# Efficacy and safety of a recombinant hepatitis E vaccine in healthy adults: a large-scale, randomised, double-blind placebo-controlled, phase 3 trial

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## Summary

**Background** **Seroprevalence data suggest that a third of the world's population has been infected with the hepatitis E virus. Our aim was to assess efficacy and safety of a recombinant hepatitis E vaccine, HEV 239 (Hecolin; Xiamen Innovax Biotech, Xiamen, China) in a randomised, double-blind, placebo-controlled, phase 3 trial.**

**Methods** **Healthy adults aged 16–65 years in, Jiangsu Province, China were randomly assigned in a 1:1 ratio to receive three doses of HEV 239 (30 μg of purified recombinant hepatitis E antigen adsorbed to 0·8 mg aluminium hydroxide suspended in 0·5 mL buffered saline) or placebo (hepatitis B vaccine) given intramuscularly at 0, 1, and 6 months. Randomisation was done by computer-generated permuted blocks and stratified by age and sex. Participants were followed up for 19 months. The primary endpoint was prevention of hepatitis E during 12 months from the 31st day after the third dose. Analysis was based on participants who received all three doses per protocol. Study participants, care givers, and investigators were all masked to group and vaccine assignments. This trial is registered with ClinicalTrials.gov, number NCT01014845.**

**Findings** **11 165 of the trial participants were tested for hepatitis E virus IgG, of which 5285 (47%) were seropositive for hepatitis E virus. Participants were randomly assigned to vaccine (n=56 302) or placebo (n=56 302). 48 693 (86%) participants in the vaccine group and 48 663 participants (86%) in the placebo group received three vaccine doses and were included in the primary efficacy analysis. During the 12 months after 30 days from receipt of the third dose 15 per-protocol participants in the placebo group developed hepatitis E compared with none in the vaccine group. Vaccine efficacy after three doses was 100·0% (95% CI 72·1–100·0). Adverse effects attributable to the vaccine were few and mild. No vaccination-related serious adverse event was noted.**

**Interpretation** **HEV 239 is well tolerated and effective in the prevention of hepatitis E in the general population in China, including both men and women age 16–65 years.**

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## Introduction

Hepatitis E virus is a major cause of sporadic and epidemic hepatitis.[1] Seroprevalence data suggest that a third of the world's population has been infected with the virus.[2] Although most cases are in developing countries, hepatitis E is no longer rare and it might be the most common type of acute viral hepatitis in industrialised countries.[3]

Clinically indistinguishable from other types of acute viral hepatitis, hepatitis E tends to be self-limited and usually does not become chronic.[4] The severity of illness increases with age; the overall case fatality ratio is estimated to be 1–3%.[5] Hepatitis E has a poor prognosis in pregnant women: mortality is 5–25%, and survivors have high rates of spontaneous abortion and stillbirth.[6,7] In patients with chronic liver disease, superinfection with hepatitis E virus often leads to a poor outcome.[8,9] Every year, 13 000–26 000 deaths are estimated in patients with chronic liver disease in industrialised countries.[10] In a continuing hepatitis E epidemic in Uganda that has caused illness in more than 10 196 people and 160 deaths, mortality was 13% in children.[11]

At least four genotypes of hepatitis E viruses have been identified.[12] Genotypes 1 and 2 were isolated from human beings and are mainly seen in developing countries. Genotypes 3 and 4 are zoonotic, with pigs being the principal reservoir; they have been identified in many sporadic cases and limited foodborne outbreaks mainly affecting middle-aged and elderly men.[13–15] Nevertheless, all hepatitis E virus associated with human diseases can be considered as belonging to one serotype.[16]

Two recombinant vaccines have undergone phase 2 clinical trials. One of the vaccines was produced in

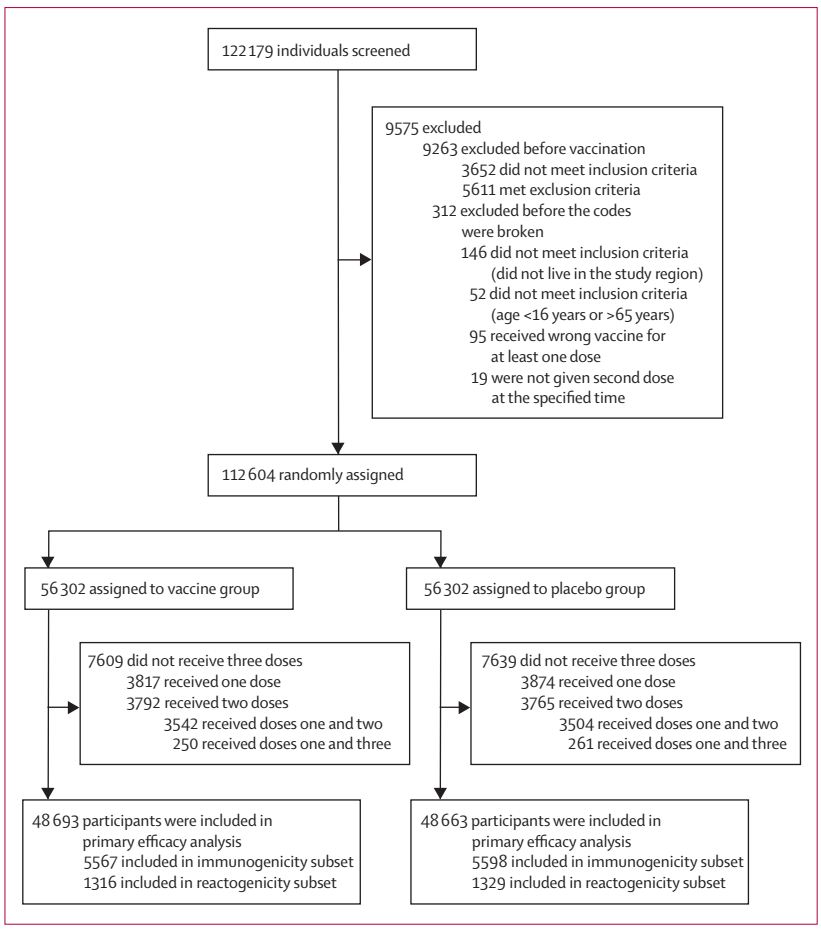


***Figure 1:*** **Trial profile**
The webappendix lists reasons for exclusion (p 30) and for non-completion (p 31).

See Online for webappendix

For the **protocol** see http://nidvd.xmu.edu.cn/HEV/protocol/Protocol_phase_3_100509.pdf

insect cells and was safe and immunogenic in young men (mean age 25·2 years; SD 6·25), providing 95% protection against hepatitis E in Nepal, where only genotype 1 hepatitis E virus had been isolated.[17] The results were encouraging but two questions remained to be answered. The first related to the safety and efficacy of the vaccine in the general population, especially in women and elderly people. The second related to the efficacy of a vaccine originally derived from hepatitis E virus genotype 1 against disease caused by heterogenic hepatitis E virus. The other candidate vaccine, HEV 239 (Hecolin; Xiamen Innovax Biotech, Xiamen, China), was produced in bacterial cells and was safe and efficacious against infection with hepatitis E virus in seronegative participants in a phase 2 trial.[18]

We undertook a randomised, double-blind, placebo-controlled, phase 3 trial to assess the efficacy and safety of HEV 239 in the general population. The trial included men and women from age 16 to 65 years, with or without antibodies against hepatitis E, from a region where both genotypes 1 and 4 cocirculate with the zoonotic genotype 4 predominating.

## Methods

### Study design and participants

This double-blind, randomised, placebo-controlled trial was done between August, 2007, and June, 2009, in Dongtai County, Jiangsu Province, China. On October, 2007, after enrolment in one township (Quindong), and before enrolment in ten other townships, the protocol was modified so that each of the 10 000 participants in one of the ten townships (Anfeng) had serum samples collected on day 0 and month 7 to assess the level of antibody protection through long-term follow-up. Independent ethics committee approval was obtained from the Ethics Committee of the Jiangsu Provincial Centre for Disease Control and Prevention (JSCDC), and the study was done in accordance with the principles of the Declaration of Helsinki, the standards of Good Clinical Practice, and Chinese regulatory requirements as stipulated by the Chinese Food and Drug Administration.

The study was designed by JSCDC and Xiamen University. Study staff at JSCDC were responsible for data collection. A sentinel hepatitis surveillance system was set up to identify incident hepatitis cases as they presented. Serial serum samples obtained from study participants were independently tested by the Chinese National Institute for the Control of Pharmaceuticals and Biological Products (NICPBP). A contract research organisation (PPD-Excel PharmaStudies, Beijing, China) monitored and ensured that the trial was done in compliance with the protocol, evaluated progress, verified that the rights of the participants were protected, and ensured that data were complete, accurate, and verifiable from source data. An independent data and safety monitoring board (DSMB) was set up to oversee the trial and ensure the safety of participants and the integrity of the data. The DSMB reviewed the clinical and laboratory data to confirm the diagnosis of hepatitis E before the group assignment (ie, vaccine *vs* placebo) of trial participants was broken.

Men and women were eligible for enrolment if they were healthy, aged 16–65 years, and understood the study procedures (detailed eligibility criteria are described in webappendix pp 2–3). Written informed consent was obtained from all participants.

### Vaccination

The preparation of HEV 239 vaccine is described elsewhere.[19] The vaccine contains 30 μg of the purified antigen adsorbed to 0·8 mg aluminium hydroxide suspended in 0·5 mL buffered saline. A licensed hepatitis B vaccine (Beijing Tiantan Biologic, Beijing, China) containing hepatitis B virus surface antigen in 0·5 mL aluminium hydroxide, was given as placebo. Vaccine doses and placebo doses were repackaged by Innovax under Good Manufacturing Practice conditions for identical appearance, but labelled with two letters each according to a random assignment. Three doses of vaccine or placebo were given intramuscularly at 0, 1, and 6 months.


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### Randomisation and masking

Trained local health-care workers enrolled the participants, and some of these health-care workers interviewed participants to assess adverse events and possible acute hepatitis later in the trial. An independent statistician prepared a permuted-block 1:1:1:1 randomisation list (with 20 codes to a block) using SAS software. The randomisation list was concealed and transferred into an immunisation management computer program through which participants were stratified by age and sex, and assigned vaccine codes. The study-group and vaccine code assignments were masked from all participants, carers, and investigators (or monitors). The integrity of the masking process was confirmed by the investigators and DSMB before the assignment of study group and vaccine codes was finally revealed. Health-care workers from JSCDC assigned participants to the study groups; they did not have any further involvement in the trial.

A subset of participants from one township was selected for active surveillance of adverse events (reactogenicity subset). Serum samples before immunisation were obtained from these participants and those from another township to establish the baseline concentration of hepatitis E virus IgG and for assessment of immunogenicity (immunogenicity subset). Fingerprint scanners and digital photographs were used to identify and track participants throughout immunisation, blood collection, and follow-up.

| | Vaccine group | Placebo group |
|---|---|---|
| Randomised participants* | 56 302 | 56 302 |
| Men | 24 511 (43·5%) | 24 567 (43·6%) |
| Age (years) | 44·14 (11·40) | 44·13 (11·40) |
| Age group (years) | | |
| 16–20 | 2520 (4%) | 2480 (4%) |
| 21–30 | 4598 (8%) | 4653 (8%) |
| 31–40 | 12 684 (23%) | 12 688 (23%) |
| 41–50 | 18 292 (32%) | 18 310 (33%) |
| 51–60 | 14 644 (26%) | 14 657 (26%) |
| 61–65 | 3564 (6%) | 3514 (6%) |
| Per-protocol population† (three doses) | 48 693 | 48 663 |
| Male to female ratio | 0·74 | 0·74 |
| Mean age (years) | 44·72, SD 11·09 | 44·68, SD 11·10 |
| Immunogenicity subset‡ | 5567 | 5598 |
| Male to female ratio | 0·64 | 0·65 |
| Mean age (years) | 45·22 (10·75) | 45·25 (10·82) |
| Anti-HEV prevalence | 47·76% (46·44–49·09) | 46·91% (45·60–48·23) |
| GMC (Wu/mL) | 0·14 (0·13–0·14) | 0·13 (0·13–0·14) |
| Reactogenicity subset§ | 1316 | 1329 |
| Men | 524 (39·8%) | 561 (42·2%) |
| Age (years) | 44·70, SD 11·23 | 44·93, SD 11·10 |

Data are number (%), mean (SD), or mean (95% CI). HEV=hepatitis E virus. GMC=geometric mean concentration. *All randomised participants who received at least one dose of vaccine or placebo. †Per-protocol population denotes all randomised participants who received three doses of vaccine or placebo. ‡Participants in the immunogenicity subset were from two townships additionally investigated for antibody response to vaccination. §Participants in the reactogenicity subset were from one township visited regularly at home by investigators to assess adverse events.

*Table 1:* **Baseline characteristics of participants**

### Hepatitis surveillance

Participants with suspected hepatitis were identified through an established active hepatitis surveillance system comprising 205 sentinels, including 162 community clinics, 30 private clinics, 11 central hospitals located in the townships, and two central hospitals in the city of Dongtai (webappendix p 32). A case of hepatitis was defined as a patient presenting with constitutional symptoms such as fatigue, loss of appetite, or both for longer than 3 days with alanine aminotransferase (ALT) exceeding 2·5-times the upper limit of normal range. Patients with abnormal concentrations of ALT were tested at first presentation by JSCDC for hepatitis A virus IgM, surface antigen of hepatitis B virus, hepatitis B virus core protein IgM, hepatitis C virus immunoglobulin, and hepatitis E virus IgM. Paired serum samples were obtained from these patients at the time of presentation and 2–6 weeks later. Serial samples were sent to the NICPBP to test for hepatitis E virus IgM and IgG, hepatitis E virus RNA, and hepatitis A virus IgM. The DSMB reviewed the clinical and laboratory results and confirmed the diagnoses of hepatitis E before unblinding. To be defined as an acute hepatitis E patient, a participant needed to fulfil three conditions: acute illness lasting for at least 3 days; abnormal serum ALT concentration 2·5-times the upper limit of normal range or greater; and positive hepatitis E virus IgM and RNA, ≥4-times increase in hepatitis E virus IgG, or both.

### Laboratory measurements

The tests for hepatitis E virus IgM were done by use of two commercial assays in parallel (Beijing Wantai, Beijing, China; MP Biomedicals, Singapore).[11,20–25] The assay for hepatitis E virus IgG used antigen more truncated than that in the vaccine antigen (Beijing Wantai, China).[20,21] Hepatitis E virus IgGs were further quantified and expressed in WHO units per mL (Wu/mL; webappendix p 2). The lower limit of hepatitis E virus IgG quantification was 0·077 Wu/mL.[26] For the analysis, the antibody concentration in samples negative for hepatitis E virus IgG were arbitrarily set at 0·0385 Wu/mL. Serum samples of patients with detectable hepatitis E virus IgM or a two times or greater rise of hepatitis E virus IgG concentration in paired samples were tested for hepatitis E virus RNA.[27] Serum samples were taken before the first vaccine dose and 1 month after the third dose from participants in the immunogenicity subset to establish concentration of hepatitis E virus IgG. Antibody concentration of 0·077 Wu/mL or greater was deemed to be a positive finding. Antibody response was defined as a greater than four-times increase of hepatitis E virus IgG in an individual's paired sera. All reagents were supplied by Beijing Wantai Biological Pharmacy Enterprise, Beijing, China.

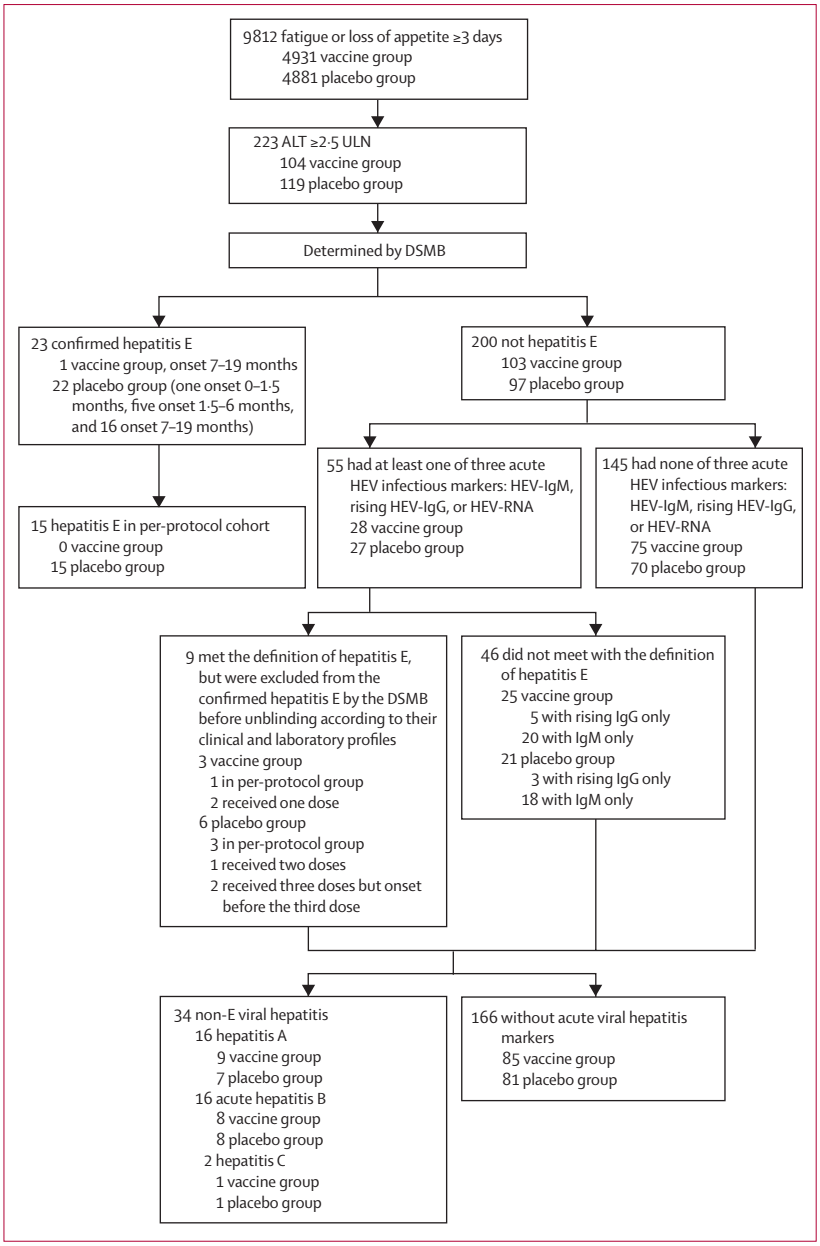


***Figure 2:*** **Flowchart of surveillance and certification of acute hepatitis E**
Sentinel hepatitis surveillance system was set up to monitor study participants for development of acute hepatitis (webappendix p 32). A case of acute hepatitis was defined as a participant who presented with constitutional signs, such as fatigue, nausea for at least 3 days, and alanine aminotransferase (ALT) exceeding 2·5-times the upper limit of normal range (ULN). Clinical and laboratory findings were assessed by an independent data and safety monitoring board (DSMB). The DSMB reviewed clinical and laboratory data to confirm the diagnosis of hepatitis E before the group assignment of trial participants was broken. HEV=hepatitis E virus.

For more on the **Medical Dictionary for Regulatory Activities** see http://www.meddramsso.com/

### Adverse events

After each dose, participants were observed for 30 min for immediate adverse reactions. Participants in the reactogenicity subset were visited at home by investigators at 6 h, 24 h, 48 h, 72 h, 7 days, 14 days, and 28 days after each dose, and observed or reported adverse effects, if any, were recorded on safety diary cards. Other participants were asked to report any adverse events to nearby clinics within 1 month after each dose. Additionally, investigators reviewed all records of admission to hospital and death to identify trial participants. Any serious adverse events were recorded throughout the study by use of the Medical Dictionary for Regulatory Activities (version 12.0).

### Statistical analysis

We estimated that the incidence of hepatitis E for adults aged 16–65 years would be about four cases per 10 000 person-years (webappendix p 1). On the assumption of a vaccine efficacy of 70%, a two-group continuity-corrected $\chi^2$ test with a one-sided significance level of 0·05 would have a power of 80% to detect a difference in incidence with 41 277 participants per group. To compensate for dropouts, 50 000 participants per group were needed.

Prespecified outcome analyses were done in eligible participants who had received at least one dose of either vaccine, and in those who received all of the three doses of the vaccines. The primary endpoint was prevention of hepatitis E in participants who received three doses of vaccine (ie, the per-protocol population) during the 12 months from the 31st day after receipt of the third dose. Vaccine efficacy and 95% CIs were calculated on the basis of the identified difference between the vaccine group and the placebo group and the accrued person-time. An exact conditional procedure was used to evaluate vaccine efficacy under the assumption that the numbers of patients with hepatitis E in the vaccine and placebo groups were independent Poisson random variables.[28] For robustness, efficacy was also assessed by use of a Cox proportional hazard model, and a log-rank test was used to compare the cumulative incidence of hepatitis E between the study groups.

Adverse events were summarised for all vaccination visits as frequencies and percentages according to study group. Proportions of events and 95% CIs (unadjusted for multiplicity) were compared between the groups by use of two-sided Fisher's exact test.

Data analysis was done with SAS software version 9.1. All reported p values are two-sided with an α value of 0·05.

### Role of the funding source

The sponsor of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

## Results

122 179 people from 11 townships attended the enrolment visit between August and October, 2007. 112 604 participants fulfilled the eligibility requirements, were randomly assigned to the study group, and received at least one dose of vaccine or placebo. 97 356 participants

| | Follow-up (month of study) | Vaccine group | | | Placebo group | | | Vaccine efficacy (95% CI) | p value |
|---|---|---|---|---|---|---|---|---|---|
| | | Number of participants/ person-years at risk | Number of cases | Incidence (per 10 000 person-years) | Number of participants/ person-years at risk | Number of cases | Incidence (per 10 000 person-years) | | |
| Per-protocol | | | | | | | | | |
| Whole group (three doses) | 7–19 | 48 693/48 594·6 | 0 | 0·0 | 48 663/48 555·1 | 15 | 3·1 | 100·0% (72·1–100·0) | <0·0001 |
| Men | 7–19 | 20 662/20 616·1 | 0 | 0·0 | 20 709/20 660·0 | 11 | 5·3 | 100·0% (60·1–100·0) | 0·001 |
| Women | 7–19 | 28 031/27 978·5 | 0 | 0·0 | 27 954/27 895·1 | 4 | 1·4 | 100·0% (–51·0 to 100·0) | 0·045 |
| Age 16–49 years | 7–19 | 30 374/30 299·5 | 0 | 0·0 | 30 355/30 276·9 | 6 | 2·0 | 100·0% (15·13–100·0) | 0·014 |
| Age 50–65 years | 7–19 | 18 319/18 295·2 | 0 | 0·0 | 18 308/18 278·2 | 9 | 4·9 | 100·0% (49·4–100·0) | 0·003 |
| First 6 months of follow-up | 7–13 | 48 693/23 981·9 | 0 | 0·0 | 48 663/23 965·8 | 6 | 2·5 | 100·0% (15·12–100·0) | 0·014 |
| Second 6 months of follow-up | 14–19 | 48 693/24 612·8 | 0 | 0·0 | 48 663/24 589·3 | 9 | 3·7 | 100·0% (49·4–100·0) | 0·003 |
| First two doses subset | 1·5–5 | 54 986/20 202·1 | 0 | 0·0 | 54 973/20 196·8 | 5 | 2·5 | 100·0% (9·1–100·0) | <0·0001 |
| Population receiving at least one dose | 7–19 | 56 302/56 104·7 | 1 | 0·2 | 56 302/56 081·2 | 16 | 2·9 | 93·8% (59·8–99·9) | 0·0001 |
| Modified subset one (all participants received at least one dose) | 0–19 | 56 302/87 354·2 | 1 | 0·1 | 56 302/87 323·2 | 22 | 2·5 | 95·5% (66·3–99·4) | <0·0001 |
| Modified subset two (participants in reactogenicity subset were excluded because of lacking follow-up data during 0–6 months) | 0–19 | 54 986/86 040·4 | 1 | 0·1 | 54 973/86 003·4 | 21 | 2·4 | 95·2% (64·6–99·4) | <0·0001 |

Person-years at risk is the cumulative follow-up years of at risk participants at the indicated timepoint. Number of at risk participants is the initial number of participants entered in the study–(cumulative hepatitis E cases+participants who had dropped out of study).

***Table 2:*** **Efficacy of recombinant hepatitis E vaccine**

received all three doses of vaccine or placebo and were included in the analysis of the primary endpoint (figure 1). Table 1 shows the baseline characteristics of study participants.

The DSMB confirmed 23 cases of hepatitis E before unblinding (figure 2); details of each case are listed in webappendix pp 4–24. Compared with the general study population, patients with hepatitis E were older (mean 51·3 years, SD 8·2; median 53, range 36–63) and more likely to be men (male-to-female ratio 2·3). The mean maximum serum ALT concentration of patients with hepatitis E was 30·8-times upper limit of normal range (SD 29·3; 18·1, 2·5–96·9), and the mean duration of illness was 57·1 days (SD 39·8; 41, 9–175). 15 patients were admitted to hospital for a mean of 24·4 days (SD 14·5; 20, 9–66). All 23 patients tested positive for hepatitis E virus IgM, 22 were positive for hepatitis E virus RNA, and 14 had a 4-times or greater increase in hepatitis E virus IgG. Of the 13 patients whose viruses were isolated for sequencing, 12 had genotype 4 and one had genotype 1. Of the eight patients who received all three doses and whose viruses underwent sequencing, all had genotype 4.

In the primary analysis population 15 participants developed hepatitis E during the 12 months from the 31st day after receipt of the third dose; all 15 were in the placebo group (table 2). Vaccine efficacy against hepatitis E was 100·0% (95% CI 72·1–100·0), and protection extended to all participants throughout the 12 months. Five participants developed hepatitis E during the 14 days after the second dose and before the third dose; all were in the placebo group. Vaccine efficacy after two doses was 100·0% (9·1–100·0).

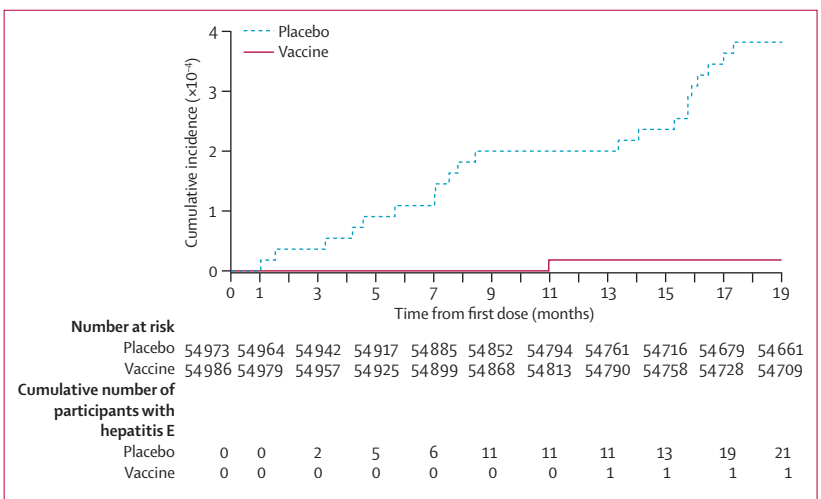


***Figure 3:*** **Cumulative incidence of hepatitis E**
Cumulative incidence in each group at an indicated time=(cumulative number of cases/cumulated following-up time for at risk participants×10 000). Number at risk=initial number of participants entered in the study–(cumulative hepatitis E cases+participants who had subsequently dropped out of study). The difference between the groups was significant (p<0·001 by log-rank test).

Most randomised participants who received at least one dose of vaccine or placebo were followed up for 19 months from the beginning of the study, and a small proportion of participants were followed up from month 7 of the study. There were 23 cases of hepatitis E during the follow-up, one in the vaccine group (the participant received one

| | Number of adverse events (rate, 95% CI) | | p value* |
|---|---|---|---|
| | Vaccine group | Placebo group | |
| **Reactogenicity subset** | | | |
| Number of participants who received more than one dose | 1316 | 1329 | .. |
| Solicited local adverse events within 72 h after each dose | | | |
| Local adverse events | 177 (13·5%, 11·65–15·41) | 94 (7·1%, 5·75–8·59) | <0·0001 |
| Local adverse events ≥grade 3 | 2 (0·2%, 0·02–0·55) | 0 (0·0%, 0·00–0·28) | 0·248 |
| Pain | 136 (10·3%, 8·74–12·11) | 73 (5·5%, 4·33–6·86) | <0·0001 |
| Pain ≥grade 3 | 0 (0·0%, 0·00–0·28) | 0 (0·0%, 0·00–0·28) | .. |
| Swelling | 30 (2·3%, 1·54–3·24) | 8 (0·6%, 0·26–1·18) | <0·0001 |
| Swelling ≥grade 3 | 2 (0·2%, 0·02–0·55) | 1 (0·0%, 0·00–0·28) | 0·248 |
| Itch | 20 (1·5%, 0·93–2·34) | 13 (1·0%, 0·52–1·67) | 0·210 |
| Itch ≥grade 3 | 0 (0·0%, 0·00–0·28) | 0 (0·0%, 0·00–0·28) | .. |
| Solicited systemic adverse events within 72 h after each dose | | | |
| Systemic adverse events | 267 (20·3%, 18·15–22·56) | 263 (19·8%, 17·68–22·03) | 0·748 |
| Systemic adverse events ≥grade 3 | 7 (0·5%, 0·21–1·09) | 4 (0·3%, 0·08–0·77) | 0·356 |
| Fever | 245 (18·6%, 16·55–20·83) | 239 (18%, 15·95–20·16) | 0·674 |
| Fever ≥grade 3 | 6 (0·5%, 0·17–0·99) | 3 (0·2%, 0·05–0·66) | 0·341 |
| Headache | 14 (1·1%, 0·58–1·78) | 8 (0·6%, 0·26–1·18) | 0·191 |
| Headache ≥grade 3 | 1 (0·1%, 0·00–0·42) | 0 (0·0%, 0·00–0·28) | 0·498 |
| Fatigue | 28 (2·1%, 1·42–3·06) | 20 (1·5%, 0·92–2·31) | 0·230 |
| Fatigue ≥grade 3 | 1 (0·1%, 0·00–0·42) | 0 (0·0%, 0·00–0·28) | 0·498 |
| **Total vaccinated cohort minus the reactogenicity subset** | | | |
| Number of participants who received more than one dose | 54 986 | 54 973 | .. |
| Solicited local adverse events within 72 h after each dose | | | |
| Local adverse events | 1532 (2·8%, 2·65–2·93) | 1051 (1·9%, 1·8–2·03) | <0·0001 |
| Local adverse events ≥grade 3 | 61 (0·1%, 0·08–0·14) | 27 (0·1%, 0·03–0·07) | <0·0001 |
| Pain | 1143 (2·1%, 1·96–2·20) | 754 (1·4%, 1·28–1·47) | <0·0001 |
| Pain ≥grade 3 | 1 (0·0%, 0·00–0·01) | 0 (0·0%, 0·00–0·01) | 1·000 |
| Solicited systemic adverse events within 72 h after each dose | | | |
| Systemic adverse events | 1068 (1·9%, 1·83–2·06) | 1045 (1·9%, 1·79–2·02) | 0·617 |
| Systemic adverse events ≥grade 3 | 60 (0·1%, 0·08–0·14) | 63 (0·1%, 0·09–0·15) | 0·786 |

(Continues on next page)

dose of the vaccine) and 22 in the placebo group (table 2). Vaccine efficacy for participants who received at least one dose was 95·5% (95% CI 66·3–99·4). There were 17 cases of hepatitis E during the 12 months from the 31st day after the receipt of the final dose (which could be the first, second, or third dose), one in the vaccine group, and 16 in the placebo group. The corresponding vaccine efficacy was 93·8% (95% CI 59·8–99·9%).

Figure 3 shows the cumulative incidence of hepatitis E in participants who were followed up for 19 months from the beginning of the study. The difference between the vaccine group and the placebo group was significant (p<0·0001).

Most adverse events were mild. Rates of serious adverse events were similar in the vaccine and placebo groups during the entire follow-up, and none were deemed by the DSMB to relate to vaccination (table 3 and webappendix pp 25–28). Participants in the reactogenicity subset were regularly interviewed by investigators after receipt of each dose to assess adverse events (table 3). In this subset, the proportion of all solicited local adverse events identified within 72 h after each dose was greater in the vaccine group (13·5%) than in the placebo group (7·1%; p<0·0001). The vaccine group also had a greater proportion of adverse reactions attributed to pain, swelling, and itching at injection sites, which were the most common local adverse events. The proportion of systemic adverse events were similar for both groups (20·3% *vs* 19·8%). On the basis of reports by participants not in the reactogenicity subset, the proportion of solicited local adverse events was higher in the vaccine group than in the placebo group (2·8% *vs* 1·9%) and the rates of solicited systemic adverse events were not significantly different between the two groups (table 3).

Serum samples were taken from 11165 participants before vaccination and 1 month after receipt of the third dose. 5494 (98·7%) of 5567 participants in the vaccine group had an increase in antibody concentration in the samples after vaccination of four times or more from that of the corresponding samples before vaccination. In the samples after vaccination, geometric mean concentration in these participants rose from 0·14 Wu/mL to 19·0 Wu/mL (95% CI 18·6–19·4). By contrast, 119 (2·1%) of 5598 par-

| | Number of adverse events (rate, 95% CI) | | p value* |
|---|---|---|---|
| | Vaccine group | Placebo group | |
| (Continued from previous page) | | | |
| **Total vaccinated cohort** | | | |
| Number of participants who received more than one dose | 56 302 | 56 302 | .. |
| Unsolicited events within 30 days after each dose† | | | |
| All | 6771 (12·0%, 11·76–12·3) | 6724 (11·9%, 11·68–12·21) | 0·666 |
| ≥Grade 3 | 839 (1·5%, 1·39–1·59) | 792 (1·4%, 1·31–1·51) | 0·241 |
| Serious adverse events within 30 days after each dose‡ | | | |
| All | 248 (0·4%, 0·39–0·50) | 245 (0·4%, 0·38–0·49) | 0·892 |
| Admission to hospital | 238 (0·4%, 0·37–0·48) | 233 (0·4%, 0·36–0·47) | 0·817 |
| Disability | 0 (0·0%, 0·00–0·01) | 0 (0·0%, 0·00–0·01) | .. |
| Death§ | 10 (0·0%, 0·01–0·03) | 12 (0·0%, 0·01–0·04) | 0·670 |
| Serious adverse events during period from month 2 to month 6 and from month 7 to month 19† | | | |
| All | 1423 (2·5%, 2·40–2·66) | 1430 (2·5%, 2·41–2·67) | 0·894 |
| Admission to hospital | 1328 (2·4%, 2·23–2·49) | 1336 (2·4%, 2·25–2·50) | 0·875 |
| Disability | 0 (0·0%, 0·00–0·01) | 0 (0·0%, 0·00–0·01) | .. |
| Death | 95 (0·2%, 0·14–0·21) | 94 (0·2%, 0·13–0·20) | 0·942 |

Grade 3 pain, headache, and fatigue were defined as prevention of normal activities; grade 3 swelling was defined as a diameter of more than 30 mm; grade 3 itch was defined as body itch; and grade 3 fever was defined as temperature greater than 39·0°C. Symptoms with frequency more than 1% in any group are listed. The webappendix details all serious adverse events (pp 25–28). *p values are two-sided and were calculated by Fisher's exact test. †Unsolicited adverse events included any adverse events that happened from day 4 to day 30 after each dose and any adverse events within 3 days after each dose but had not been listed in the diary card for registering solicited adverse events. Most often unsolicited adverse events in the study included upper respiratory tract infection, headache, fever, and gastritis. ‡The data and safety monitoring board did not deem any of the serious adverse events to be related to vaccination. §22 participants died within 30 days after each vaccination. Of the ten participants in the vaccine group that died, eight died as the result of an accident, one died of a cerebral haemorrhage, and one died of liver cancer after 10 years with chronic hepatitis B. Of 12 participants in the placebo group that died, six died as the result of an accident, three died of myocardial infarction, two died of cerebral haemorrhage, and one died of stomach cancer.

***Table 3:*** **Safety outcomes**

ticipants in the placebo group showed an antibody response and all the episodes were subclinical infection.

## Discussion

In our trial, efficacy of recombinant hepatitis E vaccine during the 12 months from the 31st day after the receipt of the third dose was 100·0% (95% CI 72·1–100·0), and protection was noted across all age and sex subgroups. Vaccination was also beneficial under less than perfect circumstances—ie, when participants did not receive all three doses. Vaccine efficacy after two doses was 100·0% (95% CI 9·1–100·0). Therefore, during a hepatitis E outbreak, or for travellers to an endemic area, protection can be quickly obtained by two vaccine doses given within 1 month.

Side-effects were few and mild and no serious adverse events related to vaccination. HEV 239 is unlikely to induce rare vaccine-related serious adverse events, because the large number of participants in the study affords a power of 85% to detect rare serious adverse events if the rate in the vaccine group is 0·03% and the rate ratio is 5·0 (webappendix p 29).

The study site is endemic for infection with hepatitis E virus, with nearly half the participants tested on day 0 being seropositive. The infection rate in the placebo group was 2·1% during the period from 0 months to 7 months. However, most of the infections seemed to be subclinical and incidence of hepatitis E was estimated to be about four per 10 000 person-years (webappendix p 1). The reason for the low attack rate is unknown. In developed countries where the zoonotic hepatitis E genotype 3 predominates, emerging data showed a moderate hepatitis E virus seroprevalence but rare autochthonous cases of hepatitis E.[3] These findings suggest that the low attack rate might be a common feature of both zoonotic genotypes, relating to a low-level, but nevertheless widespread, exposure in areas where these viruses are prevalent.

Animal studies showed that HEV 239, which is produced with a genotype 1 isolate, confers protection against both genotypes 1 and 4.[19] 12 of 13 patients with hepatitis E who were typed by sequencing, had genotype 4, all in the placebo group. Therefore, our study substantiates that the vaccine cross protects against genotype 4 in human beings, and the cross protection probably extends to other genotypes as well, given that they belong to the same serotype as the vaccine strain.

Data suggest that individuals with chronic liver disease should be prioritised for hepatitis E vaccination to prevent serious damage from infection.[8,9] However, because we excluded this group, additional study is needed to assess the benefits of HEV 239. Another limitation was the lack

of a hepatitis E case in the vaccine group, meaning that the protective antibody concentration could not be assessed. Further analysis of our serology data might provide important information on the vaccine's efficacy against subclinical infection. Both our study and the previous phase 2 study of the vaccine produced in insect cells showed substantial short-term protection; however, the duration of this protection needs further assessment.

In our trial, we found the vaccine well tolerated and efficacious for a general adult population. Further studies are needed to assess the safety and to support the benefits of the vaccine for pregnant women and for people younger than 15 years or older than 65 years.

#### Contributors

All authors contributed towards acquisition of data or statistical analyses, or interpretation of data, writing and revising the report, and final approval. F-CZ and JZ contributed equally to this work.

#### Hecolin study group

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#### Conflicts of interest

Y-LX and Y-ML are employees of the Xiamen Innovax. The other authors declare that they have no conflicts of interest.

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