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Prospective cohort study to evaluate Lassa fever incidence, symptoms and coinfection with malaria in West Africa: the Enable Lassa Research Programme ('ENABLE 1.5') – study protocol

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ABSTRACT

Introduction Lassa fever (LF), a viral haemorrhagic disease, poses a significant public health challenge in West Africa. Lassa virus infection frequently causes mild malaria-like symptoms, potentially leading to misdiagnosis and an underestimated burden. Severe LF can lead to multi-organ failure, and survivors may experience sensorineural hearing loss (SNHL). Building on the contributions of the Enable Lassa Research Programme (ENABLE 1.0), which ran in West Africa from 2020 to 2024, ENABLE 1.5 aims to further address gaps in understanding LF disease burden to inform future late-stage vaccine trials. The study will assess the incidence of symptomatic reverse transcription (RT)-PCR-confirmed LF disease, including malaria coinfection.

Methods and analysis The ENABLE 1.5 prospective cohort study will be conducted across five study sites: one in Liberia, three in Nigeria and one in Sierra Leone. Stratified cluster sampling will identify eligible individuals at the household level from communities either involved in ENABLE 1.0 or identified through recent LF surveillance as hotspots. A total of 5000 participants will be recruited, 1000 per study site (minimum) and equally stratified in the following ages: 0–5, 6–10, 11–17, 18–50 and >50 years. All participants will be followed up for 12 months. Baseline data collection will gather key variables and blood specimens from all participants, with baseline SNHL prevalence assessed at three study sites. Active follow-up of all participants will involve symptom assessments every 2 weeks and blood draws every 3 months for serological testing (IgG). Suspected LF cases will undergo thorough evaluations, including malaria rapid diagnostic testing, clinical assessments and laboratory testing, including RT-PCR and malaria blood smear microscopy.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ The true burden and epidemiology of Lassa fever (LF), a viral haemorrhagic fever endemic to West Africa, are challenging to ascertain due to a high proportion of asymptomatic and mild cases, as well as the non-specificity of some symptoms that lead to underdiagnoses and misdiagnoses.

⇒ In a previous study (called Enable Lassa Research Programme (ENABLE 1.0)), we assessed the incidences of Lassa virus (LASV) infection and LF disease in at-risk populations (excluding children under 2 years), and rates of coinfection with malaria in Benin, Guinea, Liberia, Nigeria and Sierra Leone to inform LASV late-stage clinical trial designs.

WHAT THIS STUDY ADDS

⇒ In this study, ENABLE 1.5, we include all age groups, using a more sensitive case definition and recognising non-febrile symptoms. We aim to assess the incidence rate of symptomatic reverse transcription (RT)-PCR-confirmed LF disease in Nigeria, Sierra Leone and Liberia, focusing on the utility of a predetermined set of unfavourable outcomes to determine disease severity, as well as assessing the incidence rate of RT-PCR confirmed LF and malaria coinfection.

⇒ We pay special attention to sensorineural hearing loss, a sequela affecting around a third of LF survivors.

INTRODUCTION

Lassa fever (LF) is a zoonotic acute viral haemorrhagic disease caused by the Lassa

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HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Overall, ENABLE 1.5 aims to improve our understanding of the burden of LF in West Africa, empower communities through sustained engagement and education, help guide future late-stage clinical trial designs, and elucidate potential reasons for vaccine hesitancy.

virus (LASV), causing significant disease burden in West Africa where it has remained endemic since its identification in 1969.[1] LF is transmitted primarily through contact with urine or droppings of an infected multimammate rat (*Mastomys natalensis*), although human-to-human transmission can occur through direct contact with the blood, urine, saliva or other bodily fluids of an infected person. While LF was known to exhibit a pronounced seasonal trend, with peak incidence occurring during the dry season, more recent socio-ecological shifts in West Africa could be contributing to the changing epidemiology.[2–4] All year-round transmission has been particularly marked in Liberia,[5] Nigeria[6] and Sierra Leone.[7] Although approximately 80% of individuals infected with LASV remain asymptomatic or exhibit mild symptoms, the remainder of infections result in severe disease affecting multiple organs,[1 8–10] with an overall case fatality risk of around 1% but between 17% and 70% among hospitalised patients.[11 12] Among symptomatic cases, a third of survivors endure sensorineural hearing loss (SNHL) as a sequela, resulting in permanent hearing impairment for approximately 18% of symptomatic LF survivors.[13]

Efforts to combat LF encompass surveillance, early case detection and prompt treatment, which is challenging due to LF's non-specific symptoms.[10 14] Current primary treatment involves early supportive care and the administration of the antiviral drug ribavirin.[10] Over the past three decades, several vaccine candidates demonstrated good immunogenicity and efficacy in animal models of LF. One of these candidates has now progressed to a phase II human clinical trial.[15] To aid future late-stage clinical studies, a consensus position on the core components of phase III clinical trials was developed through a multistakeholder consultation. One of the components includes a core outcome set describing unfavourable outcomes in children and adults that are being assessed to inform a composite outcome measure consisting of mortality or worsening of the patient's condition from baseline, as assessed at day 14 post-enrolment in the study.[16]

In 2019, the Coalition for Epidemic Preparedness Innovations (CEPI) launched a prospective multi-country cohort study, known as the Enable Lassa Research Programme (ENABLE), now referred to as ENABLE 1.0,[17] to address knowledge gaps identified in the WHO LF Research and Development Roadmap[18] and inform the design of future late-stage vaccine trials and vaccine delivery strategies.[17] The primary objectives included estimating the LF disease incidence, and seroprevalence and seroincidence rate of LASV infection. From 2021 to 2023, >23 000 participants were recruited across five West African countries (Benin, Guinea, Liberia, Nigeria and Sierra Leone), resulting in an overall LASV seroprevalence of approximately 30% (unpublished ENABLE 1.0 results). In addition, during the follow-up period, 39 confirmed symptomatic LF cases were observed, corresponding to an overall incidence rate of 0.96/1000 person-years (95% CI 0.68 to 1.32). The incidence ranged from 0.22 (95% CI 0.03 to 0.78) per 1000 person-years in Benin to 1.9 (95% CI 1.2 to 2.85) in Nigeria-Edo (unpublished ENABLE 1.0 results).

ENABLE 1.0 highlighted gaps in our understanding of (a) disease burden in children who are at least as susceptible to LF disease as adults, if not more so, due to their low pre-exposure to LASV; (b) LF and malaria coinfection rates and (c) prevalence and incidence of mild and moderate LF disease. Addressing these knowledge gaps is crucial to ensure that vaccine trial results are well-designed and will enhance our understanding of the natural history of LF. Finally, we identified instances of health system hesitancy across sites, which may undermine the potential uptake of future LF vaccines when they become available.

Objectives

Primary objectives

- To assess the incidence rate of symptomatic reverse transcription (RT)-PCR-confirmed LASV infection.
- To assess the incidence rate of symptomatic RT-PCR confirmed LASV infection with symptomatic malaria coinfection in Liberia, Nigeria and Sierra Leone.
- To explore the use of predetermined unfavourable LF outcomes to inform LF severity scores.

Secondary objectives

- Further assess the overall seroprevalence and seroincidence rate of LASV infection in Liberia, Nigeria and Sierra Leone, stratified by (a) site, (b) age and sex, (c) most recent previous LASV serostatus (IgG) and (d) age and most recent previous LASV serostatus.
- Assess the overall incidence rate of symptomatic RT-PCR-confirmed LASV infection in Liberia, Nigeria and Sierra Leone stratified by (a) site, (b) age and sex, (c) most recent previous LASV serostatus (IgG) and (d) age and most recent previous LASV serostatus.
- Assess the prevalence of baseline SNHL among participants in selected study site(s) in Liberia and Nigeria, stratified by (a) site, (b) age and sex, (c) baseline LASV serostatus.
- Assess the proportion of participants with incident SNHL among those with symptomatic RT-PCR-confirmed LASV infection.
- Assess the performance of clinical case definitions for suspected symptomatic LASV infection using non-febrile-driven screening.
- Assess the performance of clinical case definitions for suspected symptomatic LASV infection using non-febrile-driven screening.

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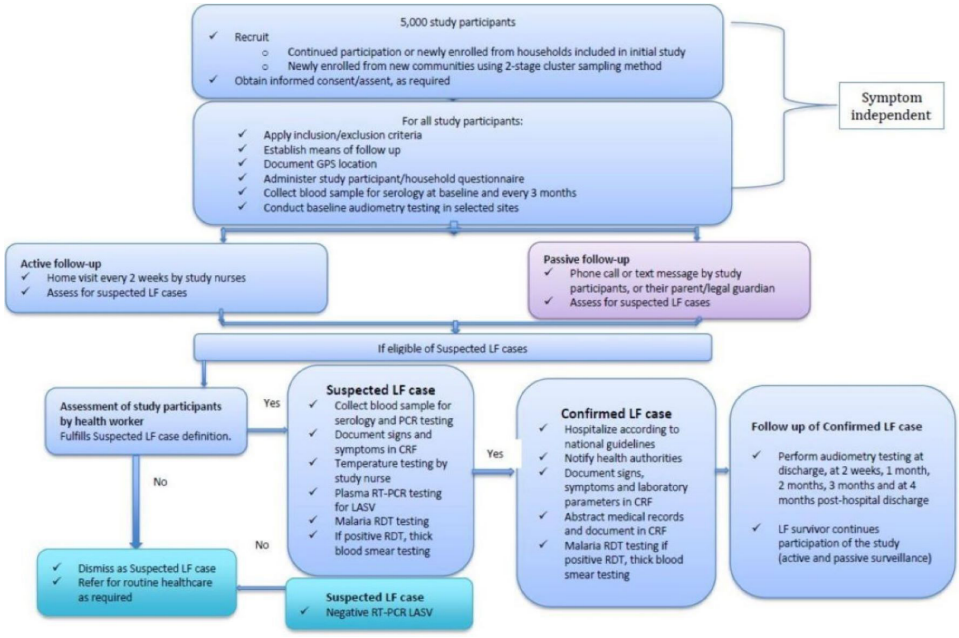

Figure 1 General flow diagram of Enable Lassa Research Programme (ENABLE 1.5). CRF, case report form; LASV, Lassa virus; LF, Lassa fever; RDT, rapid diagnostic test; RT-PCR, reverse transcriptase PCR.

- Assess attitudes and community acceptance of vaccination with a licensed LF vaccine and willingness to participate in LF vaccine and therapeutic clinical trials in each LF-endemic participating country.

Exploratory objective

To explore the feasibility of simplifying the LF severity score into a classification of mild, moderate or severe.

METHODOLOGY AND ANALYSIS

Study design, setting and study period

ENABLE 1.5 is a community-based prospective cohort study. The design of the study was informed by its predecessor ENABLE 1.0. Five sites across Liberia (Phebe Hospital, Phebe, Bong County), Nigeria (Irrua Specialist Teaching Hospital—ISTH, Irrua, Edo State; Federal Medical Centre—FMCO, Owo, Ondo State; and Alex Ekwueme Federal University Teaching Hospital Abakaliki—AEFUTHA, Abakaliki, Ebonyi State) and Sierra Leone (Kenema Government Hospital—KGH, Kenema)—which were also part of ENABLE 1.0—will participate in ENABLE 1.5[17] from Q4 2024 to Q2 2026. The target of 5000 participants (minimum N=1000 per site) will be followed for 12 months (figure 1). At each study site, a specialised team will be entrusted with the implementation, comprising field and laboratory researchers, data managers, administrative support and clinical personnel. All study sites will be led by local principal investigators.

Patient and public involvement

Prestudy activities

Leveraging insights gained from ENABLE 1.0, we will conduct a preparatory phase prior to participant and household recruitment to ensure early engagement and sensitisation activities focused on LF transmission, clinical manifestations and prevention. To enhance public and community stakeholder involvement, ENABLE 1.5 will be introduced while providing feedback to the communities on ENABLE 1.0 and its lessons learnt. Whenever possible, local LF survivors will be invited to participate in community activities and share their experiences with the disease. Participation will be entirely voluntary, and informed consent (verbal or written, as appropriate) will be obtained before participation.

Communities will be advised on rodent control measures, such as safe food storage and effective vegetation clearance. This will be accomplished through community-driven behavioural change campaigns aimed at raising awareness of LASV transmission. Concurrently, we will reinforce our support for existing public health measures, extending efforts toward environmental sanitation and promoting the use of insecticide-treated bed nets to prevent other vector-borne diseases.

To optimise sensitisation and reduce participant attrition due to more frequent blood draws (see the Data collection section), we will recruit community health workers residing within the communities as part of the field team to support study recruitment and follow-up, capitalising on their established rapport and trust. Rather than establishing new structures, we will use pre-existing community entities such as Lassa-centric ENABLE 1.0 community advisory boards (CABs). These CABs, composed of community stakeholders, will play a crucial role in garnering support and facilitating effective communication throughout ENABLE 1.5.

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Sampling strategy and recruitment

Household and participant selection

Akin to the sampling strategy employed in ENABLE 1.0,[17] stratified cluster sampling will be employed, where the stratum is the community level, and the cluster is at the household level. Communities will be either selected from those identified within ENABLE 1.0 or chosen based on recent available LF surveillance indicating hotspots. Once communities have been selected, participant sampling will be stratified by the community in proportion to population size. In communities already part of ENABLE 1.0, where a representative sample has already been selected, we will randomly select a subset of households among those that completed the study. To ensure a representative selection in new communities, households will be chosen through systematic random sampling from the exhaustive enumeration of all households in the community, obtained from recent surveys or mini-census. If these data are not available, random spatial sampling using satellite images will be used. To select participants, we will use an age-stratified approach, assigning 200 participants per site to each age group: 0–5, 6–10, 11–17, 18–50 and >50 years in each site. In each randomly selected household, the study team will recruit up to 15 participants across the five age groups, with a maximum of three participants from the 18–50 years age group, the largest demographic in the general population. This limit is intended to ensure a balanced age distribution and prevent overrepresentation of the 18–50 years age group. It also enhances the acceptability of age-stratified sampling by enabling the recruitment of both parents and children for a larger number of households. In households with more than three members in the 18–50 years age group and/or more than 15 members overall, participants will be randomly selected. However, if random selection is not acceptable to the head of household, the study team, in agreement with the head of household, will prioritise selecting children and related adults (eg, parents) to maximise the number of children enrolled while still limiting the 18–50 years age group to a maximum of three participants.

Enrolment procedures

ENABLE 1.5 investigators will identify and contact the heads of selected households. Following good clinical practice (GCP) guidelines, oral consent from the household head will be obtained before discussing study procedures. The oral consent will be documented in the study procedure checklist. If the household head is not available during the initial contact, efforts will be made to arrange a subsequent visit at a mutually convenient time. If the household head cannot be reached, after three unsuccessful attempts, the household will be ineligible for inclusion in the study. Then, the investigator will proceed to summarise the study and its aims to the household head, and each household will be provided with information on how to reduce their risk of LF, as well as on rodent control.

Table 1 List of inclusion and exclusion criteria in ENABLE 1.5

Inclusion criteria	Exclusion criteria
Females and males of all ages	ENABLE 1.0 participants with RT-PCR-confirmed symptomatic LF disease who survived (referred to as ENABLE 1.0 LF survivors) and their households
Resident (including parents/guardian of child) of the study area for 6 months preceding recruitment and expected to stay with no plans to move out or relocate until the end of the study period	Persons who may not be able to consent freely, such as persons in military service
Participant or participant's parent/guardian is willing and able to provide informed consent (and assent, as required), according to country-specific procedures	Persons who have a self-diagnosed fever at the point of recruitment
Participant or participant's parent/guardian must be able to read or comprehend either the official language of the country or local languages	Any ENABLE 1.5 study staff
Household head has granted permission for the research team to approach household member(s), and the research team may contact the participant (or their household head) during the follow-up period	Any temporary migrant worker or student whose parents are non-resident in the study communities or student whose school is not located within the study communities
Participant is willing to comply with the study procedures, including blood specimen collection	

ENABLE, Enable Lassa Research Programme; LF, Lassa fever; RT-PCR, reverse transcriptase PCR.

The household head will be asked to identify household members who may participate in the study. If household members, or parents or legal guardians if appropriate, are eligible for inclusion in the study, the investigator will inform them verbally and in writing about the study background, related procedures, benefits and potential risks. All communication will be done in the appropriate local language. Those meeting the selection criteria (table 1) will undergo a detailed briefing, covering the study's background, procedures, benefits and risks. Participants will be assured of voluntary participation and the freedom to withdraw without repercussions. They will also be informed about the mandatory reporting of 'confirmed

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LF cases' to national authorities. Following this, informed consent (and assent, if required) will be sought from all household members or children's parent/legal guardian, documented either by signature or fingerprint. For illiterate participants, the approved translated information and consent/assent form will be read out in the presence of an independent literate witness. Consistent with GCP guidelines, all participants will be allowed time to reflect before consenting.

On formal enrolment, each participant will be assigned a unique study identifier (ID), and ID card featuring barcodes to facilitate identification during the study. In addition, participants will be strongly encouraged to promptly seek medical attention for symptoms associated with LF.

Finally, participants, or their parent/legal guardian if applicable, reserve the right to withdraw from the study at any point, and the reason for withdrawal will be documented in the adequate case report form (CRF), if provided or where applicable or where available. An investigator retains the right to withdraw a participant from the study if any medical condition, event or situation arises that deems continued participation detrimental to the participant's best interest. Withdrawal may also occur if the participant subsequently meets an exclusion criterion hindering further study involvement or if consent is withdrawn during the study.

Compensation for participants' time spent providing responses and blood samples will be determined individually by each country based on community norms. The specific details of compensation will be outlined in the country-specific protocols and will require approval from the National Ethics Committees.

Data collection

Baseline data collection

On enrolment, REDCap (Research Electronic Data Capture) V.5.20.9,[19] a web-based (V.14.0.16) and a mobile-based (V.5.27.0) software will capture key variables, including GPS coordinates. Standardised questionnaires including household composition, socioeconomic situation, LF history, as well as LF knowledge and LF risk factors, will be used to gather household information, which will be methodically collected during interviews.

Baseline blood collection

Trained phlebotomists will collect 5 mL of blood from each participant at baseline (2 mL for children <2 years of age) via venepuncture at their homes. Blood samples will then be transported to the study site laboratories for further processing. Serum will be divided into two cryovials: one will be used to ascertain LASV serostatus (IgG) at baseline using the Zalgen ReLASV Pan-Lassa NP IgG ELISA kits or an alternative appropriate immunoassay, while the second sample will be stored at −80°C for potential future analyses.

Baseline audiometry testing

A baseline audiometry test will be conducted for all participants at two sites in Nigeria (ISTH Irrua and FMC Owo) and at the Liberian site (Phebe Hospital), which have the necessary staffing and infrastructure to support large-scale audiometry testing.

Given the varying cadre available to conduct hearing assessments at each site, staff from all five sites will attend centralised training, coordinated by a licensed audiologist. This training will cover the study hearing assessment procedures, use of the specific equipment items and data recording processes. The chosen equipment administers automated audiology assessments. The use of automated testing should decrease the chances of interobserver errors. In addition, only raw test values will be recorded in the database without any interpretation at the testing site, further decreasing the risk of interobserver errors. The audiologist will provide continuous quality control (QC) checks.

Study participants will be referred to dedicated health facilities for an audiometry assessment, which will evaluate the presence of SNHL. This is defined as a hearing loss of at least 30 dB in three sequential frequencies on the standard pure tone audiogram, with potential aetiologies of conductive hearing loss ruled out by physical examination and tympanometry.[20]

Follow-up of study participants

Active case detection

A study nurse will conduct household visits every two calendar weeks, within a window of ±2 days. If there is no response from the household contacts during the first attempt, at least two follow-up attempts will be made within 48 hours. After three unsuccessful attempts, the data for that visit are marked as missing, and subsequent follow-ups resume as planned.

During the biweekly home visits, a temperature check using an infrared thermometer will be conducted by the study nurse; fever will be considered if the temperature is ≥38°C. All participants will be asked whether they had signs and symptoms within the last 14 days. Blood samples will be collected if the study participant meets the suspected LF case definition (table 2).

Passive case detection

Study participants or parents/legal guardians will be encouraged to immediately report any febrile episode that has persisted for any 2 of 3 consecutive days within the last 14 days or other eligible symptoms to a study nurse either by text message or phone call and to present to a healthcare facility for further assessment. Blood samples will be collected if the study participant meets the suspected LF case definition.

Case ascertainment

A suspected LF case is defined as a self-reported febrile episode on any 2 of 3 consecutive days within the last 14 days, a temperature of ≥38°C when measured at the time

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Table 2 Suspected LF case definition

LF case definitions	Descriptions
Suspected LF case	► *A febrile episode* defined as self-reported on any 2 of 3 consecutive days within the last 14 days or a temperature of ≥38°C recorded using a thermometer at the time of the visit OR ► *At least one of the following symptoms:* – Oedema (face, neck, lower extremities) – Dizziness – Seizure – Abnormal bleeding (mouth, nose, rectum and/or vagina)* – Conjunctival/sub-conjunctival haemorrhage – Self-reported hypotension and blood pressure at less than 90/60 mm Hg (checked in the health facility) – Ringing in ears (tinnitus) or acute deafness – Jaundice – Spontaneous abortion† – Stillbirth† – General malaise – Breast engorgement† – Excessive irritability – Abnormal urine (haematuria, oliguria, cola-coloured urine) OR ► *At least two of the following symptoms:* – Headache – Cough – Vomiting – Chest or retrosternal pain – Sore throat – Abdominal pain – Muscle pain or joint pain – General weakness – Fatigue – Diarrhoea
RT-PCR confirmed LF case without malaria infection or with asymptomatic malaria coinfection (‘confirmed case’)	► A ‘suspected LF case’ plus a positive LASV RT-PCR result AND (negative malaria RDT OR a positive malaria RDT followed by a malaria blood smear with <5000 parasites/µL, respectively)
RT-PCR confirmed LF case with symptomatic malaria coinfection (‘coinfection case’)	► A ‘suspected LF case’ plus a positive LASV RT-PCR result AND a positive RDT followed by a malaria blood smear with parasitaemia ≥5000 parasites/µL[21 22]

*Bleeding includes macroscopic haematuria, melena, gingival bleeding, venous puncture point bleeding, haematochezia, epistaxis, menorrhagia.
†For pregnant women only.
LASV, Lassa virus; LF, Lassa fever; RDT, rapid diagnostic test; RT-PCR, reverse transcriptase PCR.

of assessment by a nurse, or the presence of one or more non-febrile symptoms (table 2). If a participant informs the study nurse of signs and symptoms observed within the last 14 days consistent with the suspected LF case definition, these clinical manifestations will be documented on a CRF. A trained phlebotomist will draw a whole blood sample from the participant. Blood samples will then be sent to the laboratory for analysis. All study sites have access to laboratory facilities with RT-PCR testing capacity. Trained laboratory personnel will assess the blood samples for LASV using the RealStar Lassa Virus RT-PCR kit 2.0 (Altona Diagnostics, Germany) and malaria using a malaria rapid diagnostic test (RDT). All RT-PCR-confirmed LF cases who have a positive malaria RDT result will also have a malaria blood smear microscopic test to quantify parasitaemia. Participants will be notified of their test status when they are positive for either LF or malaria, or both. A ‘suspected LF case’ will be classified as an RT-PCR-confirmed LF case if it has a positive LASV RT-PCR result and a negative malaria RDT, or if it has a positive LASV RT-PCR result, a positive malaria RDT and malaria parasitaemia of <5000 parasites/µL. Conversely, a ‘suspected LF case’ with a positive LASV RT-PCR result, a positive malaria RDT and malaria parasitaemia of ≥5000 parasites/µL will be defined as an LF-malaria co-infected case.

Confirmed LF cases and follow-up

On confirmation of an RT-PCR-confirmed LF case, infection control measures will be implemented as outlined in national and/or regional guidelines. The participant, along with their guardian if applicable, will be promptly informed, and health authorities will also be notified. For confirmed LF cases or confirmed coinfection cases, the study nurse will adhere to relevant national LF and malaria management guidelines, facilitating referral to an appropriate treatment facility. Furthermore, participants who tested RDT positive for malaria but negative for LASV via LASV RT-PCR will be appropriately referred for routine healthcare, and the participant or parent/guardian will be informed of the results of both laboratory tests if any test is positive. Confirmed LF cases will be followed up through dedicated research staff (nurses and physicians in the hospital) and data on clinical signs, symptoms, and chemistry and haematology, including IgG serostatus, will be obtained using standardised case report forms. Before hospital discharge, an audiometry test will be performed to assess for the presence of SNHL. Local authorities will assume responsibility for providing medical care in accordance with national guidelines for LF patients and costs will be covered by CEPI.

Follow-up of confirmed cases

LF confirmed cases will be monitored for sequelae through continuous active and passive follow-up.

LF survivors will undergo follow-up assessments at the hospital at discharge, and at 2 weeks, 1 month, 2 months, 3 months and 4 months post-discharge. The following information will be assessed and documented in the CRF:

► A trained health worker will conduct audiometry assessments using automated test equipment to evaluate any persistent or delayed onset of SNHL. For this study, ‘delayed SNHL’ is defined as audiometry

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indicating SNHL during follow-up but not at the time of hospitalisation, while 'persistent SNHL' is defined as audiometry indicating SNHL both at follow-up and at hospitalisation.

- The study nurse will ask the study participant an open question regarding their health status and whether they are suffering from any other sequelae.
- If any sequelae persist, the health worker will refer the study participant to appropriate routine healthcare services.

LF survivors will remain in the study, undergoing active and passive follow-up as they recover from LF.

Fatal confirmed LF cases and follow-up of community deaths

Information on fatal outcomes for confirmed LF cases will be collected using a dedicated CRF. Any confirmed LF case resulting in death within 30 days of diagnosis, or where the death is attributed to LF disease as determined by the research physician at any time following LF confirmation, will be classified as a confirmed LF case with a fatal outcome. If the study team is notified of a study participant's death in the community, they will attempt to determine the cause of death via a verbal autopsy, which will be conducted within 4 weeks following the death (see online supplemental file 1 for a summary of study procedures and the schedule of activities).

LASV infection

At baseline and every 3 months throughout the duration of ENABLE 1.5, irrespective of symptoms, a trained phlebotomist will draw a blood specimen from study participants for testing with the Zalgen ReLASV Pan-Lassa NP IgG ELISA kits or another suitable immunoassay to determine serostatus. If a sample cannot be taken during the visit, is invalid on laboratory arrival, is lost or yields an inconclusive result, a resample will be requested. Serological outcomes will be recorded and interpreted as detailed in table 3.

Table 3 Interpretation of serology at follow-up assessments

Serology at visit T	Serology at visit T+3 months	Interpretation
IgG–	IgG–	No LASV infection
IgG–	IgG+	LASV infection, seroconversion
IgG+	IgG+, fourfold increase	LASV infection, boosted infection
IgG+	IgG+, no fourfold increase	No LASV infection
IgG+	IgG–	LASV reversion
Indeterminate	IgG– or IgG+	Inconclusive
IgG– or IgG+	Indeterminate	Inconclusive

LASV, Lassa virus.

Community attitudes and acceptance nested study

A qualitative nested study methodology (currently under development) will be used to collect data on attitudes and community acceptance of vaccination with a licensed LF vaccine, as well as participation in LF vaccine and therapeutic clinical trials in each LF-endemic participating country.

Capacity strengthening

ENABLE 1.5 will address key targeted capacity strengthening needs, specifically in staff training and community engagement. Additional training in RT-PCR, malaria microscopy and ELISA techniques will be provided as required. To ensure safety, study field staff and laboratory workers who may have encountered suspected LASV cases or their blood specimens will receive comprehensive training on the proper use of personal protective equipment.

Data management

As specified in the data management plan, investigators will ensure the maintenance of records, including participant identities, original signed consent forms, questionnaires, source documents and correspondence, in compliance with local regulations and study contract specifications. Each site will receive an initiated study file, which will be regularly updated, accessible for review during monitoring, audits or inspections, and properly archived. Secure automated dashboards will be created to allow visualisation of key performance indicators for data quality, study follow-up and monitoring.

Statistical methods

Sample size

Recognising the importance of increased precision of point estimates with a larger sample size, we opted to recruit at least 1000 participants in each of the five ENABLE 1.5 sites. This decision reflects a careful balance between statistical precision and logistical feasibility. Instead of estimating the sample size for desired precision, we evaluate the expected precision of three estimators (LASV seroprevalence, LASV infection rate and LF disease rate) given a sample size of N=1000 (and N=5000 for the pooled analysis of the five sites). Precision is calculated using exact binomial CIs (lower and upper bounds of the 95% CI) with the Clopper-Pearson method and can also be expressed as relative errors, that is, half the width of the 95% CI divided by the true value of the estimator (see online supplemental file 2).

Data analyses

Descriptive analyses will be performed to understand the qualitative and quantitative data collected and the characteristics of the study participants. In general, missing data will not be imputed. However, if more than 10% of the data are missing for one or more key variables, the impact of missing data on the analysis will be assessed and the pattern of missing data will be explored. In cases of evident bias, the multiple imputation method may be

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applied in secondary exploratory analyses using variables known to predict missing data. A sensitivity analysis will compare results with the complete case analysis when multiple imputation is employed. Statistical analyses for the study's specific objectives and outcome measures (online supplemental file 3) are described in further detail in the Statistical Analysis Plan.

QC and assurance

QC and assurance will be rigorously prioritised throughout ENABLE 1.5 and will be systematically implemented across all stages of the clinical and laboratory data collection. Dedicated QC officers will be assigned to each study site, conducting undisclosed spot checks on 10% of visits during active follow-up to verify the accuracy and thoroughness of clinical status assessments. Additionally, QC officers will supervise trained study nurses who extract clinical data from medical records at the community health facilities to ensure precise documentation of clinical outcomes. The use of electronic data capture forms (e-CRFs) with integrated data validation rules will enable the clinical and QC teams to review data daily and remotely, ensuring both data completeness and consistency/plausibility.

To ensure that the quality of the clinical and laboratory data is upheld at all levels and times, quality management manuals and standard operating procedures will be developed and implemented in all study sites.

Ethics and dissemination

Limitations

This study may encounter certain potential limitations, including underestimation of LF incidence due to reliance on incomplete surveillance data to select study communities, under-reporting of symptoms owing to earlier self-treatment, participant attrition due to frequent blood draws and lack of individual serology results, and potential missing data from intensive follow-up procedures. To mitigate these risks, the study employs data triangulation for more accurate household selection, robust community engagement, transparent communication and culturally appropriate incentives to support retention. Additionally, comprehensive training in data collection and management, along with partnerships with experienced LF organisations in West Africa, will strengthen implementation, ensure quality assurance and minimise selection bias.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were involved in the design, or conduct, or reporting, or dissemination plans of this research. Refer to the Methods section for further details.

Patient consent for publication Not applicable.

Ethics approval 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 (2023) and its amendments, and any applicable national guidelines. Ethical approval has been obtained from all local ethics committees. For the three Nigerian sites (FMC Owo, AEFUTHA and ISTH), ethical approvals were obtained from the National Health Research Ethics Committee (NHREC) at the national level (Approval ID: NHREC/01/01/2007), as well as from the following institutional review boards: the Health Research Ethics Committee of the Federal Medical Centre, Owo (FMC Owo HREC) (FMCOWO/HREC/2024/061); the Health Research Ethics Committee of Alex Ekwueme Federal University Teaching Hospital, Abakaliki (AEFUTHA HREC) (NHREC/16/05/22/367); and the Health Research Ethics Committee of Irrua Specialist Teaching Hospital (ISTH HREC) (ISTH/HREC/20241006/601). For Kenema Government Hospital (KGH), Sierra Leone, approval was granted by the Sierra Leone Ethics and Scientific Review Committee (SLESRC) (SLESRC 016/08/2024). For Phebe Hospital, Liberia, approvals were obtained from both the University of North Carolina Institutional Review Board (UNC IRB) (24-1478) and the National Research Ethics Board (NREB) (FWA00021658). Informed consent was obtained from all study participants in accordance with applicable ethical guidelines. Ethical approval has been obtained from all local ethics committees. The Principal Investigator's Institutions are AEFUTHA, FMC Owo, ISTH, KGH and Phebe Hospital. We provide open-access data deposition for full transparency and accessibility to facilitate scientific inquiry. Results will be disseminated through scientific journals, conferences, health authorities and community outreach.

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Data availability statement No data are available. At the end of the study, the de-identified dataset underlying the findings will be available on request, in

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accordance with the legal framework set forth by Epicentre's data sharing policy, which ensures that data will be available upon request to interested researchers while addressing all security, legal, and ethical concerns. For data access, all readers may contact the Data Protection and Compliance Officer at dpco.archive@epicentre.msf.org.

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