PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	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
AUTHORS	Zaman, K; Dudman, Susanne; Stene-Johansen, Kathrine; Qadri, Firdausi; Yunus, Md; Sandbu, Synne; Gurley, Emily S; Overbo, Joakim; Julin, Cathinka Halle; Dembinski, Jennifer Lynn; Nahar, Quamrun; Rahman, Anisur; Bhuiyan, Taufiqur R; Rahman, Mustafizur; Haque, Warda; Khan, Jahangir; Aziz, Asma; Khanam, Mahbuba; Streatfield, Peter Kim; Clemens, John D.

VERSION 1 – REVIEW

REVIEWER	Ting Wu National Institute of Diagnostics and Vaccine Development in infectious diseases, School of Public Health, Xiamen University, China My institute received funding from Innovax to conduct the phase III clinical trial of this HEV 239 vaccine in China.
REVIEW RETURNED	04-Oct-2019

GENERAL COMMENTS	The 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" is well designed and the trial would provide important data for proving the effectiveness of the only commercialized hepatitis E vaccine (HEV 239) during pregnancy. Major comments: 1. Page 3 Line 48, dried blood spots (DBS) are used to assess immunogenicity of the vaccine. Is there any data to analyze the consistency of the antibody levels of DBS and frozen serum after different time periods of storage? Is there any standard used in the assays? 2. Page 9 Line 48, ‘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’, please make clear the time frequencies of the phone calls or household visits. 3. Page 9 Line 52, ‘women with suspected hepatitis will be tested for liver function and virological causes of hepatitis (A, B, C, E)’, please make clear whether women with suspected hepatitis E will be followed-up and take serial serum samples to test the dynamic of antigen, antibody and RNA levels of HEV, until they were completely recovered? The follow-up procedure of suspected hepatitis patients is the core part of this protocol, should be described more clearly.

	4. Page 10 Line 55, as ‘cellular immune responses on a subset of participants would be investigated’, I think serum sample should be used for this test instead of DBS, how many people would be taken serum sample instead of DBS? 5. It’s better to include more details of laboratory tests into this protocol. Minor comments: 6. Page 4 Line 37, the vaccine was licensed in China for people aged 16 years and older, not in people aged 16-65 years old.

REVIEWER	Lisa McHugh Menzies School of Health Research- Global and Tropical Health Division, Australia
REVIEW RETURNED	05-Oct-2019

GENERAL COMMENTS	Thank you for the opportunity to review this protocol. This will be a very interesting and valuable study. Given the vaccine is being transported from China, can you please advise in the protocol how the cold-chain process will be maintained. A break in the cold-chain would be of a major concern for this study, so explaining the process of how you will maintain the cold-chain process is necessary. There seems to be a discrepancy in the age of the female study participants. On the informed consent form it reads 18-39 year old, however in the protocol and flow-chart this states 16-39 years. Please clarify. Also, on the consent form it states the study participant should observe for jaundice. Presumably the term 'jaundice' will be explained to the study participants. Even though the study is not powered to analyse maternal and infant deaths, there must be some secondary outcomes analyses included to describe and/or compare adverse birth outcomes between women who received a vaccine during pregnancy and women who did not. This is an extremely important component of safety of vaccination studies that include pregnant women. Adverse birth outcomes should include the n(%) of preterm births, low birthweight or small for gestational and stillbirths at a minimum, and these need to be analysed using cox proportional-hazard ratios using the date of vaccination in pregnancy as the start of the period of time at risk, ending at birth of the infant. Good luck with this interesting study.

REVIEWER	PATRINEE TRAISATHIT Department of Statistics, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand
REVIEW RETURNED	30-Oct-2019

GENERAL COMMENTS	Dear authors, I feel that this study protocol addresses important aspects with strong points of the project, especially the number of participants who will be involved (20,745 non-pregnant women). The authors of this project describe a large randomized clinical trial for a novel recombinant hepatitis E virus (HEV) vaccine in women of child-bearing age in Bangladesh. Overall, it is a good project with potentially useful data and with an important message. The planned usage of resources is clearly described. The introduction provides a good generalized background and

	rationale of the topic and the experimental apparatus is appropriate for the study. Please note my minor comments below: 1. The choice of designing a cluster randomized trial may be worth discussing. This would help the reader to understand the study design in a more effective way. 2. The objective is clearly defined. The main focus of the project is to demonstrate the effectiveness of the HEV vaccine in preventing HEV disease during pregnancy and to determine risk factors for severe HEV infection. However, I hope the authors could highlight the importance of their findings in affecting practice. 3. Methods: The authors state that unbalanced covariates may be included in the models. I think it is important to give a definitive list of these. 4. I realize that the safety analyses will be performed using generalized estimating equations for logistic regression to account for cluster randomization. However, the definition of safety outcomes should include all local and systemic adverse events, which is currently difficult for the reader to understand. Please provide more clarity about this issue.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Reviewer Name: Ting Wu
Institution and Country: National Institute of Diagnostics and Vaccine Development in infectious diseases, School of Public Health, Xiamen University, China Please state any competing interests or state ‘None declared’: My institute received funding from Innovax to conduct the phase III clinical trial of this HEV 239 vaccine in China.

Please leave your comments for the authors below The 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" is well designed and the trial would provide important data for proving the effectiveness of the only commercialized hepatitis E vaccine (HEV 239) during pregnancy.
Major comments:
1. Page 3 Line 48, dried blood spots (DBS) are used to assess immunogenicity of the vaccine. Is there any data to analyze the consistency of the antibody levels of DBS and frozen serum after different time periods of storage? Is there any standard used in the assays?

Response: A validation study of the ELISA method for analyzing anti HEV-IgG from DBS samples was conducted in relation to the pilot study. We are currently conducting storage and stability studies on DBS after different timepoints and storage conditions. The results are analyzed and will be published in an article that is currently being written. Three standards produced from the WHO HEV-IgG standard are used in each ELISA run in order to ensure optimal performance and to convert the results into international units.

2. Page 9 Line 48, ‘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’, please make clear the time frequencies of the

phone calls or household visits.

Response: Thanks. 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.

3. Page 9 Line 52, 'women with suspected hepatitis will be tested for liver function and virological causes of hepatitis (A, B, C, E)', please make clear whether women with suspected hepatitis E will be followed-up and take serial serum samples to test the dynamic of antigen, antibody and RNA levels of HEV, until they were completely recovered? The follow-up procedure of suspected hepatitis patients is the core part of this protocol, should be described more clearly.

Response: 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.

4. Page 10 Line 55, as 'cellular immune responses on a subset of participants would be investigated', I think serum sample should be used for this test instead of DBS, how many people would be taken serum sample instead of DBS?

Response: 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.

5. It's better to include more details of laboratory tests into this protocol.

Response: Thanks. It has been added.

Minor comments:
6. Page 4 Line 37, the vaccine was licensed in China for people aged 16 years and older, not in people aged 16-65 years old.

Response: Thanks. It has been changed.

Reviewer: 2
Reviewer Name: Lisa McHugh
Institution and Country: Menzies School of Health Research- Global and Tropical Health Division, Australia Please state any competing interests or state 'None declared': None declared

Please leave your comments for the authors below Thank you for the opportunity to review this protocol.
This will be a very interesting and valuable study.

Given the vaccine is being transported from China, can you please advise in the protocol how the cold-chain process will be maintained. A break in the cold-chain would be of a major concern for this study, so explaining the process of how you will maintain the cold-chain process is necessary.

Response: 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 (mentioned in the text).

There seems to be a discrepancy in the age of the female study participants. On the informed consent form it reads 18-39 year old, however in the protocol and flow-chart this states 16-39 years. Please clarify. Also, on the consent form it states the study participant should observe for jaundice. Presumably the term 'jaundice' will be explained to the study participants.
Response: The 16-17 year-old participants will sign an assent form together with their legal guardians, while participants 18 and above will sign a consent form. Ref page 7-8 in the manuscript: "In the case of 16-17-year-old participants, their legal guardians sign an assent form in place of the consent form."
Yes, the term Jaundice will be well expalined to the study participants while taking the assent and consent by the study doctors.

- Even though the study is not powered to analyse maternal and infant deaths, there must be some secondary outcomes analyses included to describe and/or compare adverse birth outcomes between women who received a vaccine during pregnancy and women who did not. This is an extremely important component of safety of vaccination studies that include pregnant women. Adverse birth outcomes should include the n(%) of preterm births, low birthweight or small for gestational and stillbirths at a minimum, and these need to be analysed using cox proportional-hazard ratios using the date of vaccination in pregnancy as the start of the period of time at risk, ending at birth of the infant.

Response: 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.

Reviewer: 3
Reviewer Name: PATRINEE TRAISATHIT
Institution and Country:
Department of Statistics, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand Please state any competing interests or state 'None declared': None

Please leave your comments for the authors below Dear authors,

I feel that this study protocol addresses important aspects with strong points of the project, especially the number of participants who will be involved (20,745 non-pregnant women).

The authors of this project describe a large randomized clinical trial for a novel recombinant hepatitis E virus (HEV) vaccine in women of child-bearing age in Bangladesh. Overall, it is a good project with potentially useful data and with an important message. The planned usage of resources is clearly described.

The introduction provides a good generalized background and rationale of the topic and the experimental apparatus is appropriate for the study.

Please note my minor comments below:

1. The choice of designing a cluster randomized trial may be worth discussing. This would help the reader to understand the study design in a more effective way.

Response: 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' (mentioned in the text).

2. The objective is clearly defined. The main focus of the project is to demonstrate the effectiveness of the HEV vaccine in preventing HEV disease during pregnancy and to determine risk factors for severe HEV infection. However, I hope the authors could highlight the importance of their findings in affecting practice.

Response: Thanks for your comments

3. Methods: The authors state that unbalanced covariates may be included in the models. I think it is important to give a definitive list of these.

Response: The list has been included in the text.

4. I realize that the safety analyses will be performed using generalized estimating equations for logistic regression to account for cluster randomization. However, the definition of safety outcomes should include all local and systemic adverse events, which is currently difficult for the reader to understand. Please provide more clarity about this issue.

Response: It is clarified in analysis section

VERSION 2 – REVIEW

REVIEWER	Ting Wu National Institute of Diagnostics and Vaccine Development in infectious diseases, School of Public Health, Xiamen University, China My institute received funding from Innovax to conduct the phase III clinical trial of this HEV 239 vaccine in China.
REVIEW RETURNED	17-Dec-2019

GENERAL COMMENTS	I have no more comments.

REVIEWER	Lisa McHugh Menzies School of Health Research, Australia
REVIEW RETURNED	04-Dec-2019

GENERAL COMMENTS	There are some minor grammatical errors throughout the revised and original manuscript. These would be worth correcting before publication. A thorough read-through should be enough.