Receipt of hepatitis E vaccine and fetal loss in rural Bangladesh: further analysis of a double-blind, cluster-randomised, controlled trial

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Summary

Background Vaccination constitutes an attractive control measure for hepatitis E virus (HEV), a major cause of maternal and perinatal mortality globally. Analysis of pregnant participants in an effectiveness trial of the HEV vaccine HEV239 showed possible HEV239-associated fetal losses. We aimed to conduct a detailed analysis of this safety signal.

Methods In a double-blind, cluster-randomised trial, 67 villages in Matlab, Bangladesh, were randomly allocated (1:1) to two vaccine groups, in which non-pregnant women aged 16–39 years received either HEV239 (HEV239 group) or Hepa-B (a hepatitis B vaccine; control group). We implemented weekly surveillance for pregnancy detection, and follow-up of pregnancies once every 2 weeks, using physician-confirmed diagnoses to evaluate fetal loss outcomes (miscarriage [spontaneous abortion], stillbirth, and elective termination). Data from a parallel system of reproductive health surveillance in Matlab were used to clarify study diagnoses when necessary. Miscarriage was assessed only among participants whose first positive pregnancy test and vaccination date (for whichever dose was closest to the date of last menstrual period [LMP]) were before 20 weeks' gestation. We defined the following analysis periods of interest: from 90 days before the LMP until the pregnancy outcome (the proximal period); from the LMP date until the pregnancy outcome (the pregnancy period); from 90 days before the LMP until the LMP date (90 days pre-LMP period); and from enrolment until 90 days before the LMP (the distal period). Both Poisson and Cox regression models were used to assess the associations between receipt of HEV239 and fetal loss outcomes. The trial was registered with ClinicalTrials.gov (NCT02759991).

Findings Among the 19 460 non-pregnant participants enrolled in the trial, 5011 were identified as having pregnancies within 2 years following vaccination and met the criteria for analysis (2407 in the HEV239 group and 2604 in the control group). Among participants vaccinated in the proximal period and evaluated for miscarriage, miscarriage occurred in 54 (8·9%) of 607 in the HEV239 group and 32 (4·5%) of 719 in the control group (adjusted relative risk [aRR] 2·0 [95% CI 1·3–3·1], p=0·0009). Similarly, the risk of miscarriages was increased in the HEV239 group versus the control group among participants inadvertently vaccinated during pregnancy (22 [10·5%] miscarriages among 209 participants in the HEV239 group *vs* 14 [5·3%] of 266 in the control group; aRR 2·1 [95% CI 1·1–4·1], p=0·036) and among those vaccinated within 90 days pre-LMP (32 [8·0%] of 398 *vs* 18 [4·0%] of 453; 1·9 [1·1–3·2], p=0·013). No increased risk of miscarriage was observed in those who received HEV239 in the distal period (93 [5·6%] of 1647 *vs* 80 [4·5%] of 1773; 1·3 [0·8–1·9], p=0·295). Stillbirth and elective termination showed no increased risk among women administered HEV239 versus those administered Hepa-B in any of the analysis periods.

Interpretation HEV239 given shortly before or during pregnancy was associated with an elevated risk of miscarriage. This association poses a possible safety concern for programmatic use of HEV239 in women of childbearing age.

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Introduction

Effective preventive interventions are needed for hepatitis E virus (HEV) infection, which is especially virulent in pregnancy and leads to approximately 44 000 maternal deaths and 3000 stillbirths per year, mostly in south Asia and Africa.[1–3] One HEV vaccine (HEV239 [Hecolin]; Xiamen Innovax Biotech, Xiamen, China), developed and licensed in China, has proven highly efficacious against HEV when given to non-pregnant people aged 16–65 years in China, as well as non-pregnant women aged 16–39 years in Bangladesh.[4,5] However, there is insufficient evidence on the effectiveness and safety of HEV239, administered either before or during pregnancy, in protecting pregnant women against hepatitis E.[6,7] We conducted a large-scale, cluster-randomised trial of the effectiveness of HEV239

Research in context

Evidence before this study

There is scant published evidence on the relationship between administration of the only available hepatitis E virus (HEV) vaccine, HEV239, shortly before or during pregnancy and the risk of miscarriage. We systematically searched PubMed and preprint platforms for literature containing the terms "hepatitis E vaccine" or "Hecolin" or "HEV239", combined with "pregnancy" and "fetal loss", until Oct 6, 2023. Previous research on HEV239 and the risk of miscarriage was limited to just two studies conducted in China. The first, a randomised, controlled, efficacy trial targeting non-pregnant people aged 16–65 years, in which controls received a hepatitis B virus (HBV) vaccine, reported on only 37 women who had inadvertently received HEV239 during pregnancy, none of whom had a miscarriage. However, a high proportion of these women (51%) chose elective terminations, making it impossible to assess the natural outcomes of these pregnancies. Furthermore, the study lacked documentation regarding the specific timing of HEV239 administration in relation to the onset of pregnancy and used unclear methods for detection and outcome evaluations of pregnancies. The second study, a randomised trial of HEV239 versus human papillomavirus vaccine targeting non-pregnant women aged 18–45 years, found no elevation of the risk of miscarriages in the 213 women given HEV239 shortly before or during pregnancy. However, 70% of these recipients had elective terminations, precluding an evaluation of natural pregnancy outcomes.

Added value of this study

In a large-scale, double-blind, cluster-randomised, effectiveness trial of HEV239 in 19 460 women aged 16–39 years in Bangladesh, we found approximately double the risk of miscarriage among the 209 women who unintentionally received HEV239 while pregnant and the 398 women who received HEV239 within 90 days before pregnancy, as compared with those who received the HBV vaccine Hepa-B during these periods. The specificity of this risk was underscored by observations of no increased risk of miscarriage for participants given HEV239 more than the 90 days before pregnancy, and no increased risk of either stillbirth or elective termination for women administered HEV239 either before or during pregnancy. These findings came from a population with a very low rate (<2%) of elective termination and from a study with a much larger sample of pregnancies evaluable for miscarriages than earlier studies. Our findings were further strengthened by active weekly surveillance for early pregnancy detection and comprehensive, biweekly surveillance for pregnancy outcomes, use of ultrasound assessments to date the start of the majority of detected pregnancies and to document all miscarriages, and physician-confirmed diagnoses of most of the pregnancy outcomes. Analyses were additionally strengthened by control for multiple baseline risk factors for pregnancy outcomes and blinding of the data analysts to participants' group allocations. Additionally, the availability of 2 years of post-vaccination surveillance provided an ample number of subjects in whom vaccines were given long before pregnancy, serving as negative controls for the associations between vaccination and pregnancy outcomes.

Implications of all the available evidence

Administration of HEV239 shortly before or early in pregnancy seems to be associated with a significantly elevated risk of miscarriage. The biological mechanisms for such an effect are unknown and warrant further study. Regardless of the biological mechanism, our findings raise a possible safety concern for programmatic use of HEV239 in women of childbearing age.

in non-pregnant women of childbearing age living in rural Bangladesh,[8] the results of which are reported in a separate Article.[5] Analysis of this trial yielded a signal indicating possible HEV239-associated fetal loss among participants whose pregnancies were detected during the trial. Herein, we report a more detailed analysis of this relationship, taking account of the temporal relationship between HEV239 receipt and the onset of pregnancy.

Methods

Study design and participants

We conducted a phase 4, community-based, double-blind, cluster-randomised, controlled trial assessing the protection conferred by a three-dose regimen of the recombinant subunit HEV239 vaccine to non-pregnant women aged 16–39 years against HEV during post-vaccination pregnancies.[5] In the trial, conducted in the Matlab field research site of the International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b),[9] HEV239 was compared against a three-dose control vaccine, Hepa-B (Incepta Pharmaceuticals, Dhaka, Bangladesh), a recombinant subunit hepatitis B virus vaccine. Eligible participants were healthy, non-pregnant women aged 16–39 years living in any of 67 villages of the study area. Participants were followed up for 2 years after receiving the last dose.

Analysis of all pregnancies within 2 years of vaccination, using only study surveillance, revealed a possible association between receipt of HEV239 and miscarriages.[5] However, that analysis did not evaluate the temporal relationship between vaccine dosing and the onset of pregnancy, and did not consider data on pregnancies collected in parallel by the icddr,b maternal and child health (MCH) programme in Matlab. Therefore, we did an additional, detailed analysis that considered not only data for participants obtained directly by the study but also data collected through the Matlab MCH system.

The protocol[10] for the trial was approved by the Independent Ethics Committee of the icddr,b, the

Directorate General of Drug Administration (Dhaka, Bangladesh) and Regional Ethics Committee of Oslo (Norway), as well as the data protection officer at the Norwegian Institute of Public Health (NIPH). An independent data and safety monitoring board, constituted by the icddr,b, closely monitored the study design and progress. Written informed consent was obtained from all participants.

The trial was registered on ClinicalTrials.gov (NCT02759991).

Randomisation and masking

Participants in the trial were randomly assigned in clusters (villages), with stratification by population size, to one of two vaccine groups, as described.[5] Vaccines were identical in appearance; both vaccines were fill-finished into identical single-dose vials and labelled with letter codes by Incepta Pharmaceuticals. All parties were masked to group allocation.[5] To maintain blinding in this analysis, an independent statistician reassigned participants in the two vaccine groups to one of two new code letters, which were kept secret until the analyses were completed.

Procedures

Vaccination procedures in the trial are detailed in a separate Article.[5]

To exclude those who were pregnant at enrolment and at administration of each vaccine dose, any participant who reported a missed period, irregular cycles, or a delay in onset of menstruation exceeding 2 weeks from the expected date, or who expressed uncertainty about the onset date of their last menstrual period (LMP), was tested for pregnancy with a urine strip hCG rapid test kit (Hangzhou Biotest Biotech, Hangzhou, China) and was excluded if the test was positive.[11]

After enrolment, female field workers for the study contacted each study participant weekly by home visit or telephone to detect symptoms compatible with HEV infection and to refer suspected cases for diagnostic evaluation.[8] These field workers also inquired as to whether the participant had delayed onset of menstruation exceeding 2 weeks beyond the expected date and referred those with positive histories to a community health research worker (CHRW) working for icddr,b's MCH programme, which covered all 67 study villages, for a urine pregnancy test. In parallel, but not as part of the trial, the CHRWs conducted monthly visits to all households to detect pregnancies.[9,12] In cases where a study participant was identified as pregnant after receiving one or two doses of the vaccine, no further doses were administered.

Follow-up visits were made once every 2 weeks by the study's female field workers to all pregnant participants, in addition to antenatal care visits through the Matlab MCH programme and monthly follow-up visits by the CHRWs. The last pregnancy-related study visit was 14 days post-delivery. Pregnancy follow-up, supplemented by further ultrasounds when warranted (all miscarriages were ultrasound-confirmed), continued until the conclusion of pregnancy, with outcome diagnoses for the study made by a study physician. Simultaneously, the MCH programme carried out its own data collection regarding pregnancy outcomes through its routine monthly visits and care. For participants seen in the antenatal clinics during the first trimester, a gestational ultrasound was obtained. Diagnoses of outcomes in the MCH programme were made by trained female health workers.

Outcomes

To further evaluate the safety signal for HEV239-related fetal losses identified in the main analysis,[5] the current analysis focused on the occurrence of three fetal loss outcomes: miscarriage (spontaneous abortion), stillbirth, and elective pregnancy termination. Gestational age was calculated using the first day of the participant's LMP as the start date of pregnancy. Miscarriage was defined as pregnancy loss before 20 weeks' gestation (140 days) without human interference. Stillbirth was defined as fetal loss at or beyond 20 weeks' gestation (140 days). Elective termination was defined as intentional or therapeutic abortion by an artificial procedure.

Statistical analysis

Use of the trial data and Matlab MCH system data on pregnancies and pregnancy outcomes required explicit a priori algorithms to handle discrepancies between the two databases. The following principles were followed to resolve these issues and create a final, locked data file before data analysis. First, outcome diagnoses made by a study physician were deemed more reliable than those in the MCH system, which were made by non-physicians. Second, diagnoses of miscarriage versus stillbirth had to be consistent with gestational age at the time of the event (see below). Third, if no adverse outcome diagnosis was given in the study files, but one was indicated in the MCH system files, the latter was accepted (no such cases were found in the MCH data). Fourth, if the adverse outcome in the study file was not sufficiently specific (eg, "abortion"), and the diagnosis in the MCH file clarified the diagnosis (eg, "elective termination"), the latter was accepted. Fifth, a first-trimester gestational ultrasound conducted at 8–13 weeks of gestation was used to determine the date of the LMP (66% of women vaccinated during pregnancy had a first-trimester gestational ultrasound). Finally, in the absence of a gestational ultrasound, study dating of LMP, which was based on weekly interviews with participants by the study, was regarded as more reliable than that from the MCH system, which conducted interviews on a monthly basis. All pregnancies found in the MCH files had already been detected in the study surveillance.

We assembled all test-positive pregnancies with a date of LMP within 2 years after receiving the last vaccine

dose. We analysed only the initial pregnancy for each woman, as this pregnancy occurred closest to a vaccination. The start date of pregnancy was defined as the date of the LMP, while the start date of actual follow-up for pregnancy outcomes was defined as the date of first positive pregnancy test.

Each woman could receive up to three doses of vaccine during the study, thus having up to three vaccination dates. We deemed the vaccine dose received closest to the date of LMP to be biologically most relevant for analysing the impact of vaccination on pregnancy outcome and defined the date of this dose as "zero time".

Following an earlier published analysis of HEV239,[13] the association between vaccine dosing at zero time and fetal loss centred around four key pregnancy-related periods for zero time: from 90 days before the LMP until the pregnancy outcome (the proximal period); from the LMP date until the pregnancy outcome (the pregnancy period); from 90 days before the LMP until the LMP date (90 days pre-LMP period); and from enrolment until 90 days before the LMP (the distal period; figure 1).

Our prespecified primary analysis focused on the associations between receipt of HEV239 during the proximal period and fetal loss outcomes. Secondary analyses addressed these associations when zero time occurred during pregnancy, during the 90 days pre-LMP, and during the distal period. Since miscarriage is defined as the termination of pregnancy before 20 weeks of gestation, only pregnancies with a first positive pregnancy test and zero time both occurring before 20 weeks of gestation were included in the analysis of miscarriage for all periods. Since elective terminations can occur at any time and stillbirths at or after 20 weeks' gestation, this constraint was not applied in the analysis of these outcomes.

For each analysis, the HEV239 and Hepa-B groups were compared at zero time for known risk factors collected as part of the study protocol for each of the three fetal loss outcomes. We also compared the groups for the gestational age at zero time and at the first positive pregnancy test and the total number of doses received from enrolment up to and including zero time. In simple analyses, categorical variables were compared with the χ^2 test, or with Fisher's exact test when mandated by sparse data, and continuous variables were compared with the Student's t test, or the Mann–Whitney U test when data were not normally distributed, as assessed by inspection of Q-Q plots. Geographical clustering of cases was assessed by calculating intra-cluster correlation coefficients using Poisson models.

To assess the relative risk (RR) of post-zero time occurrence of fetal loss outcomes, adjusting for unequally distributed baseline variables, we used Poisson regression models with robust sandwich standard errors estimated using a jackknife approach to account for overdispersion and the design effect of cluster randomisation of villages.[14] Variables differing between the two vaccine groups in simple analyses at $p<0{\cdot}10$ at baseline were entered as independent variables, together with gestational age at the first positive pregnancy test, the stratification variable used for randomisation (village population size), and the vaccine group variable. The RR for the association between vaccination with HEV239 and each pregnancy outcome was estimated by exponentiation of the coefficient of the vaccine group variable from the fitted model, and the standard error of the coefficient was used to estimate the p value and 95% CI for each vaccine–outcome association.

We did several sensitivity analyses. First, study participants who inadvertently received vaccine doses during pregnancy had their pregnancies detected at various intervals after vaccination. Because the likelihood of detected fetal loss increases with how early in gestational age that pregnancy is recognised, this interval between vaccination and pregnancy detection creates the possibility of immortal time bias. We therefore repeated all analyses to correct for left truncation, taking the first positive pregnancy test rather than zero time as the start of follow-up. Second, we reanalysed the data using Cox proportional hazards models. After first confirming that the proportionality assumption was fulfilled for all independent variables, which were selected or forced in the same fashion as for the Poisson models, we

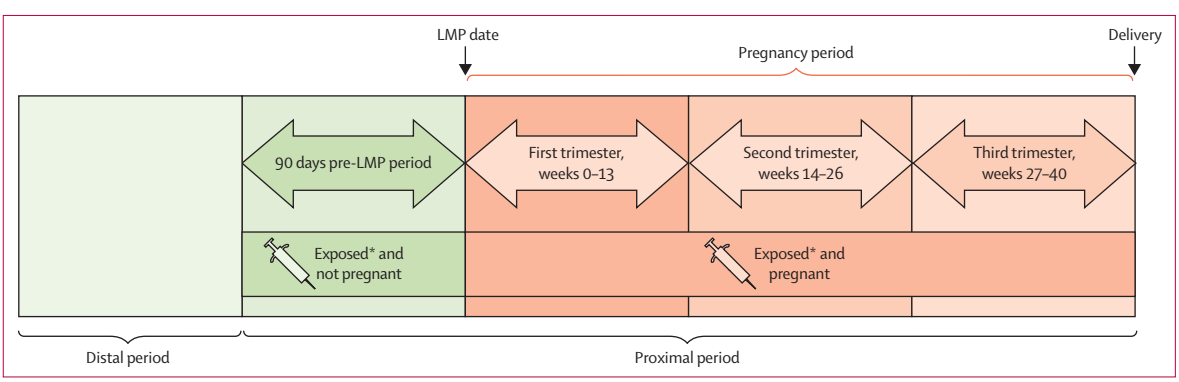

Figure 1: **Definitions of analysis periods**
LMP=last menstrual period. *Exposure refers to vaccination.

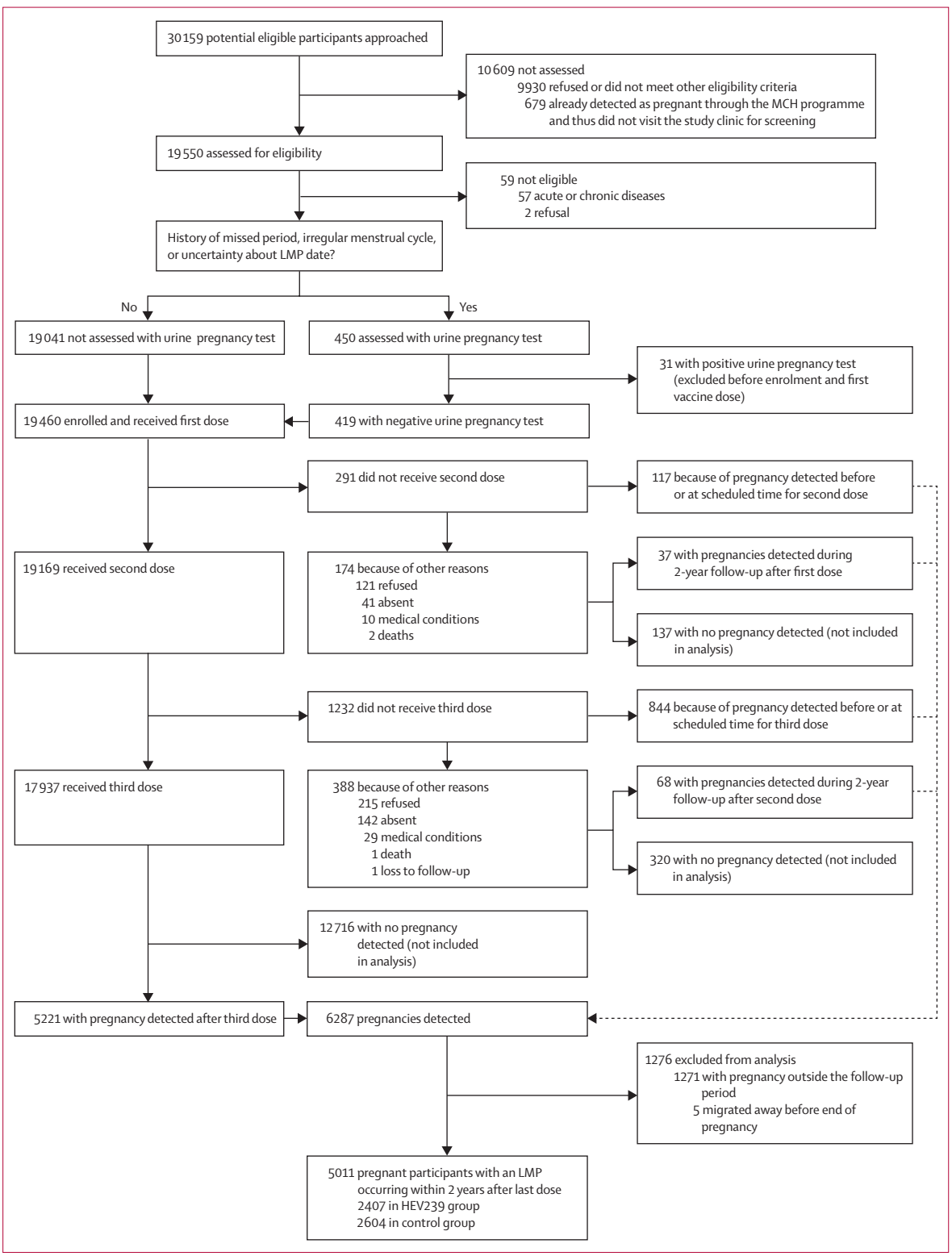

Figure 2: **Pregnancy detection at various stages throughout the main trial**
LMP=last menstrual period. MCH=maternal and child health.

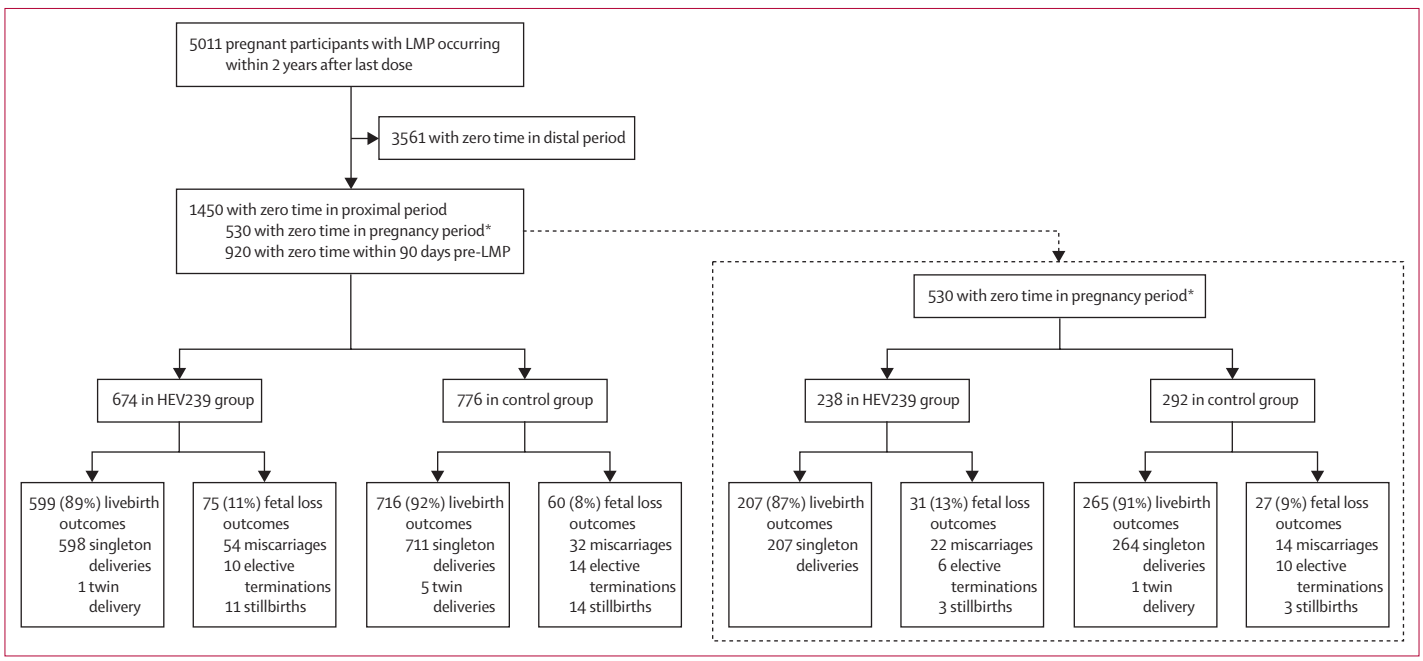

Figure 3: **Pregnancy outcomes among participants whose zero times occurred during the proximal period and during pregnancy**
LMP=last menstrual period. *Those with a zero time during the pregnancy period (shown within the dashed box) represent a subset of those with zero times in the proximal period.

exponentiated the coefficient for the vaccine group variable to estimate hazard ratios, adjusting for the design effect of cluster-randomisation using a generalised estimating equation model, assuming a working independence variance structure. Third, we considered an alternative pregnancy start date definition by using LMP plus 14 days, rather than LMP itself, and repeated all analyses. Finally, because pregnancy outcomes in the Matlab MCH system are assessed by non-physicians, in contrast to assessments in the study, we repeated the primary analysis using only the pregnancy outcomes based on study data.

See Online for appendix

Statistical analyses were conducted using STATA 15 and R (version 4.2.1). In all analyses, the threshold of statistical significance was set at $p<0·05$ (two-tailed).

Role of the funding source

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

Results

Between Oct 2, 2017, and Feb 28, 2019, the main trial enrolled a total of 19460 participants (including 9478 [48·7%] participants across 33 clusters in the HEV239 group and 9982 [51·3%] participants across 34 clusters in the control group). 6287 (32·3%) participants had at least one confirmed pregnancy during the study period until Oct 31, 2021, among whom 1271 did not meet the criteria for our analysis (with pregnancies occurring outside of the 2-year follow-up period from the last dose of vaccine) and an additional five who migrated away during pregnancy had unknown pregnancy outcomes and were excluded from the analysis. 5011 vaccinated pregnant participants who were followed up until pregnancy outcome were included in the analysis (2407 in the HEV239 group and 2604 in the control group; figure 2). 1450 of the included participants had zero times during the proximal period (figure 3): 530 during the pregnancy period and 920 within 90 days before their LMP. The zero times of the remaining 3561 women were in the distal period (appendix p 1). Recurrent pregnancies were not considered (only six occurred in participants with zero times in the primary analysis, all with livebirth outcomes).

Baseline characteristics, including variables that could potentially affect the risk of miscarriage, stillbirth, and elective termination, were consistently similar between the HEV239 and control groups in all four predefined exposure periods (tables 1, 2; appendix pp 5–14). In analyses of miscarriages, more than 95% of those with a zero time during pregnancy received HEV239 and Hepa-B doses in the first 7 weeks after their LMP (table 2). The median gestational age at first positive pregnancy test was 9 weeks (IQR 7–12) for HEV239 recipients and 10 weeks (7–13) for Hepa-B recipients with zero times in the proximal period, and over 85% of these participants had received multiple doses of either vaccine from enrolment up to and including zero time (table 1).

In total, 259 miscarriages, 98 stillbirths, and 34 elective terminations occurred among the included participants.

	HEV239 group (n=607)	Control group (n=719)	p value*
Total number of doses received from enrolment to zero time (inclusive)	..	..	0·312
One	69 (11·4%)	101 (14·0%)	..
Two	273 (45·0%)	322 (44·8%)	..
Three	265 (43·7%)	296 (41·2%)	..
Maternal age at zero time, years			
Median	23·0 (20·0 to 28·0)	24·0 (20·0 to 28·0)	0·824
Age group	..	..	0·731
<20	146 (24·1%)	164 (22·8%)	..
20–35	433 (71·3%)	526 (73·2%)	..
>35	28 (4·6%)	29 (4·0%)	..
Maternal age at first pregnancy test, years			
Median	24·0 (20·0 to 28·0)	24·0 (20·0 to 29·0)	0·803
Age group	..	..	0·387
<20	138 (22·7%)	149 (20·7%)	..
20–35	437 (72·0%)	540 (75·1%)	..
>35	32 (5·3%)	30 (4·2%)	..
Median time difference between last menstrual period and zero time, weeks	−3·0 (−8·0 to 1·0)	−3·0 (−8·0 to 1·0)	0·385
Gestational age at first positive pregnancy test, weeks			
Median	9·0 (7·0 to 12·0)	10·0 (7·0 to 13·0)	0·501
Age group	..	..	0·755
0–3	2 (0·3%)	1 (0·1%)	..
4–7	170 (28·0%)	190 (26·4%)	..
8–11	249 (41·0%)	295 (41·1%)	..
12–19	186 (30·6%)	233 (32·4%)	..
BMI at enrolment (first dose), kg/m²			
Median	22·2 (19·6 to 25·2)	22·5 (20·0 to 25·6)	0·076
BMI group	..	..	0·941
≤30	583 (96·0%)	690 (96·0%)	..
>30	24 (4·0%)	29 (4·0%)	..
History of miscarriage	..	..	0·559
Yes	51 (8·4%)	67 (9·3%)	..
No	556 (91·6%)	652 (90·7%)	..
History of induced or therapeutic abortion	..	..	0·627
Yes	16 (2·6%)	16 (2·2%)	..
No	591 (97·4%)	703 (97·8%)	..
History of hypertension in pregnancy	..	..	0·828
Yes	10 (1·6%)	11 (1·5%)	..
No	574 (94·6%)	708 (98·5%)	..
Unknown	23 (3·8%)	0	..
Parity			
Median	1·00 (0·00 to 2·00)	1·00 (0·00 to 2·00)	0·435
Parity group	..	..	0·561
0	201 (33·1%)	251 (34·9%)	..
≥1	403 (66·4%)	468 (65·1%)	..
Unknown	3 (0·5%)	0	..

(Table 1 continues in next column)

(Continued from previous column)

Data are n (%) or median (IQR). Only participants whose first positive pregnancy test and zero time were before 20 weeks' gestation are included in the analysis of miscarriage. *From Pearson's χ^2 test, Fisher's exact test, or Wilcoxon rank-sum test.

Table 1: **Baseline variables affecting the risk for miscarriage among participants whose zero time occurred during the proximal period**

Of these outcomes, 236 miscarriages, 81 stillbirths, and 32 elective terminations were determined by study physician diagnosis, and the remainder (23 miscarriages, 17 stillbirths, and two elective terminations) were reclassified using MCH data from the 42 cases labelled as "abortion", not further specified, in the HEV study database.

Among participants with zero times during the proximal period, 54 (8·9%) of 607 in the HEV239 group and 32 (4·5%) of 719 in the control group had miscarriages (adjusted [a]RR 2·0 [95% CI 1·3–3·1], p=0·0009). However, there was no significant difference between the groups in the risks of stillbirth or elective terminations in either crude or adjusted analyses (table 3).

For women with zero times during pregnancy, 22 (10·5%) of 209 in the HEV239 group and 14 (5·3%) of 266 in the control group had miscarriages (aRR 2·1 [95% CI 1·1–4·1], p=0·036), whereas no such increases in risk were observed for stillbirths or elective terminations (table 3). Similarly, for women with zero times within 90 days pre-LMP, an elevated risk of miscarriage was observed in the HEV239 group (32 [8·0%] of 398) versus the control group (18 [4·0%] of 453; aRR 1·9 [95% CI 1·1–3·2], p=0·013), with no elevation of risk for stillbirths or elective terminations (table 3). By contrast, for women with zero times in the distal period, there was no significant elevation of risk of miscarriage (93 [5·6%] of 1647 in the HEV239 group *vs* 80 [4·5%] of 1773 in the control group; aRR 1·3 [95% CI 0·8–1·9], p=0·295) or any other fetal loss outcome (table 3).

Median gestational ages at the time of miscarriage ranged from 10 weeks to 12 weeks for women with zero times in each of the four defined periods, and were similar between the HEV239 and control groups (appendix p 29). There was no evidence of geographical clustering of miscarriages among women with zero times in the proximal period (intracluster correlation coefficient 0·009 [95% CI −0·014 to 0·032]), nor was there clear evidence of seasonality of miscarriages (appendix p 3). Additionally, the RRs of miscarriage among participants with zero times in the proximal

	HEV239 group (n=209)	Control group (n=266)	p value*
Total number of doses received from enrolment to zero time (inclusive)	..	..	0·656
One	62 (29·7%)	88 (33·1%)	..
Two	64 (30·6%)	82 (30·8%)	..
Three	83 (39·7%)	96 (36·1%)	..
Maternal age at zero time, years	..	..	..
Median	23·0 (21·0–28·0)	24·0 (19·2–29·0)	0·772
Age group	..	..	0·490
<20	43 (20·6%)	67 (25·2%)	..
20–35	151 (72·2%)	182 (68·4%)	..
>35	15 (7·2%)	17 (6·4%)	..
Maternal age at first pregnancy test, years			
Median	23·0 (21·0–29·0)	24·0 (20·0–29·0)	0·838
Age group	..	..	0·419
<20	40 (19·1%)	63 (23·7%)	..
20–35	152 (72·7%)	186 (69·9%)	..
>35	17 (8·1%)	17 (6·4%)	..
Gestational age at zero time, weeks			
Median	2·00 (1·00–3·00)	2·00 (1·00–3·00)	0·636
Age group	..	..	0·262
0–3	172 (82·3%)	235 (88·3%)	..
4–7	32 (15·3%)	27 (10·2%)	..
8–11	3 (1·4%)	3 (1·1%)	..
12–19	2 (1·0%)	1 (0·4%)	..
Gestational age at first positive pregnancy test, weeks			
Median	8·0 (7·0–11·0)	8·0 (7·0–11·0)	0·962
Age group	..	..	0·520
0–3	0	0	..
4–7	77 (36·8%)	110 (41·4%)	..
8–11	81 (38·8%)	91 (34·2%)	..
12–19	51 (24·4%)	65 (24·4%)	..
BMI at enrolment (first dose), kg/m²			
Median	21·8 (19·5–25·4)	22·4 (20·0–25·4)	0·323
BMI group	..	..	0·820
≤30	197 (94·3%)	252 (94·7%)	..
>30	12 (5·7%)	14 (5·3%)	..
History of miscarriage	..	..	0·564
Yes	23 (11·0%)	25 (9·4%)	..
No	186 (89·0%)	241 (90·6%)	..
History of induced or therapeutic abortion	..	..	0·683
Yes	5 (2·4%)	8 (3·0%)	..
No	204 (97·6%)	258 (97·0%)	..
History of hypertension in pregnancy	..	..	0·770
Yes	6 (2·9%)	6 (2·3%)	..
No	198 (94·7%)	260 (97·7%)	..
Unknown	5 (2·4%)	0	..
Parity			
Median	1·00 (0·00–2·00)	1·00 (0·00–2·00)	0·607
Parity group	..	..	0·105
0	63 (30·1%)	100 (37·6%)	..
≥1	144 (68·9%)	166 (62·4%)	..
Unknown	2 (1·0%)	0	..

Data are n (%) or median (IQR). Only participants whose first positive pregnancy test and zero time were before 20 weeks' gestation are included in the analysis of miscarriage. *From Pearson's χ^2 test, Fisher's exact test, or Wilcoxon rank-sum test.

Table 2: **Baseline variables affecting the risk for miscarriage among participants whose zero time occurred during the pregnancy period**

period were elevated in the HEV239 group versus the control group throughout the calendar interval period in which these zero times occurred (appendix p 4).

Repetition of the analyses with Cox regression models yielded similar results (appendix pp 30–31). Moreover, revision of the start of follow-up from zero time to the time of the first positive pregnancy test yielded associations very similar to those in the earlier analyses (appendix pp 32–33). Analyses done after redefinition of the start of pregnancy as the LMP plus 14 days also had no impact on the findings (appendix pp 15–28, 34–37). Finally, repetition of the primary analysis using only physician diagnoses recorded in the study surveillance yielded adjusted RRs for miscarriages (2·1 [95% CI 1·4–3·2], p=0·0009), stillbirths (0·8 [0·3–2·0], p=0·614), and elective terminations (1·0 [0·4–2·2], p=0·976) nearly identical to those in the analysis that also considered MCH information (appendix p 38).

Discussion

Our analysis revealed that women who received HEV239 during pregnancy or within 90 days before pregnancy had an approximately doubled risk of miscarriage compared with women who received Hepa-B. The specificity of this elevated risk was indicated by the absence of increased risk for HEV239 recipients who were vaccinated during the period more than the 90 days before the onset of pregnancy, as well as by the absence of an increased risk of either stillbirths or elective terminations among HEV239 recipients, regardless of the timing of vaccination.

Several potential limitations require discussion. First, clusters rather than individuals were randomly assigned to the two vaccine groups, and geographically or temporally varying external exposures could have led to a spurious relationship between HEV239 and the risk of miscarriage.[15–19] However, random assignment of

	HEV239 group		Control group		Crude analysis		Adjusted analysis	
	Outcome absent	Outcome present	Outcome absent	Outcome present	RR (95% CI)	p value	RR (95% CI)	p value
Zero time during proximal period								
Miscarriage*	553/607 (91·1%)	54/607 (8·9%)	687/719 (95·5%)	32/719 (4·5%)	2·0 (1·3–3·1)	0·0017	2·0 (1·3–3·1)†	0·0009
Stillbirth	663/674 (98·4%)	11/674 (1·6%)	762/776 (98·2%)	14/776 (1·8%)	0·9 (0·4–2·0)	0·804	0·9 (0·4–2·0)†	0·801
Elective termination	664/674 (98·5%)	10/674 (1·5%)	762/776 (98·2%)	14/776 (1·8%)	0·8 (0·4–1·8)	0·637	0·9 (0·4–2·1)‡	0·817
Zero time during pregnancy								
Miscarriage*	187/209 (89·5%)	22/209 (10·5%)	252/266 (94·7%)	14/266 (5·3%)	2·0 (1·0–4·0)	0·043	2·1 (1·1–4·1)§	0·036
Stillbirth	235/238 (98·7%)	3/238 (1·3%)	289/292 (99·0%)	3/292 (1·0%)	1·2 (0·2–6·6)	0·802	1·3 (0·2–8·2)§	0·765
Elective termination	232/238 (97·5%)	6/238 (2·5%)	282/292 (96·6%)	10/292 (3·4%)	0·7 (0·3–2·0)	0·553	0·8 (0·3–2·0)§	0·574
Zero time within 90 days pre-LMP								
Miscarriage*	366/398 (92·0%)	32/398 (8·0%)	435/453 (96·0%)	18/453 (4·0%)	2·0 (1·1–3·7)	0·017	1·9 (1·1–3·2)§	0·013
Stillbirth	428/436 (98·2%)	8/436 (1·8%)	473/484 (97·7%)	11/484 (2·3%)	0·8 (0·3–2·0)	0·645	0·8 (0·3–2·3)§	0·670
Elective termination	432/436 (99·1%)	4/436 (0·9%)	480/484 (99·2%)	4/484 (0·8%)	1·1 (0·3–4·7)	0·883	1·1 (0·3–6·1)§	0·939
Zero time during distal period								
Miscarriage*	1554/1647 (94·4%)	93/1647 (5·6%)	1693/1773 (95·5%)	80/1773 (4·5%)	1·3 (0·9–1·7)	0·141	1·3 (0·8–1·9)§	0·295
Stillbirth	1693/1733 (97·7%)	40/1733 (2·3%)	1795/1828 (98·2%)	33/1828 (1·8%)	1·3 (0·8–2·0)	0·296	1·2 (0·8–1·9)§	0·309
Elective termination	1726/1733 (99·6%)	7/1733 (0·4%)	1825/1828 (99·8%)	3/1828 (0·2%)	2·5 (0·7–11·4)	0·192	2·6 (0·6–11·8)§	0·218

LMP=last menstrual period. RR=relative risk. *Only participants whose first positive pregnancy test and zero time were before 20 weeks' gestation are included in the denominators. †Adjusted for gestational age at first pregnancy test, BMI, study design effect, and stratifying variable. ‡Adjusted for gestational age at first pregnancy test, doses received between enrolment and zero time (inclusive), study design effect, and stratifying variable. §Adjusted for gestational age at first pregnancy test, study design effect, and stratifying variable.

Table 3: **Association between vaccination and fetal loss outcomes among participants whose zero time occurred during the proximal period, during pregnancy, within 90 days pre-LMP, and during the distal period**

a relatively large number of units of randomisation (n=67) helped to safeguard imbalances between the treatment groups. Furthermore, the Matlab surveillance revealed similar risks of miscarriage during the 5 years before the trial in villages assigned to the HEV239 group (768 miscarriages [10·2%] out of 7522 pregnancies) and villages assigned to the control group (768 miscarriages [9·7%] out of 7932 pregnancies; RR 1·05 [0·95–1·15], p=0·29). Furthermore, there was no elevation of risk of miscarriage in HEV239 vaccinees during the distal period, when any relationship between HEV239 and the risk of miscarriage would be implausible. The success of randomisation in ensuring highly comparable treatment groups was further underscored by the excellent balance of a large number of individual-level baseline variables between the HEV239 and control groups in analyses of all periods for zero time, although the study variables available through the study data were not exhaustive compilations of risk factors for fetal loss outcomes. Furthermore, analyses did not reveal geographical clustering of miscarriages; concurrent enrolment and follow-up of participants in the two groups helped ensure a balance in seasonal factors, such as ambient temperature, that might affect the risk of miscarriage; and analyses showed an elevation of the RR of miscarriage in HEV239 recipients throughout the calendar interval for zero times in the primary analysis. Finally, our findings analytically accounted for the design effect of cluster randomisation, as well as for the few baseline variables that were modestly imbalanced, in assessing the statistical significance of the associations. All of these considerations attest to the internal validity of our findings.

A second possible limitation is that we used Hepa-B rather than a true placebo as the control. However, we know of no evidence that Hepa-B exerts a protective effect against miscarriage when given during pregnancy or in the 90 days before pregnancy (the proximal period), but not during the period preceding the 90 days before pregnancy onset (distal period), both of which would be needed to explain our findings in the absence of an effect of HEV239. Another argument against such a protective effect is that the risk of miscarriage in the Hepa-B group in our analysis was identical for women who received Hepa-B with zero times in the distal versus the proximal periods (4·5%).

Third, although the two vaccines were identical in appearance, the administered volumes of the two different vaccines differed slightly.[5] However, the use of identical single-dose vials; the use of eight different codes corresponding to different age and vaccine combinations, which were kept secret by Incepta Pharmaceuticals, to label the vaccines; and the fact that vaccinators were not informed of the differing volumes by vaccine nor the manufacturers of the vaccines, helped to ensure that neither the vaccinators nor the participants became aware of the identities of the vaccines administered. Moreover, ascertainment of eligibility, acquisition of informed consent, and conduct of all follow-up procedures were done independently by staff who were not involved with vaccine administration and who had no access to the codes.

Fourth, among participants in both groups with zero times in the distal period and among those in the control group with zero times in the proximal period, miscarriage occurred in around 5% of pregnancies, which was lower than the rate of around 10% from Matlab MCH surveillance in the study villages before the trial.[20] However, surveillance for pregnancies and pregnancy complications in the trial was likely to have been at least as intensive as that used in the Matlab MCH system in earlier years, and no new interventions that could have affected miscarriage rates were introduced during the trial period. Furthermore, during the trial, no changes had occurred in the Matlab MCH system of pregnancy surveillance, and only around 9% of miscarriages among the total in the combined distal and proximal period analyses were identified with the Matlab MCH surveillance but not the study surveillance. Notably, the miscarriage rate for women of childbearing age reported by the Matlab MCH system for the years of the trial, 2017–21, was 10·4% (1640 miscarriages out of 15 801 pregnancies), unchanged from the previous years and supporting the assertion that no changes in the risk of miscarriage or the Matlab MCH surveillance occurred during the trial. By contrast, the rate reported by the Matlab MCH system for study participants in both groups with zero times in the distal period (120 [4·2%] of 2834) was markedly lower and nearly identical to the rate based on dual use of study and Matlab MCH surveillance in the control group of our analysis (80 [4·5%] of 1773). This suggests that younger age range for study participants (16–39 years) than for Matlab MCH surveillance (15–49 years), perhaps together with the participation of healthier women in the trial, in view of the 65% participation rate of age-eligible women in the study villages, explained the lower miscarriage rates observed in the trial compared with in earlier Matlab surveillance.[21] Additionally, our analysis defined miscarriage as pregnancy loss before 20 weeks' gestation, as is conventional, versus the criterion of before 28 weeks' gestation used by the Matlab MCH system.

Fifth, it could be argued that the association between HEV239 and miscarriage, despite being statistically significant, might have been a chance finding in the main trial analysis, resulting from multiple comparisons.[5] Although we cannot disprove this, it is noteworthy that the association between HEV239 and miscarriage was highly significant in our primary analysis (RR 2·0 [95% CI 1·3–3·1], p=0·0009), and, as expected, no association was seen in the distal period analysis. Finally, most (466 of 475) women who were inadvertently vaccinated during pregnancy received their doses in the first 7 weeks of gestation, and our study enrolled young women. Our data do not address the effects of HEV239 given later in pregnancy or to older women.

The credibility of our findings was enhanced by several strengths of study design and analysis. In addition to being a prospective study with randomised allocation and double-blinded vaccine administration and participant follow-up, the two study groups were similar with respect to several baseline variables associated with the risk of the pregnancy outcomes under study. Additionally, all pregnancies were monitored until their conclusion, with a low rate (<1%) of losses to follow-up, and the 2-year follow-up period after the last dose enabled the evaluation of associations when vaccines were given long before pregnancy, so that the evaluation of the associations during the distal period could serve as a negative control. Moreover, we implemented intensive, community-based, active surveillance both to detect pregnancies and to evaluate pregnancy outcomes. This ensured that the dating of LMPs that relied on recall was done with only 1-week recall periods, and that data on pregnancy outcomes were collected with very little delay. Dating of pregnancy onset was also enhanced by the use of first-trimester ultrasound examinations. Also, all miscarriages from the study data were confirmed by physician diagnoses and based on ultrasound examinations. In addition, the number of miscarriages yielded estimates of RR with narrow 95% CIs in our primary analysis of proximal exposure. Moreover, our findings remained unchanged in multiple sensitivity analyses redefining the start of pregnancy follow-up as the date of the first positive pregnancy test or as the date of LMP plus 14 days, using survival analysis, or considering only study diagnoses. Finally, preparation of the final dataset and conduct of the analysis was done by investigators blinded to whether women had received HEV239 or Hepa-B.

A large-scale, randomised, controlled, efficacy trial of HEV239 versus Hepa-B done in non-pregnant people aged 16–65 years in China reported no miscarriages among 37 women who were inadvertently vaccinated during pregnancy.[4] However, 19 (51%) of these women had elective pregnancy terminations, precluding assessment of the natural outcomes of these pregnancies, and the timing of the doses in relation to the onset of pregnancy was not reported.[10] Another randomised trial in China, in which the HEV239 was given as a control vaccine for evaluation of a bivalent human papillomavirus (HPV) vaccine in non-pregnant women aged 18–45 years, found no elevation of the risk of miscarriage in HEV239 recipients inadvertently vaccinated during pregnancy, or in proximal and distal period analyses, defined as in our study.[13] However, of the 213 women exposed to HEV239 during pregnancy or within 90 days before pregnancy, 149 (70%) had elective terminations, precluding assessment of the natural outcomes of these pregnancies and leaving only 64 pregnancies for assessment.[13]

Of interest, a study of a bivalent HPV vaccine revealed that the RR of miscarriage was 1·6 (95% CI 1·0–2·4) when the vaccine was administered during the 45 days before the LMP.[22] Moreover, an aggregate analysis of randomised trials of a nine-valent HPV vaccine found an increased

risk of miscarriage (RR 2·0 [95% CI 1·3–3·2]) when the vaccine was given during the period from 30 days before to 30 days after conception.[23] However, the overall evidence of an association of miscarriage with different HPV vaccines is mixed.[22]

The biological basis for our findings is not known, although immune hypersensitivity has been suggested for the elevated risk of miscarriage seen with HPV vaccines.[24] Potentially contradicting this explanation, our analyses of women who received HEV239 during the proximal period showed little difference in the RR of miscarriage by number of doses received by zero time (data not shown). The mechanisms of obstetric, placental, and fetal damage induced by natural HEV infection have been well studied and might provide insights.[25–29] Regardless of the biological mechanism, our findings raise a possible safety concern for use of HEV239 in women of childbearing age. This concern will have to be weighed against the major benefit of preventing hepatitis E in pregnant women, in whom the disease is an important cause of maternal morbidity and mortality, as well as perinatal mortality in many socioeconomically disadvantaged populations, especially in south Asia. In future, vigilant surveillance of pregnant women who have received HEV239, either during or before pregnancy, will be essential. Additionally, thorough examination of aborted fetuses and placentas might help to identify potential pathological changes in the fetus and placental–fetal interface, which could provide insight into the possible mechanisms underlying miscarriage in women following HEV239 vaccination.

Contributors

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

Declaration of interests

We declare no competing interests.

Data sharing

The data collected for this study will be accessible to other members of the scientific community upon request, in accordance with the data sharing policies of icddr,b and NIPH. To ensure the integrity of the research, standard criteria for data sharing will be employed, subject to approval by qualified researchers. To request data access, please email asma.aziz@ivi.int and kzaman@icddrb.org.

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References

1 WHO. Hepatitis E. https://www.who.int/news-room/fact-sheets/detail/hepatitis-e (accessed June 5, 2023).
2 Qian Z, Li T, Zhang Y, et al. Prevalence of hepatitis E virus and its association with adverse pregnancy outcomes in pregnant women in China. *J Clin Virol* 2023; **158:** 105353.
3 Bigna JJ, Modiyinji AF, Nansseu JR, et al. Burden of hepatitis E virus infection in pregnancy and maternofoetal outcomes: a systematic review and meta-analysis. *BMC Pregnancy Childbirth* 2020; **20:** 426.
4 Zhu FC, Zhang J, Zhang XF, et al. Efficacy and safety of a recombinant hepatitis E vaccine in healthy adults: a large-scale, randomised, double-blind placebo-controlled, phase 3 trial. *Lancet* 2010; **376:** 895–902.
5 Zaman K, Julin CH, Aziz AB, et al. Safety and effectiveness of a recombinant hepatitis E vaccine among women of childbearing age in rural Bangladesh: a phase 4, double-blind, cluster-randomised, controlled, trial. *Lancet Glob Health* 2024; **12:** e1288–99.
6 Lynch JA, Lim JK, Asaga PEP, et al. Hepatitis E vaccine—illuminating the barriers to use. *PLoS Negl Trop Dis* 2023; **17:** e0010969.
7 WHO. Hepatitis E vaccine: WHO position paper, May 2015. *Wkly Epidemiol Rec* 2015; **90:** 185–200.
8 Zaman K, Dudman S, Stene-Johansen K, et al. HEV study protocol: design of a cluster-randomised, blinded trial to assess the safety, immunogenicity and effectiveness of the hepatitis E vaccine HEV 239 (Hecolin) in women of childbearing age in rural Bangladesh. *BMJ Open* 2020; **10:** e033702.
9 Alam N, Ali T, Razzaque A, et al. Health and Demographic Surveillance System (HDSS) in Matlab, Bangladesh. *Int J Epidemiol* 2017; **46:** 809–16.
10 Wu T, Zhu FC, Huang SJ, et al. Safety of the hepatitis E vaccine for pregnant women: a preliminary analysis. *Hepatology* 2012; **55:** 2038.
11 Biotest. hCG one-step pregnancy test cassette (urine)—package insert. Hangzhou: Hangzhou Biotest Biotech, 2017.
12 Arifeen SE, Ekström EC, Frongillo EA, et al. Cohort profile: the Maternal and Infant Nutrition Interventions in Matlab (MINIMat) cohort in Bangladesh. *Int J Epidemiol* 2018; **47:** 1737–38e.
13 Zhong G, Zhuang C, Hu X, et al. Safety of hepatitis E vaccination for pregnancy: a post-hoc analysis of a randomized, double-blind, controlled phase 3 clinical trial. *Emerg Microbes Infect* 2023; **12:** 2185456.
14 Zeileis A, Köll S, Graham N. Various versatile variances: an object-oriented implementation of clustered covariances in R. *J Stat Softw* 2020; **95:** 1–36.
15 Ahmad SA, Sayed MH, Barua S, et al. Arsenic in drinking water and pregnancy outcomes. *Environ Health Perspect* 2001; **109:** 629–31.
16 Milton AH, Smith W, Rahman B, et al. Chronic arsenic exposure and adverse pregnancy outcomes in Bangladesh. *Epidemiology* 2005; **16:** 82–86.
17 Rahman A, Persson LÅ, Nermell B, et al. Arsenic exposure and risk of spontaneous abortion, stillbirth, and infant mortality. *Epidemiology* 2010; **21:** 797–804.
18 Das S, Sagar S, Chowdhury S, Akter K, Haq MZ, Hanifi SMA. The risk of miscarriage is associated with ambient temperature: evidence from coastal Bangladesh. *Front Public Health* 2023; **11:** 1238275.
19 Bhattacharya P, Hossain M, Rahman SN, et al. Temporal and seasonal variability of arsenic in drinking water wells in Matlab, southeastern Bangladesh: a preliminary evaluation on the basis of a 4 year study. *J Environ Sci Health A Tox Hazard Subst Environ Eng* 2011; **46:** 1177–84.
20 Aktar S, Nu UT, Rahman M, et al. Trends and risk of recurrent preterm birth in pregnancy cohorts in rural Bangladesh, 1990-2019. *BMJ Glob Health* 2023; **8:** e012521.
21 Risch HA, Weiss NS, Clarke EA, Miller AB. Risk factors for spontaneous abortion and its recurrence. *Am J Epidemiol* 1988; **128:** 420–30.

22 Tan J, Xiong YQ, He Q, et al. Peri-conceptional or pregnancy exposure of HPV vaccination and the risk of spontaneous abortion: a systematic review and meta-analysis. *BMC Pregnancy Childbirth* 2019; **19:** 302.

23 Moreira ED Jr, Block SL, Ferris D, et al. Safety profile of the 9-valent HPV vaccine: a combined analysis of 7 phase III clinical trials. *Pediatrics* 2016; **138:** e20154387.

24 Baril L, Rosillon D, Willame C, et al. Risk of spontaneous abortion and other pregnancy outcomes in 15–25 year old women exposed to human papillomavirus-16/18 AS04-adjuvanted vaccine in the United Kingdom. *Vaccine* 2015; **33:** 6884–91.

25 Khuroo MS. Hepatitis E and pregnancy: an unholy alliance unmasked from Kashmir, India. *Viruses* 2021; **13:** 1329.

26 Khuroo MS. Discovery of hepatitis E and its impact on global health: a journey of 44 years about an incredible human-interest story. *Viruses* 2023; **15:** 1745.

27 Qian Z, Li T, Xia Y, et al. Genotype 4 hepatitis E virus replicates in the placenta, causes severe histopathological damage, and vertically transmits to fetuses. *J Infect* 2023; **87:** 34–45.

28 Li M, Li S, He Q, et al. Hepatitis E-related adverse pregnancy outcomes and their prevention by hepatitis E vaccine in a rabbit model. *Emerg Microbes Infect* 2019; **8:** 1066–75.

29 Khuroo MS, Kamili S, Jameel S. Vertical transmission of hepatitis E virus. *Lancet* 1995; **345:** 1025–26.