Determining sero-prevalence of antibodies against Hepatitis E during an acute outbreak scenario.

Study protocol

Study Summary Table

Study design	Cross sectional population survey (s)
Study period	**Survey 1**: as early in the outbreak as possible **Survey 2**: when outbreak has reduced to <2 cases of acute jaundice per week
Study site	Open or closed setting with confirmed outbreak of Hepatitis E
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2   INTRODUCTION

2.1   HEPATITIS E AND OUTBREAKS

Hepatitis E virus (HEV) is a common cause of acute viral hepatitis throughout Asia, Africa and the Middle East. The true burden of HEV globally remains unknown, but in recent studies, the estimated global burden of HEV infection is around 20 million persons, resulting in 3.4 million cases of symptomatic illness, 70,000 deaths and 3000 stillbirths . The disease is usually self-limiting but in severe cases can develop into fulminant hepatitis which can lead to neurological sequelae, spontaneous abortions and sometimes death. The overall case fatality rate is estimated between 4-30%, but has been documented to reach up to 40% in pregnant women, particularly during the third trimester .

Large outbreaks of HEV have been reported previously from sub-Saharan Africa, mostly in refugee and IDP settings in Uganda, Sudan and Ethiopia . In 2004, Chad experienced a HEV outbreak in Goz Amer refugee camps with Sudanese refugees. Between June and August of 2004, 672 cases were reported with 211 deaths (http://www.who.int/csr/don/2004_08_19/en/).   All   these outbreaks were due to HEV genotype 1. The clinical presentation of Genotype 1 is much more severe than Genotype 3 or 4.

The epidemiology of HEV in sub Saharan Africa remains unclear and outbreaks (as mentioned above) have occurred in different time periods, acquired different epidemic curves and in some cases resulted in different clinical presentation of infection to health care. In recent outbreaks in Gambella, Ethiopia in 2014 and Bentiu, South Sudan in 2015 (data not published), despite early detection and confirmation of an HEV outbreak, the shape of the epidemic curve never reached the same levels that had been seen in previous outbreaks in Maban county in South Sudan, 2012-2013. Also, in this same Gambella outbreak, severe clinical manifestations of disease in pregnant women were rarely identified (also unpublished data). Risk factors for infection that have been identified include consumption of contaminated water, handwashing in communal handwashing facilities, caring for animals and having other household members previously presenting with jaundice .

These examples suggest that the epidemiology of disease might differ due to different initial sources of exposure, underlying immunity in the affected population, or differences in the pathogenicity of the genotype in circulation. Additionally, it is known that children rarely display any symptoms of infection, but it is unknown what role they play in the onwards transmission of disease (i.e. asymptomatic but possible infectious). This would not be unexpected considering the epidemiology and transmission dynamics of hepatitis A outbreaks which are also due to faecal-oral transmission .

Understanding the role in transmission of different age groups, in addition to their underlying immunity against further infection seems crucial. During the 2007-2009 outbreak in Uganda, preliminary sero-prevalence studies showed that between 30-57% of children under five years of age had either IgM (i.e. recent infection) or IgG (older infection) to HEV mid-way the outbreak in 2008 . During the same outbreak persons in the older age groups (≥15 years) had between 64-71% prevalence of either IgM or IgG antibodies . More recent work on the same serological samples obtained from the outbreak in Uganda 2007, 37.3% of children between 0 and 15 years of age had evidence of a recent or older infection with HEV, suggesting that

they play a potential role in the propagation of HEV outbreaks . In Maban, South Sudan, a sero prevalence survey carried out two months after the initial detection of the outbreak only showed 50% immunity (IgG seroprevalence) and was followed by a dramatic upsurge in reported cases (approximately 10,000 additional cases followed) .

The diagnosis of HEV is based on antibody detection using enzyme-linked immunosorbent assay (ELISA) [Wantai] or detection of the virus by a polymerase chain reaction (PCR) test. However, none of these two methods are available in resource-limited settings and samples have to be shipped for laboratory confirmation of an outbreak. The use of dried blood spots (DBS) for the same purpose has been tested and confirmed to work well (Singh et al. 2014), but has not been used as standard practice and requires more investigation. Preliminary experiments at Sanquin (Amsterdam, The Netherlands) have shown that anti-HEV IgM and IgG can be reliably detected from DBS using Wantai ELISAs. Additionally, the potential use of oral swabs to understand immunity for HEV has not been explored in MSF settings. Both the use of DBS and oral swabs would facilitate the collection of samples in MSF settings for outbreak confirmation and understanding immunity in an affected population. These techniques for sample collection would speed up sample collection, reduce international shipping limitations around biological materials, would require less blood or none at all (particularly important in children) and would reduce overall costs and resources required. A rapid diagnostic test (RDT) is available, Assure® Hepatitis E IgM from MP Diagnostics, which has been used by MSF in previous outbreaks with good correlation with the ELISA and this is currently being used as the first line test in the field.

2.2   JUSTIFICATION FOR THE STUDY

Considering the limited knowledge about HEV epidemiology in outbreak settings (particularly acute outbreak settings) and the list of unknowns in relation to the transmission and impact of this disease (age groups infected, age groups infectious, underlying immunity, sample collection methods for diagnosis etc.), we would like to use acute outbreak settings of HEV to contribute to the understanding of this disease. In order to answer some of these unknowns, serological studies during acute outbreak settings will provide valuable information. Data on the prevalence of IgM (recent infections) and IgG (older/past infections) against HEV during acute outbreaks of HEV will increase our understanding of which age groups (or other risk groups) are already immune to the disease, which have been infected and symptomatic, and which have been infected but are asymptomatic and possibly infectious. By comparing this information to the shape of the epidemic curve in the outbreak, and the information around the gender and age distribution of identified cases, we will inform our understanding of the behavior of this disease during outbreaks.

Also, during a serological survey, we will manage to collect different types of biological samples which can then be validated for use for either outbreak confirmation or immunity measurements in patients and thus contribute to the development of better adapted techniques and diagnostics for future HEV outbreaks in low resource and complex settings.

2.3   CURRENT OUTBREAK IN AM TIMAN PROPOSED AS FIRST LOCATION

We propose to use an existing outbreak of HEV in Am Timan, Chad, which was detected in early September 2016 to implement two serological surveys. The first serological survey will be implemented as soon as we are able to obtain Ethical Approval from MSF and from the Ministry of Health of Chad, to help us better understand at which point in the outbreak we have arrived and to inform a risk assessment on the possible future cases we might anticipate. A second serological survey would be conducted at the tail end of the outbreak (when less than two cases of acute jaundice from suspected HEV infection are reported per week), in order to determine immunity levels for HEV in this point of the outbreak.

Since 2010 we have been running a primary and secondary healthcare program. After starting off small, MSF has invested a lot in the hospital and is now key to the functionality of the Am Timan Regional Hospital. The population of Am Timam is estimated to be around 55,000 persons, but swells to approximately 70,000 during the periods of large nomadic movements (with cattle in the region).

At the time of writing, 102 cases of acute jaundice syndrome (AJS) have been identified through medical review of jaundiced patients at our hospital and health centres in Am Timam and through active case finding of jaundice in the community through outreach workers. Twelve cases (out of 38 tested; 39%) have been confirmed to be HEV infected (IgM-positive) through rapid tests. Out of 22 samples of AJS cases tested with Wantai ELISA and PCR, 12 were IgM positive (54.5%) and 10 were also positive for HEV RNA (i.e. confirming a recent infection). Of these, five samples were genotyped as Genotype 1. All serology and PCR testing was done at Sanquin in Amsterdam, the Netherlands. The geographic spread of medically reviewed AJS cases is throughout Am Timam town, and the epidemic curve (based on self-reported date of jaundice onset in medically assessed cases) suggests a point source exposure at the present time (even though this is hard to judge due to the long incubation period of HEV which can take up to 6 weeks) (see Figure 1 and 2).

Figure 1: Epidemic curve by self-reported date of onset

Epidemic Curve by reported week of AJS onset, medically reviewed cases, HEV outbreak, Am Timam, Chad, 2016
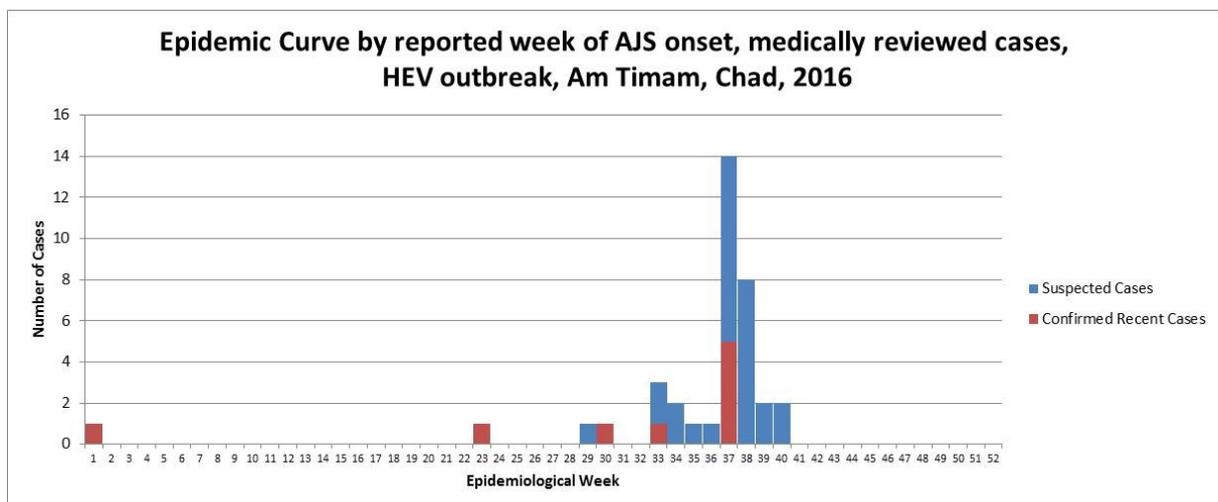
Figure 2: Geographic distribution of medically reviewed AJS cases, Am Timam, Chad, 2016
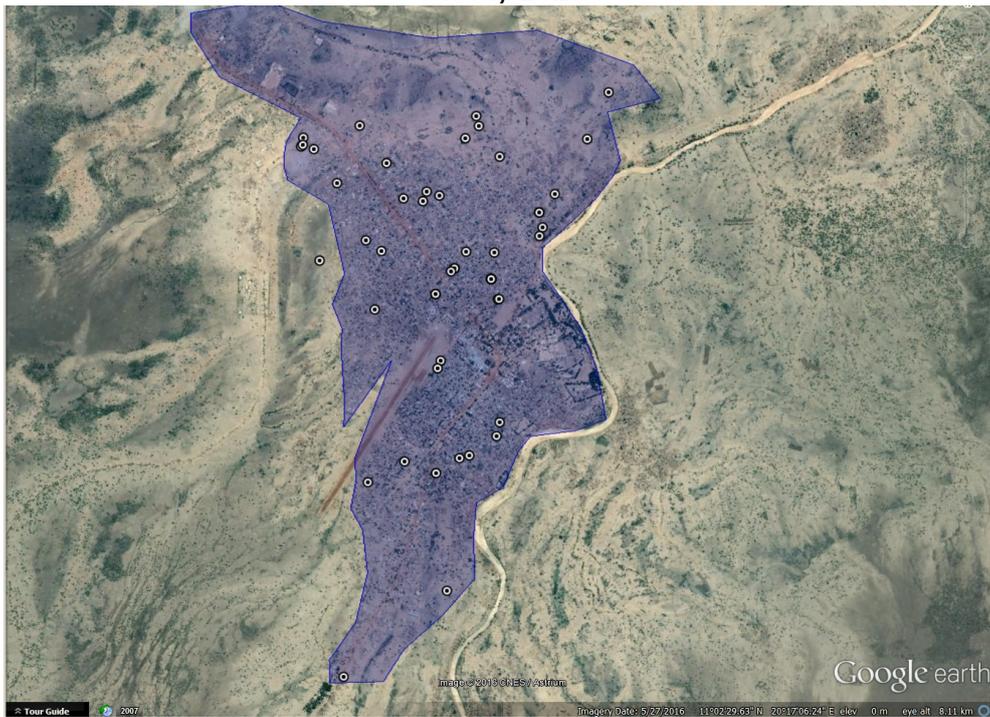
3 OBJECTIVES

3.1 PRIMARY OBJECTIVES

To estimate sero prevalence of anti-HEV antibodies (IgG and IgM) in different age groups in Am Timam, Chad

3.2 SECONDARY OBJECTIVES

- To determine individual risk factors associated with different anti-HEV antibody status during an acute outbreak;

- To determine household level risk factors associated with different anti-HEV antibody status during an acute outbreak;

- To compare the sero prevalence of anti-HEV antibodies (IgG and IgM) in different age groups at two different time periods during an acute HEV outbreak to inform our understanding of viral transmission dynamics in a population in this context;

- To determine sero prevalence in different age groups of other jaundice causing agents (malaria, hepatitis A, B and C, leptospirosis and arboviral diseases such as yellow fever, viral haemorrhagic fever, Dengue and Rift valley fever;

- To compare dried blood spots (DBS) with blood samples for detection of HEV IgM and IgG and HEV RNA through PCR;

- To compare oral swabs with blood samples for the detection of HEV IgM and IgG and HEV RNA through PCR.

4 METHODS

4.1 STUDY DESIGN

We propose to conduct a cross-sectional population-based serological survey stratified by age groups using simple random sampling through random GPS point generation.

4.2 STUDY AREA, POPULATION AND PERIOD

The population resident in Am Timam at the time of the study will be considered the study population (this might include people from the host population and people from nomadic populations that are only present for shorter periods of the year). The study area will be considered the entire inhabited part of Am Timam town (as judged from recent satellite imagery) excluding the nomadic population. The study period will be defined as soon as the respective approvals have been obtained to implement the first survey. The second survey will be implemented as soon as the number of AJS cases identified through the MSF surveillance system is less than two cases per week.

However, as this protocol is also developed for any future AJS outbreaks due to HEV infection, the study area and frequency of use of the serological study might differ. Amendments to this present protocol will be submitted prior to their implementation in settings other than Am Timam, Chad.

4.3 INCLUSION AND EXCLUSION CRITERIA

Household for inclusion into the study will be randomly selected using random GPS point generation (explained in more detail below). Thus all persons living in the randomly-selected household will be considered for inclusion in the survey. The head of the household will be asked to provide informed consent for the household questionnaire and for performing any other part of the survey in a selected household. All persons ≥18 years of age will be asked to provide informed consent for the taking of a blood sample and an oral swab. For all persons <18 years of age, their parent/caretaker will be asked to provide informed consent for the sample to be taken.

Exclusion criteria will be:
- Person in the household who is not considered a permanent resident of Am Timam and/or the household;
- Refusal to participate;
- Receiving blood or blood products within the last three months

4.4 DEFINITIONS

Definition of household

A household will be defined as a group of people who are under the responsibility of one person or head of household during the previous six months. The whole household will be included if they meet the inclusion and exclusion criteria.

Definition of head of household

The head of household is defined as follows:
- Adult household member [≥18 years], and
- Can give accurate information on all demographic and environmental questions around their household;
- Is present at the time of the survey.

A household will be excluded from the study if none of the household members fulfil all these criteria.

4.5 SAMPLE SIZE

We aim to have a sample size that is large enough to be able to stratify sero-prevalence estimates in three age groups: 0-4 years, 5-14 years, ≥15 years. Based on a recent survey conducted elsewhere in Chad (Bokoro district), the proportion of the population that represents these age groups is the following: 0-4=30%; 5-14=32%; ≥15 years =39%.

The average household size in Am Timam is estimated to be around 5.5 persons, thus in each household we would expect to find 1.65 persons 0-4 years of age, 1.76 persons 5-14 years of age and 2.1 persons ≥15 years.

The largest sample size required per age-specific stratum would be to estimate a 50% level of immunity (IgM or IgG). Based on a simple random sampling (design effect/DEFF=1) and a precision of 5%, we would require 382 persons per strata in order to have a representative sample in this survey. As the smallest age group is that of 0-4 years, we would require 232 households to achieve this sample size for this age-stratum. Response rates were 60% in previous sero-surveys for this purpose, thus we would aim to include 385 households in order to achieve the 232 households sample size. We therefore estimate that we will collect samples from between 1277 and 2100 individuals depending on the refusal rate to participate.

We accept that for the "elderly" (i.e. persons ≥45 years) we can only calculate prevalence estimates for anti-HEV antibody sero-prevalence with wider confidence intervals, but accept this limitation due to the feasibility of implementing the study.

4.6   SAMPLING

Using a recent satellite image of Am Timam, we will draw a polygon around the inhabited area of the town. Then using QGIS software we will generate 385 random GPS points. Using the satellite image, we will select houses that fall right under the randomly generated GPS point (i.e. on the roof). The closest household is not selected as this will lead to biased household selection in rural areas (where households will have a higher chance of being selected). For those points that do not land on the roof, we will generate a new set of GPS points and identify new households that are marked on their roofs by these GPS points. We will continue the process until households have been allocated for each of the 385 households that we have calculated in our sample size.

The study supervisor will go the day before to ensure that the GPS points do correspond to actual households and make note of their locations. The next day the study teams will visit those households that had been identified the day before and complete the household questionnaires as well as the blood and oral swab-sample taking. This process will be repeated each day until the complete sample of households has been completed.

For households identified to be empty at the time of the visit of the study team, the study supervisor will only generate a new random GPS point if the household is still empty at the end of that study day. If it is not empty an appointment will be made with the head of the household to repeat the visit the next day.

4.7   FIELD DATA COLLECTION

All data collection tools will be piloted in the field prior to implementation.

4.7.1   Sensitisation

The team in Am Timan will inform the the town and local MoH authorities as soon as we receive ethical review clearance from the MoH in Chad to implement the study. The information will address the objective and planned activities (and timelines of these). We also aim to inform neighbourhood authorities in the week prior to the start of the study in order to announce our presence in the town and the reason for being present.

4.7.2 *Informed signed consent for the household to participate.*

This consent form is found in Appendix 2 and will be preceded by the study team ensuring the head of households understands the information sheet in Appendix 1. Both the information sheet and informed consent form will be translated into French and back translated into English prior to use to ensure consistency of language. Also, study teams will ensure that correct translations to verbal Arabic (written Arabic is poorly understood in this population) are known prior to the study implementation to be able to translate the wording from the information sheet in French correctly.

4.7.3 Household questionnaire

This questionnaire will be administered to the household head and will cover questions related to household practices around water and sanitation, previous disease in the household members and knowledge about HEV and its manifestations. Survey teams will aim to observe the location and state of their main water collection facility, their latrines, handwashing practices, cooking facilities and ownership of soap. Additionally, the household head will be asked to report on the presence of any cases of acute jaundice in his/her household in the time since the start of the outbreak in September 2016. The questionnaire will be translated to French and back translated to English to ensure consistency of language. Additionally, verbal Arabic translations will be available to study teams in order to best explain the questions in the language best understood by the head of household. The questionnaire is available in Appendix 3.

4.7.4 Individual questionnaire
In parallel to the sample taking we will also some limited questions to household members. The questions (Appendix 5) include questions on sex, age, history of jaundice with fever and malaise, and access to care if they were sick with these symptoms in the previous 3 months. Additionally we will ask women to self report whether they are pregnant and to self-report whether they were pregnant when they presented with any of those symptoms. We will adjust the recall period of 3 months once the study is implemented to ensure that there is a key calendar date that the population can recall (i.e. religious or political celebration).

4.7.5 Verbal consent for sample taking

Each individual ≥18 years of age will be read an information sheet (Appendix 4) which explains the need to take biological samples (blood and oral swabs). They will then be asked to provide verbal consent for these biological samples to be taken. Additionally, caretakers or parents of persons <18 years of age will be asked to provide verbal consent to take samples from these individuals. Both the information sheet and verbal consent form will be translated to French and back translated into English prior to use to ensure consistency of language. Also, study teams will ensure that correct verbal Arabic translations (written Arabic is poorly understood in this population) are known prior to the study implementation to be able to verbally translate the wording from the information sheet correctly.

4.7.6 *Biological sample taking*

Whole blood, DBS and oral swab of each individual in the household will be collected. For the whole blood, 4ml of whole blood samples will be collected in sterile vacutainer tubes with Ethylene diamine tetra acetic acid (EDTA) anticoagulant. From this, the dry blood spot (DBS) samples will be prepared and a malaria rapid test, (RDT) will be performed. The sample will then be centrifuged to obtain plasma which will be sent to the reference laboratory together with the DBS samples for testing of serological status of the patient.

For children below 6 months where it may be difficult to perform a successful venepuncture, a capillary blood sample will be collected instead from the heel.

Oral swabs will also be collected from each participant using Copan Swab, Copan, Italy by rotating the swab on the inside of the cheek for 10 seconds.

All biological samples will be taken by professional healthcare staff.

4.8 LABORATORY AND VIROLOGICAL ASPECTS

4.8.1 *Sample transport*
Blood samples will be transferred immediately after collection to a vaccine carrier GioStyle 2.6L containing conditioned ice packs until they can be delivered to the laboratory. The temperature will be monitored using a LogTag®TRIX-8. After plasma preparation, 2 aliquots will be prepared per patient and stored at 2-8°C in the refrigerator awaiting shipment. Oral swabs will be re-sheathed after collection in the Universal Transport and also transported at 2-8⁰C. Dry blood spots will be transported without any temperature regulation.

All samples will be sent in batches to the reference laboratory, Sanquin Blood services (Netherlands) and Institute Pasteur (France) in triple packaging adhering to the IATA regulations for transport of dangerous goods, Class 6.2, Category B (UN3373, Biological substances).

4.8.2 *Sample processing*

All plasma samples and oral swabs (for the purpose of this calculation, approximately 2000 samples each) will be tested using the Beijing Wantai ELISA for anti-HEV IgM and IgG. Based on the IgG and IgM test results a selection of samples will be used for PCR, genotyping and for the validation of DBS and oral swabs for anti-HEV antibody testing as described below.

For two-hundred IgM positive study participants and 100 IgM and IgG negative study participants, the DBS and oral swab samples will be matched. These (DBS and oral swabs) will then be tested using Beijing Wantai ELISA for anti-HEV IgM and IgG and comparability with the results from the blood samples will be calculated. From the 200 IgM positive samples, 30 will be used to run a PCR to detect viral RNA and from these 15 samples with viable HEV RNA will be genotyped.

A suggested flow chart of the laboratory work up around the collected samples is shown below.

At the end of testing, all samples will be stored at Sanquin blood supply for a maximum period of 5 years as per WHO good clinical laboratory practice (GCLP) guidelines 16.2 and thereafter destroyed according to international standards.
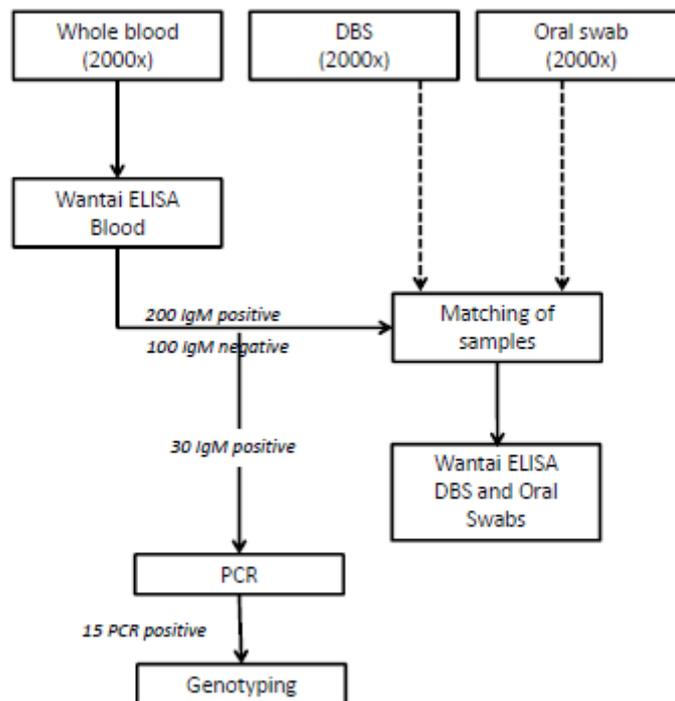
A representative panel of ~300 samples will be made with different IgG and IgM reactivity (ranging from negative to maximally positive) in order to be able to compare the sensitivity and degree of quantitative correlation between the sampling methods. The DBS and oral swabs from the selected individuals will be tested, as will samples with plasma reactivity around the cut-off (OD/CO between 0.9- and 1.1). In addition, PCR and genotyping will be performed and interpreted for IgM positive samples to confirm ELISA results and ensure that HEV genotyping data is available using methods described by Slot et al. Depending on the number of IgM positive samples, up to 30 PCR reactions and 15 genotyping assays from the PCR positive samples will be performed. PCR of DBS and oral swabs will be performed for individuals testing PCR positive. All samples will also be tested (EIA), hepatitis A, B and C, leptospirosis and arboviral diseases such as yellow fever, rift valley fever, viral haemorrhagic fever, Dengue and Chikungunya. The RDT for malaria will have already been performed in the field at the time of venous blood collection.

We will aim to get sufficient blood samples from all participants, but cannot exclude that from small children in some cases the volume of blood will be insufficient to perform the full battery of tests in this case we will prioritise malaria RDT and hepatitis E ELISA.

4.8.3 Sample results interpretation

The ELISA results interpretation will be done in the following way:

Anti-HEV IgG	Anti-HEV IgM	Interpretation
Positive	Positive	Acute HEV infection
Positive	Negative	Prior HEV infection
Negative	Positive	Acute HEV infection (PCR confirmation required)
Negative	Negative	No HEV infection

Please note for the further testing for arboviruses, ELISA testing is well known to be cross reactive for all these viruses. Diagnosis of acute infection for each specific arbovirus will therefore not be very specific.

4.9 DATA ENTRY AND ANALYSIS

All data will be entered into specifically designed Epidata databases by trained data entry clerks. All laboratory data will be matched to interview results for individuals in each household through unique participant IDs that are assigned at the time of sample collection. 10% of all entered data will be double checked by the study epidemiologist to ensure the quality of data entry.

Data cleaning will be performed using STATA version 14. Prevalence rates of anti-HEV IgM and IgG will be calculated on pooled data and for the age-specific strata. The magnitude and direction of association of household exposure factors with IgM and IgG-positivity status will be calculated using prevalence ratios (PRs) and their respective 95% confidence intervals. Multivariate logistic regression models will be constructed to remove confounding of variables and to calculate adjusted odds ratios for magnitude and direction of association between household exposures and sero-status.

For the comparison between performances of different laboratory tests we will compare qualitative and quantitative outcomes of the tests from plasma, DBS and oral swabs. Based on the results, alternative cut-off values may be defined to improve the correlation between sampling methods as described by Singh et al. .

5 COMMUNITY ENGAGEMENT

Since the outbreak was detected by MSF hospital staff in Am Timam in early September 2016, the team have implemented extensive community engagement activities. These have included:
- Active tour of health structures in the city to alert medical staff on signs and symptoms of HEV and to refer patients with serious medical conditions to the hospital;

- Employment of specifically trained community health workers (CHWs) to do active case finding on a house to house basis for acute jaundice syndrome, including the identification of pregnant women. These are also responsible for the delivery of hygiene and sanitation messages to all households to encourage prevention of infection as well as targeted distributions of soap to households with identified suspected cases of HEV infection and households with pregnant women;
- Establishment of bucket chlorination activities to reduce possible transmission through water based sources at main water collection locations throughout the city.
- Active follow up of all identified suspected cases by a specifically trained outreach team to conduct a more specific assessment of the household, water collection/use practices and current sanitation facilities in these specific households to better understand the current population that is affected by this outbreak.

MSF is engaging directly with Ministry of Health staff working in the Hospital of Am Timam and strengthening their clinical management support of cases of HEV that are admitted. Also, healthcare staff working in other health structures in and around Am Timam town have been engaged and encouraged to facilitate case identification and referral of suspected cases. Finally at the Ndjamena level, MSF has advocated since the start of the outbreak with Ministry of Health officials and their stakeholder (i.e. World Health Organisation), to visit Am Timan and conduct their own assessment of the situation (this visit happened in the last week).

6   ETHICAL ISSUES
- The study will be conducted in accordance with the Council for International Organisations of Medical Sciences (CIOMS) International Ethical Guidelines for Biomedical Research Involving Human Subjects and International Ethical Guidelines for Epidemiological Studies .
- The study protocol will be submitted to the Ethics Review Board of MSF. It will also be presented to the MoH of Chad for approval.
- Am Timam town authorities and health authorities will be informed of the intention to perform the study and request their collaboration.
- MSF-OCA commits to sharing study results with everybody who has participated in the study. The MSF medical team will decide about the best venues to display the aggregate results to the communities that were involved in the survey.
- The MSF medical responsible in the field will advise the study team on the referral practices when finding sick people in specific households. Additionally, all persons with malaria RDT positive tests will receive immediate treatment or will be referred to the hospital for treatment (depending on the severity of their clinical presentation). All treatment will be free of charge.
- Written informed consent will be sought from all heads of households/caretakers participating in the study as explained before.
- All data collected will only be identifiable by an ID number (which is established by the epidemiologist to identify households and individuals in households). These numbers will not be linked to individual's names and thus will be anonymous both in the paper collection format as well as in the

electronic database. On the information sheet provided to the head of the household, the study team will provide the household number and the individual ID numbers that correspond to the individuals in their household. The household head can request to add the names to each respective ID number. This list will remain with the household head and not the study team. When laboratory data have become available the study team will make every effort to communicate back their laboratory results to individual household members.

- No information at household level will be able to be traced to that household as the GPS point used for its identification will be kept separately from all files related to the data collected at that household. All data collected in paper format will never include names/addresses or phone numbers. Thus in absence of the GPS point information will never be able to be traced back to the household in question.
- All data will be used for the specific use of this study. Data sharing in MSF is considered to be open. Therefore data from this study will only be shared with third parties if an official request for the data is made, the request is approved by the Medical Director and a data sharing agreement is signed.
- All households and all household members have the option to refuse to participate in the study without penalty.

6.1 RISKS AND BENEFITS OF THE STUDY AND CONTINGENCY PLANS

The proposed sero prevalence surveys will provide crucial epidemiological information to better understand the dynamics of HEV outbreaks and individual risk factors associated with infection (not necessarily with disease). This information will provide much needed additional evidence to inform responses in the settings where MSF works.

Household members that choose to participate in the study will benefit from knowing their sero positivity status for HEV and other infections, which can be considered a benefit in the setting of an acute outbreak. ELISA and PCR results will be available between two and three months after samples arrive in the reference laboratory while Malaria results will be issued on site at time of blood collection.

For any study participants that is identified to have an acute infection with hepatitis A, B or C during the laboratory testing, we will refer them for further medical evaluation through the existing possibilities that are present in Am Timan and the Chadian MoH system. MSF does not provide treatment for hepatitis B and C in our projects at this time.

Minimal risks (possible pain, discomfort and phlebitis at site of blood draw and time inconvenience) are associated with taking blood samples and completing survey questionnaires.

7 COLLABORATION

This study will be carried out in collaboration between MSF-OCA and to the extent possible, with the national and district level Ministry of Health representatives. MSF will also collaborate with Sanquin Blood services as detailed in the annexed Material and data transfer agreement.

MSF-OCA is the study sponsor and is responsible for the funding. Sanquin blood supply will cover part of the cost related to testing at their reference laboratory. It is in charge of the field part of the study, the analysis and report writing. Permission for publication must be obtained from MSF-OCA and the MoH.

Study results will belong to MSF-OCA and the MoH of Chad.

8   IMPLEMENTATION OF THE STUDY IN THE FIELD

This study will require the establishment of a parallel study team to prepare the serological study, implement it and follow up with households that participated to provide their laboratory results.

8.1   HUMAN RESOURCE REQUIREMENTS

This study will run in parallel with the existing project and will function as a separate project from Am Timam in order to ensure adequate resources are dedicated to it.

We anticipate that the following human resources would be required to implement this study:
- 1 International Study Coordinator (Epidemiologist) (3 months in the field, 3 months in Amsterdam for analysis of data)
- 1 International Medical expat (3 months in the field)
- 1 Data entry clerk recruited in Am Timam (3 months)
- Lab sample storage and transport coordinator (3 months)
- Four study teams consisting of (2 months):
  - Team lead (medically trained)
  - Questionnaire and note recorder
  - Medical staff/nurse to facilitate Malaria treatment
  - Phlebotomy staff to take blood and saliva samples supervised by the flying lab expat in Chad.

The best recruitment strategy would be to target staff from Ndjamena and pay for their transport and accommodation in Am Timan for the duration of the study. This will ensure that we recruit qualified staff in a short period.

8.2   SUPERVISION AND TRAINING

The coordination of the study implementation will be under the responsibility of the Study Coordinator. This person will liaise with the Project Coordinator (PC) and Medical Team Lead (MTL) in the field in order to implement the study. They will also have direct lines to the Medical Coordinator and the primary investigators in Amsterdam HQ to resolve any technical concerns.

Study teams will each consist of two medical staff (one team lead and one phlebotomist) as well as someone who ensures the correct completion of questionnaires and sample registers.

The study team, once complete, will have a 5-day training period as a team and further on their specific tasks.

8.3   SUGGESTED MSF SUPPORT IN THE FIELD

In Chad (at mission level), the following support will be required:

- Ensuring ethical approval is correctly requested and obtained;
- Ensuring all links with sample transport companies are established and protocols are followed to ship samples internationally out of the country;
- Appropriate storage conditions exist in order to store samples in Ndjamena prior to transport to the Netherlands;
- Ensuring supply of sufficient laboratory materials for sample taking and storage;
- Possibly recruitment of staff to be relocated to Am Timam for the duration of the project.

In Am Timam (at project level), the following support will be required:
- Administrative and financial support to the Study Coordinator to ensure appropriate hiring of study teams, contracts and payments are done in accordance with MSF rules and regulations in the country;
- Provision and maintenance of 1-2 cars for the duration of the study to ensure study teams can move around sufficiently and without too many complications;
- Photocopying of study materials and stationary needs for study teams and the study coordinator;
- Provision of office space and laboratory storage space for the duration of the study and sufficiently accommodating for the entire study team.
- Identifying supply and treatment options for all study participants that are identified to be malaria positive by RDT testing during the survey (i.e. malaria point in the hospital or health centres where MSF works or by providing medication and materials to the medical person in the team to provide on-site treatment).

8.4 TRAINING OF THE STUDY TEAM AND PRE-TESTING OF THE QUESTIONNAIRES

The study teams will all be trained together for a period of 5 days. During this time, the study teams will review the objectives of the study, the protocol and the process of GPS sampling of households. Additionally, they will pilot all the information sheets, the informed consent forms and questionnaires to ensure they are clear.

9 TIMEFRAME STUDY IMPLEMENTATION

Activity	Estimated time/duration
Protocol agreed by HQ and mission	2 weeks (starting mid October 2016)
Submission for MSF ERB	Start November 2016
French translation requested	First week November 2016
MSF ERB First Reply (fast tracked)	End November 2016
MSF ERB approval obtained	Mid December 2016
Search for study coordinator	End November 2016
Submission to Chadian ERB approval	End November 2016
Chadian ERB approval obtained	Early January 2017
Study coordinator arrives in country	Early January 2017
Preparation/training of study teams and sampling	January 2017

Implementation of first seroprevalence study	February 2017 (3-4 weeks data collection)
Preliminary results samples (blood)	Mid March 2017
Preliminary analysis survey data	End April 2017

10 APPENDICES

Appendix 1: Information sheet Head of Household

Appendix 2: Written Informed consent Head of Household

Appendix 3: Household level questionnaire

Appendix 4: Information sheet household member

Appendix 5: Verbal consent biological samples and hepatitis E questions

Appendix 6: Material and data transfer agreement

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