LASSA FEVER GENERIC PROTOCOL

PROSPECTIVE MULTI-SITE COHORT STUDY TO ESTIMATE INCIDENCE OF INFECTION AND DISEASE DUE TO LASSA FEVER VIRUS IN WEST AFRICAN COUNTRIES

Protocol version	1.0 (FINAL)
Date	05 March 2020
Funding body	CEPI, Coalition for Epidemic Preparedness Innovations, Marcus Thranes gate 2, 0473 Oslo, Norway
Prepared by	P95 Epidemiology and Pharmacovigilance, Koning Leopold III laan 1, 3001 Heverlee, Belgium

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

CEPI	The Coalition for Epidemic Preparedness Innovations
CRF	Case report form
CT	Clinical trial
EERG	Epidemiology Expert Reference Group (part of programme governance structure)
ELISA	Enzyme-linked immunosorbent serologic assay
FGD	Focus group discussion
FIND	Foundation for Innovative New Diagnostics
GPS	Global positioning system
HCW	Health care worker
ICF	Informed consent form
IEC	Information education communication
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IEC	Independent Ethics Committee
IRB	Institutional Review Board
KAB	Knowledge, attitudes and behaviors
LASV	Lassa Virus
LF	Lassa Fever
NCDC	Nigeria Center for Disease Control
PPE	Personal protective equipment
PSC	Programme Steering Committee (part of programme governance structure)
RDT	Rapid diagnostic test
RT-PCR	Real-time polymerase chain reaction
R&D	Research and development
SAC	Scientific advisory committee
SAP	Statistical analysis plan
SNHL	Sensorineural hearing loss
SOP	Standard operating procedures
TBD	To be decided
WHO	World Health Organization

1 PROJECT SUMMARY INFORMATION

Location of the project: The project is planned to be carried out in five Lassa Fever (LF) endemic countries (Nigeria, Benin, Sierra Leone, Liberia, Guinea) in order to allow an appropriate representation of incidence of LF disease or Lassa Virus (LASV) infection in affected countries in west Africa.

Principal Investigators: Names and Institutions for each country

Project funder: Coalition for Epidemic Preparedness Innovations (CEPI)

CEPI will serve as the funding body of this study. The country-specific partner institutions, each represented by a Principal Investigator, will serve as the study sponsors of their individual country studies. It is the responsibility of the sponsor in each country to ensure proper monitoring of the study, data ownership and compliance with all applicable regulatory guidelines and laws.

2 BACKGROUND

2.1 EPIDEMIOLOGY OF LASSA FEVER

Lassa fever (LF)

LF has been known since the 1950s, but LASV was not identified until 1969, when two missionary nurses died from it in the town of Lassa in Nigeria. Found predominantly in west Africa, it has the potential to cause tens of thousands of deaths.[1] LASV, the causative agent of LF, is a zoonotic pathogen, meaning that humans generally become infected from contact with excreta or tissues from infected animals, most common the Natal multimammate mouse, *Mastomys natalensis.* The reservoir animals infected with LASV do not become ill, but they can shed the virus in their urine and faeces.[2] Evidence has shown that other rodent species found in the rural homes, bushes and agriculture farmlands in sub-Sahara Africa may also act as hosts for the virus, especially *Hylomyscus pamfi, Mastomys erythroleucus,* and *Mus baoulei* species[3,4], though their role in transmission to humans is not well understood. These rodents reproduce at a high rate and prefer to live near humans, especially where food items are kept. Rodents are also consumed by individuals as "bush meat" in many settings in West Africa.[5]

LASV and genetic diversity

LASV belongs to the family Arenaviridae and has an enveloped, ambisense, RNA genome. The genome consists of two segments, a small (S) segment and a large (L) segment. The S segment encodes the glycoprotein precursor (GPC), which is expressed on the envelope of the virus in a trimeric state.[6] The S segment also encodes the nucleoprotein (NP) in the opposite direction, which encapsulates the viral genome. The L segment encodes the viral matrix protein (Z) and the viral RNA-dependent RNA polymerase (L).[7]

Genetic sequencing studies have established that LASV likely originated in Nigeria and has been in circulation in the country for over a thousand years.[8] Next-generation sequencing (NGS) has also recognized four main lineages, with Lineages I, II, and III appearing to be restricted to isolates from Nigeria and exhibiting the greatest variability, while lineage IV circulates in Guinea, Sierra Leone, Liberia, Mali, and Cote d'Ivoire.[9] Lineage IV is the most studied lineage, and additional lineages V and

VI have been described. A new yet to be typed lineage was recently isolated in Togo.[10] Recent studies using next-generation sequencing show higher LASV genome diversity than previously assumed, up to 32% and 25% for the L and S RNA segments, further underlying the high variability of the virus.[11] In addition, an NCBI protein Basic Local Alignment Search Tool (BLAST) analysis of the LASV Josiah strain showed that glycoprotein ([GPC] NP_694870) and nucleoprotein ([NP] NP_694869. 1) varied in percent identity from 91 to 99% and 86 to 99%, respectively, with full-length protein sequences of the other LASV protein sequences in GenBank.[12]

This high variability of the virus presents a significant challenge for the development of diagnostic assays and developing vaccines that will cover all the clades of the virus.

Transmission

The virus is transmitted to humans most commonly via direct contact with or ingestion of items contaminated with infected rodent excreta. Person-to-person transmission also occurs through contact with infected blood or body secretions.[1] Contact with the virus may also occur when a person inhales tiny particles in the air contaminated with infected rodent excretions. This aerosol or airborne transmission may occur during cleaning activities, such as sweeping. Casual contact (including skin-to-skin contact without exchange of body fluids) does not spread LASV. Person-to-person transmission is common in health care settings (called nosocomial transmission) where proper personal protective equipment (PPE) is not available or not used. LASV may also be spread through contaminated medical equipment, such as reused needles.[1,15] LF occurs in all age groups and in both men and women.[16] No studies have been done to determine the degree to which environmental contamination through urination in an open space, or exchange of body fluids (semen) through sexual intercourse contribute to LF transmission. Most evidence shows that the rodent to man route is the main driver of human infection. Viability of the LF virus in the environment has not been well studied.[17]

Geographical distribution

Since the isolation of the LASV in 1969, the disease has continued to cause seasonal morbidity and mortality with sporadic outbreaks. It is estimated that there are over 300,000 infections annually in west Africa, making LASV a primary cause of haemorrhagic fever worldwide.[18] LASV has caused numerous outbreaks and is endemic to Benin (diagnosed for the first time in November 2014), Guinea, Liberia, Mali (diagnosed for the first time in February 2009), Sierra Leone, and Nigeria, but there is strong evidence of LASV infections throughout much of west Africa (Figure 1).[1]

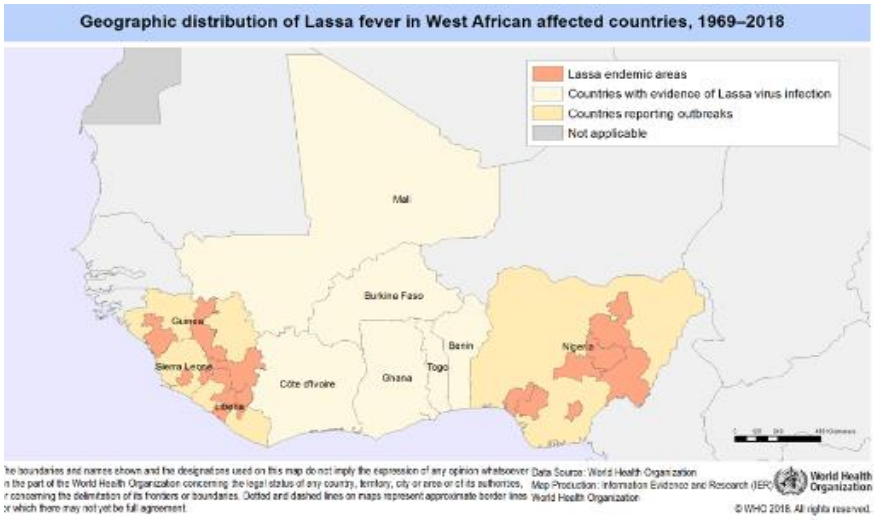


Figure 1. Lassa fever geographical distribution in west Africa, 1969-2018.[1]

Seasonality

Presentation of cases used to be highest during the dry season (January to March) and lowest during the wet season (May to November). However, data from Kenema, Sierra Leone (1999-2002) showed that admissions were highest during the change from the dry to the wet season (May to November). This change might be related partly to population movements during the civil unrest in Sierra Leone and overcrowding among refugees.[19] More recently, in Nigeria the Lassa season has become more prolonged and has generated more cases, including more severe and fatal cases, than usual. These larger and more widespread outbreaks may be attributed to increasing urbanization bringing rodents in previously rural areas to newly urbanized settlements, a favourable prevailing climatic condition for rodent breeding occasioned by an increase in annual rainfall volume since 2015, and the subsequent improvement in crops yield (food) translating into an increase in the population of rodents and reservoir of the disease.[17]

2.2 POPULATION AT RISK OF LASSA FEVER

Risk factors for primary (zoonotic) transmission are still unclear and possibly linked to housing and hunting and consumption of rodents.[19,20] LF occurs in all age groups and both sexes. Persons at greatest risk are those living in rural areas where *Mastomys* rats are usually found, especially in communities with poor sanitation or crowded living conditions. Health workers (including laboratory workers) are at risk if caring for LF patients without proper barrier nursing and infection prevention and control practices.[21] Mapping of LF affected areas using predictive models estimated that approximately 80% of the surface area of both Sierra Leone and Liberia, 50% of Guinea, 40% of Nigeria, 30% of each of Côte d'Ivoire, Togo, and Benin, and 10% of Ghana are at risk of transmission and outbreaks.[22] Risk factors for primary (zoonotic) transmission are still unclear and possibly linked to housing and hunting and consumption of rodents.[20,21] LF occurs in all age groups and both sexes. Persons at greatest risk are those living in rural areas where *Mastomys* are usually found, especially in communities with poor sanitation or crowded living conditions.

2.3 MORBIDITY, MORTALITY AND INFECTION DUE TO LASV

In west Africa, it has been estimated that there are about 100,000- 300,000 cases of LF and 5,000 deaths from LF annually.[24] However, these are extrapolations from a longitudinal study conducted in Sierra Leone over 30 years ago and the true public health burden of disease is unknown. The absence of good surveillance networks in affected countries coupled with the difficulty in establishing a diagnosis due to lack of rapid and reliable diagnostic kits means most non-severe cases are likely to be misclassified and managed as cases of malaria, typhoid or influenza. This under-reporting is also likely responsible for the difference in case fatality rates (CFR) between community-acquired (1-2%) and hospital-acquired (15-20%) infections since the actual disease incidence of community-acquired LF is unknown.[25]

Since 2010, there has been an increase in reported cases of LF and geographical spread of the disease. Increased awareness of health care workers, increased diagnostic capability and the adoption of a new data collection tool have been adduced as possible reasons behind the upsurge in cases.[35] Although the overall CFR is about 1% in all patients with LF (when asymptomatic and mildly symptomatic patients are included), mortality has been reported to be 20% or higher among patients hospitalized with severe illness.[1,25] Death usually occurs within 14 days of onset in fatal cases. During pregnancy, high rates of maternal death (29%) and fetal and neonatal loss (87%) have been recorded, with one hospital in Sierra Leone attributing 25% of all maternal deaths to LF.[26] The prevalence of antibodies to the virus in the population has been estimated to range from 8 to 52% in Sierra Leone,[27] 4 to 55% in Guinea[28] and 13 to 37% in Nigeria.[29] Seropositivity has also been found in the Central African Republic, Democratic Republic of the Congo, Mali, and Senegal.[30]

2.4 CLINICAL ASPECTS OF LASSA FEVER

Symptoms

When transmitted to humans, LASV infection is asymptomatic in most cases (80%); however, 20% of infections have clinical symptoms and may result in severe haemorrhagic fever with multi-organ failure (such as liver, kidneys).[7] The incubation period of LF is typically 6-21 days with accompanying headache, fever, muscle/joint pain, diarrhea, vomiting, elevated liver enzymes (AST and ALT) and haematocrit. Symptoms indicative of a poor prognosis include oedema of the face and neck, abdominal and retrosternal pain, enlarged lymph nodes, and/or haemorrhage in the conjunctiva or mucosal surfaces.[18]

Complications/ sequelae

Shock, seizures, tremor, disorientation, and coma may be seen in the later stages of disease.[1] A third of survivors suffer from sensorineural hearing loss (SNHL) as sequelae. This results in a permanent hearing loss in approximately 18% of survivors.[32] A prospective case-control study of LF established in Sierra Leone found complications among LF survivors included mucosal bleeding (17%), bilateral or unilateral eighth-nerve deafness (4%), and pleural (3%) or pericardial (2%) effusion.[7] A prospective case–control study of LF patients seen with acute SNHL was conducted between July 2007 and April 2009 at Irrua Specialist Teaching Hospital Nigeria. Patients with other acute febrile illnesses were used as control. Out of the 37 confirmed cases of LF, 5 (13.5%) and none (0%) of the control developed early-onset SNHL ($p = 0.03$).[33] A recent literature review of studies published between 1972 and 1996 summarized clinical findings of SNHL in LF patients highlighting the association between LASV infection and SNHL as well as the potential mechanisms for LF-induced SNHL. Analyses revealed that an average of 33.2% (4%–75%) of LF survivors developed unilateral or bilateral SNHL. These SNHL cases were identified at 10 to 15 days after the onset of LF symptoms or at the

convalescent stage of the disease and is not dependent on viremia levels, administration of ribavirin, severity of disease, or liver enzyme levels, suggesting that it could be due to an immune-mediated mechanism. However, further research is required to confirm whether SNHL is caused by direct viral damage, immune-mediated damage, or a combination of both.[34]

2.5 CASE MANAGEMENT OF LASSA FEVER

Diagnosis

Because the symptoms of LF are so varied and non-specific, clinical diagnosis is often difficult, especially early in the course of the disease. LF is difficult to distinguish from other viral haemorrhagic fevers (such as Ebola virus disease) as well as other diseases that cause fever, including malaria, shigellosis, typhoid fever and yellow fever. Definitive diagnosis requires testing that is available only in reference laboratories.[1]

Laboratory testing

Laboratory specimens may be hazardous and appropriate special precautions should be in place when handling them. LASV infections can only be confirmed using the following tests: 1. reverse transcriptase polymerase chain reaction (RT-PCR) assay; 2. antibody enzyme-linked immunosorbent assay (ELISA, seroconversion or presence of antibodies consistent with acute infection); 3. antigen detection tests; 4. virus isolation by cell culture.[1]

Treatment and prophylaxis

Currently, there are no approved vaccines or therapeutics for the treatment or prevention of LASV with the questionable exception of the off-label use of ribavirin.[18,35] Early supportive care with rehydration has been associated with improved survival but supported by limited data. While ribavirin is commonly used in the treatment of LASV, its role has been called into question.[36] There is no evidence to support the role of ribavirin as post-exposure prophylactic treatment for LF.[26]

Prevention of Lassa Fever

Prevention of LF relies on community engagement and promoting hygienic conditions to discourage rodents from entering homes. In healthcare settings, staff should consistently implement standard infection prevention and control measures when caring for patients to prevent nosocomial spread of infections.[1]

3 RATIONALE

CEPI is funding the development of multiple Lassa vaccine candidates. A crucial step to assess the feasibility and prepare for potential future Lassa vaccine efficacy trials is to gather epidemiological data on the background rates of infection and disease due to LASV in endemic areas, which are needed to assess the sample size requirements of Lassa vaccine efficacy trials. This is necessary because the incidence and spatial distribution of LF is likely to be significantly underestimated based on existing data, due to gaps in diagnostics, surveillance and access to health services. The planned prospective multisite cohort study will provide incidence estimates of infection and disease due to LASV in multiple sites in west African countries to inform the design of future vaccine trials and Lassa vaccination strategy, when suitable vaccines become available. Conduct of the study will also help to strengthen site and investigator capacity to conduct vaccine trials, as well as to address several gaps identified in the World Health Organization (WHO) Lassa Fever Research and Development (R&D) Roadmap.

This core protocol defines the key elements for a multi-country cohort study. Studies based on the same core protocol will be conducted in multiple sites across several countries in west Africa, which will enable LF epidemiology to be assessed within each country and to be compared between sites and countries. The primary goal is to assess the feasibility of future vaccine efficacy trials, identify areas in which these trials might be optimally conducted and to inform the trial design.

4 AIM AND OBJECTIVES

4.1 AIM

The aim of this project is to conduct a prospective multi-country epidemiological cohort study that will assess LF disease and LASV infection incidence to determine the feasibility of future Phase IIb or III clinical trials for assessing the efficacy of LF vaccine candidates.

Further, strategic aims will be to:

1. Inform the design of future clinical trials.
2. Identify sites for future clinical trials.
3. Assess and strengthen the site and investigator capability and capacity to conduct future clinical trials.

4.2 STUDY OBJECTIVES

The primary objectives are:

1. To assess the incidence rate of symptomatic confirmed LF separately for each Lassa-endemic country participating in this study.

2. To estimate the incidence rate of LASV infection separately for each Lassa-endemic country participating in the study.

The secondary objectives are:

1. ***Related to primary objective 1* (*LF disease cohort*):**
    1. To assess the incidence rate of symptomatic confirmed LF
        - Overall in Lassa-endemic geographical areas
        - Separately for each recruitment site
    2. To assess the age-specific incidence rates of symptomatic confirmed LF
        - Overall in Lassa-endemic geographical areas
        - Separately for each country
        - Separately for each recruitment site
    3. To assess the monthly incidence rate of symptomatic confirmed LF
        - Overall in Lassa-endemic geographical areas
        - Separately for each country
        - Separately for each recruitment site
    4. To assess the LASV clade-specific incidence rates of symptomatic confirmed LF
        - Overall in Lassa-endemic geographical areas
        - Separately for each country
        - Separately for each recruitment site
    5. To assess the baseline seropositivity prevalence
        - Overall in Lassa-endemic geographical areas
        - By country
        - By site

6. To assess the association between baseline seropositivity and the occurrence of symptomatic confirmed LF
    - Overall in Lassa-endemic geographical areas
    - By country
    - By site
7. To describe the clinical course of symptomatic confirmed LF
    - Overall in Lassa-endemic geographical areas
    - By country
    - By site
8. To determine the case fatality rate (CFR) among symptomatic confirmed LF cases
    - Overall in Lassa-endemic geographical areas
    - By country
    - By site
9. To determine the proportion of Sensorineural Hearing Loss (SNHL) among patients with symptomatic confirmed LF
    - Overall in Lassa-endemic geographical areas
    - By country
    - By site
10. To determine the proportion of LF survivors patients with delayed or persistent SNHL
    - Overall in Lassa-endemic geographical areas
    - By country
    - By site
11. To describe clinical course and outcome of symptomatic confirmed LF cases stratified by risk groups
    - Pregnant women
    - Children
    - Elderly
12. To assess the incidence rates of co-infection of malaria in confirmed LF cases
    - Overall in Lassa-endemic geographical areas
    - By country
    - By site
13. To assess the role of selected risk factors for symptomatic confirmed LF disease
    - Overall in Lassa-endemic geographical areas
    - By country
14. To determine the proportion of lab confirmed LF among 'acute febrile illness cases'
    - Overall in Lassa-endemic geographical areas
    - By country
    - By site
15. To define different levels of severity of symptomatic confirmed LF cases

2. Related to primary objective 2 (LASV infection cohort)

16. To estimate the incidence rate of LASV infection
    - Overall in Lassa-endemic geographical areas
    - By site

17. To assess the baseline prevalence of LASV-specific antibodies (= 'seropositives', 'seropositivity')
    - Overall in Lassa-endemic geographical areas
    - By country
    - By site
18. To estimate the age-specific incidence rate of LASV infection
    - Overall in Lassa-endemic geographical areas
    - By country
    - By site
19. To assess the age-specific prevalence of 'seropositives' at baseline and over time
    - Overall in Lassa-endemic geographical areas
    - By country
    - By site
20. To assess the role of selected risk factors for LASV infection at baseline and over time
    - Overall in Lassa-endemic geographical areas
    - By country
    - By site
21. To assess the age-specific incidence of seroreversion
    - Overall in Lassa-endemic geographical areas
    - By country
    - By site

5 STUDY DESIGN

5.1 STUDY DESCRIPTION

The overall study consists of two study components: i) a ***LF disease cohort*** to assess the incidence rate of symptomatic LF cases (as confirmed by PCR); and ii) a ***LASV infection cohort*** to estimate the incidence rate of LASV infection (as assessed by seroconversion). Consortia will either implement one or both study components in their respective study sites. If consortia implement both components, the (smaller) *LASV infection cohort* will be drawn as a nested cohort from the (larger, main) *LF disease cohort*.

The following are provided below to summarise key aspects of the study that are described in detail in this document:

1. **Tables 1a and 1b** summarise the main study activities as they relate to the *LF disease cohort* and the *LASV infection cohort*. Activities have been organised in sequence of implementation (rows, top to bottom) and by key study events (columns, left to right).
2. **Figure 2** provides an overall flow diagram of the study and its activities. It is a visual representation of the key information provided in tables 1a and 1b and illustrates how the *LF disease cohort* and the *LASV infection cohort* relate to each other.

Detailed study procedures for the two study components are described separately in sections 6 (*LF disease cohort*) and 7 (*LASV infection cohort*) of this protocol.

Table 1a. Summary of study procedures and schedule of activities – *LF Disease Cohort*

Cohort study participants involved →			**All**			**'Acute febrile illness cases'**	**'Confirmed LF cases'**			
Timing of activity →		**Before study start**	**Day 0** *enrolment*	**Every 2 weeks** *active surveillance*	**Continuous** *passive surveillance + assessments*	**Presentation to study health facility**	**Upon confirmation** *RT-PCR positive*	**During hospitalisation**	**At 4 months post discharge**	**undocumented community death**
Activity	**Responsible for/Conducted by**									
Define site and select localities (clusters) for inclusion in study.	Study co-ordinator	X								
Hiring and training of site study personnel as required	Study co-ordinator	X								
Community engagement and sensitization of HCWs and community	Study co-ordinator	X								
Ensure lab tests, sample storage, and a chain of custody for specimens are in place	Study co-ordinator	X								
Testing of study tools and procedures	Study co-ordinator	X								
Use two-stage cluster sampling method to select households for study inclusion	Fieldworker team leader	X								
Assess eligibility for study inclusion	Fieldworkers		X							

Request informed consent/assent[+]	Fieldworkers		X							
Document household GPS location	Fieldworkers		X							
Perform baseline data collection & complete questionnaire	Fieldworkers		X							
Document household communication means for follow-up	Fieldworkers		X							
Obtain 5mL whole blood specimen for serology and storage for possible future LF antibody testing	Fieldworkers / Phlebotomist		X							
Contact household heads for study participant status check –	Fieldworkers			X						
Encourage household heads / participants to contact fieldworker and/or present to study health centre immediately when febrile for ≥2 consecutive nights	Fieldworkers			X						
Assess fulfilment of acute febrile illness case' definition, and refer to study health centre as required.	Fieldworker / Health worker			X[§]	X[§]					
Document signs and symptoms at the study health centre	Health worker					X				

Obtain 5ml whole blood specimen for LF confirmation (RT-PCR; plasma) and malaria testing (RDT; 15µL), at study health centre or study participant home -	Fieldworker / Phlebotomist / Health worker					X				
Notify health authorities of 'confirmed LF case'	Health worker						X			
Initiate specific treatment according to national guidelines and transfer to LF-specific hospital or treatment centre as indicated.	Health worker						x			
Obtain aliquot from patient's blood sample for genome sequencing and send to appropriate laboratory.	Health worker						X			
From medical records, extract clinical signs and symptoms, laboratory parameters and audiometry data and enter in case report form (CRF)	Fieldworker/Health worker							X *upon admission + document most severe signs and symptoms*		
Document clinical outcome in CRF	Fieldworker/Health worker							X		
Perform audiometry testing	Health worker							X	X	
Enquire about other sequelae and document in CRF	Fieldworker								X	

+ If the site is implementing both the LF disease cohort and the LASV infection cohort, the first 1000 study participants recruited into the LF disease cohort should be enrolled into the LASV infection cohort. Additional informed consent/assent should be obtained as required.

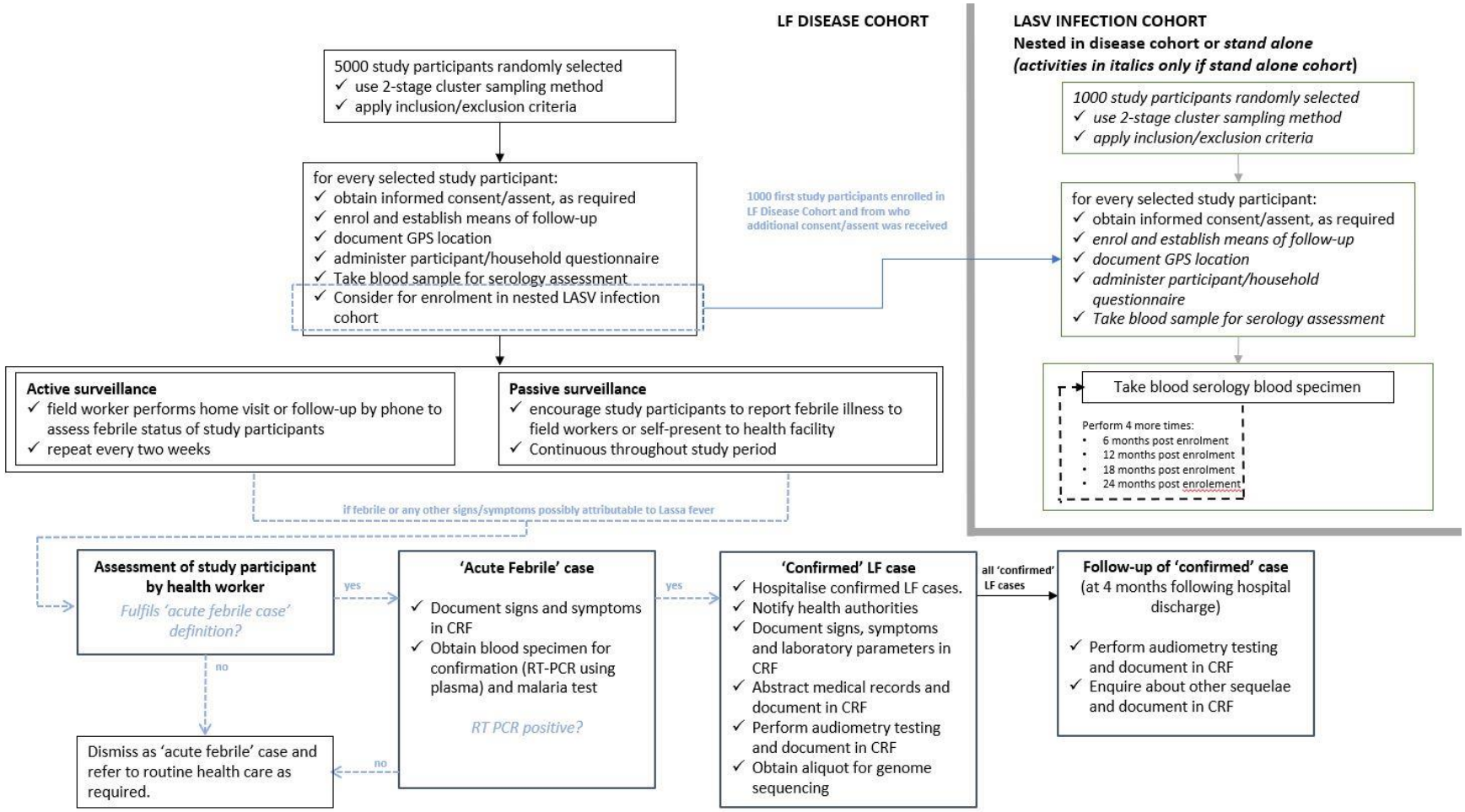
§ Assessment to be carried out by a study fieldworker or health worker when active/passive surveillance activities identify a study participant with signs/symptoms possibly attributable to LF disease.

Table 1b. Summary of study procedures and schedule of activities – LF Infection Cohort (if nested cohort, grey fields will already have been carried out)

Cohort study participants →			**All**				
Visit **Timing of activity →**		**Before study start**	**'Visit 1'** **Day 0** *enrolment*	**'Visit 2'** **Day 0 plus 6 months (±2 weeks)**	**Visit 3'** **Day 0 plus 12 months (±2 weeks)**	**Visit 4'** **Day 0 plus 18 months (±2 weeks)**	**'Visit 5'** **Day 0 plus 24 months (±2 weeks)**
Activity	**Responsible for/Conducted by**						
Define site and select localities (clusters) for inclusion in study.	Study co-ordinator	X					
Hiring and training of site study personnel as required	Study co-ordinator	X					
Community engagement and sensitization of HCWs and community	Study co-ordinator	X					
Ensure lab tests, sample storage, and a chain of custody for specimens are in place	Study co-ordinator	X					
Testing of study tools and procedures	Study co-ordinator	X					
Use two-stage cluster sampling method to select households for study inclusion	Fieldworker team leader		X				
Assess eligibility for study inclusion	Fieldworker		X				
Request informed consent/assent	Fieldworker		X				
Document household GPS location	Fieldworker		X				
Perform baseline data collection & complete questionnaire	Fieldworker		X				
Document household/individual communication means for follow-up	Fieldworker		X				
Obtain 5mL whole blood specimen for serology and storage for possible future LF antibody testing -	Fieldworker / Phlebotomist / Health worker		X				
Obtain whole blood specimen for serology	Fieldworker / Phlebotomist			X	X	X	X

+ If the site is implementing both the LF disease cohort and the LASV infection cohort, the first 1000 study participants recruited into the LF disease cohort should be enrolled into the LASV infection cohort. Additional informed consent/assent should be obtained as required.

Figure 2. General flow diagram for the LF epidemiology study.

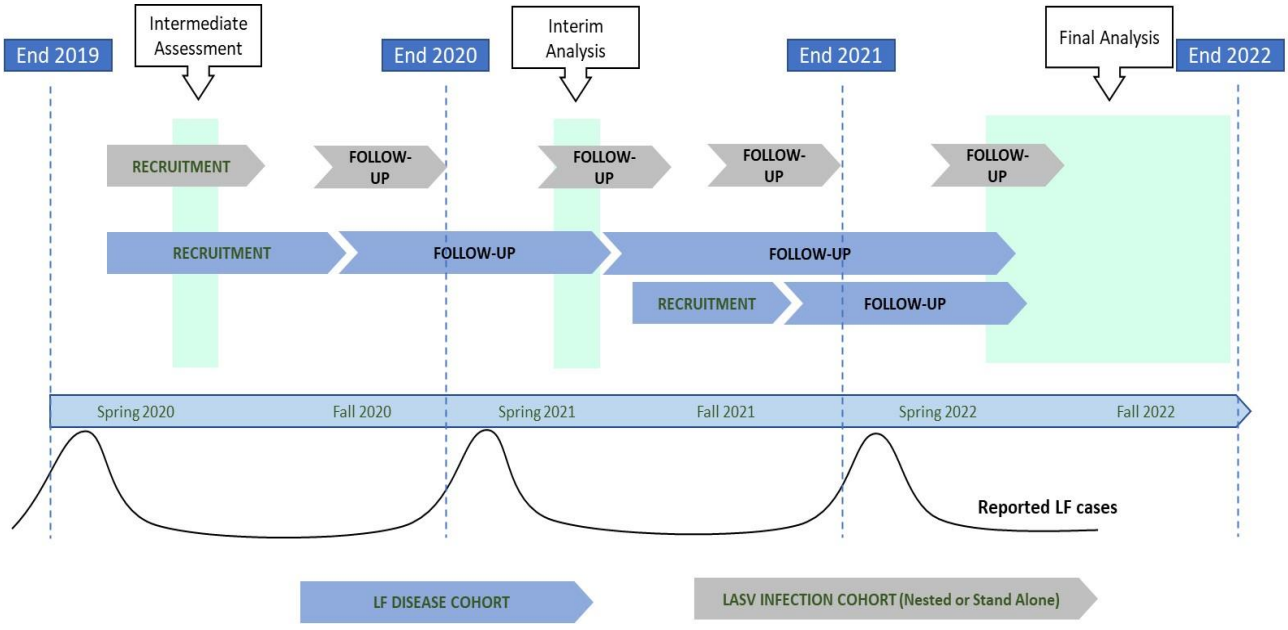


5.2 STUDY PERIOD

Recruitment will commence in (estimated) early-mid 2020, as soon as all required approvals have been obtained and site initiation (see section 5.7) has been performed by the implementing partner (a referral-level healthcare facility from which the study will be coordinated). Initial recruitment will continue until the targeted sample sizes (see section 9.1) have been attained. Recruitment should be done outside of the high LASV transmission season (preferred recruitment period is from end of March to end of November). Recruitment will be monitored continually, with steps taken to increase recruitment where needed. Additional recruitment may be considered following an interim analysis in the spring of 2021 but will not extend beyond the start of the 2021-2022 LASV high transmission season (November 2021). All study participants will therefore be followed over a period of 12 (minimum) to 24 (maximum) months.

Figure 3 provides an overall timeline for the study and shows how the timing of the study and its two cohorts (*LF disease cohort* and *LASV infection cohort*) relate to the LF high transmission seasons in west Africa. The timeline also shows the timing of key planned analytic activities (see section 9.2.2): an intermediate assessment (estimated April-June 2020), an interim analysis (estimated April 2021) and the final analysis at the end of the study. Exact dates of intermediate assessment and analysis will depend on study start date and progress.

Figure 3. Estimated timeline of the CEPI Lassa epidemiology program.



5.3 STUDY POPULATION

The study will be conducted at study sites where available data suggests that the risk of LASV infection is relatively high. The populations studied should be relatively stable, with little expected inward or outward migration.

5.4 INCLUSION CRITERIA

1. Healthy females and males ≥ 2 years;
2. Resident of the study area for six months preceding recruitment, and expected to stay in the area for the majority of the time until the end of the 2020-2021 LASV high transmission season (30th April 2021);
3. Able and willing to provide informed consent (and assent, as required), according to country-specific procedures.
4. Able to understand the language (official language of the country or local languages) or have a surrogate available who can translate;
5. Household head has granted permission for household member to be approached by research team (if necessary);
6. Suitable measures for future study contacts via household head and secondary household contact person are in place (mobile phone, fixed telephone number).

5.5 EXCLUSION CRITERIA

1. Persons unwilling to comply with any of the study procedures, including blood specimen collection;
2. Persons indicated by the respective household head as not eligible
3. Any individual who for medical or social reasons cannot be enrolled in the study;
4. Persons who may not be able to consent freely, such as persons in military service;
5. Any other reason which in the discretion of the study personnel would interfere with a person's ability to participate in the study (e.g. mental illness)
6. Any study staff and study site personnel (relatives of study staff are not excluded).

5.6 STUDY SITES

One or more sites will be included per country. A ***'site'*** consists of a well-defined geographical area encompassing the ***'localities'*** (communities, hamlets, villages, towns) situated in that area, as well as all health facilities that provide healthcare services there. Sites selected should have the following, predicated on available data:

- Include multiple rural and peri-urban localities known to be affected by Lassa;
- Be located within the catchment area of a referral-level healthcare facility from which the study will be coordinated (***'implementing partners'***). Staff at this referral-level healthcare facility will be experienced in diagnosing and treating LF. These healthcare facilities should have appropriate laboratory diagnostic infrastructure equipped for LASV testing, including experienced staff in LASV laboratory testing. Where site-level LASV testing is not possible, specimens will be sent to a national reference laboratory for investigation. Other health facilities besides the implementing partner located in the sites will be fully aware of the study and will follow defined study procedures regarding follow-up and referral of study participants as necessary.

5.7 PRE-STUDY ACTIVITIES (SITE INITIATION)

Information, engagement and sensitization of healthcare workers (HCWs) and communities will be essential before recruitment starts. Health information, education, communication (IEC) and sensitization of the HCWs and community about LF transmission, manifestations and prevention are described below:

a) **Key stakeholders' involvement**:
   - Key administrative and political authorities, chiefs, civil society and religious leaders, traditional healers, teachers, medical staff, etc. in the district, sub-district and community levels will be identified and engaged early before the start of the study. The support and buy-in of these key stakeholders will be beneficial and will lead to a research friendly environment. Continuous re-evaluation of stakeholder relationship will be strongly encouraged, as each stakeholder might have different priorities and motives which might be incompatible with the scientific rigor and adherence to the study protocol.

b) **Sensitization of the HCWs** in the health clinics and hospitals:
   - To prevent delayed case detection by the health staff at the hospital/health-care facility and to prevent nosocomial LF transmission, it is critical to continuously sensitize HCWs on LF. Health authorities, as part of the study teams, should provide training and mentorship of HCWs according to WHO guidelines on LF case detection, diagnosis and control in order to maintain high LF index of suspicion, an adherence of HCWs to use protective measures (use of PPE) while providing care, and on prompt treatment of diagnosed cases and supportive care[37].

c) **Sensitization of communities**:
   - Whenever possible, local LF survivors from the communities will be invited to participate in community engagement, i.e. to share their experience of LF illness with the community.
   - People should be advised about rodent control: appropriate raw and cooked food storage in rodent-proof containers, to clear bush and trash around houses, to avoid bush burning which drives rodents into homes, to reduce rodent population by setting traps in and around homes, to block all rat hideouts and any holes in the house through which rats can access the house, to avoid hunting rats for consumption, to keep cats to kill rats as an alternative etc.
   - Behavioural change campaigns could be organized to educate people in the communities about the risk of transmission of LASV with a view to improving their health-seeking behaviour by reporting early to the health-care facility, not only to benefit from treatment at the early stage but also to reduce the period they spend in the community transmitting the virus.
   - Existing public health measures to prevent other infectious diseases such as environmental sanitation and use of insecticide treated bed nets for the prevention of malaria may also be promoted as part of the community engagement.
   - Whenever possible, community health workers or community liaison officers will be recruited and involved in the study as they live in the communities and already have a good mastery and relationship with the potential study participants or their parent / legal guardian. They should be individuals who have already gained the trust of these communities.
   - The utilisation of already existing community structures such as community advisory boards (CABs), community action groups, or community working groups is advised instead of forming new ones for the purpose to disseminate project information and facilitate access to

community networks. CABs are made up of community stakeholders and are essential to the success of the study as they build support from within communities involved in the study and sustain overall efforts. CAB members serve as a liaison between study participants and the study team to promote dialogue, ensure that the study team receives continuous community feedback, and provide the community with regular study updates.

In addition, and in line with the study's third aim, a capacity strengthening working group will be established with the aim of defining, harmonising, and facilitating capacity strengthening where needed to help carry out epidemiological studies and future clinical trials. See section 8.1 for more details.

6 STUDY PROCEDURES – LF DISEASE COHORT

6.1 CASE DEFINITIONS AND OUTCOME MEASURES

The aim of the *LF disease cohort* study component is to assess the incidence rate of symptomatic LF cases through the implementation of active and passive surveillance activities. Data on LF cases, signs and symptoms will be collected based on the case definitions described below. Data will be collected in a standardized manner to allow refinement and performance assessment of case definitions for future epidemiological and clinical trials.

6.1.1 LASSA FEVER CASE DEFINITIONS

Two case definitions will be used in this study: ‘‘acute febrile illness’ and ‘confirmed LF’ case. The ‘‘acute febrile illness’ case definition is purposely broad to maximize case finding. The ‘confirmed LF’ case definition will be used as the primary outcome for the main analysis.

The two case definitions are as follows:

1. ***‘Acute febrile illness case’****:* self-reported fever of >48 hours duration (lasting at least two consecutive nights) + 1 of the signs/symptoms listed in the following table:

Table 2. list of signs and symptoms associated with Lassa fever disease.[38]

1. Headache	2. Abnormal bleeding (from mouth, nose, rectum and/or vagina)
3. Chest pain	4. Oedema of the neck and/or face
5. Muscle or joint pain	6. Conjunctival or sub-conjunctival haemorrhage
7. Vomiting	8. Jaundice
9. Cough	10. Spontaneous abortion
11. Sore throat	12. Buzzing in ears or acute deafness
13. Abdominal pain	14. Hypotension

2. ***‘Confirmed LF case’****:* an ‘acute febrile illness case’ + positive Lassa RT-PCR result.

6.1.2 OUTCOME MEASURES

The incidence rate of confirmed symptomatic LF (primary objective 1 and secondary objectives 1, 2, 3, 4, 5): the number of ‘confirmed LF’ cases per 1,000 person-years of follow-up in the symptomatic disease cohort, overall and stratified by country, site, age groups, gender, clade, and baseline serostatus.

Clinical course of LF (secondary objectives 6, 10): percentage of 'confirmed LF' cases with selected laboratory anomalies, clinical signs and symptoms, requiring certain interventions and complications, duration of hospitalization overall and stratified by risk groups.

Case fatality rate (secondary objective 7): percentage of 'confirmed LF' cases dying within 30 days of diagnosis or attributable to LF disease as assessed by the treating clinician at any point past confirmation.

Occurrence of SNHL (secondary objective 8): the percentage of all 'confirmed LF' cases with SNHL assessed by audiometry prior to discharge. SNHL will be defined as hearing loss of at least 30dB in three sequential frequencies in the standard pure tone audiogram, where a physical examination has excluded conductive hearing loss.[38]

Occurrence of delayed or persistent SNHL and possible other sequelae (secondary objective 9): the percentage of all LF survivors with SNHL at 4 months after hospital discharge. Delayed SNHL is defined as audiometry consistent with SNHL at follow-up but not at hospitalization. Persistent SNHL is defined as audiometry consistent with SNHL at hospitalization and at follow-up. In addition to audiometry, there will be an open question enquiring about the health status of the study participant to ask whether they are suffering from any other sequelae.

Prevalence of symptomatic confirmed LF co-infected with malaria parasites (secondary objective 11): the percentage of all 'confirmed LF' cases among whom presence of malaria parasites assessed by antigen rapid diagnostic test (RDT) is detected at the time of LF diagnosis.

Assessment of risk factors for symptomatic confirmed LF (secondary objective 12): the association between the incidence rate of symptomatic LF disease and pre-specified characteristics of the study subjects, expressed as an incidence rate ratio (IRR).

The incidence rate of 'acute febrile illness' (secondary objective 13): the number of 'acute febrile illness' episodes per 1,000 person-years of follow-up in the symptomatic disease cohort, overall and stratified by country and site.

Severity (secondary objective 14): in the absence of a well-established severity scoring system, a severity scoring system for use in clinical trials will be developed from clinical and laboratory information collected from 'confirmed LF' cases, in collaboration with WHO.

6.2 STUDY PROCEDURES

6.2.1 HOUSEHOLD AND PARTICIPANT SELECTION

The methods used to select geographical areas and households will mean all eligible households in the defined population have an equal chance to be selected at random to participate. A **two-stage cluster sampling method** will be used for random selection of localities and households to include in the study, the methods for which will be adapted to sites based on the study population, availability of electricity, maps and other technologies, and the capacities of the field workers.

First, the consortia will select the localities, second, they will select households in each locality, from which study participants will be enrolled. The following is an ***example*** of a two-stage cluster sampling strategy that could be used, although consortia can adapt the sampling strategy to their specific settings:

1. A list of all localities and their population sizes based on most recent census data will be obtained from regional health authorities. This list will constitute the study's sampling frame for selection of localities to include.
2. Using the sampling frame, localities will be selected with a probability proportional to their respective population sizes. The selected localities will constitute the study's clusters.
3. The WHO's Expanded Programme on Immunization (EPI) sampling method will be used to select households within each cluster. '***Households***' will be defined as persons living in the same dwelling, sharing meals and sleeping quarters.
   The EPI sampling method can be summarized as follows:
   - The investigator will start at a central point of the cluster and randomly select a direction from that point by spinning a pen or bottle.
   - The investigator will count the number of dwellings from the centre to the edge of the cluster and randomly choose a dwelling as the starting point.
   - Within the selected dwelling, all individuals that make up the household and who fulfil the study eligibility criteria will be selected for study enrolment.
   - From the starting dwelling, the next nearest dwelling will be visited in turn.
   - This process will be repeated until the cluster size has been achieved.

6.2.2 STUDY PARTICIPANT ENROLMENT

Enrolment will consist of the following actions:

1. For each selected household, the investigator will identify the household head and summarise the study and its aims to them. If the household head is not present at the time of first contact, the investigator should return to the household within 24 hours for a second attempt. If the household head remains unavailable, the household will be dismissed for inclusion in the study.
2. Each household will be provided information on how to reduce their risk of LF, as well as on rodent control.
3. The investigator will request the household head to identify those household members who should be considered for participation.
4. Starting with the household head, potential study participants within the selected household will be individually checked by the investigator against the eligibility criteria.
5. If eligibility criteria are fulfilled, the investigator will inform eligible household members (either individually or at household level) verbally in local language and in writing about the study background, related procedures, benefits and potential risks:
   - The potential study participant -or parent / legal guardian if appropriate- will be informed that their participation is voluntary and that they will be free, without justification, to withdraw from the study at any time without repercussions.
   - The potential study participant -or parent / legal guardian if appropriate- will be informed that every '*confirmed LF case*' will be notified to national authorities under the International Health Regulations (IHR) requirements.

- The potential study participant will be informed about the aims of the study and its procedures (e.g. how often they will be contacted/visited and what information and specimens will be collected from them) to ensure that the study and its expectations are well understood.

6. Informed consent (and assent, as required) will be sought from each individual or their parent/legal guardian for recruitment in the *LF disease cohort* either by signature or fingerprint. The **consent and assent forms** for the disease cohort are available in appendices 1 to 3.
7. If informed consent (and assent, as required) is provided, the individual will be given an individual study identifier and will be formally enrolled as a study participant for the study's *LF disease cohort.*
8. For enrolled study participants, the following actions will be undertaken:
    - Baseline study participant information will be collected by fieldworkers interviewing the individual or their parent / legal guardian if appropriate, and a baseline blood sample will be taken (see sections 6.2.4 and 6.2.5, respectively).
    - If the site is implementing the *LASV infection cohort* study component in addition to the *LF disease cohort* study component, the study participant will also be considered for inclusion in the former (see section 7.2.1 for further details).
    - Study participants (or their parent / legal guardian as appropriate) will be encouraged to attend (or take their charge to) their local healthcare facility or the implementing partner's referral-level health facility if the participant has a fever that lasts more than two consecutive nights (*passive surveillance)*.
9. If one or more household members were successfully enrolled as study participants:
    - Household-level information will be collected (see section 6.2.4),
    - The household head and one secondary household contact will be requested to provide contact information (e.g. mobile phone number, precise household location) for active follow-up purposes (*active surveillance*).

6.2.3 PARTICIPANT WITHDRAWAL

Participants or their parent / legal guardian if appropriate may withdraw consent and discontinue participation in the study at any time, with no effect on their medical care or access to treatment. If a participant or their parent/legal guardian has withdrawn consent, the reason for withdrawal will be documented and all information already collected will be retained for analysis. However, no further efforts will be made to obtain or record additional information regarding the participant.

6.2.4 BASELINE DATA COLLECTION

An electronic data collection tool will be used by the fieldworker to collect all baseline data from participants upon enrolment (see section 8.2 for more details on data collection procedures). All data variables to be collected at baseline are described in the **Questionnaire and Case Report Form (CRF).** In summary, the following data will be collected at this time:

- GPS position and description of the household
- A standardized **household questionnaire**, including questions on:
    - Household composition (number and description of household members)

        - Socioeconomic status of the household as indicated by combining data about dwelling structure and amenities, occupation and ownership of assets
        - Household member LF history and exposure to potential risk factors
    - **Participant questionnaire**: to obtain demographic data of recruited household members (age, sex, date, education, ethnicity, occupation)

Each household and study participant will automatically be assigned unique identification numbers which will be used for respondent identification for further data collection and data analysis purposes.

6.2.5 BASELINE BLOOD SPECIMEN COLLECTION

Upon study entry, a baseline blood specimen will be collected from all *LF disease cohort* study participants by venepuncture from peripheral veins. A total volume of 5mL of whole blood will be taken. Baseline blood specimens will be processed by the implementing partner to extract serum into two separate cryovials. One of the cryovial samples will be used to evaluate LASV serostatus (IgG) at baseline for all study participants. The second one will be stored at -20°C for possible future assessments, e.g. antibody testing in case additional tests become available.

Specimen collection and LASV laboratory testing procedures are described in section 8.4. and will be described in further detail in a **laboratory analysis plan**.

6.2.6 FOLLOW-UP OF STUDY PARTICIPANTS

The follow-up of study participants enrolled in the *LF disease cohort* will consist of the implementation of active and passive surveillance activities as described below.

1. Active surveillance

- Field workers will conduct active surveillance for all study participants by contacting (either by telephone or home visit) household heads or secondary household contact persons every two weeks, within a window of +/- 2 days.
- During these '*contact events*' household contact persons will be asked to report on the status of all household members enrolled in the study ('*status check*').
- Status checks will include confirmation of domicile (whether the study participant remains part of the household), an assessment of the presence of a febrile episode and other clinical signs and symptoms defined in the 'acute febrile illness' case definition, a query about any illness or visits to a health facility during the preceding two weeks, and a reminder to report to a designated local health facility in case of subsequent fever that persists for ≥ two nights. The status of any 'confirmed LF cases' that have been discharged and returned to the household in the course of the study will also specifically be queried, including any possible deaths.
- If household contacts are unresponsive to the initial status check attempt, at least two further follow-up contact attempts will be performed within 48 hours, at least one of which will be a household visit. In case of three unsuccessful status check attempts, the data of that biweekly contact event will be documented as missing and the subsequent follow-up contact event will be scheduled as planned.
- If a household cannot be contacted for a minimum of two or more consecutive contact events (= a total of six consecutive missed status checks), the household will be considered lost to follow-

up (study consortia may adapt this number to be more appropriate for the setting in which they will implement the study).
- If study participants (or their parent / legal guardian, as appropriate) report symptoms or signs possibly attributable to LF during the active follow-up, either the study field worker will assess fulfillment of the 'acute febrile illness' case definition criteria, or the study participant will be referred to a nearby health facility for such assessment.
- Infection control and personal protection measures will be taken as appropriate (see section 8.2).
- If formally identified as a 'acute febrile illness case' at the health centre or by a medically qualified study field worker, further work-up (as described below) will be performed.
- If criteria for 'acute febrile illness' are not fulfilled, the study participant will be referred as appropriate for further medical follow-up and routine care provision.

2. Passive surveillance

- Study participants, or their parent / legal guardian if appropriate, will be encouraged to immediately report any febrile episode that has persisted for at least 2 consecutive nights to study field workers (e.g. by text message, phone call) and present to a health care facility for further assessment as per study guidelines.
- Infection control and personal protection measures will be taken as appropriate in such health facilities (see section 8.2).
- If the study participant is classified as a 'acute febrile illness', the healthcare facility will refer the patient to a study health facility as appropriate, or alert a study field worker who will ensure further work-up is performed.
- If criteria for 'acute febrile illness' are not fulfilled, the study participant will be either be treated as appropriate or referred as appropriate for routine care provision.

6.2.7 WORKUP OF ACUTE FEBRILE ILLNESS CASES

When a study participant has been classified as a 'acute febrile illness case', the following actions will be undertaken:

- A health worker will interview the 'acute febrile illness case' to document clinical signs and symptoms using the standardised CRF.
- A trained phlebotomist or trained health worker will draw a 5mL whole blood specimen in EDTA tube from the 'acute febrile illness case' and send the sample to the laboratory.
- A trained laboratory researcher will process and test the whole blood specimen taken from the 'acute febrile illness case' for the presence of LASV using the RT-PCR Altona Real Star Kit 2.0 (see section 6.2.8). In addition, this whole blood specimen will also be used for malaria testing purposes, as described below.
- A laboratory researcher will use an antigen rapid diagnostic test (RDT) to assess the presence of malaria parasites. RDT confirmation via microscopy is not required for study purposes and will be at the discretion of the treating clinician (additional detail outlined in the **laboratory analysis plan**). Any study participants found to be RDT positive for malaria and RT-PCR negative for LASV infection will be referred as appropriate for routine healthcare provision.
- A study fieldworker, health worker or laboratory technician will inform the 'acute febrile illness case' of the results of both laboratory tests.

- Depending on their clinical presentation, the authorized health care worker will decide whether to hospitalize the patient for any reason.
- Study field staff or laboratory workers likely to be in contact with 'acute febrile illness cases' or their blood specimens will be trained and instructed to use PPE as appropriate (see section 8.2.).

6.2.8 CONFIRMATION OF LASV

LASV confirmation testing will be performed for all 'acute febrile illness cases' identified in the *LF disease cohort*. The 5 mL whole blood specimen will be taken with appropriate PPE as described in section 8.2, either at the implementing partner's reference-level healthcare facility or a satellite healthcare facility located in its catchment area. If the patient is unable to leave their home, the blood draw will be performed on the premises. In case blood specimens are taken at home or at a satellite healthcare facility, the specimen will be transported to the implementing partner's referral-level healthcare facility as described in the **laboratory analysis plan** for testing. LASV confirmation testing procedures are described in further detail in section 8.4.1. If the patient had a blood specimen taken at a health facility and was not hospitalized based on clinical presentation, the study participant will return home pending the outcome of the LASV confirmation test. Once the result is known, a fieldworker will go to the patient's household to inform of the outcome of the test. For LASV confirmed cases, the fieldworker will follow the relevant national LF management guidelines in terms of referral to appropriate hospital or LF treatment facility.

6.2.9 FOLLOW-UP OF CONFIRMED LF CASES

When a study participant has been classified as a 'confirmed LF case' by a study investigator, the following actions will be undertaken:

- Infection control and personal protection measures will be taken as appropriate (see section 8.2).
- In case of positive RT-PCR result, the individual participant and parent / legal guardian if appropriate, as well as responsible health authorities, will be notified immediately.
- Local authorities will be responsible for provision of medical care as well as isolation and infection control measures as indicated in accordance with national and / or regional guidelines.
- A laboratory technician will aliquot a sample of the blood specimen taken for RT-PCR confirmation for genome sequencing purposes. The laboratory technician will send the aliquot to the appropriate facility for sequencing.
- The provided care will be consistent with WHO standard of care guidelines for LF patients[36]. Data on pre-defined clinical signs and symptoms as well as laboratory parameters (including blood haematology and chemistry laboratory parameters like liver function tests, creatinine) and IgM/IgG will be collected directly (from the participant) or indirectly (from hospital records) for the duration of the hospitalization. Detailed information of the data to be obtained during hospitalisation is included in the CRF (see supplementary file A).
- An audiometry test will be performed before hospital discharge to assess for possible presence of SNHL.

6.2.10 FOLLOW-UP OF CONFIRMED CASES AFTER HOSPITAL DISCHARGE

Upon hospital discharge, LF survivors will be followed up at their premises for 4 months after discharge. The following will be assessed and documented in the CRF:

- A trained health worker will take audiometry measurements to asses for any persistent or delayed onset SNHL. For this purpose, '*delayed SNHL*' is defined as audiometry consistent with SNHL at follow-up but not at hospitalization, and '*persistent SNHL*' is defined as audiometry consistent with SNHL at follow-up and at hospitalization.
- In addition to audiometry, the health worker will pose the study participant an open question enquiring about their health status and whether they are suffering from any other sequelae.
- If sequelae persist, the study participant will be referred by the health worker as appropriate for routine healthcare provision.

6.2.11 FATAL CONFIRMED LF CASES AND FOLLOW-UP OF COMMUNITY DEATHS

- Information on fatal outcome for 'confirmed LF' cases will be obtained from medical records. All 'confirmed LF' cases who die within 30 days of diagnosis or whose death was attributed to LF disease as assessed by the treating clinician at any point following LF confirmation will be considered a 'confirmed LF case' with fatal outcome.

6.2.12 STUDY PROCEDURES IN AN OUTBREAK SITUATION

- In the case of a LF outbreak being declared by the responsible health authorities, all precautions will be taken to support and not to interfere with outbreak control measures.
- Clinical care and outbreak control have priority over research procedures and there should be no competition for resources.

7 STUDY PROCEDURES – LASV INFECTION COHORT

7.1 OUTCOME DEFINITIONS AND OUTCOME MEASURES

The aim of the *LASV infection cohort* study component is to estimate the incidence rate of LASV infection, as assessed by seroconversion. Data on study participant LASV serostatus will be collected based on the definitions and outcome measures described below.

7.1.1 LASV INFECTION COHORT: SEROSTATUS DEFINITIONS

***'Seropositive'*:** presence of Lassa-specific serum antibodies in either IgG or IgM-ELISA

***'Seronegative'*:** absence of Lassa-specific serum antibodies in either IgG or IgM-ELISA]

LASV infection: a documented change from seronegative to seropositive status ('*seroconversion*')

LASV reversion: a documented change from seropositive to seronegative status ('*seroreversion*')

At baseline, only IgG status will be assessed and documented. Any study participants found to be IgG+ will be considered seropositive at baseline. At the four 6-monthly follow-up occasions both IgG and IgM will be documented and interpreted as follows:

Table 3. Interpretation of serology at 6-monthly follow-ups

Baseline serology	Follow-up serology	interpretation
IgG -	IgM+ / IgG+	LASV Infection, timing uncertain
IgG -	IgM- / IgG+	LASV infection, not recent
IgG -	IgM+ / IgG-	LASV infection, recent
IgG +	IgG – (independent of IgM)	LASV reversion

7.1.2 OUTCOME MEASURES: LASV INFECTION COHORT

The incidence rate of LASV infection (secondary objectives 15 and 17): the number of LASV infections (seroconversion) per 1,000 person-years of follow-up in the incidence of infection cohort, overall and stratified by country, site and age groups.

Assessment of seroprevalence (secondary objective 16 and 18): the percentage of subjects found to be seropositive at baseline (IgG+) out of the number of subjects tested for baseline seropositivity overall and stratified by country, site, age groups.

Assessment of risk factors for LASV infection: the association between the incidence rate of LASV infection and prespecified characteristics of the study participants or their households (**as listed in the questionnaires**), expressed as an IRR.

Assessment of factors associated with seropositivity: by estimation of the ratio of the odds of being seropositive at baseline in prespecified risk groups to the odds in the study population without the prespecified risk, expressed as an odds ratio (OR).

7.2 STUDY PROCEDURES

7.2.1 PARTICIPANT SELECTION

Two different scenarios inform the participant selection procedure for the *LASV infection cohort*:

Scenario A: The *LASV infection cohort* study component will be implemented in the proposed site *in combination with* the *LF disease cohort* study component. In this scenario, study participants for the (nested) *LASV infection cohort* are selected progressively as they are enrolled in the (main) *LF disease cohort* until the targeted sample size has been achieved.

Scenario B: the *LASV infection cohort* study component will be implemented *by itself* in the proposed site. In this scenario, study participants are selected using a **two-stage cluster sampling method** as described in section 6.2.1.

7.2.2 STUDY PARTICIPANT ENROLMENT

In scenario A

- For details for enrolment of participants in the *LF disease cohort* – see section 6.2.2
- Until the targeted sample size for the nested *LASV infection cohort* has been achieved, the investigator will ask every study participant that is enrolled in the *LF disease cohort* (or their parent / legal guardian, if appropriate) to also take part in the nested *LASV infection cohort*.
- The potential *LASV infection cohort* study participant or their parent / legal guardian, if appropriate, will be informed about the aims of the *LASV infection cohort* study component and its procedures.
- Additional informed consent will be sought from the participant or their parent / legal guardian, if appropriate (and assent, as required), for recruitment in the *LASV infection cohort*.
- The **consent and assent forms** for the infection cohort are available in appendices 4 to 6.
- If informed consent (and assent, as required) is provided, the individual will be formally enrolled as a study participant for the nested *LASV infection cohort* in addition to the *LF disease cohort*.

In scenario B

Recruitment will commence in (estimated) early-mid 2020, as soon as all required approvals have been obtained and site initiation (including community engagement activities; see section 5.7) has

been performed by the implementing partners. Initial recruitment will continue until the targeted sample sizes (see section 9.1) have been attained. Recruitment should be done outside of the high LASV transmission season. Preferred recruitment period is from end of March to end of November. Recruitment will be monitored continually, with steps taken to increase recruitment where needed. Additional recruitment will not extend beyond the start of the 2021-2022 LASV high transmission season (30th of November 2021). All study participants will therefore be followed over a period of 12 (minimum) to 24 (maximum) months. Enrolment will consist of the following actions:

1. For each selected household, the investigator will identify the household head and summarise the study and its aims to them.
2. Each household will be provided information on how to reduce their risk of LF, as well as on rodent control.
3. The investigator will request the household head to identify those household members who meet the study's eligibility criteria.
4. Starting with the household head, potential study participants within the selected household will be individually checked by the investigator against the eligibility criteria.
5. If eligibility criteria are fulfilled, the following actions will be undertaken:
    - The potential study participant or their parent / legal guardian, as appropriate, will be informed that their participation is voluntary and that they will be free, without justification, to withdraw from the study at any time without repercussions.
    - The potential study participant or their parent / legal guardian, as appropriate, will be informed about the aims of the study and its procedures (e.g. how often they will be contacted/visited and what information and specimens will be collected from them) to ensure that the study and its expectations are well understood.
    - Informed consent (and assent, as required) will be sought for recruitment in the *LASV infection cohort*.
    - The **consent and assent forms** for the infection cohort are available in appendices 4 to 6.
6. If informed consent (and assent, as required) is provided, the individual will be formally enrolled as a study participant for the study's *LASV infection cohort* and the following actions will be undertaken:
    - Baseline study participant information will be collected by a field worker interviewing the individual or their parent/legal guardian, as appropriate, and a baseline blood sample will be taken (see sections 6.2.4 and 6.2.5, respectively).
    - The household head and one secondary household contact will be requested to provide contact information (e.g. mobile phone number, precise household description and location) for study follow-up purposes.

7.2.3 PARTICIPANT WITHDRAWAL

Participants or their parent / legal guardian, as appropriate, may withdraw consent and discontinue participation in the study at any time, with no effect on their medical care or access to treatment. If a participant or their parent / legal guardian, as appropriate, has withdrawn consent, the reason for withdrawal will be documented and all information already collected will be retained for analysis. However, no further efforts will be made to obtain or record additional information regarding the participant.

7.2.4 BASELINE DATA COLLECTION

An electronic data collection tool will be used by the fieldworker to collect all baseline data from participants upon enrolment (see section 8.2. for more details on data collection procedures). All data variables to be collected at baseline are included in the **questionnaire/CRF**. In summary, the following data will be collected at this time:

- GPS position of study household
- **A standardized household questionnaire**, including questions on household composition (number and description of household members)
- **A participant questionnaire**: For Demographic data of household members (age, sex, date, education, ethnicity, occupation)

For recruitment under scenario B, each household and study participant will automatically be assigned unique identification numbers which will be used for further data collection and data analysis purposes.

7.2.5 BASELINE BLOOD SPECIMEN COLLECTION

Upon study entry, a trained fieldworker, phlebotomist or health worker will take a baseline blood specimen will be collected from all *LASV infection cohort* study participants by venepuncture from peripheral veins. A total volume of 5 ml whole blood will be taken. Baseline blood specimens will be processed by implementing partner laboratory technicians to obtain two aliquots of separated serum specimens. One aliquot of the serum specimen will be used to evaluate LASV serostatus (IgG) at baseline for all *LASV infection cohort* study participants. The second specimen will be stored at -20°C for possible future assessments, e.g. antibody testing in case additional LF tests become available. Participants who are already part of the *LF disease* cohort will not be required to provide an additional blood sample for serostatus testing.

Specimen collection and laboratory testing procedures are described in more detail in section 8.4, as well as in the **laboratory analysis plan**.

7.2.6 FOLLOW-UP OF STUDY PARTICIPANTS

Following enrolment, a follow-up blood specimen will be taken from all study participants enrolled in the *LASV infection cohort* at 6, 12, 18 and 24 months post-baseline. These samples will also undergo serological testing, as described in section 8.4.2.

8 GENERAL STUDY PROCEDURES

8.1 ASSESSMENT OF GAPS AND PREPAREDNESS FOR FUTURE CLINICAL TRIALS

To address the study's third aim, a capacity strengthening working group will be established to define and harmonise the capacity of the study sites to carry out epidemiological studies and future clinical trials, and as a longer-term effect strengthen research and national surveillance capabilities.

During the preparation for these epidemiological studies the working group will:

- Map resources available in each study site to carry out the epidemiological studies (infrastructure, project management, human resources, training courses, relevant existing study SOPs)
- Define minimal requirements for the capacity to carry out the *LF disease* and *LASV infection* cohort studies in terms of human resources and training needs, infrastructure and materials
- Define prioritized capacity strengthening and training needs of the sites based on site assessment and interactions with consortia
- Prepare capacity strengthening plans for each site based on the Integrated Epidemiology Project Plans, which are site assessments reports and input from site representatives.

During the epidemiology studies the working group will:

- Develop training materials for the sites.
- Implement training activities.
- Agree minimal requirements to carry out Phase III clinical trials in terms of infrastructure, human resources and materials.

8.2 INFECTION CONTROL AND PERSONAL PROTECTION MEASURES

PPE measures are the responsibility of study sites and should be used in line with national guidelines, with procurement support provided by CEPI.

Any staff member involved in taking or handling blood samples or providing care to study participants will be instructed in appropriate use of PPE according to national guidelines.

According to the WHO[1], staff in health-care settings should always apply standard precautions as infection prevention and control measures when caring for patients, regardless of their presumed diagnosis. These include proper hand hygiene, respiratory hygiene, use of standard PPE (such as gloves, face masks, aprons etc) to protect against splashes or contact with infected materials and the application of safe injection practices. HCWs caring for patients with 'confirmed LF' should apply extra infection control measures (additional precautions as appropriate) to prevent contact with patient's blood and body fluids and contaminated surfaces or materials such as clothing and bedding, as detailed in the national guidelines of each country.

Any field study staff that had drawn blood from a 'confirmed LF case' should be monitored daily by thermometer for fever for three weeks post blood draw. If fever occurs during this period, they will be admitted to the implementing partner's health facility for further investigation and care.

8.3 DATA COLLECTION

Electronic data collection will be used to record all required study data on each participant, from baseline interviews in households, follow up activities by telephone or in person, and extracting data from medical records for acute febrile illness and confirmed LF cases. The electronic data collection tool will be device agnostic to allow for data entry using smartphones, tablets, laptops or desktop computers, whichever option is more convenient for a given data entry occasion (whether in the field, a health facility or a laboratory environment). Hard copies of all data collection forms will also be available in case electronic data capture tools are unavailable due to technical reasons. Any data captured using paper forms will be entered electronically afterwards, as will be clearly described in the **data management plan**.

A standard electronic version of the **questionnaire and case report form (eCRF)** will be developed and used by all sites for the core variables. The same data capture methods will be used across all sites to allow pooled analyses and comparability between sites.

Upon enrolment, the GPS position of participating households will be recorded, as well as data on selected baseline characteristics including household composition, demographics, and brief medical history. During the follow up of participants, pre-defined data on self-reported clinical signs and symptoms will be collected. Clinical data will also be obtained from medical records of health centres within the communities by trained field workers to assess/document outcomes for 'acute febrile illness'' and 'confirmed' cases, as well as for source data verification purposes. All variables are described in the **questionnaire and CRF**, which can be found in supplementary file A. Data elements and collection will be fully described in the **data management plan**.

8.4 SPECIMEN COLLECTION AND LABORATORY TESTING

All blood specimens for LASV testing will be collected, processed, stored and shipped in a comparable manner at all sites. Venepuncture in young children will be performed by experienced nurses or phlebotomists. All laboratory procedures are described in detail in a separate **laboratory analysis plan**, which includes detailed info on the following:

- Blood sampling procedures;
- Maintaining an appropriate chain of custody for specimens
- specimen aliquots for storage and/or centralized testing;
- ELISA and RT-PCR testing procedures;
- description of the study site personnel who will collect and manipulate blood/plasma specimens;
- information on material provided for optimal specimen collection;
- description of basic common lab standard operating procedures (SOPs);
- description on building specific technical capacity and training of biomedical engineers;
- description on maintaining resources for maintaining lab functions.

Table 4 summarises the occasions on which blood is drawn during the study, the purpose of the blood specimen, and the amount to be drawn.

Table 4. Overview of study blood draw events.

Study event	Amount of whole blood drawn	Purpose
LF disease cohort baseline and LASV infection cohort baseline	5mL whole blood in dry (SST) tube.	• Obtain serum specimen for documenting baseline LASV serostatus (IgG) • Obtain serum specimen for possible assessment of future LF diagnostic tests.
LASV infection cohort follow-up	5mL whole blood in dry (SST) tube	• Obtain serum specimen for documenting LASV serostatus (IgG and IgM) and establish possible seroconversion
'Acute febrile illness case' identified	5mL whole blood in EDTA tube	• Obtain plasma specimen for RT-PCR test • Obtain plasma aliquot for genome sequencing RT-PCR+ specimens. • From whole blood specimen obtain (15µL) for malaria RDT

8.4.1 RT-PCR CONFIRMATION TESTING

The blood (plasma) specimen will be tested for LASV by using the RT-PCR Altona RealStar® Lassa Virus RT-PCR Kit 2.0. Laboratory staff will be trained as required. All implementing partners will have access to laboratory facilities with RT-PCR capacities, either on-site or through collaboration with the respective national reference laboratory. Additional capacity building in performing RT-PCR tests will be provided as required.

8.4.2 SEROLOGICAL TESTING

Serological testing will be performed on i) blood specimens taken from all *LF disease cohort* study participants upon enrolment (see section 6.2.5), and ii) serum from blood specimens taken from all *LASV infection cohort* study participants upon enrolment and at subsequent 6-monthly follow-up intervals (see sections 7.2.5 and 7.2.6).

Serological testing will be done by a validated IgG, IgM ELISA, which at the time of writing is still under evaluation by the Foundation for Innovative New Diagnostics (FIND). The test is expected to be validated and available from (estimated) May 2020. If the validation data will not be available by the start of the study, specimens will be stored and tested as soon as the validated ELISA is available.

8.4.3 GENOME SEQUENCING

High LASV genome diversity was observed previously in west Africa and several RNA genome sequencings (complete and partial) have been performed on the various LASV strains. Up to 27% sequence diversities resulting in four major LASV lineages (lineages I-IV) [40] have been observed. Currently there are however still major LASV genome sequencing data gaps, which we want to address in this study. Aliquots obtained from all LASV positive specimens will be sequenced. Redeemer's University in Nigeria will be responsible for LASV positive samples collected in Nigeria, Benin and Liberia. Kenema Hospital in Sierra Leone will be responsible for sequencing samples collected in Sierra Leone.

9 STATISTICAL METHODS

All statistical methods are fully described in a written and approved **statistical analysis plan** (SAP).

9.1 SAMPLE SIZE

To estimate the incidence of symptomatic infections with a 95% CI of 7.6 – 13.2 for an incidence of 10/1,000 person-years or a 95% CI of 0.4 – 2.3 for an incidence of 1/1,000 person-years, 5000 subjects need to be recruited per country (or per site per country in those with multiple sites).

To estimate the incidence of LASV infection with a 95% CI of 0.5 – 1.8 for an incidence of 1/100 person-years or a 95% CI of 8.3 – 12.0 for an incidence of 10/100 person-years, 1000 subjects need to be recruited per country (or per site per country in those with multiple sites).

To guide the selection of a reasonable sample size, we calculated the sample size for different precision levels considering the incidence of LF to be between 0,001% and 3% (Table 5) and seroprevalence between 1% and 50% (Table 6). For an incidence rate of 0.1% over the two-year period (or 1/1.000), the precision would range from 0.04% (or 0.4/1.000) to 0.23% (or 2.3/1.000). For an incidence rate of 1% over the two-year period, the precision would range from 0.76 % to 1.32%.

The sample size does not take into account the baseline seropositivity rate. If the seropositivity rate in the general population is high, this will likely diminish the pool of susceptible individuals and decrease the incidence rates of LF. Sites are encouraged to consider ways of mitigating this issue by considering ways of enrolling more subjects if high seropositivity rates are observed at baseline. Sites could also consider recruiting patients from a different geographic area. An assessment will be made during the interim analysis.

Table 5. Sample size estimates – Incidence rate (%: per 100 persons over 2-year period)

Sample size	**N=200**		**N=1,000**		**N=5,000**		**N=25,000**	
Incidence rate (%)	Lower 95% CI	Upper 95% CI	Lower 95% CI	Upper 95% CI	Lower 95% CI	Upper 95% CI	Lower 95%CI	Upper 95% CI
0.001 %	0.00	1.89	0.00	0.39	0.00	0.08	0.00	0.02
0.01 %	0.00	1.90	0.00	0.40	0.00	0.10	0.00	0.03
0.1 %	0.01	2.08	0.02	0.56	0.04	0.23	0.07	0.15
1 %	0.28	3.57	0.54	1.83	0.76	1.32	0.84	1.13
3 %	1.38	6.39	2.11	4.25	2.56	3.51	2.80	3.22

Confidence intervals (lower and upper limits 95%CI) were calculated using the Wilson method. [41]

Table 6. Sample size estimates – Seroprevalence rate (%: per 100 persons)

Sample size	N=200		N=1,000		N=5,000		N=25,000	
Seroprevalence rate (%)	Lower 95% CI	Upper 95% CI	Lower 95% CI	Upper 95% CI	Lower 95% CI	Upper 95% CI	Lower 95% CI	Upper 95% CI
1 %	0,3	3,6	0,5	1,8	0,8	1,3	0,9	1,1
5 %	2,7	9,0	3,8	6,5	4,4	5,6	4,7	5,3
10 %	6,6	14,9	8,3	12,0	9,2	10,9	9,6	10,4
50 %	43,1	56,9	46,9	53,1	48,6	51,4	49,4	50,6

Confidence intervals (lower and upper limits 95%CI) were calculated using the Wilson method. [41]

9.2 DATA ANALYSES

9.2.1 GENERAL CONSIDERATIONS

Descriptive analyses will be performed on an ongoing basis to gain an understanding of the qualitative and quantitative nature of the data collected and the characteristics of the study participants studied. Statistical analyses for the study's specific objectives (see section 4.2) and outcome measures (see sections 6.1.2 and 7.1.2) will be described in further detail in the **statistical analysis plan** (SAP).

In general, missing data will not be imputed and the data will be analysed as they are recorded in the **questionnaire/CRF**. However, if more than 10% of data is missing for one or more key variable, the impact of missing data on the analysis will be discussed, and the pattern of missing data will be explored. If there is evidence of bias in the missing data, and variables that are considered good predictors of the missing data are available, the multiple imputation method at the study level may be used to replace missing values as secondary exploratory analyses. If the multiple imputation method is used, a sensitivity analysis will be carried out comparing results from the complete case analysis (where records with missing data will be dropped) and the full set analysis (with imputed data).

9.2.2 INTERIM ANALYSES

Based on ongoing descriptive analyses, a first structured look at the data as they accrue will be performed between (estimated) April and August 2020 assuming that at least one site will have started recruitment by then. The aim is to evaluate and possibly revise study procedures and methods if necessary. This **intermediate assessment** will serve as a first input into the clinical trial designs and will for example serve to:

1. Assess the practicability / efficiency of the 'acute febrile illness' case definition used for screening purposes. The outcome of this analysis may lead to a refinement of the case definition used for screening for 'acute febrile illness' cases.
2. Assess the recruitment strategy: If the recruited study population does not reflect overall population characteristics (e.g. age distribution) or the population considered at high risk for

LASV infection (e.g. rural population) the recruitment strategy may be revised accordingly. High baseline seropositivity might also cause to review the recruitment strategy and potentially recruit in a different area.

A pre-defined complete **interim analysis** will be undertaken at the end of the first full Lassa season (in April 2021). The main purpose of this interim analysis is to analyse data collected after the first full Lassa season for the primary objectives and key selected secondary objectives (defined in the SAP). The aim is to assess the probability that the continuation of the study during the second season will provide additional knowledge on the first primary objective. The outcome of this analysis may lead to:

1. a suspension of the study, if it is demonstrated that the incidence of symptomatic LF disease is too low to justify continuation (futility)
2. a suspension of the study, if it demonstrated that the incidence of symptomatic LF disease is sufficiently high and well defined after one single season (success)
3. an adaptation of the study design (e.g. additional recruitment and / or continued follow-up, shift of the geographical focus of recruitment), if it is demonstrated that the incidence of symptomatic LF disease will not be sufficiently well defined under the current design.

Decisions on amendment or suspension of the study following interim analysis will be made by CEPI in conjunction with the study Programme Steering Committee (PSC), with advice sought from the Epidemiology Expert Reference Group (EERG), Council of Public Health Authorities (CPHA), and other stakeholders as necessary.

9.2.3 LIMITATIONS OF RESEARCH METHODS

Clinical records in most facilities in Africa are not electronic but paper based, making them prone to error. Data extracted from such paper-based records for study purposes would inherently include any errors as they appear in the clinical records. If the clinical records have not documented LF cases clearly and consistently, this could lead to underestimation of LF incidence.

The sampling method for each site will have to be carefully specified in advance, given that sampling method may affect the sample size estimation. Without a rigorous sampling plan the estimates from the study may be biased (selection bias).

There is always a risk of low recruitment rates and potential loss to follow-up. These will be assessed during the intermediate assessment. If necessary, recruitment and participant retention strategies will be reviewed.

9.3 DATA REPORTING

An interim study report will follow the interim analysis, and a final study report will be issued following completion of the study. The study report will encompass a description of the complete study population, laboratory outcomes and analyses as described in the SAP.

10 DATA MANAGEMENT

This study will be implemented at country / site level under the responsibility of the respective implementing partner and its principal investigator. Additional support will be provided by pre-defined consortium partner institutions. CEPI reserves the right to perform monitoring activities or delegate respective activities to a third party.

10.1 DATA COLLECTION AND EXTRACTION

Optimal data collection methods and a **study monitoring plan** that is appropriate for the study design will be developed and implemented centrally. Data collection and extraction processes will be described in detail in a separate **data management plan.** This will be created before data collection begins and will describe all functions, processes, and specifications for data collection, cleaning and validation.

Whenever possible, electronic data collection (capture) will be performed and will include programmable edits to obtain immediate feedback if data are missing, out of range, illogical or potentially erroneous. If study data will be obtained on paper (for example, if electronic devices malfunction), the data from the paper questionnaires will be entered retrospectively directly into an electronic data capture system by study site staff. Dual data entry procedures or regular data consistency checks will be implemented to minimise possible data entry errors on these occasions. All electronic data will be backed up nightly. All changes or corrections to entered and stored data should be justified and documented in an audit trail. High data quality standards will be maintained, and processes and procedures utilised to ensure that the data are as clean and accurate as possible when presented for analysis.

10.2 FILE RETENTION AND ARCHIVING

To enable evaluations and/or audits from regulatory authorities or CEPI, the investigators agree to keep records, including the identity of all participants, all original signed informed consent forms (ICF)s and assent forms, copies of all paper-based questionnaires used, source documents and adequate documentation of relevant correspondence (e.g., letters, meeting minutes, telephone calls reports). The records should be retained by the investigator according to local regulations, or as specified in the study contract, whichever is longer.

Each site will receive a **study site file** at study initiation which contains all documents necessary for the conduct of the registry and will be updated throughout the study. This file must be available for review in the event the site is selected for monitoring, audits, or inspections and must be safely archived for a time period to be specified in the study contract. Documents to be archived include the patient enrolment log and the signed informed consent form (ICFs). In the event that archiving of the file is no longer possible at the site, the site will be instructed to notify CEPI.

10.3 MONITORING

A **study monitoring plan** that is appropriate for the study design will be developed and implemented and will include description of monitoring procedures and frequency of monitoring visits.

Monitoring will be performed to examine compliance with the protocol and adherence to the data collection procedures in accordance to the **data management plan** in order to assess the accuracy and completeness of submitted data, and to verify that records and documents are being properly maintained for the duration of the study.

10.4 CHANGES TO THE PROTOCOL

Any proposed changes will need to be cleared with CEPI before proceeding. If approval given, any changes introduced to the protocol after initial ethics approval will be done in adherence to guidelines as set out by the relevant Independent Ethics Committee (IEC)/Institutional Review Board (IRB). Changes to the protocol will be documented in written protocol amendments. Major (i.e., changes affecting primary objectives) amendments will usually require submission to the relevant IEC/ IRB for approval or favourable opinion and to the relevant regulatory authorities, if applicable. In such cases, the amendment will be implemented only after approval or favourable opinion has been obtained.

Minor (non-substantial) protocol amendments, including administrative changes, will be filed at each participating site and will be submitted to the relevant IRB/IEC or regulatory authorities where required by pertinent regulations. Any amendment that could have an impact on the patient's agreement to participate in the study requires the patient's re-consent prior to continued participation in the study.

10.5 PUBLICATION POLICY

Any publication of the results from this study must be consistent with the study publication policy and guided by the Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Writing and Editing for Biomedical Publication of the International Committee of Medical Journal Editors (ICMJE), updated April 2010. The rights and responsibilities of the Investigator(s) with regard to publication of the results of this study/registry are described in CEPI's equitable access policies, as well as in contracts with the Investigators, and through a study-wide data sharing and publication policy now under development.

11 ETHICAL AND REGULATORY CONSIDERATIONS

To ensure the quality and integrity of research, this study will be conducted under the International Ethical Guidelines on Epidemiological Studies issued by the Council for International Organizations of Medical Sciences (CIOMS, 2009), the Declaration of Helsinki and its amendments, and any applicable national guidelines.

11.1 PATIENT INFORMATION AND INFORMED CONSENT

An ICF must be signed by the participant (or parent / legal guardian for minors) prior to enrolment in the study (appendices 1 to 6). Informed assent forms will be completed by minors old enough to understand the proposed study and specimen collection procedures – the age of which will be determined by national guidelines of each participating country. For illiterate participants the information and consent/assent form will be read out in the presence of an independent literate witness. The participant will put their fingerprint on the form to indicate their willingness to participate. The witness will sign the consent/assent form to confirm that the participant understands the study processes, has had the opportunity to ask questions and is consenting of their own free will. The study file for each participant should document the informed consent process and that written informed consent was obtained prior to participation in the study.

Informed consent will also indicate that any confirmed LASV infection will be notified to national authorities under the International Health Regulations (IHR) requirements. If applicable, ICF will be provided in a certified translation of the local language. In case of illiteracy, a specific procedure depending on the country will have to be applied (e.g. witnessed consent, consent documented by thumb print).

A copy of each signed ICF must be provided to the participant. All signed and dated ICFs must remain in each participant's study file and must be available for verification at any time. The ICF should be revised whenever there are changes to procedures outlined in the informed consent or when new information becomes available that may affect the willingness of the participant to participate. For any updated or revised ICFs, the study file for each participant should document the informed consent process and that written informed consent was obtained for the updated/revised ICF for continued participation in the study.

11.2 STUDY PARTICIPANT CONFIDENTIALITY

In order to maintain patient confidentiality, each participant will be assigned a unique identifier upon study enrolment. This participant identifier will be used in place of patient name for the purpose of data analysis and reporting. All parties will ensure protection of participant personal data and will not include participant names on any reports, publications, or in any other disclosures, except where required by law.

In accordance with local regulations in each of the countries, participants will be informed about data handling procedures and asked for their consent. Data protection and privacy regulations will be observed in capturing, forwarding, processing, and storing patient data. Every effort will be made to protect participant confidentiality according to the Directive 95/46/EC on the protection of individuals, and in compliance with Safe Harbor privacy principles.

The database will be housed at a physically and logically secure computer system in accordance with a written security policy. The system meets approved established standards for the security of health information and is validated. The system also meets the standards of the International Committee on Harmonisation (ICH) guideline E6R1 regarding electronic study data handling and is available for audit upon request. Participant confidentiality will be strictly maintained.

11.3 CLINICAL CARE FOR STUDY PARTICIPANTS

The responsibility for supportive or specific treatment for malaria (in case of positive RDT) or LF (in case RT-PCR confirmed) lies with the local health care authority of each study site, which will have to follow national guidelines. General support will be provided by CEPI depending on local needs. National guidelines generally call for hospitalisation and isolation of all confirmed LF cases and all measures are to be taken to prevent human to human transmission. WHO care guidelines should be followed as possible.[37]

11.4 INDEPENDENT ETHICS COMMITTEE/INSTITUTIONAL REVIEW BOARD

Consistent with local regulations and prior to enrolment of participants at a given site, the study protocol will be submitted together with its associated documents to the responsible independent ethics committee (IEC)/institutional review board (IRB) for its review.

Participant enrolment will not start at any site before the implementing partner has obtained written confirmation of a favourable opinion/approval from the relevant central or local IRB/IEC. The IRB/IEC will be asked to provide documentation of the date of the meeting at which the favourable opinion/approval was given that clearly identifies the study, the protocol version, and the ICF version reviewed.

Before implementation of any substantial changes to the protocol, protocol amendments will also be submitted to the relevant IRB/IEC in a manner consistent with local regulations. Pertinent safety information will be submitted to the relevant IECs during the course of the study in accordance with local regulations and requirements. It is the responsibility of the investigator to have prospective approval of the study protocol, protocol amendments, and informed consent forms, and other relevant documents, if applicable, from their local IRB/IEC and provide documentation of approval to CEPI. All correspondence with the IRB/IEC should be retained in the Investigator File.

Should the study be terminated early for any unanticipated reason, the investigator will be responsible for informing the IRB/IEC of the early termination.

REFERENCES

1. WHO. World Health Organization. Lassa fever 2018. https://www.who.int/emergencies/diseases/lassa-fever/en/ (accessed 5 December 2018).

2. Monath TP, Newhouse VF, Kemp GE. Lassa virus isolation from Mastomys natalensis rodents during an epidemic in Sierra Leone. *Science* 1974; **185**(4147): 263-5.

3. Olayemi A, Cadar D, Magassouba N, et al. New Hosts of The Lassa Virus. *Sci Rep* 2016; **6**: 25280.

4. Yadouleton A, Agolinou A, Kourouma F, et al. Lassa Virus in Pygmy Mice, Benin, 2016-2017. *Emerg Infect Dis* 2019; **25**(10): 1977-9.

5. Bonwitt J, Kelly AH, Ansumana R, et al. Rat-atouille: A Mixed Method Study to Characterize Rodent Hunting and Consumption in the Context of Lassa Fever. *Ecohealth* 2016; **13**(2): 234-47.

6. Schlie K, Maisa A, Lennartz F, Stroher U, Garten W, Strecker T. Characterization of Lassa virus glycoprotein oligomerization and influence of cholesterol on virus replication. *J Virol* 2010; **84**(2): 983-92.

7. McCormick JB, King IJ, Webb PA. A case-control study of the clinical diagnosis and course of Lassa fever. *Journal of Infectious Diseases* 1987; **155**(3): 445-55.

8. Andersen KG, Shapiro BJ, Matranga CB, et al. Clinical Sequencing Uncovers Origins and Evolution of Lassa Virus. *Cell* 2015; **162**(4): 738-50.

9. Safronetz D, Lopez JE, Sogoba N, et al. Detection of lassa virus, mali. *Emerging Infectious Diseases* 2010; **16**(7): 1123-6.

10. Whitmer SLM, Strecker T, Cadar D, et al. New Lineage of Lassa Virus, Togo, 2016. *Emerg Infect Dis* 2018; **24**(3): 599-602.

11. Lukashevich IS. Advanced vaccine candidates for Lassa fever. *Viruses* 2012; **4**(11): 2514-57.

12. Raabe V, Koehler J. Laboratory diagnosis of Lassa fever. *Journal of Clinical Microbiology* 2017; **55**(6): 1629-37.

13. Hallam HJ, Hallam S, Rodriguez SE, et al. Baseline mapping of Lassa fever virology, epidemiology and vaccine research and development. *NPJ Vaccines* 2018; **3**: 11.

14. Lukashevich IS, Pushko P. Vaccine platforms to control Lassa fever. *Expert Review of Vaccines* 2016; **15**(9): 1135-50.

15. CDC. Centre for Disease Prevention and Control. Lassa fever fact sheet. 2018. https://www.cdc.gov/vhf/lassa/pdf/factsheet.pdf (accessed 6 December 2018.

16. Ogbu O, Ajuluchukwu E, Uneke CJ. Lassa fever in West African sub-region: An overview. *Journal of Vector Borne Diseases* 2007; **44**(1): 1-11.

17. Gibb R, Moses LM, Redding DW, Jones KE. Understanding the cryptic nature of Lassa fever in West Africa. *Pathogens and Global Health* 2017; **111**(6): 276-88.

18. Bausch DG, Hadi CM, Khan SH, Lertora JJL. Review of the literature and proposed guidelines for the use of oral ribavirin as postexposure prophylaxis for lassa fever. *Clinical Infectious Diseases* 2010; **51**(12): 1435-41.

19. Wilson ME. Infectious diseases: an ecological perspective. *BMJ* 1995; **311**(7021): 1681-4.

20. Lo Iacono G, Cunningham AA, Fichet-Calvet E, et al. Using modelling to disentangle the relative contributions of zoonotic and anthroponotic transmission: the case of lassa fever. *PLoS Negl Trop Dis* 2015; **9**(1): e3398.

21. Bonner PC, Schmidt WP, Belmain SR, Oshin B, Baglole D, Borchert M. Poor housing quality increases risk of rodent infestation and Lassa fever in refugee camps of Sierra Leone. *Am J Trop Med Hyg* 2007; **77**(1): 169-75.

22. WHO. World Health Organization. Lassa Fever Fact Sheets. 2017. https://www.who.int/en/news-room/fact-sheets/detail/lassa-fever (accessed 21 December 2018).

23. Fichet-Calvet E, Rogers DJ. Risk maps of lassa fever in West Africa. *PLoS Neglected Tropical Diseases* 2009; **3**(3).

24. WHO. World Health Organization. Technical guidance on Lassa fever. 2018. https://www.who.int/emergencies/diseases/lassa-fever/technical-guidance/en/ (accessed 11 December 2018).

25. Ajayi NA, Nwigwe CG, Azuogu BN, et al. Containing a Lassa fever epidemic in a resource-limited setting: Outbreak description and lessons learned from Abakaliki, Nigeria (January-March 2012). *International Journal of Infectious Diseases* 2013; **17**(11): 1011-6.

26. WHO. World Health Organization Lassa Fever Nigeria Report. 2018. http://www.who.int/csr/don/23-march-2018-lassa-fever-nigeria/en/. (accessed 5 December 2018).

27. Price ME, Fisher-Hoch SP, Craven RB, McCormick JB. A prospective study of maternal and fetal outcome in acute Lassa fever infection during pregnancy. *British Medical Journal* 1988; **297**(6648): 584-7.

28. McCormick JB, Webb PA, Krebs JW. A prospective study of the epidemiology and ecology of Lassa fever. *Journal of Infectious Diseases* 1987; **155**(3): 437-44.

29. Lukashevich LS, Clegg JCS, Sidibe K. Lassa virus activity in Guinea: Distribution of human antiviral antibody defined using enzyme-linked immunosorbent assay with recombinant antigen. *Journal of Medical Virology* 1993; **40**(3): 210-7.

30. Tomori O, Fabiyi A, Sorungbe A, Smith A, McCormick JB. Viral hemorrhagic fever antibodies in Nigerian populations. *Am J Trop Med Hyg* 1988; **38**(2): 407-10.

31. Richmond JK, Baglole DJ. Lassa fever: Epidemiology, clinical features, and social consequences. *British Medical Journal* 2003; **327**(7426): 1271-5.

32. CDC. Centre for Disease Prevention and Control. Management of patients with suspected viral hemorrhagic fever. 1988. http://www.cdc.gov/MMWR/preview/mmwrhtml/00037085.htm. (accessed 10 Dec 2018).

33. Ibekwe TS, Okokhere PO, Asogun D, et al. Early-onset sensorineural hearing loss in Lassa fever. *Eur Arch Otorhinolaryngol* 2011; **268**(2): 197-201.

34. Mateer EJ, Huang C, Shehu NY, Paessler S. Lassa fever-induced sensorineural hearing loss: A neglected public health and social burden. *PLoS Negl Trop Dis* 2018; **12**(2): e0006187.

35. Warner BM, Safronetz D, Stein DR. Current research for a vaccine against Lassa hemorrhagic fever virus. *Drug Des Devel Ther* 2018; **12**: 2519-27.

36. Eberhardt KA, Mischlinger J, Jordan S, Groger M, Gunther S, Ramharter M. Ribavirin for the treatment of Lassa fever: A systematic review and meta-analysis. *Int J Infect Dis* 2019; **87**: 15-20.

37. WHO. Technical guidance on Lassa fever. 2019. https://www.who.int/emergencies/diseases/lassa-fever/technical-guidance/en/ (accessed 14th November 2019).

38. Bausch D. Surveillance Study of Acute Febrile Viral Diseases in Kenema Government Hospital. Definition for a Suspected Case of Lassa, Yellow Fever, Dengue, and West Nile Fever (personal communication). 2019.

39. The Brighton Collaboration SNHL Working Group. Definition of sensorineural hearing loss. 2019.

40. Bowen MD, Rollin PE, Ksiazek TG, et al. Genetic diversity among Lassa virus strains. *J Virol* 2000; **74**(15): 6992-7004.

41. Wilson EB. Probable inference, the law of succession, and statistical inference. *Journal of the American Statistical Association* 1997; **22**: 209-12