

ORIGINAL ARTICLE

Long-Term Efficacy of a Hepatitis E Vaccine

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ABSTRACT

BACKGROUND

Hepatitis E virus (HEV) is a leading cause of acute hepatitis. The long-term efficacy of a hepatitis E vaccine needs to be determined.

METHODS

In an initial efficacy study, we randomly assigned healthy adults 16 to 65 years of age to receive three doses of either a hepatitis E vaccine (vaccine group; 56,302 participants) or a hepatitis B vaccine (control group; 56,302 participants). The vaccines were administered at 0, 1, and 6 months, and the participants were followed for 19 months. In this extended follow-up study, the treatment assignments of all participants remained double-blinded, and follow-up assessments of efficacy, immunogenicity, and safety were continued for up to 4.5 years.

RESULTS

During the 4.5-year study period, 60 cases of hepatitis E were identified; 7 cases were confirmed in the vaccine group (0.3 cases per 10,000 person-years), and 53 cases in the control group (2.1 cases per 10,000 person-years), representing a vaccine efficacy of 86.8% (95% confidence interval, 71 to 94) in the modified intention-to-treat analysis. Of the participants who were assessed for immunogenicity and were seronegative at baseline, 87% of those who received three doses of the hepatitis E vaccine maintained antibodies against HEV for at least 4.5 years; HEV antibody titers developed in 9% in the control group. The rate of adverse events was similar in the two groups.

CONCLUSIONS

Immunization with this hepatitis E vaccine induced antibodies against HEV and provided protection against hepatitis E for up to 4.5 years. (Funded by the Chinese Ministry of Science and Technology and others; ClinicalTrials.gov number, NCT01014845.)

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HEPATITIS E VIRUS (HEV) IS A COMMON cause of acute hepatitis worldwide.^{1,2} HEV infection occurs in two distinct epidemiologic patterns.³ The most common pattern is waterborne infection, which is caused by HEV genotype 1 or 2 and occurs mainly in resource-limited countries, often in large, protracted outbreaks or in sporadic cases associated with high mortality among pregnant women.⁴⁻⁶ The other pattern is transmission from animals and humans, which is caused by HEV genotype 3 or 4 and occurs widely in both resource-limited and developed countries.^{1,7-9} Rein et al.¹⁰ estimated the incidence of hepatitis E in areas in which HEV genotype 1 is endemic to be 3.3 million cases per year, resulting in 70,000 deaths and 3000 stillbirths. This may be an underestimation, because a recent study suggests that, in Bangladesh alone, hepatitis E is responsible for more than 1000 deaths per year among pregnant women.¹¹ An estimation of the burden of disease in developed countries has not been performed. Nevertheless, HEV infection is an important cause of acute viral hepatitis in developed countries.^{12,13}

HEV infection has a wide spectrum of clinical manifestations.⁷ In most affected patients, symptomatic infection leads to an acute hepatitis that is indistinguishable from other forms of acute viral hepatitis, is usually self-limited, and does not become chronic, except in severely immunocompromised persons.^{2,14} Severe courses of acute hepatitis E have been described in pregnant women,¹¹ elderly men,⁸ and persons with preexisting chronic liver disease.¹⁵ Hepatitis E infection is associated with an estimated overall case fatality rate of 1 to 3%¹⁶; the rate increases to 5 to 25% among pregnant women.^{17,18} Extrahepatic manifestations — including pancreatitis, arthritis, aplastic anemia, and neurologic complications — have been associated with autochthonous HEV in Europe.^{1,19}

Basic sanitation is the first line of defense against HEV infection. However, during recent outbreaks in southern Sudan²⁰ and northern Uganda,⁴ typical interventions did not prevent additional infections. Thus, a safe and effective vaccine is needed. Vaccine development has been facilitated by the observation that all major genotypes of HEV in humans belong to the same serotype.²¹ Two recombinant hepatitis E vaccines developed from HEV genotype 1, by GlaxoSmithKline and Xiamen Innovax Biotech, have had short-term efficacy in clinical trials.^{9,22,23} The

latter vaccine, with the commercial name of Hecolin, has been in use in China since 2012. However, the long-term efficacy of this hepatitis E vaccine has not yet been determined.

METHODS

PARTICIPANTS

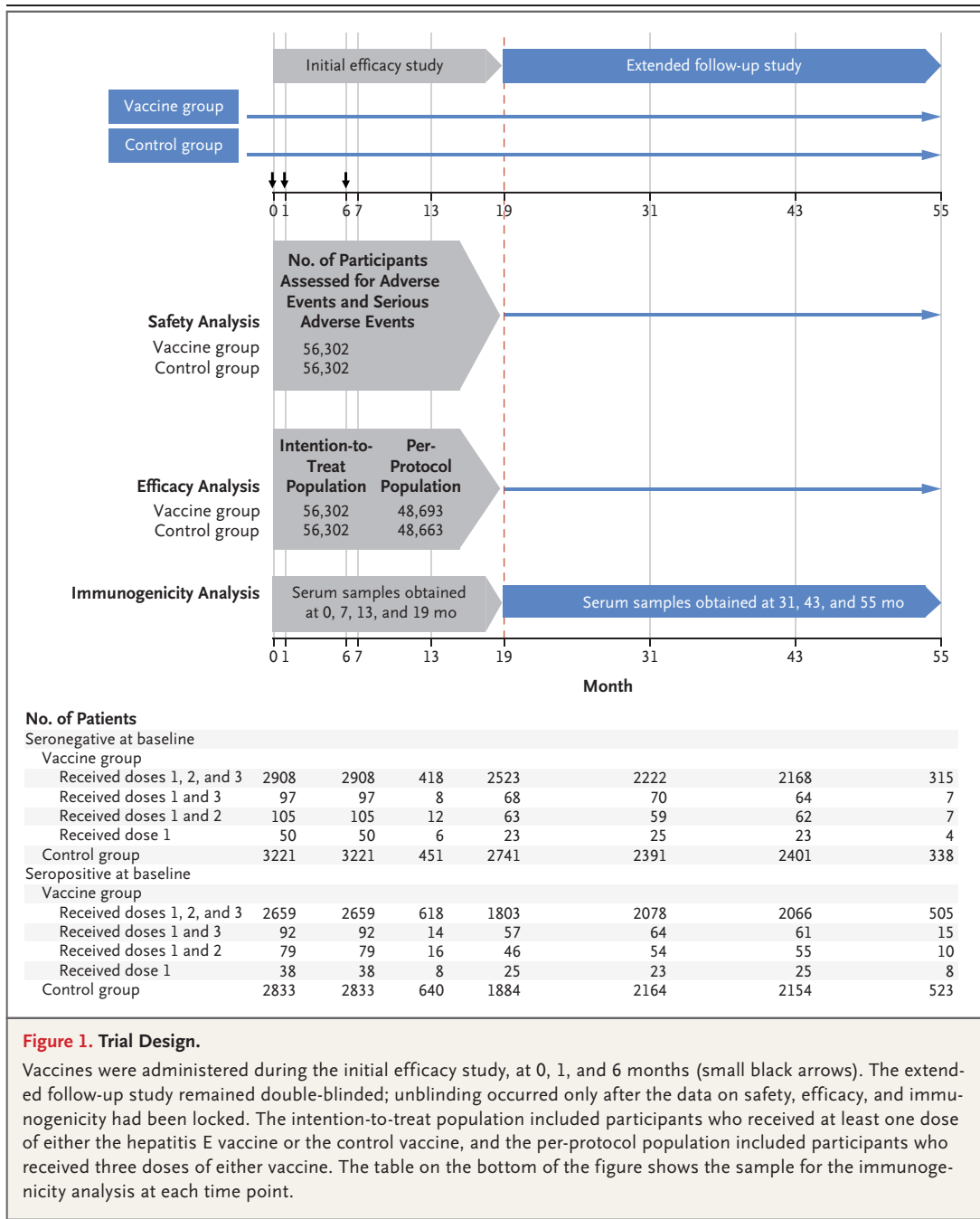
In 2007, we enrolled 112,604 healthy adults 16 to 65 years of age in a randomized, placebo-controlled, phase 3 clinical trial to determine the short-term efficacy of the hepatitis E vaccine Hecolin (Xiamen Innovax Biotech).²³ In month 19 of the study, data analyses were completed for registration purposes, and the results led to the licensure of Hecolin in China. In this extended follow-up study, the treatment assignments of all participants remained double-blinded, and follow-up was continued for a total of 54 months. Fingerprint scanners and digital photographs were used to identify and track participants throughout the assessments of immunization, blood collection, and follow-up. Written informed consent was obtained from each participant.

STUDY OVERSIGHT

Approval for the study was obtained from the ethics committee of the Jiangsu Provincial Center for Disease Control and Prevention. All the authors vouch for the completeness and accuracy of the data and analysis and for the fidelity of the study to the protocol (available with the full text of this article at NEJM.org). Xiamen Innovax Biotech donated the hepatitis E vaccine, provided funding for the control vaccine and all the laboratory assays, and was involved in protocol development, periodic auditing of study conduct, management of serious adverse events, and analysis of the causality of serious adverse events. There is no agreement between Xiamen Innovax Biotech and the authors regarding confidentiality of the data.

PROCEDURES

Figure 1 shows the overall study design. In the initial efficacy study, participants were randomly assigned to receive three doses of either the hepatitis E vaccine (vaccine group) or a hepatitis B vaccine (Beijing Tiantan Biological Products) (control group) and were followed for the occurrence of hepatitis E and serious adverse events.²³ The loss of participants to follow-up is summa-



rized in Table S1 in the Supplementary Appendix, available at NEJM.org. We assessed vaccine immunogenicity by testing serum samples for IgG antibodies against HEV; the samples were obtained before vaccination and at regular intervals after vaccination from participants living in 2 of the 11 townships involved in the study. The participants, investigators, study personnel, and per-

sonnel from Xiamen Innovax Biotech who were directly involved in the study remained unaware of the group assignments throughout the study. Unblinding occurred only after the data on safety, efficacy, and immunogenicity had been locked.

Suspected cases of hepatitis were identified through a well-established hepatitis surveillance system.²³ Participants were informed and remind-

ed in a timely manner of the benefits of free laboratory testing for hepatitis and the study policy of medical reimbursement for expenses related to HEV infection. They were encouraged to be seen by a doctor when they had persistent hepatitis-like symptoms, such as jaundice, anorexia, fatigue, and epigastric pain. Virtually all the health care centers in the study area were involved in the surveillance program; the study area comprised 205 village and private clinics located in the 11 participating townships of Dongtai, as well as hospitals in each township and in the city. As outlined in Figure S1 in the Supplementary Appendix, the alanine aminotransferase (ALT) level was measured in all patients who presented to any of the clinical sites with hepatitis-like symptoms that had been present for 3 days or more. Patients with an ALT level that was at least 2.5 times as high as the upper limit of the normal range (i.e., 40 U per liter in men and 31 U per liter in women) received a diagnosis of acute hepatitis. Sequential serum samples were obtained from these patients and sent to central laboratories for testing. A diagnosis of HEV infection was made on the basis of the presence of at least two of the following three markers: a positive test for IgM antibodies against HEV, a positive test for HEV RNA, and a level of IgG antibodies against HEV that was at least four times as high as a level measured previously at any time during the same illness. Cases of HEV infection that occurred before month 19 were confirmed by the data and safety monitoring board.²³ From month 19 through month 54, the diagnosis of HEV infection was confirmed by the investigators before unblinding according to the presence of the markers described above.

LABORATORY TEST RESULTS

With the use of commercial enzyme-linked immunosorbent assay (ELISA) kits (Beijing Wantai Biological Pharmacy), with the assay performed according to the manufacturer's instructions, specimens were tested for IgM and IgG antibodies against HEV, IgM antibodies against hepatitis A virus, IgM antibodies against hepatitis B virus core antigen, hepatitis B virus surface antigen, and antibodies against hepatitis C virus. Levels of IgG antibodies against HEV were measured in World Health Organization units (WU), which were calculated by a comparison of the measured level with a reference level of the as-

say; the detection limit of the assay was 0.077 WU per milliliter. Participants were tested for HEV RNA if they had a detectable level of IgM antibodies against HEV or a level of IgG antibodies against HEV that was two times as high as a level measured previously at any time during the same illness.²³

During the extended follow-up period, we reviewed all local hospitalization records and death records to identify trial participants who may have been hospitalized or had other adverse events. In analyzing the safety of the vaccine, data on adverse events that were coded as hepatitis E were excluded from the safety analysis.

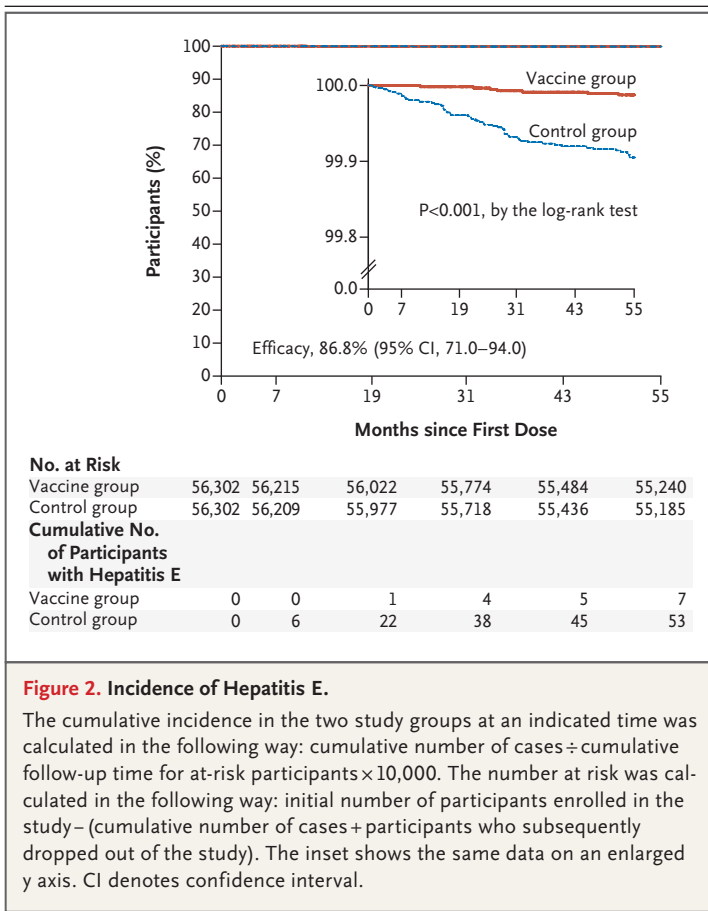
STATISTICAL ANALYSIS

Efficacy was estimated with the use of data collected during both the initial study and the extended follow-up study. We defined three sets of efficacy analyses: a per-protocol analysis, which included data collected from 1 month after the last vaccination (month 7) through month 54 from participants who received three doses of either the hepatitis E vaccine or the control vaccine; an intention-to-treat analysis, which included data collected from month 7 through month 54 from participants who received at least one dose of either vaccine; and a modified intention-to-treat analysis, which included data collected from baseline through month 54 from participants who received at least one dose of either vaccine.

Vaccine efficacy and 95% confidence intervals were calculated on the basis of the observed difference between the vaccine group and the control group and the accrued person-years. An exact conditional procedure was used to evaluate vaccine efficacy under the assumption that the numbers of patients with hepatitis E in the vaccine and control groups were independent Poisson random variables.²⁴ To test the robustness of the results, we also assessed the vaccine efficacy using a Cox proportional-hazards model and compared the cumulative incidence of hepatitis E between the study groups with a log-rank test.

For the analysis of immunogenicity, we included participants who received at least one dose of a vaccine and who had available serologic test results at baseline, month 7, and at least one later time point. In calculating the geometric mean concentrations, we arbitrarily set seronegative samples at 0.04 WU per milliliter.

Long-term safety was assessed according to



the rate of serious adverse events in the intention-to-treat population. The rates and 95% confidence intervals (unadjusted for multiple comparisons) were compared between the groups with the use of a two-sided Fisher's exact test.

The data analyses were performed with the use of SAS software, version 9.1 (SAS Institute). All reported P values are two-sided, and values of less than 0.05 were considered to indicate statistical significance. To maintain the study blinding, an independent external statistician performed the efficacy analysis.

RESULTS

EFFICACY

Participants who were enrolled in the initial efficacy study were followed for 54 ± 4.2 months (Fig. 1, and Table S1 in the Supplementary Appendix). A total of 60 cases of hepatitis E were confirmed, including 23 in the initial study²³ and 37 in the extended study. The cumulative incidence

of hepatitis E was significantly lower in the vaccine group than in the control group ($P < 0.001$ in the modified intention-to-treat analysis) (Fig. 2); 53 of the cases were detected in the control group, and 7 were detected in the vaccine group (Table 1). Of the 7 participants in the vaccine group who had hepatitis E, 3 had received all three doses of the vaccine, 1 had received two doses, and 3 had received one dose. The illness in these participants was moderate or mild; no deaths or episodes of liver failure were recorded. There were no notable differences between the two groups with respect to clinical disease manifestations. (Details of the clinical features of the cases of hepatitis E that occurred during the extended study are provided in Tables S2 and S3 in the Supplementary Appendix.) Laboratory test results among participants who had hepatitis E were generally similar in the two groups, although a difference in the avidity of antibodies was seen during the acute phase. Participants in the vaccine group in whom hepatitis E developed had antibodies with high avidity, a finding that is consistent with the anamnestic response in persons with previous immunity; in contrast, the majority of participants in the control group in whom hepatitis E developed had antibodies with low avidity, a finding that is consistent with the antibody response in persons without previous immunity (Fig. S2 and Table S4 in the Supplementary Appendix). Of the 29 participants whose viral isolates were sequenced, 26 were infected with HEV genotype 4, and 3 were infected with HEV genotype 1.

The analyses showed significant efficacy of the hepatitis E vaccine through month 54 (Table 2). Efficacy did not decrease during the extended follow-up study. Vaccine efficacy was 86.8% (95% confidence interval [CI], 71.0 to 94.0) in the modified intention-to-treat analysis, 85.1% (95% CI, 67.1 to 93.3) in the intention-to-treat analysis, and 93.3% (95% CI, 78.6 to 97.9) in the per-protocol analysis.

IMMUNOGENICITY

We determined the geometric mean concentrations of IgG antibodies against HEV and the seropositivity rates before and after vaccination and plotted them according to the immune status of the participants at baseline (Fig. 3). Of the 5567 participants in the vaccine group who were assessed for immunogenicity, 52% were seroneg-

Table 1. Characteristics of Participants with Hepatitis E.*

Characteristic	Vaccine Group (N=7)	Control Group (N=53)	P Value
Age — yr	47±11.2	54±9.2	0.07
Sex — no. of participants			0.67
Male	6	37	
Female	1	16	
HEV genotype — no. of participants			0.37
Genotype 1	1	2	
Genotype 4	3	23	
Peak alanine aminotransferase†	13.6±3.5 x ULN	23.3±2.6 x ULN	0.32
Length of symptomatic course — days	49±29.6	51±21.9	0.53
Mean (95% CI) peak level of IgM antibodies against HEV — signal over cutoff	4.3 (1.6–11.4)	8.5 (7.1–10.3)	0.09
Mean (95% CI) peak level of IgG antibodies against HEV — WU/ml	15.8 (1.2–215.2)	57.4 (37.8–87.3)	0.51
Mean (95% CI) avidity of IgG antibodies in samples obtained during the acute phase — %‡	64 (50–77)	6 (4–8)	<0.001

* Plus–minus values are means ±SD. CI denotes confidence interval, and WU World Health Organization units.

† Alanine aminotransferase levels were measured in units per liter. The upper limit of the normal range (ULN) is 40 U per liter in men and 31 U per liter in women.

‡ The test for avidity of IgG antibodies against HEV was conducted on serum samples collected during the acute phase, before day 60 of the illness (see Fig. S2 and Table S4 in the Supplementary Appendix). Avidity was the residual antibody level in the presence of 5M urea expressed as a percentage of the residual antibody level in the absence of urea. A residual antibody level greater than 60% indicates high avidity, a level of 40 to 60% indicates moderate avidity, and a level lower than 40% indicates low avidity. Of the participants who had hepatitis E, 1 of the 7 in the vaccine group and 5 of the 53 in the placebo group were not tested for avidity because not enough samples were available.

ative at baseline, of whom 99.9% underwent seroconversion after vaccination; 87% of those who were seronegative at baseline and received three doses of the hepatitis E vaccine remained seropositive at the end of month 54 (i.e., the beginning of month 55) (Table S5 in the Supplementary Appendix). The seroconversion rates were similar among participants who were seronegative at baseline and received either one, two, or three doses of the hepatitis E vaccine. From month 7 through month 43, antibody levels were higher in participants who received three doses of the hepatitis E vaccine than in those who received two doses; antibody levels were lowest in participants who received only one dose of the vaccine. Participants who were seropositive at baseline had a response to a single dose of the vaccine (Fig. 3).

SAFETY

A similar number of participants in the vaccine group and the control group reported serious adverse events (Tables S6 and S7 in the Supplementary Appendix). None of the serious adverse

events were judged by the principal investigator (who remained masked to treatment assignment) to be related or possibly related to the hepatitis E vaccine.

DISCUSSION

The hepatitis E vaccine we tested provided sustained protection against hepatitis E for up to 4.5 years after the first vaccination. The immune response persisted in participants with and those without preexisting immunity to HEV. No concerns about the safety of the vaccine were identified.

Vaccine efficacy did not appear to decrease over time. In addition to one breakthrough infection that was observed in a vaccinated participant during the initial study, six more breakthrough infections occurred in vaccinated participants during the extended follow-up study. The immune responses to the vaccine in participants with breakthrough infections are unknown, which makes it impossible to determine the level of anti-

Table 2. Vaccine Efficacy in the Modified Intention-to-Treat, Intention-to-Treat, and Per-Protocol Analyses.

Analysis	Vaccine Group			Control Group			Vaccine Efficacy % (95% CI)	P Value
	Person-Yr at Risk*	Cases of Hepatitis E	Rate	Person-Yr at Risk*	Cases of Hepatitis E	Rate		
	number	no./10,000 person-yr (95% CI)	number	no./10,000 person-yr (95% CI)				
Modified intention-to-treat								
0–18 mo	87,354	1	0.1 (0.0–0.6)	87,323	22	2.5 (1.6–3.8)	95.5 (66.3–99.4)	<0.001
0–30 mo	143,249	4	0.3 (0.1–0.7)	143,173	38	2.7 (1.9–3.6)	89.5 (70.5–96.2)	<0.001
0–42 mo	198,873	5	0.3 (0.1–0.6)	198,751	45	2.3 (1.7–3.0)	88.9 (72.0–95.6)	<0.001
0–54 mo	254,380	7	0.3 (0.1–0.6)	254,198	53	2.1 (1.6–2.7)	86.8 (71.0–94.0)	<0.001
Intention-to-treat								
7–18 mo	56,105	1	0.2 (0.0–1.0)	56,081	16	2.8 (1.6–4.6)	93.8 (52.9–99.2)	<0.001
7–30 mo	112,000	4	0.4 (0.1–0.9)	111,931	32	2.9 (2.0–4.0)	87.5 (64.7–95.6)	<0.001
7–42 mo	167,624	5	0.3 (0.1–0.7)	167,509	39	2.3 (1.7–3.2)	87.2 (67.5–95.0)	<0.001
7–54 mo	223,131	7	0.3 (0.1–0.6)	222,956	47	2.1 (1.5–2.8)	85.1 (67.1–93.3)	<0.001
19–54 mo	167,026	6	0.4 (0.1–0.8)	166,875	31	1.9 (1.3–2.6)	80.1 (53.7–91.9)	<0.001
Per-protocol								
7–18 mo	48,595	0	0.0 (0.0–0.0)	48,555	15	3.1 (1.7–5.1)	100.0 (72.1–100.0)	<0.001
7–30 mo	97,031	1	0.1 (0.0–0.6)	96,946	30	3.1 (2.1–4.4)	96.7 (75.6–99.6)	<0.001
7–42 mo	145,262	1	0.1 (0.0–0.4)	145,122	37	2.6 (1.8–3.5)	97.3 (80.3–99.6)	<0.001
7–54 mo	193,409	3	0.2 (0.0–0.5)	193,197	45	2.3 (1.7–3.1)	93.3 (78.6–97.9)	<0.001
19–54 mo	144,814	3	0.2 (0.0–0.6)	144,642	30	2.1 (1.4–3.0)	90.0 (67.3–97.0)	<0.001

* The number of person-years at risk was defined as the cumulative number of follow-up years of the at-risk participants at the indicated time point.

body protection. In a previous study in which we assessed the association between antibody level and the risk of subclinical hepatitis E infection,⁹ we estimated that a marginal antibody level (0.077 to 0.25 WU per milliliter) may prevent infection in 74% of persons exposed to HEV. In addition, the antibody levels that were present in affected persons before infection varied widely. These findings suggest that host factors other than antibody level might play an important role.

All the participants in the vaccine group in whom hepatitis E developed had antibodies with high or moderate avidity during the acute phase, a finding that is consistent with the anamnestic response in persons with previous immunity. In contrast, most of the participants in the control group in whom hepatitis E developed had antibodies with low avidity, a finding that is consistent with the antibody response in persons without previous immunity (Table S4 and Fig. S2 in

the Supplementary Appendix). These findings suggest that, in some situations, the virus can evade the immunity induced by vaccination in the same manner that it can evade naturally acquired immunity, as reported previously.⁹

The sustained vaccine efficacy observed in this study was associated with persistent immunity. The level of vaccine-induced antibodies decreased quickly in the first 2 years after vaccination and subsequently decreased more slowly. Most of the vaccinated participants had detectable antibodies up to 4.5 years after the first vaccination.

The antibody levels induced by two doses of the hepatitis E vaccine were slightly lower than the levels induced by three doses. Previous reports have suggested that the first two doses of the hepatitis E vaccine prevent illness and infection in the 5 months after vaccination, until the third dose is given.^{23,25} These findings raised interest in the use of a two-dose schedule in future

Figure 3. Geometric Mean Concentrations of IgG Antibodies against Hepatitis E Virus and Seropositivity Rates among Participants Who Were Assessed for Immunogenicity.

In calculating the geometric mean concentrations, we arbitrarily set seronegative samples at 0.04 WU per milliliter (dashed lines). I bars indicate 95% confidence intervals. WU denotes World Health Organization units.

vaccination strategies; nevertheless, this concept needs to be proven in specifically designed efficacy trials.

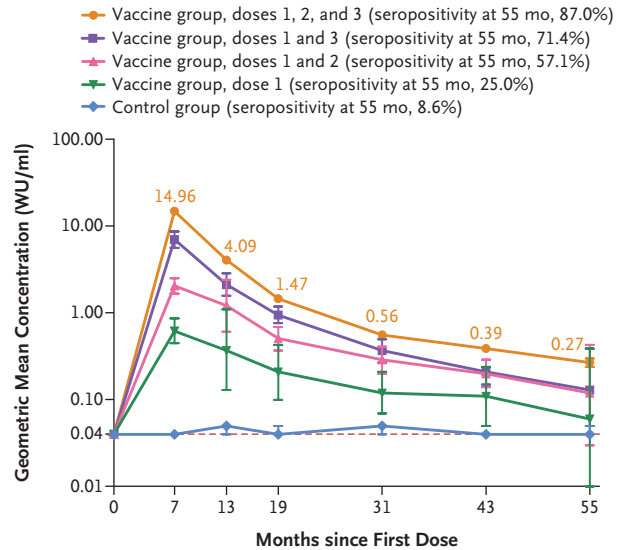
Fluctuation in the sensitivity for disease surveillance is always a concern in a long-term study. In this study, the surveillance system covered all the clinical sites and hospitals in the study area. In addition, the surveillance data, including the number of patients with hepatitis-like symptoms and the proportion of patients with abnormal ALT levels, remained stable during the study period (Table S7 in the Supplementary Appendix). Furthermore, the majority of the participants continued to live in the study area and were covered by the surveillance system throughout the study. These factors suggest that fluctuations in outcome measurement did not introduce a significant bias in the analyses.

There are several limitations of the current study. First, the majority of cases of hepatitis E were caused by HEV genotype 4. The efficacy of this vaccine against the other HEV genotypes remains to be explored, but it is generally recognized that all HEV genotypes belong to the same serotype and that one hepatitis E vaccine can protect against infection with any HEV genotype.²¹ Second, the lack of regular follow-up assessments might have caused us to miss some cases of hepatitis E in participants who did not seek medical care. Third, the majority of the long-term safety data were collected from the local medical insurance system; hence, the effect of the vaccination according to data recorded in other medical systems cannot be assessed.

In conclusion, immunization with the hepatitis vaccine we tested induced a sustained level of antibodies and protection against hepatitis E for up to 4.5 years.

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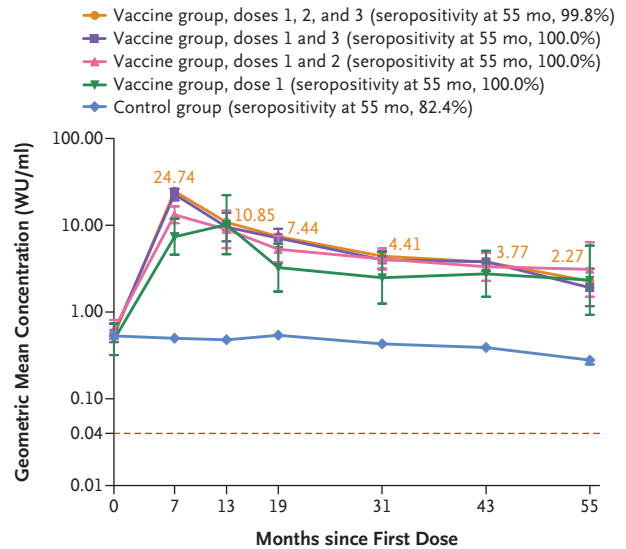
A Participants Who Were Seronegative at Baseline



No. of Seropositive Participants

Vaccine group	0	2904	418	2512	2165	1996	274
Control group	0	103	30	151	158	165	29
Total No.							
Vaccine group	2908	2908	418	2523	2222	2168	315
Control group	3221	3221	451	2741	2391	2401	338

B Participants Who Were Seropositive at Baseline



No. of Seropositive Participants

Vaccine group	2659	2658	618	1801	2074	2059	504
Control group	2833	2760	629	1834	2066	1970	466
Total No.							
Vaccine group	2659	2659	618	1803	2078	2066	505
Control group	2833	2833	640	1884	2164	2154	523

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.
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