Safety and effectiveness of a recombinant hepatitis E vaccine in women of childbearing age in rural Bangladesh: a phase 4, double-blind, cluster-randomised, controlled trial

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Summary

Background Hepatitis E virus (HEV) leads to high mortality in pregnant women in low-income countries. We aimed to evaluate the safety of a HEV vaccine and its effectiveness in preventing hepatitis E during pregnancy.

Methods In this phase 4, double-blind, cluster-randomised trial, 67 villages in Matlab, Bangladesh, were randomised 1:1 to receive HEV239 (a recombinant HEV vaccine) or a control vaccine (Hepa-B, a hepatitis B vaccine), using block randomisation with random number tables and blocks of size eight, stratified by cluster population size. Eligible non-pregnant women (aged 16–39 years) were vaccinated intramuscularly on day 0, at 1 month, and at 6 months, and followed up for 2 years after the last immunisation. The primary endpoint was hepatitis E in the pregnant, per-protocol population (those who received all three doses within 2 days of the scheduled dates), while safety was a secondary endpoint, assessed in the intention-to-treat (ITT) population (participants who received at least one dose). Solicited adverse events were recorded for the first 7 days after each dose, and unsolicited events until 2 years after a participant's final dose. Pregnancy-related safety outcomes were assessed in the pregnant ITT population. This study is registered with ClinicalTrials.gov (NCT02759991).

Findings Between Oct 2, 2017, and Feb 28, 2019, 19 460 participants were enrolled and received either HEV239 (9478 [48·7%] participants, 33 clusters) or Hepa-B (9982 [51·3%] participants, 34 clusters), of whom 17 937 (92·2%) participants received three doses and 17 613 (90·5%) were vaccinated according to protocol (8524 [48·4%] in the HEV239 group and 9089 [51·6%] in the control group). No pregnant participants were confirmed to have hepatitis E in either treatment group. HEV239 showed a mild safety profile, similar to Hepa-B, with no difference in the proportion of solicited adverse events between groups and no severe solicited events. Pain was the most common local symptom (1215 [12·8%] HEV239 recipients and 1218 [12·2%] Hepa-B recipients) and fever the most common systemic symptom (141 [1·5%] HEV239 recipients and 145 [1·5%] Hepa-B recipients). None of the serious adverse events or deaths were vaccine related. Among pregnant participants, the HEV239 group had a higher risk of miscarriage (136 [5·7%] of 2407 pregnant participants) compared with the control group (102 [3·9%] of 2604; adjusted odds ratio 1·54 [95% CI 1·15–2·08]).

Interpretation The effectiveness of HEV239 in pregnant women remains uncertain. HEV239 was safe and well tolerated in non-pregnant women, but findings regarding miscarriage warrant further investigation.

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Introduction

Hepatitis E virus (HEV) is a major cause of acute viral hepatitis worldwide, causing endemic disease or outbreaks in low-income countries with poor sanitation.[1] Clinically, hepatitis caused by HEV resembles other types of acute viral hepatitis and is typically self-limiting.[2] Severity increases with age, with a projected 1–3% fatality rate.[3] Africa and the Indian subcontinent are the most frequently affected areas.[1] Pregnant patients face a high mortality rate of 5–25%, and survivors have high rates of miscarriage (spontaneous abortion) and stillbirth.[4,5] HEV infection can also have poor outcomes in patients with chronic liver disease and those who are immunocompromised.[6]

HEV, a member of the Hepeviridae family, comprises eight genotypes under a single serotype, of which four primarily infect humans.[7] HEV1 and HEV2 cause outbreaks in humans, while the zoonotic HEV3 and HEV4 cause sporadic cases in humans. The remaining genotypes (HEV5–8) are predominantly found in animals.[8]

The recombinant HEV vaccine HEV239 (Hecolin; Xiamen Innovax Biotech, Xiamen, China) underwent a large phase 3 clinical study in China, in which the

Research in context

Evidence before this study

HEV239 (Hecolin; Xiamen Innovax Biotech, Xiamen, China) is the only licensed hepatitis E virus (HEV) vaccine. We searched PubMed for English-language articles published between Jan 1, 2001, and Aug 2, 2023, using the following search terms: Hecolin and or HEV efficacy and safety. We found 27 articles and selected the seven articles describing clinical trials of HEV239. This vaccine, based on HEV genotype 1 (HEV1), was well tolerated and safe in healthy adults in early phase trials. A large phase 3 trial in China including more than 100 000 individuals aged 16–65 years showed that the vaccine had a good safety profile and high efficacy against hepatitis E (100% after three doses). The HEV cases in that trial were predominantly due to genotype 4, indicating the vaccine's capacity for genotype-heterologous protection. Notably, the vaccine showed high immunogenicity, with seroconversion rates of 98–100% among vaccinees. However, previous studies lacked statistical power to sufficiently demonstrate the vaccine's efficacy in preventing hepatitis E in women of childbearing age. Further research was necessary to explore the vaccine's efficacy specifically in this population who could potentially benefit the most from vaccination.

Added value of this study

The current study was designed to explore the knowledge gap relating to the efficacy of HEV239 in women of childbearing age, and particularly those who become pregnant after vaccination. However, no HEV infections were detected among pregnant participants in either treatment group. Secondary analyses provided evidence that HEV239 is effective in women of childbearing age. Furthermore, we found high immunogenicity of HEV239 after both two and three doses. This is the first study to show that HEV239 protects against HEV1. Importantly, a safety signal emerged from this study regarding an increased risk of miscarriage in the HEV239 group, which demands further attention.

Implications of all the available evidence

The available evidence suggests that both two and three doses of HEV239 are immunogenic, with three doses being effective against HEV infection and disease, including in women of childbearing age. The knowledge gap regarding its efficacy in preventing hepatitis E in pregnant women remains. The safety signal identified in our study warrants further investigation. Additionally, future studies should explore the effectiveness of a two-dose schedule, which might offer a more feasible and cost-effective vaccination strategy.

observed efficacy after three doses was 100% (95% CI 72·1–100·0), and adverse events related to the vaccine were mild.[9] HEV239 is developed from HEV1 and showed cross-protection in the Chinese study, wherein HEV4 was prominent. Data are lacking on protection of the vaccine against HEV1–3. To evaluate efficacy against all genotypes that commonly cause illness in humans, further testing in diverse geographical locations is necessary. In south Asia, including Bangladesh, the most common circulating human strain is HEV1.[8,10,11]

WHO's Strategic Advisory Group of Experts has not recommended the HEV239 vaccine in endemic areas due to several knowledge gaps,[12] and has thus called for more research on efficacy and safety in specific populations, such as pregnant women.[13,14]

Several studies from the south Asian region report high numbers of maternal and neonatal HEV-related deaths, highlighting the need to reduce the burden in this region.[15,16] As estimates show that Bangladesh alone has around 1000 HEV-related maternal deaths annually,[17] and the epidemiology aligns with other parts of south Asia,[15] a trial on HEV239 in Bangladesh would provide relevant results for the estimated 1·5 billion south Asians at risk for HEV infection.

We conducted a phase 4 clinical trial in a rural area in Bangladesh, where previous serosurveillance studies have shown that HEV infections are common,[17] to evaluate the safety of the vaccine and its effectiveness in women of childbearing age for preventing hepatitis E during pregnancy.

Methods

Study design

This cluster-randomised, double-blind trial was conducted in Matlab, in rural Bangladesh, where the International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b) has maintained a field research site (The Matlab Health and Research Centre) for more than 60 years with a continuing health and demographic surveillance system (HDSS) covering a population of approximately 240 000 in 67 villages.[18] A maternal, child health, and family planning intervention programme operates in the research site, from 41 fixed site clinics.

We chose a cluster-randomised design because this trial was intended as an effectiveness trial and because this design enabled us to better replicate public health practice.[19] The real-world impact, acceptability, and feasibility of a vaccine when administered under practical public health circumstances cannot be adequately assessed with an individually randomised design but can be effectively analysed through a cluster-randomised approach.[19]

The study protocol[20] was approved by the Independent Ethics Committee of the icddr,b, the Directorate General of Drug Administration (Dhaka, Bangladesh) and Regional Ethics Committee of Oslo (Norway), as well as

the data protection officer at the Norwegian Institute of Public Health (NIPH). An independent data and safety monitoring board, constituted by the icddr,b, closely monitored the study design and progress. Written informed consent was obtained from all participants.

This study was registered on ClinicalTrials.gov (NCT02759991).

Participants

Eligible participants were non-pregnant, healthy women aged 16–39 years from the 67 villages in the study area, identified via the Matlab HDSS and informed of the study through home visits. Those who visited the fixed site clinic and provided written informed consent were assessed for eligibility by a study physician. Exclusion criteria included current pregnancy, serious acute or chronic disease, infectious disease, recent receipt of vaccine or immunoglobulin within the 2 weeks preceding enrolment, temporal fever above 38°C, or history of allergic vaccine reaction. The eligibility criteria are detailed in the trial protocol (appendix 1 p 1). Eligibility was reassessed before each dose.

Randomisation and masking

The two recombinant subunit vaccines in the study were HEV239 (Hecolin) and a hepatitis B virus (HBV) control vaccine (Hepa-B; Incepta Pharmaceuticals, Dhaka, Bangladesh), both with aluminium hydroxide adjuvant. The two vaccines were identical in appearance, fill-finished into identical single-dose vials, and labelled with letter codes by Incepta Pharmaceuticals. The volume of HEV239 was the same for all ages (0·5 mL), while the dose of Hepa-B was 0·5 mL for participants aged 16–18 years and 1 mL for older participants. To enhance masking of the vaccinators, eight different letter codes were used to label the vials. Each vaccine type had two codes for each of the two age groups (16–18 years and 19–39 years). Randomisation was stratified by population size, and villages (clusters) were iteratively randomised (1:1) to receive HEV239 or Hepa-B in blocks of eight, corresponding to the four two-letter code combinations for each group (appendix 1 p 2). An independent statistician, not further involved in the trial, used a table of random numbers to perform the randomisation.

All parties were masked to group allocation, except for vaccine administrators, who could be aware of the differing volumes of the vaccines. However, they were only involved in vaccination and the differing volumes of the vaccines were not mentioned in the training of the staff. The identities of the codes were known only by staff at Incepta Pharmaceuticals. Staff who ascertained eligibility and obtained informed consent for participation, as well as staff who conducted post-dosing surveillance, were not involved in the administration of vaccine, and hence were also kept blinded. Finally, data analysis was done by researchers blinded to the agent received by each participant.

Procedures

Enrolment and vaccination were conducted at the fixed site clinics in the HDSS area and occurred concurrently across all study villages in both groups (appendix 1 pp 1–3). Each vaccine was administered intramuscularly into the deltoid muscle of either arm at 0, 1, and 6 months, with proper cold-chain maintenance. Post vaccination, participants were observed for 30 min and received home visits for 7 consecutive days to inquire about local reactions and solicited systemic symptoms, followed by unsolicited events from 8 days until 2 years after a participant's final dose of vaccine. To standardise the reporting of serious adverse events, medical terms used by researchers were analysed according to the Medical Dictionary for Regulatory Activities terminology (MedDRA version 19.1).

Two monitors, one each from the icddr,b and NIPH, conducted seven site visits from November, 2017, until the COVID-19 nationwide lockdown in March, 2020. They assessed procedures of inclusion and informed consent, staff resources, source documents, the trial master file, drug storage, auditing and monitoring of stock including checking of expiry dates, laboratory equipment, and general study conduct, including home visits and vaccination. Serious adverse event reports were continuously evaluated. Over 11% of case report forms were monitored either on site or by copies transferred electronically.

See Online for appendix 1

Participants were surveilled for serious adverse events, hepatitis, and pregnancy for 2 years post-vaccination last dose, both passively and actively. For passive surveillance, participants were given immunisation cards and instructed to instantly contact study staff if they had serious adverse events, or if they had jaundice or specified symptoms for 3 days or more, including fatigue, loss of appetite, abdominal discomfort, abdominal pain in the right upper quadrant, nausea, or vomiting. Suspected cases of hepatitis were diagnosed by study physicians at the Matlab study clinic, and those patients were tested for liver function (albumin, alanine aminotransferase [ALT], international normalised ratio, and bilirubin) and underwent virological assessments for hepatitis (anti-hepatitis A virus [HAV] IgM, HBsAg, anti-HBc IgM, anti-hepatitis C virus [HCV], and anti-HEV IgM; appendix 1 p 4).

Active surveillance involved weekly check-ins by field workers through home visits to identify pregnancies and screen for clinical hepatitis. The procedures for detecting pregnancies at enrolment and during the weekly visits are outlined in a separate Article[21] addressing pregnancy outcomes. During periods of COVID-19 lockdown, follow-up was primarily done through telephone calls. All information was captured in individual case report forms.

Dried blood spots were prepared from capillary blood from all participants before vaccination and approximately 30 days after the last dose. The dried blood spot samples were analysed in pairs for anti-HEV IgG (Wantai, Beijing, China) according to the method previously validated by Øverbø and colleagues.[22] IgG values were standardised to WHO units per mL (WU/mL) via a five-parameter logistic function, using the WHO reference reagents (code 95/584). Suspected cases of HEV infection were analysed by a rapid anti-HEV IgM test detecting anti-HEV IgM in an immunochromatographic assay (Wantai, Beijing, China), total anti-HEV IgG and IgM (Wantai, Beijing, China) by ELISA, and HEV RNA using RealStar HEV RT-PCR kit 2.0 (Altona, Hamburg, Germany), according to the manufacturers' recommendations. Serum samples were considered positive for IgM or IgG in the ELISA assays if the optical density of the relevant analyte exceeded the manufacturer's cutoff value of 1. For dried blood spots, the optical density had to exceed 1·6 to be considered positive.[22] For HEV RNA positive samples, whole-genome sequencing was conducted using baits enrichment. The extracted RNA was prepared by DNase-treatment before library preparation using the KAPA RNA HyperPrep kit (Roche, Basel, Switzerland). Further, the samples were normalised based on cycle threshold values and pooled into the HyperCap Target enrichment using VirCap EZ share developer probes (Roche). Finally, samples were sequenced using MiSeq Reagents kit v3 (Illumina, San Diego, CA, USA) according to the manufacturer's recommendations. Sequences were genotyped based on reference mapping using Tanoti.

Outcomes

The primary efficacy endpoint was hepatitis E in pregnant women, defined by the presence of both an illness duration of more than 2 days and an abnormal serum ALT level (>2·5 times the upper limit of normal), with infection confirmed by detection of anti-HEV IgM or HEV RNA in the serum, or by seroconversion, defined by a 4-times or higher increase of anti-HEV IgG concentration in paired sera, with the last sample being anti-HEV IgG positive. Total vaccine protection was defined as reduction of the incidence of hepatitis E in recipients of complete regimens of HEV239 vaccine in HEV239 clusters relative to recipients of complete regimens of Hepa-B vaccine in control clusters. Predefined secondary outcomes in pregnant women were altered immune response and maternal and perinatal mortality associated with maternal jaundice.

The secondary efficacy outcome was hepatitis E in non-pregnant participants. Other secondary outcomes were immunogenicity in all participants (HEV IgG response 1 month after the last vaccine dose), safety in all participants as defined in the study protocol (appendix 1 p 5), and viral load and subtypes. The secondary outcomes perceptions about vaccine acceptability and operational challenges in the delivery of the vaccine will be evaluated in separate reports.

Statistical analysis

For the sample size calculations, the following assumptions were made: women aged 16–39 years represented 20% of the general population, with an estimated 22% being HEV IgG positive at baseline.[15,16] Further, we assumed that among recipients of three vaccine doses, 20% would become pregnant and complete their pregnancies and 6% of seronegative pregnant participants would become infected, with 35% of these infections being symptomatic. The expected protective efficacy of a three-dose regimen of the HEV239 vaccine against symptomatic infections would exceed 95% at $p<0·05$ (two-tailed). We anticipated a 15% loss in person-time due to migration and a 5% refusal rate, with a design effect of 2. To achieve 80% power, we required a baseline enrolment of 20 745 women.[20]

Safety was assessed in the intention-to-treat (ITT) population, defined as all participants who received at least one dose of either HEV239 or Hepa-B vaccine. In the safety analyses, we adjusted for age and BMI due to their relevance in influencing health outcomes and to control for potential confounding. The primary efficacy endpoint was assessed in the per-protocol population, which included participants vaccinated with all three doses of their respective vaccine within 2 days before or after the scheduled date. Finally, the immunogenicity dataset constituted all samples collected according to protocol.

To compare the incidence of hepatitis E between the study groups, we estimated incidence rate ratios (IRRs) by dividing the incidence rate in the HEV239 group by that in the control group. p values and 95% CIs were calculated using exact Poisson regression models, with accrued person-time as the offset for individual-level analyses and cluster size as the offset for cluster-level analyses. Vaccine effectiveness was calculated as $(1-\text{IRR})\times 100\%$. We used a log-rank test to assess whether there was a significant difference in the hepatitis E rate between the two groups. To assess differences in immunological outcomes, we used the χ^2 test for categorical data and the Student's t test on log-transformed continuous data. We also reported frequencies and percentages of safety outcomes, including solicited and unsolicited adverse events after each vaccination.

To compare the risk of adverse events between the HEV239 and Hepa-B groups, while accounting for clustered data, we calculated odds ratios (ORs) with a logistic regression model, using the generalised estimating equations approach to fit the model to account for dependencies within clusters. The McNemar test was used to compare the risk of adverse events after the second and third doses of the HEV239 vaccine. $p<0·05$ was considered statistically significant. All statistical analyses were done with Stata (version 17.0).

Role of the funding source
The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

In total, 19 460 women were enrolled in the trial and constituted the ITT population (figure). Recruitment lasted from Oct 2, 2017, until Feb 28, 2019. It ended before reaching the target population of 20 745, as the enrolment process was more time-consuming than anticipated and had to be finalised 6 months ahead of the Hecolin vaccine's expiration date (September, 2019).

33 villages (9478 [48·7%] participants) were randomly allocated to receive the HEV239 vaccine and 34 (9982 [51·3%] participants) to receive the Hepa-B vaccine. 17 937 (92·2%) participants received the third dose. 17 613 (90·5%) participants were vaccinated according to schedule (within 2 days) and included in the per-protocol analyses (8524 [48·4%] in the HEV239 group and 9089 [51·6%] in the control group). All participants were followed up for 2 years to detect pregnancies, development of acute hepatitis, and adverse events. The end of the study period was Oct 31, 2021.

Baseline demographics are detailed in table 1. In the ITT population, mean age was 25·9 years (SD 7·08) in the HEV239 group and 26·0 years (7·02) in the control group. Mean BMI was 23·0 kg/m² (4·15) in the HEV239 group and 22·9 kg/m² (4·07) in the control group. 5011 participants became pregnant within 2 years of their last vaccination (2407 [25·4%] of 9478 in the HEV239 group and 2604 [26·1%] of 9982 in the control group). 3758 participants had at least one pregnancy after three doses (1829 [19·3%] in the HEV239 group and 1929 [19·3%] in the control group). We used rigorous procedures to exclude pregnant women at enrolment and at each vaccine dose, as described in a separate Article.[21] Nonetheless, 238 (2·5%) participants in the HEV239 group and 292 (2·9%) in the control group were inadvertently vaccinated during pregnancy, mostly during the first weeks of gestation.

The total seroprevalence of HEV IgG before vaccination (day 0) was 40·4% (95% CI 39·7–41·0), with a geometric mean titre of 0·33 WU/mL (0·33–0·34; table 2). Seroconversion between day 0 and day 210, was observed in 97·1% (96·8–97·5) of HEV239-vaccinated participants in the ITT population and 97·7% (97·3–98·0) of HEV239-vaccinated participants in the per-protocol group. In the control group, seroconversion occurred in 10·3% (9·7–11·0) of the ITT population, corresponding to an annual incidence of 17·9% (calculated as 10·3% / 210 days × 365 days), assuming homogeneous occurrence of infection during each season of the year.

During follow-up, 2839 participants (14·6%) reported possible hepatitis symptoms to the study team and underwent urgent medical examination at 3395 visits by a study physician (appendix 1 p 4). Among them, 238 individuals, 16 of whom were pregnant, were suspected to have hepatitis and received further laboratory testing (appendix 1 pp 6–14). The laboratory results revealed six cases of hepatitis E, all among non-pregnant participants, as well as 16 cases of HAV disease, 35 cases of HBV disease, and two cases of HCV disease. The remaining symptomatic participants had unknown

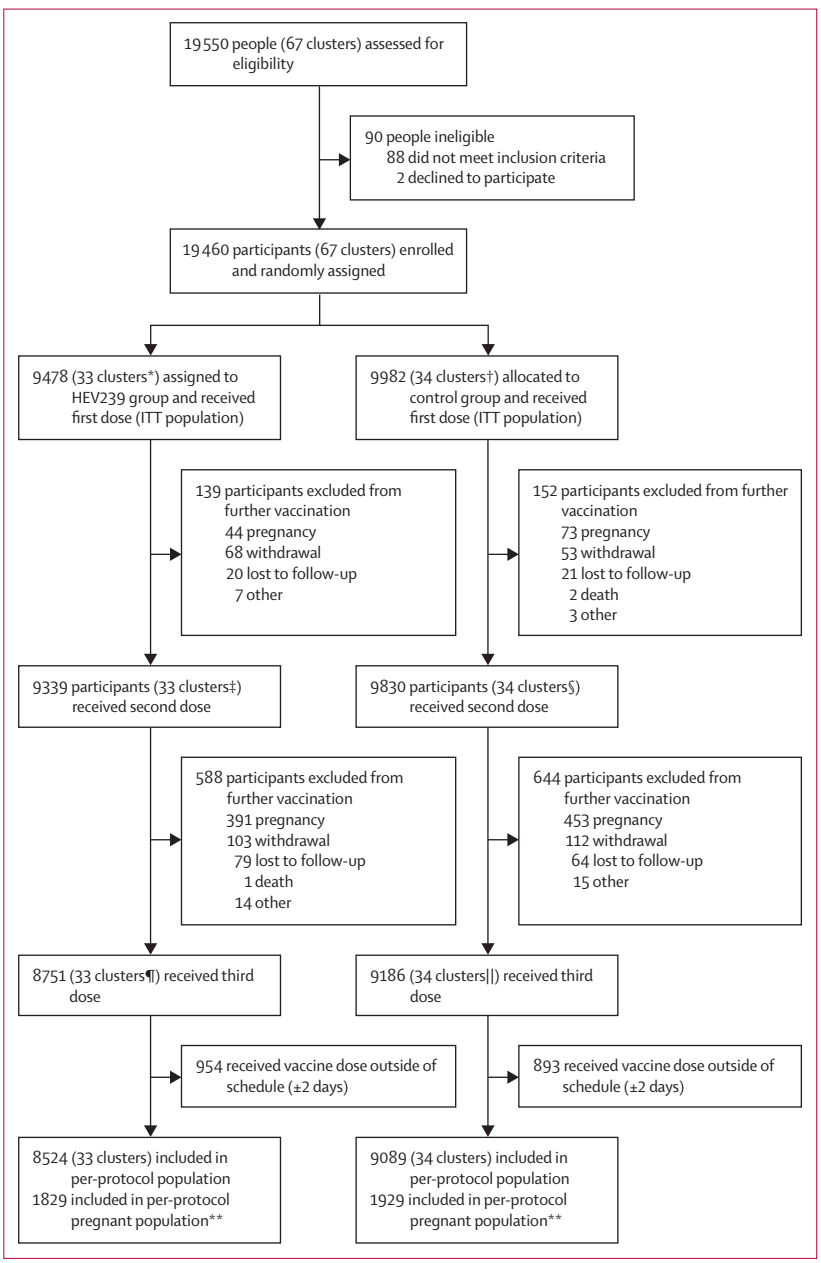

Figure: **Trial profile**
ITT=intention-to-treat. *Mean 287 participants per cluster (SD 285; range 40–1280). †Mean 294 participants per cluster (SD 295; range 16–1562). ‡Mean 283 participants per cluster (SD 281; range 39–1280). §Mean 289 participants per cluster (SD 291; range 16–1534). ¶Mean 265 participants per cluster (SD 266; range 37–1197). ||Mean 270 participants per cluster (SD 270; range 13–1415). **Participants with pregnancy start date (date of last menstruation) of at least one pregnancy after the date of their 3rd dose.

	HEV239 group	Control group
Cluster factors		
Number of clusters	33	34
Participants per cluster		
Mean (SD)	287 (285)	294 (295)
Range	40–1280	16–1562
Population density per km²		
Mean (SD)	2096 (2417)	1674 (1005)
Range	354–13 269	294–4660
Participant factors, intention-to-treat population		
Number of participants	9478	9982
Age, years		
16–18	1882 (19·9%)	1939 (19·4%)
19–29	4380 (46·2%)	4631 (46·4%)
30–39	3216 (33·9%)	3412 (34·2%)
Mean (SD)	25·9 (7·08)	26·0 (7·02)
BMI, kg/m²		
<18·5	1343 (14·2%)	1417 (14·2%)
18·5–24·9	5271 (55·6%)	5577 (55·9%)
25·0–29·9	2305 (24·3%)	2491 (25·0%)
≥30·0	559 (5·9%)	497 (5·0%)
Mean (SD)	23·0 (4·15)	22·9 (4·07)
Participant factors, per-protocol population		
Number of participants	8524	9089
Age, years		
16–18	1686 (19·8%)	1779 (19·6%)
19–29	3862 (45·3%)	4113 (45·3%)
30–39	2976 (34·9%)	3197 (35·2%)
BMI, kg/m²		
<18·5	1206 (14·1%)	1298 (14·3%)
18·5–24·9	4712 (55·3%)	5060 (55·7%)
25·0–29·9	2092 (24·5%)	2276 (25·0%)
≥30·0	514 (6·0%)	455 (5·0%)

Table 1: **Baseline characteristics of the clusters and participants**

aetiology. Since there were no confirmed cases of hepatitis E among pregnant participants, we found no difference in the primary outcome. This meant we did not assess differences in the prespecified secondary endpoints of altered immune response in pregnant women and maternal and perinatal mortality associated with maternal jaundice.

The secondary efficacy outcome of vaccine effectiveness in the general study population could be assessed based on the six non-pregnant participants with hepatitis E (appendix 1 pp 15–16). These infections were all in the control group but occurred in six different villages and at different timepoints (appendix 1 p 17). All the cases resolved without any serious sequelae. Four of these samples were confirmed genotype HEV1f by whole-genome sequencing, one sample was untypeable, and one had insufficient material for typing. One of the HEV cases was detected after the second dose of vaccine and five cases after the third dose; four cases were in the per-protocol group. Effectiveness of HEV239 against symptomatic hepatitis E was 100% (95% CI 10·6 to 100; p=0·036) in the ITT population and 100% (–63·3 to 100) in the per-protocol population. We observed only minor changes in these numbers when adjusting for age and BMI or analysing the results at the village (cluster) level (table 3).

The solicited local adverse events mainly appeared within the first day after vaccination for both vaccine groups (median 1 day [IQR 0–1] in both groups). Most solicited systemic events also started shortly after vaccination (median 1 day [1–3] in the HEV239 group and 1 day [0–3] in the control group). The solicited adverse events mainly resolved within the 7-day solicitation period (appendix 1 p 18). There was no apparent difference in the duration of solicited events between vaccine groups for local events (median 2 days [2–3] for both groups) or systemic events (3 days [2–4] for both groups).

The proportion of participants with solicited adverse events after any dose did not differ between the HEV239 group (14·7%) and the control group (13·8%; table 4). In both groups, the majority of solicited adverse events were mild, and no severe solicited adverse events were reported. Pain was the most common local symptom, recorded in 1215 (12·8%) participants in the HEV239 group and in 1218 (12·2%) in the control group. The risk of itching tended to be higher in the HEV239 group (34 [0·4%]) than in the control group (12 [0·1%]), although not significantly (adjusted OR [aOR] 2·55 [95% CI 0·95–6·84]). A higher proportion of moderate systemic events was seen in the HEV239 group (1·95 [1·03–3·70]), but the incidence was low in both the HEV239 group (19 [0·2%] cases) and control group (nine [0·1%]). Fever was the most frequently observed systemic symptom, reported in 141 (1·5%) cases in the HEV239 group and 145 (1·5%) in the control group (table 4). There was no significant difference between the vaccine groups in any of the solicited events, except for headache (aOR 2·48 [95% CI 1·14–5·38]), which occurred in 41 (0·4%) participants in the HEV239 group and 18 (0·2%) in the control group. The proportions of participants reporting unsolicited adverse events between days 8 and 28 and between day 29 and 2 years after the last vaccine dose were similar between the groups (table 4).

All serious adverse events were assessed as unrelated to vaccination, except for one possibly related case in the HEV239 group, in which breathing difficulty started shortly after the first vaccine dose. This patient recovered fully within 3 days, and no adverse event occurred after the next dose. The number of deaths (12 deaths [0·1% of participants] occurring 69–575 days after the last dose of HEV239 and 16 deaths [0·2%] occurring 28–660 days after the last dose of Hepa-B) and admissions to hospital (43 [0·5%] in the HEV239 group and 55 [0·6%] in the control group) were not significantly different between

	Day 0			Day 210			p value*
	HEV239 group	Control group	Overall	HEV239 group	Control group	Overall	
Anti-HEV IgG seroprevalence							
Total (ITT population)							
Number of participants	9475†	9982	19 457	8523	9089	17 612‡	..
Positive (%)	3911 (41·3% [40·3–42·3])	3939 (39·5% [38·5–40·4])	7850 (40·4% [39·7–41·0])	8429 (98·9% [98·7–99·1])	2944 (32·4% [31·4–33·4])	..	<0·0001§
Seropositive day 0	..	..	..	3233 (99·2% [98·9–99·5])	2136 (63·2% [61·5–64·8])	6639	<0·0001§
Seronegative day 0	..	..	..	5195 (98·7% [98·4–99·0])	808 (14·2% [13·3–15·1])	10 972	<0·0001§
Received only one dose							
Number of participants	139	152	291	44	68	112	..
Positive (%)	86 (61·9% [53·5–69·6%])	94 (61·8% [53·8–69·3])	180 (61·9% [56·1–67·3])	41 (93·2% [81·3–98·6])	38 (55·9% [43·3–67·9])	..	<0·0001§
Seropositive day 0	..	..	..	21 (95·5% [77·2–99·9])	27 (73·0% [55·9–86·2])	59	0·0320§
Seronegative day 0	..	..	..	20 (90·9% [70·8–98·9])	11 (35·5% [19·2–54·6])	53	<0·0001§
Received only two doses							
Number of participants	586	644	1230	82	91	173	..
Positive (%)	410 (70·0% [66·1–73·6])	404 (62·7% [58·9–66·4])	814 (66·2% [63·5–68·8])	80 (97·6% [91·5–99·7])	55 (60·4% [49·6–70·5])	..	<0·0001§
Seropositive day 0	..	..	..	52 (100% [93·2–100])	40 (70·2% [56·6–81·6])	109	<0·0001§
Seronegative day 0	..	..	..	28 (93·3% [77·9–99·2])	15 (44·1% [27·2–62·1])	64	<0·0001§
Received three doses (per-protocol population)							
Number of participants	7209	7773	14 982	7014	7629	14 643	..
Positive (%)	2681 (37·2% [36·1–38·3])	2767 (35·6% [34·5–36·7])	5448 (36·4% [35·6–37·1])	6944 (99·0% [98·7–99·2])	2271 (29·8% [28·7–30·8])	..	<0·0001§
Seropositive day 0	..	..	..	2530 (99·3% [98·8–99·6])	1639 (61·1% [59·2–63·0])	5231	<0·0001§
Seronegative day 0	..	..	..	4414 (98·9% [98·5–99·2])	632 (12·8% [11·9–13·7])	9412	<0·0001§
Anti-HEV IgG geometric mean titre¶							
Total (ITT population)	0·34 (0·33–0·35)	0·32 (0·31–0·33)	0·33 (0·33–0·34)	74·62 (72·83–76·45)	0·27 (0·26–0·28)	..	<0·0001‖
Seropositive day 0	2·03 (1·96–2·10)	2·01 (1·94–2·09)	2·02 (1·97–2·07)	86·55 (83·49–89·71)	0·77 (0·73–0·82)	..	<0·0001‖
Seronegative day 0	0·10 (0·10–0·10)	0·10 (0·10–0·10)	0·10 (0·10–0·10)	68·06 (65·91–70·29)	0·15 (0·14–0·15)	..	<0·0001‖
Received only one dose	0·79 (0·58–1·07)	0·76 (0·57–1·03)	0·77 (0·63–0·96)	3·37 (2·10–5·44)	0·63 (0·40–0·99)	..	<0·0001‖
Seropositive day 0	2·82 (2·23–3·57)	2·70 (2·13–3·41)	2·75 (2·34–3·25)	5·76 (2·94–11·32)	1·16 (0·63–2·13)	..	0·0010‖
Seronegative day 0	0·10 (0·10–0·10)	0·10 (0·10–0·10)	0·10 (0·10–0·10)	1·97 (1·03–3·78)	0·30 (0·16–0·57)	..	0·0001‖
Received only two doses	1·00 (0·87–1·15)	0·70 (0·61–0·80)	0·83 (0·75–0·91)	20·67 (14·90–28·67)	0·75 (0·51–1·11)	..	<0·0001‖
Seropositive day 0	2·71 (2·45–2·99)	2·23 (2·01–2·47)	2·46 (2·28–2·64)	29·79 (20·99–42·28)	1·10 (0·69–1·75)	..	<0·0001‖
Seronegative day 0	0·10 (0·10–0·10)	0·10 (0·10–0·10)	0·10 (0·10–0·10)	10·97 (5·90–20·39)	0·40 (0·21–0·77)	..	<0·0001‖
Received three doses (per-protocol population)	0·30 (0·29–0·31)	0·29 (0·28–0·30)	0·29 (0·29–0·30)	77·88 (75·92–79·89)	0·25 (0·24–0·26)	..	<0·0001‖
Seropositive day 0	1·93 (1·85–2·01)	1·90 (1·82–1·99)	1·93 (1·87–1·99)	93·29 (89·79–96·92)	0·71 (0·66–0·76)	..	<0·0001‖
Seronegative day 0	0·10 (0·10–0·10)	0·10 (0·10–0·10)	0·10 (0·10–0·10)	70·25 (67·95–72·62)	0·14 (0·14–0·15)	..	<0·0001‖

(Table 2 continues on next page)

the groups (table 4), and all were deemed unrelated to vaccination. Two instances of pregnancy-related death were reported, one in each vaccine group; in both cases, the deaths were associated with childbirth but resulted in livebirths.

The risk of solicited adverse events was similar after the first and second doses of HEV239 (appendix 1 p 19). Notably, the third dose of HEV239 was associated with a significant increase in the risk of any solicited adverse event compared with the second dose (risk ratio 2·79 [95% CI 2·48–3·15]). Specific events that showed this increased risk included both local events such as pain and swelling, and systemic events, dominated by fever. However, a similar pattern was also seen for the control group; thus, the only significant difference in solicited adverse events between the HEV239 and control groups after the third dose was the occurrence of headache, a relatively rare event (appendix 1 p 20).

39 cases of autoimmune-mediated conditions were reported during follow-up, (appendix 1 p 21), with five cases in the HEV239 group and nine cases in the control group emerging within the first 90 days. In the HEV239 group, three cases (one each of diabetes, goitre, and hypothyroidism) appeared after the second vaccine

	Day 0			Day 210			p value*
	HEV239 group	Control group	Overall	HEV239 group	Control group	Overall	
(Continued from previous page)							
Seroconversion, %**							
Total (ITT population)	..	..	..	8278 (97·1% [96·8–97·5])	940 (10·3% [9·7–11·0])	..	<0·0001§
Seropositive day 0	..	..	..	3089 (94·8% [94·0–95·5])	160 (4·7% [4·1–5·5])	..	<0·0001§
Seronegative day 0	..	..	..	5189 (98·6% [98·2–98·9])	780 (13·7% [12·8–14·6])	..	<0·0001§
Received only one dose	..	..	..	26 (61·4% [46·0–74·8])	14 (20·6% [11·7–32·1])	..	<0·0001§
Seropositive day 0	..	..	..	7 (31·8% [13·9–54·9])	3 (8·1% [1·7–21·9])	..	0·018§
Seronegative day 0	..	..	..	19 (86·4% [65·1–97·1])	11 (35·5% [19·2–54·6])	..	0·0002§
Received only two doses	..	..	..	72 (87·8% [78·6–93·4])	19 (20·9% [13·7–30·6])	..	<0·0001§
Seropositive day 0	..	..	..	44 (84·6% [71·9–93·1])	4 (7·0% [2·0–17·0])	..	<0·0010§
Seronegative day 0	..	..	..	28 (93·3% [77·9–99·2])	15 (44·1% [27·2–62·1])	..	<0·0001§
Received three doses (per-protocol population)	..	..	..	6851 (97·7% [97·3–98·0])	713 (9·4% [8·7–10·0])	..	<0·0001§
Seropositive day 0	..	..	..	2442 (95·8% [95·0–96·6])	106 (4·0% [3·2–4·8])	..	<0·0001§
Seronegative day 0	..	..	..	4409 (98·8% [98·4–99·1])	607 (12·3% [11·4–13·2])	..	<0·0001§

Data are n, n (% [95% CI]), or point estimate (95% CI). All participants included above were within the ITT population. Immunogenicity data constitute all samples collected according to protocol. HEV=hepatitis E virus. ITT=intention to treat. *For the difference in outcome between the HEV239 group and control group at day 210. †Three participants were excluded due to lack of serological data on day 0. ‡One participant had a missing IgG status on day 0, but a valid (positive) result on day 210. §From χ^2 test. ¶Anti-HEV IgG antibody concentration in WHO units per mL. ||From Student's *t* test on log-transformed data. **Seroconversion was defined as a 4-times increase of anti-HEV IgG between day 0 and day 210, together with a positive result on day 210.

Table 2: **Seroprevalence, concentrations, and seroconversion of anti-HEV IgG according to number of vaccine doses received, days after vaccination, and vaccine group**

	Follow-up (months)	HEV239 grou				Control group				Vaccine effectiveness, % (95% CI)*	p value
		Participants	Person-years at risk	Cases	Incidence	Participants	Person-years at risk	Cases	Incidence		
Intention-to-treat population											
Individuals	1–30	9478	23 688	0	0·0†	9982	24 955	6	2·4†	100% (10·6 to 100)	0·036
Adjusted‡	1–30	9478	23 688	0	0·0†	9982	24 955	6	2·4†	100% (10·8 to 100)	0·036
Villages§	1–30	33	82·5	0	0·0	34	85	6	0·07	100% (10·5 to 100)	0·037
Per-protocol population¶											
Individuals	8–30	7291	18 228	0	0·0†	7859	15 713	4	2·6†	100% (–63·3 to 100)	0·145
Adjusted‡	8–30	7291	18 228	0	0·0†	7859	15 713	4	2·6†	100% (–63·3 to 100)	0·145
Villages§	8–30	33	82·5	0	0·0	34	85	4	0·05	100% (–59·6 to 100)	0·139

Incidence is presented as cases per person-year, or per 10 000 person-years where indicated. p values are median unbiased estimates from exact Poisson regression. *Effectiveness was calculated as 100% × (1 – [incidence in HEV239 group / incidence in control group]). †Per 10 000 person-years. ‡Adjusted for age and BMI. §Adjusted for cluster size. ¶Restricted to participants who received three vaccine doses according to protocol.

Table 3: **Effectiveness of the HEV239 vaccine**

dose and two cases (one of Bell's palsy and one of hypothyroidism) after the third dose. In the control group, four cases (two of goitre, one of thyrotoxicosis, and one of Bell's palsy) were reported after the first vaccine dose, three cases (two of hypothyroidism and one of Bell's palsy) after the second dose, and two cases (one each of thyrotoxicosis and Bell's palsy) after the third dose.

When categorising all adverse events during follow-up by System Organ Class in accordance with MedDRA and comparing risk (table 5), there was a significant difference in risk of events within the System Organ Class category pregnancy, puerperium, and neonatal disorders between the groups (aOR 1·33 [95% CI 1·08–1·65]). This difference appeared to be attributable to the HEV239 group having a higher risk of miscarriage (136 [5·7%] of 2407 in the HEV239 group and 102 [3·9%] of 2604 in the control group; aOR 1·54 [95% CI 1·15–2·08]; table 5). A significant difference between groups was also found for conditions under the hepatobiliary disorder category (0·67 [0·46–0·97]). Upon closer examination of prevalent subdiagnoses (including HAV, HBV, and cholelithiasis), no significant differences in risk of these individual subdiagnoses were observed between the vaccine groups (data not shown).

Discussion

The findings from this study indicate that a three-dose regimen of HEV239 is highly immunogenic and effective

compared with the control vaccine in women of reproductive age in a region with endemic HEV. However, no difference in effect between the two vaccines in pregnant women could be found, as HEV cases in our study were few and only occurred in the non-pregnant population. Adverse events were generally mild and transient, with no serious adverse events attributed to the HEV239 vaccine. However, a significantly higher risk of miscarriage was observed in the HEV239 group than in the control group.

The vaccine effectiveness observed in our study is consistent with previous research on HEV239 in a Chinese population by Zhu and colleagues.[9] While that study showed the efficacy of HEV239 in providing cross-protection against HEV4, effectiveness data from our study show that HEV239 is also effective against HEV1, as expected considering that the vaccine is based on this HEV genotype.

Our study further shows the immunogenicity of HEV239 following vaccine doses administered at day 0, 1 month, and 6 months, with seroconversions and HEV IgG concentrations positively correlated with the numbers of vaccine doses received. These findings corroborate the results obtained in our study conducted in nearby villages in 2017[23] and similar findings from Zhu and colleagues' study.[9] Although a high immune response was observed after two and three doses, suggesting potential protection against hepatitis E, the effectiveness of a two-dose regimen remains uncertain due to the absence of HEV infections in recipients who received only two doses. Future research should focus on the effectiveness of a two-dose regimen, ideally in a randomised clinical trial conducted during an outbreak. Notably, HEV239 was employed in South Sudan's 2020 HEV outbreak among displaced people, albeit using a three-dose schedule.[24]

The safety profile of HEV239 observed in this study, characterised by mostly mild and transient adverse events, aligns with previous findings.[9] However, a noteworthy safety signal concerning an increased risk of miscarriage in the HEV239 group warrants further investigation. In contrast to our findings, a post-hoc analysis of a Chinese study of HPV vaccine, in which HEV239 was used as a control, showed no difference in adverse pregnancy outcomes.[25] We did an additional study to thoroughly investigate the data on fetal losses during pregnancy in the HEV239 group.[21]

Considering that autoimmune-mediated conditions are more prevalent in women than men,[26] we monitored for such conditions during follow-up. If these diseases were vaccine-induced, a spike in incidence would be expected shortly after vaccination. However, the incidence of such conditions was low in both vaccine groups during the first 90 days after any dose.

The study has a number of strengths. Conducted in an area within the HDSS, we easily identified a substantial number of eligible participants. Existing trust in the icddr,b field staff, built over years of previous vaccine

	HEV239 group (n=9478)	Control group* (n=9982)	Crude analysis		Adjusted analysis	
			OR*	p value	OR†	p value
Solicited adverse events, days 0 to 7						
Local and systemic	1392 (14·7%)	1373 (13·8%)	0·84 (0·66–1·06)	0·14	0·87 (0·64–1·18)	0·37
Mild	1335 (14·1%)	1317 (13·2%)	0·83 (0·65–1·05)	0·12	0·87 (0·64–1·19)	0·39
Moderate	80 (0·8%)	70 (0·7%)	1·14 (0·70–1·85)	0·60	1·11 (0·70–1·77)	0·66
Severe	0	0	..	..	..	..
Local	1258 (13·3%)	1250 (12·5%)	0·82 (0·64–1·05)	0·12	0·87 (0·62–1·21)	0·40
Mild	1211 (12·8%)	1196 (12·0%)	0·82 (0·64–1·05)	0·12	0·88 (0·64–1·20)	0·47
Moderate	62 (0·7%)	62 (0·6%)	0·96 (0·53–1·73)	0·89	0·88 (0·50–1·60)	0·67
Severe	0	0	..	..	..	..
Pain	1215 (12·8%)	1218 (12·2%)	0·81 (0·63–1·04)	0·10	0·86 (0·62–1·19)	0·35
Swelling or induration	52 (0·5%)	54 (0·5%)	0·94 (0·48–1·84)	0·87	0·83 (0·39–1·77)	0·64
Redness	25 (0·3%)	27 (0·3%)	0·98 (0·5–1·93)	0·95	1·01 (0·51–2·02)	0·98
Itching	34 (0·4%)	12 (0·1%)	2·55 (0·94–6·87)	0·065	2·55 (0·95–6·84)	0·064
Systemic	216 (2·3%)	201 (2·0%)	1·14 (0·77–1·68)	0·50	1·15 (0·78–1·70)	0·48
Mild	198 (2·1%)	193 (1·9%)	1·08 (0·72–1·62)	0·70	1·09 (0·73–1·64)	0·67
Moderate	19 (0·2%)	9 (0·1%)	2·1 (1·06–4·14)	0·032	1·95 (1·03–3·70)	0·040
Severe	0	0	..	..	..	..
Fever	141 (1·5%)	145 (1·5%)	1·09 (0·73–1·62)	0·69	1·10 (0·74–1·64)	0·64
Headache	41 (0·4%)	18 (0·2%)	2·49 (1·16–5·35)	0·021	2·48 (1·14–5·38)	0·020
Nausea or vomiting	44 (0·5%)	42 (0·4%)	1·01 (0·56–1·81)	0·98	1·03 (0·57–1·83)	0·93
Asthenia or fatigue	55 (0·6%)	54 (0·5%)	1·05 (0·53–2·08)	0·89	1·08 (0·54–2·14)	0·83
Myalgia	5 (0·1%)	6 (0·1%)	0·87 (0·26–2·87)	0·82	0·85 (0·26–2·84)	0·80
Allergic reaction	3 (<0·1%)	3 (<0·1%)	0·69 (0·1–4·97)	0·71	0·84 (0·11–6·52)	0·86
Malaise	0	0	..	..	..	..
Dizziness	0	1 (<0·1%)	..	..	..	..
Syncope	0	0	..	..	..	..
Unsolicited adverse events, days 8 to 28						
Any	115 (1·2%)	117 (1·2%)	0·97 (0·66–1·43)	0·87	0·98 (0·67–1·44)	0·92
Severe	8 (0·1%)	8 (0·1%)	1·14 (0·35–3·64)	0·83	1·05 (0·34–3·31)	0·93
Unsolicited adverse events, 29 days to 2 years						
Any	3156 (33·3%)	2918 (29·2%)	1·07 (0·89–1·3)	0·46	1·12 (0·92–1·37)	0·25
Severe	55 (0·6%)	71 (0·7%)	0·84 (0·52–1·38)	0·50	0·84 (0·52–1·37)	0·49
Serious adverse events within 2 years						
All	53 (0·6%)	70 (0·7%)	0·80 (0·50–1·29)	0·36	0·81 (0·50–1·33)	0·41
Admission to hospital	43 (0·5%)	55 (0·6%)	0·81 (0·47–1·40)	0·45	0·83 (0·47–1·47)	0·52
Death‡	12 (0·1%)	16 (0·2%)	0·57 (0·28–1·17)	0·13	0·66 (0·33–1·30)	0·23

Data are n (%) or OR (95% CI). ORs were estimated with a logistic regression model. The effect of clustering was accounted for by using a generalised estimating equations approach. OR=odds ratio. *Due to within-cluster dependencies, the crude OR is not equivalent to odds among participants in the HEV239 vaccine group relative to odds among participants in the control group. †Adjusted for age, BMI, and number of doses. ‡Includes four cases of maternal death unrelated to vaccination; one case was in the HEV239 group and three cases in the control group, of which two of the control group cases were reported by telephone after migration from the study area and were therefore lost to follow-up of their pregnancies.

Table 4: **Number of participants with adverse events and serious adverse events after any vaccine dose, intention-to-treat population**

	HEV239 group (n=9478)	Control group (n=9982)	Crude analysis		Adjusted analysis	
			OR*	p value	Adjusted OR†	p value
Blood and lymphatic disorders	32 (0·3%)	44 (0·4%)	0·70 (0·37–1·32)	0·27	0·71 (0·37–1·34)	0·29
Endocrine disorders	12 (0·1%)	20 (0·2%)	0·61 (0·22–1·65)	0·33	0·63 (0·23–1·69)	0·36
Eye disorders	2 (<0·1%)	5 (0·1%)	..	..	..	..
Gastrointestinal disorders	1083 (11·4%)	976 (9·8%)	1·22 (0·95–1·57)	0·13	1·21 (0·94–1·55)	0·14
General disorders and administration site conditions	2271 (24·0%)	2096 (21·0%)	0·98 (0·80–1·20)	0·87	1·04 (0·82–1·30)	0·76
Hepatobiliary disorders	31 (0·3%)	52 (0·5%)	0·67 (0·45–0·98)	0·038	0·67 (0·46–0·97)	0·036
Immune system disorders	5 (0·1%)	6 (0·1%)	0·89 (0·28–2·85)	0·85	0·84 (0·25–2·84)	0·79
Infections and infestations	37 (0·4%)	48 (0·5%)	0·80 (0·50–1·28)	0·35	0·80 (0·50–1·28)	0·35
Injury, poisoning, and procedural complications	37 (0·4%)	47 (0·5%)	0·74 (0·44–1·27)	0·28	0·76 (0·45–1·29)	0·31
Musculoskeletal and connective tissue disorders	68 (0·7%)	60 (0·6%)	1·17 (0·76–1·80)	0·49	1·19 (0·78–1·83)	0·42
Neoplasms benign, malignant, and unspecified	2 (<0·1%)	4 (<0·1%)	..	..	..	..
Nervous system disorders	108 (1·1%)	103 (1·0%)	1·04 (0·63–1·71)	0·89	1·04 (0·64–1·71)	0·87
Psychiatric disorders	11 (0·1%)	14 (0·1%)	0·81 (0·38–1·76)	0·60	0·80 (0·37–1·72)	0·56
Pregnancy, puerperium, and neonatal disorders‡	228/2407 (9·5%)	196/2604 (7·5%)	1·32 (1·06–1·64)	0·014	1·33 (1·08–1·65)	0·0086
Miscarriage (spontaneous abortion)	136/2407 (5·7%)	102/2604 (3·9%)	1·52 (1·13–2·05)	0·0061	1·54 (1·15–2·08)	0·0042
Elective termination	17/2407 (0·7%)	16/2604 (0·6%)	1·15 (0·58–2·28)	0·14	1·14 (0·58–2·27)	0·14
Abortion, not further specified	23/2407 (1·0%)	19/2604 (0·7%)	1·50 (0·75–2·97)	0·25	1·45 (0·73–2·88)	0·29
Stillbirth	40/2407 (1·7%)	45/2604 (1·7%)	1·07 (0·75–1·53)	0·69	1·10 (0·78–1·55)	0·58
Other§	13/2407 (0·5%)	18/2604 (0·7%)	0·79 (0·41–1·55)	0·50	0·77 (0·40–1·48)	0·44
Renal and urinary disorders	76 (0·8%)	75 (0·8%)	1·09 (0·75–1·59)	0·65	1·08 (0·75–1·55)	0·68
Reproductive system and breast disorders	37 (0·4%)	41 (0·4%)	0·74 (0·42–1·30)	0·30	0·78 (0·44–1·40)	0·41
Respiratory, thoracic, and mediastinal disorders	139 (1·5%)	115 (1·2%)	1·00 (0·61–1·66)	0·99	1·07 (0·65–1·76)	0·80
Skin and subcutaneous tissue disorders	13 (0·1%)	5 (0·1%)	2·79 (0·91–8·51)	0·072	2·92 (0·95–8·94)	0·061
Surgical and medical procedures	17 (0·2%)	15 (0·2%)	1·19 (0·56–2·50)	0·65	1·18 (0·57–2·42)	0·66
Vascular disorders	11 (0·1%)	9 (0·1%)	0·66 (0·18–2·41)	0·53	0·84 (0·26–2·69)	0·77

Events are summarised by System Organ Class in accordance with MedDRA. Data are n (%) or OR (95% CI). ORs were estimated with a logistic regression model, with the control group as the reference group. The effect of clustering was accounted for by using a generalised estimating equations approach. OR=odds ratio. *Due to within-cluster dependencies, the crude OR is not equivalent to odds among participants in the HEV239 group relative to odds among participants in the control group. †Adjusted for age, BMI, and number of doses. ‡Pregnancy, puerperium, and neonatal disorders, and the listed subcategories, are presented for the 5011 women who became pregnant within 2 years of receiving their last vaccine dose; as each participant could have had multiple pregnancies and thus multiple pregnancy outcomes, the numbers can exceed the total number of pregnancy, puerperium, and neonatal disorders. §Includes four cases of congenital anomalies in the control group (two of which resulted in elective terminations and two were discovered after birth [sick newborn]); this category thus overlaps with the other subcategories above.

Table 5: **Number of participants with adverse events within 2 years of receipt of final vaccine dose, intention-to-treat population**

studies, likely boosted the participation rate. Regular follow-up ensured thorough monitoring and meticulous data collection on both pregnancies and suspected cases of hepatitis. Additionally, local field workers familiar with the community dynamics and dialects facilitated data collection and minimised cultural barriers.

A limitation of the study is the lack of serum collection from participants after 2 years of follow-up for detecting subclinical HEV infections via anti-HEV IgG seroconversion. As this trial was not designed for this, a follow-up study is ongoing to investigate the anti-HEV IgG seroconversion rate in a subgroup of participants in the control group. Although the study was slightly underpowered because the desired target sample size was not met, this limitation was compensated for by the number of participants who completed three vaccine doses according to protocol (90%), which surpassed our expectation of 60% completion.

Although the number of pregnancies among the participants in our study exceeded our assumptions, an unexpectedly low number of HEV cases were identified, with none among the pregnant participants. Our comprehensive follow-up strategy identified 238 cases of suspected hepatitis; however, the COVID-19 pandemic might have affected case investigations. The stringent infection control measures could have contributed to fewer HEV cases, and the transition to telephone-based surveillance during lockdown might also have influenced HEV detection. Several other interconnected factors could also explain the low number of HEV infections. First, the study population constituted young, healthy, adult women, who typically have milder or asymptomatic infections, pregnancy excepted. The precise rate of symptomatic HEV infection is unknown, and our pre-trial assumption of 35% was probably an overestimation. Second, the absence of reported outbreaks during the

study period suggests different exposure dynamics and virus strains from outbreak conditions. Evidence indicates endemic presence in low-income countries of HEV genotypes 3 and 4, which are often underdiagnosed due to their reduced virulence and the infrequent testing for these strains.[27,28] Third, our study revealed a very high seropositivity rate of 40·4% in the population at baseline, nearly double our initial estimation of 22%, which was based on data published in 2010.[17] This discrepancy might result from the use of less sensitive anti-HEV assays in the previous study than in our study.[29] Thus, the high baseline seropositivity rate in our participants might have acted as a protective barrier against symptomatic HEV infection. Other factors to consider are improved sanitation, safer water sources, and socioeconomic advancements in the study area, as well as the absence of flooding during the study period. These factors might have collectively contributed to a reduced risk of waterborne diseases, including HEV.[30]

The indicated annual HEV incidence rate of 17·9% among controls suggests a substantial circulation of HEV within the community during the study. The temporal distribution of the six HEV cases further implies persistent transmission within the area. Notably, the occurrence of a large HEV outbreak 200 km away from our study area during the follow-up period[10] underscores the continued risk of HEV infections in rural Bangladesh.

Our trial was conducted in participants aged 16 years and older, in accordance with the vaccine manufacturer's recommendation. Data from trials using HEV239 in children younger than 16 years is needed, as a potential target group is younger girls.[12]

In a position paper from 2015, WHO recommended considering HEV vaccination to control outbreaks, including in pregnant women.[12,31] This study is the first large effectiveness trial of HEV239 in a low-income country and was done in non-pregnant women of reproductive age. The vaccine's robust immunogenicity and its ability to provide protection against HEV in a HEV1-endemic area have profound implications for global HEV-prevention strategies. The demonstrated effectiveness and immunogenicity of HEV239 suggest its potential role in outbreak responses and highlight its versatility in various epidemiological contexts. However, the increased rate of miscarriage in the HEV239 group found in our study should warrant precautionary measures.

Contributors

KZ, CHJ, SD, KS-J, SS, WH, and JØ wrote the first draft, and further revisions were done by KZ, JDC, IL, ESG, CHJ, JØ, KS-J, ABA, and SD. JØ, IL, ABA, and CHJ did the statistical analysis. JDC, KZ, MY, SD, KS-J, FQ, SS, JØ, JLD, CHJ, and ESG contributed to study design. KZ, JDC, ABA, SD, KS-J, JØ, SS, WH, JLD, and CHJ were involved in trial management. KZ, ABA, MK, MR, TRB, and WH were responsible for managing the field teams and logistics of the study. All authors read and approved the manuscript. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication. KZ, CHJ, SD, SS, JLD, ABA, and JØ directly accessed and verified the underlying data reported in the manuscript.

Equitable partnership declaration

The authors of this paper have submitted an equitable partnership declaration (appendix 2). This statement allows researchers to describe how their work engages with researchers, communities, and environments in the countries of study. This statement is part of *The Lancet Global Health*'s broader goal to decolonise global health.

See **Online** for appendix 2

Declaration of interests

We declare no competing interests.

Data sharing

The data collected for this study will be accessible to other members of the scientific community upon request, in accordance with the data-sharing policies of icddr,b and Norwegian Institute of Public Health (NIPH). To ensure the integrity of the research, standard criteria for data sharing will be employed, subject to approval by qualified researchers. For data access, please contact KZ (kzaman@icddrb.org).

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