# Efficacy, safety, and immunogenicity of an oral recombinant *Helicobacter pylori* vaccine in children in China: a randomised, double-blind, placebo-controlled, phase 3 trial

*Ming Zeng*, Xu-Hu Mao*, Jing-Xin Li, Wen-De Tong, Bin Wang, Yi-Ju Zhang, Gang Guo, Zhi-Jing Zhao, Liang Li, De-Lin Wu, Dong-Shui Lu, Zhong-Ming Tan, Hao-Yu Liang, Chao Wu, Da-Han Li, Ping Luo, Hao Zeng, Wei-Jun Zhang, Jin-Yu Zhang, Bo-Tao Guo, Feng-Cai Zhu, Quan-Ming Zou*

## Summary

**Background** ***Helicobacter pylori*** **is one of the most common gastric pathogens, affecting at least half the world's population, and is strongly associated with gastritis, peptic ulcer, gastric adenocarcinoma, and lymphoma. We aimed to assess the efficacy, safety, and immunogenicity of a three-dose oral recombinant *H pylori* vaccine in children in China.**

**Methods** **We did this randomised, double-blind, placebo-controlled, phase 3 trial at one centre in Ganyu County, Jiangsu Province, China. Healthy children aged 6–15 years without past or present *H pylori* infection were randomly assigned (1:1), via computer-generated randomisation codes in blocks of ten, to receive the *H pylori* vaccine or placebo. Participants, their guardians, and study investigators were masked to treatment allocation. The primary efficacy endpoint was the occurrence of *H pylori* infection within 1 year after vaccination. We did analysis in the per-protocol population. This trial is registered with ClinicalTrials.gov, number NCT02302170.**

**Findings** **Between Dec 2, 2004, and March 19, 2005, we randomly assigned 4464 participants to either the vaccine group (n=2232) or the placebo group (n=2232), of whom 4403 (99%) participants completed the three-dose vaccination schedule and were included in the per-protocol efficacy analysis. We extended follow-up to 3 years. We recorded 64 events of *H pylori* infection within the first year (14 events in 2074·3 person-years at risk in the vaccine group *vs* 50 events in 2089·6 person-years at risk in the placebo group), resulting in a vaccine efficacy of 71·8% (95% CI 48·2–85·6). 157 (7%) participants in the vaccine group and 161 (7%) participants in the placebo group reported at least one adverse reaction. Serious adverse events were reported in five (<1%) participants in the vaccine group and seven (<1%) participants in the placebo group, but none was considered to be vaccination related.**

**Interpretation** **The oral recombinant *H pylori* vaccine was effective, safe, and immunogenic in *H pylori*-naive children. This vaccine could substantially reduce the incidence of *H pylori* infection; however, follow up over a longer period is needed to confirm the protection of the vaccine against *H pylori*-associated diseases.**

**Funding** **Chongqing Kangwei Biological Technology.**

Published **Online** July 1, 2015 http://dx.doi.org/10.1016/S0140-6736(15)60310-5

See **Online/Comment** http://dx.doi.org/10.1016/S0140-6736(15)60579-7

*Contributed equally

**Jiangsu Provincial Center for Disease Control and Prevention, Nanjing, Jiangsu Province, China** (F-C Zhu MSc, J-X Li MSc, Y-J Zhang BS, L Li MSc, Z-M Tan BS)**; National Institute for Food and Drug Control, Beijing, China** (M Zeng PhD, B Wang PhD, Z-J Zhao PhD, H-Y Liang MSc)**; College of Pharmacy, Third Military Medical University, Chongqing, China** (Prof Q-M Zou PhD, Prof X-H Mao PhD, Prof G Guo PhD, D-S Lu BS, Prof C Wu PhD, Prof P Luo PhD, Prof H Zeng PhD, W-J Zhang MSc, J-Y Zhang PhD, B-T Guo, PhD)**; Kangwei Biological Technology, Chongqing, China** (W-D Tong MSc)**; and Ganyu County Center for Disease Control and Prevention, Lianyungang, Jiangsu Province, China** (D-L Wu BS, D-H Li BS)

Correspondence to:
Dr Feng-Cai Zhu, Jiangsu Provincial Center for Disease Control and Prevention, Nanjing 210009, Jiangsu Province, China
**jszfc@vip.sina.com**

or

Prof Quan-Ming Zou, College of Pharmacy, Third Military Medical University, Chongqing 400038, China
**qmzou2007@163.com**

## Introduction

*Helicobacter pylori*, a gram-negative spiral bacterium originally found in the human gastric epithelium, is one of the most common gastric pathogens.[1–3] *H pylori* affects more than half the world's population, causing gastritis, peptic ulcer, gastric adenocarcinoma, and lymphoma.[4,5] In China, more than 600 million individuals are infected with *H pylori*.[6] In 1994, WHO classified *H pylori* as a class I human carcinogen.[7] Discovery of the pathogen was awarded a Nobel Prize in 2005, in view of its importance in gastroenterology.[8,9]

Efforts to develop an effective vaccine against *H pylori* began in the early 1990s.[10,11] Findings have shown that protection against *H pylori* infection can be achieved both prophylactically and therapeutically in animals, but previous trials of *H pylori* vaccine candidates have all been at an early stage, with no real efficacy reported.[10,12,13] An oral recombinant *H pylori* vaccine using urease B subunit fused with heat-labile enterotoxin B subunit was developed by Third Military Medical University and Chongqing KangWei Biotechnology in China. The vaccine has been assessed in phase 1 and phase 2 clinical trials for preliminary safety, immunogenicity, and optimum dose (unpublished). Here, we report phase 3 findings of the efficacy, safety, and immunogenicity of this novel three-dose oral recombinant *H pylori* vaccine in children in China.

## Methods

### Study design and participants

We did this randomised, double-blind, placebo-controlled, phase 3 trial at one centre in Ganyu County, Jiangsu Province, China. Prospective participants were recruited from 12 local schools. Eligible participants were healthy children without past or present *H pylori* infection. We excluded children who were positive for *H pylori* infection in ELISA or breath tests. The appendix provides full details of the exclusion criteria.The study was approved by the institutional review board of Jiangsu Provincial Center of Disease Control and Prevention, and done in

See **Online** for appendix

accordance with the Declaration of Helsinki, Good Clinical Practice, and Chinese regulatory requirements. Guardians of participants provided written informed consent for the initial 1 year study; separate written informed consent was provided before participants entered the extended follow-up. The full protocol is available online.

For the **protocol** see http://jscdc.cn/jgzn/zzjg/kyjyk/wjtz/201505/t20150514_46658.html

### Randomisation and masking

Participants were randomly assigned (1:1), via computer-generated randomisation codes in blocks of ten, to receive either the *H pylori* vaccine or placebo. Vaccine and placebo were identical in appearance, with a randomisation code number on each dose as the only identifier. Eligible participants were assigned a sequential number according to their sequence of enrolment and received vaccine or placebo labelled with the same numbers. Participants, their guardians, and study investigators were masked to treatment allocation until after final database lock, but assignment was concealed from individuals involved in generation of the randomisation list.

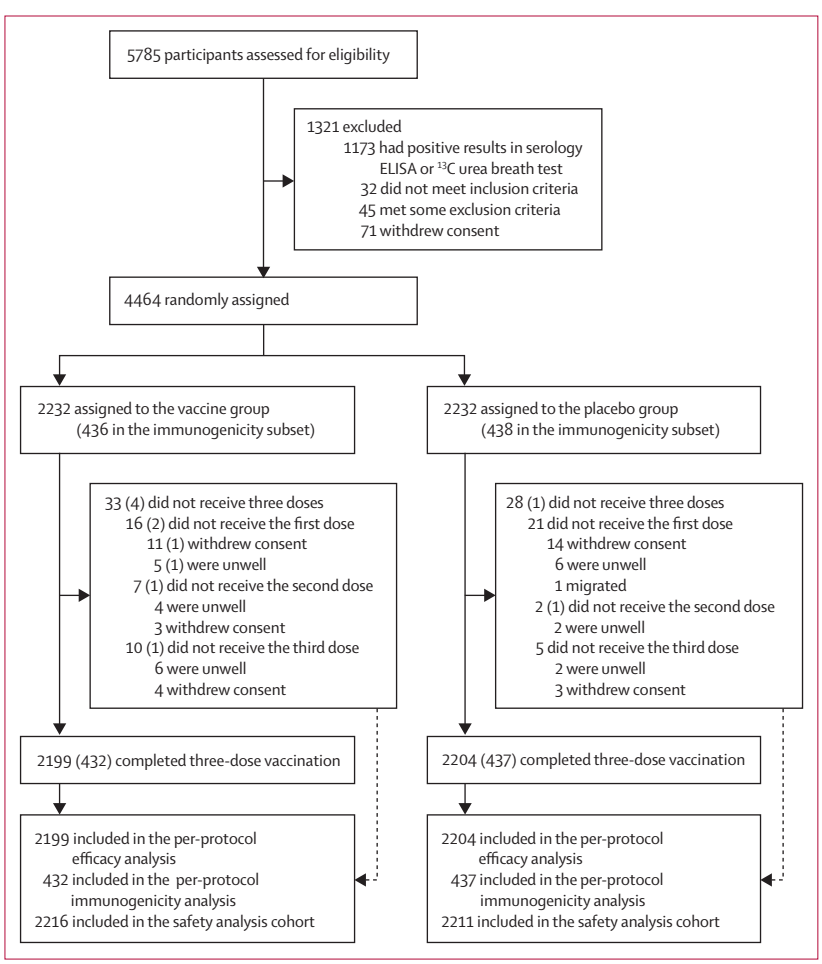


***Figure 1:*** **Trial profile**
Numbers in parentheses indicate participants in the immunogenicity subset.

### Procedures

Past or present *H pylori* infection was confirmed by a two-step process:[14,15] first, serum antibodies against cytotoxin-associated gene A and heat-shock protein 58 of *H pylori* were measured with a commercial indirect ELISA diagnostic kit (Bell Biological Engineering, Beijing, China). We then did $^{13}$C urea breath tests in children with negative ELISA results, with a commercial $^{13}$C urea breath test kit (Boran Pharmaceutical, Beijing, China; appendix). Participants who received all three doses of assigned treatment were included in the initial 1 year surveillance for vaccine efficacy. We then extended follow-up to 3 years.

The *H pylori* vaccine was developed with DNA recombination technology. Each dose of vaccine contains 15 mg fusion proteins consisting of urease B subunit (gene derived from *H pylori* 9803) and heat-labile enterotoxin B subunit (gene derived from *E coli* H44815). The fusion proteins were purified by ion-exchange chromatography combined with gel filtration chromatography, with a purity of more than 80% and an endotoxin contamination of less than 5 EU per dose. Placebo contains only the vaccine excipients (eg, mannitol, EDTA-$Na_2$), but with no fusion protein. Both vaccine and placebo were formulated as lyophilised powder.

The oral vaccination was given on day 0, 14, and 28. Participants fasted for at least 2 h and were given 80 mL of buffer solution, containing 2·8 g sodium bicarbonate and 1·1 g sodium citrate, 2 min before the oral vaccination. Participants then took one dose of the *H pylori* vaccine or placebo, which was fully dissolved in 30 mL of 25–30°C distilled water immediately before ingestion. We monitored participants for adverse reactions for at least 30 min after each dose. Solicited reactions, including fever, headache, dizzy, and some gastrointestinal disorders, were recorded in diary cards by participants or their guardians for the next 3 days. We documented serious adverse events that happened during the first year of the study.

We obtained blood and saliva samples from all participants immediately before the first dose and 1 month after the third dose. Additionally, participants from Chengtou central school and Zhucundian school in Chengtou town were selected for inclusion in a subset for immunogenicity and antibody persistency, donating blood and saliva samples at months 6 and 12 in the first year of the study, and at months 24 and 36 of extended follow-up. Serum samples were sent to Chinese National Institutes for Food and Drug Control to measure specific anti-urease B subunit IgG in serum by ELISA and IgA in saliva by avidin-biotin complex ELISA.[15]

We visited participants at months 4, 8, and 12, and then months 24 and 36 after the third dose, to assess the occurrence of *H pylori* infection events. At each timepoint, we obtained breath samples from fasting participants for the breath tests. Participants who had positive breath tests then provided blood samples for ELISA tests. An *H pylori* infection event could be confirmed on the basis

of positive results in both the two separate tests. To estimate the immunological correlates of protection against *H pylori* infection, we selected two infection-free participants matched for age and sex with each laboratory-confirmed *H pylori* event in the first year of follow-up to form an event-control subset. The matched infection-free participants were confirmed by negative breath-test results throughout the 1 year of follow-up, and by negative serology ELISA results at months 1 and 12 after the third dose.

### Outcomes

The primary efficacy endpoint was occurrence of *H pylori* infection within the first 12 months after vaccinations. The secondary efficacy endpoint for was the occurrence of *H pylori* infection during follow-up. Immunogenicity endpoints were geometric mean titre (GMT), conversion rate (defined as at least a four-fold increase in antibody titres), and geometric mean fold increase of serum IgG and salivary IgA against UreB. Safety endpoints were the incidence of solicited adverse reactions and serious adverse events in each group.

### Statistical analysis

With an assumed incidence of *H pylori* infection of roughly 2·0%, a minimum of 1374 eligible participants per group would be needed to achieve more than a 90% power to detect vaccine efficacy of 70% with the lower boundary of the 95% CI greater than zero. The sample size of the immunogenicity subset provided adequate power to show the difference between the vaccine and placebo groups.

We reported 1 year vaccine efficacy in a per-protocol population who had received all three doses of assigned treatment. We reported overall vaccine efficacy by combining the first year data with that for the extended follow-up. We defined the unadjusted vaccine efficacy as 1 minus the ratio of event rates in the vaccine group to that in the placebo group. Event rate was calculated as the number of events divided by the total follow-up in years and was expressed as per 100 person-years. We applied Cox proportional hazards regression models to estimate adjusted vaccine efficacy.[15] Analyses of immunogenicity were done in the per-protocol immunogenicity subset who complied with three-dose immunisations and had serum and saliva antibody titres available before the first dose and 1 month after the third dose. Participants who donated blood and saliva samples during follow-up were included in the assessment of antibody persistency. We estimated the immunological correlates of protection in the event-control subset by calculation of specificity (defined as the proportion of the *H pylori*-infected children with antibody titres lower than the protective level) and sensitivity (defined as the proportion of the uninfected children with titres greater than the level) at month 1 after the third dose.[16] We assessed the safety profile of the vaccine in all participants who received at least one dose of the vaccine or placebo. We used Student's *t* test for log-transformed antibody titres and Fisher's exact or $\chi^2$ for categorical data. We did analyses with SAS (version 9.1).

This trial is registered with ClinicalTrials.gov, number NCT02302170.

### Role of the funding source

The funder of the study participated in study design, but had no role in data collection, data analysis, data interpretation, or writing of the report. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

## Results

Figure 1 shows the trial profile. Between Dec 2, 2004, and March 19, 2005, we randomly assigned 4464 participants to either the vaccine group (n=2232) or the placebo group (n=2232), of whom 4403 (99%) participants completed the three-dose vaccination schedule and were included in the per-protocol efficacy analysis (figure 1). 869 (99%) of 874 participants assigned to the immunogenicity subset were included in the per-protocol immunogenicity analysis (figure 1). 2024 (92%) participants in the vaccine group and 2059 (93%) participants in the placebo group completed the first year of follow up. *H pylori* naivety was associated with younger age (from 849 [82%] of 1032 children aged 6–7 years to 1188 [76%] of 1573 children aged 12–15 years) and male sex (2825 [81%] of 3489 boys *vs* 1787 [78%] of 2296 girls; appendix). Although the proportion of boys was higher than the proportion of girls, the sex ratios across the treatment groups were similar (table 1).

| | Vaccine group | Placebo group |
|---|---|---|
| **Efficacy cohort** | | |
| N | 2199 | 2204 |
| Age (years) | 9·2 (1·7) | 9·2 (1·7) |
| Sex | | |
| Male | 1338 (61%) | 1349 (61%) |
| Female | 861 (39%) | 855 (39%) |
| **Safety cohort** | | |
| N | 2216 | 2211 |
| Age (years) | 9·2 (1·7) | 9·2 (1·7) |
| Sex | | |
| Male | 1349 (61%) | 1352 (61%) |
| Female | 867 (39%) | 859 (39%) |
| **Immunogenicity subset** | | |
| N | 432 | 437 |
| Age (years) | 9·1 (1·8) | 9·0 (1·7) |
| Sex | | |
| Male | 277 (64%) | 279 (64%) |
| Female | 155 (36%) | 158 (36%) |

Data are mean (SD), or n (%), unless otherwise indicated.

***Table 1:*** **Baseline demographic characteristics**

| | Vaccine group | | | | Placebo group | | | | Efficacy (%)* | Adjusted efficacy (%)† | p value‡ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Participants (n)§ | Events (n)¶ | Person-years at risk‖ | Event rate** | Participants (n)§ | Events (n)¶ | Person-years at risk‖ | Event rate** | | | |
| Month 4 | 2165 | 4 | 720·9 | 0·6 (0·2–1·4) | 2176 | 16 | 724·6 | 2·2 (1·3–3·6) | 74·9 (22·1–93·9) | 75·0 (25·1–91·6) | 0·0133 |
| Month 8 | 2186 | 10 | 1403·6 | 0·7 (0·3–1·3) | 2192 | 36 | 1416 | 2·5 (1·8–3·5) | 72·0 (42·4–87·6) | 72·0 (43·6–86·1) | 0·0004 |
| Month 12 | 2199 | 14 | 2074·3 | 0·7 (0·4–1·1) | 2204 | 50 | 2089·6 | 2·4 (1·8–3·1) | 71·8 (48·2–85·6) | 71·9 (49·1–84·4) | <0·0001 |
| Month 24 | 2199 | 24 | 3588·3 | 0·7 (0·4–1·0) | 2204 | 72 | 3589·6 | 2·0 (1·6–2·5) | 66·7 (46·4–79·9) | 66·7 (47·1–79·0) | <0·0001 |
| Month 36 | 2199 | 30 | 4582·3 | 0·7 (0·4–0·9) | 2204 | 85 | 4541·6 | 1·9 (1·5–2·3) | 65·0 (46·4–77·7) | 64·9 (46·8–76·9) | <0·0001 |

Data in parentheses are 95% CIs. *Unadjusted, defined as 1 minus the incidence rate ratio. †Adjusted for sex and age in Cox proportional hazards regression models. ‡Calculated by Cox proportional hazards regression models with adjustment of sex and age. §Cumulative number of participants who attended scheduled visits and received all three vaccine doses, and were included in the per-protocol efficacy cohort. ¶Cumulative number of laboratory confirmed *H pylori* infection events. ‖Cumulative follow-up years of at-risk participants from month 1 after the third dose to either the time of first *H pylori* infection event or to indicated timepoint. **Number of events divided by person-years at risk.

***Table 2:*** **Cumulative vaccine efficacy against *Helicobacter pylori* infection in the per-protocol efficacy cohort**

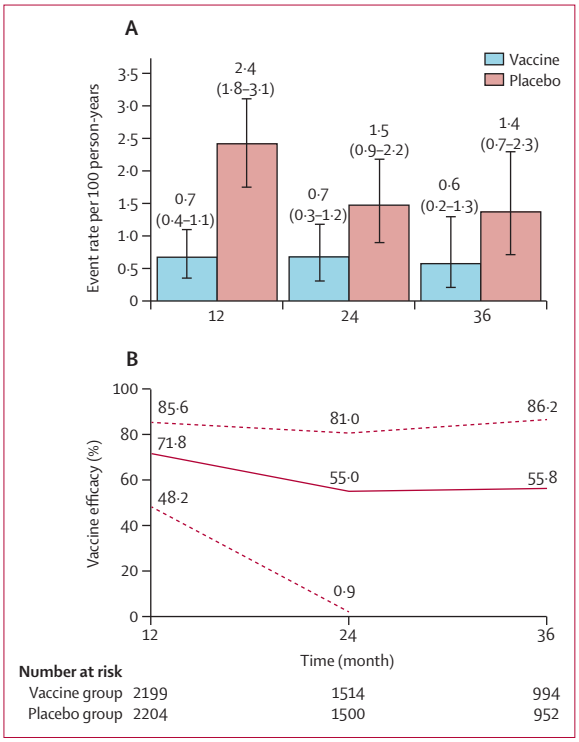


***Figure 2:*** **Event rate of *Helicobacter pylori* infection (A) and vaccine efficacy (B), by surveillance year**

Error bars and data in parentheses in panel A show 95% CIs. Event rate per 100 person-years was calculated as the number of events divided by the person-years in each year. The solid line in panel B indicates the point estimates of efficacy and the dotted lines indicate 95% CIs. Vaccine efficacy was truncated at 0% as the lower limit.

During the extended follow-up, we tested 3014 (68%) participants for *H pylori* infection at month 24 and 1946 (44%) participants at month 36. Participants who attended the visits at months 24 and 36 had a younger average age (8·7 and 8·0 years, respectively) than those in the initial efficacy cohort (p<0·0001), but no substantive imbalance in demographic characteristics of the participants was noted across the treatment groups (appendix).

During the first year of follow-up, we recorded 64 confirmed events of *H pylori* infection, with 14 events in 2074·3 person-years at risk in the vaccine group and 50 events in 2089·6 person-years at risk in the placebo group (table 2). Of these events, 63 events were positive in both the breath test and the serology ELISA. One participant in the placebo group had positive results in the breath tests at months 8 and 12, but refused to donate a blood sample for the serology ELISA. Because consecutive positive breath tests could suggest a persistent *H pylori* infection, we included this participant as an event of *H pylori* infection before unmasking (appendix). The event rate of *H pylori* infection was significantly lower in participants in the vaccine group than in those in the placebo group, resulting in a vaccine efficacy of 71·8% (95% CI 48·2–85·6) in the first year (table 2, figure 2). When the cohort was further stratified by age, sex, and township, the point estimates of vaccine protection were numerically, albeit not significantly, lower in girls and older children (≥10 years) at enrolment (appendix).

During extended follow-up, we recorded an additional 32 events of *H pylori* infection (n=10 in the vaccine group *vs* n=22 in the placebo group) at month 24, and 19 events (n=6 *vs* n=13) at month 36 (table 2; appendix). On the basis of these data, vaccine efficacy was 55·0% (95% CI 0·9–81·0) in the second year and 55·8% (–24·7 to 86·2) in the third year after vaccinations (figure 2). The event rate of the placebo group varied from 2·4 to 1·4 per 100 person-years, whereas that of the vaccine group remained around 0·7 per 100 person-years during the 3 years (figure 2). Despite the waning of efficacy after the first year, the *H pylori* vaccine significantly reduced infection events throughout the whole study period (table 2). Robust vaccine efficacy against *H pylori* infection was shown after adjustment for sex and age (table 2).

The baseline concentrations of anti-urease B subunit IgG in serum were similar between the vaccine and placebo groups (figure 3; appendix). The *H pylori* vaccine elicited a significantly higher immune response than did placebo (figure 3; appendix), with a GMT of

389·4 (95% CI 355·5–426·6) and a seroconversion rate of 86·1% (82·5–89·2) in participants in the vaccine group versus 72·2 (67·1–77·6) and 4·6% (2·8–7·0), respectively, in those in the placebo group, at month 1 after the third dose (figure 3; appendix). Concentrations of serum-specific antibodies in the vaccine group remained high, with no significant waning during the first year of follow-up (appendix).

Before first dose, participants in the placebo group had a significantly higher GMT of salivary IgA; however, participants in the vaccine group had a significantly stronger salivary IgA response after vaccination, with a conversion of 74·1% (95% CI 69·7–78·1) and geometric mean fold increase of 3·9 (3·6–4·2; figure 3, appendix). The immune response in the intention-to treat subset for immunogenicity was similar to that in the per-protocol subset (appendix). We recorded a moderate correlation between serum IgG and salivary IgA titres, both before and at later timepoints after vaccinations, with a Pearson correlation coefficient of 0·4 and 0·6, respectively ($p<0·0001$). During extended follow-up, specific antibody titres decreased to 187·8 (175·5–200·9) in serum and 4·8 (4·5–5·2) in saliva at month 24, then to 175·1 (161·5–189·9) in serum and 5·2 (4·7–5·8) in saliva at month 36 (figure 3, appendix). Both serum IgG and salivary IgA of vaccine group waned over time, but still remained significantly higher than the concentrations in the placebo group throughout the 3 years (appendix).

On the basis of the event-control subset, consisting of 64 events and 128 event-free participants, participants with an event of *H pylori* infection had higher serum IgG and salivary IgA at month 1 (appendix). The decreases in serum IgG and salivary IgA at month 1 were associated with an elevated infection risk in separate logistic regression models, with odds ratios of 1·5 (95% CI 1·1–1·9) and 1·4 (1·0–2·0), respectively. In the estimation of the immunological correlates of vaccine protection against *H pylori* infection, we noted little variation in the sum of specificity and sensitivity values for 1 month serum IgG titres of 1:200 to 1:400, and salivary IgA of 1:8 to 1:16 (appendix).

157 (7%) of 2216 participants in the vaccine group and 161 (7%) of 2211 participants in the placebo group reported at least one adverse reaction, within 3 days after vaccinations (table 3). All the adverse reactions were mild and resolved within 24 h. The most common reaction was vomiting, followed by fever and headache. Slightly more patients in the vaccine group reported abdominal bloating, but incidence of other adverse reactions did not differ significantly between the two treatment groups (table 3). Five (<1%) participants in the vaccine group and seven (<1%) participants in the placebo group reported serious adverse events during the first year of follow-up (table 3, appendix). One of these events was a fatal drowning; the other events resolved after hospital admission (table 3). No serious adverse event was considered to be related to the study drug.

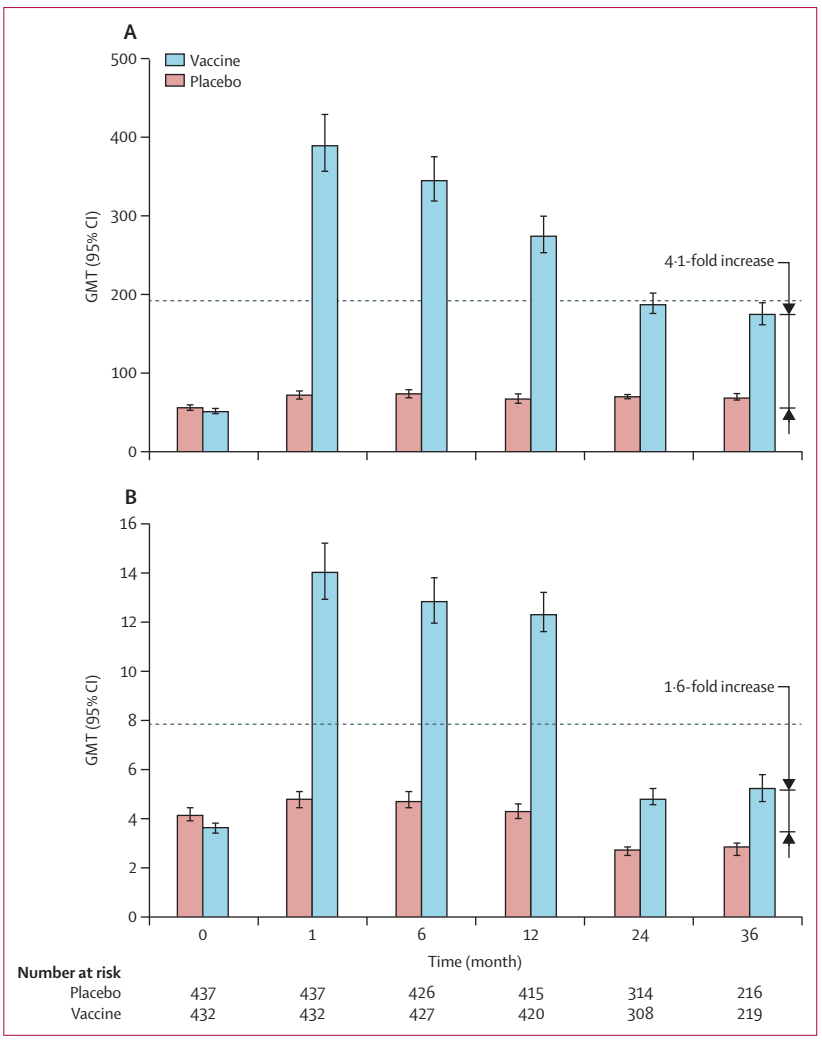


***Figure 3:*** **Specific anti-urease B subunit serum IgG (A) and salivary IgA (B) response in the per-protocol immunogenicity subset**

Error bars show 95% CIs. We defined conversion rate as the proportion of participants with at least a four-fold increase in post-vaccination antibodies. The dashed line in panel A represents a serum antibody titre of 1:200 and in panel B represents a salivary antibody titre of 1:8. See appendix for detailed data. GMT=geometric mean titre.

## Discussion

In our study, oral administration with the *H pylori* vaccine provided good protection against *H pylori* infection in children aged 6–15 years up to 1 year after vaccination. Although point estimates of the vaccine's protectiveness at later timepoints showed a mild waning of efficacy, overall protection was sustained up to 3 years.

Several clinical trials with other *H pylori* vaccine candidates have been done to test the safety, immunogenicity, and possible efficacy. In Kotloff and colleagues' phase 1 trial of an oral inactivated whole-cell *H pylori* vaccine in adults with or without *H pylori* infection, the inactivated whole-cell vaccine could not eradicate *H pylori* in the infected adults, but a significant immune response was recorded in participants with no *H pylori* infection.[17] Clinical trials

| | Vaccine group (n=2216) | Placebo group (n=2211) | p value |
|---|---|---|---|
| **Serious adverse events within the first of the study** | | | |
| Any | 5 (<1%) | 7 (<1%) | 0·5605 |
| Death | 1 (<1%) | 0 | 1·0000 |
| **Solicited adverse reactions within 0–3 days** | | | |
| Any | 157 (7%) | 161 (7%) | 0·7997 |
| Gastrointestinal reactions | 121 (5%) | 118 (5%) | 0·8559 |
| Vomiting | 52 (2%) | 67 (3%) | 0·1596 |
| Stomach ache | 21 (1%) | 14 (1%) | 0·2375 |
| Diarrhoea | 27 (1%) | 25 (1%) | 0·7865 |
| Abdominal bloating | 35 (2%) | 21 (1%) | 0·0427 |
| Other systematic reactions | | | |
| Fever | 48 (2%) | 57 (3%) | 0·3678 |
| Headache | 40 (2%) | 35 (2%) | 0·5670 |
| Dizzy | 25 (1%) | 14 (1%) | 0·0780 |

Data are n (%).

***Table 3:*** **Serious adverse events and adverse reactions in the safety cohort**

with *Salmonella typhi*-based or *Salmonella typhimurium*-based vaccines expressing *H pylori* urease showed a poor immunogenicity in *H pylori*-free adults.[18–20] An *H pylori* vaccine candidate with recombinant urease and heat-labile enterotoxin at a dose of 0–2·5 μg was assessed in *H pylori*-free adults and had a good safety and immunogenicity profile, eliciting humoral and cellular mucosal immune responses.[21,22] In another study with a therapeutic vaccine in *H pylori*-infected adults, investigators assessed various doses of urease with 5 μg heat-labile enterotoxin.[23] Although no evidence of eradication of *H pylori* infection was recorded, urease immunisation induced a significant decrease in gastric *H pylori* density. However, diarrhoea was noted as an adverse reaction associated with heat-labile enterotoxin, which might be attributed to its A subunit.[24]

Findings from the above studies showed that the *H pylori* urease might be a promising antigen for *H pylori* vaccine, but with a need for an efficient mucosal adjuvant, optimised formulations, and delivery systems. By contrast, the formulation of our novel *H pylori* vaccine with urease B subunit fused with heat-labile enterotoxin B subunit has three characteristics: (1) the dose of antigen is large, which is necessary for an oral vaccine; (2) we used heat-labile enterotoxin as mucosal adjuvant, which is non-toxic and could be safely used in a high dose; (3) the urease B subunit and heat-labile enterotoxin B subunit were fused in a 1:1 ratio. In preclinical studies, we identified that this fusing protein worked as an efficient delivery system with the ability to deposit in mucosal lymphoid tissue in BALB/c mice given the $^{125}$I-labeled recombinant heat-labile enterotoxin B subunit and urease B subunit protein.[25] The urease B subunit and heat-labile enterotoxin B subunit fused protein might enhance antigen presentation by binding to ganglioside GM1 on enterocytes and activating antigen-presenting cells, such as dendritic cells. Similar enhancement of antigen-presenting function has been noted in other antigen–heat-labile enterotoxin B subunit fused vaccines, including an intranasal trivalent inactivated influenza vaccine.[26]

The estimates of vaccine efficacy during extended follow-up in our study have some uncertainties. On one hand, the extended follow-up was not initially planned and the loss of participants resulted in a reduction in power to identify the trend of protection. Participants who remained in the extended follow-up were younger than those who dropped out. The higher dropout rate of older children was largely because they graduated from local schools and moved out of the town for higher education. In view of the slightly higher point-estimated vaccine protection in the younger age group compared with the older group, the vaccine efficacy of the extended follow-up might be somewhat overestimated.

On the other hand, we included *H pylori*-naive participants who were confirmed as so by the serology ELISA and $^{13}$C urea breath test before enrolment, but completion of these tests took about 3 weeks. Moreover, completion of the three-dose immunisation schedule took a further 4 weeks. Although we could not exclude the possibility that participants might be exposed to *H pylori* before they received vaccine-induced protection, we calculated the efficacy on the basis of the assumption that no *H pylori* infection arose before month 1 after the third dose, which might cause an underestimation.

Both the serology ELISA and $^{13}$C urea breath test are highly sensitive and specific methods that have been used in previous clinical trials of *H pylori* vaccines.[14] The two methods were sequentially applied to screen children with *H pylori* infection at enrolment, enhancing the sensitivity and minimising the likelihood of false-negative diagnoses. Additionally, we combined the two methods to confirm an *H pylori*-associated event after vaccination to achieve a high specificity. Generally, the results of the breath test and serology ELISA were consistent throughout the study.

Although the role of antibodies in the protection against *H pylori* is still controversial, and several studies have suggested that cell-mediated immune response might also play an important part,[27,28] our findings show a rise in serum IgG and salivary IgA after vaccination. 1 month serum anti-urease B subunit IgG of 1:200 and salivary anti-urease B subunit IgA of 1:8 seemed to be optimum markers for a protection against *H pylori* infection. However, the correlates of protection might vary dependent on the different formulation of vaccines, endpoints measured, and individual characteristics being studied.[29] The waning trend of the *H pylori* vaccine-elicited antibodies might suggest a need for a booster dose in later years.

Generally, the safety profile of *H pylori* vaccine was good, with only a few mild adverse reactions reported, which was similar to the profile of placebo. However, the

**Panel: Research in context**

**Systematic review**

We searched PubMed up to Nov 13, 2014, with the terms "*Helicobacter pylori*" and "vaccine" for clinical trial reports, with no date or language restrictions. Only seven articles reported the safety and immunogenicity of prophylactic *Helicobacter pylori* vaccines in early clinical trials. Previous studies showed that the *H pylori* urease was safe and immunogenic.

**Interpretation**

As far as we are aware, this study is the first phase 3, randomised, double-blind placebo-controlled trial assessing the efficacy of a novel oral recombinant *H pylori* vaccine against *H pylori* infection. In addition to the good protective efficacy of the vaccine, our study also provides robust evidence of the vaccine's immunogenicity and safety. Furthermore, three-dose immunisation with the *H pylori* vaccine elicited a significant immune response in terms of serum IgG and salivary IgA, and provided sustained protection against *H pylori* infection in children up to 3 years. On the basis of these findings, this vaccine could substantially reduce the incidence of *H pylori*-associated gastritis, gastric ulcer, duodenal ulcer, and gastric adenocarcinoma. Longer follow-up is needed to provide direct evidence of vaccine protection against *H pylori*-associated diseases.

number of participants in this study might not be enough to identify rare vaccine-related adverse events. Long-term follow-up is needed in much larger populations, more diverse groups, and more diverse settings in a post-licensure surveillance programme to identify any rare adverse events associated with this *H pylori* vaccine.

Although this study was done in one centre, with all participants recruited from one region, which could compromise the generalisability of the results, we assessed the vaccine in children, whereas all previous trials with prophylactic or therapeutic *H pylori* vaccines have been done in adults. Considering the high proportion of *H pylori* infection in adults, most of them had already been exposed to *H pylori*. Assessment of prophylactic *H pylori* vaccine in *H pylori*-free adults could be biased because the recruited participants were very likely to have resistance to *H pylori*.

The screening data in this study showed that roughly 20% of children had already been infected with *H pylori*, showing a need to vaccinate younger children before the exposure to *H pylori*. However, this vaccine might not be suitable for children of this age because of the volume of each dose—80 mL of buffer solution and 30 mL of dissolved vaccine—which might be too large a quantity for young children to take in several minutes. Other routes of delivery or better formulation of the *H pylori* vaccine might need to be investigated.

Although several studies have shown evidence of the therapeutic effects of urease,[23,30] we only assessed the preventive efficacy of the *H pylori* vaccine. Future studies should assess whether this vaccine exerts any therapeutic effects against *H pylori* by reducing bacterial load. Eradication of *H pylori* might not be feasible by immunisation with the present formulation of prophylactic *H pylori* vaccine; however, an effective therapeutic vaccine could be more applicable to the general population because of the high proportion of adults with *H pylori* infection.

In conclusion, this oral recombinant *H pylori* vaccine was effective, safe, and immunogenic (panel). The sustained vaccine protection against *H pylori* infection up to 3 years suggests that this vaccine could substantially reduce the incidence of *H pylori*-associated gastritis, gastric ulcer, duodenal ulcer, and gastric adenocarcinoma. However, longer follow-up in the vaccinated cohort would be warranted to provide direct evidence of the vaccine protection against *H pylori*-associated diseases. Furthermore, booster doses at appropriate timing might be needed for long-term protection.

**Contributors**

Q-MZ, F-CZ, MZ, and X-HM designed the study and the study protocol, and contributed to the critical review and revision of the report. MZ was principal investigator of this trial. F-CZ was the coprincial investigator of this trial. MZ and BW contributed to the study protocol and led the laboratory analyses. GG, CW, HZ, J-YZ contributed to acquisition of data, drafting of the manuscript, study supervision, and revision of the report. LL, Y-JZ, D-LW, Z-MT, D-HL, D-SL, PL, and W-JZ led and participated in the site work, including the recruitment, data collection, and data interpretation. BW, Z-JZ, H-YL contributed to the laboratory analyses, data interpretation, and literature search. W-DT monitored the trial. B-TG and J-XL contributed to the data analysis. All authors reviewed and approved the final version of the report. MZ and X-HM contributed equally to this work.

**Declaration of interests**

W-DT is an employee of Kangwei biological technology. All other authors declare no competing interests.

**Acknowledgments**

This study is funded by Chongqing Kangwei Biological Technology. We thank the investigators from Third Military Medical University, Jiangsu Provincial Center for Disease Control and Prevention, National Institutes for Food and Drug Control, and Ganyu County Center for Disease Control and Prevention for contributions to the site work of the trial.

**References**

1 Warren JR, Marshall B. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. *Lancet* 1983; **1:** 1273–75.
2 Brown LM. *Helicobacter pylori*: epidemiology and routes of transmission. *Epidemiol Rev* 2000; **22:** 283–97.
3 Parsonnet J, Friedman GD, Vandersteen DP, et al. *Helicobacter pylori* infection and the risk of gastric carcinoma. *N Engl J Med* 1991; **325:** 1127–31.
4 Atherton JC. The pathogenesis of *Helicobacter pylori*-induced gastro-duodenal diseases. *Annu Rev Pathol* 2006; **1:** 63–96.
5 Cave DR. How is *Helicobacter pylori* transmitted? *Gastroenterology* 1997; **113** (6 suppl): S9–14.
6 The Team of Collaboration of *Helicobacter pylori* research in China. Prevalence of *Helicobacter pylori* infection in China. *Mod Digest Interven* 2010; **15:** 265–70 (in Chinese).
7 IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Schistosomes, liver flukes and *Helicobacter pylori*. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Lyon, 7–14 June 1994. *IARC Monogr Eval Carcinog Risks Hum* 1994; **61:** 1–241.

8 Megraud F. A humble bacterium sweeps this year's Nobel Prize. *Cell* 2005; **123:** 975–76.

9 Parsonnet J. Clinician-discoverers—Marshall, Warren, and *H pylori*. *N Engl J Med* 2005; **353:** 2421–23.

10 Del Giudice G, Malfertheiner P, Rappuoli R. Development of vaccines against *Helicobacter pylori*. *Expert Rev Vaccines* 2009; **8:** 1037–49.

11 Salama NR, Hartung ML, Muller A. Life in the human stomach: persistence strategies of the bacterial pathogen *Helicobacter pylori*. *Nat Rev Microbiol* 2013; **11:** 385–99.

12 Corthesy B, Boris S, Isler P, Grangette C, Mercenier A. Oral immunization of mice with lactic acid bacteria producing *Helicobacter pylori* urease B subunit partially protects against challenge with *Helicobacter felis*. *J Infect Dis* 2005; **192:** 1441–49.

13 Malfertheiner P, Schultze V, Rosenkranz B, et al. Safety and immunogenicity of an intramuscular *Helicobacter pylori* vaccine in noninfected volunteers: a phase I study. *Gastroenterology* 2008; **135:** 787–95.

14 Malfertheiner P, Megraud F, O'Morain CA, et al. Management of *Helicobacter pylori* infection—the Maastricht IV/Florence Consensus Report. *Gut* 2012; **61:** 646–64.

15 Chinese Medical Association Digestive Disease Branch. *Helicobacter pylori* Consensus Report (2003, Anhui Tongcheng). *Mod Digest Interven* 2004; **9:** 59–60 (in Chinese).

16 Barrett PN, Berezuk G, Fritsch S, et al. Efficacy, safety, and immunogenicity of a Vero-cell-culture-derived trivalent influenza vaccine: a multicentre, double-blind, randomised, placebo-controlled trial. *Lancet* 2011; **377:** 751–59.

17 Kotloff KL, Sztein MB, Wasserman SS, Losonsky GA, DiLorenzo SC, Walker RI. Safety and immunogenicity of oral inactivated whole-cell *Helicobacter pylori* vaccine with adjuvant among volunteers with or without subclinical infection. *Infect Immun* 2001; **69:** 3581–90.

18 DiPetrillo MD, Tibbetts T, Kleanthous H, Killeen KP, Hohmann EL. Safety and immunogenicity of phoP/phoQ-deleted Salmonella typhi expressing *Helicobacter pylori* urease in adult volunteers. *Vaccine* 1999; **18:** 449–59.

19 Angelakopoulos H, Hohmann EL. Pilot study of phoP/phoQ-deleted Salmonella enterica serovar typhimurium expressing *Helicobacter pylori* urease in adult volunteers. *Infect Immun* 2000; **68:** 2135–41.

20 Aebischer T, Bumann D, Epple HJ, et al. Correlation of T cell response and bacterial clearance in human volunteers challenged with *Helicobacter pylori* revealed by randomised controlled vaccination with Ty21a-based Salmonella vaccines. *Gut* 2008; **57:** 1065–72.

21 Sougioultzis S, Lee CK, Alsahli M, et al. Safety and efficacy of *E coli* enterotoxin adjuvant for urease-based rectal immunization against *Helicobacter pylori*. *Vaccine* 2002; **21:** 194–201.

22 Banerjee S, Medina-Fatimi A, Nichols R, et al. Safety and efficacy of low dose *Escherichia coli* enterotoxin adjuvant for urease based oral immunisation against *Helicobacter pylori* in healthy volunteers. *Gut* 2002; **51:** 634–40.

23 Michetti P, Kreiss C, Kotloff KL, et al. Oral immunization with urease and *Escherichia coli* heat-labile enterotoxin is safe and immunogenic in *Helicobacter pylori*-infected adults. *Gastroenterology* 1999; **116:** 804–12.

24 Sixma TK, Pronk SE, Kalk KH, et al. Crystal structure of a cholera toxin-related heat-labile enterotoxin from E. coli. *Nature* 1991; **351:** 371–77.

25 Guo TS, Zou QM, Guo G, et al. In vivo distribution of $^{125}$I-labeled rLTB-UreB in M ice administered with the drug by oral Route. *Chin J Biologicals* 2003; **16:** 215–17 (in Chinese).

26 da Hora VP, Conceicao FR, Dellagostin OA, Doolan DL. Non-toxic derivatives of LT as potent adjuvants. *Vaccine* 2011; **29:** 1538–44.

27 Garhart CA, Nedrud JG, Heinzel FP, Sigmund NE, Czinn SJ. Vaccine-induced protection against *Helicobacter pylori* in mice lacking both antibodies and interleukin-4. *Infect Immun* 2003; **71:** 3628–33.

28 Ermak TH, Giannasca PJ, Nichols R, et al. Immunization of mice with urease vaccine affords protection against *Helicobacter pylori* infection in the absence of antibodies and is mediated by MHC class II-restricted responses. *J Exp Med* 1998; **188:** 2277–88.

29 Plotkin SA. Immunologic correlates of protection induced by vaccination. *Pediatr Infect Dis J* 2001; **20:** 63–75.

30 Leal-Herrera Y, Torres J, Perez-Perez G, et al. Serologic IgG response to urease in *Helicobacter pylori*-infected persons from Mexico. *Am J Trop Med Hyg* 1999; **60:** 587–92.