icddr,b	**RRC APPLICATION FORM**

RESEARCH PROTOCOL **Number: PR-16014** **Version No. 2.4** **Version date: 07-05-2021**	**FOR OFFICE USE ONLY**			
	RRC Approval:	☒ Yes	☐ No	Date:17.2.2016
	ERC Approval:	☒ Yes	☐ No	Date:28.4.2016
	AEEC Approval:	☐ Yes	☐ No	Date:
	External IRB Approval	☐ Yes	☐ No	Date:
	Name of External IRB: _____			

Protocol Title: * (maximum 250 characters including space**)** An effectiveness trial to evaluate the protection of pregnant women with a hepatitis E vaccine in rural Bangladesh and the risk factors for severe hepatitis E infection

Short Title: (maximum **100** characters including space) HEV vaccine study in Bangladesh

Key Words: *Hepatitis E vaccine, Effectiveness study, Pregnant women, Bangladesh

Name of the Research Division Hosting the Protocol:*

☐ Health Systems and Population Studies Division (HSPSD)
☐ Nutrition and Clinical Services Division (NCSD)
☒ Infectious Diseases Division (IDD)
☐ Maternal and Child Health Division (MCHD)
☐ Laboratory Sciences and Services Division (LSSD)
☐ Other (specify)

Has the Protocol been Derived from an Activity:* ☒ No ☐ Yes (please provide following information):

Activity No. :
Activity Title:
PI:
Grant No.: Budget Code: Start Date: End Date:

icddr,b Strategic Priority/ Initiative (SP 2015-8):* (check all that apply**)**

☒ Reducing maternal and neonatal mortality
☐ Controlling enteric and respiratory infections
☐ Preventing and treating maternal and childhood malnutrition
☐ Detecting and controlling emerging and re-emerging infections
☐ Achieving universal health coverage
☐ Examining the health consequences of climate change
☐ Preventing and treating non-communicable diseases

Research Phase (4 Ds):* (check all that apply**)**

☐ Discovery
☐ Development
☒ Delivery
☒ Evaluation of Delivery

Anticipated Impact of Research:* (check all that apply**)**

☒ Knowledge Production
☒ Capacity Building
☒ Informing Policy
☒ Health and Health Sector Benefits
☐ Economic Benefits

Which of the Sustainable Development Goal This Protocol Relates to?:*
(Please visit:http://www.icddrb.net.bd/jahia/Jahia/..... for selecting SDG Code(s) code 3

☒ Yes (please select SDGs)
☐ No

Does this Protocol Use the Gender Framework:*
(Please visit:http://www.icddrb.net.bd/jahia/Jahia/pid/684 for Gender Alanysis Tool with instructions)

☒ Yes (please complete Gender Analysis Tool)
☐ No

If 'no' is the response, its reason(s) in brief:

Will this Research Specifically Benefit the Disadvantaged (economically, socially and/or otherwise**):** ☐ Yes ☒ No

Does this Protocol use Behaviour Change Communication: ☐ Yes ☒ No

Principal Investigator (Should be icddr,b staff):* Sex ☐ Female ☒ Male
(Position, phone no, extension no, cell, and email address):
Dr. K Zaman, Senior Scientist and Epidemiologist
kzaman@icddrb.org
Do you have ethics certification? ☐ No ☒ Yes (please attach in your CV below)
Do you have RBM training certification? ☐ No ☐ Yes (please attach the certificate with CV below)

Primary Scientific Divis
IDD

Co-Principal Investigator(s) Internal: Sex ☐ Female ☒ Male
Dr. John David Clemens
Executive Director
icddr,b,
Tel: 880 2 9827001-10, Ext. 3100

Dr. Firdausi Qadri (F), Senior Director, IDD, icddr,b
Signature or written consent of Co-PI: _____
(electronic signature or email or any sort of written consent)
[if more than one, please copy and paste this row for additional Co-PIs]

Do you have ethics certification? ☐ No ☒ Yes (please attach in your CV below)
Do you have RBM training certification? ☐ No ☐ Yes (please attach the certificate with CV below)

Primary Scientific Divis
Programme of the Co-P

Approval of the Respect
Director/ Programme H

(Signature

Co-Principal Investigator(s) - External: Sex ☒ Female ☐ Male
Dr. Susanne G. Dudman
Address (provide full official address, including land phone no(s), extension no. (if any), cell phone number, an address).
Department of Virology, Division of Infection Control, P.O.Box 4404 Nydalen
Oslo, Norway; Email: Susanne.Gjeruldsen.Dudman@fhi.no
Signature or written consent of Co-PI: _____
(electronic signature or email or any sort of written consent)
[if more than one, please copy and paste this row for additional Co-PIs]

Co-Investigator(s) - Internal: Sex ☐ Female ☐ Male
Dr. Emily Gurley (F), Dr. Md Yunus (M), Dr. Peter Kim Streatfield (M), Dr. Quamrun Nahar (F), Dr. Md. Anisur Rahman (M), Mustafizur Rahman (M)
(Position, phone no, extension no, cell, and email address):
Signature or written consent of Co-I: _____
(electronic signature or email or any sort of written consent)
[if more than one, please copy and paste this row for additional Co-Is

Do you have ethics certification? ☐ No ☒ Yes (please attach in your CV below)
Do you have RBM training certification? ☐ No ☐ Yes (please attach the certificate with CV below)

Primary Scientific Divis
Co-I

Approval of the Respec
Director/ Programme H

(Signature

Co-Investigator(s) – External: Sex ☐ Female ☐ Male
Address (provide full official address, including land phone no(s), extension no. (if any), cell phone number, a address):
Dr. Kathrine Stene-Johansen (F), Dr. Synne Sandbu (F), Dr .Sara V. Watle (F), Dr. Tor Stra
Norwegian Institute of Public Health, Oslo, Norway
Health, Oslo, Norway
Dr. Jahangir Khan, (M); Sweden
Dr. Kaiissar Mannor (M), ideSHi, Bangladesh
Signature or written consent of Co-I: _____
(electronic signature or email or any sort of written consent)
[if more than one, please copy and paste this row for additional Co-Is]

Student Investigator(s) - Internal: Sex ☐ Female ☐ Male (Position, phone no, extension no, cell, and email address **):** Signature or written consent of Student Investitor: _____ (electronic signature or email or any sort of written consent) Have ethics certificate? ☐ No ☐ Yes (If Yes, please attach to your CV below)	Students Affiliation _____ Approval of the Respective Senior Director/ Programme Head (Signature)

Student Investigator(s) - External: Sex ☐ Female ☒ Male
Address (provide full official address, including land phone no(s), extension no. (if any), cell phone number, and email address):
Dr. Joakim Ø verbø,Norwegian Institute of Public Health, Oslo, Norway

Signature or written consent of Student Investitor: _____
(electronic signature or email or any sort of written consent)

Collaborating Institute(s): Please provide full official address

Institution # 1

Country	Norway
Contact person	Susanne Gjeruldsen Dudman
Department (including Division, Centre, Unit)	Department of Virology
Institution (with official address)	Norwegian Institute of Public Health
Directorate (in case of GoB i.e. DGHS)	
Ministry (in case of GoB)	

Institution # 2

Country	China
Contact person	Steven Gao
Department (including Division, Centre, Unit)	
Institution (with official address)	XIAMEN Innovax BIOTECH CO.Ltd
Directorate (in case of GoB i.e. DGHS)	
Ministry (in case of GoB)	

Institution # 3

Country	Bangladesh	
Contact person	Abdul Muktadir	
Department (including Division, Centre, Unit)		
Institution (with official address)	Incepta Pharmaceuticals 40 Shahid Tajuddin Ahmed Sarani Tejgaon I/A, Dhaka-1208. Bangladesh Phone: (+88 02) 8891688 - 703	
Directorate (in case of GoB i.e. DGHS)		
Ministry (in case of GoB)		

Institution # 4

Country	Bangladesh	
Contact person	Dr. Kaiissar Mannor	
Department (including Division, Centre, Unit)		
Institution (with official address)	ideSHi /CMBT (Institute for Developing Science & Health Initiatives) ideSHi /CMBT , IPH Bhaban (2nd floor), Shaheed Tajuddin Ahmed Sarani, Mohakhali Dhaka – 1212, Bangladesh	
Directorate (in case of GoB i.e. DGHS)		
Ministry (in case of GoB)		

Note: If less than or more than three collaborating institutions, please delete or insert blocks as needed.

Contribution by the Members of the Scientific Team:

Members' Name	Contribution								
	Research idea/ concept	Study design	Protocol writing	Respond to external reviewers' comments	Defending at IRB	Developing data collection Tool(s)	Data Collection	Data analysis/ interpretation of results	Manuscript writing
Dr. K. Zaman	☒	☒	☒	☒	☒	☒	☒	☒	☒
Dr. John D Clemens	☒	☒	☒	☒	☒	☒	☒	☒	☒
Dr. Susanne Gjeruldsen Dudman	☒	☒	☒	☒	☐	☒	☐	☒	☒
Dr Emily Gurley	☒	☒	☒	☒	☒	☒	☒	☒	☒
Dr Firdausi Qadri	☒	☒	☒	☒	☒	☒	☒	☒	☒
Dr. Md Yunus	☒	☒	☒	☒	☒	☒	☒	☒	☒
Dr. Peter Kim Streatfield	☒	☒	☒	☒	☒	☒	☒	☒	☒
Dr . Quamrun Nahar	☒	☒	☒	☒	☒	☒	☒	☒	☒
Dr. Jahangir Khan	☒	☒	☒	☒	☐	☒	☒	☒	☒
Dr. Md. Anisur Rahman	☐	☐	☐	☐	☒	☒	☒	☒	☒
Dr..Md.Mustafizur Rahman	☒	☒	☒	☒	☒	☒	☒	☒	☒
Dr. Shafiqul Alam Sarker	☒	☒	☒	☒	☒	☒	☒	☒	☒
Dr. Kathrine Stene-Johansen,	☒	☒	☒	☒	☐	☒	☒	☒	☒
Dr. Synne Sandbu	☒	☒	☒	☒	☐	☒	☒	☒	☒
Dr. Sara Viksmoen Watle	☐	☒	☒	☒	☐	☒	☒	☒	☒
Dr. Kaiissar Mannor	☐	☒	☐	☐	☐	☐	☒	☒	☒
Dr. Asma Binte Aziz	☒	☒	☒	☒	☒	☒	☒	☒	☒

Study Population: Sex, Age, Special Group and Ethnicity

Research Subject:
- ☒ Human
- ☐ Animal
- ☐ Microorganism
- ☐ Other (specify): _____

Sex:
- ☐ Male
- ☒ Female
- ☐ Transgender

Age:
- ☒ 0 – 4 years
- ☐ 5 – 10 years
- ☒ 11 – 17 years
- ☒ 18 – 64 years
- ☐ 65 +

Special Group:
- ☐ Pregnant Women
- ☐ Fetuses
- ☐ Prisoners
- ☐ Destitutes
- ☐ Service Providers
- ☐ Cognitively Impaired
- ☐ CSW
- ☐ Expatriates
- ☐ Immigrants
- ☐ Refugee
- ☐ Others (specify): _____

Ethnicity:
- ☒ No ethnic selection (Bangladeshi)
- ☐ Bangalee
- ☐ Tribal group
- ☐ Other (specify): _____

NOTE: It is icddr.b's policy to include men, women, children and transgender in its research projects involving participation of humans, unless there is strong justification(s) for their exclusion.

Consent Process: (Check all that apply**)**

- ☒ Written
- ☐ Oral
- ☐ Audio
- ☐ Video
- ☐ None

Language:

- ☒ Bangla
- ☐ English
- ☐ Other (specify: _____

Project/Study Site: (Check all that apply**)**

- ☐ Chakaria
- ☐ Bandarban
- ☐ Dhaka Hospital
- ☐ Kamalapur Field Site/HDSS
- ☐ Mirpur (Dhaka)
- ☒ Matlab DSS Area
- ☐ Matlab non-DSS Area
- ☒ Matlab Hospital
- ☐ Mirzapur
- ☐ Bianibazar (Sylhet)
- ☐ Kanaighat (Sylhet)
- ☐ Jakigonj (Sylhet)
- ☐ Other community in Dhaka
  Name: _____
- ☐ Other sites in Bangladesh
  Name: _____
- ☐ Multi-national Study
  Name of the country_____

Project/Study Type: (Check all that apply)

- ☐ Case Control Study
- ☒ Clinical Trial (Hospital/Clinic/Field)*
- ☐ Community-based Trial/Intervention
- ☐ Cross Sectional Survey
- ☐ Family Follow-up Study
- ☒ Longitudinal Study (cohort or follow-up)
- ☐ Meta-analysis
- ☐ Programme Evaluation
- ☐ Programme (Umbrella Project)
- ☐ Prophylactic Trial
- ☐ Record Review
- ☐ Secondary Data Analysis
  Protocol No. of Data Source: _____
- ☐ Surveillance/Monitoring
- ☐ Systematic Review
- ☐ Other (specify): _____

***Note**: International Committee of Medical Journal Editors (ICMJE) defines Clinical Trial as "*Any research project that prospectively assigns human participants to intervention and comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome*".

PI of the RRC- and ERC-approved Clinical Trials should provide necessary information to IRB Secretariat (Research Administration) for registration and uploading into relevant websites (usually at the https://register.clinicaltrials.gov/). They should also provide relevant information to the IRB Secretariat in the event of amendment/modification after their approval by RRC and ERC.

Biological Specimen:

a) Will the biological specimen be stored for future use?	☒Yes ☐No ☐ Not applicable
b) If the response is 'yes', how long the specimens will be preserved?	____5_____ years
c) What types of tests will be carried out with the preserved specimens?	Microbiological and Immunological tests
d) Will the consent be obtained from the study participants for use of the preserved specimen for other initiative(s) unrelated to this study, without their re-consent?	☒Yes ☐No ☐ Not applicable
e) Will the specimens be shipped to other country/ countries? If yes, name of institution(s) and country/ countries?	☒Yes ☐No ☐ Not applicable
f) If shipped to another country, will the surplus/unused specimen be returned to icddr,b? If the response is 'no', then the surplus/unused specimen must be destroyed.	☐Yes ☒No ☐ Not applicable
g) Who will be the custodian of the specimen at icddr,b?	Dr. K. Zaman
h) Who will be the custodian of the specimen when shipped outside Bangladesh?	Dr. Susanne Gjeruldsen Dudman. Norway

i) Who will be the owner(s) of the specimens?	icddr,b and NIPH
j) Has a MoU been signed with regards to collection, storage, use and ownership of specimen? If the response is 'yes', please attach a copy of the MoU.. If the response is 'no', appropriate justification should be provided for not signing a MoU.	☐Yes ☒No☐ Not applicable

Proposed Sample Size:
Sub-group (Name of subgroup e.g. Men, Women) and Number

Name	Number	Name	Number
(1) Pilot phase	100	(3)	
(2) Main phase	20,745	(4)	
		Total sample size	20,845

Determination of Risk: Does the Research Involve (Check all that apply**)**

- ☐ Human exposure to radioactive agents?
- ☐ Foetal tissue or abortus?
- ☐ Investigational new device?
  Specify: _____
- ☐ Existing data available from Co-investigator?
- ☐ Human exposure to infectious agents?
- ☒ Investigational new drug?
- ☐ Existing data available via public archives/sources?
- ☐ Pathological or diagnostic clinical specimen only?
- ☐ Observation of public behaviour?
- ☐ New treatment regime?

Will the information be recorded in such a manner that study participants can be identified from the information directly or through identifiers linked to the study participants?	Yes ☒	No ☐
Does the research deal with sensitive aspects of the study participants' sexual behaviour, alcohol use or illegal conduct such as drug use?	Yes ☐	No ☒
Could information on study participants, if available to people outside of the research team:		
a) Place them at risk of criminal or civil liability?	Yes ☐	No ☒
b) Damage their financial standing, reputation or employability, or social rejection, or lead to stigma, divorce etc.?	Yes ☐	No ☒

Do you consider this research: (check one)

☐ Greater than minimal risk	☒ No more than minimal risk	☐ Only part of the diagnostic test

Note: Minimal Risk: The probability and the magnitude of the anticipated harm or discomfort to participants is not greater than those ordinarily encountered in daily life or during the performance of routine physical, psychological examinations or tests, e.g. the risk of drawing a small amount of blood from a healthy individual for research purposes is no greater than when the same is performed for routine management of patients.

Risk Group of Infectious Agent and Use of Recombinant DNA				
a) Will specimens containing infectious agent be collected?	☒ Yes	☐ No	☐ Not applicable	
b) Will the study involve amplification by culture of infectious agents?	☒ Yes	☐ No	☐ Not applicable	
c) If response to questions (a) and/or (b) is 'yes', to which Risk Group (RG) does the agent(s) belong? (Please visit http://www.icddrb.net.bd/jahia/Jahia/pid/684 to review list of microorganism by Risk Group)	☐ RG1	☒ RG2	☐ RG3	☐ RG4
d) Does the study involve experiments with recombinant DNA?	☐ Yes	☒ No	☐ Not applicable	

Does the study involve any biohazards materials/agents or microorganisms of risk group 2, 3, or 4 (GR2, GR-3 or GR4)?

☒ Yes ☐ No

[If the response is 'yes'] I, (print name of the PI) affirm that we will use the standard icddr,b laboratory procedures for biosafety of the hazardous materials/agents or microorganisms in the conduction of the study.

[redacted]

Signature of the Principal Investigator

26/1/16
Date

Dissemination Plan: [please explicitly describe the plans for dissemination, including how the research findings would be shared with stakeholders, identifying them if known, and the mechanism to be used; anticipated type of publication (working papers, internal (institutional) publication, international publications, international conferences/seminars/workshops/ agencies. [Check all that are applicable]

Dissemination type	Response		Description (if the response is a yes)
Seminar for icddr,b scientists/ staff	☐ No	☒ Yes	Multiple icddr,b scientists will be working on this clinical trial. The results will be shared in internal icddr,b seminars.
Internal publication	☒ No	☐ Yes	
Working paper	☒ No	☐ Yes	
Sharing with GoB (e.g. DGHS/ Ministry, others)	☐ No	☒ Yes	The clinical trial has implications for HEV vaccine policy in Bangladesh and globally. Therefore, the results will be shared with GoB.
Sharing with national NGOs	☐ No	☒ Yes	The results will be shared with national NGOs.
Presentation at national workshop/ seminar	☐ No	☒ Yes	The results will be presented at National workshop/seminar.
Presentation at international workshop/ conference	☐ No	☒ Yes	The clinical trial has implications for HEV vaccine policy. Therefore, the results will be shared in international conferences.
Peer-reviewed publication	☐ No	☒ Yes	Multiple peer-reviewed publications are expected.
Sharing with international agencies	☐ No	☒ Yes	As the trial has global HEV vaccine policy implications, the results will be shared with international agencies.
Sharing with donors	☐ No	☒ Yes	The results of this study will be shared through presentations and peer review publications.
Policy brief	☐ No	☒ Yes	A policy brief will be prepared
Other			
Other			

Funding:

Is the protocol fully funded?	☒ Yes	☐ No
If the answer is yes, please provide sponsor(s)'s name	1. GLOBVAC 2. Bill and Melinda Gates Foundation for the sub-study	
	2.	
Is the protocol partially funded?	☐ Yes	☐ No
If the answer is yes, please provide sponsor(s)'s name	1.	
	2.	

If fund has not been identified:

Is the proposal being submitted for funding?	☐ Yes	☐ No
If yes, name of the funding agency	1.	
	2.	

Conflict of interest:
Do any of the participating investigators and/or member(s) of their immediate families have an equity relationship (e.g. stockholder) with the sponsor of the project or manufacturer and/or owner of the test product or device to be studied or serve as a consultant to any of the above?

☒ No | ☐ Yes (please submit a written statement of disclosure to the Executive Director, icddr,b)

Proposed Budget:

Dates of Proposed Period of Support

(Day, Month, Year - DD/MM/YY)

Beginning Date : April 1, 2016

End Date : March 31, 2020

Cost Required for the Budget Period ($)

Years	Direct Cost	Indirect Cost	Total Cost
Year-1	861,126	86,113	947,239
Year-2	807,826	80,783	888,609
Year-3	772,157	77,216	849,373
Year-4	436,197	43,620	479,817
Year-5			
Total	2,877,319	287,732	3,165,0351

Certification by the Principal Investigator:

I certify that the statements herein are true, complete and accurate to the best of my knowledge. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. I agree to accept the responsibility for the scientific conduct of the project and to provide the required progress reports including updating protocol information in the NAVISION if a grant is awarded as a result of this application.

I also certify that I have read icddr,b Data Policies and understand the PIs' responsibilities related to archival and sharing of research data, and will remain fully compliant to the Policies. (Note: The Data Policies can be found here: http://www.icddrb.org/who-we-are/data-policies)

[redacted]

Signature of PI

26/1/16

Date

Approval of the Project by the Division Director of the Applicant:

The above-mentioned project has been discussed and reviewed at the Division level.

[redacted]

Name of the Division Director

[redacted]

Signature

26/1/16

Date of Approval

Table of Contents

☐ Check here if appendix is included

Project Summary

[The summary, within a word limit of 300, should be stand alone and be fully understandable.]

Principal Investigator: K Zaman
Research Protocol Title: An effectiveness trial to evaluate the protection of pregnant women with a hepatitis E vaccine in rural Bangladesh and the risk factors for severe hepatitis E infection
Proposed start date: April 1, 2016 Estimated end date: March 31, 2020

Background (brief):

a. Burden:

Hepatitis E virus (HEV) infection is the commonest cause of acute hepatitis disease in South Asia, including Bangladesh. Population-based studies, some with verbal autopsies from Bangladesh showed that 19-27% of maternal deaths and 7-13% of neonatal deaths, respectively, were associated with acute onset of jaundice as a complication of pregnancy, with at least 60% of these acute icteric episodes being due to HEV.

b. Knowledge gap:

There are no data showing that HEV vaccination can prevent hepatitis E disease in pregnant women, the group that could benefit most from vaccination.

c. Relevance:

There are currently no effective treatments for pregnant women who contract HEV. Considering that approximately 40% of maternal deaths and 45% of neonatal deaths globally occur in HEV-endemic South Asia, efforts to reduce the burden of maternal and neonatal mortality from HEV in the South Asia region could have a major global impact. HEV vaccines may offer one option for prevention of maternal mortality attributed to HEV. Safe and effective HEV vaccines have been developed, such as the HEV 239 (Hecolin^{TM}) vaccine manufactured by Innovax in China.

Hypothesis (if any):

- Vaccination of women of childbearing age with Hecolin will decrease HEV disease incidence and thus HEV-related complications among vaccinated pregnant women and their children.
- Hecolin is safe and immunogenic in women of childbearing age.
- Hecolin is effective in protecting against HEV genotype 1.
- Vaccination of women against HEV is feasible and cost-effective in Bangladesh
- Dried blood spot and salivary samples (approx 2 ml) are suitable for diagnosing HEV infections and evaluating immunity against HEV.
- High viral load, abnormal immune response, and certain subtypes of HEV are all risk factors for serious disease.

Objectives:

Primary

- To determine the effectiveness of hepatitis E virus (HEV) vaccine given in women of child bearing age in preventing HEV disease during pregnancy among women in rural Bangladesh

Secondary objectives:

- To determine the safety and immunogenicity of HEV vaccine in Bangladeshi women of childbearing age
- To measure the effectiveness of HEV vaccine in preventing HEV disease in non-pregnant Bangladeshi women of childbearing age

- To estimate serological correlates of protection
- 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.
- To analyze the persistance of vaccine induced serum antibody responses

Methods:
We propose a cluster-randomized double-blinded effectiveness trial to assess the feasibility, acceptability, protective impact, and cost-effectiveness of immunization of women of childbearing age with the Hecolin^{TM} HEV vaccine as a strategy to prevent clinical hepatitis HEV during pregnancy in rural Bangladesh.

The trial will be conducted in Matlab where icddr,b has maintained a Maternal, Child Health & Family Planning intervention programme (MCH-FP) since 1978. All 67 villages in the MCH-FP area will be randomized by village at a 1:1 ratio to receive either HEV vaccine or the control vaccine (hepatitis B). All non-pregnant women aged 16-39 years in the study area will be eligible for receipt of vaccine. We require a sample size of 20,745 women enrolled at baseline, which will be feasibly assembled in the population of the Matlab field site. The primary outcome will be clinical HEV disease among pregnant women. All women enrolled in the study will be visted every week to screen for clinical hepatitis and pregnancy. Women who would become pregnant following vaccination with confirmed pregnancies will be visited every 2 weeks to collect information about pregnancy outcomes. Women with clinical hepatitis will have liver function and virological diagnostic testing done to confirm HEV infection. Our proposed project also includes transfer of production technology for this vaccine from China to a high quality Bangladesh vaccine manufacturer (Incepta) in providing low cost HEV vaccine for public health programmes in Bangladesh.Pretesting (100 participants) will be done before proceeding to large scale trail. This pretesting will look mainly the safety and immunogenicity.

Outcome measures/variables:
Confirmed- HEV diseases among pregnant women, safety, immunogenicity, confirmed HEV diseases in non-pregnant women, viral load and subtypes/mutations, altered immune response, maternal and perinatal mortality associated with maternal jaundice, perceptions about vaccine acceptability.

Description of the Research Project

Hypothesis to be tested:

In a hypothesis testing research proposal, briefly mention the hypothesis to be tested and provide the scientific basis of the hypothesis, critically examining the observations leading to the formulation of the hypothesis.

Does this research proposal involve testing of hypothesis: ☐ No☐ Yes (describe below)

Does this research proposal involve testing of hypothesis: ☐ No☒ Yes (describe below)

- Vaccination of women of childbearing age with Hecolin will decrease HEV disease incidence and thus HEV-related complications among vaccinated pregnant women and their children.
- Hecolin is safe and immunogenic in women of childbearing age.
- Hecolin is effective in protecting against HEV genotype 1.
- Vaccination of women against HEV is feasible, acceptable and cost-effective in low-income countries.
- Dried blood spot and salivary samples (approx 2 ml) are suitable for diagnosing HEV infections and evaluating immunity against HEV.
- High viral load, abnormal immune response, and certain subtypes of HEV are all risk factors for serious disease.

Specific Objectives:

Describe the specific objectives of the proposed study. State the specific parameters, gender aspects, biological functions, rates, and processes that will be assessed by specific methods.

Primary objectives:

- To determine the effectiveness of hepatitis E virus (HEV) vaccine given in women of child bearing age in preventing HEV disease during pregnancy among women in rural Bangladesh

Primary endpoint: confirmed- HEV diseases among pregnant women

Secondary objectives:

- To determine the safety and immunogenicity of HEV vaccine in Bangladeshi women of childbearing age
- To measure the effectiveness of HEV vaccine in preventing HEV disease in non-pregnant Bangladeshi women of childbearing age
- To estimate serological correlates of protection
- 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.
- To analyze the persistance of vaccine induced serum antibody responses

Secondary endpoint: Safety, immunogenicity, confirmed HEV diseases in non-pregnant women, viral load and subtypes/mutations, altered immune response, maternal and perinatal mortality associated with maternal jaundice, operational challenges in the delivery of the vaccine and acceptance of vaccine by the users.

Background of the Project including Preliminary Observations:

Provide scientific validity of the hypothesis based on background information of the proposed study and discuss previous works on the research topic, including information on sex, gender and diversity (ethnicity, SES) by citing specific references. Critically analyze available knowledge and discuss the questions and gaps in the knowledge that need to be filled to achieve the proposed aims. If there is no sufficient information on the subject, indicate the need to develop new knowledge.

1.1 Background – Disease

1.1.1 Epidemiology and laboratory diagnosis: Hepatitis E virus (HEV) can cause acute hepatitis, sometimes leading to acute liver failure and death. Globally, it is probably the most common cause of acute hepatitis (Kamar, 2012), estimated to be responsible for 20 million symptomatic hepatitis cases, 70 000 deaths and 3000 stillbirths per year.
HEV is prevalent all over the world. It has four genotypes and two distinct geographical patterns. Genotype 1 (HEV-1), and less frequently genotype 2 (HEV-2) dominate in many low income countries (LIC) causing both large outbreaks and sporadic cases of acute hepatitis. These genotypes infect only humans and can be transmitted by the oro-faecal route, usually via contaminated water in areas with poor sanitation. Genotypes 3 (HEV-3) and 4 (HEV-4) are often found in high and middle income countries and are transmitted zoonotically from animal reservoirs, causing sporadic cases. The main reservoirs seem to be both wild and domestic pigs, although HEV is found in a variety of mammals. The epidemiological difference related to genotype and outcome is poorly understood. It is a public health problem in both rich and poor countries.

Laboratory diagnosis of HEV is done by serology (anti-HEV IgM and IgG) and polymerase chain reaction (PCR). IgM anti-HEV antibodies can be detected during the first few months after HEV infection, whereas IgG anti-HEV antibodies represent either recent or remote exposure. The presence of HEV RNA indicates current infection, (Aggarwal R, 2013). The virus can be further characterized by sequencing and/or growing the virus in culture. The current diagnostic tools are not suitable to identify acute infections in LICs and need to be optimized for the local situation. Treatment of HEV disease is mostly supportive care for serious cases. Ribavirin and Pegyleted interferon have proven effectiveness in some cases, but are both contraindicated during pregnancy.

1.1.2 HEV and pregnancy: The propensity of HEV-1 and HEV-2 to kill pregnant women and their fetuses or newborns in LICs distinguishes HEV from the other hepatitis viruses. Although the overall case fatality ratio is 1 to 3%, it is 5-25% among pregnant women in LICs (Kamar, 2014). The reason for this increased mortality is still unclear, as is the reason why pronounced pregnancy-related mortality is only seen in LICs. It may be a result of greater virulence of the human-adapted HEV-1 and HEV-2 genotypes, increased susceptibility of pregnant women in developing countries where these genotypes are endemic, or the epidemiological conditions of exposure to these viruses in theses settings (Krain, 2014). Membrane rupture, miscarriages, stillbirths and high neonatal morbidity and mortality are also associated with HEV-infections in pregnancy (Patra. 2007). Population-based studies with verbal autopsies in Bangladesh showed that 19-27% of maternal deaths and 7-13% of neonatal deaths were associated with acute onset of jaundice as a complication of pregnancy, with at least 60% of these acute icteric episodes likely due to HEV (Gurley 2012).

1.1.3 Protective immunity: Little is known about the long-term immunity of HEV. A study from China on HEV4 concluded that naturally and vaccine acquired immunity gives 75% protection against infection and alleviates the severity of the disease caused be re-infection (Huang, 2014). There are no data on the protection from natural-infection or vaccine acquired immunity to other HEV genotypes.

1.2 Rationale for the Study

99% of maternal and neonatal mortality occurs in the developing world which also carries the greatest burden of HEV. Efforts to reduce the burden of maternal and neonatal mortality from HEV could have a major global impact.

The current scientific gaps concerning HEV infection are many. In October 2013, WHO established the SAGE Working Group on Hepatitis E for reviewing the evidence with respect to the epidemiology, burden and implementation of a vaccine against HEV. In their recommendations from 2014 the importance and promising results of the Chinese vaccine were noted, but several knowledge gaps pointed out. Effectiveness data that show that the vaccine can be feasibly delivered to women of childbearing age, and that the vaccine can reduce the burden of HEV disease in pregnancy in a cost-effective manner is necessary before the vaccine can be implemented in LICs (Uddin, 2013). The project will contribute this important data.

It is imperative to understand the determinants of the clinical course of HEV and factors associated with resistance to repeat infection. Deciphering the nuances of individual risk and host-related factors will help in improving identification, preventive precautions, and secondary or tertiary management of highly vulnerable individuals. In order to document the global disease burden of HEV it is important to have rapid and reliable diagnostic tests, as many currently available tests give suboptimal results. Some of the main problems of microbiological testing in developing countries are related to sampling and transportation of blood samples because of a shortage in trained personnel to take samples and a lack of facilities for processing blood and maintaining frozen samples. Saliva could potentially be an alternative to blood samples for HEV-testing, which would be safer, easier and cheaper, making HEV-testing more available in developing countries. Antibodies against different pathogens are often found in high titres in saliva, but very little data is available on the presence of HEV antibodies in saliva.

The effectiveness of a HEV-vaccine needs to be tested in a population with high endemicity to be conducted in a reasonable time period. Bangladesh is suitable for this type of study. Recently, it has been estimated that there are 1000 HEV-related maternal deaths per annum in Bangladesh alone (Labrique, 2012). This study will be conducted at Matlab, a field study area of the International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b). A recent study from this region showed that 22% of the population had been previously infected with HEV, and the incidence of seroconversion was 60 per 1000 person years (Labrique, 2010). Further, we have documented baseline data on HEV incidence and burden of disease in Bangladesh (Gurley, 2012, Labrique, 2012). Importantly, the epidemiology of HEV in Bangladesh is similar to other parts of South Asia, so results from the trial will be relevant for the estimated 1.5 billion South Asians at risk for HEV. Genotype 1, which is homologous to the Chinese vaccine antigen, dominates in Bangladesh so there is reason to believe that Hecolin will be as effective in Bangladesh as in the Chinese trial where genotype 4 is endemic. Matlab has been the site for many successful large-scale vaccine field trials in the past, including trials of oral cholera vaccine (Clemens, 1991) and rotavirus vaccine (Zaman, 2010), in addition to several large-scale studies of pregnancy (Persson, 2012). Matlab is thus suited for undertaking the trial described in this protocol.

Bangladesh has historically been a pioneer in vaccine introduction, and the close partnership between icddr,b and the Bangladesh government has played an important role in the deployment of new generation vaccines into public health programs. The government recognizes the seriousness of HEV disease, especially in pregnant women, and has expressed interest in the potential introduction of HEV vaccine in its public sector programs, as the projected impact will be high. However, the feasibility of the delivery of the HEV vaccine and acceptance of vaccine by the users are not known.

Summary of findings from previous studies

The vaccine has been shown to be safe and highly efficacious against HEV disease in a large phase III trial in China with over 100.000 participants (100% after 3 doses of vaccine, 95% CI: 72.1-100.0), where it is commercially manufactured and licensed sale (Zhu, 2010). The majority of cases in this trial were due to genotype 4, indicating that the vaccine was able to confer genotype-heterologous protection. The vaccine was highly immunogenic with seroconversion rates of 98-100% among vaccinees (Zhang, 2014). The study did not provide sufficient data (not powered) to show efficacy in preventing HEV disease during pregnancy. Pregnant women are most vulnerable to HEV-infection and have the greatest impact of an effective vaccine.

Summary of known and potential risks

In phase 1, 2 and a large phase 3 study conducted by the manufacturer, preclinical toxicity of HEV239 was assessed and Hecolin was well tolerated and safe for use in healthy adults. AEs were mainly local with pain, swelling and itching at the injection site. The rate of AEs was similar in the study groups and the placebo groups (receiving a Hepatitis B vaccine). AEs of grade 3 or more were reported only very rarely, and include injection site swelling, fever and headache.

An extended follow-up study from the phase 3 study collecting data on safety for up to 4.5 years after the first vaccine dose, showed the number of reported SAEs (4792 vs 4667; p=0.179) and the number of participants with one or more SAE (4602 vs 4490; p=0.221) to be comparable between the vaccine and placebo groups. None of the SAEs was judged by the principal investigator (who reminded masked to treatment assignment) to be related or possibly related to the hepatitis E vaccine (Zhang, 2015).

Phase III retrospective cohort study - pregnant women

There are limited data on safety of Hecolin® on maternal and fetal outcomes following use during pregnancy.

Following completion of the phase III clinical trial (Zhu, 2010), it was found that 37 women in the HEV 239 vaccine group (out of 31,791) and 31 women in the placebo group (out of 31,735) were either pregnant upon commencement of the study or became pregnant during the trial, even though pregnancy was an exclusion criterion for this study. Data for this group of participants were reviewed carefully (Wu, 2012). The 37 women in the HEV 239 vaccine group had received 53 vaccine doses (22 one dose, 14 two doses and one three doses). The vaccine was well tolerated in the pregnant women with only one woman reporting grade 1 injection site pain. The rate of AEs was similar in the pregnant women who had inadvertently received HEV 239 vaccine and the vaccinated non-pregnant women. Half (19; 51.3%) of the pregnant women in the HEV 239 group underwent elective abortion; the rate was 45.2% in the placebo group. No spontaneous abortions occurred in the vaccine group and the remaining 18 babies, delivered either by normal vaginal delivery (n=7) or caesarean section (n=11), were as healthy as those in the control group (vaginal delivery n=7; caesarean delivery n=10); none of the babies had any congenital abnormality. Birth weights (3573.5±356.7 g vs. 3565.6±531.6 g), lengths (50.7±1.3 cm vs. 50.8±1.5 cm) and gestational ages (276.2±7.6 d vs. 276.6±7.1 d) of the babies born to the mothers in the vaccine group and the placebo group were comparable.

Global Advisory Committee on Vaccine Safety (GACVS) reviewed the safety of Hecolinin its meeting held in June 2014 (World Health Organization, 2014), and concluded as follows:

In summary, available safety data on Hecolin® derived from Phase 1, 2 and 3 clinical trials in healthy subjects are reassuring. However, GACVS noted that there are no safety data in paediatric subjects (<16 years of age), the elderly (>65 years of age), persons with underlying diseases or conditions such as those who are immunosuppressed persons or have liver disease and thus recommended that studies be conducted to assess the safety of Hecolin® in these subpopulations. Any follow-up of those inadvertently vaccinated in pregnancy during the HEV trial should be useful to assess safety in this group. The committee also noted that there are as yet no studies to evaluate the safety and immunogenicity of Hecolin® when given concomitantly with other vaccines. In addition, GACVS

recommended that a Phase 4 post-marketing study be conducted once the vaccine is in more widespread use to further assess the safety profile of Hecolin® , in particular with regard to serious and rare adverse events.

Research Design and Methods

Describe the research design and methods and procedures to be used in achieving thespecific aims of the research project. If applicable, mention the type of personal protective equipment (PPE), use of aerosol confinement, and the need for the use BSL2 or BSL3 laboratory for different part of the intended research in the methods.. Define the study population with inclusion and exclusion criteria, the sampling design, list the important outcome and exposure variables, describe the data collection methods/tools, and include any follow-up plans if applicable. Justify the scientific validity of the methodological approach (biomedical, social, gender, or environmental).

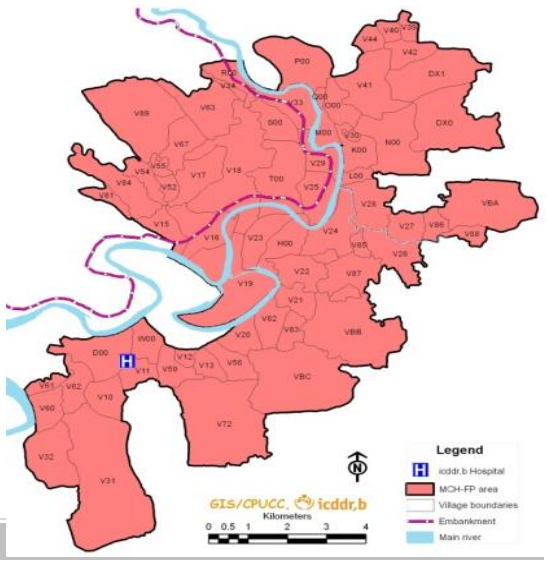
Also, discuss the limitations and difficulties of the proposed procedures and sufficiently justify the use of them.

Study population participant enrolment

Selection of Study Site and Population

The study will be conducted in a rural Bangladesh area called Matlab with a population of over 220,000, where icddr,b has maintained a field research site since 1963. A health and demographic surveillance system (HDSS) which consists of regular cross-sectional censuses and longitudinal registration of vital events, has been maintained in the area since 1966 (icddr,b; 2006). Each of the 22 community health research workers (CHRW) in the MCH-FP area covers a population of about 5800, visiting each household every months to record vital events and to collect health information. CHRWs conduct spot urine tests for pregnancy for women who have missed their last menstrual periods; systematic queries and home urine pregnancy testing by the CHRWs routinely identify pregnancies by 8-12 weeks' gestation. Health services, including immunization for children <5 years of age and women of childbearing age, are provided by another an additional CHRW at each of 41 fixed site clinics (FSC). A Maternal, Child Health & Family Planning intervention program (MCH-FP) is also operating in this area from 41 FSC providing health services and systematic queries pregnancy testing and immunization (Koenig et al, 1998). EPI (Expanded Programme on Immunization) coverage in the villages is more than 90%. Detailed information on all individuals included in the MCH-FP are available and includes sex, exact date of birth, date of marriage, migrations, immunization history, pregnancy outcome, date of death etc. The study will be conducted in the MCH-FP area of Matlab. In 2012, the mid-year population in the MCH-FP area of Matlab was 116,377. There were 2,994 pregnancies, 2,605 live births, 364 miscarriages (121.6/1000 live births) and 25 still births (8.4/1,000 live births) during 2012 (icddr,b 2014). The infant and neonatal mortality rates were 20.5 and 15.6 per 1,000 live births, respectively. The maternal mortality ratio was 119/100,000 live births. More than 80% of deliveries took place in healthcare facilities.

The Map Showing Villages of MCH-FP area, Matlab



General Study Design

The study will be a cluster-randomized double blinded trial to evaluate the efficacy, effectiveness and cost-effectiveness of vaccinating women of reproductive age with three doses of the study vaccine (Hecolin®). HEV disease among pregnant women as the primary outcome. The study design will also allow for investigating possible relationship between host and viral factors against the severity of HEV disease and vaccine efficacy. The feasibility and acceptability of the HEV vaccine will be assessed using qualitative methodology.

In cluster randomised controlled trials, groups of people, rather than individuals, are randomly allocated to the interventions under investigation. The 'unit of allocation' in this trial is a village.
A cluster randomized controlled study has been selected as the method of choice to conduct a vaccine effectiveness trial (Clemens, 1996). A vaccine effectiveness trial is a randomized trial designed to evaluate vaccine impact, vaccine acceptability and programmatic feasibility when the vaccine is given under realistic public health conditions. As such conditions are not achievable in an individually randomized design, but can be achieved with cluster randomized design, the latter design is selected. Our trail's primary aim is to measure total vaccine protection. We are aware that the trial may not be adequately powered to measure indirect and overall vaccine protection, which are considered secondary aims. The trial investigators are very familiar with designing and conducting cluster-randomized vaccine trails.

Pregnant women are most vulnerable to HEV-infection and have the greatest impact of an effective vaccine. Effectiveness of the vaccine in pregnant women has not been investigated, which is one of the main reasons for this study to be conducted. Hence, the vaccine is not licensed for pregnant women. Some fertilewomen are likely to become pregnant within the timeframe of our study, and some of these will have been vaccinated, thus providing necessary data for this group.

HEV can not be distinguished from other viral hepatitis infections without specific virological diagnostics. Characterization of the virus is important to document the effect of the vaccine on different circulating HEV - genotypes.

The inclusion of participants will take place during six months. Vaccines will be administered as a 3-dose regimen on day 0, at one month, and six months. The follow up period will be average of 2 years following the third dose of vaccine.

Study participants will be non-pregnant women who were randomly allocated to receive HEV vaccine or HBV vaccine at Day 0, 1 month and 6 months. In the ongoing study blood was collected at baseline (day 0) and 1 month after dose 3 (7 month).

Inclusion Criteria

- Women aged 16-39 years at the time of the first vaccination
- Living in the MCH-FP area of Matlab

Exclusion Criteria

Participants will be excluded from the study if they meet any of the following criteria:
For dose 1:

- Pregnancy (visible or verbal report on date of last menstruation or urine test) *
- History of severe allergic reaction to a vaccine or a vaccine component
- Having other vaccine or immunoglobulin within two weeks
- Serious chronic disease (as assessed by medical officer)
- Acute and chronic infectious disease
- Fever > 38 ℃ (temporarily)

*Women with a history of missed periods, irregular cycles, or uncertain last menstrual period (LMP) date will be tested for pregnancy using a urine strip test before enrollment and vaccination. If the test is negative, vaccination will proceed. If the test is positive, or if the participant refuse, they will be excluded from enrollment and vaccination.

Participants will be excluded from further vaccination if they meet any of the five first of above mentioned exclusion criteria, but will still be eligible for ITT analysis and participating in the study

until the end. In such cases the blood sample for measuring vaccine response will be taken one month after the last vaccine dose.

After 1st dose or after 2nd dose of vaccine if a participant becomes pregnant then surveillance will continue, but no further doses of vaccine will be given according to the study protocol exclusion criteria.

Obtaining informed consent

The eligible women will be approached in their homes and will be asked to come to the Fixed site clinic (FSC) for enrolment in the study.
Theinvestigatorordesignatewilldescribetheprotocoltothepotentialparticipantface-to-face. The process will ensure that potential participants have an understanding of the potential risks and benefit, or lack of benefit, of the vaccine, the study procedures (including maintaining confidentiality and anonymity), their right to refuse and/or withdraw from the study at any point without affecting the health services or care they receive, and without having to disclose a reason for their refusal or withdrawal.
Participants have opportunity to inquire about the details of the study. Recruitment is based on voluntary participation and the participants can decline at any time. Designated study staff will review the consent form with potential participants who meet study eligibility criteria. The consent form will also be verbally explained in the appropriate local language.The participants can also request destruction of the dataconcerning them.
Incidental abnormal findings of health importance for the participants will becommunicated.
Confidentiality of personal identifiers will be maintained by keeping names noted inprimary data instruments in secure files and by removing names from the computerized dataset for analysis
The participants to be enrolled will sign a consent and assent (in case of 16-17 years old participant) form informing them of the nature of the study according to the Declaration of Helsinki and the ICH Harmonized Tripartite Guidelines for Good Clinical Practices. The Informed consent forms will be archived at the Matlab Hospital.
In accordance with the principles of the current revision of the Declaration of Helsinki, a volunteer has the right to withdraw from the study at any time and for any reason, and is not obliged to give his or her reasons for doing so. The Investigator may withdraw the volunteer at any time in the interests of the volunteer's health and wellbeing**.**
The group will continue to follow up all the vaccinees, with their agreement, until the end of the study. The reason for withdrawal will be recorded in the CRF. If withdrawal is due to an AE, appropriate follow-up visits or medical care will be arranged, with the agreement of the volunteer, until the AE has resolved, stabilized or a non-trial related causality has been established.

Pretesting Before proceeding a large scale trial, we will perform pretesting of the study. We will look mainly at/on the safety and also immunogenicity of this vaccine with this small sample size. This will be done outside of the MCH-FP area but within the HDSS area. The sample size for the pretesting phase will be 25 female and 25 male from the HEV and HBV group respectively (estimated assuming 70% seroresponse in vaccine group vs. 10% in control, $p<0.05$, 2 tailed, 0.9 power). The sample will be randomly selected among nonpregnant women aged 16-39 years and male will be randomely selected from the age group of 16-39 years. We will observe each participant for 30 minutes after each vaccination. After each dose field worker will visit daily for 7 days at their homes enquiring about any untoward events. Blood will be collected before vaccination and 1 month after the second dose. Process for determining immunogenity will be same as described in the protocol.

We want to find out the strength and longevity of the immune reaction to a two-dose schedule compared to the standard three-dose schedule of the vaccine for the pilot study. We will collect one blood sample

(maximum 3ml) from all participants of pilot study (100) to determine the immune response and an additional 9 ml blood from the 10 persons previously selected to assess the cellular response approximately 2 years after first vaccination.

To better understand the persistence of immunological responses 2 years (24 to35 months) after the last dose of the vaccine we will do a sub-study under this main study. We will sequentially enrol for this addon study from the list of original enrollment and identify eligible participants who received 3 doses of vaccine and completed follow up 2 years (24 to 35 months) and also who received 1 or 2 doses of vaccine (incomplete schedule).We will include total 1,760 (440 participants in each group) HEV vaccine study participants for this sub-study among those who are 1) HEV seronegative at baseline and received HEV vaccine, 2) those who were HEV seropositive at baseline and received HEV vaccine, 3) who were HEV seropositive at baseline and received HBV vaccine, 4) participants who are HEV seronegative at baseline and received HBV vaccine (pure control group).

Enrolment

Following informed consent obtained by the medical officer, nurses will administer vaccine to the participants. All enrolled women in the study will be provided with an immunization card with identification numbers and with a phone number to call if they become ill with jaundice or missed periods (>2 weeks weeks from the expected start date) between the weekly home visits by the study field workers.The phone number will be answered by a study staff member who will make household visits to screen for suspected hepatitis regardless of whether or not the woman is pregnant. Regular SMS messages will be sent to remind participants to report cases of jaundice (>80% of households have access to a cellphone). All women whose pregnancies are detected through the weekly visits by study field workers or routine MCH-FP visits will be visited every 2 weeks to collect information about pregnancy outcomes and to screen for clinical hepatitis. A copy of the consent form and participant information sheet will also be provided to the participant before concluding the enrolment visit.

Study vaccination

For this study Hecolin and Hepa-B is defined as Investigational Medicinal Products (IMP).

Administration of vaccine

On vaccination day, vaccine will be administered according to manufacturer's instructions and local study logistics. The vaccine will be administered intramuscularly in the deltoid muscle of either arm, avoiding broken skin or injuries. It is recommended, but not required, that the injection be administered into the non-dominant arm.
Participants will be observed at the vaccination site for 30 minutes after the vaccine is administered to monitor for any immediate reactions.

Dose modification

There will be no dose modification. Previous large scale study in China used this dose successfully (Zhu et al, 2010).

Concomitant interventions

Any interventions required to treat a disease or condition in an enrolled participant will be allowed.
Prohibited interventions are vaccinations with a vaccine against HEV other than the study vaccine.
All concomitant medication used by the participant will be recorded in the participant's file and CRF.

Drug Accountability

The responsible site personnel will confirm receipt of study drug and will use the study drug only within the framework of this clinical study and in accordance with this protocol. Receipt, distribution, return, and destruction (if any) of the study drug must be properly documented according to the sponsor's agreed and specified procedures.

Drug Labelling

The investigational product will have a label permanently affixed to the outside and will be labeled according with ICH GCP and national regulations, stating that the material is for clinical trial / investigational use only and should be kept out of reach of children.

Information on study interventions

Interventions

Intervention treatment

Hecolin® is based on a 239 amino acid long recombinant HEV peptide, termed HEV 239, corresponding to amino acids 368-606 of open reading frame 2 (ORF2) which encodes the capsid protein of HEV. The amino acid sequence is derived from a genotype 1 Chinese HEV strain Hecolin® . It was developed and is produced by Xiamen Innovax Biotech CO., LTD (Innovax). The vaccine is expressed in Escherichia coli, and is purified to >95% homogeneity (Zhang 2014). The vaccine contains 30 μg of the purified protein absorbed to 0.8mg of aluminium hydroxide suspended in 0.6 ml of buffered saline.

Control treatment

A commercial hepatitis B vaccine (Hepa-B®) produced by InceptaVaccine Ltd. will be used as a control vaccine. Each 0.5 ml dose (for age 0-18 yrs) contains 10 μg of hepatitis B surface antigen absorbed on Alluminium Hydoxide gel equivalent to Al3+ 0.25 mg. Each 1.0 ml dose (for age 19 yrs and above).

Allocation of intervention and control

All 67 villages in the MCH-FP area will be randomized by village at a 1:1 ratio to receive either HEV vaccine or the control vaccine according to randomization. We will use hepatitis B vaccine (HBV) as the control vaccine because it will be of benefit to the target population and because the dosing regimen is the same for both vaccines. Since the volume of the vaccine in each dose is different for the two vaccines (HEV 0.6 ml and HepaB 0.5 ml for 16-18 years & 1 ml for 19 years and above), to maintain blinding vaccinators will be kept unaware of these differences, and no one involved with vaccination will participate in follow-up or adverse event assessment of study participants.. The two vaccines will be filled in identical, coded, single dose vials. Vaccines will be administered as a 3-dose regimen on day 0, at one month, and six months. Bangladesh introduced HBV for children <5 years into the EPI program in 2005, so women aged ≥16 years have not been routinely vaccinated against HBV and could benefit from receiving this vaccine.

Randomization and blinding procedures

Incepta will receive bulk vaccine for the trial from Innovax, and will package both the HEV and control (hepatitis B) vaccines in identical presentations. The investigational product will have a label permanently affixed to the outside and will be labelled according with ICH GCP and national regulations, stating that the material is for clinical trial/investigational use only and should be kept out of reach of children.

An independent statistician will ensure randomization.

Storage and handling

Hecolin should be stored at 2℃ to 8℃, out of direct sunlight, and has an approved shelf life of 36 months under appropriate storage conditions. The vaccine is stable for at least 45 months.

Visits to participants

Eligibility and vaccination visits

All women aged 16-39 have already been identified through existing Matlab HDSS system. The women will be approached at their homes by a study team. A standardized form will be used to assess eligibility according to the criteria.. If found eligible for the study the women will be asked to come to the FSC for enrolment in the study.

After each vaccination dose, the participant will be observed for 30 minutes and a field worker will visit daily for 7 consequtive days at their homes enquiring about any untoward events. They will also be asked to report if they have any local reactions (i.e., erythema, swelling, induration and pain at the injection site), systemic reactions (i.e., nausea, malaise, myalgia, arthralgia, headache) and fever within 7 days after vaccination.

Procedures for the vaccination visit, including recording immediate post vaccination adverse events, are described in SOP "Post Vaccination Observation" Procedure and SOP "Immediate Post Vaccination Reaction Management Procedure".

Post vaccination scheduled visits

All participants will have a blood sample taken one month after the last dose of the vaccine.
All women whose pregnancies are detected through the weekly home visits by the study field workers or routine MCH-FP visits will be visited every 2 weeks to collect information about pregnancy outcomes and to screen for clinical hepatitis.

Close of study visits in the pregnant cohort.

The last study visit to pregnant women included will be 14 days after birth. The participant's general health status will be recorded and information on any changes in the participant's occupation, residence or other important time-varying factors will be collected at this visit. Information on pregnancy outcome and health status of the child will be recorded. Participants will be advised there will be no further scheduled study visits but that they should inform the study team by telephone of any possible serious adverse events or signs of hepatitis.

Close of call

The detection for pregnancies and hepatitis surveillance will end atleast 2 years after the last dose of vaccination is given.

Criteria for Participant Discontinuation

Participants may be discontinued from study treatment and assessments at any time. Specific reasons for discontinuing a participant for this study are:

- Voluntary discontinuation by the participant who is at any time free to discontinue his/her participation in the study, without prejudice to further treatment.
- Safety reason as judged by the Principal Investigator
- Major protocol deviation
- Incorrect enrolment ie, the participant does not meet the required inclusion/exclusion criteria for the study
- Participant lost to follow-up

Procedures for Discontinuation

Participant Discontinuation

Participant will be offered a clinical assessment for safety prior to leaving the study, consistent with the assessments done during follow-up visits. Furthermore, SAEs will be cared for and followed through until a cause has been established, even if the participant is withdrawn from the study.

Trial Discontinuation

The whole trial may be discontinued at the discretion of the PI or the sponsor in the event of any of the following:

- Occurrence of AEs unknown to date in respect of their nature, severity and duration
- Medical or ethical reasons affecting the continued performance of the trial
- Difficulties in the recruitment of participants
- Cancellation of drug production

The sponsor and principal investigator will inform all investigators, the relevant Competent Authorities and Ethics Committees of the termination of the trial along with the reasons for such action. If the study is terminated early on grounds of safety, the Competent Authorities and Ethics Committees will be informed within 15 days.

Safety Monitoring and reporting

The investigator is responsible for the detection and documentation of events meeting the criteria and definition of an adverse event (AE) or serious adverse event (SAE). Each participant will be instructed to contact the investigator immediately should they manifest any signs or symptoms they perceive as serious.
The methods for collection of safety data are described below.

Definitions

Adverse Event (AE)

An AE is any untoward medical occurrence in a participant administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment.
An adverse event (AE) can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.
The term AE is used to include both serious and non-serious AEs.

If an abnormal laboratory value/vital sign are associated with clinical signs and symptoms, the sign/symptom should be reported as an AE and the associated laboratory result/vital sign should be considered additional information that must be collected on the relevant CRF.

Serious Adverse Event (SAE)

Any untoward medical occurrence that:

- Results in death
- Is life-threatening, an AE is life threatening if the participant was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe.
- Requires in-participant hospitalization or prolongation of existing hospitalization. In general, hospitalization signifies that the participant has been detained (usually involving at least 24 h stay) at the hospital or emergency ward for treatment that would not have been appropriate in the physician's office or outparticipant setting.
- Results in persistent or significant disability or incapacity. An AE is incapacitating or disabling if the event results in a substantial disruption of the participant's ability to carry out normal life functions. This definition is not intended to include experiences of relatively minor medical significance such as headache, nausea, vomiting, diarrhoea, influenza, injection site reactions and accidental trauma (e.g. sprained ankle).
- Is a congenital abnormality or birth defect in the offspring of the study participant.
- Is an important medical event that may jeopardize the subject or may require medical intervention to prevent one of the outcomes listed above. Examples of such treatments are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization.

Medical and scientific judgment is to be exercised in deciding on the seriousness of a case. Important medical events may not be immediately life-threatening or result in death or hospitalization, but may jeopardize the subject or may require intervention to prevent one of the listed outcomes in the definitions above. In such situations, or in doubtful cases, the case should be considered as serious. Hospitalization for administrative reason (for observation or social reasons) is allowed at the investigator’s discretion and will not qualify as serious unless there is an associated adverse event warranting hospitalization.
Hospitalization for either elective surgery related to a pre-existing condition which did not increase in severity or frequency following initiation of the study, or for routine clinical procedures (including hospitalization for "social" reasons) are not considered as SAEs. When in doubt as to whether “hospitalization” occurred or was necessary, the AE will be considered serious.
The definition of a routine clinical procedure is a procedure, which may take place during the study period and should not interfere with the study vaccine administration or any of the on-going protocol specific procedures. If anything untoward occurs during an elective procedure and satisfies any of the criteria for SAE, this will be documented and reported.

Time Period for Reporting AE and SAE

For each participant the standard time period for collecting and recording AE and SAEs will begin at the time of the first vaccination and will continue for at least 1 year following the last dose of study treatment for each participant.
During the course of the study all AEs and SAEs will be followed up actively and passively for each participant; events will be followed up to resolution, unless the event is considered by the investigator to

be unlikely to resolve due to underlying disease. Every effort will be made to obtain a resolution for all events, even if the events continue after study completion.

Recording of Adverse Events

If the participant has experienced adverse event(s), the investigator will record the following information in the CRF:

- The nature of the event(s) will be described by the investigator in precise standard medical terminology (i.e. not necessarily the exact words used by the participant).
- The duration of the event will be described in terms of event onset date and event ended data.
- The intensity of the adverse event: Mild/ Moderate / Severe; according to Common Terminology Criteria for Adverse Events version 4.0 (CTCAE).
- The Investigator will assess the causality of all SAEs, using the following question: "Is there a reasonable possibility that the SAE may have been caused by the study vaccine?" After assessment of causality, the investigator will classify the SAE as defined below:

  Unrelated:

  There is not a temporal relationship to investigational product administration (too early, or late, or investigational product not taken), or there is a reasonable causal relationship between non-investigational product, concurrent disease, or circumstance and the AE.

  Unlikely:

  There is a temporal relationship to investigational product administration, but there is not a reasonable causal relationship between theinvestigational product and the AE.

  Possible:

  There is reasonable causal relationship between the investigationalproduct and the AE. Dechallenge information is lacking or unclear.

  Probable:

  There is a reasonable causal relationship between the investigational product and the AE. The event responds to dechallenge. Rechallenge is not required.

  Definite:

  There is a reasonable causal relationship between the investigational product and the AE.

- Action taken

- The outcome of the adverse event – whether the event is resolved or still ongoing.

If an event meets the criteria to be determined "serious" (see definition of SAE); it will be examined by the investigator (and DSMB) to determine all contributing factors applicable to each SAE. These contributing factors will be documented and reported appropriately. Every effort will be made by the investigator to explain each SAE and assess its causal relationship, if any, to administration of the study vaccine.

Reporting Procedure

AEs and SAEs

All adverse events and serious adverse events that should be reported as above will be recorded in the participant's CRF.
All participants will be instructed to contact the investigator team immediately, should the participant show any signs or symptoms perceived as serious during the period extending from enrolment to the study end. Monitoring will be done for events requiring medical treatment or hospitalization, or deaths. Whenever possible, SAEs will be documented in terms of a diagnosis or syndrome rather than multiple symptoms that are clearly manifestations of the same diagnosis/syndrome. In case signs and symptoms are reported by the participant, a medical diagnosis will be obtained by the investigator. If a diagnosis cannot be obtained then enter each sign or symptoms as separate events. Pre-existing conditions or signs and/or symptoms (including any which are not recognized at study entry but are recognized during the study period) present in a participant prior to enrolment will be recorded in the medical history in the participant's CRF. SAEs that occur after participant is enrolled in the study, but prior to first vaccination, will be documented in the medical history and pre-existing conditions sections of the CRF, and may necessitate delay or cancellation of vaccination for that participant.
SAEs in all participants will be collected through an SAE form (structured questionnaire) administered at scheduled study visits and by participant initiated reports between assessments. SAEs will be followed up to resolution, and cessation of treatment or end of study, irrespective of severity or whether or not they are considered vaccination-related.
SAEs observed by the study team or reported by the participant or participant's relatives will be evaluated by the investigator and recorded in the Serious Adverse Event page of the participant's CRF. The diagnosis, date and time (where appropriate) of onset, outcome, severity and relationship to vaccination will be established. Details of any treatment given will be recorded appropriately.
The initial SAE report will be followed by a full summary utilizing the SAE Report Form detailing relevant aspects of the adverse events in question. Where applicable, information from relevant hospital case records and autopsy reports will be obtained.
SAEs must be reported by the investigator to the sponsor within 24 hours after the site has gained knowledge of the SAE. Every SAE must be documented by the investigator on the SAE pages. The Serious Adverse Event Report Form must be completed, signed and sent to the sponsor. The initial report shall promptly be followed by detailed, written reports if necessary. The initial and follow-up reports shall identify the trial subjects by unique code numbers assigned to the latter.
The sponsor keeps detailed records of all SAEs reported by the investigators and performs an evaluation with respect to seriousness, causality and expectedness.

Annual Safety Report

Once a year throughout the clinical trial, the sponsor will provide the Competent Authority with an annual safety report. The format will comply with national requirements.

Clinical Study Report

The adverse events and serious adverse events occurring during the study will be discussed in the safety evaluation part of the Clinical Study Report.

SAE Reporting to relevant bodies

The Pharmacovigilance Coordinator will coordinate the safety monitoring and reporting in the study. The PI will report SAEs to local IRB/EC following local law and IRB/EC requirements. The PI will also report SAEs to the vaccine manufacturer. Adequate documentation will be provided to the Sponsor showing that the IRB/EC and manufacturer have been properly notified.

The Pharmacovigilance Coordinator will report SAEs to DSMB, as required. A detailed description of DSMB functions and responsibilities and guidelines on the transmission flow of SAEs is provided in the DSMB charter. Notification of SAE to concerned National Regulatory Authorities will follow national law requirements

Management of SAEs

SAE will be managed in line with current Good Medical Practice. Management of any SAE will be recorded in the CRF. Medical assistance, consultation and follow up will be done at the Matlab Hospital. Participants may also be referred to the local FSC for follow up.

Pregnancy

To ensure participant safety and the safety of the unborn child, each pregnancy in a participant who has been vaccinated during the study period will be reported to the sponsor within one week of the site learning of its occurrence. The pregnancy will be followed to determine outcome, including spontaneous or voluntary termination, details of birth, and the presence or absence of any birth defect, congenital abnormalities, or maternal and /or newborn complications. The pregnancy follow-up will be done until conclusion of of the pregnancy even if the intended duration of safety follow-up for the trial has ended. This will be performed by the study field research assistants and female field workers.

Data management and monitoring

Case Report Forms (CRFs)

The designated investigator staff will enter the data required by the protocol into the Case report forms (CRF) through interviewing and also reviewing medical records. The Principal Investigator is responsible for assuring that data entered into the CRF is complete, accurate, and that entry is performed in a timely manner. The signature of the investigator will attest the accuracy of the data on each CRF. If any assessments are omitted, the reason for such omissions will be noted on the CRFs. Corrections, with the reason for the corrections will also be recorded.

Source Data

The records for each participant should contain information which is important for the participant's safety and to fulfil the requirement that critical study data should be verifiable.
A health and demographic surveillance system has been maintained in the area for the last 50 years. Detailed information of all individuals are available which include sex, exact date of birth, date of marriage, migrations, immunization history, pregnancy outcome, date of death etc.

Study Monitoring

The investigator will be visited on a regular basis to check the following:

- Informed consent process
- Reporting of adverse events and all other safety data
- Adherence to protocol
- Maintenance of required regulatory documents
- Study Supply accountability
- Facilities and equipments
- Data completion on the CRFs including source data verification (SDV).

The monitor will review the relevant CRFs for accuracy and completeness and will ask the site staff to adjust any discrepancies as required.

Sponsor's representatives (e.g. monitors, auditors) and/or competent authorities will be allowed access to source data for source data verification in which case a review of those parts of the hospital records relevant to the study may be required.

Data and Safety Monitoring Board

The study will be monitored by an Independent Data and Safety Monitoring Board (DSMB) constituted by icddr,b with input from the Ethical Review Committee (ERC). The DSMB will include representation outside of icddr,b. The DSMB is expected to convene once prior to the start of the study after ethical and regulatory approval of the study protocol. The DSMB is expected to convene meeting after the completion of the pretesting, at the start of the study, near the middle of the study and at study completion. We will present the data of pretesting phase in DSMB before further proceeding of the study.The committee will be responsible for the monitoring and review of the safety and conduct of the trial. There will be also a DSMB at the NIPH.

Confidentiality

The investigator shall arrange for the secure retention of the participant identification and the code list. The study documentation (CRFs, Site File etc) shall be retained and stored during the study and for 15 years after study closure. All information concerning the study will be stored in a safe place inaccessible to unauthorized personnel.

Database management

All the data are kept in a database at icddr,b where each study participant will have an unique identification number. Data security procedures ensure the prevention of unauthorized access to or loss of participant data and storage (including timeframe) during and after the trial. The system includes processes to promote data quality (eg, double data entry; range check for data values).

STUDY MANAGEMENT

Investigator Delegation Procedure

The icddr,b principal investigator is responsible for making and updating a "delegation of tasks" listing all the involved study staffs and their role in the project. He will ensure that appropriate training relevant to the study is given to all of these staffs, and that any new information of relevance to the performance of this study is forwarded to the staff involved.

Protocol Adherence

Investigators ascertain they will apply due diligence to avoid protocol deviations.
All significant protocol deviations will be recorded and reported in the Clinical Study Report (CSR).

Study Amendments

If it is necessary for the study protocol to be amended, the amendment and/or a new version of the study protocol (Amended Protocol) must be notified to and approved by the Competent Authority and the Ethics Committee according to EU and national regulations.

Audit and Inspections

Authorized representatives of a Competent Authority and Ethics Committee may visit the centre to perform inspections, including source data verification. Likewise the representatives from sponsor may visit the center to perform an audit. The purpose of an audit or inspection is to systematically and independently examine all study-related activities and documents to determine whether these activities

were conducted, and data were recorded, analyzed, and accurately reported according to the protocol, Good Clinical Practice (ICH GCP), and any applicable regulatory requirements. The principal investigator will ensure that the inspectors and auditors will be provided with access to source data/documents.

Ethical and regulatory requirements

The study will be conducted in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with ICH/Good Clinical Practice and applicable regulatory requirements. Registration of participant data will be carried out in accordance with national personal data laws.

Ethics Committee Approval

The study protocol, including the participant information and informed consent form to be used, must be approved by the ethics committee in Bangladesh and Norway before enrolment of any participants into the study.

The icddr,b principal investigator is responsible for informing the ethics committee of any serious and unexpected adverse events and/or major amendments to the protocol as per national requirements.

Study registration

The study will be registered in www.clinicaltrials.gov.

Participant Identification

The investigator is responsible for keeping a list of all participants (who have received study treatment or undergone any study specific procedure) including date of birth and personal number, full names and last known addresses.

The participants will be identified in the CRFs by ID number.

Trial sponsorship and financing

The study is sponsored by GLOBVAC funds from the Norwegian Research Council.

Trial insurance

As a standard insurance policy of clinical trails of icddr,b.

Publication policy

Upon study completion and finalization of the study report the results of this study will be submitted for publication.

The results of this study will also be submitted to the Competent Authority and the Ethics Committee according to national regulations.

All personnel who have contributed significantly with the planning and performance of the study may be included in the list of authors.

Sample Size Calculation and Outcome (Primary and Secondary) Variable(s)

Clearly mention your assumptions. List the power and precision desired. Describe the optimal conditions to attain the sample size. Justify the sample size that is deemed sufficient to achieve the specific aims.

Sample size: The sample size for the study has been calculated based on the following assumptions (data from Matlab HDSS and HEV research studies). The goal will be to estimate total vaccine protection in a per protocol analysis. Women 16-39 years constitute 20% of the general population, and 22% will be HEV seropositive at baseline (we assume that these women will be protected against HEV

infection); post dose 3 follow up will range from 22-28 months (average 25 months). During post dose 3 follow up, 16% of women (followed over a mean of 16 months) will become pregnant and will 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, 3806 are expected to become pregnant after dose 3 and 3073 to be followed to term (follow up of the remaining pregnancies will be right censored by termination of follow up); 6% of seronegative pregnant women will become infected during pregnancy of which 35% of infections will be symptomatic; the protective efficacy of a 3-dose regimen of HEV vaccine against symptomatic infections is $\geq$ 95%, $p<0.05$ (2 tailed); there will be 15% loss of person-time due to migration out; 10% of women will not be included due to pregnancy at baseline, the refusal rate will be 5%; another 10% will not complete 3 doses for other reasons (e.g intercurrent pregnancy) and the design effect will be 2. To achieve 0.8 power, we will need a sample size of 20,745 women to be enrolled at baseline (60% are assumed to receive all 3 doses which takes into account of 10% baseline pregnancy, 5% refusal, 15% loss to follow up and 10% for other reasons e.g. intercurrent pregnancy), derived from a total population of 116,377, which can be assembled from the MCH-FP area population in Matlab (Labrique et al, 2010; icddr,b 2012). Although not a primary outcome, our study will have 90% power to evaluate $\geq$95% protective efficacy against HEV disease among non-pregnant women. Although the ultimate goal of any future vaccination campaign would be to prevent maternal and perinatal mortality, the sample size for our study will not be able to directly evaluate these outcomes with adequate power due to the low perinatal, maternal death ratios in Matlab. However, given the substantial evidence linking maternal and perinatal mortality to HEV disease in pregnancy, demonstration of vaccine protection against confirmed HEV disease in pregnancy will provide strong evidence that vaccination will likely prevent maternal and perinatal deaths. The table below shows the results of the sensitivity analysis of sample size with different assumptions, maintaining 0.8 power and a design effect of 2.

Incidence rate of symptomatic HEV among pregnant women followed to term	Baseline sero-positive	Receive 3 doses	Loss of follow up	Vaccine effectiveness	Sample size
0.42%	22%	90%	15%	95%	20745
0.40%	22%	90%	15%	95%	21788
0.42%	27%	90%	15%	95%	22167
0.42%	22%	85%	15%	95%	21966
0.42%	22%	90%	20%	95%	22042
0.42%	22%	90%	15%	90%	23811

Endpoints

Primary endpoints

Confirmed HEV disease among pregnant women

Illness that lasted for at least 3 days; and abnormal

of serum ALT level of $\geq$ 2.5 ULN; and detection of IgM anti-HEV in serum collected within 1 month after onset; and /or the presence of HEV RNA and/or $\geq$ 4 folds rise of IgG anti-HEV levels in paired sera.

Total vaccine protection is defined as reduction of the incidence of HEV disease in recipients of complete regimens of HEV vaccine in HEV vaccine clusters relative to recipients of complete regimens of HBV vaccine in HBV vaccine clusters.

Secondary endpoints

Endpoints- safety, immunogenicity, confirmed HEV diseases in non-pregnant women, viral load and subtypes/mutations, altered immune response, maternal and perinatal mortality associated with maternal jaundice, perceptions about vaccine acceptability.

Safety: Serious Adverse Events (SAEs) will be assessed. A detailed clinical description using standardized forms will be provided, including symptoms, the date and time of onset, first observation, diagnosis, end of episode and final outcome, as recommended by the Brighton collaboration.

Confirmed HEV diseases in non-pregnant women: clinical and laboratory criteria as described. Maternal and perinatal mortality associated with maternal jaundice as recorded in the local participant register.

DATA COLLECTION, MEDICAL EXAMINATION, BIOLOGICAL SAMPLING AND LABORATORY ANALYSES

Data collection: Health register data for study participants and their pregnancy outcome.

Biological sampling: A blood sample (max 3 ml) will be collected at the inclusion and one month after the last vaccine dose for all participants. Blood and saliva samples will be taken for participants when presenting with acute hepatitis. Saliva will be collected in sterile containers in amounts of approx 2 ml and stored in a -20 degrees freezer.
All cases of acute hepatitis will be investigated both clinically and by laboratory diagnostics for the presence of markers of viral hepatitis (A, B, C or E). The clinical symptoms and signs included and the laboratory analyses are described in SOP and will include diagnostics for confirmation of HEV.
Laboratory work will be conducted both in Bangladesh and Norway. All blood samples will be transported to the Matlab hospital. icddr,b laboratory will perform ELISA tests for HEV and HEV PCR an well as other analyses. In addition biochemical parameters with possible relation to vaccine response or failure and HEV infection will be analysed. Samples from the acute hepatitis cases will be transported to the Department of Virology at NIPH for further characterisation. Thus, the samples will be sent abroad and will be stored for 5 years.
During pre-testing 10 participants (among 100 participants) and during main study 50 participants (among 20,745 participants) will be selected randomly for blood collection (9 ml) before and 1 month after last vaccine dose. We need to validate DBS method with plasma samples using ELISA .We will compare the results and confirm that DBS method is suitable for HEV antibody testing (IgG and IgM) as conducted by using direct plasma samples. During main study these results (PBMC and DBS etc) will also be compared with acute hepatitis cases.

In this sub-study another whole blood sample (3 ml) will be collected 2 years (24 to 35 months) after the last dose (dose 3) for those who completed full schedule and also who received 1 or 2 doses of vaccine (incomplete schedule).

Laboratory Methodology

HEV RNA detection method: Viral RNA will be extracted using QIAamp viral RNA extraction kit (Qiagen, Hilden, Germany). HEV RNA will be detected using real time PCR. Negative and positive controls will be included in each run. For details, see the latest version of the Lab Analysis Plan.

Surveillance

Two parallel surveillance for clinical hepatitis and pregnancy detection will be conducted. All enrolledwomen in the study will be provided with an immunization card with identification numbers and with a phone number to call if they become ill with jaundice or missed periods (>2 weeks weeks from the expected start date) between the weekly home visits by the study field workers. The phone number will be answered by a study staff member who will make household visits to screen for suspected hepatitis, regardless of whether or not the woman is pregnant. Jaundice is recognized by lay language as "*Jaundeesh"* in the study area. More than 80% of households have access to a cell phone. Regular SMS messages will be sent to remind participants to report cases of jaundice. All women whose pregnancies are detected through the weekly home visits by the study field workers or routine MCH-FP visits will be visited every 2 weeks to collect information about pregnancy outcomes and to screen for clinical hepatitis. Laboratory diagnosis will be undertaken in both types of surveillance for any woman with acute onset of jaundice of any duration or with illnesses lasting for at least 3 days with at least three of the following symptoms: fatigue, loss of appetite, abdominal discomfort, abdominal pain in the right upper quadrant, nausea or vomiting (Shrestha, 2007, Clayson 1995). Suspected cases will be referred to Matlab hospital for clinical and laboratory examination. Surveillance for hepatitis will continue for approximately 2 years after the completion of all vaccinations.

Acute hepatitis cases with negative HEV diagnostics will be further investigated for other possible causes (other hepatitis such as hepatitis B).

Piloting of HEV Surveillance

We want to establish a surveillance system for detection of HEV disease through identifying hepatitis E virus (HEV) disease among patients with Hepatitis in adult female aged 16- 39 years before starting the main phase of the study.

Vaccine immune responses

A blood sample will be taken from all participants at baseline and one month after the last dose of the vaccine. Paired specimens from each individual will be tested together. Seroconversion will be determined using ELISA method (Zhu et al, 2010) and the rates will be compared between recipients of HEV vaccine and HBV vaccine. Serological immune correlates of protection will be determined by two methods: 1) comparing post-HEV vaccination titres in recipients of a complete regimen of HEV vaccine who develop HEV disease versus recipients who do not 2) the method which estimates protective level of antibodies by comparing the relative risk of the disease outcome using the reverse cumulative distribution curves of antibody responses in vaccinees and comparison group participants (Klugman 1996).

Titers of serum IgG anti-HEV antibodies, as well as of different isotypes for IgG (G1-G4) of these antibodies will be determined (following Wantai kit guidelines, China) at the different time points (baseline day 0, 1 month after dose 3 and at 2 years (24 to 35 months) after dose 3 or who received 1 or 2 doses).

Evaluation of specimen types for acute hepatitis cases

The feasibility of using dried blood spots (DBS) for detection of HEV RNA and HEV antibodies will be evaluated (Lee CE eta al, 2011; Mohamed S et al, 2013) taking into account storage conditions (time and temperature) and sensitivity. EDTA-plasma, capillary blood on DBS and saliva samples (approx 2 ml) from 50 participants with established active HEV infection and 50 vaccinated participants will be collected for this project. Standard HEV ELISA anti-HEV IgM/IgG and PCR will be optimised and evaluated for the different types of material using plasma samples as gold standard.

Evaluation of risk factors and the natural course of HEV disease
A blood and stool sample will be collected form jaundiced patients. 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 include viral load, HEV subtypes,other hepatitis infections, antibody titer, cellular response, cytokines, alanine transaminase (ALT), INR and albumin.. The patients symtoms and signs will be recorded regularly during the disease by health workers.
The following possible risk factors for severity of the disease will be looked for: Occupation, age, height, weight, body mass index, sanitation system, water use pattern, Anti-HEV IgG antibody level, immunological factors, viral load and viral sequence.
Pregnant women will be interviewed for other information (any complications during previous pregnancies, foetal loss etc.).

Incidence of HEV during pregnancy: All with confirmed symptomatic and asymptomatic HEV disease during pregnancy, matched with possible risk factors.

Mortality and morbidity of HEV disease during pregnancy: Natural course,clinical outcome and laboratory results of HEV disease during pregnancy will be recorded.

Pregnancy outcome in HEV infected pregnant women : Data on pregnancy outcome, including complications during delivery and health status of mother and child, will be collected on all participants and analysed together with records of eventual HEV disease and type, time and number of vaccine doses.

Economic analysis
Cost-effectiveness analysis (CEA) and cost-benefit analyses (CBA) will be carried out for HEV vaccine delivery to women in this setting taking the societal perspective (Cartwright, 2005). The cost of vaccination will thus be estimated and compared with its outcomes in terms of health gain (DALYs averted/QALYs gained) and economic gain (cost-saved from the society) (Manca et al. 2015; Murray and Lopez, 2013). Cost of vaccination (total cost, cost per vaccinated woman, marginal cost for vaccination) will be calculated for applying in both CEA and CBA. Costing of any intervention is concerned about systematic collection of information about resources used, categorizing the resources and analysing their costs (Drummond et al. 2005). Ingredient and budgetary approach will be utilized for this costing exercise considering the program perspective. All inputs, for instance, staff time, use of space, medical equipment, shared administrative costs, will be identified by visiting the site, interviewing the relevant key personnel and reviewing the documents of the vaccination program. Identified inputs will then be classified into capital and recurrent items as well as into fixed and variable items. Annuitization of capital items (like, medical equipment, computer) will be done using the lifetime of such items for estimating their annual equivalent costs. In order to adjusting for the differential in timing of resources used, discounting rate of 3% will be applied for base analysis and 0% and 5% for sensitivity analysis. Cost per vaccinated will be calculated by dividing the total costs with the total number of vaccinated women.

Feasibility and acceptability of the vaccine
Operational feasibility of introduction of HEV vaccine will be assessed by in-dpeth interviews with health workers (15-20) (who will motivate women to bring to the health centre for vaccination) and health care providers (15-20) (providing vaccines to the study women). These interviews will help to identify operational barriers in motivating women for vaccination and in the storage and administration of vaccines. Acceptability of the vaccine will be assessed by conducting in-depth interviews with vaccine acceptors and non-acceptors. A total of 30-40 interviews will be conducted - half of which will be conducted with acceptors and the rest will be conducted with the non-acceptors.

Organization of the study

Study coordination

NIPH

The team at NIPH consist of Dr. Susanne G. Dudman, Dr. Kathrine Stene-Johansen, Dr. Synne Sandbu, Dr. Sara V. Watle. In addition, three researchers financed by funds from the Norwegian Research Council will be assigned to the project; Dr. Joakim Ø verbø, Dr.Cathinka H. Julin
and Dr. Jennifer L.Dembinski. NIPH will constitute a steering committee including representatives from the research partners, and will be be chaired by Specialist Director Vaccines Dr. Ingeborg AabergeMD, PhD of the Norwegian Institute of Public Health (NIPH).

icddrb

The team at icddr,b will be headed by Dr. Zaman supported by other investigators.
icddr,b will implement the study in collaboration with NIPH.

Sykehuset Innlandet

Professor Tor Strand will be collaborating researcher from this site.

ideSHi

Dr. Kaise Mannor of ideSHi will be collaborating researcher from this site.

Study timeline

The overall timeline for the study and milestones are shown in table :

Activities	2016			2017			2018			2019		
Regulatory approvals/site preparation and training												
MoU/contract between NIPH, icddr,b, Innovax and Incepta												
Pretesting, Enrollment/vaccination of participants												
Pregnancy follow up, HEV surveillance												
Data collection and Lab analyses												
Data analysis, dissemination, report and manuscript writing												

Capacity building and technology transfer in Bangladesh

An important additional component of the proposed project will be the establishment of technology transfer of the vaccine from Innovax to a highly qualified Bangladesh vaccine producer, Incepta, for production of low-cost vaccine in Bangladesh for government immunization programmes. There is already established contact between these two producers. Incepta will receive bulk vaccine for the trial from Innovax and will package both the HEV and control (hepatitis B) vaccines in identical presentations. The agreement on technology transfer for fill finish and production in Bangladesh is in process.

Data Analysis

Describe plans for data analysis, including stratification by sex, gender and diversity. Indicate whether data will be analysed by the investigators themselves or by other professionals. Specify what statistical software packages will be used and if the study is blinded, when the code will be opened. For clinical trials, indicate if interim data analysis will be required to determine further course of the study.

Statistical methods and data analysis

Statistical Analysis

We will measure total vaccine protection as our primary objective in a per protocol analysis of three-dose vaccine recipients, using analytic methods to adjust for the design effect of cluster randomization (Halloran, 1997; Donner 2000). In this analysis we will compare the incidence of confirmed HEV disease in pregnant women who have been recipients of a complete three dose regimen of HEV vaccine or HBV vaccine and in whom the HEV disease episode begins at least 14 days after the third dose of vaccine. We will attempt to assess the indirect vaccine protection (defined as protection of non-HEV vaccinees in HEV vaccine clusters) and overall vaccine protection (defined as protection of all members of HEV vaccine clusters relative to all members of HBV vaccine clusters), but are aware that our trial may not be adequately powered for these analyses. Adverse events, immune responses to vaccines, and other outcomes will employ tests appropriate for comparison of categorical and dimensional outcomes, adjusted for the design effect of cluster randomization. All statistical tests will be evaluated at $P<0.05$, two tailed.

In one main analysis (**Analysis 1**), we will compare waning of serum titers of IgG anti-HEV antibodies elicited by the third dose of HEV, measured 2 years (24 to 35 months) later after dose 3, among those who are 1) HEV seronegative at baseline and received HEV vaccine, 2) those who were HEV seropositive at baseline and received HEV vaccine, 3) who were HEV seropositive at baseline and received HBV vaccine, 4) participants who are HEV seronegative at baseline and received HBV vaccine (pure control group).

In a second main analysis (**Analysis 2**), we will also compare the decline in the attributable percentage of HEV seropositive (% HEV vaccinees who are IgG HEV serum antibody seropositive minus % of HBV vaccinees who are HEV seropositive) among those who are HEV seronegative at baseline until two years after the third dose.In secondary analyses we will conduct similar analyses of serum IgG subclass anti-HEV antibodies, as well as similar analyses among persons who only received only the first dose, or only the second dose of either vaccine or two doses due to receipt of incomplete vaccine regimens.

For cost-effectiveness analysis, cost per DALY averted and QALYs gained will be compared with Gross-Domestic Product per capita threshold for classifying the intervention into very cost-effective, cost-effective and not cost-effective. The vaccination program will be very cost-effective, cost-effective or not cost-effective if the cost-effectiveness ratio estimate is 1, 2 and 3 times less than the GDP per capita of the reporting country cost-effectiveness ratio (WHO 2003).

For cost-benefit analysis, the monetary benefits will be calculated by estimated costs saved (corresponding to cost of illness) due to HEV infection aversion through vaccination. Net benefit (total benefits minus total vaccination cost) and benefit-cost ratio (BCR) will be calculated, where positive value of net benefit and value more than 1 of BCR will indicate the acceptability of the vaccination against HEV.

The cost analysis will give the opportunity for modelling the costs for scaling up the vaccination program on the national level.

Data Safety Monitoring Plan (DSMP)

All clinical investigations (research protocols testing biomedical and/or behavioural intervention(s)) should include the Data and Safety Monitoring Plan (DSMP). The purpose of DSMP is to provide a framework for appropriate oversight and monitoring of the conduct of clinical trials to ensure the safety of participants and the validity and integrity of the data. It involves involvement of all investigators in periodic assessments of data quality and timeliness, participant recruitment, accrual and retention, participant risk versus benefit, performance of trial sites, and other factors that can affect study outcome.

Data Safety and Monitoring

A Data Monitoring Board (DSMB) will be tasked with monitoring the conduct of the trial, review the accumulating trial data and in particular reports of SAEs (related and not related, irrespective of severity) and make recommendations as appropriate. The study will be discontinued according to a priori defined stopping rules.

Ethical Assurance for Protection of Human rights

Describe the justifications for conducting this research in human participants. If the study needs observations on sick individuals, provide sufficient reasons for using them. Indicate how participants' rights will be protected, and if there would be benefit or risk to each participants of the study. Discuss the ethical issues related to biomedical and social research for employing special procedures, such as invasive procedures in sick children, use of isotopes or any other hazardous materials, or social questionnaires relating to individual privacy. Discuss procedures safeguarding participants from injuries resulting from study procedures and/or interventions, whether physical, financial or social in nature. [Please see Guidelines]

The study will be conducted in accordance with all applicable regulatory requirements.
The study will be conducted in accordance with the ICH Guideline for Good Clinical Practice (GCP), all applicable subject privacy requirements and the guiding principles of the Declaration of Helsinki. The study has been designed and will be conducted in accordance with the ICH Harmonized Tripartite Guideline for clinical investigation of medicinal products in the paediatric population (ICH E11) and all other applicable ethical guidelines.

Conduct of the study includes, but is not limited to, the following:
Institutional Review Board (IRB)/Independent Ethics Committee (IEC) review and approval of study protocol and any subsequent amendments and participants informed consent.

Use of Animals

Describe if and the type and species of animals to be used in the study. Justify with reasons the use of particular animal species in the research and the compliance of the animal ethical guidelines for conducting the proposed procedures.

Not Applicable

Collaborative Arrangements

Describe if this study involves any scientific, administrative, fiscal, or programmatic arrangements with other national or international organizations or individuals. Indicate the nature and extent of collaboration and include a letter of agreement between the applicant or his/her organization and the collaborating organization.

The Norwegian Institute of Public Health (NIPH) is a driving force in improving the health, quality of life and legal protection of the population. NIPH a wide range of internationally recognized scientific expertise reflected in participation in 26 projects in FP7 and an ERC grant in 2013. The NIPH also collaborates with the WHO, EU, EEA, sister institutions, universities, organizations and health authorities in low and middle income countries on global health issues. The NIPH is currently involved in several vaccine-trials abroad and nationally, including Ebola vaccine in Guinea, meningococcal vaccine in Ethiopia and rotavirus vaccine in India and Norway.

Incepta Pharmaceuticals- Established in 1999, one of the largest pharmaceutical industry is involved in producing vaccine and drugs.

Xiamen University and Innovax Biotech in China developed HEV 239 (Hecolin™) vaccine.

ideSHi- Optimization and validation of DBS ELISA will be performed from samples of HEV patient's serum those are being collected for completion of PhD Project of Dr. Rosy Sultana, who is working at our collaborating institute ideSHi. These optimization and validation of the assay will be performed in both places and is very much needed for understanding and assay of samples of vaccine participants from HEV pilot project. Optimization and validation of the DBS ELISA assay will help to conduct a large scale study to see the vaccine efficacy and thus help to prevent maternal and perinatal mortality.

Facilities Available

Describe the availability of physical facilities at site of conduction of the study. If applicable, describe the use of Biosafety Level 2 and/or 3 laboratory facilities. For clinical and laboratory-based studies, indicate the provision of hospital and other types of adequate participant care and laboratory support services. Identify the laboratory facilities and major equipment that will be required for the study. For field studies, describe the field area including its size, population, and means of communications plus field management plans specifying gender considerations for community and for research team members.

The International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b) has large multi-disciplinary international and national scientific research staff. Existing field, hospital, laboratory and office facilities will be used for this study. icddr,b scientists have conducted a variety of vaccine studies (influenza, pneumococcal, cholera, Shigella, rotavirus, Polio, Japanese encephalitis etc.).

Field site

The study will be conducted in rural Matlab. icddr,b has been maintaining a field research centre at Matlab for about fifty years. Due to the presence of ongoing health and demographic surveillance system (HDSS), clinic and laboratory facilities, effective referral systems and well-established infrastructure at Matlab, it offers excellent research facilities for this study. The HDSS is a regularly updated information system on the approximate population of 220,000.

Laboratory facilities

icddr,b and ideSHi laboratory facilities will be used. icddr,b will provide necessary support with reagents for the validation of the assay at ideSHi.

Literature Cited

Identify all cited references to published literature in the text by number in parentheses. List all cited references sequentially as they appear in the text. For unpublished references, provide complete information in the text and do not include them in the list of Literature Cited. There is no page limit for this section, however, exercise judgment in assessing the "standard" length.

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Gender Framework

In Relation to HEV:	Are there sex differences in	How do biological differences between women and men influence their :	How do the different roles and activities of men and women affect their	How do gender norms / values affect men and women's	How do access to, and control over resources affect men and women's
Vulnerability: **Incidence Prevalence (male/female)**	*No significant sex difference reported in incidence or prevalence of HEV.* *Severity of infection increases in pregnancy*	*Nothing.*	*Men may drink more contaminated food and water from outside of their homes/work place. They may have higher chance of infection*	*Due to job nature men may be more exposed to infection and chance of developing diseases*	
Health seeking behaviour	*Men may seek care from hospital/medical practitioners while women mostly from traditional healers*	*Nothing known*	*Men may work outside and take care from health services*	*In many cases men get priority because of higher status.*	*Economic factors are responsible in seeking treatment. Women have little control over household income. They may be non-user of services.*
Ability to access health services	*Men have more access to health services*	*Not applicable*		*Routine treatment of hepatitis in pregnancy is hampered by the range of cultural, social and economic factors. Women more likely to use traditional healers*	
Experience with health services and health providers	*Female participants had more difficulty in getting services*	*Not applicable*	*Women, as mothers, are the target of programmes to get children to health centres*	*Women may refuse examination by male providers; resulting delayed diagnosis.*	*Gender, a factor because women have less control over household income*
Preventive and Treatment options, responses to treatment or rehabilitation	*Hepatitis E Vaccine is not licensed in many countries including Bangladesh. It is an*	*Non-involvement of women in vaccine trials may result*	*Women may have less in getting preventive and treatment care.*	*Preventive programmes are usually community based; where social norms mean that women cannot*	*Financial considerations are usually important whether a full course of treatment is completed – links to women's lesser access to income*

In Relation to HEV:	Are there sex differences in	How do biological differences between women and men influence their :	How do the different roles and activities of men and women affect their	How do gender norms / values affect men and women's	How do access to, and control over resources affect men and women's
	effective vaccine in males.			*participate;*	
Outcome of health problem	*Female mortality due to Heaptitis is higher than male. Death from Hepatitis E may occur 20-25% in pregnant women*	*Poor prognosis during pregnancy*	*Different studies have shown higher mortality among females*	*Biologically bad outcomes in females, may not be due to treatment seeking patterns*	*Poor outcomes for pregnant women*
Consequences (economic & social, including attitudinal)	Economically affects the family as females are worst sufferer	*Biologically bad outcome in females*			*Poor outcomes in females*

Check-List

Check-list for Submission of Research Protocol
For Consideration of the Research Review Committee (RRC)
[Please check all appropriate boxes]

1. Has the proposal been reviewed, discussed and cleared by all listed investigators?

   ☒ Yes ☐ No

   If the response is No, please clarify the reasons:

2. Has the proposal been peer-reviewed externally?

   ☒ Yes ☐ No ☐ External Review Exempted

   If the response is 'No' or "External Review Exempted", please explain the reasons:

   If the response is "Yes", please indicate if all of their comments have been addressed?

   ☒ Yes (please attach)

   ☐ No (please indicate reason(s)):

3. Has the budget been reviewed and approved by icddr,b's Finance?

   ☒ Yes ☐ No (reason): _____

4. Has the Ethics Certificate(s) been attached with the Protocol?

   ☒ Yes ☐ No

   If the answer is 'No', please explain the reasons:

[redacted]

Signature of the Principal Investigator

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 randomely 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 randomely 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 consequtive 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, 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

Assent Form
Pretesting (male and female) and main study for female (16 -17 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 randomely 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 randomely 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 consequtive 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, 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.

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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 or left thumb impression of Parent	Date
_____	_____
Signature of the impartial witness	Date
_____	_____
Signature of the PI or his representative	Date

Informed Consent Form
Piloting of HEV Surveillance for female (18-39 years old)

Protocol No.PR- 16014	Version No. 2.1	Date: 22.06.2017

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 this pilot surveillance is to identify hepatitis E virus (HEV) infection among patient with Hepatitis in adult female (18- 39 years) in rural Bangladesh and to establish a formal surveillance system for detection of HEV disease.

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. Bangladesh is an ideal setting to conduct this surveillance because of the high number of HEV cases. Results from this surveillance will help to understand the process of surveillance system which we will be doing in the main phase.

Why invited to participate in the study?

We are inviting you to participate in this HEV surveillance as you are living in Matab HDSS area and you are afemale of age 18-39 years.

Methods and procedures

Study staff will visit your house once in every two weeks and would want to know about some information regarding illness as follows:

- Do you have Jaundice (Yellow coloration of skin or Eye) of any duration with any of these following symptoms:

1. Nausea or Vomiting
2. Fatigue
3. Loss of appetite
4. Abdominal Discomfort
5. Right upper abdominal pain

If you have any of these above symptoms then study staff will refer you to Matlab hospital for care and if needed they will collect blood and stool sample for testing to detect the type of hepatitis and assess liver function status.

Risk and benefits

When we take blood, there may be mild, but short lasting pain at the needle site in the arm. The risk of infection is very small because we will clean skin thoroughly and use only new sterile needles.

Privacy, anonymity and confidentiality

We assure that the privacy, anonymity and confidentiality of data/information identifying you will be strictly maintained.

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

Assent Form
Piloting of HEV Surveillance for female (16 -17 years old)

Protocol No.PR- 16014	Version No. 2.1	Date: 22.06.2017

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

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

Purpose of the research

The purpose of this pilot surveillance is to identify hepatitis E virus (HEV) infection among patient with Hepatitis in adult female (16- 17 years) in rural Bangladesh and to establish a formal surveillance system for detection of HEV disease.

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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. Bangladesh is an ideal setting to conduct this surveillance because of the high number of HEV cases. Results from this surveillance will help to understand the process of surveillance system which we will be doing in the main phase.

Why invited to participate in the study?

We are inviting you to participate in this HEV surveillance as you are living in Matab HDSS area and you are afemale of age 16-17 years.

Methods and procedures

Study staff will visit your house once in every two weeks and would want to know about some information regarding illness as follows:

- Do you have Jaundice (Yellow coloration of skin or Eye) of any duration with any of these following symptoms:

6. Nausea or Vomiting
7. Fatigue

8. Loss of appetite
9. Abdominal Discomfort
10. Right upper abdominal pain

If you have any of these above symptoms then study staff will refer you to Matlab hospital for care and if needed they will collect blood and stool sample for testing to detect the type of hepatitis and assess liver function status.

Risk and benefits

When we take blood, there may be mild, but short lasting pain at the needle site in the arm. The risk of infection is very small because we will clean skin thoroughly and use only new sterile needles.

Privacy, anonymity and confidentiality
We assure that the privacy, anonymity and confidentiality of data/information identifying you will be strictly maintained.

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

Thank you for your cooperation

Assent of participants:

I have read this consent form and/or the study staff has read the consent form to me. The study staff has informed me, to my understanding, about the purpose of this study and its procedures, its risk and benefits, and my rights as a participant in the research.

_____ _____
Signature or left thumb impression of participant Date

Consent of parent / Legal Guardian:

As Parent or legal guardian , I authorize _____(Participant's name) to become a participant in this surveillance described above.

_____ _____
Signature or left thumb impression of Parent/Legal Guardian Date