Vaccine xxx (xxxx) xxx

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Immunogenicity and safety of a two-dose regimen with hepatitis E virus vaccine in healthy adults in rural Bangladesh: A randomized, double-blind, controlled, phase 2/pilot trial

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ARTICLE INFO

Article history:
Received 26 November 2021
Received in revised form 27 December 2022
Accepted 28 December 2022
Available online xxxx

Keywords:
Hepatitis E virus
Vaccine
Safety
Feasibility
Immunogenicity
HEV 239
Low-income countries

ABSTRACT

Background: Hepatitis E virus (HEV) is a major cause of acute viral hepatitis worldwide and it contributes to considerable maternal and neonatal mortality and morbidity in many low-income countries like Bangladesh. A three-dose regimen of a vaccine against HEV (HEV 239) has shown promising results in China. The effects and safety of this vaccine in other populations and with different dosing regimens remains uncertain.

Objectives: Investigate the immune response and safety of a two-dose regimen with the HEV 239 vaccine among healthy adults. Examine the feasibility of conducting a larger HEV 239 vaccine trial in rural Bangladesh.

Methods: One-hundred healthy men and non-pregnant women 16–39 years old were randomized in a 1:1 ratio to receive two doses of either the study (HEV) or control (Hepatitis B virus, HBV) vaccine (at 0, 1 month). Blood samples were collected at day 0, day 60 and 2 years after vaccination. The primary endpoints were the proportion and severity of adverse events up to 2 months after dose one and the longitudinal shift in anti-HEV IgG levels from day 0 to day 60 and 2 years after vaccination.

Results: Adverse events to HEV 239 were comparable to the control vaccine, mild in severity and resolved within one to nine days. All participants in the study group seroconverted and achieved high levels of HEV IgG antibodies that remained positive for two years in all but one. A *T*-cell response was detected one month after HEV 239 vaccination.

Conclusion: Our results show that two doses of the HEV 239 vaccine produces broad and likely functional immune responses against HEV that remain for at least two years. The safety profile was acceptable and a phase four study of HEV 239 in rural Bangladesh is feasible.

ClinicalTrials.gov Identifier: NCT02759991.

Abbreviations: HEV, hepatitis E virus; HBV, hepatitis B virus; AE, adverse event; icddr,b, International Centre for Diarrhoeal Disease Research, Bangladesh; low-income country, LIC; WU/ml, World Health Organization (WHO) units per milliliter; GMT, geometric mean titer.

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https://doi.org/10.1016/j.vaccine.2022.12.064
0264-410X/

1. Introduction

Hepatitis E is a disease caused by the Hepatitis E virus (HEV). Several genotypes with different clinical and epidemiological characteristics have been identified, out of which HEV genotype 1 (HEV1), HEV2, HEV3, and HEV4 cause most of the human infections [1]. HEV1 dominates in low-income countries (LIC), where HEV infection is a major cause of acute hepatitis [1].

HEV is enterically transmitted mainly by faecally contaminated drinking water and can lead to large waterborne outbreaks in areas with poor sanitation. The virus can cause hepatitis E disease, which presents as acute, self-limiting hepatitis [1]. Pregnant women in LIC are at risk of more severe disease with a high morbidity and a mortality rate of around 25 % [2]. Other conditions, such as chronic liver disease, may also predispose for more severe outcomes. Prevention can be challenging in areas with poor access to clean water, and treatment options are few and primarily supportive [3]. Therefore, vaccination is needed to protect vulnerable populations, especially pregnant women, against severe disease and death. Bangladesh is an ideal country to study the effectiveness of vaccination, as it is a country with a high HEV seroprevalence rate (49.5 %) [4] and the infection is estimated to account for 11–15 % of maternal deaths and 4–7 % of neonatal deaths [5].

The HEV 239 vaccine (Hecolin®, Xiamen Innovax Biotech Co., ltd., China) is based on virus-like particles similar to HEV, contributing to stronger B and *T*-cell responses than traditional subunit vaccines [6]. Zhu et al. studied the vaccine in a large phase 3 clinical study, where >110,000 healthy men and women aged 16–65 years were given three doses of either the HEV vaccine or a hepatitis B virus (HBV) vaccine over six months [7]. The HEV vaccine showed a 100 % (95 % CI: 72–100) efficacy against HEV infection with minor adverse events. Despite promising results from this study, the vaccine is still only licensed in China and recently also in Pakistan. Due to limitation on effectiveness and safety data on different populations, the WHO HEV working group of the Strategic Advisory Group of Experts (SAGE) has not recommended the vaccine so far. However, more data as the basis for a global recommendation would contribute to a much higher impact of the vaccine in areas with high HEV disease burden [8].

A regimen of three doses using the HEV 239 vaccine has been shown to elicit a long-lasting and robust antibody response, which exceeds both the peak level and duration of the response measured in convalescent serum [9]. Analyses of a subgroup of the participants in the Chinese phase 3 trial that received only two doses of the vaccine indicated that two doses might yield an immune response sufficient for protection against HEV infection in most recipients [9]. However, the correlation between antibody level and risk of disease is uncertain. Chang et al. indicated that natural or vaccine-induced IgG antibody levels as low as 0.07–0.25 WHO units/ml (WU/ml) might offer substantial protection against infection [10].

Over a six-month period, a three-dose vaccine schedule requires little migration and compliance, and may be challenging to implement in many settings. A reduced and shorter vaccine schedule would make vaccination campaigns against HEV more feasible and affordable.

Broad functional HEV-specific CD4+ and CD8 + *T*-cell responses have been detected in patients following an acute HEV infection [11]. To our knowledge, no investigation of any HEV vaccine induced *T*-cell response has been previously conducted in humans. One study by Wu et al. showed strong Th-1 and Th-2 responses in mice [12]. The role of *T*-cell immunity in protection against HEV disease is not well understood, but likely plays a role in protecting against more severe disease similarly as shown with other viral infections [13]. The cellular response to the vaccine is also critical when considering the risk of Th2-type immunopathology, as described for other viral vaccines [14].

This study aimed to evaluate the immunogenicity and safety of a two-dose vaccine schedule with HEV 239 in a population with a high burden of HEV1 infection. This study also served as a pilot study in preparation for a larger phase 4 clinical trial described by Zaman et al. [15].

2. Material and methods

2.1. Study population and design

This study is a randomized, double-blinded, controlled, phase 2 trial conducted in two villages (Sepaikandi and Naburkandi) in Matlab, Bangladesh (see fig S1 in appendix). The participants were vaccinated from May to July 2017, and the observation period continued for two years after the second dose. The villages are part of a health and demographic surveillance system operated by the International Centre for Diarrhoeal Disease Research, Bangladesh (icddr, b). One hundred healthy men and non-pregnant women aged 16 to 39 years old were planned for recruitment. Before inclusion, informed consent was obtained from adult participants. For participants aged 16–17 years old, assent was obtained, and their parents signed the informed consent. An independent data and safety monitoring board (DSMB) was set up to oversee the trial and ensure the participants' safety and integrity of the data. The DSMB reviewed the adverse events data to determine the relatedness of the adverse events with the study vaccines.

The main inclusion criteria were:

- Women/men aged 16–39 years at the time of the first vaccination and
- Who lived in the study area and willingly provided informed consent

The main exclusion criteria were:

- Pregnancy (visible or verbal report on date of last menstruation or urine test)
- History of severe allergic reaction to a vaccine or a vaccine component
- Having other vaccines or immunoglobulin within two weeks
- Serious chronic diseases
- Acute and chronic infectious disease
- Fever > 38 °C (temporary exclusion)

2.2. Endpoints

The primary endpoints were the proportion and severity of adverse events recorded up to 2 months after dose one and the longitudinal shift in anti-HEV IgG levels from day 0 to day 60 and 2 years after vaccination. Secondary endpoints included: anti-HEV IgG titers in participants who were seropositive and seronegative at baseline, *T*-cell responses measured at day 0 and 60, seroconversion or a fourfold increase in anti-HEV IgG between day 60 and two years (a sign of HEV infection), and the feasibility of conducting an HEV vaccine study in rural Bangladesh.

2.3. Randomization and blinding

The participants were randomized individually to receive either the study (HEV) or control (HBV) vaccine. Four different letter codes were given to each vaccine and randomization was done by an independent statistician, ensuring a 50–50 divide between the HEV vaccine group and the control group.

Upon completion of this study, all samples were analyzed for HEV antibodies and HBV surface antibodies (anti-HBs).

As this study also served as a pilot study, the vaccine letter codes were the same as in the main study. Therefore, it was important not to unblind the ongoing phase 4 study when analyzing the pilot study data. An independent statistician re-coded the eight vaccine codes for this study to avoid this. The study scientists analyzing the pilot study data received the new codes only. These new codes were generated (by the independent statistician) based on the results from the HEV and HBV antibody measurements, and each new code could be linked to one of the two vaccines with a high degree of certainty, revealing the vaccine status of the pilot study participants.

2.4. Vaccines

HEV 239 was developed by Xiamen Innovax Biotech Co., ltd, China. The company produced and donated the vaccine in bulk, free of charge, for this trial. The vaccine is based on a 239 amino acid long recombinant HEV1 peptide, which encodes the capsid protein [16]. The vaccine is expressed in Escherichia coli and is purified to > 95 % homogeneity. The vaccine contains 30 μg of the purified protein absorbed to 0.8 mg of aluminium hydroxide suspended in 0.5 ml of buffered saline. The dosing regimen for the HEV vaccine is the same (0. 5 ml) for persons aged 16–18 and for those aged 19 years and above. The control vaccine is a commercial hepatitis B vaccine (Hepa-B®) produced by Incepta Vaccine ltd, Bangladesh. Participants aged 19 and above received 1 ml dose Hepa-B® which contains 20 μg of hepatitis B surface antigen adsorbed on Aluminium Hydroxide gel equivalent to Al3 + 0.5 mg in 1 ml of buffered saline. Participants aged 16–18 received half a dose of Hepa-B® (0.5 ml) according to recommendations from the Directorate General of Drug Administration, Bangladesh. It was thus theoretically possible for the vaccine administrators (nurses) delivering the vaccines to distinguish between the two vaccines for the age group 16–18 by looking at the dose, however they were not involved in any further follow-up of the study participants. All participants, investigators, monitors, and field staff were blinded to the identities of the participants.

Both vaccines were tested and filled in identical vials according to ICH-GMP and labeled with one of the randomization codes by Incepta.

Two doses of HEV 239 vaccine or Hepa-B® were administered intramuscularly in the deltoid muscle at days 0 and 30.

HBV vaccination is likely to provide health benefits to the participants in the control group as most adults in Bangladesh have not recieved any HBV vaccines.

2.5. Safety/adverse events

Participants were observed by study health care workers for 30 min following vaccination and visited daily for seven days to record any adverse events. They were also given information on adverse events and a phone number to call should any appear. If field workers noted any medical concerns of a participant, the participant was referred to a medical doctor for appropriate care. All AEs were reported in the case report forms (appendix) according to the given criteria.

See the published study protocol by Zaman et al. [15] for further details.

2.6. Sampling/laboratory

Venous blood was collected from all participants before vaccination (day 0) and at day 60 and 2 years after the first vaccine dose. Plasma was separated and stored at −80 °C until analysis.

Additionally, blood samples for investigating cellular immune responses were collected from a subgroup of 10 randomized participants. The collection was done before vaccination and one month after the second dose. Peripheral blood mononuclear cells (PBMC) were isolated by Ficoll-Paque density gradient centrifugation (Ficoll-Paque Premium 1.077; GE Healthcare) using SepMate 50 ml tubes (Stemcell Technologies) following the manufacturer's instructions. Cells were cryopreserved and stored at −150 °C in 25 % fetal calf serum/10 % dimethyl sulfoxide (DMSO)/65 % AIM-V media (Gibco; Thermo Fisher Scientific, Waltham, MA) until analysis.

2.7. Serology

Anti-HEV IgG was measured using Wantai HEV IgG ELISA (Beijing Wantai, China) according to the manufacturer's instructions. The same batch was used for all analysis.This ELISA kit detects antibodies induced after infection with relevant genotypes and after vaccination with HEV 239. All samples from one individual were run in the same ELISA setup (paired samples). Serial dilutions of the WHO reference reagent for HEV antibody (WHO 95/584) were included in each ELISA run. The results were converted to WHO units/ml (WU/ml) using a five-parameter logistic function [17] with the results from the WHO reference reagents (Code 95/584) as standards. Samples were deemed positive if the OD was above the cut-off (OD/CO) value calculated according to the manufacturer's instructions (OD/CO > 1 corresponding to 0.2 WU/ml in our analyses). The lower limit of quantification was an OD/CO of 0.03, corresponding to 0.06 WU/ml. Samples with an OD/CO below 0.03 were set to 0.06 WU/ml. Samples with values above the limit of quantification (25 OD/CO) were diluted to obtain quantifiable results. A positive serological response was defined as either a negative to positive IgG change (seroconversion) or a four-fold increase in IgG-titer in an individual's paired samples.

Anti-HBs were measured on samples collected before and one month after vaccination using Abbott Architect anti-HBs assay according to manufacturer's instructions (Abbott Diagnostics Division, Rungis, France). For this assay, samples with an anti-HBs titer $\geq$ 10mIU/mL were deemed positive according to WHO criteria [18].

2.8. FluoroSpot assay (IFN-γ / IL-4)

HEV-specific IFN-γ and IL-4 *T*-cell responses were measured by an *ex vivo* FluoroSpot assay according to the manufacturer's instructions (Human IFN-γ/IL-4 FluoroSpotPLUS kit, Mabtech, Sweden). In short, 200 000 PBMCs in AIM-V medium (Gibco, Thermo Fisher Scientific, Norway) with anti-CD28 co-stimulator (included in the kit) were added to each well in pre-coated plates. Antigenic stimulants used were as follows: negative control (DMSO in AIM-V media to match the peptide pool), positive control (anti-CD3, included in the kit), and the JPT peptide pool (PepMix™ HEV (ORF2), JPT Peptide Technologies, Germany) at 5 μg/mL in DMSO/AIM-V. Plates were incubated for 20 h at 37 °C in a humidified incubator with 5 % CO_2 and developed the following day. The plates were read using a CTL S6 Ultra V ImmunoSpot analyzer (Cellular Technology Limited, Shaker Heights, OH). Data were analyzed using Microsoft Excel and GraphPad Prism software (GraphPad Software, Inc.). The cut-off level of spot forming units (SFU) was calculated as the average number of SFU in the negative control wells. Samples with no positive control response were excluded. Background values from the negative control were subtracted from those achieved after antigen stimulation. Zero values were set to 1 for calculation purposes.

2.9. Statistics

The sample size for the trial was not based on a statistical power calculation but practical and clinical considerations. Dependent variables included: AEs, anti-HEV IgG status (positive/negative) and level (WU/ml), and HEV-specific IFN-γ and IL-4 response. These were analyzed for possible correlations to the independent variables: vaccine status (HEV or HBV vaccine) and anti-HEV IgG status at day 0 (positive/negative). Antibody values were summarized using the geometric mean and 95 % confidence intervals based on log-transformed values of WU/ml. For categorical data, the Pearson Chi-square test was used for immune results, and the mid-p Fisher exact test was used for adverse events. P-values below 0.05 were considered statistically significant. Multiple linear regression models and student t-tests were used for continuous data. Data analyses were done by STATA (16) and Excel (2016).

2.10. Ethical approvals and funding

The study was approved by the icddr,b Research Review Committee (RRC) and the Ethical Review Committee (ERC), the Directorate General of Drug Administration (DGDA) in Bangladesh, and the Regional Ethics Committee (REC) in Norway. The Norwegian Research Council funded the project through the GLOBVAC funds (project number: 248143). The study was done by the principles of the Declaration of Helsinki, the standards of Good Clinical Practice, and the regulatory requirements of Bangladesh. ClinicalTrials.gov Identifier: NCT02759991.

3. Results

3.1. Participants

Out of the 112 persons evaluated for participation, 100 were successfully enrolled in the study and randomized to receive two doses of either the study (HEV) or the control (HBV) vaccine (Fig. 1). The mean age of the participants was 26 years (range 16–39), and the two groups appear balanced on all major baseline characteristics (Table 1). All 100 participants received two vaccine doses and had blood collected on days 0 and 60. Twelve participants (seven from the HEV group and five from the HBV group) did not provide the last blood collection after 2 years. The reasons for study discontinuation were lost to follow-up and voluntary withdrawal. Additional blood for PBMC analysis was collected from 10 randomly selected participants on days 0 and 60.

3.2. Adverse events

Safety analysis was completed for all 100 participants. During the 2 month follow-up period, there were no serious adverse events (SAE). However, 12 adverse events (AE) were reported (Table 2), but were mild in severity and resolved within one to nine days. There was no significant difference between AEs reported in the HEV vaccine group and the HBV vaccine group.

3.3. Immune results

3.3.1. Humoral response

Seroprevalence of HEV IgG prior to vaccination was 33 % (95 % CI 23.9–43.1), with a geometric mean titer (GMT) of 0.23 WU/ml (95 % CI 0.16–0.35) overall and 3.3 WU/ml (95 % CI 2.1–5.4) in seropositive and 0.06 WU/ml in seronegative participants. Seroconversion or a fourfold increase of antibody titer was observed in 100 % of the HEV vaccine group between day 0 and 60 with a GMT of 49.5 at day 60, compared to 10 % in the HBV vaccine group with a GMT of 0.21 at day 60 ($p < 0.001$ for differences in seroconversion and GMT) (see Fig. 2).

Two years later, the GMT was reduced to 6.4 (84 % reduction) in the HEV group compared to day 60, but nearly all remained IgG positive while the HBV group remained stable with a GMT of

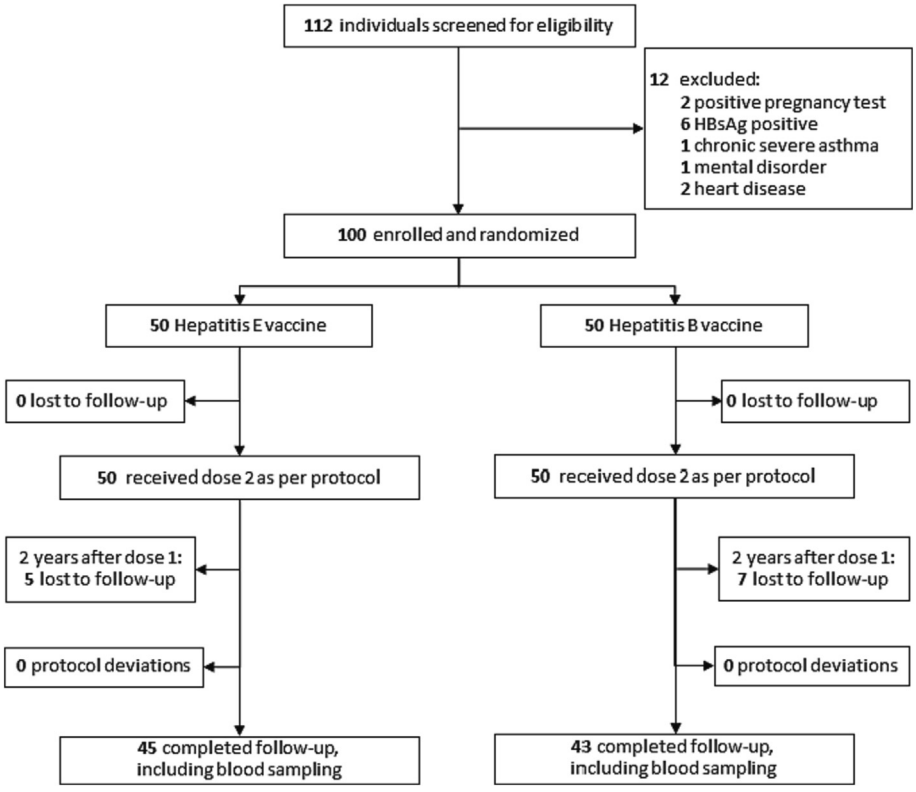

Fig. 1. Trial Profile.

Table 1
Baseline characteristics of the participants.

	Total	HEV 239	Control (HBV)	P- Value [a]
Enrolled participants, n	100	50	50	1.0
Men, n (%)	52 (52.0)	28 (56.0)	24 (48.0)	0.423
Women, n (%)	48 (48.0)	22(44.0)	26 (52.0)	
Mean age for Men, Years (SD)	26.1 (9.6)	26.0 (6.7)	26.1 (6.8)	0.958
Age Groups for Men, Years				0.045
16–18, n (%)	10 (19.2)	6 (21.0)	4 (17.0)	
19–24, n (%)	12 (23.1)	9 (32.0)	3 (12.0)	
25–30, n (%)	12 (23.1)	8 (29.0)	4 (17.0)	
31–39, n (%)	18 (34.6)	5 (18.0)	13 (54.0)	
Mean Age for Women, Years (SD)	25.6 (9.7)	25.3 (6.8)	25.8 (6.9)	0.802
Age Groups for Women, Years				0.283
16–18, n (%)	10 (20.8)	4 (18.2)	6 (23.1)	
19–24, n (%)	14 (29.2)	4 (18.2)	10 (38.5)	
25–30, n (%)	10 (20.8)	5 (22.7)	5 (19.2)	
31–39, n (%)	14 (29.2)	9 (40.9)	5 (19.2)	
Participants completed vaccination (2 doses), n (%)	100 (100)	50 (100)	50 (100)	1.0
BMI Men (Kg/m^2), Mean (SD)	21.1 (4.7)	21.1 (3.3)	21.1 (3.3)	1.0
BMI Women (Kg/m^2), Mean (SD)	21.2 (4.7)	21.2 (3.3)	21.0 (3.3)	0.835
Anti-HEV IgG, n (%)	33 (33.0)	20 (40.0)	13 (26.0)	0.137

[a] , p-values values were determined using the chi-squared test.

Table 2
Adverse Events reported in the study.

Symptoms	HEV 239 (N = 50)	Control (HBV) (N = 50)	Time of onset	Duration	Relationship with study vaccine	P-value[a]
Pain at injection site	1 (2 %)	1 (2 %)	Day 0 of dose 1	2 days	Related	0.75
Bruising at injection site	1 (2 %)	0	Day 0 of dose 1	4 days	Related	0.50
Headache	0	1 (2 %)	1 day after dose 1	1 day	Possibly related	0.50
Urinary tract infection	1 (2 %)	2 (4 %)	12–15 days after dose 1 (HEV group) and 4 days after dose 2 (HBV group)	3 days and 6 days, respectively	Not related	0.62
Fever	2 (4 %)	0	3–7 days before dose 2	3 days	Not related	0.25
Body ache	1 (2 %)	0	3 days before dose 2	3 days	Not related	0.50
Acute otitis media	1 (2 %)	0	9 days after dose 2	9 days	Not related	0.50
Vomiting	1 (2 %)	0	3 days before dose 2	3 days	Not related	0.50

[a] , The p-values were determined using mid-p Fisher's exact test.

0.22. One vaccinated participant had IgG levels below cut-off (0.18 WU/ml) and was deemed anti-HEV negative.

3.3.2. Effect of prior infection on the humoral response

There was a difference in the proportion of baseline HEV seropositive participants between the HEV vaccine group (40 %) and the control group (26 %), and almost all these initial seropositive participants remained seropositive throughout the study period (Table 3). Twenty participants in the HEV vaccine group were seropositive at baseline, and compared to the seronegative participants, they obtained a 9.5-fold higher increase in GMT at day 60. This difference was further increased to 22.6-fold higher two years later ($p < 0.001$). The mean HEV IgG titer reduction in vaccinated participants was significantly lower in baseline seropositive participants than HEV naïve participants (68.9 % versus 85.8 % reduction, $p = 0.01$) (see Table 3).

Multiple linear regression was used to test if HEV IgG levels at two months and 24 months were significantly predicted by the participant's vaccine status when controlling for IgG levels at day zero. A significant ($p < 0.001$) regression equation was found for day 60 ($F(2, 97) = 60.15$, $R^2 = 0.55$) and 24 months ($F(2, 85) = 28.30$, $R^2 = 0.40$). It was found that baseline IgG level (WU/ml) significantly predicted IgG levels 2 months later ($\beta = 16.27$, $p < 0.001$) and 24 months later ($\beta = 1.72$, $p < 0.001$). HEV vaccination significantly predicted IgG levels 2 months later ($\beta = 93.76$, $p < 0.001$) and 24 months later ($\beta = 16.20$, $p < 0.001$).

Anti-HEV IgG (WU/ml) measured in participants receiving HEV 239 vaccine (yellow) and control (HBV) vaccine (green) before vaccination (Day 0), 30 days after the second vaccine dose (Day 60), and two years after the second dose. The y-axis is split in 0–100 WU/ml in the lower part - and 200–1000 WU/ml in the upper part of the figure. The black line represents Geometric Mean Titer (GMT) with the 95 % confidence interval in red.

3.3.3. Seroconversion between day 60 and 2 years

Five participants in the HBV group (and none in the HEV group) had a higher than fourfold increase in IgG titer between day 60 and 2 years, indicating an HEV infection during this period (see figure S3 in appendix). This may point to a protective effect of HEV 239 against HEV infection ($p = 0.04$, mid-p Fisher's exact), although such a measure of vaccine efficacy against infection is uncertain as the correlation between a fourfold increase in antibodies and HEV infection in a two year period after vaccination (with high initial antibody titers) are unknown.

3.3.4. Cellular response

We measured a significant HEV viral capsid-specific IFN-γ and IL-4T-cell response in 2 of 6 baseline samples, and at least one

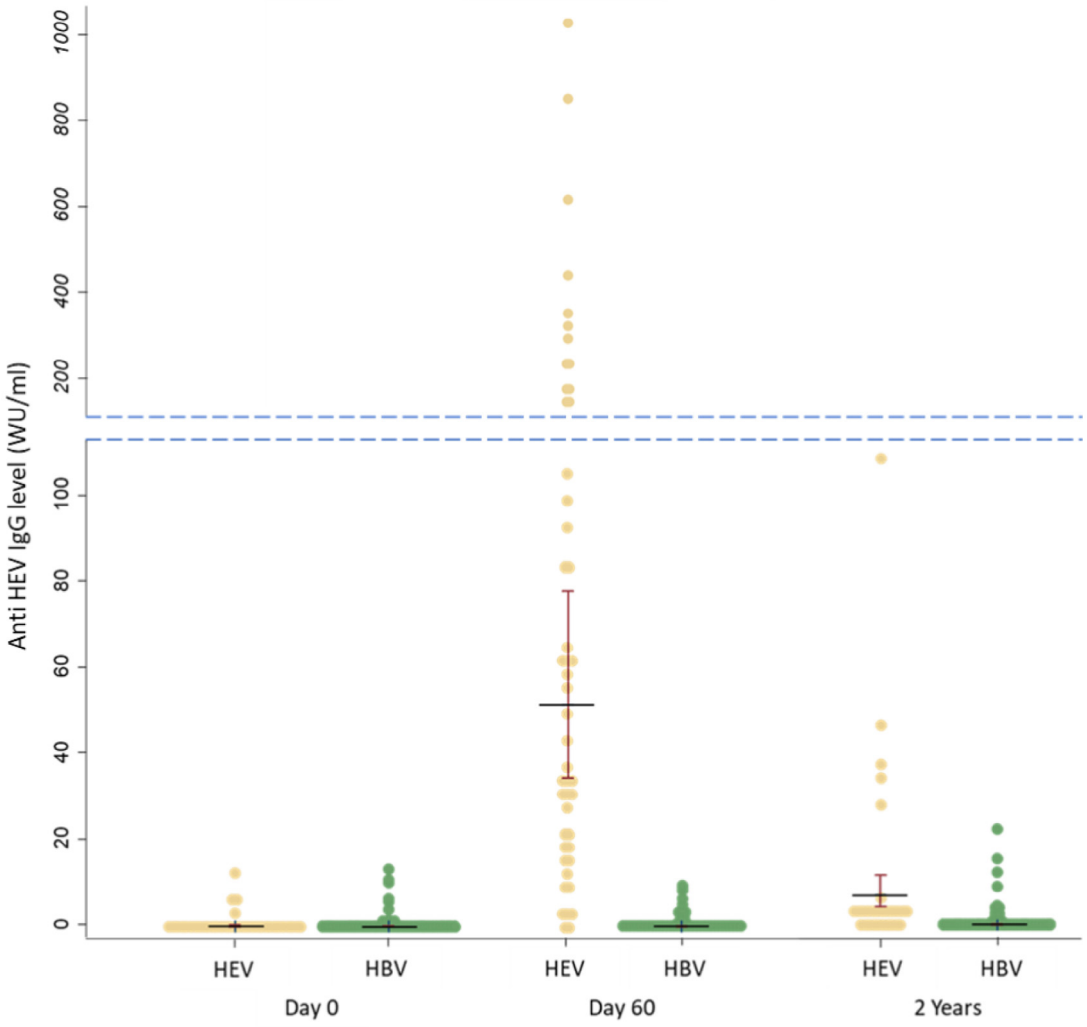

Fig. 2. Antibody responses at different time points in the phase 2 trial.

remained positive on day 60 (Table 4). One sample corresponded to a participant with a positive IgG level of 1.34 WU/ml, while the other participant had an IgG level below cut-off but still quantifiable. HEV 239 induced a significant IFN-γ and IL-4 response in 3/3 participants (see supplement Fig. 2a in appendix). However, due to low cell numbers, the *T*-cell response could not be measured in some PBMC samples (marked "no cells" in Table 4).

3.4. Feasibility

We tested protocols and procedures for all stages of the trial, including training a large, experienced field team of over 100 staff in study-specific processes such as vaccine transportation (cold chain management), vaccination techniques, safety follow-ups, reporting of adverse events, sample collection and storage, data management, and quality control. Two trial monitors scrutinized the study elements and found no major deviations from the study protocols.

4. Discussion

This study is the first trial of the HEV 239 vaccine conducted in an area where HEV1 is highly endemic. The participants were recruited from a region severely affected by HEV and in need of effective measures against HEV disease. We found that the HEV 239 had an acceptable safety profile and elicited a strong antibody response that remained positive for two years in almost all participants. Our results indicate a robust *T*-cell response after vaccination which compliments the serological results from this and previous HEV 239 vaccine studies.

Pre-existing antibodies prior to vaccination in our study were associated with higher and more stable antibody responses. This is in line with similar findings by Zhang et al. [9] and might indicate a superior vaccine response in previously infected individuals.

The observed *T*-cell responses likely contribute to the protective effects of the vaccine and may offer long-term protection against severe disease and heterologous genotype protection. Similar to responses after natural infection, the vaccine appears to induce both IFN-γ and IL-4 producing *T*-cells against parts of the capsid protein, indicating a balanced Th-1 and Th-2 response. However, the small sample size analyzed for cellular responses requires a cautious interpretation of the results. Further investigation is needed to fully understand the *T*-cell responses after vaccination.

Almost all IgG measurements two years post-vaccination were above the upper correlate of protection of 0.22 IU/ml suggested by Zhang et al. [10] and HEV 239 vaccinated individuals would thus be expected to have protection from HEV infection in this period. There are serological signs of a lower rate of reinfection in the

Table 3
Serological results from anti HEV IgG measurements.

Anti-HEV IgG	HEV 239			Control (HBV)			p-value (total, seropositive, seronegative)
	Total	Seropositive at baseline	Seronegative at baseline	Total	Seropositive at baseline	Seronegative at baseline	
Population (n)							
Day 0	50	20	30	50	13	37	
Day 60	50	20	30	50	13	37	
2 years [a]	43	19	24	45	10	35	
% Seropositive (95 % CI) [b]							
Day 60	100 (92.9–100)	100 (83.2–100)	100 (88.4–100)	34 (21.2–48.8)	92 (60.3–98.9)	14 (5.7–28.9)	<0.001, 0.208, <0.001
2 years	98 (87.7–99.9)	100 (82.4–100)	95 (75.1–99.4)	36 (21.9–51.2)	100 (69.2–100)	11 (3.2–26.7)	<0.001, 1.0, <0.001
GMT (95 % CI) (Wu/ml) [c]							
Day 0	0.34 (0.18–0.64)	4.1 (2.1–8.0)	0.06 (0.06–0.07)	0.16 (0.10–0.26)	2.4 (1.17–5.05)	0.06 (0.06-0.0.06)	0.060, 0.292, 0.163
Day 60	49.5 (31.5–77.7)	189.3 (122.3–293.1)	20.2 (12.6–32.5)	0.21 (0.12–0.35)	2.0 (0.78–4.9)	0.09 (0.06–0.14)	<0.001, <0.001, <0.001
2 years	6.4 (3.6–11.3)	36.1 (23.2–56.4)	1.6 (1.0–2.5)	0.22 (0.12–0.40)	2.3 (1.0–5.1)	0.11 (0.07–0.19)	<0.001 < 0.001, <0.001
Seroconversion or fourfold increase % (95 % CI) [d]							
Day 0 –..day 60	100 (92.9–100)	100 (83.2–100)	100 (88.4–100)	10 (3.3–21.8)	0 (0–24.7)	14 (4.5–28.8)	<0.001 < 0.001, <0.001
Day 60 –.0.2 years	0 (0–0.08)	0 (0–17.7)	0 (0–14.3)	11 (3.7–24.1)	10 (0.3–44.5)	11 (3.2–26.7)	0.040 0.172, 0.081

GMT, Geometric mean titer;95% CI, 95% Confidence interval; WU/ml, antibody values in WHO units per ml.
[a] , The number of participants in each group declined at 2 years due to 12 participants lost to follow-up.
[b] , The p-values were determined using chi-squared test.
[c] , The p-values were determined using student-*t* test on log transformed data.
[d] , The p-values were determined using mid-p Fisher's exact test.

HEV vaccine group than the HBV vaccine group, which supports this assumption (see figure S3), but this cannot be used to draw any conclusions on vaccine effectiveness.

One HEV participant was seronegative 2 years post-vaccination, while the antibody level was still quantifiable (0.18WU/ml). The significance of low antibody titers 2 years post-vaccination is uncertain, as both memory B and *T*-cells could offer a substantial level of protection against disease and especially serious disease. However, the *T*-cell response in this participant was also lower than for the other participants suggesting an overall reduced immune response to the vaccine with possible subsequent lower protection.

Together our results indicate that two doses of HEV 239 may offer lasting protection against HEV in a majority of vaccine recipients. As such, a shorter vaccination period with fewer vaccine doses is especially relevant for chaotic settings such as refugee camps where large HEV outbreaks are known to occur [19].

Table 4
Antibody and *T*-cell response in participants randomly selected for additional sampling.

Day 0 (prior to vaccination)		Day 60 (30 days after last vaccination)	
Antibody level (WU/ml)	*T*-cell response	Antibody level (WU/ml)	T cell response
HEV 239			
0.06	No cells [a]	17.5	No cells [a]
0.06	0	1.8	IL-4 and IFN-γ
0.06	No cells [a]	36.3	IL-4 and IFN-γ
0.06	0	60.0	No cells [a]
0.06	No cells [a]	100.7	No cells [a]
0.06	No cells [a]	2.2	IL-4 and IFN-γ
Control (HBV)			
0.07	IL-4 and IFNγ	0.07	IL-4 and IFN-γ
0.06	0	0.06	0
0.06	0	0.06	0
1.34	IL-4 and IFNγ	1.1	No cells [a]

[a] Insufficient amount of cells for FluoroSpot analyses.

The seroprevalence measured at baseline in our study of 33 % (95 % CI 23.9–43.1) is lower than 46 % (95 % CI 43.5–49.8) found by Kmush et al. who used the same ELISA-assay as in our study to investigate samples collected from participants from the same area of Bangladesh between 2004 and 2005[20]. This might indicate a decrease in HEV cases in the Matlab area of Bangladesh during the last decade.

This pilot study also aided in preparing and testing the logistics and management plan required for proper field implementation of a large clinical trial in this rural setting. Based on this, the main trial was deemed feasible, and we embarked on enrolling 20,000 non-pregnant women of reproductive age.

There are several limitations to our study. The small sample size makes our study unlikely to detect any but the most common AEs. Although we show a robust immune response to the HEV vaccine, the level and kind of immunity (B-cell, *T*-cell, or both) needed for protection against HEV disease are still unclear. We show a likely protective effect of HEV 239 against HEV infection based on seroconversion rate, but this is uncertain as an initial rise in antibodies due to infection could be masked by a subsequent decline before blood sampling at two years. The lack of active hepatitis surveillance prevented us from connecting seroconversion signs to possible acute HEV disease. Also, it is uncertain how the antibody levels in the previous HEV vaccinated individuals would respond to an acute HEV infection later on. The limited number of participants with enough viable PBMC for analysis made it difficult to interpret *T*-cell responses to the vaccine other than simply establishing their presence. We have yet to determine the reason behind the low cell count in many samples, as the PBMC samples had high viability after thawing. Therefore, we assume that a loss of cells occurred somewhere, possibly due to a faulty cell counting machine, or a calculation error. More extensive studies with longer and more active observation periods are needed to further investigate the efficacy and duration of protection after two vaccine doses of HEV 239.

In conclusion, the HEV 239 vaccine appears safe and effective. We demonstrate a strong antibody response after two doses of

HEV 239 that remained detectable for two years and a vaccine-induced *T*-cell response. This indicates a broad and likely functional protection against HEV infection and disease.

Data availability

Data will be made available on request.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We acknowledge with gratitude the Research Council of Norway for supporting this research through the GLOBVAC program (grant number: 248143/H10. We wish to convey our thanks to the participants in this trial. The authors thank all the members of the research team at icddr,b who helped with participant recruitment, vaccination, sampling and follow up, and the laboratory team who performed laboratory procedures. The local clinical monitor (Wasif Ali Khan) is thanked for monitoring the trial. The authors also thank the data safety monitoring board (Kazi Zulifiquer Mamun, S M Shamsuzzaman, Saria Tasnim, Md. Nur Haque Alam, and Hanne M. Nøkleby), and the steering committee (Shams El Arifeen, Rashidul Haque, Ingeborg Aaberge and Geir Bukholm) for their important contributions throughout the trial. A special thanks to Innovax for their donation of the HEV vaccine. NIPH is the sponsor for this clinical trial.

Joakim Overbo reports financial support was provided by Research Council of Norway. Cathinka Julin reports financial support was provided by Research Council of Norway.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2022.12.064.

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