Effectiveness of a hepatitis E vaccine against medically-attended symptomatic infection in HBsAg-positive adults from a test-negative design study

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The effectiveness of the hepatitis E vaccine in high-risk groups, such as chronic hepatitis B (CHB) patients, remains understudied. A key clinical manifestation of CHB is the persistent positivity of hepatitis B surface antigen (HBsAg). We conducted a test-negative design study involving 2,926 HBsAg-positive individuals (born 1941–1991; median age 49.0; male-to-female ratio of 1.4), identified through a hepatitis surveillance system, as part of the phase 3 trial (NCT01014845) of the recombinant hepatitis E vaccine HEV 239 (Hecolin). This system monitored suspected hepatitis cases and performed diagnoses across 11 townships in Dongtai, Jiangsu, China, from 2007 to 2017. Vaccine effectiveness of HEV 239 was assessed by comparing vaccination status between confirmed 96 hepatitis E cases and 2830 test-negative controls, using logistic regression adjusted for sex and age. We found that HEV 239 vaccination was associated with a reduced risk of hepatitis E among HBsAg-positive individuals, with an estimated effectiveness of 72.1% [95% confidence interval (CI) 11.2–91.2], and 81.5% (95% CI 35.9–94.6) among phase 3 trial participants. Our findings show that HEV 239 is highly effective in HBsAg-positive adults, supporting its future recommended use in this population.

Hepatitis E virus (HEV) stands as a prominent cause of viral hepatitis, constituting a global health concern[1]. Despite being largely self-limiting, HEV infection can lead to severe complications, particularly among individuals with pre-existing chronic liver diseases (CLD), immunocompromised patients, and pregnant women[2,3]. Meta-analysis results have revealed significantly elevated risks of liver failure (OR: 5.5, 95% CI: 1.5-20.1) and mortality (OR: 5.0, 95% CI: 1.9-13.3) in CLD patients with HEV superinfection compared to those without[4]. This issue is

further exacerbated by the considerable overlap of hepatitis B virus (HBV) and HEV endemicity in many Asian countries, where HEV infection is an important trigger of acute-on-chronic liver failure[5,6]. Additionally, the reactivation of chronic hepatitis B (CHB) led by acute HEV superinfection[7,8], and adverse outcomes among organ transplant recipients or HIV-infected patients caused by HBV and HEV dual-infection[9–11], have been reported in high-income countries.

The recombinant hepatitis E vaccine, HEV239 (Hecolin; Xiamen Innovax Biotech, Xiamen, China), has been the sole vaccine available for hepatitis E since 2012[12,13]. Hecolin has demonstrated excellent long-term efficacy, safety, and immunogenicity in adults[13–15], and shown to be safe and immunogenic in hepatitis B carriers or CHB as well[16,17]. However, the lack of additional efficacy or effectiveness data in CLD or CHB patients hinders the prioritization of this vaccine for these populations, leaving a critical gap in hepatitis E prevention strategies targeting the vulnerable groups[5,18,19].

In areas of high endemicity, such in China, HBV is transmitted mostly perinatally from infected mothers to neonates, while the risk of progression from acute to chronic HBV infection is 90% when infection occurs in infants[20]. It is estimated that ~93 million individuals in China are living with HBV infection, with an HBsAg prevalence of about 8% among those aged 15–59 years[21,22]. And the vast majority (about 90%) of hepatitis B reported in China are clinically diagnosed as CHB[23,24]. Given that hepatitis B surface antigen (HBsAg) positivity is one of the clinical manifestations of CHB and considering the large population of adult CHB in China, here we conducted a test-negative design study to assess the vaccine effectiveness (VE) of Hecolin against symptomatic hepatitis E among adults who tested positive for HBsAg, representing individuals at high risk of CHB.

Results

In the phase 3 trial of Hecolin and its extended follow-up study (NCT01014845)[13], a cumulative total of 313,536 suspected hepatitis events were noted over a 10 year period (from August, 2007 to October, 2017) in the study area, of which 12,787 were found to have alanine aminotransferase (ALT) ≥ 2.5 upper limit of normal (ULN), and 12,707 completed the laboratory testing for HEV-related markers. Through the preliminary hepatitis typing testing, a total of 3282 events were found to be positive for HBsAg, and finally 2926 individuals born between 1941 and 1991 were identified and included in the analysis of this study, with a median age of 49.0 years [interquartile range (IQR) 40.0–58.0] and a male-to-female ratio of 1.4. 96 out of 2926 (3.3%) individuals with positive HBsAg were confirmed as hepatitis E cases and categorized as test-positive cases, while the remaining 2830 were categorized as test-negative controls (Fig. 1). The distribution of acute infection detection markers among the tested population is detailed in Supplementary Table 1. No non-trial participants were found to have received any dose of Hecolin during the observation period. To assess the VE of Hecolin in HBsAg-positive adults, we compared vaccination status between confirmed hepatitis E cases and controls, using logistic regression adjusted for sex and age.

In the analysis of clinical characteristics comparing test-positive cases with test-negative controls (Table 1), we observed that the median age at onset of hepatitis-like symptoms in cases was 53.5 years (IQR 44.0–63.0), which was significantly older (P = 0.0028) compared to controls (49.0 years, IQR 40.0–58.0); this difference primarily arose from the unscreened non-trial participants. Although there was no significant difference in sex distribution between the two groups overall, among trial participants the proportion of male test-positive cases was significantly higher (75% vs 51%, P = 0.0350). Furthermore, ALT levels were significantly higher in test-positive cases (15.2 ULN vs 5.6 ULN, P < 0.0001), irrespective of whether they were trial or non-trial participants. Taking the test-negative controls as reference, we found a significant association between Hecolin vaccination status and a reduced proportion of hepatitis E [Crude odds ratio (OR): 0.30, 95%

confidence interval (CI) 0.09-0.94]. After controlling for sex and age, the corresponding estimated VE for at least one dose was 72.1% (95% CI 11.2–91.2). Moreover, when restricted to the 600 trial participants, the estimated VE appeared higher. Specifically, the VE of at least one dose of Hecolin among trial participants was 81.5% (95%CI 35.9–94.6) (Table 2). The specific age distributions of test-positive cases and test-negative controls are detailed in Supplementary Table 2.

Discussion

In this study, we demonstrate that Hecolin confers long-lasting (2007–2017) and substantial protection against hepatitis E in populations positive for HBsAg. To our knowledge, prior studies have not evaluated the effectiveness of the hepatitis E vaccine in HBsAg-positive populations, who are considered at high risk for CHB. While the question of whether HBsAg-positive individuals or those with CLD exhibit a higher prevalence of HEV infection remains debatable[25–28], it is widely recognized that HEV superinfection in CLD patients is associated with more severe clinical outcomes[29–31]. The United States and several European countries have advocated for hepatitis A vaccination in CLD patients to reduce the disease burden of dual-infection[32,33]. However, in some countries especially in Asia where the incidence of hepatitis E is higher than that of hepatitis A, there have been no plans to promote hepatitis E vaccination among CLD patients, primarily due to a lack of data on the safety and efficacy or effectiveness of Hecolin in this population. Given that CLD patients represent a high-risk group that could benefit significantly from the hepatitis E vaccine, clinical data supporting the use of Hecolin in these patients is urgently needed[18]. The findings regarding the high effectiveness of Hecolin among HBsAg-positive individuals here provide valuable insights, which implied promising prospects for hepatitis E vaccine deployment in such population, especially within regions grappling with high incidence of CHB or CLD.

Intriguingly, we observed that the VE was 9.4% lower when non-trial participants were included compared to when they were excluded, suggesting potential confounding factors. Trial-participants were required to have no history of hepatitis B or E at enrollment; and subsequent antibody assessments revealed no differences in baseline anti-HEV IgG positivity rates between the vaccine and placebo groups (Supplementary Fig. 1), supporting the point that both groups had similar HEV exposure risks after screening and randomization. In contrast, the non-trial participants were not pre-screened, and their unknown infection history and disease status likely contributed to the observed discrepancy in the VE results. For instance, studies of SARS-CoV-2 vaccines using test-negative designs have shown that failing to account for prior infection history can underestimate VE[34]. Notably, compared to traditional case-control or cohort designs, the test-negative design used in this study is less susceptible to bias arising from differences in healthcare-seeking behavior between cases and controls[35,36]. However, the potential influence of other unmeasured or unknown confounders cannot be ruled out.

Previous studies have linked acute HEV superinfection with accelerated hepatocytes damage and liver failure in patients with CHB, as evidenced by elevated transaminases and deteriorating liver function, respectively[37]. Nevertheless, the impact of HEV superinfection in HBV DNA replication and its underlying mechanisms remain unclear. A concept that hepatocytes injury is predominantly triggered by HEV and only a trivial effect from HBV in hepatocytes injury has been proposed by several researchers[37–39]. Cheng et al. reported that HBV infection was dormant during acute HEV infection, and the profound clinical effect during coinfection was significantly triggered by HEV[38]. In our study, we also observed elevated ALT levels among individuals co-infected with HEV and HBV compared to those with HBV mono-infection, supporting this intriguing point. Conversely, a few studies argue that HBV exerts a dominant effect in coinfection or that HEV has no impact on HBV DNA replication[25,40]. Thus, further research is warranted to elucidate the underlying mechanisms of the immune-

mediated responses initiated by both HBV and HEV viruses, which may enhance our understanding of the effects of dual infection and lead to improved strategies of prevention and treatment.

Some limitations must be considered in this study. First, the hepatitis surveillance system was initially designed to identify hepatitis E, which resulted in notable shortcomings in diagnosing other types of hepatitis. CHB is often defined by HBsAg positivity for >6 months but we did not continuously monitor this in our study. Consequently, it was almost impossible to accurately differentiate between acute and chronic infections of hepatitis B based on the available laboratory data. Second, the limited number of observed hepatitis E cases was insufficient to support further stratified analyses, including stratification by time period, age, or sex groups. Additionally, since the vast majority of vaccine recipients included in the analysis completed the three-dose regimen, it is currently not possible to compare the VE for individuals among different dosage groups. Third, beyond the limitation of unknown infection history and disease status in the non-trial population, this study did not explore the impact of environmental exposures, socioeconomic factors, baseline liver function before infection, or missing data on

the outcomes. It should be noted that 10 years of case surveillance in the study region revealed that over 90% of hepatitis E were caused by HEV genotype-4 (with no genotype-1 cases observed since 2012)[13]. This zoonotic genotype is primarily associated with individual dietary hygiene practices and occupational exposure. Last, it is generally recognized that case-control studies achieve optimal power when vaccination coverage ranges between 20% and 80%[41]. In this study region, however, the Hecolin coverage rate was ~19%, potentially reducing the statistical power of the analysis.

In conclusion, this test-negative design study observed a notable effectiveness of Hecolin against hepatitis E in HBsAg-positive populations, shedding light on the potential expansion of vaccine applicability to broader populations. However, further studies are warranted to enhance the evidence regarding the efficacy or effectiveness and safety of Hecolin in individuals with pre-existing CLD.

Methods
Study design and setting
This test-negative design study was done as part of the long-term follow-up for a single-centre, double-blind, randomized, placebo-
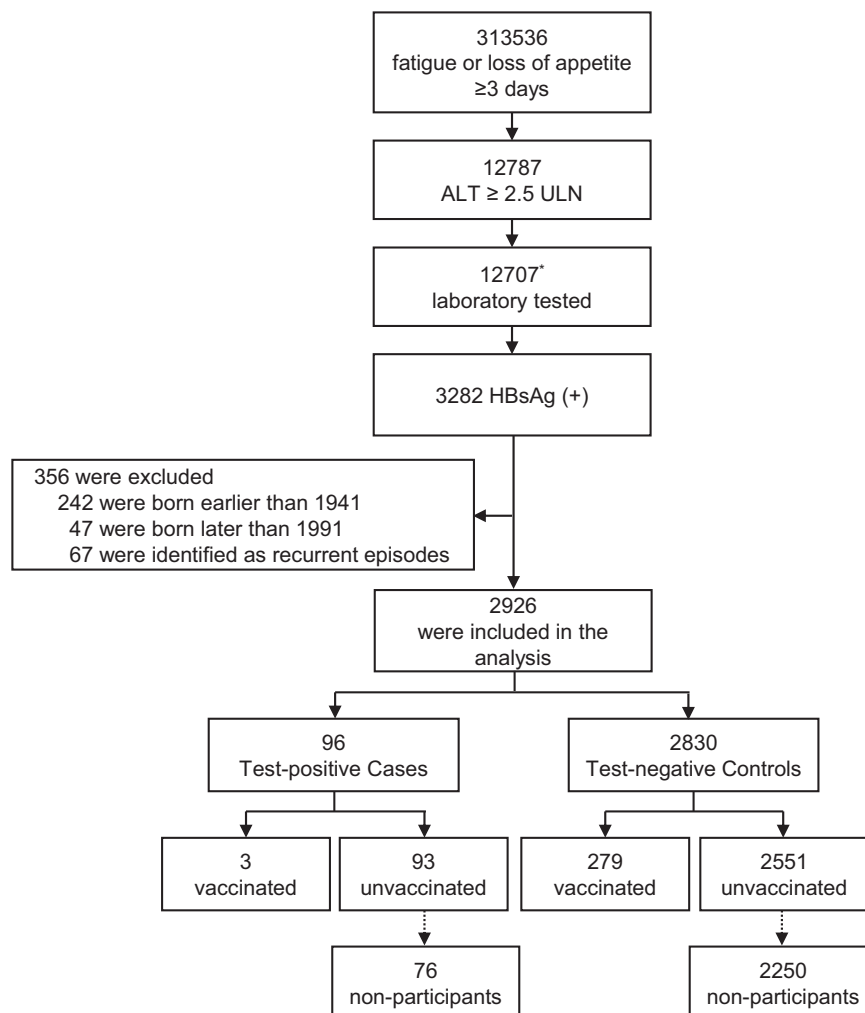

Fig. 1 | Study flow diagram. ALT alanine aminotransferase, ULN upper limit of normal, HEV hepatitis E virus, HBsAg hepatitis B surface antigen. *Hepatitis E-related laboratory testing was not completed for 80 events because of insufficient sample availability. Individuals exhibiting hepatitis-like symptoms (fatigue or loss of appetite) lasting for three or more consecutive days underwent ALT testing. Suspected hepatitis cases were defined as those with ALT levels at least 2.5 times the ULN, indicating acute hepatitis. Preliminary hepatitis typing tests, including assays for HBsAg, hepatitis B core antibody (HBcAb), hepatitis A virus IgM antibody,

hepatitis C virus IgG antibody and anti-HEV IgM, was performed on the first serum sample from each suspected case. A second serum sample was required to be collected 2–6 weeks later and also tested for anti-HEV IgM. Any positive result for anti-HEV IgM in either of the two samples prompted regular follow-up sampling until ALT levels normalized or symptoms resolved. All serial serum samples were sent to a central laboratory for hepatitis E diagnosis. A positive diagnosis for hepatitis E was made if at least two acute HEV markers were present (i.e., HEV RNA, IgM anti-HEV, and a fourfold or greater increase in IgG anti-HEV).

controlled, phase 3 trial of Hecolin in adults in Dongtai County, Jiangsu Province, China, spanning from August, 2007 to October, 2017 (ClinicalTrial.gov, NCT01014845)[13–15]. As previously described, between 22 August and 6 November, 2007, a total of 112,604 adults aged 16–65 years from 11 townships were enrolled and randomly assigned (stratified by age and sex) in a 1:1 ratio to received three doses of Hecolin or placebo (hepatitis B vaccine)[14]. Among these participants, all individuals from the Anfeng and Qindong townships were selected as the immunogenicity subgroup for the phase 3 clinical trial, based on considerations such as sample size and feasibility, to evaluate the immunogenicity and immune persistence[13–15].

At the beginning of the Hecolin phase 3 trial, a comprehensive active hepatitis surveillance system, including 205 clinical sentinel sites (village clinics, private clinics, town hospitals, municipal hospitals, etc.), was established to cover the entire study region and maintained for 10 years post-vaccination. Details of the surveillance methods have been described previously[13–15]. Briefly, individuals exhibiting hepatitis-like symptoms (fatigue or loss of appetite) lasting three or more consecutive days underwent alanine aminotransferase (ALT) testing. Suspected hepatitis cases were defined as those with ALT levels at least 2.5 times higher than the upper limit of normal (ULN), indicating acute hepatitis. A preliminary hepatitis typing testing, including tests for HBsAg, HBcAb, anti-HAV IgM, anti-HCV IgG and anti-HEV IgM, was performed on the first serum sample from each suspected hepatitis case. A second serum sample was required to be collected 2–6 weeks later and also tested for anti-HEV IgM. Any occurrence of anti-HEV IgM positivity in either of the two samples prompted regular follow-up sampling until ALT levels normalized or symptoms resolved. All serial serum samples were sent to a central laboratory for testing HEV-related markers (HEV RNA, IgM and IgG anti-HEV). Suspected cases with detectable anti-HEV IgM antibody or anti-HEV IgG antibody levels at least twice as high as any previous sample during the same illness episode underwent HEV RNA testing.

To evaluate the effectiveness of Hecolin in HBsAg-positive individuals, this test-negative design study focused on the HBsAg-positive population identified through hepatitis typing within the surveillance system. Confirmed hepatitis E cases were classified as test-positive, while non-confirmed cases were classified as test-negative.

Participants

Through the hepatitis surveillance system, Dongtai County conducted hepatitis case monitoring for over 10 years, covering ~290,000 permanent residents born between 1941 and 1991 (the target population of the phase 3 trial of Hecolin). Following the large-scale phase 3 trial, the Hecolin vaccination coverage rate in the target population (born between 1941 and 1991) was ~19%. Participants with health-seeking behaviors entered the test-negative design study through the surveillance system, including the following two groups: a. Individuals who participated in the Hecolin phase 3 trial and met the inclusion criteria, including being aged 16–65 years in 2007, having no history of hepatitis B or E, no immunodeficiency, no severe chronic diseases, and meeting other trial-specific eligibility requirements; b. Local residents of study region who did not participate in the Hecolin clinical trial, received no interventions, and were within the same age range as trial participants.

Sex information was determined based on identity cards (trial participants) or medical records (non-trial participants). Site investigators employed personal identity information, such as fingerprints and photographs, obtained from phase 3 trial to identify whether suspected cases observed in the surveillance system were indeed trial-participants. For trial-participants, Hecolin vaccination status was obtained from the electronic records of the phase 3 clinical trial, while

Table 1 | Characteristics of HBsAg-positive individuals with or without hepatitis E

Characteristic	Total			Trial-participants			Non-trial-participants		
	Test-positive cases (N = 96)	Test-negative controls (N = 2830)	P-value	Test-positive cases (N = 20)	Test-negative controls (N = 580)	P-value	Test-positive cases (N = 76)	Test-negative controls (N = 2250)	P-value
Age at the onset of symptom-Median (IQR), years	53.5 (44.0–63.0)	49.0 (40.0–58.0)	0.0028	55.0 (44.0–59.0)	51.5 (44.0–59.0)	0.4379	52.0 (44.5, 63.0)	48.0 (38.0, 57.0)	0.0034
Sex—n (%)									
Male	61 (63.5)	1637 (57.8)	0.2660	15 (75.0)	296 (51.0)	0.0350	46 (60.5)	1341 (59.6)	0.8714
Female	35 (36.5)	1193 (42.2)		5 (25.0)	284 (49.0)		30 (39.5)	909 (40.4)	
Peak alanine aminotransferase—GM ± SD, ×ULN	15.2 ± 3.1	5.6 ± 2.1	<0.0001	25.1 ± 2.5	5.4 ± 2.2	<0.0001	13.3 ± 3.1	5.6 ± 2.1	<0.0001
HEV vaccination history—n (%)									
No	93 (96.9)	2551 (90.1)	0.0279	17 (85.0)	301 (51.9)	0.0035	76 (100.0)	2250 (100.0)	NA
Yes	3 (3.1)	279 (9.9)		3 (15.0)	279 (48.1)		0 (0)	0 (0)	
Interval between the onset of symptom and last dose—Median (IQR), months	40.2 (21.3, 79.5)	62.3 (40.0, 86.9)	0.4379	40.2 (21.3, 79.5)	62.3 (40.0, 86.9)	0.4379	-	-	-
1-dose	0 (0.0)	20 (0.7)	0.8298	0 (0.0)	20 (3.4)	0.8298	-	-	-
2-dose	0 (0.0)	11 (0.4)		0 (0.0)	11 (1.9)		-	-	-
3-dose	3 (3.1)	248 (8.8)		3 (15.0)	248 (42.8)		-	-	-

IQR interquartile range, GM geometric mean, SD standard deviation, ULN upper limit of normal, HEV hepatitis E virus, NA not applicable. '-' indicates that no data was available for analysis. The characteristics of cases and controls were compared using the Wilcoxon rank-sum tests for age and the interval between the onset of symptom and last vaccine dose; t-tests with logarithmic transformation for peak alanine aminotransferase levels, and χ^2 tests for categorical variables including sex, vaccination history, and the number of doses. All reported P-values were two-sided with a significance level set at 0.05.

Table 2 | Vaccine Effectiveness against hepatitis E in the HBsAg-positive population

Hepatitis E vaccination history	N	No. of cases	No. of controls	Crude OR (95%CI)	aOR* (95%CI)	Vaccine effectiveness % (95%CI)	P-value
Total							
Unvaccinated	2644	93	2551	-	-	-	-
Vaccinated with at least one dose	282	3	279	0.30 (0.09, 0.94)	0.28 (0.09, 0.89)	72.1 (11.2, 91.2)	0.0307
Only trial participants							
Unvaccinated	318	17	301	-	-	-	-
Vaccinated with at least one dose	282	3	279	0.19 (0.06, 0.66)	0.19 (0.05, 0.64)	81.5 (35.9, 94.6)	0.0078

OR odd ratio, *aOR* adjusted OR, *CI* confidence interval. *Adjusted for sex and age. The unconditional logistic regression models were utilized to compute the crude OR, aOR, the corresponding 95% CIs and P-values. All reported P-values were two-sided with a significance level set at 0.05.

for non-trial-participants, the vaccination status would be check by the local Immunization Information System. It is important to note that although Hecolin became commercially available in China in 2012, its initial uptake (self-paid vaccination) was low due to insufficient public awareness and education. Furthermore, the timing of vaccine introduction varied across urban and rural areas with different economic levels. Prior to performing this study, investigators reviewed local vaccination records and consulted regional sales, revealing that there was no self-paid vaccination of Hecolin in Dongtai from 2012 to 2017.

In addition, for the trial-participants in the immunogenicity subgroup, serum samples were collected at baseline and at multiple time points post-vaccination. The details of the serological follow-up have also been previously reported[13–15].

Definitions

We defined confirmed hepatitis E cases (i.e., the test-positive group) as individuals who sought medical care and met the following criteria: symptoms persisting for at least 3 days, ALT level ≥ 2.5 ULN, and at least two of the following: positive HEV RNA, positive anti-HEV IgM antibody, and a fourfold or greater increase in anti-HEV IgG antibody concentrations[13]. Suspected hepatitis cases that were tested but did not fully meet these criteria were defined as the test-negative group.

Laboratory measurements

ALT concentrations were quantified by the Dongtai Centre for Disease Control and Prevention (CDC) or the municipal hospitals using commercial assay kits (BioSino, Beijing, China) in international units per litre of blood (units/L), and reported as multiples of the ULN. According to the kit instructions, the ULN values stand at 40 units/L for men and 31 units/L for women[13,15]. Hepatitis typing and anti-HEV IgG antibody detection were conducted using commercial ELISA kits (Beijing Wantai Biological Pharmacy, Beijing, China; see Supplementary Table 3 for detailed performance data of the kits) following standardized procedures provided by the manufacturer. Preliminary hepatitis typing, involving serum samples from all suspected hepatitis cases, was performed by Dongtai CDC, while HEV-related markers testing for the final diagnosis of hepatitis E, as well as immunogenicity measurements for the subgroup, were done by the central laboratory. HBsAg and anti-HEV IgM levels were expressed as a ratio of signal to cutoff (s/co), with a positive result defined as ≥ 1.0 s/co[13,42]. Concentrations of anti-HEV IgG were determined in parallel with WHO reference reagent (NIBSC code 95/584) and expressed in WHO unit (WU) per mL[13].

Statistical analysis

All suspected hepatitis patients with HBsAg positivity were included in this study and categorized into two groups according to their diagnosis results for hepatitis E (i.e., test-positive cases and test-negative controls). The characteristics of cases and controls were compared using χ^2 tests, t-tests with logarithmic transformation or Wilcoxon rank-sum tests. In the test-negative design analyses, unconditional logistic regression models were utilized to compute the odds ratio (OR) and its corresponding 95% confidence intervals (CIs) to assess the association between vaccination and the status of being a case or control. The potential confounders such as age (at suspected symptom onset) and sex were included as covariates in the models to evaluate adjusted OR (aOR). Vaccine effectiveness (VE) estimates were then derived as (1-aOR) × 100%.

All analyses were performed using SAS software (version 9.4), and missing data were not imputed. Reported P-values were two-sided with a significance level (α) of 0.05.

Ethics declaration

This work was approved by the ethics committee of the Jiangsu Provincial Center for Disease Control and Prevention and Xiamen University. Written informed consent was obtained from each participant of the Hecolin clinical trial, while the requirement for non-participants included in the analysis to provide informed consent was waived due to the study's observational nature without any deviation from the local medical practice, and all data for the analysis were anonymized.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

The individual-level raw data in this study were collected as part of a clinical trial and subject to confidentiality restrictions. The processed data are available under restricted access. Researchers may submit a detailed study proposal (non-commercial purpose) to access processed data from 6 months to 36 months after publication, directed to zhangj@xmu.edu.cn or yingyingsu@xmu.edu.cn. The corresponding authors will promptly review the request and determine whether the requested data can be shared, and will respond within 8 weeks of receiving the request. Any data that can be shared will be released via a material transfer agreement. The data will be available for 1 year once access has been granted.

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Author contributions

J.Z. and N.X. conceived of the study. H.J., X.Z., Y.W., C.Y., D.L. and X-F.Z. were investigators. Z.Z., X.H., J.L., K.Z., M.L. and L.C. were responsible for sample testing or management. S.H. and Y.S. managed the data. C.Z. performed the statistical analysis. C.Z. and X.L. drafted the manuscript, and J.Z., Y.S., T.W. and Y.H. critically revised it.

Competing interests

The authors declare no competing interests.

Additional information

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