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# Design of a cluster-randomized, blinded trial to assess the safety, immunogenicity and effectiveness of the hepatitis E vaccine HEV239 (Hecolin®) in women of childbearing age in rural Bangladesh



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# Design of a cluster-randomized, blinded trial to assess the safety, immunogenicity and effectiveness of the hepatitis E vaccine HEV239 (Hecolin®) in women of childbearing age in rural Bangladesh

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## ABSTRACT:

### Introduction

Hepatitis E virus (HEV) is a leading cause of acute viral hepatitis in the developing world and is a public health problem, in particular among pregnant women, where it may lead to severe or fatal complications. A recombinant HEV vaccine, 239 (Hecolin® (Xiamen Innovax Biotech.Xiamen, China), is licensed in China, but WHO calls for further studies to evaluate the safety and immunogenicity of this vaccine in vulnerable populations, and to evaluate protection in pregnancy. We are therefore conducting a phase IV trial to assess the effectiveness, safety, and immunogenicity of the HEV 239 vaccine when given in women of childbearing age in rural Bangladesh, where HEV infection is endemic.

### Methods and analysis

Enrollment of a target of approximately 20,000 non-pregnant women, aged 16-39, started on October 2, 2017 in Matlab, Bangladesh. Sixty-seven villages were randomized by village at a 1:1 ratio to receive either the HEV vaccine or the control vaccine (hepatitis B vaccine). A 3-dose vaccination series at 0, 1, and 6 months is ongoing, and women are followed up of for 24 months. The primary outcome is confirmed HEV disease among pregnant women. After vaccination participants are requested to report information about clinical hepatitis symptoms. Participants who become pregnant are visited at their homes every 2 weeks to collect information about pregnancy outcome and to screen for clinical hepatitis. All suspected hepatitis cases undergo laboratory testing for diagnostic evaluation. The incidence of confirmed HEV disease among pregnant and non-pregnant women will be compared between the HEV vaccinated and control groups, safety and immunogenicity of the vaccine will also be evaluated.

## Ethics and dissemination

The protocol was reviewed and approved by the icddr,b Research Review Committee (RRC) and Ethical Review Committee (ERC), and the Directorate General of Drug Administration (DGDA) in Bangladesh, and by the Regional Ethics Committee (REC) in Norway. This article is based on the protocol version 2.2 dated 29.06.2017. We will present the results through peer-reviewed publications and at international conferences.

## Trial registration

The trial is registered at clinicaltrials.gov with the registry name «Effectiveness Trial to Evaluate Protection of Pregnant Women by Hepatitis E Vaccine in Bangladesh» and the identifier NCT02759991.

## STRENGTHS AND LIMITATIONS OF THE STUDY:

- This trial is conducted in a defined population covered by a long-term demographic surveillance registry, in a site that has a well-established clinical and laboratory infrastructure and effective referral systems.
- Women are visited in their homes for 7 days following vaccination to follow up and record details of any adverse events.
- Active surveillance of acute hepatitis is conducted through home visits every 2 weeks to all the pregnant participants during their pregnancy.
- Dried blood spots (DBS) are used to assess immunogenicity of the vaccine, simplifying blood collection and making storage of 40 000 samples feasible in a community setting.
- A limitation of this trial design is the lack of blood sampling at the end of follow up, thus we will not be able to explore the occurrence of asymptomatic hepatitis E.

# INTRODUCTION

## Background and rationale

Hepatitis E virus (HEV) is an enteric RNA virus causing outbreaks or endemics in developing countries with poor sanitation and is one of the leading causes of acute hepatitis worldwide.[1] Pregnant women are particularly vulnerable to HEV infection, with a high rate of maternal mortality, miscarriage, premature delivery, and stillbirth.[2]

An effective vaccine could prevent symptomatic HEV infection in vulnerable people. Only two HEV vaccines have been evaluated in human clinical trials, namely rHEV (GlaxoSmithKline), and HEV 239 (Hecolin®, Xiamen Innovax Biotech Co.,Ltd.,China), of which only the latter has undergone further commercial development.[3] Zhu et al. studied this vaccine in a large phase III clinical study in China, where 100,000 healthy men and women (aged 16-65 years) received either the HEV vaccine or a hepatitis B (HBV) vaccine.[4] They found more than 90 % protection against symptomatic HEV infection. The adverse events related to the vaccine were few and mild, and there were no vaccine-related serious adverse events. The vaccine is currently only licensed in China for people 16-65 years old, and is recommended for individuals with a high risk of HEV infection.

The WHO Strategic Advisory Group of Experts made a working group to review the evidence on the HEV 239 vaccine and make recommendations for its use. They concluded that knowledge gaps prevents recommendation of the vaccine in endemic countries, and that further studies should evaluate the safety and immunogenicity of this vaccine in children, the elderly, persons with underlying diseases or conditions such as immunosuppression or liver disease, and immunogenicity and protection in pregnant women. [3 5]

HEV comprises eight genotypes, of which mainly four infect humans.[6] HEV genotypes 1 and 2 dominate human infections in developing countries. Genotypes 3 and 4 primarily infect animals that can further transmit the virus to humans, causing illness in both developed and developing countries. Genotype 4 predominates in mainland China, where the previous phase III vaccine trial was conducted, but there are limited data on protection of the vaccine against the other genotypes. A small study investigated immunogenicity against genotypes 1-4 after vaccination with p239 and found that in humans, IgG antibodies reacted slightly stronger against genotypes 1 and 2 versus 3 and 4, which could be due to the presence of genotype-specific neutralizing antibodies.[7] However, the vaccine still needs to be tested in other geographical areas to fully evaluate efficacy against all the genotypes that frequently cause illness in humans.[3]

We are currently conducting a phase IV cluster-randomized clinical trial (2017- ongoing) with the HEV 239 vaccine to provide more data on the effectiveness of the vaccine on genotype 1 and the outcome in pregnant women, and on safety of the vaccine. The trial is conducted in a rural area of Bangladesh where genotype 1 is predominant, and includes women aged 16-39, allowing us to evaluate effectiveness and immunogenicity of the vaccine among women who become pregnant following vaccination.

## Objectives

### Primary objective

- To determine the effectiveness of the HEV 239 vaccine given to women of childbearing age in rural Bangladesh in preventing HEV disease during pregnancy

### Secondary objectives:

- To determine the safety and immunogenicity of the HEV 239 vaccine in Bangladeshi women of childbearing age

- To measure the effectiveness of the HEV 239 vaccine in preventing HEV disease in non-pregnant Bangladeshi women of childbearing age

### Trial design

This study is a phase IV cluster-randomized, double blinded trial on the safety and effectiveness of three doses of the HEV 239 vaccine in women of childbearing age (16-39) in Bangladesh. Participants are sampled at day 0 and day 210 and followed up as described in Table 1.

## METHODS AND ANALYSIS

### Study setting

The study field site for this clinical trial is located in Matlab in rural Bangladesh, where HEV is endemic.[8] icddr,b has maintained a field research site in this area for more than fifty years with an ongoing health and demographic surveillance system covering the entire population. The icddr,b field site comprises 67 villages, with a total population of about 116,000 and a well-established infrastructure including health care services (hospital and local health clinics), laboratory facilities, and effective referral systems.

### Randomization and blinding

All 67 villages were randomized by village at a 1:1 ratio to receive either HEV vaccine or HBV vaccine. All participants, investigators and field staff are blinded. The vaccine administrators, however, cannot be blinded since the dose of the licensed HBV vaccine in Bangladesh is different for women below and above 18 years, while the HEV vaccine dose is identical for all age groups. Each village is allocated to two vaccine codes (one for 16-18 years

and another for 19-39 years) with eight codes in total; two codes for each vaccine in each age group. To achieve similar distributions of the codes for the two vaccines in different age groups, computerized allocation was employed taking the population size of the villages into consideration. The randomization was rerun until the groups were balanced in size (<200 in difference). An independent statistician from Johns Hopkins University performed the randomization. The vials were labeled with the eight respective codes by Incepta Pharmaceuticals Ltd., Bangladesh, who fill-finished and bottled the vaccine shipped in bulk from China.

The treatment allocation code is being securely kept under lock and key and may be broken by the investigator only in case of a Serious Adverse Events (SAEs) for which knowledge of the participant's treatment assignment may influence her or the clinical care or if the event is unexpected and suspected to be causally related to the investigational product. Code breaking will be reported to the clinical monitor and Data Safety and Monitoring Board (DSMB) within 24 hours. The participant may continue in the study with protocol-specified follow-up despite unblinding, unless she fulfils any protocol-defined exclusion criteria.

## Recruitment and enrolment

All eligible women in the study area, identified through existing Matlab surveillance system, are being approached at their homes by designated study staff who inform about the nature of the study according to the International Conference on Harmonization of Good Clinical Practice (ICH-GCP) in their local language, and the participants may inquire about the details of the study. Eligibility is further assessed according to the inclusion and exclusion criteria in table 2.

A urinary pregnancy test is offered to women who have missed a period. Eligible participants visit a fixed site clinic for enrolment, where they sign a consent form. In the case of

16-17-year-old participants, their legal guardians sign an assent form in place of the consent form. A Case Report Form (CRF) with an identification number is then created for each participant. Confidentiality of personal identifiers is maintained by keeping names noted in primary data instruments in secure files and by removing names from the computerized dataset for analysis. A flow chart of anticipated participant recruitment is shown in Figure 1.

## Withdrawal

The participants and/or their legal guardians are informed that participation is voluntary, and that they may withdraw consent at any time, without giving a reason and without prejudice to further treatment. Participants who withdraw may demand destruction of samples and deletion of data concerning themselves. Participants may be discontinued from the study by the investigator at any time in case of safety reasons or significant protocol deviations. The reason for withdrawal will be recorded in the CRF. If withdrawal is due to an adverse event, appropriate follow-up will be arranged.

## Interventions

The HEV 239 vaccine is based on a 239 amino acid long recombinant HEV peptide, corresponding to amino acids 368-606 of open reading frame 2 that encodes the capsid protein of HEV. The amino acid sequence is derived from a genotype 1 Chinese HEV strain. HEV 239 was developed and is produced by Innovax. The vaccine is expressed in *Escherichia coli*,[9] and contains 30 µg of the purified protein absorbed to 0.8 mg of aluminium hydroxide suspended in 0.5 ml of buffered saline. The control vaccine is a commercial hepatitis B vaccine (Hepa-B®) produced by Incepta. Each 0.5 ml dose (for age 16-18 yrs) contains 10 µg of hepatitis B surface antigen absorbed on aluminium hydroxide gel equivalent to $Al^{3+}$ 0.25 mg. A 1.0 ml dose for older

persons contains 20 µg of hepatitis B surface antigen absorbed on aluminium hydroxide gel equivalent to $Al^{3+}$ 0.5 mg. We chose this control vaccine for the following reasons: it will benefit the target population who have not received this vaccine through the child immunization program; the dosing regimen is the same as the HEV vaccine; and it was the control vaccine in the phase III trial,[4] facilitating comparison with previous results.

Innovax donated bulk HEV vaccine for the trial, while Incepta filled the HBV and HEV vaccines in identical, single dose vials, respectively. Both bulk vaccines and finished products were quality tested and labelled according to ICH-Good Manufacturing Practice (GMP).

The vaccines are administered intramuscularly in the deltoid muscle of either arm, in a 3-dose regimen on day 0, at one month, and at six months (Table 1).

## Follow up/Surveillance

The full study period will last for 2 ½ years after enrollment of the final participant. (**Table 1)**

### Hepatitis surveillance

Each participant get an immunization card with identification numbers and a phone number, and are instructed to contact the investigator immediately if they experience jaundice or have any of the following symptoms for at least three days: fatigue, loss of appetite, abdominal discomfort, abdominal pain in the right upper quadrant, nausea, or vomiting. This passive surveillance for hepatitis is ongoing throughout the full study period for all participants.

The participants are reminded by cell phone to report cases of jaundice (>80% of households have cell phone access). Study staff make household visits to screen for signs of hepatitis regardless of whether the woman is pregnant or not, and women with suspected hepatitis will be tested for liver function and virological causes of hepatitis (A, B, C, E). All participants

who become pregnant during the study period are visited every two weeks to collect information about pregnancy outcomes and to screen for clinical hepatitis. The last study visit to pregnant women is 14 days after delivery, to record information on the health status of the child.

### Adverse events surveillance

After each vaccination dose, participants are observed for 30 minutes and visited at their homes by a field worker for seven consecutive days. During these visits, participants are specifically asked about any local reactions (e.g. erythema, swelling, induration and pain at the injection site) and systemic symptoms (e.g. nausea, malaise, myalgia, arthralgia, headache, fever). Participants are also asked to report any significant symptoms after the last vaccine dose, i.e.at least 2 years until the last study visit. After this visit, they are advised to report to the study team about any possible serious adverse events or signs of hepatitis, even after completion of the study.

## Outcomes

The primary outcome is clinical HEV disease among pregnant women. HEV disease is defined by illness lasting for at least 3 days, abnormal serum ALT level of ≥ 2.5 times upper limit of normal, detection of IgM anti-HEV in serum collected within 1 month after onset, and/or the presence of HEV RNA and/or ≥ 4 fold rise of IgG anti-HEV levels in paired sera. All acute hepatitis cases are also tested for the presence of markers of other viral hepatitis (A, B, C). Secondary outcomes are confirmed HEV disease in non-pregnant women, safety, and immunogenicity. Safety outcomes include all local and systemic adverse events, which are recorded in the participant case report form during the study period. Investigation of vaccine induced immune responses will include anti-HEV IgG on all participants, together with additional antibody and cellular responses on a subset of participants. A possible protective anti-

HEV IgG level will be sought. An antibody response to vaccination will be defined as a ≥ 4 fold rise of IgG anti-HEV levels in an individual's post vaccination sample (one month after the last dose) compared to baseline sample.[4]

## Sample size and power calculations

The sample size was calculated to estimate total vaccine protection of pregnant participants in a per protocol analysis (Figure 1). The following assumptions are based on data from Matlab health and demographic surveillance system and previous HEV research studies from the region.[8] We expect that 10% of women will not be included because of baseline pregnancy, and that the refusal rate will be 5%. After randomization, there will be 15% loss of person-time because of migration out of the study site, and 10% will not complete three vaccine doses for other reasons. During follow up after the last vaccine dose, we assume that 16% of women (followed over a mean of 16 months) will become pregnant and reach term before the end of surveillance; an additional 9% of women (followed over a mean of 9 months) will be followed for an average of half full term (4.5 months). This predicts that 20% of the dose 3 recipients will have "completed" pregnancies, 3,806 are expected to become pregnant after dose 3 and 3,073 to be followed to term. Follow up of the remaining pregnancies will be right censored by termination of follow up. We expect 15% of the pregnant dose 3 recipients to be lost to follow up because of migration out, while 5% are expected to withdraw from the study. We expect the design effect for this cluster randomized trial to be 2. We assume that 22% of the participants are HEV seropositive at baseline, indicating protection against HEV infection.[8] Furthermore, six percent of seronegative pregnant women are expected to become infected during pregnancy,[8] of which we assume 35% to be symptomatic. [8] The protective efficacy of a 3-dose regimen of HEV vaccine against symptomatic infections is >95%.[4] We therefore need a sample size of 20,745

women at baseline to achieve 80% power at P<.05 (two-tailed) for the analyses in pregnant women. Further, our study will have 90% power to show >95% protective efficacy against HEV disease among non-pregnant women. Our study is not powered to directly evaluate perinatal and maternal death, because of the low rates of these outcomes in Matlab. However, given the substantial evidence linking maternal and perinatal mortality to HEV disease in pregnancy,[2] demonstration of vaccine protection against confirmed HEV disease in pregnancy will provide strong evidence that vaccination is likely to prevent maternal and perinatal deaths.

## Data collection, management and analysis

The field staff are entering the required data into the paper-based CRFs through interviewing during vaccination and home visits, and by reviewing medical records when applicable. The data are further transcribed to an electronic data capturing system (developed by icddr,b using Oracle data base) within a week of the clinic visit. This system will automatically check data to detect errors and inconsistencies. The data in the system are reviewed weekly by the analyst programmer, and any data deleted from the main database will be saved in a shadow table. Data are stored in the Oracle database system in a central server at Matlab. An electronic database backup is made weekly by the data management team, and the final database is sent to the icddr,b data archive system.

Health registry data for study participants and their previous pregnancy outcome are also being collected. Field staff and medical officers are checking these data with the family record book. They are checking the participant data with the eligible participant list after every visit. The data analyst manager is verifying entered data biweekly. Any inconsistencies are resolved with the field staff and medical officers.

All completed CRFs and other documents are stored in a locked cabinet with limited personnel access. The CRF register in the data management center at Matlab keeps track of CRF movement between the file cabinet and the data management center. The log book contains the columns; participant ID, receipt date, visit number, number of pages, name of staff, purpose of file movement, return date, name of staff, and any relevant remarks. All informed consent forms are being filed and kept separately in a locked cabinet. Data are entered in electronic data base. A dedicated data management team will be responsible for data entry, cleaning, analysis and data archiving in de-identified way.

Blood samples are being analyzed by icddr,b and/or Norwegian Institute of Public Health (NIPH), according to predefined standard operating procedures. Additional blood will be stored in the research biobank at icddr,b in case re-analysis is needed.

## Statistical methods

### Analyses

In the primary analysis, the risk of confirmed HEV disease in pregnant women who received the HEV vaccine will be compared to the risk in women who received the HBV vaccine using Cox regression with shared frailty,[10] to adjust for the design effect of cluster randomization. Demographic and other baseline characteristics will be compared between the HEV and HBV vaccination clusters, for all participants, and separately for pregnant participants, to assess the degree to which randomization was achieved. Unbalanced covariates may be included in the models. Subgroup analyses will be performed to evaluate effectiveness in participants who are negative/positive at baseline for anti-HEV IgG antibodies, respectively, and subgroup analyses for effectiveness will also be performed per number of doses received. Additionally, subgroup analyses will be carried out to explore effectiveness in participants within different BMI and age

intervals. Safety analyses will be performed using generalized estimating equations (GEE) for logistic regression to account for cluster randomization.

### Analysis sets

The full analysis set (FAS) population will include all randomized participants who received at least one dose of either the study or the control vaccine. The per protocol (PP) population will include participants who were randomized and received three doses of the study or control vaccine, respectively, and provided blood samples according to the protocol schedule (Table 1). Pregnant FAS and PP populations are defined as participants from the respective populations with confirmed pregnancies during the study period. The primary effectiveness analysis will be performed on the pregnant PP population. Secondary effectiveness analyses will be performed on the pregnant FAS population, as well as the non-pregnant FAS and PP populations, with the same methods as the primary analysis. Safety analyses will be performed on the FAS population.

## Data and safety monitoring

### Data monitoring

An independent Data Safety and Monitoring Board (DSMB) with no competing interests was appointed to provide the icddr,b Ethical Review Committee (ERC) with an overall scientific, safety and ethical appraisal of the study. The DSMB also informs this committee regarding the progression of the study, with special attention to the safety of the participants. They convene at least once annually, and make recommendations directly to the ERC Chairperson. As described

in the ERC guidelines, the principal investigator prepares a report to the DSMB before each meeting, describing the accumulated adverse events and serious adverse events and a summary of the current data for inclusion, progress, and deviations from the protocol or planned procedures. Any reports from the monitor regarding quality control are also included. The DSMB is also responsible for detailed reviews of all the serious adverse events. More details can be found in the DSMB Charter included in the Trial Master file in the trial office in Bangladesh.

No interim analyses have been planned, and no stopping guidelines have been created for the trial. In the event of serious safety issues, the DSMB will meet to provide recommendations regarding termination of the trial. The steering committee will have the final decision to terminate the study.

## Harms

All adverse events observed or reported are logged in the relevant participant CRF by the study staff. The following information is registered: description of the adverse event (in precise standard medical terminology), time of onset and resolution, severity (mild/ moderate/ severe; according to Common Terminology Criteria for Adverse Events version 4.0, outcome, assessment of the causality with study drug, and action taken with study drug.

All serious adverse events are communicated to the DSMB within 24 hours after awareness by the study staff. Serious adverse event reports are initially sent to the Chairperson of the DSMB and the ERC with copy to sponsor/principal investigator. All adverse events and serious adverse events are being followed up to resolution unless the event is considered by the investigator to be unlikely to resolve due to underlying disease. We will endeavor that all events are resolved, even if they continue after study completion.

### Auditing

The sponsor (NIPH) has appointed an independent local clinical monitor to ensure that the study is conducted according to protocol, standard operating procedures, GCP and regulatory requirements, and to verify that investigators are collecting and reporting quality data. In addition, a monitor from NIPH is auditing the study. The monitors are periodically monitoring on-site, including at study initiation and at close-out. The monitors check the informed consent process, reporting of adverse events and other safety data, CRF completeness, and adherence to the study protocol for at least 10 % of the study participants randomly distributed between the 67 study villages. They also monitor maintenance of regulatory documents, study supply accountability, facilities and equipment.

## Patient and Public Involvement statement

The public was not involved in the development of the research question nor the study design.

## ETHICS AND DISSEMINATION

### Protocol amendments

Any significant amendments and/or new versions of the study protocol will be notified to and approved by the competent authority and the ethics committees in both countries according to EU and national regulations before changes are implemented.

### Confidentiality

All study-related information is being kept confidential, under lock and key, or on secure servers in case of digital information. The study monitor, the ERC, and any law-enforcing agency

will have access to this information only in the event of necessary inspection. The samples may be sent outside the country for analysis, and preserved for 5 years where applicable, however, any personal identifiable information will be held and processed under secured conditions with access limited to pre-identified staff. Remaining blood samples will be disposed of after completing testing for all participants in the study.

## Access to data

The study principal investigator, the co-investigators in the study, and the data analyst manager will have access to the final trial dataset.

## Dissemination policy

Upon study completion and finalization of the study report, we will submit the study results to the competent authority and ethics committees according to national regulations, and publish the results in peer-reviewed journals. We will report the trial in accordance with the CONSORT guidelines, with authorship based on the ICMJE recommendations.

## Acknowledgments

icddr,b acknowledges with gratitude to the Research Council of Norway for supporting this research through the GLOBVAC program. icddr,b is also grateful to the Governments of Bangladesh, Canada, Sweden and the UK for providing core/unrestricted support. We thank all the members of the research team at icddr,b who helped with participant recruitment, vaccination, sampling and follow up, and the laboratory team at icddr,b who performed laboratory procedures. The local clinical monitor (Dr. Wasif Ali Khan) is thanked for monitoring the trial. We also thank the data safety monitoring board (Prof. Kazi Zulifiquer Mamun, Prof S M Shamsuzzaman, Prof Saria Tasnim, Dr. Md. Nur Haque Alam, and Dr. Hanne M. Nøkleby), and the steering

committee (Dr. Shams El Arifeen, Dr. Rashidul Haque, Dr. Ingeborg Aaberge, and Prof. Dr. Geir Bukholm) for their important contributions throughout the trial. A special thanks to Innovax for their donation of the HEV vaccine.

NIPH is the sponsor for this clinical trial (Contact name: Susanne Dudman. Address: Norwegian Institute of Public Health, Postboks 222 Skøyen, 0213 Oslo, Norway).

We presented a poster of the study at 10th Conference on Global Health and Vaccination (GLOBVAC) Research in Trondheim, Norway in 2017.

## Funding statement

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## Authors' contributions

JDC, KZ, SD, KSJ, FQ, MY, SS, JØ, ESG, JLD, QN, AR, PKS, JK, and CHJ contributed to study design, KZ, JDC, AA, MK, SD, KSJ, JØ, SS, TRP, MR, WH, JLD and CHJ were involved in trial management. CHJ, KSJ, SD, JDC, KZ, SS, JLD, JØ, and ESG contributed in manuscript writing and editing. KZ, AA, MK, WH responsible for managing the field teams/logistics of the study. All authors read and approved the final manuscript.

## Competing interests statement

The authors reports no conflicts of interest in this work.

## Ethics approval

This trial is approved by the icddr,b Research Review Committee (RRC) and Ethical Review Committee (ERC), and by the Directorate General of Drug Administration (DGDA) in Bangladesh. Further, it has been approved by the Regional Ethics Committee (REC) in Norway, This article refers to protocol 2.2 dated 22.06.2017. The registration number of this trial is NCT02759991.

## Appendices

- Informed consent and assent.

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**Table 1. Participant timeline in the study.**

| | Contact with participant | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Visit 1 | Visit 2 | Visit 3-9 | Visit 10 | Visit 11-17 | Visit 18 | Visit 19-25 | Visit 26 | Visit 27 |
| Days | -1 | 0 | 1-7 | 30* | 31-37* | 180* | 181-187* | 210* | 2½ years |
| ENROLMENT | | | | | | | | | |
| Invitation visit | X | | | | | | | | |
| Eligibility screening | X | | | X | | X | | | |
| Informed consent | | X | | | | | | | |
| Demographics (age, height, weight, BMI), medications/medical history | | X | | X | | X | | X | X |
| INTERVENTIONS | | | | | | | | | |
| Vaccination | | X | | X | | X | | | |
| Blood sample† | | X | | | | | | X† | |
| SURVEILLANCE | | | | | | | | | |
| Hepatitis surveillance (active and passive) | Throughout the study period | | | | | | | | |
| Pregnancy surveillance | Throughout the study period | | | | | | | | |
| ASSESSMENTS | | | | | | | | | |
| Pregnancy home visit | Every two weeks | | | | | | | | |
| Physical exam | | | | | | | | | X |
| HARMS/SAFETY | | | | | | | | | |
| Immediate reactions | | X | | X | | X | | | |
| Home visit | | | X | | X | | X | | X |
| SAE | At any time following 1st vaccine dose | | | | | | | | |
| Participant reporting of AEs | At any time following 1st vaccine dose | | | | | | | | |
| Withdrawal | At any time following enrolment | | | | | | | | |

*±2 days

† Dried blood spots (DBSs) are collected before vaccination and one month after last vaccine dose, or earlier if off-study

**Abbreviations:** AE, Adverse Event, BMI; Body Mass Index, SAE; Serious Adverse Events

**Table 2** HEV Bangladesh study inclusion and exclusion criteria

| |
|---|
| Inclusion criteria |
| - Healthy non-pregnant female, aged 16-39 at time of first vaccination |
| - Living in the iccdr,b field site in Matlab, Bangladesh |
| - Willingly giving written informed consent |
| Exclusion criteria |
| - Pregnancy (visible or verbal report on date of last menstruation or urine for pregnancy test) |
| - History of severe allergic reaction to a vaccine or a vaccine component |
| - Received another vaccine or immunoglobulin within two weeks |
| - Serious chronic diseases (medical assessment) |
| - Acute or chronic infectious disease (medical assessment) |
| - Fever > 38 °C (axillary temperature) |

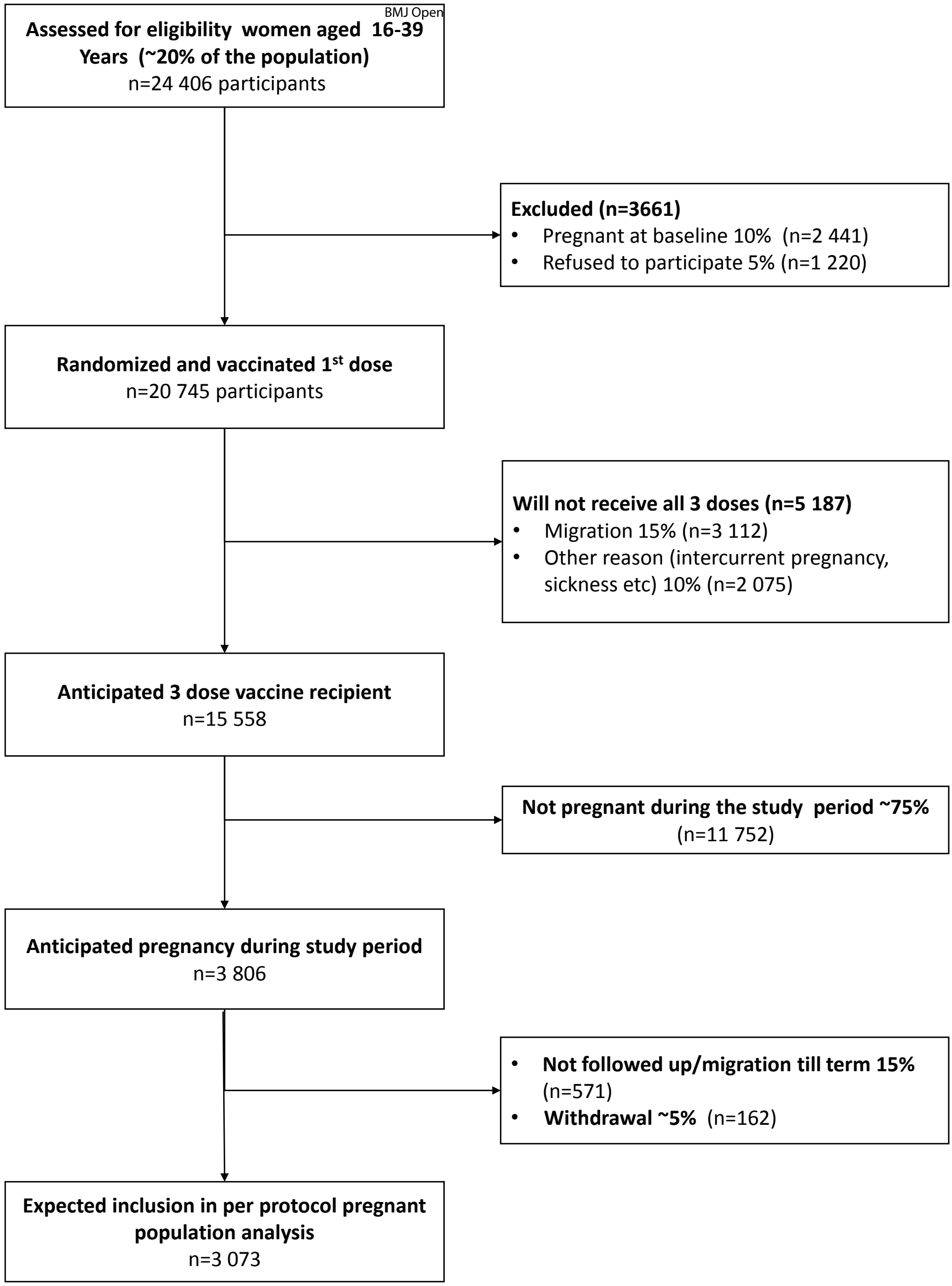


**Figure 1:** Anticipated participants enrollment flow chart

Forskningsrådet

# Assessment of grant application submitted to the Research Council of Norway

## Grant application

| | |
|---|---|
| Project number | 248143 |
| Project title | An effectiveness trial to evaluate protection of pregnant women by a HEV vaccine in Bangladesh and risk factors for severe HEV infection. |
| Project manager | Dudman, Susanne Gjeruldsen |
| Project Owner | Norwegian Institute of Public Health |
| Programme/Activity | Global helse- og vaksin.forskn |
| Case officer | Åse Marit Kristiansen |



## Confirmation

By completing and submitting this form, I / we confirm the following (applies for the individual referee or the referee panel):

| | |
|---|---|
| - I am /We are qualified to assess this application. See Regulations on Impartiality and Confidence in the Research Council of Norway. | Yes |
| - I/We have read and understood both the criteria I/we have been asked to use for assessing the application and the description of the scale of marks. The scale of marks is to be applied as an absolute scale, i.e. marks are to be determined for each grant application independently and not relative to other applications that the panel/referee is assessing. | Yes |
| - I/We understand and accept the guidelines for assessing applications for the Research Council of Norway. See Guidelines for referees/panels who assess applications for the Research Council of Norway. | Yes |
| - I am/We are qualified to conduct this assessment. | Yes |

Forskningsrådet

# Summary of marks

| Criterion | Mark |
|---|---|
| Scientific merit | 6 |
| The project manager and project group | 6 |
| Implementation plan and resource parameters | A |
| Dissemination and communication of results | B |
| Overall assessment of the referee/panel | 6 |

| Special points to consider | Answer |
|---|---|
| Relevance relative to the call for proposals | Very good |
| International cooperation | Very good |
| Ethical perspectives | Yes |

Forskningsrådet

# Criteria

## Scientific merit

How would you rank the project's scientific merit?

This criterion gives an indication of the essential, fundamental aspects of the research project. The scientific merit of a project will be assessed in relation to the following points:
* Originality in the form of scientific innovation and/or the development of new knowledge.
* Whether the research questions, hypotheses and objectives have been clearly and adequately specified.
* The strength of the theoretical approach, operationalisation and use of scientific methods.
* Documented knowledge about the research front.
* The degree to which the scientific basis of the project is realistic.
* The scientific scope in terms of a multi- and interdisciplinary approach, when relevant.

This is a very interesting and ambitious project which aims to conduct a large RCT of a novel recombinant HepE vaccine in women of child bearing age in Bangladesh. It represents a collaboration with a well established clinical trials centre with a strong track record and brings together vaccines manufactured in China and Bangladesh. If successful it would be a landmark project. The amount of funding requested is - as one might expect - substantial. The arguments that the study could potentially fill an important knowledge gap concerning a significant public health problem and would potentially lead to a significant advance in preventative care are convincing. The project is very ambitious and complex and aspects of the full protocol may need detailed evaluation - possibly even site visits - beyond the scope of this review panel process.

Specific issues:

1. Why is the design cluster- rather than individually randomised? Assessment of indirect effects appear to be largely beyond the scope and power of the study. Clusters may be organisationally easier but run the risk of introducing bias. Also classically cluster randomised studies are powered on the number of clusters although in this case - since individual protection is the main endpoint - the approach taken to sample size calculation may be appropriate. Nevertheless the choice to design the study this way needs clarification.

2. It will be critical to ensure that good manufacturing practice standards are adequate. HepE vaccine is to be imported from China and repackaged in Bangladesh alongside locally produced HepB vaccine. This is no trivial matter. If either vaccine is not handled perfectly and thus rendered inactive or suboptimal then the whole study will be undermined. Also the process of effective blinding and related QC monitoring would be critical. The funder would need to be thoroughly satisfied about the robustness of these aspects.

3. It is clear from the introductory summary that there are significant uncertainties around the epidemiology and diagnostic tests for HepE. This in turn raises uncertainties over the power of the study and the security of the primary endpoint. The funder might want some more concrete data on the performance of the lab methodologies to be used when in the hands of these investigators and applied in this population before proceeding. Perhaps the developmental work described in section 3.3.9 might usefully be done before the trial. In addition there may be a need for more concrete epidemiological data on prevalence and incidence in this population to reconfirm that the study is adequately powered and the capacity of the research team reliably to be able to ascertain cases.

4. Recruitment feasibility. The authors estimate they can recruit 20745 out of 23275 eligible subjects. Does pilot work need to be done to ensure such ambitious targets can be reached? Clearly this is an experienced site likely to have a good sense of what can be achieved - but against such a large investment one would want to be as confident as possible that under recruitment would not be a problem.

5. Safety assessment - adverse event reporting. Since the vaccine has only been used in one previous large scale study, this will be very important. The proposal focuses on the first 7 days post dose. Some effective mechanism of

Forskningsrådet

blinded passive or active SAE reporting also needs to be in place throughout the study.

6. Is HepB vaccine (control vaccine) in routine use in this population to any extent? If so how might this impact on recruitment?

7. Is the proportion of the population already seropositive/immune to HepE taken into account in the power calculation?

Selected mark : 6 - Excellent

The project's objectives, research questions and hypotheses are very clearly presented and are based on an excellently formulated and highly original project concept. The project is in the forefront of its field and will contribute to scientific innovation as well as generate important new knowledge. The project is of excellent quality, with no significant weak points. Publications in leading scientific journals in the field are highly likely.

## The project manager and project group

How would you rank the qualifications of the project manager and project group?

This criterion gives an indication of the qualifications of the project manager and project group. The project manager and project group will be assessed in relation to the following points:
* Project management
* Expertise and experience within the field of research
* Publication record
* Experience with national and international collaboration on projects
* Experience with supervision of students and younger researchers
* The degree to which the project manager and project group are part of a research environment that has the competence and resources needed to ensure the success of the project

The project lead and his centre have an established track record. Nevertheless aspects of the feasibility of this project in this site would need to be evaluated carefully for an investment of this size.

Selected mark : 6 - Excellent

The project manager and/or research/project group is/are qualified at a high international level, has/have contacts within the foremost national and international research environments and will be able to play an important role in ensuring the success of the project.

Forskningsrådet

## Implementation plan and resource parameters

How well-suited are the implementation plan and resource parameters in relation to the project?

This criterion gives an indication of whether the plan for project implementation is satisfactory, and whether the planned use of resources in the project is well-suited for the tasks in the project, based on assessment of the following elements:
* Plans for project implementation, including breakdown into work packages/sub-projects, milestones and deliverables.
* Need for personnel resources, as listed in terms of work time distributed by work packages, sub-projects or milestones.
* Need for other resources (such as equipment, data collection, field work), distributed by work packages/sub-projects or milestones.

The assessment is not to be linked to any scientific risk.

In principle the plan sounds feasible. Several aspects, including those listed above, would need evaluating carefully.

Selected mark : A - Very good
The project plan and planned use of resources are very clearly described and are well-suited to the tasks in the project.

## Dissemination and communication of results

How would you rank the quality of the dissemination and communication plans?

This criterion gives an indication of the quality of the dissemination and communication plans for the project. Dissemination and communication of results will be assessed in relation to the following points:
* Plans for scholarly publication, dissemination and other communication activities.
* Plans for popular science dissemination and communication activities vis-à-vis the general public as well as users of the project results, including planned use of channels and measures.
* Plans for ensuring that important users (in industry, community life and public administration) are incorporated into/take part in dissemination activities for the project.

When assessing dissemination and communication plans, importance should be attached to the level of detail provided and how realistic the plans are.

Plans look fine

Selected mark : B - Good
The project's dissemination and communication plans are satisfactory.

Forskningsrådet

## Overall assessment of the referee/panel

How does the project rank in terms of the referee's/panel's overall assessment?

This criterion indicates the overall view of the referee/panel, based on the specific criteria which they have been asked to assess.

A potentially important, exciting and very ambitious project. If taken forward, some additional evaluative work would need to be done to ensure its success.

Selected mark : 6 - Excellent
A project at a very high international level and of great national and international interest. Publications in leading journals are expected. The researchers are among the leaders in their field.

Forskningsrådet

# Special points to consider

| Special points to consider | Answer |
|---|---|
| Relevance relative to the call for proposals | Very good |
| International cooperation | Very good |
| Ethical perspectives | Yes |

## Comments to special points to consider

No obvious ethical issues

# Informed Consent Form
## Pretesting (male and female) and main study for female (18-39 years old)

| Protocol No.PR- 16014 | Version No. 2.2 | Date: 29.06.2017 |
|---|---|---|

Protocol Title: **An effectiveness trial (phase IV) to evaluate protection of pregnant women by Hepatitis E virus (HEV) vaccine in Bangladesh and risk factors for severe HEV infection.**

Investigator's name: K. Zaman
Organization: icddr,b

### Purpose of the research

The purpose of the main study is to test the effectiveness of hepatitis E virus (HEV) vaccine in preventing HEV disease during pregnancy among women in rural Bangladesh and to determine risk factors for severe HEV infection. Before starting the study, a pretesting phase will be conducted to confirm the feasibility and safety of vaccination and test the procedures.

### Background

Hepatitis E virus (HEV) infection is a major cause of liver inflammation worldwide and is the commonest cause of acute liver disease in South Asia, including Bangladesh. HEV spreads via contaminated drinking water and food. Pregnant women and their babies bear the greatest burden from HEV, since HEV infection causes high numbers of disease and death both in pregnant women and their babies. There are currently no effective medicines to prevent or treat HEV infection. So efforts to reduce the numbers of HEV infections in pregnancy in the South Asia region could have a major global impact. A newly developed vaccine from China (Innovax), has been shown to be safe and effective, but data showing that it can protect pregnant women are lacking. Bangladesh is an ideal setting to conduct this trial, because of the high number of HEV cases. Results from this trial will also be relevant for people living in many other countries affected by HEV.

### Why invited to participate in the study?

We are inviting you to participate in this HEV vaccine study in order to demonstrate how well the vaccine can prevent HEV infection in pregnancy and in the general population .We will invite 20,745 non-pregnant women aged 16-39 years in the main phase.

### Methods and procedures

### Pretesting:

We will do pretesting for 25 female and 25 male for HEV and HBV group respectively aged 16-39 years. They will receive 3 doses of HEV or HBV vaccine. We will take blood sample (maximum 3 ml) before vaccination and one month after the second dose of the vaccine. Among 100 participants, 10 participants will be randomly selected for 9 ml blood collection and a finger prick blood sample.

**Main phase:**

- We will give you either HEV vaccine or Hepatitis B vaccine (HBV) vaccine which will be determined by lottery.
- You will not know which vaccine you will receive.
- Vaccines will be administered as a 3-dose regimen on day 0, at one month, and six months.
- We would also like to take finger prick blood sample (maximum 300 micro litre) before vaccination and one month after the last dose of the vaccine. Among 20,745 participants, 50 participants will be randomly selected for 9 ml blood collection.
- We may take a saliva sample (approx 2 ml).
- After each vaccination dose, you will be observed for 30 minutes and a field worker will visit daily for 7 consecutive days at your home enquiring about any untoward events.
- You will also be asked to report if you have any local reactions (i.e., erythema, swelling, induration and pain at the injection site), systemic reactions (i.e., nausea, malaise, myalgia, arthralgia, and headache) and fever within 7 days after vaccination.

- You will be provided with an immunization card having study and HDSS identification numbers and with a phone number to call if you become ill with jaundice or similar symptoms.
- The phone number will be answered by our study staff member who will visit you to screen for suspected hepatitis.
- Regular SMS messages will be sent by cell phone to remind you to report about jaundice.

- We will do laboratory diagnostic tests if you have jaundice of any duration or illnesses lasting for at least 3 days with at least three of the following symptoms: abnormal tiredness, loss of appetite, stomach discomfort or pain, nausea or vomiting. In such cases, you will be referred to Matlab hospital for examination of hepatitis, including blood and stool tests.

**Risk and benefits**

When we take blood, there may be mild, but short lasting pain. The risk of infection is very small because we will clean skin thoroughly and use only new sterile needles.
If you receive the HEV-vaccine, you are expected to be benefitted with protection against HEV disease, particularly during pregnancy. If you receive the HBV-vaccine as part of the control group, it will protect you and your new-borns against HBV.
The project will give knowledge on how to reduce the burden of HEV in a highly endemic area struggling with diseases spread by water. The results will be especially beneficial for groups at particular risks who are eligible for a future HEV vaccine program.

**Privacy, anonymity and confidentiality**
We assure that the privacy, anonymity and confidentiality of data/information identifying you will be strictly maintained. We would keep all medical information, description of treatment, and results of the laboratory tests performed on you confidential, under lock and key. None other than the investigators of this research; possible study monitor; the Ethical Review Committee (ERC) of icddr,b; and any law-enforcing agency in the event of necessity would have an access to the information. We want to inform you that data/samples related to the study may be sent outside the country for analysis, and preserved for 5 years where applicable; however, any

personal identifiable information will be held and processed under secured conditions, with access limited to pre-identified staff of that organisation.

**Future use of information**

Information collected from this study may be used in future research. In such cases, anonymous or abstracted information and data may be supplied to other researchers, which should not conflict with or violate the maintenance of privacy, anonymity and confidentiality of information identifying you in any way.

After we have completed the blood testing for all participants in the study, any remaining samples will be disposed of.

**Right not to participate and withdraw**

Your participation in the study is voluntary, and you have the sole authority to decide for or against your participation. You are also able to withdraw your participation any time during the study, without stating any cause. Refusal to take part in or withdrawal from the study will involve no penalty or loss of care, benefits or attention. Even if you do not want to join this study, or if you withdraw from the study, you will still get all health services from Matlab Health Research Centre.

**Principle of compensation**

There are no costs to you for participating; all study procedures will be performed with no cost to you. You will not receive any compensation for your participation. In case of Serious Adverse Events related to the vaccine you will be given proper treatment and referred to hospital. If you develop fulminant hepatitis you will be referred to appropriate hospital for better management. All treatment cost will be covered by the study.

**Answering your questions/ Contact persons**

We will happily provide you further information about the study. You may communicate with the principal investigator of the study at the contact address given below.

Principal Investigator: K. Zaman
Tel: 880 1713047100

Please contact the IRB Secretariat in case you have any questions or want to know more about your rights and benefits as a study participant.

IRB Secretariat:
M. A. Salam Khan
Phone No: 9886498 or PABX 9827001-10 Extension. 3206

If you agree to our proposal of enrolling you in our study, please indicate that by putting your signature or your left thumb impression at the specified space below

Thank you for your cooperation

| | |
|---|---|
| Signature or left thumb impression of participant | Date |
| Signature of the impartial witness | Date |
| Signature of the PI or his representative | Date |


SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

| Section/item | Item No | Description | |
|---|---|---|---|
| **Administrative information** | | | |
| Title | 1 | Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym | P1 (Title page) |
| Trial registration | 2a | Trial identifier and registry name. If not yet registered, name of intended registry | P3 (Abstract) |
| | 2b | All items from the World Health Organization Trial Registration Data Set | See online reg details at clinicaltrials.orgg |
| Protocol version | 3 | Date and version identifier | P3 (Abstract) |
| Funding | 4 | Sources and types of financial, material, and other support | P18 (Funding statement) |
| Roles and responsibilities | 5a | Names, affiliations, and roles of protocol contributors | P18 (Authors contributions) |
| | 5b | Name and contact information for the trial sponsor | P17-18 (Acknowl.) |
| | 5c | Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority | P18 (Funding statement) |
| | 5d | Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee) | P16 (Auditing), P17-18 (Acknowl), P12-13 (Data coll, man. and analysis) |
| **Introduction** | | | |
| Background and rationale | 6a | Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms | P4-5 (Introduction) |
| | 6b | Explanation for choice of comparators | P8-9 (Interventions) |
| Objectives | 7 | Specific objectives or hypotheses | P5-6 (Objectives) |

| | | | |
|---|---|---|---|
| Trial design | 8 | Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, | P6 (Trial Design) |
| **Methods: Participants, interventions, and outcomes** | | | |
| Study setting | 9 | Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be | P6 (Study Setting) |
| Eligibility criteria | 10 | Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, | Table 2 |
| Interventions | 11a | Interventions for each group with sufficient detail to allow replication, including how and when they will be | P8-9 (Interventions) |
| | 11b | Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or | P8 (Withdrawal) |
| | 11c | Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests) | NA |
| | 11d | Relevant concomitant care and interventions that are permitted or prohibited during the trial | NA |
| Outcomes | 12 | Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm | P10-11 (Outcomes) |
| Participant timeline | 13 | Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended | P7-8 (Recruit. and enroll.) + Table 1 |
| Sample size | 14 | Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size | P11-12 (Sample Size and Power C.) |
| Recruitment | 15 | Strategies for achieving adequate participant enrolment to reach target sample size | P7-8 (Recruit. and enrol.) |
| **Methods: Assignment of interventions (for controlled trials)** | | | |
| Allocation: | | | |

| | | | |
|---|---|---|---|
| Sequence generation | 16a | Method of generating the allocation sequence (eg, computer- generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign | P6-7 (Random. and Blind.) |
| Allocation concealment mechanism | 16b | Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned | P6-7 (Random. and Blind.) |
| Implementation | 16c | Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions | P6-7 (Random. and Blind.) |
| Blinding (masking) | 17a | Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how | P6-7 (Random. and Blind.) |
| | 17b | If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial | P6-7 (Random. and Blind.) |
| **Methods: Data collection, management, and analysis** | | | |
| Data collection methods | 18a | Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection | P12-13 (Data coll., man, and an.) + P10 (Outcomes) |
| | 18b | Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention | Table 1 |
| Data management | 19 | Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol | P12-13 (Data coll., man, and an.) |
| Statistical methods | 20a | Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol | P13 (Stat. Methods-Analyses) |
| | 20b | Methods for any additional analyses (eg, subgroup and adjusted analyses) | P13 (Stat. Meth. - Analyses) |
| | 20c | Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple | P14 (Stat. Meth-Analyses Sets) |

| | | | |
|---|---|---|---|
| **Methods: Monitoring** | | | |
| Data monitoring | 21a | Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. | P14-15 (Data Monitoring) |
| | 21b | Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial | P14-15 (Data Monitoring) |
| Harms | 22 | Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial | P15 (Harms) |
| Auditing | 23 | Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor | P16 (Auditing) |
| **Ethics and dissemination** | | | |
| Research ethics | 24 | Plans for seeking research ethics committee/institutional review board (REC/IRB) approval | P19 (Ethics Approval) |
| Protocol amendments | 25 | Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators) | P16 (Protocol Amendments) |
| Consent or assent | 26a | Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32) | P7-8 (Recruit. and Enrol. |
| | 26b | Additional consent provisions for collection and use of participant data and biological specimens in ancillary | NA |
| Confidentiality | 27 | How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial | P7-8 (Recr. and enr.) P16-17 (Confidentiality) |
| Declaration of | 28 | Financial and other competing interests for principal investigators for the overall trial and each study site | P19 (Comp. int. statement) |
| Access to data | 29 | Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators | P17 (Access to Data) |
| Ancillary and post-trial care | 30 | Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation | P15 (Harms) |

| | | | |
|---|---|---|---|
| Dissemination policy | 31a | Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing | P17 (Dissemination Policy) |
| | 31b | Authorship eligibility guidelines and any intended use of professional writers | NA |
| | 31c | Plans, if any, for granting public access to the full protocol, participant- level dataset, and statistical code | NA |
| **Appendices** | | | |
| Informed consent | 32 | Model consent form and other related documentation given to participants and authorised | Appendix 1 |
| Biological specim | 33 | Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if | P10-11 (Outcomes) |

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

# HEV study protocol : Design of a cluster-randomized, blinded trial to assess the safety, immunogenicity and effectiveness of the hepatitis E vaccine HEV239 (Hecolin®) in women of childbearing age in rural Bangladesh



| | |
|---|---|
| Journal: | *BMJ Open* |
| Manuscript ID | bmjopen-2019-033702.R1 |
| Article Type: | Protocol |
| Date Submitted by the Author: | 28-Nov-2019 |
| Complete List of Authors: | Zaman, K; International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), Infectious Disease Division;<br>Dudman, Susanne; University of Oslo, Institute of clinical Medicine; Norwegian Institute of Public Health (NIPH), Division of Infection Control and Environmental Health<br>Stene-Johansen, Kathrine; Norwegian Institute of Public Health (NIPH), Division of Infection Control and Environmental Health<br>Qadri, Firdausi ; International Centre for Diarrhoeal Disease Research<br>Yunus, Md; International Centre for Diarrhoeal Disease Research Bangladesh<br>Sandbu, Synne; Norwegian Institute of Public Health (NIPH), Division of Infection Control and Environmental Health<br>Gurley, Emily; International Centre for Diarrhoeal Disease Research Bangladesh; Johns Hopkins University Bloomberg School of Public Health<br>Overbo, Joakim; Norwegian Institute of Public Health (NIPH), Division of Infection Control and Environmental Health<br>Julin, Cathinka; Norwegian Institute of Public Health (NIPH), Division of Infection Control and Environmental Health<br>Dembinski, Jennifer; Norwegian Institute of Public Health (NIPH), Division of Infection Control and Environmental Health<br>Nahar, Quamrun; International Centre for Diarrhoeal Disease Research Bangladesh<br>Rahman, Anisur ; International Centre for Diarrhoeal Disease Research, Maternal and Child Health Division<br>Bhuiyan, Taufiqur; International Centre for Diarrhoeal Disease Research Bangladesh<br>Rahman, Mustafizur; International Centre for Diarrhoeal Disease Research Bangladesh<br>Haque, Warda; International Centre for Diarrhoeal Disease Research Bangladesh<br>Khan, Jahangir; Liverpool School of Tropical Medicine, Health Economics; International Centre for Diarrhoeal Disease Research Bangladesh, Health Systems and Population Studies Division<br>Aziz, Asma; International Centre for Diarrhoeal Disease Research Bangladesh<br>Khanam, Mahbuba; International Centre for Diarrhoeal Disease Research Bangladesh<br>Streatfield, Peter ; International Centre for Diarrhoeal Disease Research |

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# HEV study protocol : Design of a cluster-randomized, blinded trial to assess the safety, immunogenicity and effectiveness of the hepatitis E vaccine HEV239 (Hecolin®) in women of childbearing age in rural Bangladesh

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## ABSTRACT:

### Introduction

Hepatitis E virus (HEV) is a leading cause of acute viral hepatitis in the developing world and is a public health problem, in particular among pregnant women, where it may lead to severe or fatal complications. A recombinant HEV vaccine, 239 (Hecolin® (Xiamen Innovax Biotech.Xiamen, China), is licensed in China, but WHO calls for further studies to evaluate the safety and immunogenicity of this vaccine in vulnerable populations, and to evaluate protection in pregnancy. We are therefore conducting a phase IV trial to assess the effectiveness, safety, and immunogenicity of the HEV 239 vaccine when given in women of childbearing age in rural Bangladesh, where HEV infection is endemic.

### Methods and analysis

Enrollment of a target of approximately 20,000 non-pregnant women, aged 16-39, started on October 2, 2017 in Matlab, Bangladesh. Sixty-seven villages were randomized by village at a 1:1 ratio to receive either the HEV vaccine or the control vaccine (hepatitis B vaccine). A 3-dose vaccination series at 0, 1, and 6 months is ongoing, and women are followed up of for 24 months. The primary outcome is confirmed HEV disease among pregnant women. After vaccination participants are requested to report information about clinical hepatitis symptoms. Participants who become pregnant are visited at their homes every 2 weeks to collect information about pregnancy outcome and to screen for clinical hepatitis. All suspected hepatitis cases undergo laboratory testing for diagnostic evaluation. The incidence of confirmed HEV disease among pregnant and non-pregnant women will be compared between the HEV vaccinated and control groups, safety and immunogenicity of the vaccine will also be evaluated.

## Ethics and dissemination

The protocol was reviewed and approved by the icddr,b Research Review Committee (RRC) and Ethical Review Committee (ERC), and the Directorate General of Drug Administration (DGDA) in Bangladesh, and by the Regional Ethics Committee (REC) in Norway. This article is based on the protocol version 2.2 dated 29.06.2017. We will present the results through peer-reviewed publications and at international conferences.

## Trial registration

The trial is registered at clinicaltrials.gov with the registry name «Effectiveness Trial to Evaluate Protection of Pregnant Women by Hepatitis E Vaccine in Bangladesh» and the identifier NCT02759991.

## STRENGTHS AND LIMITATIONS OF THE STUDY:

- This trial is conducted in a defined population covered by a long-term demographic surveillance registry, in a site that has a well-established clinical and laboratory infrastructure and effective referral systems.
- Women are visited in their homes for 7 days following vaccination to follow up and record details of any adverse events.
- Active surveillance of acute hepatitis is conducted through home visits every 2 weeks to all the pregnant participants during their pregnancy.
- Dried blood spots (DBS) are used to assess immunogenicity of the vaccine, simplifying blood collection and making storage of 40 000 samples feasible in a community setting.
- A limitation of this trial design is the lack of blood sampling at the end of follow up, thus we will not be able to explore the occurrence of asymptomatic hepatitis E.

# INTRODUCTION

## Background and rationale

Hepatitis E virus (HEV) is an enteric RNA virus causing outbreaks or endemics in developing countries with poor sanitation and is one of the leading causes of acute hepatitis worldwide.[1] Pregnant women are particularly vulnerable to HEV infection, with a high rate of maternal mortality, miscarriage, premature delivery, and stillbirth.[2]

An effective vaccine could prevent symptomatic HEV infection in vulnerable people. Only two HEV vaccines have been evaluated in human clinical trials, namely rHEV (GlaxoSmithKline), and HEV 239 (Hecolin®, Xiamen Innovax Biotech Co.,Ltd.,China), of which only the latter has undergone further commercial development.[3] Zhu et al. studied this vaccine in a large phase III clinical study in China, where 100,000 healthy men and women (aged 16-65 years) received either the HEV vaccine or a hepatitis B (HBV) vaccine.[4] They found more than 90 % protection against symptomatic HEV infection. The adverse events related to the vaccine were few and mild, and there were no vaccine-related serious adverse events. The vaccine is currently only licensed in China for people 16 years and older , and is recommended for individuals with a high risk of HEV infection.

The WHO Strategic Advisory Group of Experts made a working group to review the evidence on the HEV 239 vaccine and make recommendations for its use. They concluded that knowledge gaps prevents recommendation of the vaccine in endemic countries, and that further studies should evaluate the safety and immunogenicity of this vaccine in children, the elderly, persons with underlying diseases or conditions such as immunosuppression or liver disease, and immunogenicity and protection in pregnant women. [3 5]

HEV comprises eight genotypes, of which mainly four infect humans.[6] HEV genotypes 1 and 2 dominate human infections in developing countries. Genotypes 3 and 4 primarily infect animals that can further transmit the virus to humans, causing illness in both developed and developing countries. Genotype 4 predominates in mainland China, where the previous phase III vaccine trial was conducted, but there are limited data on protection of the vaccine against the other genotypes. A small study investigated immunogenicity against genotypes 1-4 after vaccination with p239 and found that in humans, IgG antibodies reacted slightly stronger against genotypes 1 and 2 versus 3 and 4, which could be due to the presence of genotype-specific neutralizing antibodies.[7] However, the vaccine still needs to be tested in other geographical areas to fully evaluate efficacy against all the genotypes that frequently cause illness in humans.[3]

We are currently conducting a phase IV cluster-randomized clinical trial (2017- ongoing) with the HEV 239 vaccine to provide more data on the effectiveness of the vaccine on genotype 1 and the outcome in pregnant women, and on safety of the vaccine. The trial is conducted in a rural area of Bangladesh where genotype 1 is predominant, and includes women aged 16-39, allowing us to evaluate effectiveness and immunogenicity of the vaccine among women who become pregnant following vaccination.

## Objectives

### Primary objective

- To determine the effectiveness of the HEV 239 vaccine given to women of childbearing age in rural Bangladesh in preventing HEV disease during pregnancy

### Secondary objectives:

- To determine the safety of HEV vaccine in Bangladeshi women of childbearing age

- To determine the immunogenicity of HEV vaccine in Bangladeshi women of childbearing age
- To determine the effectiveness of HEV vaccine in preventing HEV disease in non-pregnant Bangladeshi women of childbearing age
- To assess the anti-HEV IgG levels before and one month after the last dose of vaccine for primary vaccine response and record if any HEV disease occurs.
- To assess the feasibility, acceptability and cost-effectiveness of HEV vaccination of women of childbearing age in rural Bangladesh
- To investigate acute HEV cases virologically, clinically and immunologically in relation to outcome.

## Trial design

This study is a phase IV cluster-randomized, double blinded trial on the safety and effectiveness of three doses of the HEV 239 vaccine in women of childbearing age (16-39) in Bangladesh. In this Phase IV study we will assess the safety, acceptability, immunogenicity and effectiveness of Hepatitis E vaccine. It is not required to conduct a phase III study in Bangladesh when it has already been conducted elsewhere and the results are published, which showed that the vaccine is safe and highly efficacious. In a cluster randomized trial groups of people rather than individuals are randomly allocated to the interventions under investigations. The unit of allocation in this trail is a 'Village'. Participants are sampled at day 0 and day 210 and followed up as described in Table 1.

## METHODS AND ANALYSIS

### Study setting

The study field site for this clinical trial is located in Matlab in rural Bangladesh, where HEV is endemic.[8] icddr,b has maintained a field research site in this area for more than fifty years with an ongoing health and demographic surveillance system covering the entire population. The icddr,b field site comprises 67 villages, with a total population of about 116,000 and a well-established infrastructure including health care services (hospital and local health clinics), laboratory facilities, and effective referral systems.

### Randomization and blinding

All 67 villages were randomized by village at a 1:1 ratio to receive either HEV vaccine or HBV vaccine. All participants, investigators and field staff are blinded. The vaccine administrators, however, cannot be blinded since the dose of the licensed HBV vaccine in Bangladesh is different for women below and above 18 years, while the HEV vaccine dose is identical for all age groups. Each village is allocated to two vaccine codes (one for 16-18 years and another for 19-39 years) with eight codes in total; two codes for each vaccine in each age group. To achieve similar distributions of the codes for the two vaccines in different age groups, computerized allocation was employed taking the population size of the villages into consideration. The randomization was rerun until the groups were balanced in size (<200 in difference). An independent statistician from Johns Hopkins University performed the randomization. The vials were labeled with the eight respective codes by Incepta Pharmaceuticals Ltd., Bangladesh, who fill-finished and bottled the vaccine shipped in bulk from China.

The treatment allocation code is being securely kept under lock and key and may be broken by the investigator only in case of a Serious Adverse Events (SAEs) for which knowledge of the participant's treatment assignment may influence her or the clinical care or if the event is unexpected and suspected to be causally related to the investigational product. Code breaking will be reported to the clinical monitor and Data Safety and Monitoring Board (DSMB) within 24 hours. The participant may continue in the study with protocol-specified follow-up despite unblinding, unless she fulfils any protocol-defined exclusion criteria.

## Recruitment and enrolment

All eligible women in the study area, identified through existing Matlab surveillance system, are being approached at their homes by designated study staff who inform about the nature of the study according to the International Conference on Harmonization of Good Clinical Practice (ICH-GCP) in their local language, and the participants may inquire about the details of the study. Eligibility is further assessed according to the inclusion and exclusion criteria in table 2.

A urinary pregnancy test is offered to women who have missed a period. Eligible participants visit a fixed site clinic for enrolment, where they sign a consent form. In the case of 16-17-year-old participants, their legal guardians sign an assent form in place of the consent form. A Case Report Form (CRF) with an identification number is then created for each participant. Confidentiality of personal identifiers is maintained by keeping names noted in primary data instruments in secure files and by removing names from the computerized dataset for analysis. A flow chart of anticipated participant recruitment is shown in Figure 1.

## Withdrawal

The participants and/or their legal guardians are informed that participation is voluntary, and that they may withdraw consent at any time, without giving a reason and without prejudice to further treatment. Participants who withdraw may demand destruction of samples and deletion of data concerning themselves. Participants may be discontinued from the study by the investigator at any time in case of safety reasons or significant protocol deviations. The reason for withdrawal will be recorded in the CRF. If withdrawal is due to an adverse event, appropriate follow-up will be arranged.

## Interventions

The HEV 239 vaccine is based on a 239 amino acid long recombinant HEV peptide, corresponding to amino acids 368-606 of open reading frame 2 that encodes the capsid protein of HEV. The amino acid sequence is derived from a genotype 1 Chinese HEV strain. HEV 239 was developed and is produced by Innovax. The vaccine is expressed in *Escherichia coli*,[9] and contains 30 μg of the purified protein absorbed to 0.8 mg of aluminium hydroxide suspended in 0.5 ml of buffered saline. The control vaccine is a commercial hepatitis B vaccine (Hepa-B®) produced by Incepta. Each 0.5 ml dose (for age 16-18 yrs) contains 10 μg of hepatitis B surface antigen absorbed on aluminium hydroxide gel equivalent to $Al^{3+}$ 0.25 mg. A 1.0 ml dose for older persons contains 20 μg of hepatitis B surface antigen absorbed on aluminium hydroxide gel equivalent to $Al^{3+}$ 0.5 mg. We chose this control vaccine for the following reasons: it will benefit the target population who have not received this vaccine through the child immunization program; the dosing regimen is the same as the HEV vaccine; and it was the control vaccine in the phase III trial,[4] facilitating comparison with previous results.

Innovax donated bulk HEV vaccine for the trial, while Incepta filled the HBV and HEV vaccines in identical, single dose vials, respectively. Both bulk vaccines and finished products were quality tested and labelled according to ICH-Good Manufacturing Practice (GMP).

Innovax donated bulk vaccine to Incepta, Bangladesh maintaining cold chain through courier. Incepta prepared the HEV single dose vaccine vials. We maintained proper cold chain from Incepta to field sites.

The vaccines are administered intramuscularly in the deltoid muscle of either arm, in a 3-dose regimen on day 0, at one month, and at six months (Table 1).

## Follow up/Surveillance

The full study period will last for 2 ½ years after enrollment of the final participant. (**Table 1)**

### Hepatitis surveillance

Each participant get an immunization card with identification numbers and a phone number, and are instructed to contact the investigator immediately if they experience jaundice or have any of the following symptoms for at least three days: fatigue, loss of appetite, abdominal discomfort, abdominal pain in the right upper quadrant, nausea, or vomiting. This passive surveillance for hepatitis is ongoing throughout the full study period for all participants. After each dose of vaccine field staff visited participant's household daily for 7 days. Then all participants are visited by field staff for hepatitis surveillance weekly and will be continued till the end of the study. All women who became pregnant after any dose are visited every 2 weeks to collect information about pregnancy outcomes and to screen for clinical hepatitis. In addition message on hepatitis symptoms are also being sent on mobile phones to all participants (>80% of households have cell phone access). Suspected cases will be referred to Matlab hospital for

clinical and laboratory examination including tests of liver function and virological causes of hepatitis (A, B, C, E), and eventual treatment. If HEV disease is confirmed by the presence of anti HEV IgM or HEV RNA, blood samples will be analysed for relevant biochemical, microbiological and immunological markers. This includes viral load, HEV subtypes, other hepatitis infections, antibody titer, cellular immune response, cytokines, alanine transaminase (ALT), INR and albumin. Blood samples will be taken at least two times a week until recovery In order to assess the dynamic in viral and host factors during the illness. The patient's symptoms and signs will also be recorded regularly in this period. The last study visit to pregnant women is 14 days after delivery, to record information on the health status of the child.

### Adverse events surveillance

After each vaccination dose, participants are observed for 30 minutes and visited at their homes by a field worker for seven consecutive days. During these visits, participants are specifically asked about any local reactions (e.g. erythema, swelling, induration and pain at the injection site) and systemic symptoms (e.g. nausea, malaise, myalgia, arthralgia, headache, fever). Participants are also asked to report any significant symptoms after the last vaccine dose, i.e.at least 2 years until the last study visit. After this visit, they are advised to report to the study team about any possible serious adverse events or signs of hepatitis, even after completion of the study.

## Outcomes

The primary outcome is clinical HEV disease among pregnant women. HEV disease is defined by illness lasting for at least 3 days, abnormal serum ALT level of ≥ 2.5 times upper limit of normal, detection of IgM anti-HEV in serum collected within 1 month after onset, and/or the presence of HEV RNA and/or ≥ 4 fold rise of IgG anti-HEV levels in paired sera. All acute

hepatitis cases are also tested for the presence of markers of other viral hepatitis (A, B, C). Secondary outcomes are confirmed HEV disease in non-pregnant women, safety, and immunogenicity. Safety outcomes include all local and systemic adverse events, which are recorded in the participant case report form during the study period. Investigation of vaccine induced immune responses will include anti-HEV IgG on all participants, together with additional antibody and cellular responses in plasma and PBMC samples taken from a subset of 50 participants. A possible protective anti-HEV IgG level will be sought. An antibody response to vaccination will be defined as a $\geq 4$ fold rise of IgG anti-HEV levels in an individual's post vaccination sample (one month after the last dose) compared to baseline sample.[4] Data on pregnancy outcome, including complications during delivery and health status of mother and child, will be collected on all participants on a Pregnancy Report Form and analysed together with records of eventual HEV disease and type, time and number of vaccine doses.

## Sample size and power calculations

The sample size was calculated to estimate total vaccine protection of pregnant participants in a per protocol analysis (Figure 1). The following assumptions are based on data from Matlab health and demographic surveillance system and previous HEV research studies from the region.[8] We expect that 10% of women will not be included because of baseline pregnancy, and that the refusal rate will be 5%. After randomization, there will be 15% loss of person-time because of migration out of the study site, and 10% will not complete three vaccine doses for other reasons. During follow up after the last vaccine dose, we assume that 16% of women (followed over a mean of 16 months) will become pregnant and reach term before the end of surveillance; an additional 9% of women (followed over a mean of 9 months) will be followed

for an average of half full term (4.5 months). This predicts that 20% of the dose 3 recipients will have "completed" pregnancies, 3,806 are expected to become pregnant after dose 3 and 3,073 to be followed to term. Follow up of the remaining pregnancies will be right censored by termination of follow up. We expect 15% of the pregnant dose 3 recipients to be lost to follow up because of migration out, while 5% are expected to withdraw from the study. We expect the design effect for this cluster randomized trial to be 2. We assume that 22% of the participants are HEV seropositive at baseline, indicating protection against HEV infection.[8] Furthermore, six percent of seronegative pregnant women are expected to become infected during pregnancy,[8] of which we assume 35% to be symptomatic. [8] The protective efficacy of a 3-dose regimen of HEV vaccine against symptomatic infections is >95%.[4] We therefore need a sample size of 20,745 women at baseline to achieve 80% power at P<.05 (two-tailed) for the analyses in pregnant women. Further, our study will have 90% power to show >95% protective efficacy against HEV disease among non-pregnant women. Our study is not powered to directly evaluate perinatal and maternal death, because of the low rates of these outcomes in Matlab. However, given the substantial evidence linking maternal and perinatal mortality to HEV disease in pregnancy,[2] demonstration of vaccine protection against confirmed HEV disease in pregnancy will provide strong evidence that vaccination is likely to prevent maternal and perinatal deaths.

## Data collection, management and analysis

The field staff are entering the required data into the paper-based CRFs through interviewing during vaccination and home visits, and by reviewing medical records when applicable. The data are further transcribed to an electronic data capturing system (developed by icddr,b using Oracle data base) within a week of the clinic visit. This system will automatically

check data to detect errors and inconsistencies. The data in the system are reviewed weekly by the analyst programmer, and any data deleted from the main database will be saved in a shadow table. Data are stored in the Oracle database system in a central server at Matlab. An electronic database backup is made weekly by the data management team, and the final database is sent to the icddr,b data archive system.

Health registry data for study participants and their previous pregnancy outcome are also being collected. Field staff and medical officers are checking these data with the family record book. They are checking the participant data with the eligible participant list after every visit. The data analyst manager is verifying entered data biweekly. Any inconsistencies are resolved with the field staff and medical officers.

All completed CRFs and other documents are stored in a locked cabinet with limited personnel access. The CRF register in the data management center at Matlab keeps track of CRF movement between the file cabinet and the data management center. The log book contains the columns; participant ID, receipt date, visit number, number of pages, name of staff, purpose of file movement, return date, name of staff, and any relevant remarks. All informed consent forms are being filed and kept separately in a locked cabinet. Data are entered in electronic data base. A dedicated data management team will be responsible for data entry, cleaning, analysis and data archiving in de-identified way.

Blood samples are being analyzed by icddr,b and/or Norwegian Institute of Public Health (NIPH), according to predefined standard operating procedures. Additional blood will be stored in the research biobank at icddr,b in case re-analysis is needed.

## Statistical methods

### Analyses

In the primary analysis, the risk of confirmed HEV disease in pregnant women who received the HEV vaccine will be compared to the risk in women who received the HBV vaccine using Cox regression with shared frailty,[10] to adjust for the design effect of cluster randomization. Demographic and other baseline characteristics will be compared between the HEV and HBV vaccination clusters, for all participants, and separately for pregnant participants, to assess the degree to which randomization was achieved. Unbalanced covariates (e,g. occupation, age, body mass index, hepatitis B disease, Socio-economic information, education, sanitation and water use, distance from river, season, baseline HEV IgG antibodies) may be included in the models. Subgroup analyses will be performed to evaluate effectiveness in participants who are negative/positive at baseline for anti-HEV IgG antibodies, respectively, and subgroup analyses for effectiveness will also be performed per number of doses received. Additionally, subgroup analyses will be carried out to explore effectiveness in participants within different BMI and age intervals. Safety analyses of all local and systemic adverse events (e,g pain, swelling, redness, fever, myalgia, headache) will be performed using generalized estimating equations (GEE) for logistic regression to account for cluster randomization

### Analysis sets

The full analysis set (FAS) population will include all randomized participants who received at least one dose of either the study or the control vaccine. The per protocol (PP)

population will include participants who were randomized and received three doses of the study or control vaccine, respectively, and provided blood samples according to the protocol schedule (Table 1). Pregnant FAS and PP populations are defined as participants from the respective populations with confirmed pregnancies during the study period. The primary effectiveness analysis will be performed on the pregnant PP population. Secondary effectiveness analyses will be performed on the pregnant FAS population, as well as the non-pregnant FAS and PP populations, with the same methods as the primary analysis. Safety analyses will be performed on the FAS population.

## Data and safety monitoring

### Data monitoring

An independent Data Safety and Monitoring Board (DSMB) with no competing interests was appointed to provide the icddr,b Ethical Review Committee (ERC) with an overall scientific, safety and ethical appraisal of the study. The DSMB also informs this committee regarding the progression of the study, with special attention to the safety of the participants. They convene at least once annually, and make recommendations directly to the ERC Chairperson. As described in the ERC guidelines, the principal investigator prepares a report to the DSMB before each meeting, describing the accumulated adverse events and serious adverse events and a summary of the current data for inclusion, progress, and deviations from the protocol or planned procedures. Any reports from the monitor regarding quality control are also included. The DSMB is also responsible for detailed reviews of all the serious adverse events. More details can be found in the DSMB Charter included in the Trial Master file in the trial office in Bangladesh.

No interim analyses have been planned, and no stopping guidelines have been created for the trial. In the event of serious safety issues, the DSMB will meet to provide recommendations regarding termination of the trial. The steering committee will have the final decision to terminate the study.

## Harms

All adverse events observed or reported are logged in the relevant participant CRF by the study staff. The following information is registered: description of the adverse event (in precise standard medical terminology), time of onset and resolution, severity (mild/ moderate/ severe; according to Common Terminology Criteria for Adverse Events version 4.0, outcome, assessment of the causality with study drug, and action taken with study drug.

All serious adverse events are communicated to the DSMB within 24 hours after awareness by the study staff. Serious adverse event reports are initially sent to the Chairperson of the DSMB and the ERC with copy to sponsor/principal investigator. All adverse events and serious adverse events are being followed up to resolution unless the event is considered by the investigator to be unlikely to resolve due to underlying disease. We will endeavor that all events are resolved, even if they continue after study completion.

## Auditing

The sponsor (NIPH) has appointed an independent local clinical monitor to ensure that the study is conducted according to protocol, standard operating procedures, GCP and regulatory requirements, and to verify that investigators are collecting and reporting quality data. In addition, a monitor from NIPH is auditing the study. The monitors are periodically monitoring on-site, including at study initiation and at close-out. The monitors check the informed consent

process, reporting of adverse events and other safety data, CRF completeness, and adherence to the study protocol for at least 10 % of the study participants randomly distributed between the 67 study villages. They also monitor maintenance of regulatory documents, study supply accountability, facilities and equipment.

## Patient and Public Involvement statement

The public was not involved in the development of the research question nor the study design.

## ETHICS AND DISSEMINATION

The protocol has been approved by the icddr,b Ethical review committee and Regional Ethics Committee in Norway

## Protocol amendments

Any significant amendments and/or new versions of the study protocol will be notified to and approved by the competent authority and the ethics committees in both countries according to EU and national regulations before changes are implemented.

## Confidentiality

All study-related information is being kept confidential, under lock and key, or on secure servers in case of digital information. The study monitor, the ERC, and any law-enforcing agency will have access to this information only in the event of necessary inspection. The samples may be sent outside the country for analysis, and preserved for 5 years where applicable, however, any personal identifiable information will be held and processed under secured conditions with access

limited to pre-identified staff. Remaining blood samples will be disposed of after completing testing for all participants in the study.

## Access to data

The study principal investigator, the co-investigators in the study, and the data analyst manager will have access to the final trial dataset.

## Dissemination policy

Upon study completion and finalization of the study report, we will submit the study results to the competent authority and ethics committees according to national regulations, and publish the results in peer-reviewed journals. We will report the trial in accordance with the CONSORT guidelines, with authorship based on the ICMJE recommendations.

## Acknowledgments

icddr,b acknowledges with gratitude to the Research Council of Norway for supporting this research through the GLOBVAC program. icddr,b is also grateful to the Governments of Bangladesh, Canada, Sweden and the UK for providing core/unrestricted support. We thank all the members of the research team at icddr,b who helped with participant recruitment, vaccination, sampling and follow up, and the laboratory team at icddr,b who performed laboratory procedures. The local clinical monitor (Dr. Wasif Ali Khan) is thanked for monitoring the trial. We also thank the data safety monitoring board (Prof. Kazi Zulifiquer Mamun, Prof S M Shamsuzzaman, Prof Saria Tasnim, Dr. Md. Nur Haque Alam, and Dr. Hanne M. Nøkleby), and the steering committee (Dr. Shams El Arifeen, Dr. Rashidul Haque, Dr. Ingeborg Aaberge, and Prof. Dr. Geir Bukholm) for their important contributions throughout the trial. A special thanks to Innovax for their donation of the HEV vaccine.

NIPH is the sponsor for this clinical trial (Contact name: Susanne Dudman. Address: Norwegian Institute of Public Health, Postboks 222 Skøyen, 0213 Oslo, Norway).

We presented a poster of the study at 10th Conference on Global Health and Vaccination (GLOBVAC) Research in Trondheim, Norway in 2017.

## Funding statement

The Research Council of Norway supported this study through the GLOBVAC program, project number 248143. Xiamen Innovax Biotech Co., Ltd., China provided the bulk HEV 239 vaccine. SGD is supported by both NIPH and University of Oslo, KSJ and JLD are additionally supported by NIPH, who also provided additional support from statisticians, technical engineers, vaccine experts and access to laboratory facilities and instruments. The design, management, analysis and reporting of the study are entirely independent of the vaccine manufacturers.

## Authors' contributions

JDC, KZ, SD, KSJ, FQ, MY, SS, JØ, ESG, JLD, QN, AR, PKS, JK, and CHJ contributed to study design, KZ, JDC, AA, MK, SD, KSJ, JØ, SS, TRP, MR, WH, JLD and CHJ were involved in trial management. CHJ, KSJ, SD, JDC, KZ, SS, JLD, JØ, and ESG contributed in manuscript writing and editing. KZ, AA, MK, WH responsible for managing the field teams/logistics of the study. All authors read and approved the final manuscript.

## Competing interests statement

The authors reports no conflicts of interest in this work.

## Ethics approval

This trial is approved by the icddr,b Research Review Committee (RRC) and Ethical Review Committee (ERC), and by the Directorate General of Drug Administration (DGDA) in Bangladesh. Further, it has been approved by the Regional Ethics Committee (REC) in Norway, This article refers to protocol 2.2 dated 22.06.2017. The registration number of this trial is NCT02759991.

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**Table 1. Participant timeline in the study.**

| | Contact with participant | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Visit 1 | Visit 2 | Visit 3-9 | Visit 10 | Visit 11-17 | Visit 18 | Visit 19-25 | Visit 26 | Visit 27 |
| Days | -1 | 0 | 1-7 | 30* | 31-37* | 180* | 181-187* | 210* | 2½ years |
| ENROLMENT | | | | | | | | | |
| Invitation visit | X | | | | | | | | |
| Eligibility screening | X | | | X | | X | | | |
| Informed consent | | X | | | | | | | |
| Demographics (age, height, weight, BMI), medications/medical history | | X | | X | | X | | X | X |
| INTERVENTIONS | | | | | | | | | |
| Vaccination | | X | | X | | X | | | |
| Blood sample† | | X | | | | | | X† | |
| SURVEILLANCE | | | | | | | | | |
| Hepatitis surveillance (active and passive) | Throughout the study period | | | | | | | | |
| Pregnancy surveillance | Throughout the study period | | | | | | | | |
| ASSESSMENTS | | | | | | | | | |
| Pregnancy home visit | Every two weeks | | | | | | | | |
| Physical exam | | | | | | | | | X |
| HARMS/SAFETY | | | | | | | | | |
| Immediate reactions | | X | | X | | X | | | |
| Home visit | | | X | | X | | X | | X |
| SAE | At any time following 1st vaccine dose | | | | | | | | |
| Participant reporting of AEs | At any time following 1st vaccine dose | | | | | | | | |
| Withdrawal | At any time following enrolment | | | | | | | | |

*±2 days

† Dried blood spots (DBSs) are collected before vaccination and one month after last vaccine dose, or earlier if off-study

**Abbreviations:** AE, Adverse Event, BMI; Body Mass Index, SAE; Serious Adverse Events

**Table 2** HEV Bangladesh study inclusion and exclusion criteria

| |
|---|
| Inclusion criteria |
| - Healthy non-pregnant female, aged 16-39 at time of first vaccination |
| - Living in the iccdr,b field site in Matlab, Bangladesh |
| - Willingly giving written informed consent |
| Exclusion criteria |
| - Pregnancy (visible or verbal report on date of last menstruation or urine for pregnancy test) |
| - History of severe allergic reaction to a vaccine or a vaccine component |
| - Received another vaccine or immunoglobulin within two weeks |
| - Serious chronic diseases (medical assessment) |
| - Acute or chronic infectious disease (medical assessment) |
| - Fever > 38 °C (axillary temperature) |

**Figure Legend**

Anticipated participants enrollment flow chart

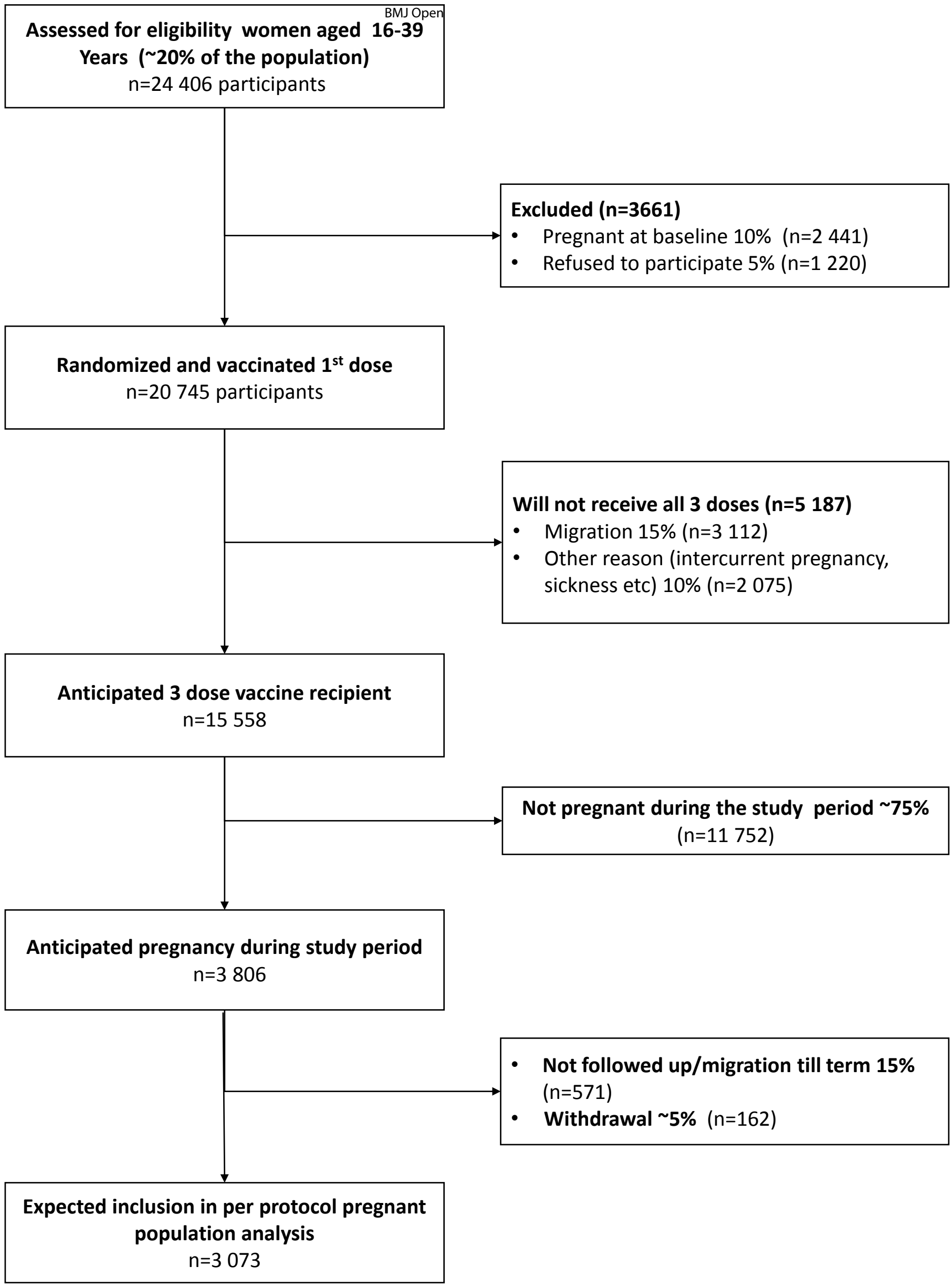


**Figure 1:** Anticipated participants enrollment flow chart


SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

| Section/item | Item No | Description | |
|---|---|---|---|
| **Administrative information** | | | |
| Title | 1 | Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym | P1 (Title page) |
| Trial registration | 2a | Trial identifier and registry name. If not yet registered, name of intended registry | P3 (Abstract) |
| | 2b | All items from the World Health Organization Trial Registration Data Set | See online reg details at clinicaltrials.orgg |
| Protocol version | 3 | Date and version identifier | P3 (Abstract) |
| Funding | 4 | Sources and types of financial, material, and other support | P18 (Funding statement) |
| Roles and responsibilities | 5a | Names, affiliations, and roles of protocol contributors | P18 (Authors contributions) |
| | 5b | Name and contact information for the trial sponsor | P17-18 (Acknowl.) |
| | 5c | Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority | P18 (Funding statement) |
| | 5d | Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee) | P16 (Auditing), P17-18 (Acknowl), P12-13 (Data coll, man. and analysis) |
| **Introduction** | | | |
| Background and rationale | 6a | Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms | P4-5 (Introduction) |
| | 6b | Explanation for choice of comparators | P8-9 (Interventions) |
| Objectives | 7 | Specific objectives or hypotheses | P5-6 (Objectives) |

| | | | |
|---|---|---|---|
| Trial design | 8 | Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, | P6 (Trial Design) |
| **Methods: Participants, interventions, and outcomes** | | | |
| Study setting | 9 | Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be | P6 (Study Setting) |
| Eligibility criteria | 10 | Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, | Table 2 |
| Interventions | 11a | Interventions for each group with sufficient detail to allow replication, including how and when they will be | P8-9 (Interventions) |
| | 11b | Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or | P8 (Withdrawal) |
| | 11c | Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests) | NA |
| | 11d | Relevant concomitant care and interventions that are permitted or prohibited during the trial | NA |
| Outcomes | 12 | Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm | P10-11 (Outcomes) |
| Participant timeline | 13 | Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended | P7-8 (Recruit. and enroll.) + Table 1 |
| Sample size | 14 | Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size | P11-12 (Sample Size and Power C.) |
| Recruitment | 15 | Strategies for achieving adequate participant enrolment to reach target sample size | P7-8 (Recruit. and enrol.) |
| **Methods: Assignment of interventions (for controlled trials)** | | | |
| Allocation: | | | |

| | | | |
|---|---|---|---|
| Sequence generation | 16a | Method of generating the allocation sequence (eg, computer- generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign | P6-7 (Random. and Blind.) |
| Allocation concealment mechanism | 16b | Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned | P6-7 (Random. and Blind.) |
| Implementation | 16c | Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions | P6-7 (Random. and Blind.) |
| Blinding (masking) | 17a | Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how | P6-7 (Random. and Blind.) |
| | 17b | If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial | P6-7 (Random. and Blind.) |
| **Methods: Data collection, management, and analysis** | | | |
| Data collection methods | 18a | Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection | P12-13 (Data coll., man, and an.) + P10 (Outcomes) |
| | 18b | Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention | Table 1 |
| Data management | 19 | Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol | P12-13 (Data coll., man, and an.) |
| Statistical methods | 20a | Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol | P13 (Stat. Methods-Analyses) |
| | 20b | Methods for any additional analyses (eg, subgroup and adjusted analyses) | P13 (Stat. Meth. - Analyses) |
| | 20c | Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple | P14 (Stat. Meth-Analyses Sets) |

| | | | |
|---|---|---|---|
| **Methods: Monitoring** | | | |
| Data monitoring | 21a | Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. | P14-15 (Data Monitoring) |
| | 21b | Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial | P14-15 (Data Monitoring) |
| Harms | 22 | Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial | P15 (Harms) |
| Auditing | 23 | Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor | P16 (Auditing) |
| **Ethics and dissemination** | | | |
| Research ethics | 24 | Plans for seeking research ethics committee/institutional review board (REC/IRB) approval | P19 (Ethics Approval) |
| Protocol amendments | 25 | Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators) | P16 (Protocol Amendments) |
| Consent or assent | 26a | Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32) | P7-8 (Recruit. and Enrol. |
| | 26b | Additional consent provisions for collection and use of participant data and biological specimens in ancillary | NA |
| Confidentiality | 27 | How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial | P7-8 (Recr. and enr.) P16-17 (Confidentiality) |
| Declaration of | 28 | Financial and other competing interests for principal investigators for the overall trial and each study site | P19 (Comp. int. statement) |
| Access to data | 29 | Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators | P17 (Access to Data) |
| Ancillary and post-trial care | 30 | Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation | P15 (Harms) |

| | | | |
|---|---|---|---|
| Dissemination policy | 31a | Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing | P17 (Dissemination Policy) |
| | 31b | Authorship eligibility guidelines and any intended use of professional writers | NA |
| | 31c | Plans, if any, for granting public access to the full protocol, participant- level dataset, and statistical code | NA |
| **Appendices** | | | |
| Informed consent | 32 | Model consent form and other related documentation given to participants and authorised | Appendix 1 |
| Biological specim | 33 | Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if | P10-11 (Outcomes) |

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.