *Vaccine*. 2014 Feb 12;32(8):996-1002. doi: 10.1016/j.vaccine.2013.11.104. Epub 2013 Dec 14.
38. Yang HH, Wu CG, Xie GZ, Gu QW, Wang BR, Wang LY, Wang HF, Ding ZS, Yang Y, Tan WS, Wang WY, Wang XC, Qin M, Wang JH, Tang HA, Jiang XM, Li YH, Wang ML, Zhang SL, Li GL. Efficacy trial of Vi polysaccharide vaccine against typhoid fever in south-western China. *Bull World Health Organ*. 2001;79(7):625-31.