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A Phase 1, Double-Blinded, Placebo-Controlled Clinical Trial to Evaluate the Safety and Immunogenicity of HEV-239 (Hecolin) Vaccine in Healthy US Adults

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Background. Establishing the safety and immunogenicity of a hepatitis E virus vaccine in multiple populations could facilitate broader access and prevent maternal and infant mortality.

Methods. We conducted a phase 1, randomized, double-blinded, placebo-controlled (4:1 vaccine to placebo) trial of 30 μg HEV-239 (Hecolin, Xiamen Innovax Biotech Company Limited, China) administered intramuscularly in healthy US adults aged 18–45 years. Participants were vaccinated on days 1, 29, and 180. Participants reported solicited local and systemic reactions for 7 days following vaccination and were followed through 12 months after enrollment for safety and immunogenicity (IgG, IgM).

Results. Solicited local and systemic reactions between treatment and placebo group were similar and overall mild. No participants experienced serious adverse events related to HEV-239. All participants receiving HEV-239 seroconverted at 1 month following the first dose and remained seropositive throughout the study. HEV-239 elicited a robust hepatitis E IgG response that peaked 1 month following the second dose (geometric mean concentration [GMC], 6.16; 95% confidence interval [CI], 4.40–8.63), was boosted with the third dose (GMC, 11.50; 95% CI, 7.90–16.75) and persisted through 6 months.

Conclusions. HEV-239 is safe and elicits a durable immune response through at least 6 months after the third dose in healthy US adults.

Clinical Trials Registration. NCT03827395.

Keywords. hepatitis E; acute hepatitis; viral hepatitis; immunization; vaccination.

Hepatitis E virus (HEV) is the leading cause of acute viral hepatitis worldwide, estimated to cause 20 million infections and 70 000 deaths each year [1–3]. Although the virus is globally distributed, it is highly endemic in several countries in Asia and Africa, where the seroprevalence approaches 50% [3, 4]. Outbreaks of HEV are associated with fecal contamination of water supplies and poor sanitation and cause a significant public health burden in resource-limited countries [5, 6]. HEV typically causes sporadic cases in high-income countries, but the disease burden is likely underestimated due to limited surveillance and lack of standardized testing methods [7]. Clinical manifestations range from asymptomatic infection to fulminant hepatitis, and immunocompromised individuals can experience chronic hepatitis with progression to liver cirrhosis and hepatocellular carcinoma [8]. Adults are more likely to develop symptomatic disease than children, although high mortality rates in young children and infants have been described [9, 10]. In particular, mortality rates up to 30% have been described in pregnant women infected with HEV, with increased risk for fulminant liver failure, obstetric complications, and fetal loss [9, 11]. Importantly, there are currently no available treatments for acute HEV, and prevention is primarily based on improved hygiene and sanitation measures. Infection with HEV results in cross-reactive antibodies against all 4 genotypes [9] and appears to confer long-term protection against symptomatic disease, suggesting that a potential HEV vaccine would confer significant public health benefit [12].

HEV-239 (Hecolin, Xiamen Innovax Biotech Company Limited, China) is the only HEV vaccine currently available and licensed in China. A phase 3 randomized, double-blinded,

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placebo-controlled trial evaluated the safety and efficacy of HEV-239 in healthy subjects 16–65 years of age regardless of baseline HEV seropositivity status [13]. Subjects were randomized to receive 3 doses of either HEV-239 or placebo (a licensed hepatitis B vaccine) administered intramuscularly (IM) at 0, 1, and 6 months. Overall, HEV-239 was well-tolerated and conferred 100% protection against hepatitis E infection after administration of either 2 doses (95% confidence interval [CI], 9.1–100.0) or 3 doses (95% CI, 72.1–100.0) of vaccine [13]. The study was limited by a low event rate and high baseline anti-HEV prevalence, ranging from 44%–49% in study participants [12]. While promising, the broader generalizability of this study is limited given varying distribution of HEV genotypes based on geographic location and differences in population HEV prevalence and attack rates, which may impact vaccine efficacy rates.

A vaccine against HEV that has undergone safety and immunogenicity testing in diverse settings could broaden access to provide protection for travelers, mitigate outbreaks, and prevent maternal, fetal, and infant morbidity and mortality. Although HEV-239 is licensed in China, more comprehensive immunologic characterization of the kinetics of response, including in the setting of a distinct HEV-naive population, would expedite the licensure and use of this vaccine in other countries. Given the significant global burden of HEV and lack of preventative and therapeutic strategies, a safe and effective HEV vaccine is urgently needed.

METHODS

Trial Design and Participants

Healthy men and nonpregnant women, aged 18–45 years (inclusive) were recruited to enroll into this double-blinded, randomized, single-center, placebo-controlled phase 1 clinical trial of HEV-239. Inclusion criteria included subjects in good general health without acute illness, with screening laboratory values within normal limits, and negative serology for HEV (immunoglobulin G [IgG] and immunoglobulin M [IgM]). Subjects were excluded who had chronic liver disease, prior HEV infection, travel within the past 90 days or intended travel to Asia, the Middle East, Africa, Central America, or to an area with an active HEV outbreak, or immunodeficiency. Subjects who met all enrollment criteria were randomized at a ratio of 4:1 to receive 3 doses of HEV-239 vaccine or placebo administered on days 1, 29, and 180. Subjects were followed through 12 months for safety and to determine the durability of immunological responses. This study was approved by the institutional review board at Emory University.

Study Product

The HEV-239 vaccine was manufactured by Xiamen Innovax Biotech Company Limited (China). HEV-239 is composed of a truncated HEV capsid protein corresponding to amino acid residues 368–606 of a genotype 1 HEV strain that assembles into virus-like particles. This region of the HEV capsid protein includes the predominant HEV neutralizing epitope (amino acid residues 459–607) [14]. The vaccine contains 30 μg of purified protein adsorbed to 0.8 mg of aluminum hydroxide suspended in 0.5 mL of phosphate-buffered saline with thimerosal. The finished product is a white suspension that is packaged in sterile prefilled syringes. The dose was selected based on data from the previous phase 3 study conducted in China. Placebo was 0.5 mL normal saline given IM into the deltoid muscle.

Data Collection

Subjects were observed for 30 minutes after vaccination. All adverse events (AEs) were captured, including solicited local (injection site) and systemic reactions. AEs were graded for severity (Supplementary Methods) from mild to severe and assessed for relationship to the study product. The occurrence of solicited injection site and systemic reactogenicity events were measured from the time of study vaccination through day 8 after each vaccination. These events were ascertained through use of an electronic memory aid, a telephone call occurring 3 days after each vaccine dose, and clinic visits at 1 week and 2 weeks after each vaccine dose. Unsolicited AEs were collected from vaccination through 4 weeks after each vaccination. Serious adverse events (SAEs) were defined as an AE that resulted in a life-threatening event, hospitalization, or important medical event in view of the site principal investigator or sponsor. SAEs were collected from the time of the first study vaccination through the last study visit (day 360). Phlebotomy for safety laboratories were conducted prior to and at 7 days after each dose of vaccine.

Assessment of Antibody Response

Qualitative HEV-specific serum IgM and IgG were measured using enzyme-linked immunosorbent assays (ELISAs) for samples collected on days 1 (before dose 1), 8, 15, 29 (before dose 2), 36, 43, 57, 180 (before dose 3), 187, 194, 208, and 360. Quantitative HEV-specific serum IgG titers were also measured using ELISA for samples collected at all time points. The primary immunogenicity end point was the percentage of subjects showing ≥4-fold rise in serum HEV IgG concentration by ELISA from baseline. The secondary immunogenicity end points were the number of subjects with HEV IgM and IgG seroconversion by ELISA and IgG geometric mean concentrations by ELISA.

Qualitative IgM and IgG ELISA

Samples were tested in duplicate for HEV IgM and IgG using Wantai HEV ELISA kits (WE-7196 and WE-7296, respectively; Wantai BioPharm) according to manufacturer instructions

without deviation. Results were reported for each duplicate as positive, negative, or borderline according to the kit's criteria. Samples with initially borderline results were repeated once. Note was made of samples that appeared hemolytic or lipemic, and these samples were repeated to validate their initial results. Plates were also repeated if they failed quality controls as specified by the manufacturer. Seroconversion was defined as a change from seronegative to seropositive in the qualitative assay.

Quantitative Anti-HEV IgG Titer by ELISA

To quantify HEV IgG titer according to World Health Organization (WHO) standard units, the Wantai HEV-IgG ELISA kits were used in conjunction with the WHO reference reagent for HEV antibody (National Institute for Biological Standards and Control 95/584). Samples were initially screened in duplicate according to manufacturer protocol with the following deviations: an 8-point, 1.5-fold dilution series of the WHO reference standard was prepared with a starting concentration of 1.2 U/mL in phosphate-buffered saline; 10 μL of this dilution series was plated in duplicate on each IgG screening plate.

Data from WHO reference standard samples on each IgG screening plate were used to produce a standard curve by linear regression in GraphPad Prism. Samples whose A_{450} values fell below the A_{450} lowest point on the dilution curve were considered below the lower limit of quantification of 0.077 U/mL and assigned a value of 0.0385 U/mL. For samples with A_{450} values falling within the range of the standard curve, these values were used to interpolate a concentration using the standard curve and reported in U/mL.

Samples with A_{450} values above the standard curve range during screening were repeated on a 6-point, 4-fold dilution series prepared in phosphate-buffered saline starting with undiluted sample. These samples were further diluted 11-fold as standard for the kit. The 11-fold to 11 264-fold dilution series were run alongside the WHO reference standard according to manufacturer protocol. All dilution points with A_{450} values falling within the range of the standard curve were used to interpolate a concentration and average reported in U/mL. Samples with a difference between maximum and minimum titer for all replicates greater than 50% of the average were repeated (ie, if [max-min]/mean > 50%). Plates were also repeated if they failed quality control as specified by the manufacturer.

Statistical Analysis

Safety analyses included all subjects who received at least 1 dose of study product. Immunogenicity analyses for the modified intention-to-treat (mITT) population included all subjects who received at least 1 dose of study product and contributed both pre- and at least 1 poststudy vaccination blood sample for immunogenicity testing for which valid results were reported. Immunogenicity data from testing performed at the final study visit that were out of visit window were excluded from the per protocol (PP) analyses. Demographics were summarized with descriptive statistics. Safety events were summarized as the number and percentage of subjects experiencing the event type. For quantitative IgG ELISAs, the geometric mean concentrations (GMCs) and geometric mean fold rises (GMFRs) were determined. For the qualitative ELISAs, the number and percentage of subjects with HEV IgM and IgG seroconversion were described. Ninety-five percent CIs for the percentage of subjects showing ≥4-fold rise in serum HEV IgG concentration from baseline (defined as the change in quantitative titer compared to baseline) and the percentage of subjects with seroconversion (defined as crossing the threshold from negative to positive in the qualitative assay) from baseline in serum HEV IgG and IgM determined by ELISA were calculated using the Clopper-Pearson exact method.

Modifications Due to the Coronavirus Disease 2019 Pandemic

Emory University temporarily suspended nonessential noncoronavirus disease 2019 (non–COVID-19) research clinic visits during the spring of 2020. The COVID-19 pandemic prevented most participants from coming to their day 360 visits within the study window. On-site visits resumed in the summer of 2020. Due to being out of window, the immunogenicity data from blood samples collected at these visits were excluded from the PP day 360 analyses but included in the mITT analyses.

For the mITT analysis, a mixed effects model was used to estimate what the HEV IgG concentration would have been for each subject if their day 360 visit occurred on the target study day. All subjects with an out-of-window day 360 visit were included along with their observed after dose 3 peak antibody concentration and subsequent visit concentrations. Because antibody concentration declines over time, holding these visits at later dates would be expected to result in lower observed concentrations. The model included fixed effects for time and treatment group, an interaction term between time and treatment group, and a random effect for the intercept for each subject. The relationship between HEV IgG concentration and time was found to be nonlinear and therefore a $\log_{10}$ transformation was applied. An unstructured covariance matrix was used. No other imputations were performed to account for missing data. All data analyses and presentations were conducted with SAS software, version 9.4 (SAS Institute).

RESULTS

Study Participants

Fifty-three healthy adults were screened to enroll 25 into the study (Figure 1) to receive vaccine (n = 20) or placebo (n = 5) between May and August 2019. Of those enrolled, 76% were female, 72% were White, 20% Black, 8% Asian, and 8% Hispanic

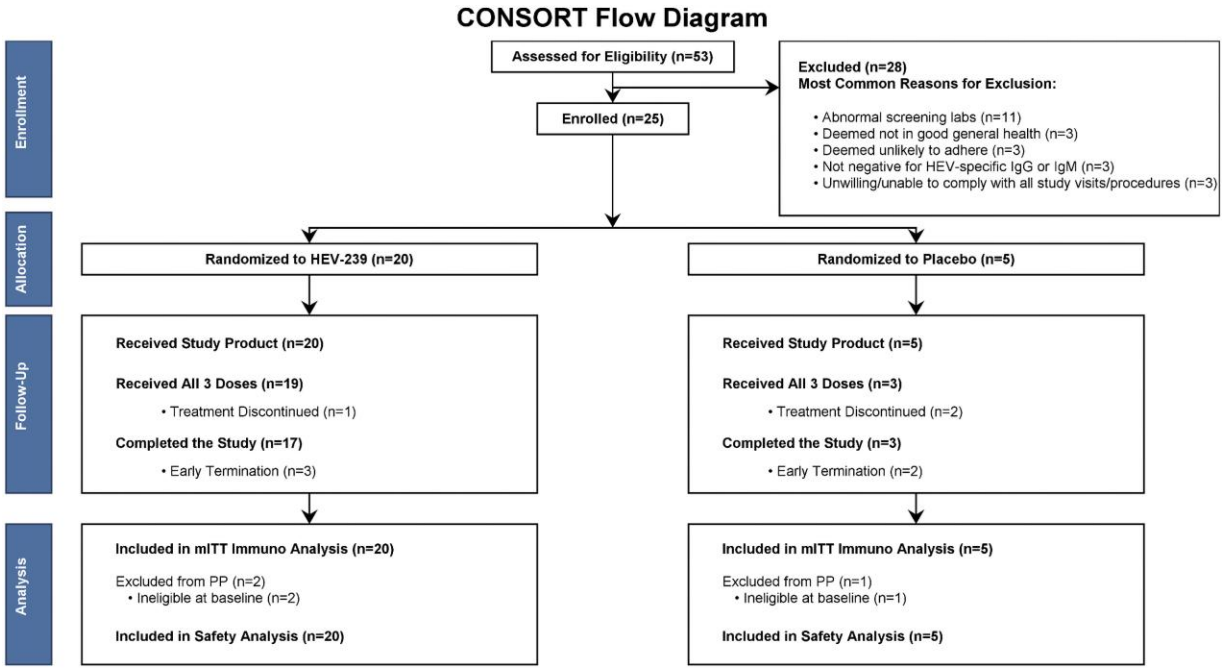

Figure 1. CONSORT diagram. Abbreviations: HEV, hepatitis E virus; Ig, immunoglobulin; mITT, modified intention to treat; PP, per protocol.

Table 1. Demographic and Baseline Characteristics

Variable	Characteristic	HEV-239 (n = 20)	Placebo (n = 5)	All Subjects (n = 25)
Sex	Male	5 (25)	1 (20)	6 (24)
	Female	15 (75)	4 (80)	19 (76)
Ethnicity	Not Hispanic or Latino	19 (95)	4 (80)	23 (92)
	Hispanic or Latino	1 (5)	1 (20)	2 (8)
Race[a]	Asian	2 (10)	0 (0)	2 (8)
	Black or African American	3 (15)	2 (40)	5 (20)
	White	15 (75)	3 (60)	18 (72)
Age, y	Median (IQR)	30 (24.0–33.5)	35 (27.0–36.0)	31 (24.0–35.0)
	Range	20–44	23–43	20–44

Data are No. (%) except where indicated.

Abbreviations: IQR, interquartile range; n, number of subjects.

[a]No enrolled subjects self-identified as "American Indian or Alaska Native," "Native Hawaiian Other Pacific Islander," or "multiracial," or declined to have their race reported.

(Table 1). The median age was 31 years (interquartile range [IQR], 24–35 years). Age, ethnicity, race, and the percentage of men and women were generally similar between HEV-239 and placebo recipients. All 25 subjects (100%) received the first dose, 23 subjects (92%) received the second dose, and 22 subjects (88%) received the third dose. Two of the subjects that did not receive the second and third dose were in the placebo arm. Of those that did not receive the third dose, 1 became ineligible due to initiation of a new medication and 2 because of travel/relocation to an HEV-endemic area. Twenty subjects (80%) completed follow-up through the final study visit, despite the COVID-19 pandemic.

Vaccine Reactogenicity and Safety

Local solicited events were experienced by 19 of 20 subjects (95%) who received HEV-239 and 3 of 5 subjects (60%) who received placebo (Figure 2). The most common local event was mild tenderness, observed in 85% (17 subjects) of those that received HEV-239 versus 40% (2 subjects) in the placebo group. Systemic solicited events were experienced by 85% (17 subjects) who received HEV-239 and 80% (4 subjects) who received placebo (Figure 2). Headache was the most common symptom and was reported by 11 subjects (55%) in the HEV-239 group versus 3 subjects (60%) in the placebo group. One subject (5%) in the HEV-239 group experienced grade 3 malaise (significant

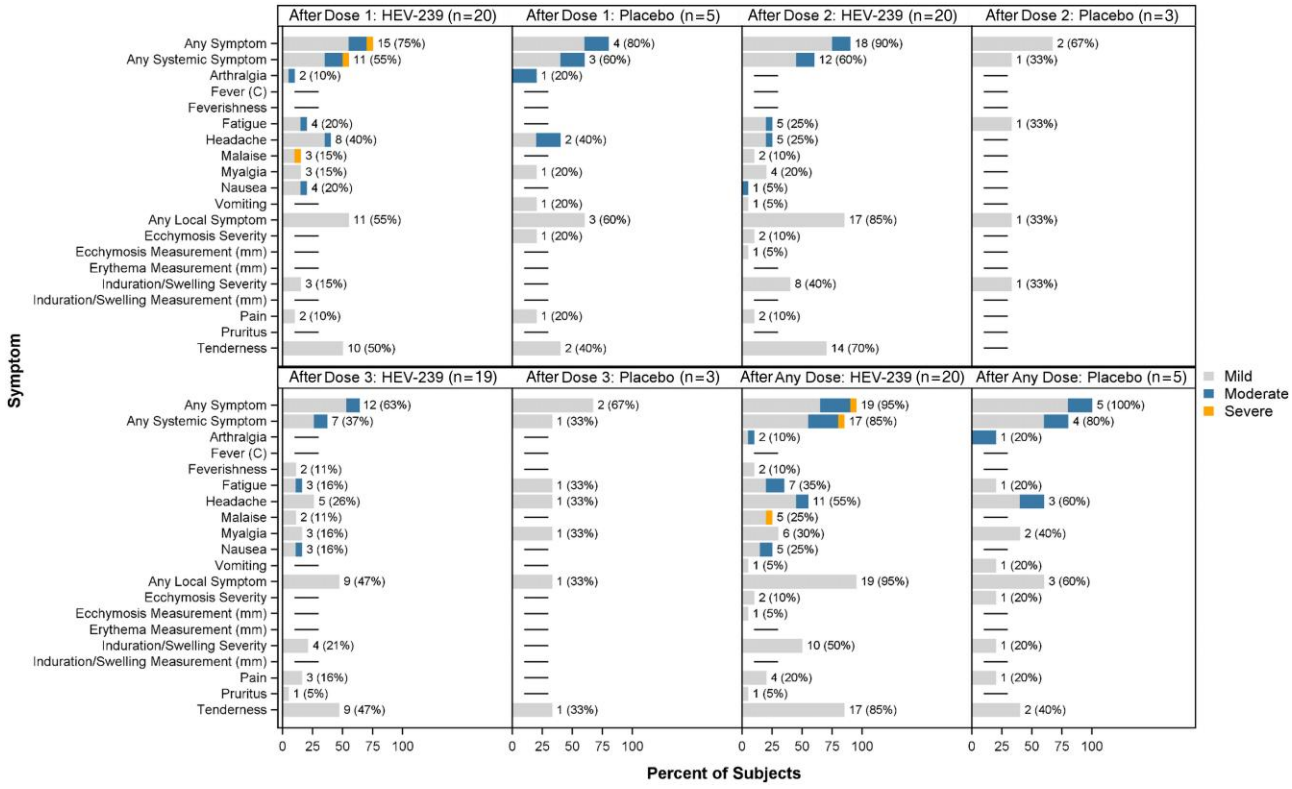

Figure 2. Solicited local and systemic adverse reactions observed after receipt of HEV-239 or placebo.

interference that prevents daily activity) on day 8 following the first dose, which was considered unrelated to the study product (alternative etiology of upper respiratory infection). All other solicited events, both systemic and local, were of mild or moderate severity. Ten subjects who received HEV-239 (50%) and 1 subject who received placebo (20%) experienced at least 1 unsolicited AE, all considered unrelated to the study product. Most abnormal laboratory values were graded as mild. Two subjects experienced a moderate hemoglobin decline after HEV-239, 1 at day 8 after study vaccination 2 and 1 at day 8 after vaccination 3. Both events resolved in follow-up and were deemed unrelated to the study product. One unrelated SAE of dysmenorrhea occurred in the placebo group. No pregnancies and no vaccine-related SAEs occurred during the study.

Immunogenicity Outcomes

All participants were seronegative at baseline. Minimal HEV IgG change was observed at day 8, but 65% (95% CI, 41%–85%) of subjects who received HEV-239 in the mITT population achieved ≥4-fold rise in serum HEV IgG concentration at day 15 (Table 2). In the HEV-239 group, 100% (95% CI, 83%–100%) of subjects in the mITT population achieved ≥4-fold rise in serum HEV IgG concentration at day 29, which was maintained through day 360. Peak GMC (11.50 U/mL; 95% CI, 7.90–16.75) and peak GMFR (298.8; 95% CI, 205.17–435.06) for the HEV-239 group were observed at day 208 for the mITT population (Figure 3 and Supplementary Figure 1). No placebo subjects achieved ≥4-fold rise in serum HEV IgG concentration at any time point. The percentage of subjects with HEV IgG seroconversion by qualitative ELISA from the baseline mirrored the responses observed for the primary end point (≥4-fold rise in serum HEV IgG concentration by quantitative ELISA). Increases were observed in the GMCs by 1 week after the second and third vaccine doses, which declined at 5 months after dose 2 (3.8; 95% CI, 2.66–5.41) and at 6 months after dose 3 (predicted 4.3; 95% CI, 2.79–6.56).

In the prespecified secondary immunogenicity end points, the highest percentage of subjects to achieve IgM seroconversion occurred at day 43 with 16% (95% CI, 3%–40%) of subjects in the mITT population (data not shown). IgM seroconversion was not observed in any placebo recipients at any time point.

DISCUSSION

HEV is a significant public health burden and the leading cause of viral hepatitis worldwide, with no effective treatments or preventative therapeutics. While an HEV vaccine has been licensed for use in China, an HEV vaccine is not available for the majority of the at-risk population, including immunocompromised

Table 2. Serum HEV IgG GMC, GMFR, and Seroresponse (≥4-Fold Rise) Results by Time Point and Treatment Group, mITT Population

Time Point	Statistic	HEV-239 (N = 20)	Placebo (N = 5)
Day 8[a]	n	20	5
	GMC (95% CI)	0.04 (NC)	0.04 (NC)
	GMFR[b] (95% CI)	1.00 (NC)	1.00 (NC)
	4-fold rise[c] (95% CI)	0 (0–17)	0 (0–52)
Day 15	n	20	5
	GMC (95% CI)	0.25 (.13–.48)	0.04 (NC)
	GMFR[b] (95% CI)	6.49 (3.39–12.43)	1.00 (NC)
	4-fold rise[c] (95% CI)	65 (41–85)	0 (0–52)
Day 29 (dose 2)	n	20	5
	GMC (95% CI)	1.44 (.91–2.29)	0.04 (NC)
	GMFR[b] (95% CI)	37.53 (23.73–59.35)	1.00 (NC)
	4-fold rise[c] (95% CI)	100 (83–100)	0 (0–52)
Day 36	n	20	3
	GMC (95% CI)	3.35 (2.18–5.13)	0.04 (NC)
	GMFR[b] (95% CI)	86.92 (56.68–133.30)	1.00 (NC)
	4-fold rise[c] (95% CI)	100 (83–100)	0 (0–71)
Day 43	n	19	3
	GMC (95% CI)	5.89 (3.82–9.10)	0.04 (NC)
	GMFR[b] (95% CI)	153.11 (99.20–236.31)	1.00 (NC)
	4-fold rise[c] (95% CI)	100 (82–100)	0 (0–71)
Day 57	n	20	5
	GMC (95% CI)	6.16 (4.40–8.63)	0.04 (NC)
	GMFR[b] (95% CI)	159.93 (114.17–224.02)	1.00 (NC)
	4-fold rise[c] (95% CI)	100 (83–100)	0 (0–52)
Day 180 (dose 3)	n	19	5
	GMC (95% CI)	3.79 (2.66–5.41)	0.04 (NC)
	GMFR[b] (95% CI)	98.52 (69.06–140.54)	1.00 (NC)
	4-fold rise[c] (95% CI)	100 (82–100)	0 (0–52)
Day 187	n	18	3
	GMC (95% CI)	5.53 (3.73–8.20)	0.04 (NC)
	GMFR[b] (95% CI)	143.60 (96.81–213.02)	1.00 (NC)
	4-fold rise[c] (95% CI)	100 (81–100)	0 (0–71)
Day 194	n	18	3
	GMC (95% CI)	9.62 (6.28–14.75)	0.04 (NC)
	GMFR[b] (95% CI)	249.92 (163.03–383.12)	1.00 (NC)
	4-fold rise[c] (95% CI)	100 (81–100)	0 (0–71)
Day 208	n	19	3
	GMC (95% CI)	11.50 (7.90–16.75)	0.04 (NC)
	GMFR[b] (95% CI)	298.77 (205.18–435.06)	1.00 (NC)
	4-fold rise[c] (95% CI)	100 (82–100)	0 (0–71)
Day 360 (predicted)	n	17	3
	GMC (95% CI)	4.28 (2.79–6.56)	0.05 (.01–.19)
	GMFR[b] (95% CI)	111.06 (72.36–170.45)	1.36 (.37–5.02)
	4-fold rise[c] (95% CI)	100 (80–100)	0 (0–71)
Day 360 (observed)	n	17	3
	GMC (95% CI)	2.92 (1.87–4.56)	0.05 (.01–.23)
	GMFR[b] (95% CI)	75.92 (48.66–118.45)	1.40 (.33–5.99)
	4-fold rise[c] (95% CI)	100 (80–100)	0 (0–71)

Values for GMC below lower limit of detection are assigned a value of 0.04 U/mL.

Abbreviations: CI, confidence interval; GMC, geometric mean fold rise; GMFR, geometric mean fold rise; HEV, hepatitis E virus; IgG, immunoglobulin G; mITT, modified intention to treat; N, number of subjects in the mITT population; n, subjects with results at visit; NC, not calculable.

[a]All participants had undetectable antibodies at baseline.

[b]GMFR represents the geometric mean fold rise in antibody compared to before dose 1.

[c]Four-fold rise represents the percentage of subjects with at least a 4-fold rise in antibody compared to before dose 1.

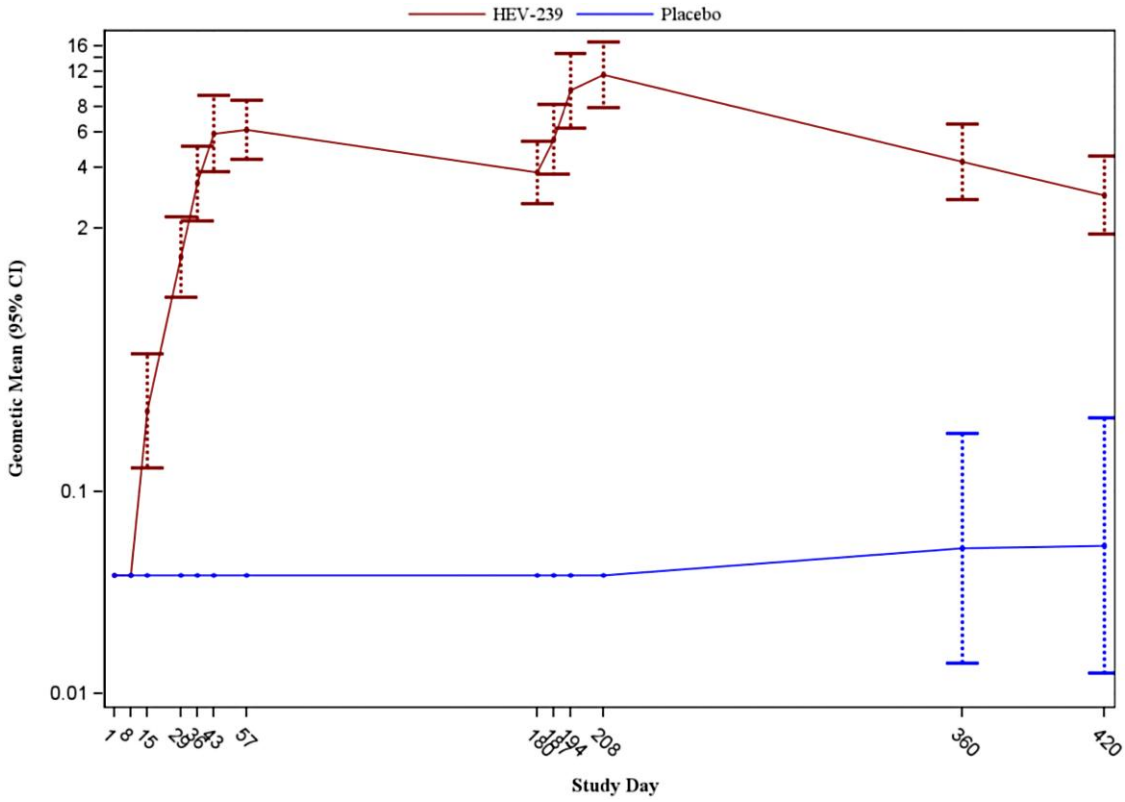

Figure 3. Immunogenicity of HEV-239 versus placebo; immunoglobulin G (IgG) geometric mean concentration, modified intention-to-treat population.

individuals and pregnant women in other resource-limited countries. Extrapolation of vaccine efficacy data from prior studies conducted in China is difficult due to differences in HEV genotype distribution around the world, baseline HEV seroprevalence, and the potential for varying vaccine responses in diverse populations. Therefore, this phase 1 study assessed the safety, tolerability, and immunogenicity of HEV-239 in healthy US adults. Overall, the vaccine was safe and well tolerated. Reactogenicity, both local and systemic, was generally mild and transient. The most frequent symptom was tenderness at the injection site. No participants experienced SAEs related to HEV-239. The safety profile was similar to that observed in the prior phase 2 and 3 studies conducted in China [13, 15].

The HEV-239 vaccine was immunogenic, eliciting a strong IgG response with seroconversion in all recipients by 4 weeks after the first dose that persisted throughout the duration of follow-up. IgG responses peaked 1 month following the second dose and were boosted after the third dose. IgG antibodies persisted through 6 months following the third dose, although some decline was observed. IgM levels peaked at day 43 for subjects in the treatment group. Immunogenicity data from this study were similar to immunogenicity observed in the phase 2 and 3 studies conducted in China [13, 15]. In the phase 3 study, protection against HEV infection was observed in participants receiving at least 1 dose of HEV-239, with efficacy of 93.8% (95% CI, 59.8%–99.9%) [13]. A follow-up efficacy study subsequently conducted through 4.5 years after vaccination found a vaccine efficacy of 86.8% (95% CI, 71%–94%), with vaccine recipients experiencing mild to moderate illness [16]. In those enrolled in the immunogenicity subset that were seronegative prior to vaccination, 87% who received 3 doses of HEV-239 maintained antibodies against hepatitis E through 4.5 years. In baseline seropositive participants from the phase 3 study conducted in China, HEV seropositivity persisted in greater than 99% of HEV-239 recipients through 5.5 years [17]. Altogether, these data indicated that 3 doses of HEV-239 vaccination elicits durable protection in both HEV seronegative and seropositive individuals.

Data from the phase 2 and 3 studies suggest that a 2-dose series might be as effective as a 3-dose series, which has the potential benefit of affording faster and broader protection in an outbreak scenario. We observed robust IgG responses after the 2-dose schedule used in this study (vaccination on days 1 and 29). In the phase 2 study, IgG GMCs were similar at the 6-month postdose time point between subjects who completed a 2-dose series (doses at days 1 and 180) and the 3-dose series (doses at days 1, 30, and 180) [15]. While about half of the participants in the phase 3 study were seropositive at baseline, the long-term follow-up study assessed immunogenicity in the seronegative group and showed only slight differences between

IgG concentrations in the group receiving 3 doses instead of 2 doses over 55 months following first dose of HEV-239 [13, 16]. While offering 2 doses of HEV-239 would be more efficient in outbreaks or for travel preexposure prophylaxis, additional data are needed to compare a 2-dose versus 3-dose series and to determine the optimal timing of the second dose to elicit an optimal anamnestic response.

The HEV-239 vaccine is derived from the HEV genotype 1, which is an exclusively human pathogen transmitted by the fecal-oral route and associated with outbreaks. Although other HEV genotypes are responsible for infection in humans, infection with HEV results in cross-reactive antibodies against all 4 genotypes [18], which appear to confer long-term protection against symptomatic disease [12]. Thus, a vaccine that is effective against 1 genotype is expected to provide cross-protection against other genotypes [19]; however, data showing efficacy against all 4 genotypes are limited. Of the 7 cases of hepatitis E in vaccine recipients in the phase 3 study, 3 were genotype 4, 1 was genotype 1, and the remaining 3 cases were not sequenced for genotype identification [16]. Future, larger studies conducted globally in areas where different HEV genotypes circulate are needed to confirm whether HEV-239 provides similar vaccine efficacy against all genotypes.

There were several limitations of this study. The study had a small sample size and was conducted at a single center. There is no known correlate of protection against hepatitis E infection, so additional studies are needed to evaluate whether antibody concentration correlates with prevention of symptomatic infection. Serological assays used to determine anti-HEV levels also vary between studies, which limits the ability to compare results across trials. The COVID-19 pandemic impacted the final study visit due to local restrictions, limiting the ability to conduct the final in-person study visits and requiring a modified intention to treat analysis and statistical extrapolation. Participant loss to follow-up may have biased our final immunogenicity results, although 20 (80%) did complete their final study visit. Finally, studies in pregnant and immunocompromised individuals, which were excluded from this study, are needed as these groups experience disproportionate disease severity and mortality.

In conclusion, this phase 1 study demonstrated that HEV-239 is safe, well-tolerated, and resulted in a robust immune response that persisted over 6 months in healthy US adults. Although additional studies are needed to assess safety in other populations and to define optimal number and timing of doses, these data support the further development of HEV-239 to prevent the global burden of HEV infections.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Data availability. Data available upon reasonable request.

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Potential conflicts of interest. E. J. A has consulted for Pfizer, Sanofi Pasteur, GSK, Janssen, Moderna, and Medscape; serves on a safety monitoring board for Kentucky BioProcessing, Inc and Sanofi Pasteur; serves on a data adjudication board for WCG and ACI Clinical; and is now an employee of Moderna, Inc. His institution receives funds to conduct clinical research unrelated to this article from MedImmune, Regeneron, PaxVax, Pfizer, GSK, Merck, Sanofi-Pasteur,

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