Safety of hepatitis E vaccine in pregnancy: an emulated target trial following a mass reactive vaccination campaign in Bentiu internally displaced persons camp, South Sudan

Robin C Nesbitt, Andrew S Azman, Vincent Kinya Asilaza, Jessie K Edwards, Priscillah Gitahi, Patrick Nkemenang, Jetske Duncker, Melat Haile, Primitive Gakima, Joseph F Wamala, Fredrick Beden Loro, Duol Biem, Nelly Staderini, Manuel Albela, Monica Rull, John Rumunu, Iza Ciglenecki, Etienne Gignoux

Summary

Background Epidemic forms of hepatitis E cause high mortality among pregnant people, with case fatality risks over 30% and adverse fetal outcomes. In 2022, the first mass reactive vaccination campaign against hepatitis E was conducted in South Sudan with the HEV239 vaccine. We aimed to assess whether vaccination against hepatitis E in pregnancy increases the risk of fetal loss in a cohort of vaccinated and unvaccinated pregnant people.

Methods In this emulated target trial, an exhaustive pregnancy census was conducted in Bentiu internally displaced persons camp after the second of three vaccination rounds. Women and girls aged 14–45 years with no current jaundice or acute illness were eligible for participation. Individuals who consented were revisited 28 days after their delivery date to document the pregnancy outcome. We used an emulated target trial framework to address biases inherent in observational studies. We matched vaccinated to unvaccinated participants on age, gestational age, and vaccination propensity score and estimated cumulative incidence functions for fetal loss in vaccinated compared to unvaccinated women in a competing risks framework using the Aalen-Johansen estimator.

Findings Between May 16 and June 30, 2022, 3421 participants were enrolled and followed up for inclusion in analysis. Among 2741 women who had a pregnancy outcome after the start of the vaccination campaign, 67 (2·4%) were vaccinated before conception, 2036 (74·3%) were vaccinated during pregnancy, and 638 (23·2%) were not vaccinated. Among the 2407 women retained in the matched analyses, the cumulative risk of fetal loss among individuals vaccinated during pregnancy was 7·2% (95% CI 5·6–8·7) compared with 6·1% (3·7–9·2) among unvaccinated individuals, implying a risk ratio of 1·2 (95% CI 0·7–1·9).

Interpretation No evidence of increased risk of fetal loss was found among individuals vaccinated during pregnancy.

Funding Médecins Sans Frontières.

Lancet Glob Health 2024; 12: e1881–90

Epicentre, Paris, France (R C Nesbitt DrScHum, E Gignoux MPH); **Médecins Sans Frontières, Geneva, Switzerland** (A S Azman PhD, M Haile PhD, P Gakima MD, N Staderini MPH, M Albela MSc, M Rull MD, I Ciglenecki MD); **Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD, USA** (A S Azman); **Geneva Centre for Emerging Viral Diseases and Division of Tropical and Humanitarian Medicine, Geneva University Hospitals, Geneva, Switzerland** (A S Azman); **Médecins Sans Frontières, Juba, South Sudan** (V Kinya Asilaza MSc, P Gitahi MPH, P Nkemenang MD, J Duncker MD); **University of North Carolina, Chapel Hill, NC USA** (J K Edwards PhD); **World Health Organization, Juba, South Sudan** (J F Wamala PhD, F B Loro MPH); **Ministry of Health, Juba, South Sudan** (D Biem MBBS, J Rumunu MD)

Correspondence to: Dr Robin C Nesbitt, Epicentre, Paris 75019, France **robin.nesbitt@epicentre.msf.org**

Introduction

Hepatitis E virus genotypes 1 and 2 (g1/g2) are transmitted through the fecal–oral pathway and affect populations with poor access to clean water and insufficient sanitation infrastructure. In the general population, 5–30% of HEV g1/g2 infections are thought to be symptomatic, and case fatality ratios are less than 4%.[1] The major risk with g1 infection occurs in pregnancy, particularly in the second or third trimester where case fatality risks of up to 31% have been documented for the pregnant person,[2,3] in addition to a high risk of fetal loss.[4]

The only licensed vaccine against hepatitis E is a recombinant vaccine with a three-dose schedule, registered for use among people aged 16 years and older (HEV239). Clinical trials reported a 100% vaccine efficacy in the first year, few mild adverse events,[5] and 93% efficacy at 4·5 years.[6] Given the substantial, documented risk to pregnant people with hepatitis E virus infection, and the low expected risk posed by a recombinant vaccine, WHO recommended in 2015 that the hepatitis E vaccine be considered to prevent and mitigate outbreaks, including among pregnant people,[7] and further reiterated this recommendation in 2021.[8]

Although pregnant people are more at risk of death or fetal loss due to hepatitis E virus infection than other individuals are, evidence of vaccine safety in pregnancy is sparse. Pregnant people have been excluded from hepatitis E vaccine clinical trials; however, some data are available from inadvertently vaccinated pregnant people in studies using HEV239. In the original phase 3 trial of HEV239 in China,[9] 37 pregnant people were inadvertently vaccinated with HEV239. No serious adverse events, non-elective fetal loss, or congenital malformations occurred, suggesting no abortive or teratogenic effects.[9] Similarly, 66 pregnant people were inadvertently vaccinated with HEV239 in a phase 3 trial of an HPV vaccine in China[10] in which HEV239 was used as control and no serious adverse reactions were documented. A phase 4 safety trial

Research in context

Evidence before this study

On Jan 24, 2024, we searched PubMed with the search terms: (((hepatitis e) AND (preg*)) AND ((vaccine) OR (vaccination)) AND (safety)), with no date or language restrictions. The search yielded 30 records. All titles and abstracts were screened, and full texts were read for five records that described studies on the safety of hepatitis E vaccines among human pregnant people. Two records were excluded after review: one protocol of a phase 4 safety trial of HEV239 for non-pregnant female individuals aged 16–39 years in Bangladesh, with no results published at the time of writing. The second reported feasibility results of a reduced schedule of the hepatitis E vaccine in Bangladesh, where pregnant people were excluded. Three relevant papers reported results from two primary clinical trials. A 2012 paper from Wu and colleagues reported a post-hoc analysis of the original phase 3 trial of HEV239, where 37 pregnant people were inadvertently vaccinated with the hepatitis E vaccine. No serious adverse events, non-elective fetal loss, or congenital malformations occurred. Two records reported results on a phase 3 clinical trial of an HPV vaccine (Cecolin) where the hepatitis E vaccine (HEV239) was used as a comparator vaccine. One record reports a post-hoc analysis of the main study where 66 people were inadvertently vaccinated during pregnancy with HEV239 and 74 with Cecolin; no vaccine-related serious adverse reactions were reported, and the incidence of serious adverse events in the two groups was not statistically different.

Added value of this study

This study was conducted alongside the first use of the HEV239 vaccine in a mass reactive vaccination campaign in South Sudan, where pregnant women were intentionally offered the vaccine. Before this study, the evidence was limited to approximately 100 inadvertently vaccinated people in clinical trials in China. We present real-world evidence on pregnancy outcomes among over 2000 vaccinated and unvaccinated pregnant people during an hepatitis E outbreak, where this vaccine has the potential for an immediate impact on mortality. We used an emulated target trial framework to control for biases inherent in observational data and found no increased risk of fetal loss associated with vaccination.

Implications of all the available evidence

All available evidence suggests that the HEV239 vaccine is safe in pregnancy, although this evidence comes from post-hoc trial analyses and observational data in an hepatitis E outbreak. The high risk of death and fetal loss due to infection provides a strong rationale for vaccine use among pregnant people in outbreak settings, and the evidence on safety should strengthen confidence in existing WHO recommendations for vaccine use.

of HEV239 for non-pregnant people aged 16–39 years[11] was conducted in Bangladesh and, in a secondary analysis published in 2024, investigators found that people vaccinated with HEV239 very early in their pregnancy (median 2 weeks) or within the 90 days before conception had a higher incidence of miscarriage compared with those receiving the control Hepatitis B vaccine.[12]

The South Sudan Ministry of Health and Médecins Sans Frontières implemented a mass reactive vaccination campaign against hepatitis E using HEV239 in response to an hepatitis E virus genotype 1 outbreak in the Bentiu internally displaced persons (IDP) camp, Unity State, South Sudan.[13] The vaccination campaign took place in three rounds in March 22–30, April 19–26, and Oct 4–25, 2022. All residents aged 16–40 years of Bentiu IDP camp (26 848 people) were eligible for vaccination, including pregnant people. A post-campaign survey found self-reported coverage with one or more dose was 86%, two or more doses was 73%, and three-dose coverage was 58% among the vaccine eligible population.[14] We conducted a cohort study to compare pregnancy outcomes among female individuals who were vaccinated and those who were not.

Methods

Study design

This emulated target trial was based in Bentiu IDP camp between May 16, 2022, and April 25, 2023. Ethics approval was granted for this study from the Médecins Sans Frontières Ethical Review Board (approval number 2167) and by the South Sudan Ministry of Health Research Ethics Review Board (54/27/09/2022). Participants had to be a woman or girl aged 14–45 years and living in the study area for inclusion. Gender data were collected by asking the head of household how many women lived there.

Census and follow-up methods

We conducted a household census in Bentiu IDP camp from May 16 until June 30, 2022—19 days after the second vaccination round ended on April 26, 2022—to enrol female individuals into a pregnancy cohort. A team went systematically door-to-door in Bentiu camp using the camp management address system.

Heads of households were approached for verbal consent to discuss household composition, and if consented, to provide the number of female individuals aged 14–45 years residing in their household. Interviewers then asked each woman individually for verbal consent to ask about their pregnancy status. If they consented, we asked whether they had been pregnant at any point in time between Jan 1, 2022, and the day of the interview. If yes, we asked for written informed consent. In the case of illiteracy, participants were asked to mark a left thumb impression and an additional signature from a witness was obtained; the witness confirmed that the participant

was fully informed. We also asked household heads about any female individuals residing in their household who had been pregnant during this period, but who had left or died before the time of the interview. We also asked whether the woman had had jaundice (using locally appropriate terminology referring to yellow colouring of the sclera) or was diagnosed with hepatitis E during the pregnancy period.

We collected information on vaccination status, and pregnancy history at enrolment. If the individual already had a pregnancy outcome (ie, livebirth, miscarriage, or stillbirth), all information was collected at the enrolment interview. If they were pregnant at the enrolment interview, we scheduled a follow-up visit 28 days after the estimated delivery date. If they were no longer residing in Bentiu IDP camp at the follow-up visit, a senior member of the household with knowledge of the pregnancy was delegated to give the interview on their behalf.

Vaccination status was self-reported; to help participants recall the hepatitis E vaccine specifically, interviewers specified the vaccination campaign dates and locations, and showed photographs of the vaccine and vaccination card during the interview. When available, photographs were taken of vaccination cards. For all currently or previously pregnant individuals, the start of pregnancy was defined as first day of last menstrual period, which was obtained from the participant's antenatal care card. If an antenatal care card was not available, participants reported estimated gestational age and we calculated last menstrual period and estimated delivery date using 280 days as the expected gestational age for full term. Pregnancy outcome and outcome date were self-reported and cross-checked on the postnatal section of the antenatal care card, if available. If either pregnancy start or outcome date were missing, they were calculated using the other date available (appendix pp 8–9). We used a composite fetal loss outcome (miscarriage and stillbirth) for exposure at any time during pregnancy, and miscarriage alone in a subgroup analysis of exposure in the first trimester. Type of fetal loss was self-reported and checked in the postnatal section of the antenatal care card, if available.

The hepatitis E vaccine was only available during fixed campaign periods. After data collection, we cross-checked and corrected vaccination dates following an algorithm and using the actual campaign dates to minimise errors (appendix pp 8–9). Additionally, to ensure accurate recording, we randomly verified vaccination dates for 100 individuals per round by comparing the dates entered on the forms with photos of vaccination cards taken by the surveyor during the interviews.

Emulated target trial framework

To help overcome the potential biases involved with observational studies, we used an emulated target trial framework, where the protocol for the ideal randomised clinical trial that would have been conducted is specified, and then emulated with observational data.[15,16] To account for the imbalance in the distribution of risk factors for fetal loss and gestational age between vaccinated and unvaccinated people, we matched vaccinated to unvaccinated people at a 1:1 ratio on maternal age, gestational age (using calendar week of conception), and propensity to be vaccinated at each vaccination round using a propensity score.

We aimed to answer two causal questions and therefore specify two target trials (panel). The primary target trial aims to assess whether vaccination against hepatitis E during pregnancy increases the risk of fetal loss. The second target trial aims to assess whether vaccination against hepatitis E before conception increases the risk of fetal loss.

Cohort and risk periods

Participants were categorised according to the timing of vaccine exposure relative to their pregnancy. For the first target trial on exposure to hepatitis E vaccine during

***Panel*: Framework for target trials**

The details of the implementation of the framework to our data is provided in the appendix (p 10).

Eligibility criteria:

- HEV239 vaccine target trial 1, pregnancy: currently pregnant people, any gestational age
- HEV239 vaccine target trial 2, before conception: people planning to become pregnant in the next 3 months
- Other criteria: resident of Bentiu internally displaced persons camp, aged 16–40 years, and no current jaundice or acute illness

Treatment strategies
At least one dose of HEV239 or placebo

Treatment assignment
Randomly assigned to HEV239 or placebo

Time zero (start of follow-up)
Assignment to HEV239 or placebo first dose

End of follow-up
Pregnancy outcome (miscarriage, stillbirth or livebirth) or Feb 28, 2023

Outcomes
Miscarriage (fetal loss before 20 weeks), stillbirth (fetal loss after 20 weeks), or composite fetal loss outcome (either miscarriage or stillbirth)

Causal contrast of interest
Per-protocol and intention to treat

Analyses
Estimate risk in each group and compare with risk ratios

See Online for appendix

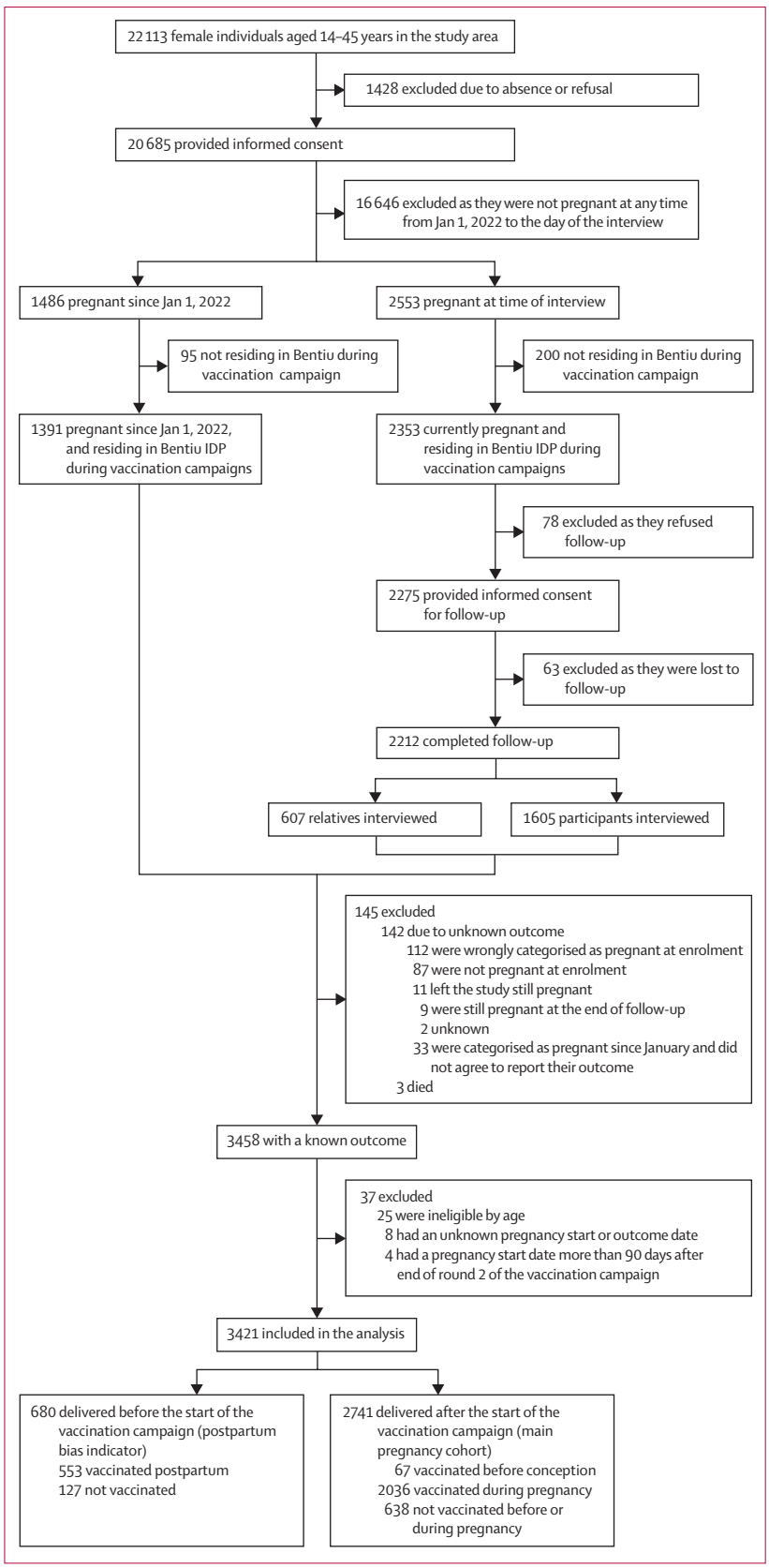

Figure 1: **Trial profile**
IDP=internally displaced persons.

pregnancy, people who were pregnant at any point during the vaccination campaign period of March 22 to April 26, 2022 were included. For the second target trial on exposure up to 3 months before conception, people who were vaccinated before conception were included. This group consisted of women who received the vaccine during the campaign (March 22 to April 26, 2022) and who declared themselves as pregnant during enrolment (between May 16 and June 30, 2022), with their pregnancy starting after receiving the vaccine. A subgroup of unvaccinated female individuals with a pregnancy start within the same calendar time range as women vaccinated before conception were used as a comparison group (March 25 to June 26, 2022).

Vaccination after end of pregnancy cannot, by definition, influence fetal outcome. To understand potential differences between female individuals who were vaccinated and those who were not, we conducted a bias indicator analysis including those who had a pregnancy outcome before the vaccination campaigns (postpartum exposure), between Jan 1 and March 22, 2022—the start of the first vaccination campaign round.

Statistical analysis

Descriptive statistics are reported using mean (SD), and median (minimum–maximum) for continuous variables and frequency and proportions for categorical variables. We assessed the significance of associations between categorical variables using χ^2 tests, and parametric student's two sample t tests for continuous variables. In our analysis we count pregnant people at the level of pregnancy; twins count as one pregnancy and at least one fetal loss is counted as a fetal loss for that pregnancy.

In both target trial matched analyses, vaccinated female individuals were matched to unvaccinated female individuals in two steps: the first step was to identify the unvaccinated female individuals who (1) had a pregnancy that started in the same week (within 1 week either side), and (2) were of the same maternal age (within 1 year) as the vaccinated individual. For the primary target trial, we additionally required that unvaccinated people were pregnant at the time when the matched vaccinated pregnant person received their first vaccination (enrolment for emulated trial). An unvaccinated person could be matched with more than one vaccinated person (ie, with replacement). Multiple unvaccinated people fit these conditions for each vaccinated person. People who were vaccinated for the first time during the second or third round in April and October, 2022, were considered unvaccinated and eligible to be matched as unvaccinated until their vaccination date, when both members of the vaccinated pair were censored. We used inverse probability of censoring weighting to try to correct for

this informative censoring. After vaccination, the previously unvaccinated person became eligible to be matched as a vaccinated person.

The second step was to select the most similar unvaccinated person among possible matches using a propensity score for vaccination at either the first or the second vaccination rounds. We estimated a propensity score for the outcome of vaccination at the first and the second vaccination rounds separately using logistic regression with variables associated with vaccination. The propensity score model included the following linear covariates: maternal age, jaundice during pregnancy, week and year of conception, education, antenatal care attendance (yes *vs* no where yes is at least one visit at enrolment and no is no visits), number of previous pregnancies, number of previous fetal losses (ie, miscarriages and stillbirths) and whether the date of outcome was known or extrapolated from date of conception. Date of outcome was extrapolated as 276 days from conception for livebirth, 70 days for miscarriage, and 196 days for stillbirth. The model used to estimate the inverse probability of censoring weights employed the same set of covariates.

Among matched people, we used the Aalen–Johansen estimator to estimate the cumulative incidence functions for adverse outcomes among vaccinated and unvaccinated people, using the time of vaccination to index a time origin for each matched pair. Fetal loss was considered the event of interest and livebirth a competing event. A subgroup analysis was done considering only people vaccinated within the first 90 days of pregnancy and their matched individuals, looking separately at miscarriage as an outcome alone. We used quantile bootstrapping to estimate 95% CIs on the cumulative incidence functions with 1000 iterations.

We conducted sensitivity analyses varying the range of age and gestational age for matching, as well as the variables included in the propensity score, and considering only people who could confirm vaccination with a card.

Role of the funding source

Employees of the study funder, Médecins Sans Frontières, and its research affiliate, Epicentre, were involved in study design, data collection, data analysis, data interpretation, and writing of the report.

Results

The team addressed heads of household at 11 197 (92·2%) out of 12 139 shelters listed for inclusion in the study. In total, 20 685 (93·5%) of 22 113 female individuals aged 14–45 years provided informed consent (figure 1). Among them, 1486 (7·2%) had a pregnancy outcome between Jan 1, 2022, and their enrolment interview (in the timeframe of May 16 to June 30, 2022), and 2553 (12·3%) were pregnant at interview. Among the people who were pregnant at interview, 2353 (92·2%) were residing in Bentiu at the time of the vaccination campaign, and

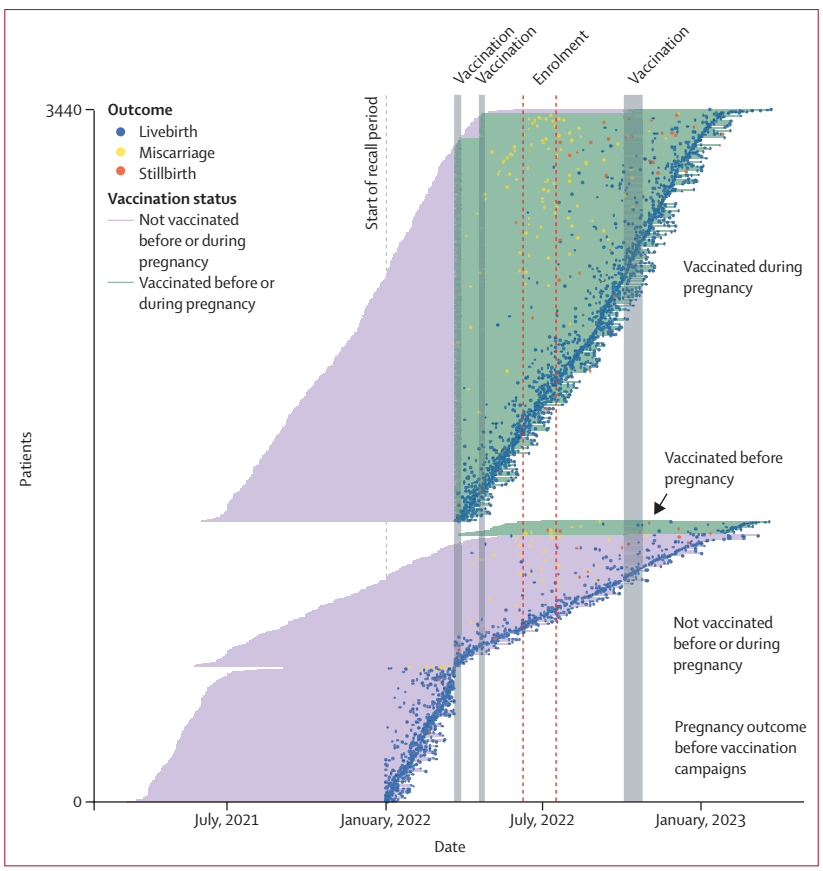

Figure 2: **Timeline of pregnancies within the full cohort**
Each horizontal line represents an enrolled participant's pregnancy start and end (dot), based on vaccination status (colour). Green lines represent vaccinated person-time and purple represents unvaccinated person-time. Dots represent pregnancy outcomes, with blue representing livebirths, orange representing miscarriages, and red representing stillbirths. Dashed lines represent the period of enrolment and transparent grey bars represent vaccination round periods. The recall period for pregnancy started on Jan 1, 2022.

2275 (96·6%) of them agreed to a follow-up visit after their expected delivery date. The team conducted a follow-up interview for 2212 (97·2%) participants and obtained outcome information from 2100 (94·9%) of those people. Among the participants who had previously been pregnant, 1391 (93·6%) were residing in Bentiu at the time of a vaccination campaign. Among them, 33 (2·4%) refused to report their pregnancy outcome. We therefore had outcome information reported for 3458 women in total. 37 (1·1%) individuals were excluded due to age eligibility, unknown pregnancy dates, or pregnancy outside of vaccination period, resulting in 3421 (98·9%) women included in the analysis. Among the 2741 (80·1%) participants who had a pregnancy outcome after the start of the vaccination campaign, 67 (2·4%) were vaccinated before conception, 2036 (74·3%) were vaccinated during pregnancy, and 638 (23·2%) were not vaccinated (figure 2).

Data quality assessment revealed that, among the 300 individuals whose vaccination card photos were taken by the surveyor during the interview (100 per round

	Not vaccinated during pregnancy (n=638)	Vaccinated during pregnancy (n=2036)	Overall (n=2674)	p value
Mean age enrolment, years (SD)	25·1 (6·1)	25·4 (6·2)	25·3 (6·2)	0·23
Median age at enrolment, years (min–max)	24·0 (16·0–40·0)	25·0 (16·0–40·0)	25·0 (16·0–40·0)	..
Gestational age at enrolment				
Before conception	0	3 (0·2%)	3 (0·1%)	0·0005
First trimester	50 (7·8%)	208 (10·2%)	258 (9·7%)	..
Second trimester	204 (32·0%)	780 (38·3%)	984 (36·8%)	..
Third trimester	154 (24·1%)	500 (24·6%)	654 (24·5%)	..
Postpartum	230 (36·1%)	545 (26·8%)	775 (29·0%)	..
Attended antenatal care	507 (79·5%)	1783 (87·6%)	2290 (85·6%)	0·0005
Antenatal care card available at interview	303 (47·5%)	1222 (60·0%)	1525 (57·0%)	0·59
Jaundice during pregnancy	8 (1·3%)	6 (0·3%)	14 (0·5%)	0·019
Malaria during pregnancy	245 (38·4%)	753 (37·0%)	998 (37·3%)	0·54
Vaccination confirmed by card	NA	1367 (67·1%)	..	..
Pregnancy outcome circumstance				
During transport	1 (0·2%)	2 (0·1%)	3 (0·1%)	0·0045
Health facility	551 (86·4%)	1858 (91·3%)	2409 (90·1%)	..
At home	76 (11·9%)	154 (7·6%)	230 (8·6%)	..
Traditional birth attendant	6 (0·9%)	12 (0·6%)	18 (0·7%)	..
Other	4 (0·6%)	10 (0·5%)	14 (0·5%)	..
Complication during delivery	53 (8·3%)	209 (10·3%)	262 (9·8%)	0·12
Caesarean section	4 (0·6%)	9 (0·4%)	13 (0·5%)	0·65
Gravidity				
Mean (SD)	2·35 (2·17)	2·57 (2·15)	2·52 (2·16)	0·029
Median (min–max)	2·0 (0·0–9·0)	2·0 (0·0–11·0)	2·0 (0·0–11·0)	..
Previous fetal loss				
Mean (SD)	0·11 (0·38)	0·13 (0·46)	0·13 (0·44)	0·33
Median (min–max)	0·0 (0·0–3·0)	0·0 (0·0–6·0)	0·0 (0·0–6·0)	..
Fetal outcome				
Livebirth	598 (93·7%)	1892 (92·9%)	2490 (93·1%)	0·80
Miscarriage	29 (4·6%)	107 (5·3%)	136 (5·1%)	..
Stillbirth	11 (1·7%)	37 (1·8%)	48 (1·8%)	..

Data are n (%) unless otherwise specified. NA=not applicable.

Table 1: **Characteristics of cohort by vaccination status during pregnancy**

of vaccination), seven (2·3%) of the recorded dates on the forms were inconsistent, 18 (6·0%) differed by within 3 days, and 275 (91·5%) were consistent.

The mean age at enrolment was 25·4 years (SD 6·2) for people vaccinated during pregnancy, and 25·1 (SD 6·1) for unvaccinated people (table 1). Antenatal care attendance was higher among participants vaccinated during pregnancy (87·6%) than among unvaccinated participants (79·5%; p=0·0005; table 1). The mean number of previous pregnancies was 2·57 (SD 2·15) among those vaccinated during pregnancy and 2·35 (SD 2·17) among those not vaccinated (p=0·029). 14 (0·5%) participants reported jaundice during pregnancy overall; however, more unvaccinated participants reported jaundice (eight, 1·3%) than vaccinated (six, 0·3%; p=0·019), suggesting a protective effect of vaccination.

Most participants (90·1%) had their pregnancy outcome in a health facility, and this care-seeking behaviour differed by vaccination status (86·4% unvaccinated *vs* 91·3% vaccinated during pregnancy, p=0·0045). Approximately 10% of participants self-reported a complication during delivery (eg, postpartum haemorrhage, infection or sepsis, or obstructed labour), and less than 1% had a caesarean section. Overall, 15 (0·6%) of women had twin pregnancies, all with concordant fetal outcomes including two (13·3%) pregnancies with twin fetal loss.

People vaccinated before conception (n=67) were slightly older than unvaccinated women with similar gestational age (date of conception between March 25 and June 26, 2022; n=44) with a mean age of 26·2 years (SD 6·76) for vaccinated individuals and 25·1 years (6·2) for unvaccinated individuals; appendix p 2). 30 (44·8%) women vaccinated before conception had attended antenatal care by their enrolment visit compared with 22 (50%) unvaccinated women with similar gestational ages (appendix p 2). 52 (77·6%) women vaccinated before conception had an outcome in a facility compared with 31 (70·5%) unvaccinated, and 13 (19·4%) vaccinated before conception had an outcome at home compared with 11 (25%) unvaccinated (appendix p 2). None of these individuals, vaccinated or unvaccinated, reported jaundice during their pregnancy.

Participants who had a pregnancy outcome before the vaccination campaign (appendix p 3)—the bias indicator population—were similar to those who had an outcome afterwards. Their mean age at enrolment was 25·7 years, and 616 (90·6%) attended antenatal care during pregnancy. Within this population, women vaccinated were slightly more likely to have attended antenatal care compared to unvaccinated women (506 [91·5%] *vs* 110 [86·6%], p=0·09). Six (0·9%) of 680 women who had a pregnancy outcome before the vaccination campaign had jaundice (explained as yellow-coloured eyes) or were diagnosed with hepatitis E during pregnancy.

Three (0·1%) participant deaths were documented during follow-up. According to relatives, two of the individuals who died were not vaccinated and one received two doses during their pregnancy (appendix p 1). Specific causes of death and exact dates were unknown by the family members, and we were unable to obtain medical records.

The proportion of fetal loss was 7·1% (144 of 2036) among those vaccinated during pregnancy and 6·3% (40 of 638) among unvaccinated individuals (p=0·54; table 1). Among individuals vaccinated before conception, 23·9% (16 of 67) had fetal loss, compared with 20·5% (nine of 44) among unvaccinated women whose pregnancy started within the same time range (p=0·82; appendix p 2). Among those in the bias indicator analysis, 1·6% (two of 127) of unvaccinated

	Vaccinated		Unvaccinated		Risk ratio (95% CI)
	Matched/total	Risk (95% CI)	Matched/total	Risk (95% CI)	
Pregnancy	1934/2036	7·2% (5·6–8·7)	474/638	6·1% (3·7–9·2)	1·2 (0·7–1·9)
Before conception	45/67	20·0% (6·3–34·5)	21/638	20·0% (5·4–52·5)	1·0 (0·2–3·3)
Bias indicator (postpartum)	460/553	0·4% (0·0–1·2)	107/127	0·0% (0·0–0·0)	NA

NA=not applicable.

Table 2: **Cumulative incidence (risk) of fetal loss by vaccination status and exposure period after matching**

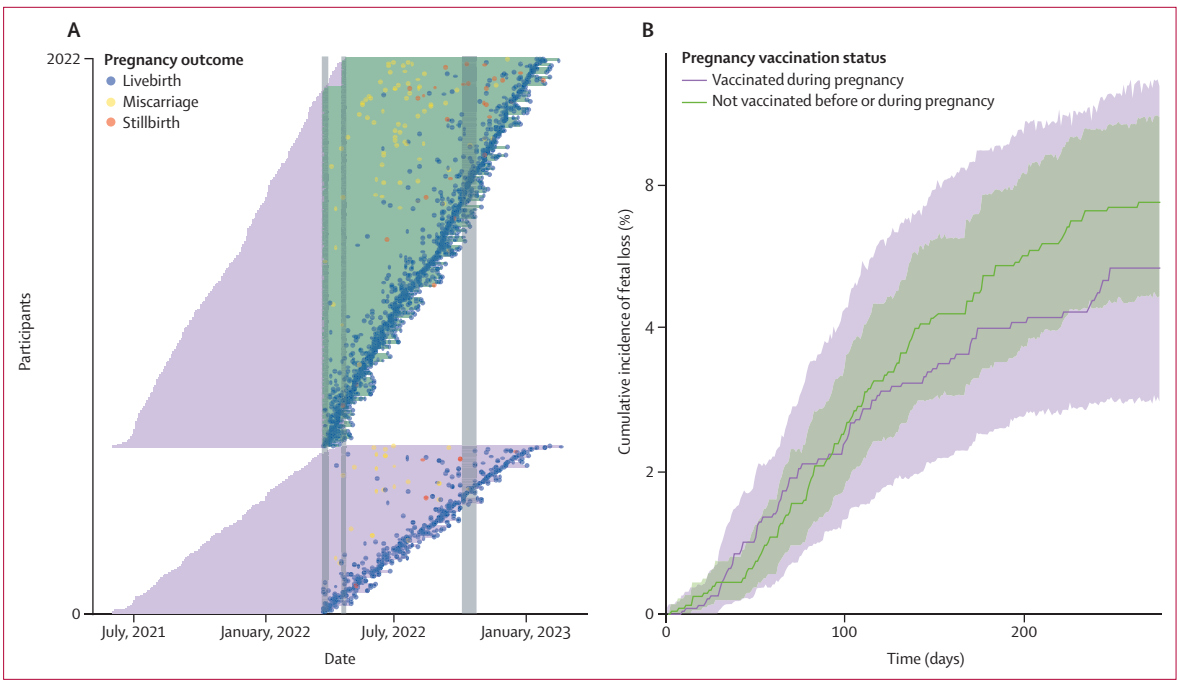

Figure 3: **Timeline (A) and cumulative Incidence curves for fetal loss (B) for matched women in pregnancy cohort emulated target trial 1**
Each horizontal line represents an enrolled participant's pregnancy start and end (dot), based on vaccination status (colour). (A) Green lines represent person-time vaccinated and purple lines represent person-time unvaccinated. Grey vertical bars represent the vaccination campaign periods. (B) Green lines represent the cumulative incidence of fetal loss among people vaccinated during pregnancy and purple lines represent cumulative incidence among those unvaccinated. Bootstrap 95% CIs are shown in shaded area.

individuals had fetal loss compared with 3·0% (16 of 553) of vaccinated people (p=0·84, appendix p 3).

Participants who reported jaundice during their pregnancy were twice as likely to have a fetal loss than those not reporting jaundice (two [14·3%] of 14 *vs* 198 [7·3%] of 2727), although this difference was not statistically significant (p=0·62).

In the primary analysis, 1928 vaccinated individuals were matched to 479 unvaccinated individuals, with each unvaccinated individual matched to a median of two (range 1–11) vaccinated individuals. Among the 1928 control participants who were initially unvaccinated, 509 remained unvaccinated until the second round in April and were censored when they received the vaccination at the second round. These 509 individuals had a median gestational age at the time of censoring of 136 days (IQR 80–256). They contributed a median time of 27 days (IQR 21–32) to the cumulative incidence risk. We estimated a cumulative risk of fetal loss of 7·2% (95% CI 5·6–8·7) for those who received at least one dose during pregnancy and a cumulative risk of 6·1% (3·7–9·2) for unvaccinated individuals (table 2, figure 3), implying a risk ratio of 1·2 (95% CI 0·7–1·9). In an analysis restricted to participants with fewer than 90 days gestation at exposure, we estimated a cumulative risk of 10·5 (95% CI 8·0–14·4) for miscarriage among vaccinated individuals and 11·7 (6·4–18·8) for miscarriage among unvaccinated individuals, giving a risk ratio of 0·9 (95% CI 0·5–1·9; appendix p 4).

In the secondary analysis, we matched 45 vaccinated to 21 unvaccinated participants. Each unvaccinated participant was matched to a median of one (range 1–6) vaccinated participant. We estimated a risk of fetal loss among individuals vaccinated before conception of 20·0% (95% CI 6·3–34·5) and a risk of fetal loss of

20·0% (5·4–52·5) among unvaccinated individuals, implying a risk ratio of 1·0 (95% CI 0·2–3·3; table 2).

In the bias indicators analysis, we were unable to compute the cumulative incidence risk ratio for postpartum exposure due to the absence of events in the unvaccinated groups after matching. In sensitivity analyses on the effect of different matching criteria on the estimated risk ratio for both target trials, we found no qualitative differences compared with the main analyses, with no evidence of increased risk of fetal loss among vaccinated women (appendix pp 4–5). We similarly found no evidence of increased risk of fetal loss among vaccinated participants in the sensitivity analyses considering vaccination status according to vaccination card (appendix p 6).

Discussion

We found no evidence of increased risk of fetal loss following vaccination during or before pregnancy in a cohort of vaccinated and unvaccinated people self-reporting known pregnancies following a mass reactive vaccination campaign during an outbreak of hepatitis E. Sensitivity analyses using different criteria on matching and propensity score showed similar results, as did subgroup analysis of the risk of vaccination during first trimester on miscarriage, the most sensitive time for fetal development.

South Sudan ranks among the lowest on maternal health indicators globally: nationally less than 20% of women have access to skilled attendance during delivery, estimates of the national maternal mortality ratio range from 789–1223 deaths per 100 000 pregnancies, and the stillbirth rate was estimated in 2015 at 30·1 per 1000 total births.[17] Given this mortality ratio, we could have expected between 26 and 41 participant deaths, and more than 100 stillbirths in this cohort—far more than the three participant deaths and 53 stillbirths we documented. In Bentiu IDP camp, pregnant people have better access to delivery care than many other parts of the country, with several primary health-care facilities within the camp and a referral system to the hospital at the periphery. Approximately 90% of participants in our cohort delivered in a health facility, which probably contributes to the lower documented number of deaths and fetal losses than expected nationally. Despite the hepatitis E outbreak and poor access to clean water and sanitation facilities, maternal health indicators in Bentiu appear better than much of the rest of the country.

We applied a matched target trial emulation methodology to account for biases inherent in observational data, specifying two target trials for two causal questions on receiving the vaccine or not before conception and receiving the vaccine or not during pregnancy. The emulated target trial framework has been used in numerous studies of different interventions during pregnancy, including antibiotic use,[15] antiretroviral therapy,[18] and COVID-19 vaccination.[16,19,20] By matching on age, gestational age, and propensity score, we attempted to control for the difference in baseline risk of fetal loss between vaccinated and unvaccinated participants. This methodology does not entirely replicate the randomisation or rigour of a randomised controlled trial and it is possible that the analysis is subject to residual bias and confounding. To understand the potential for bias in this study, we compared the proportion of livebirths between vaccinated and unvaccinated individuals resident of Bentiu IDP camp who delivered before the vaccination campaigns and found that they were similar. Criticism of matched target trial emulation studies points to a loss of precision because unmatched study participants are not included in matched analyses.[21] However, compared with previous studies, this primary target trial analysis, even after matching, had a large sample size and we were able to include 1934 (94·9%) of 2036 vaccinated women and 474 (74·3%) of 638 unvaccinated women.

Determination of exposure to vaccine during pregnancy relied on specific dates of conception, delivery, and vaccination. Antenatal care cards were available for 57·0% of individuals included in the pregnancy exposure analysis and vaccination cards were available for 67·1% of vaccinated individuals. These dates were self-reported for participants without cards, meaning that vaccine exposure during pregnancy might have been misclassified both due to imprecise recall (non-differential misclassification) and through intentional misreporting that could be related to pregnancy outcomes (differential misclassification). To minimise this potential bias, we cross-checked and corrected vaccination dates according to actual dates of the vaccination campaigns. Additionally, we randomly verified the vaccination dates entered on the form during the interviews against the vaccination card photos taken by the surveyor during the interview. We accepted interview responses from a senior member of the household at follow-up for approximately 600 participants, which could have resulted in misclassification of the pregnancy outcome; we attempted to account for this by including an indicator variable in the propensity score for whether the outcome was known or extrapolated, and we also conducted a sensitivity analysis excluding those who were not found at follow-up which gave similar results.

Due to concerns about acceptability and feasibility of pregnancy tests in the context of poor sanitation facilities and privacy, we relied on self-reported pregnancy status during enrolment. Therefore, it is likely that we missed women in their first trimester, who did not know they were pregnant or who did not yet feel comfortable reporting their pregnancy. If there was an effect of the vaccine on safety during a very early period of pregnancy, when the fetus might be most sensitive,[22] this study probably would not have been able

to accurately detect this and the overall results would be biased towards the null. However, the census was conducted 3–8 weeks after the end of the second round of the vaccination campaign, women who could have been vaccinated while unknowingly pregnant therefore had some time to realise and report their pregnancies before the enrolment interview. Furthermore, comparing the cumulative incidence risk of fetal loss across groups (20·0% among women vaccinated before conception and enrolled early in their pregnancy, 7·2% among women vaccinated during pregnancy and enrolled later in pregnancy, and 0·4% among women vaccinated postpartum and enrolled after their pregnancy ended) suggests that increasing recall period duration when asking retrospectively about pregnancy status and outcome reveals fewer pregnancy losses, and that we might have missed the inclusion of women who experienced early pregnancy losses in this cohort. However, early pregnancy loss did not appear to be differential with respect to vaccination status.

Before the vaccination campaign in Bentiu IDP camp and this cohort study, evidence on the safety of the hepatitis E vaccine in pregnancy came from analyses of inadvertent exposure of approximately 100 people in two clinical trials conducted by the vaccine manufacturer. We present data from over 2000 pregnant people vaccinated during an outbreak. We found no evidence for increased risk of fetal loss with vaccination in a crude analysis, nor after using a robust analytical method to account for possible differences in underlying risk between groups. Hepatitis E circulates in areas where sanitation and surveillance systems are weak,[23] and it is often deaths among pregnant women that alert health authorities to the start of hepatitis E outbreaks.[24,25] The evidence here on the safety in pregnancy of this efficacious vaccine can strengthen confidence in its use among pregnant women to prevent and mitigate outbreaks.

Contributors

RCN, EG, ASA, and IC conceptualised the study. RCN, VKA, and EG accessed, curated, and verified the data. RCN, EG, MA, and ASA did the formal analysis. RCN, EG, ASA, IC, and JKE designed the methods. RCN, PGi, PN, and JD were project administrators. VKA, DB, JR, and IC supervised the study. RCN, ASA, and EG wrote the original draft. RCN, ASA, VKA, JKE, PGi, PN, JD, MH, PGa, JFW, FBL, DB, NS, MA, MR, JR, IC, and EG reviewed and edited the paper. All authors had full access to all the study data if desired and had final responsibility for the decision to submit for publication.

Declaration of interests

Médecins Sans Frontières provided support in the form of salaries for ASA, VKA, PGi, PN, JD, MH, PGa, NS, MA, MR, and IC and indirectly provided salary support for Epicentre employees RCN and EG. All other authors declare no competing interests.

Data sharing

The minimal data set underlying the findings of this paper are available on request, in accordance with the legal framework set forth by Médecins Sans Frontières (MSF) data sharing policy (Karunakara U, *PLoS Med* 2013). MSF is committed to sharing and disseminating health data from its programmes and research in an open, timely, and transparent manner to promote health for populations while respecting ethical and legal obligations towards patients, research participants, and their communities. The MSF data sharing policy ensures that data will be available upon request to interested researchers while addressing all security, legal, and ethical concerns. All readers can contact the MSF generic address data.sharing@msf.org, or the Epicentre generic address epimail@epicentre.msf.org to request the data that can be shared with researchers subject to the establishment of a data sharing agreement to provide the legal framework for data sharing.

Acknowledgments

We thank the community of Bentiu IDP camp and the pregnant people who participated in this study. We thank all the study team members who conducted the interviews. We thank the Bentiu IDP camp management and health partners, including the Unity State Ministry of Health and primary health care centres for their support. The study was funded by the Operational Centre of Geneva of MSF. MSF programmatic funding covered all other costs associated with the study, including transportation, accommodations, materials, and equipment.

References

1 Lhomme S, Marion O, Abravanel F, Izopet J, Kamar N. Clinical manifestations, pathogenesis and treatment of hepatitis E virus infections. *J Clin Med* 2020; **9:** 331.
2 Al-Shimari FH, Rencken CA, Kirkwood CD, Kumar R, Vannice KS, Stewart BT. Systematic review of global hepatitis E outbreaks to inform response and coordination initiatives. *BMC Public Health* 2023; **23:** 1120.
3 Boccia D, Guthmann JP, Klovstad H, et al. High mortality associated with an outbreak of hepatitis E among displaced persons in Darfur, Sudan. *Clin Infect Dis* 2006; **42:** 1679–84.
4 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.
5 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.
6 Zhang J, Zhang XF, Huang SJ, et al. Long-term efficacy of a hepatitis E vaccine. *N Engl J Med* 2015; **372:** 914–22.
7 WHO. WHO position paper on hepatitis E vaccines. 2015. https://www.who.int/publications-detail-redirect/WER9018-185-200 (accessed March 7, 2023)
8 WHO. Meeting of Strategic Advisory Group of Experts on Immunization, October 2021: conclusions and recommendations. *Week Epidemiol Rec* 2021; **96:** 613–32.
9 Wu T, Zhu FC, Huang SJ, et al. Safety of the hepatitis E vaccine for pregnant women: a preliminary analysis. *Hepatology* 2012; **55:** 2038.
10 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.
11 Zaman K, Julin CH, Aziz AB, et al. Safety and effectiveness of a recombinant hepatitis E vaccine in women of childbearing age in rural Bangladesh: a phase 4, double-blind, cluster-randomised, controlled trial. *Lancet Glob Health* 2024; **12:** e1288–99.
12 Aziz AB, Dudman S, Julin CH, et al. Receipt of hepatitis E vaccine and fetal loss in rural Bangladesh: further analysis of a double-blind, cluster-randomised, controlled trial. *Lancet Glob Health* 2024; **12:** e1288–99.
13 Ciglenecki I, Rumunu J, Wamala JF, et al. The first reactive vaccination campaign against hepatitis E. *Lancet Infect Dis* 2022; **22:** 1110–11.
14 Nesbitt RC, Asilaza VK, Gignoux E, et al. Vaccination coverage and adverse events following a reactive vaccination campaign against hepatitis E in Bentiu displaced persons camp, South Sudan. *PLoS Negl Trop Dis* 2024; **18:** e0011661.
15 Caniglia EC, Zash R, Fennell C, et al. Emulating target trials to avoid immortal time bias—an application to antibiotic initiation and preterm delivery. *Epidemiology* 2023; **34:** 430–38.
16 Hernández-Díaz S, Huybrechts KF, Chiu YH, Yland JJ, Bateman BT, Hernán MA. Emulating a target trial of interventions initiated during pregnancy with healthcare databases: the example of COVID-19 vaccination. *Epidemiology* 2023; **34:** 238–46.

17 Lawn JE, Blencowe H, Waiswa P, et al. Stillbirths: rates, risk factors, and acceleration towards 2030. *Lancet* 2016; **387:** 587–603.

18 Caniglia EC, Zash R, Jacobson DL, et al. Emulating a target trial of antiretroviral therapy regimens started before conception and risk of adverse birth outcomes. *AIDS* 2018; **32:** 113–20.

19 Hernán MA, Wang W, Leaf DE. Target trial emulation: a framework for causal inference from observational data. *JAMA* 2022; **328:** 2446–47.

20 Dagan N, Barda N, Biron-Shental T, et al. Effectiveness of the BNT162b2 mRNA COVID-19 vaccine in pregnancy. *Nat Med* 2021; **27:** 1693–95.

21 Pearce N, Vandenbroucke JP. Are target trial emulations the gold standard for observational studies? *Epidemiology* 2023; **34:** 614–18.

22 Alwan S, Chambers CD. Identifying human teratogens: an update. *J Pediatr Genet* 2015; **4:** 39–41.

23 Azman AS, Ciglenecki I, Wamala JF, et al. Hepatitis E should be considered a neglected tropical disease. *PLoS Negl Trop Dis* 2019; **13:** e0007453.

24 Gurley ES, Hossain MJ, Paul RC, et al. Outbreak of hepatitis E in urban Bangladesh resulting in maternal and perinatal mortality. *Clin Infect Dis* 2014; **59:** 658–65.

25 Kim JH, Nelson KE, Panzner U, Kasture Y, Labrique AB, Wierzba TF. A systematic review of the epidemiology of hepatitis E virus in Africa. *BMC Infect Dis* 2014; **14:** 308.