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Review Article **Open Access**

A review of the recent advances on Lassa fever with special reference to molecular epidemiology and progress in vaccine development

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Abstract:

Lassa fever, a viral hemorrhagic fever caused by the Lassa virus (LASV), is endemic in West Africa and is associated with high morbidity and mortality. At least three of the four proposed seven lineages of LASV are found in Nigeria, where the multimammate rat, *Mastomys natalensis*, serves as the primary reservoir. Endemic countries report approximately 200,000 infections and 5,000 deaths annually, with Nigeria experiencing thousands of infections and hundreds of deaths including healthcare workers. The aim of this review is to provide scientific information for better understanding of the evolutionary biology, molecular epidemiology, pathogenesis, diagnosis, and prevention of Lassa fever in Nigeria and other endemic regions worldwide, which can lead to improved control efforts and reduce morbidity and mortality from recurrent epidemics. To achieve this aim, observational studies such as case series, cross-sectional and cohort studies published between December 2017 and September 2022 were searched for on various online databases including Google Scholar, Africa Journals Online (AJOL), Research Gates, PubMed, PMIC, NCDC, and WHO websites. Although the origin and evolutionary history, and the transmission dynamics of Lassa virus have been revealed through recent molecular epidemiological studies, the factors that drive the evolution of the virus remain unclear. Genetic changes in the viral genome may have enabled the virus to adapt to humans. Diagnosis of Lassa fever has also advanced from basic serological tests to more sophisticated methods such as quantitative real time polymerase chain reaction (qRT-PCR) and sequencing, which are particularly useful for identifying outbreak strains. Several vaccines, including recombinant vesicular stomatitis virus (rVSV), virus-like particle (VLP), and DNA-based vaccines, have shown promise in animal models and some have progressed to phase 2 clinical trials. Preventing and controlling Lassa fever is critical to safeguard the health and well-being of affected communities. Effective measures such as rodent control, improved sanitation, and early detection and isolation of infected individuals are essential for reducing transmission. Ongoing research into the genetic and ecological factors that drive the evolution of Lassa virus is necessary to reduce the impacts of Lassa fever.

Keywords: Lassa fever; recent advances; molecular epidemiology; evolutionary history; vaccine

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Une revue des avancées récentes sur la fièvre de la Lassa avec une référence particulière à l'épidémiologie moléculaire et aux progrès du développement des vaccins

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Résumé:

La fièvre de Lassa, une fièvre hémorragique virale causée par le virus de Lassa (LASV), est endémique en Afrique de l'Ouest et est associée à une morbidité et une mortalité élevées. Au moins trois des quatre lignées proposées de LASV se trouvent au Nigeria, où le rat multimammaire, *Mastomys natalensis*, sert de réservoir principal. Les pays endémiques signalent environ 200,000 infections et 5,000 décès par an, le Nigéria connaissant des milliers d'infections et des centaines de décès, y compris des travailleurs de la santé. L'objectif de cette revue est de fournir des informations scientifiques pour une meilleure compréhension de la biologie évolutive, de l'épidémiologie moléculaire, de la pathogenèse, du diagnostic et de la prévention de la fièvre de Lassa au Nigeria et dans d'autres régions endémiques du monde, ce qui peut conduire à des efforts de contrôle améliorés et réduire la morbidité et la mortalité des épidémies récurrentes. Pour atteindre cet objectif, des études observationnelles telles que des séries de cas, des études transversales et de cohorte publiées entre décembre 2017 et septembre 2022 ont été recherchées sur diverses bases de données en ligne, notamment Google Scholar, Africa Journals Online (AJOL), Research Gate, PubMed, PMIC, Sites Web du NCDC et de l'OMS. Bien que l'origine et l'histoire évolutive, ainsi que la dynamique de transmission du virus de Lassa aient été révélées par des études épidémiologiques moléculaires récentes, les facteurs qui déterminent l'évolution du virus restent flous. Des modifications génétiques du génome viral pourraient avoir permis au virus de s'adapter à l'homme. Le diagnostic de la fièvre de Lassa est également passé des tests sérologiques de base à des méthodes plus sophistiquées telles que la réaction quantitative en chaîne par polymérase en temps réel (qRT-PCR) et le séquençage, qui sont particulièrement utiles pour identifier les souches épidémiques. Plusieurs vaccins, y compris le virus recombinant de la stomatite vésiculeuse (rVSV), les particules pseudo-virales (VLP) et les vaccins à base d'ADN, se sont révélés prometteurs dans des modèles animaux et certains ont progressé vers des essais cliniques de phase 2. La prévention et le contrôle de la fièvre de Lassa sont essentiels pour préserver la santé et le bien-être des communautés touchées. Des mesures efficaces telles que le contrôle des rongeurs, l'amélioration de l'assainissement et la détection et l'isolement précoces des personnes infectées sont essentielles pour réduire la transmission. Des recherches continues sur les facteurs génétiques et écologiques qui déterminent l'évolution du virus de Lassa sont nécessaires pour réduire les impacts de la fièvre de Lassa.

Mots-clés: Lassa fever; avancées récentes; épidémiologie moléculaire; histoire évolutive; vaccin

Introduction:

Lassa virus (LASV), the causative agent of Lassa fever, belongs to the family Arenaviridae. Arenaviruses are rodent-borne viruses which are classified as segmented negative-sense RNA (nsRNA) viruses and are phylogenetically closely related to other segmented nsRNA viruses belonging to Bunyaviridae and Orthomyxoviridae (1). These three virus families are basically similar in terms of intracellular replication. This is based on serological cross-reactivity, phylogenetic relatedness, and geographical distribution (1,2) All arenaviruses are further sub-divided into the Old World and New World virus complexes. The New World arenavirus complex comprises viruses that circulate in North America i. e. Whitewater Arroyo (WWAV), Tamiami (TAMV), and Bear Canyon (BCNV) viruses, and South America i. e. Tacaribe (TACV), Junin (JUNV), Machupo (MACV), Guanarito (GTOV), and Sabia (SABV) viruses. The Old-World complex includes arenaviruses that circulate in Africa, Europe, and Asia i. e. lymphocytic choriomeningitis (LCM) and LASV viruses (3).

The Lassa virus uses various methods to attach to and infect particular cells, while avoiding the host immune system. The observed bleeding diathesis is due to the effects of proinflammatory cytokines and capillary endothelial injury. Severe manifestations of the disease are also caused by cardiovascular, renal, and central nervous system involvement (4). Mortality is highest in pregnant women and children (5). Sequelae include deafness, alopecia, and pericarditis.

Current diagnostic techniques for Lassa fever include serological tests such as ELISA which is highly sensitive and specific but cannot be used for detection of current acute infection. The reverse transcriptase polymerase chain reaction (RT-PCR) is currently the acceptable 'gold standard' test, which can detect low levels of viraemia, and is useful for follow-up of patients and early detection of outbreaks (6). Virus isolation is also a recog nized 'gold standard' test but is very expensive to set-up especially in endemic regions where the burden is highest. Currently, there are about 34 different vaccine candidates in various stages of development with the vector-based recombinant vesicular stomatitis virus (rVSV), virus-like particle (VLP) based, and DNA-based vaccines showing the greatest promise (7-11).

The objective of this review is to examine the latest advancements in the biology, epidemiology, clinical characteristics, diagnosis, management, and vaccines for Lassa fever. This will assist in enhancing our efforts to manage the disease, resulting in decreased morbidity and mortality rates from repeated outbreaks of Lassa fever.

Methodology and Results:

We performed online literature searches for studies referring to the epidemiology, biology, pathogenesis, diagnosis, and prevention of Lassa fever in Nigeria primarily, then in other endemic regions, and lastly globally.

The search engines used include Google Scholar, Africa Journals Online (AJOL), Research Gates, PubMed/PMIC, Nigeria Center for Disease Control (NCDC), and WHO websites. We searched for observational studies such as case series, cross-sectional and cohort studies published between December 2017 and September 2022, using keywords and Boolean search terms; 'Lassa virus', 'Lassa fever', 'recent advances on Lassa fever', 'Lassa fever vaccines', and 'diagnosis of Lassa fever'

Information obtained was summarized for relevant key points and findings were included in this review. The total number of full-text and peer-review publications on Lassa fever cases were 1385 articles. After deduplication, review of articles for eligibility, and secondary search, 128 articles were considered eligible for full text revision (Fig 1).

Discussion:

Biology of Lassa virus

Lassa virus is an enveloped, single-stranded, negative-sense, bi-partite ribonucleic acid (RNA) virus which belongs to the family Arenaviridae (12,13). The virus is spherical with an average diameter of 110-130 nanometers, and in cross-section, they show 'grainy particles' (ribosomes acquired from host cells) and thus the Latin name "arena", which means "sandy". The RNA genome of the virus has four encoded proteins; nucleoprotein (NP) and glycoprotein precursor (GP) on the Small (S) segment, and RNA-dependent RNA-polymerase (L) and matrix RING Zinc-finger protein (Z) on the Large (L) segment (13,14). Through nucleic acid sequencing of Lassa virus, identification and molecular characterization of seven LASV strains were possible. These include strain Josiah, originating from Sierra Leone (15), strain Nigeria (16) and strain LP (6,17) both from Nigeria, and strain AV imported into Germany by a traveller who had visited Ghana, Côte D'Ivoire, and Burkina Faso (18).

Three strains are found in Nigeria, with the fourth found in Guinea, Liberia, and Sierra Leone. The prototype LP strain isolated by Buckley and Casals in 1969 from Eastern Nigeria occupied the most basal lineage I. Strains isolated from Southern Central and Northern Central Nigeria were placed in lineage II and III, respectively, and the largest group of strains from Guinea, Liberia, and Sierra Leone occupied lineage IV (19). A fifth lineage, which falls between III and IV, has been proposed for the AV strain isolated from a patient that was infected (presumably) in Ghana or Ivory Coast (18,19). However, more recent lineage VI, originating from Togo (20). A new lineage has been discovered in the recent Nigerian Lassa fever outbreak and if confirmed will make a total of 7 lineages (21). The understanding of genetic variability of LASV is important for the designation of diagnostic molecular assays and more importantly for the development of universally acceptable vaccines for Lassa fever for use in different geographical settings.

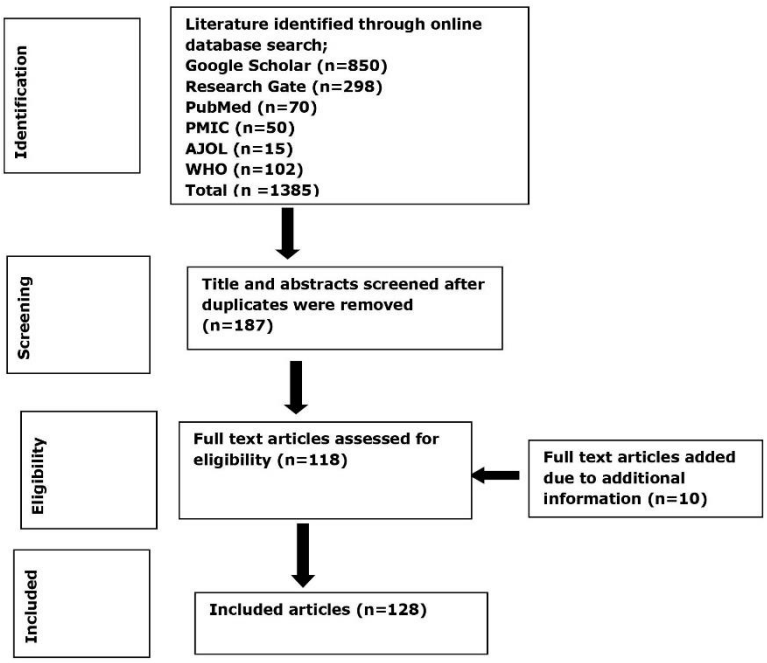

Fig 1: Process of selection of publications (PRISMA guide) used for the review

Fig 2: Pictures of the multimammate rat *Mastomys natalensis*

Mode of transmission of Lassa virus

The "multimammate rat" *Mastomys natalensis* has been considered to be the animal reservoir for LASV (Fig 2). This rodent is abundant in West Africa and breeds productively (22). The infection of these rats is in-utero which remain infected throughout their life. They do not become ill, but the virus is being shed in their urine and faeces. Other rodent reservoirs discovered include *Mastomys erythroleucus* and *Hylomyscus pamfi* (23).

Primarily, transmission of infections to humans occurs from direct or indirect contact with LASV-infected rodents (24). Those at the greatest risk of acquiring LASV infection are those living in rural areas where the *Mastomys* rodents are usually found, especially in communities with poor sanitation or crowded living conditions (25-27). The *Mastomys* rodents invade homes of humans during the dry season in search of food. Direct contact with urine, faeces, blood or meat from LASV-infected *Mastomys* rodents can lead to infection (27). The infection is usually spread through the respiratory or gastrointestinal systems after direct or indirect contact with animal excrements. Inhalation of microscopic infectious particles (aerosol) is thought to be the most common mode of acquiring infection (4).

The disease has been proven to spread from person to person, posing a threat to healthcare personnel (28). Although it has been observed that the virus is present in the semen, transmission by sexual routes remains debatable (29). Lassa fever has been reported from sexual transmission months after recovery from acute disease (30). Although no study has established the transmission by breast milk, the high level of viremia suggests its possibility (31).

Epidemiology of Lassa fever

Lassa fever is a zoonotic acute viral illness is endemic in parts of West Africa including Sierra Leone, Liberia, Guinea and Nigeria. Neighboring countries are also at risk because the animal vector lives throughout the region. Although Lassa fever is endemic in the above countries, isolated cases occur in Cote D'Ivoire, Burkina Faso, Mali and Benin Republic. Peak incidence occurs during dry season (November-April) (Fig 3).

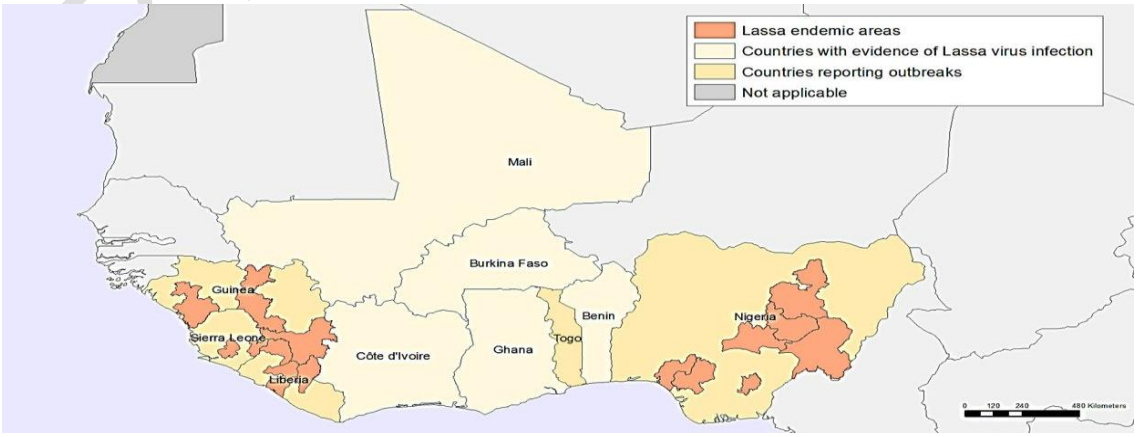

Fig 3: Geographical distribution of Lassa fever in West Africa
Map available at: https://www.afro.who.int/health-topics/lassa-fever (33)

Fig 4: Regions and areas with major outbreaks of Lassa fever (33)

The number of LASV infections per year in West Africa is estimated at 100,000 to 300,000, with approximately 5,000 deaths. Unfortunately, such estimates are crude, because surveillance for cases of the disease is not uniformly performed. In some areas of Sierra Leone and Liberia, it is known that 10%-16% of people admitted to hospitals every year have Lassa fever, which indicates the serious impact of the disease on the population of this region (32).

The prevalence of antibodies to the virus in the population is 8-52% in Sierra Leone (33), 4-55% in Guinea (34), and 21% in Nigeria (35). Seropositivity has also been found in the Central African Republic, Democratic Republic of the Congo, Mali, and Senegal (Fig 4) (32). Staff from the UK Department for International Development, the International Committee of the Red Cross, and the United Nations Mission in Sierra Leone have succumbed to the disease. Cases have occurred in travelers returning to Britain, the Netherlands, Germany, and export to countries outside Africa such as UK, US, Germany and Netherlands (18,36-38).

Epidemiology of Lassa fever in Nigeria

Lassa fever surveillance in Nigeria is conducted through the Integrated Disease Surveillance and Response (IDSR) platform. Information on Lassa fever flows from the health facilities, through the ward focal persons to the Local Government Area (LGA) Disease Surveillance and Notification Officers (DSNOs), to the State DSNOs, to the State Epidemiologist and then to the NCDC and Federal Ministry of Health (FMoH). All States in Nigeria including the Federal Capital Territory (FCT) report through the IDSR (39). Weekly reports on number of confirmed cases and deaths from Lassa fever are published in the Lassa fever weekly epidemiological reports by the NCDC (Table 1).

Historically, outbreaks of Lassa fever occur during the dry season (November to April), however, in recent years, cases have also occurred during the rainy season. Each year, Nigeria records dozens of confirmed cases and deaths including those of health care workers (HCWs). In Nigeria, majority of the cases (>70%) are from three States of Ondo, Edo, and Bauchi. Other States affected are Benue, Taraba, Kogi, Oyo, Ebonyi, Kaduna, Plateau, Cross River, Katsina, Nasarawa, Gombe, Enugu and Delta (34).

Table 1: Reported cases of Lassa fever in Nigeria from 2017-2022 (35)

Year	Suspected cases	Confirmed cases	Probable cases	Deaths (confirmed cases)	CFR (%)	HCW
2017	733	143	-	-	-	-
2018	3498	633	20	171	27.0	45
2019	5057	833	19	174	20.9	20
2020	6791	1189	14	244	20.5	47
2021	4654	510	6	102	20.0	10
2022	3542	681	28	127	18.6	51

CFR: Case fatality rate; HCW: Healthcare worker

In 2018, the NCDC reported the largest ever number of cases in Nigeria with over 600 confirmed cases and over 170 deaths (Table 1). Analysis of 77 LASV genomes from patients suggests that the surge was due to cross-species transmission from local rodents carrying various viral variants. The outbreak was not caused by a single virus variant or sustained by human-to-human transmission. Major rivers appeared to act as barriers to the migration of the rodent reservoir, leading to significant viral diversity based on geographic location (21).

A phylogenetic analysis of 14 genomes from the 2018 outbreak showed that the strains responsible were not novel and no clustering, indicating there were several sources from which zoonotic transmission occurred and were not due to a surge in human-to-human transmission (21). In 5 of the 14 sequenced LASV, intra-host single nucleotide variants (SNVs) were detected at a minor allele frequency >5%, suggesting that the virus underwent intra-host novo mutations (21). When LASV from the northern, south-western, and eastern Nigeria were compared using phylogenetic analysis, the patterns from the three regions remained very distantly related suggesting the hosts in those regions were geographically confined (21). Since then, cases had been on the increase with 2020 being the worst hit within the period under review, with 1189 confirmed cases and 244 deaths, including over 45 healthcare workers (35).

Morbidity and mortality of Lassa fever

Sensorineural hearing deficit is a feature of Lassa fever, which occur in 29% of hospitalized confirmed cases compared with 0% in febrile controls (8,36). In the general population, 81% of those who experienced sudden deafness had antibodies to LASV versus 19% of matched controls. There is no apparent relation between the severity of viral illness, initial hearing loss, or subsequent recovery (40).

Although the actual incidence of Lassa fever in Nigeria is unknown, the case fatality rate, which is the number of deaths divided by the number of confirmed cases multiplied by a factor of 100 (41), ranges from 3% to 42%, and has over the last two years remained between 20%-25% (33,35,39) with 15-20% case fatality rate among hospitalized patients. There are 300,000 to 500,000 cases per year, with approximately 5,000 deaths (42-45).

Pathogenesis of Lassa fever

The incubation period of Lassa fever ranges from 6 to 21 days in roughly 80% of persons who are infected. The condition is asymptomatic or moderately symptomatic, while 20% of infected persons will have severe multisystem disease (46,47). The antigen-presenting cells are the primary target of the virus upon entering into the host cells (48) but the virus infects most tissues in humans culminating in multi-systemic malfunctions and immunosuppression of host innate interferon (IFN) response through inhibition of interferon regulatory factor-3 (IRF-3) expression (49) through its nucleoprotein. The virus employs alpha-dystroglycan in establishing itself into targeted cells mostly macrophages, dendritic cells, and endothelial cells which are the points of commencement of its replication (50). In addition to preventing IFN production by infected cells, LASV inhibits the cells of the immune system, preventing the secretion of pro-inflammatory cytokines including tumor necrosis factor (TNF)-α, IL-6, and IL-1β, unlike the symptoms observed in other haemorrhagic fevers (50). It has been reported that LASV has exonuclease activity only targeted at double-stranded RNAs, which mostly inhibits the responses of IFN. This is achievable via assimilation of pathogen-associated molecular patterns (PAMPs), which help LASV to circumvent the immune response of the host (51).

The blood vessels are the tissues mostly afflicted and LASV replicates in the endothelial cells of blood vessels culminating in capillary injury. Bleeding might be observed in many organs such as hepatocyte, intestine, myocardium, lungs as well as the brain (52). Unregulated expression of cytokine referred to as "cytokine storm", similar to what is seen in SARS-CoV-2 and sepsis, could be another possible mechanism of Lassa fever pathogenesis.

There is paucity of autopsy findings due to religious, societal stigma and practices associated with the dead in many regions. However, few pathologic investigations of reported cases in human subjects after post-mortem examination shows injury in the hepatocyte, adrenal glands as well the spleen (46). Other studies on the analysed histologic results of the hepatocyte revealed marked necrotic eosinophils and parenchymal cells accompanied by penetration of eosinophils in the sinusoids, while analysis of spleen samples shows necrosis of eosinophils, depletion of lymphoid, deposition of fibrin, and shrinking of the white pulp as well as infiltration of mononuclear cells and lymphocytes (46).

Clinical features of Lassa fever

The clinical disease begins as a flu-like illness characterized by fever, general weakness, and malaise, which may be accompanied by cough, sore throat, and severe headache (4). Gastrointestinal manifestations

such as nausea, vomiting, and diarrhoea are also common (4). Although, haemorrhagic manifestations are not significant feature of Lassa fever, perturbation of vascular function is likely to be central to Lassa fever-associated pathobiology, since the signs of increased vascular permeability, such as facial oedema and pleural and pericardial effusions, indicate a poor prognosis for the disease outcome. Recovery from Lassa fever generally begins within 8 to 10 days of disease onset (4). In severe cases, the condition of the patient deteriorates rapidly between the 6th and 10th day of illness with severe pulmonary oedema, acute respiratory distress, clinical signs of encephalopathy, sometimes with coma and seizures, and terminal shock. Bleeding from mucosal surfaces is often observed; however, it is usually not of a magnitude to produce shock by itself (53). Sensorineural deafness is commonly observed in patients in the late stages of disease or in early convalescence in survivors (54).

In severe cases, cardiovascular symptoms appear including mucosal bleeding, melaena, and signs of vascular permeability such as oedema of the face and neck, pleural effusions, ascites, and shock accompanied by a sudden fall in blood pressure, hypovolaemia, vasoconstriction of peripheral blood vessels, and a decrease in urinary output. Some patients develop neurological symptoms including meningeal irritation, convulsions, disturbances of consciousness, and ataxia. Patients that are likely to die go into shock between the 7^{th} and 14^{th} day of illness. Death usually occurs during the second or third week of illness (4,55-57).

Lassa fever is severe in pregnancy, causing fetal death in about 80% of pregnant women. It is characterized by a high maternal mortality rate (up to 30%) and more than 90% fetal mortality rate during the third trimester (5,58). Lassa fever is a significant cause of paediatric hospitalizations in some areas of West Africa. The disease in children is often characterized by enlargement of the liver and the spleen, vomiting, convulsions, cough and malaise. It has been associated with the "swollen baby" syndrome in children under 2 years of age in Liberia, a presentation that is characterized by generalized edema, abdominal distension, and bleeding (55, 59). Deafness has been described in 20% of patients with Lassa fever. It develops during the second week of illness and involves the 8^{th} cranial nerve, and may be unilateral or bilateral, temporary or permanent (4,54). Minor complications of Lassa Fever occur in some patients who develop pericarditis, which resolves within 7 to 10 days, while others develop transient alopecia during convalescence (57,60).

Laboratory diagnosis of Lassa fever

The symptoms of Lassa fever can be nonspecific and are similar to other viral haemorrhagic fevers, such as Ebola, and can include fever, headache, muscle pain, weakness, diarrhoea, vomiting, and abdominal pain (29,60,61). In some cases, the disease can also lead to severe bleeding and death. Accurate diagnosis is thus key to management. There are numerous methods used for the diagnosis of Lassa fever as enumerated below.

Lassa fever serological test:

This involves testing a patient's blood for antibodies against LASV. Immunofluorescence was the more widely used test before enzyme-linked immunosorbent assay (ELISA) (34). ELISA is now more commonly used for this purpose, and antibodies detected can be used to confirm a past or current infection (62,63). The ELISA test uses specific viral antigens to detect the presence of antibodies in the blood of patients who have been infected with LASV (64). ELISA test is highly sensitive and specific, which makes it useful for detecting early infections. However, it is important to note that a positive ELISA test does not necessarily indicate an active infection (54,65). This is because antibodies can persist in the blood for months or even years after a person has recovered from an infection, therefore a positive result may indicate a past infection rather than a current one (65).

Another serological test that can be used to diagnose Lassa fever is the indirect fluorescent antibody (IFA) test (66). The IFA fell into disuse largely because of the low specificity in certain populations (67). This test is similar to the ELISA test in that it uses a viral antigen to detect the presence of antibodies in a patient's blood. However, instead of using enzymes to detect the binding of antibodies to the antigen, the IFA test uses a fluorescent dye. This makes it more sensitive (although less specific) than the ELISA test and allows for the detection of low levels of antibodies in the blood (65).

A combination of tests and clinical presentation is needed for the final diagnosis (68,69). In addition, the timing of testing is important. The ELISA test, for example, is useful for early detection of Lassa fever, but it may not be positive during the first week of symptoms (70). The antibody response of the patient may not have risen enough to be detectable, therefore, it should be combined with other methods such as PCR. Serological tests do not necessarily indicate active infection and should be accompanied by other diagnostic methods and clinical presentation for accurate diagnosis (69).

Antigen detection:

This involves testing for the presence of specific viral antigens in the blood or other body fluids of a patient, which can be used to diagnose Lassa fever (70,71). There are different types of antigen detection methods, but one common type is the rapid diagnostic test (RDT), which is simple and easy to perform at the point of care such as hospitals or clinics, rather than in specialized laboratories (68). One example of RDT for Lassa fever is the Lassa antigen rapid diagnostic test (LASV Ag-RDT), which is a lateral-flow based assay that detects LASV antigens in the blood of suspected patients (68). It is an easy to use, point-of-care diagnostic test that provides results in less than 30 minutes.

The LASV Ag-RDT test strip or casette contains a sample pad, a test line, and a control line. To use the test, a small sample of the patient's blood is collected and applied to the sample pad. The blood then flows along the strip or cassette, passing over the test line and the control line. The advantage of antigen detection is that it can be done quickly at point-of-care, and it can be used as a screening test in areas where Lassa fever is prevalent. However, antigen detection tests are less sensitive than other diagnostic methods such as PCR and ELISA, and they may not be able to detect the virus in patients who are in the early stages of infection or have low levels of the virus in their blood (69,72). It should be noted that positive antigen detection results should be confirmed by a more specific and sensitive methods such as RT-PCR or virus isolation. Also, it is important to consider the clinical presentation along with the laboratory results for accurate diagnosis. A combination of tests and clinical presentation is needed for the final diagnosis.

Reverse transcriptase polymerase chain reaction (RT-PCR) assay:

The reverse transcriptase polymerase chain reaction (RT-PCR) is a technique that can be used to diagnose Lassa fever by detecting the genetic materials of LASV in a patient's blood, urine, or other body fluids. The starting material for diagnosing Lassa fever by RT-PCR is the patient's blood, urine, or other body fluid, and isolating the viral RNA using automated or manual genome extraction techniques. Following extraction, the viral RNA is converted into cDNA using reverse transcriptase, following which the cDNA is amplified by PCR (73). Here, specific primers are designed to target regions of the LASV cDNA. When the primers bind to the targeted regions of the cDNA, the enzyme polymerase replicates the cDNA, creating many copies of the targeted LASV regions. The primers used in LASV PCR are designed to specifically bind to specific conserved regions of the LASV genome (74). The forward primer is designed usually to bind to the region of the viral RNA that codes for the virus nucleoprotein (NP) or polymerase (L) genes while the reverse primer is designed usually to bind the region that codes for the viral glycoprotein (GP) gene (75).

The specificity of the primers ensures that the PCR reaction only amplifies the viral genes of interest and not non-viral or human genes. Different primers targeting different viral genes can be used for specific or broad detection of LASV (6,75). Primers targeting the L-gene would only be specific for LASV, while primers targeting the GP gene could be used to detect other arenaviruses such as Lujo and lymphocytic choriomeningitis virus (76). While primer design is a critical step in the PCR process, and it is crucial to use primers that are specific for LASV to avoid cross-reactivity with other arenaviruses. The design of primers is also influenced by factors such as the genetic variability of the virus and the sensitivity and specificity of the test (77).

The amplified products are analysed by gel electrophoresis or other methods to detect the presence of the LASV. The RT-PCR test is highly specific and sensitive, and it can detect very low levels of the virus in a patient's blood or other body fluids. It is useful not just for diagnosis, but also for monitoring of the disease progression, follow-up of patients and early detection of outbreaks (69,73). RT-PCR is considered the 'gold standard' diagnostic method for Lassa fever, and a positive result is considered confirmatory of an infection (77). However, there are limitations of RT-PCR, such as the need for specialized laboratory settings, reagents and equipment and also the need for specific safety measures.

Several studies have reported successful use of loop-mediated isothermal amplification (LAMP) assay for diagnosis of Lassa fever from various sample types, including blood, urine, and saliva. This technique has been shown to have comparable or higher sensitivity than conventional PCR methods. However, LAMP assay also has some limitations, including the potential for nonspecific amplification and the need for careful primer design to avoid cross-reactivity with closely related viral species. Additionally, LAMP products can be difficult to quantify accurately, which may limit its use in monitoring treatment response (78-80).

Strand displacement assay (SDA) is a sensitive and specific nucleic acid amplification technique that has been used for the diagnosis of Lassa fever (80,81). The assay relies on the use of two target-specific oligonucleotide probes, a capture probe, and a

signal probe, both labeled with a fluorophore and a quencher (82). The SDA has several advantages over other nucleic acid amplification techniques. It is highly specific, as the hairpin structure ensures that the signal probe only binds to the target sequence (82). Additionally, the isothermal nature of the amplification process eliminates the need for thermocycling equipment, making it easier to use in resource-limited settings. Despite its advantages, the SDA has some limitations, including a lower sensitivity than quantitative RT-PCR and a higher susceptibility to the presence of inhibitors in clinical specimens. However, SDA is a promising diagnostic tool for Lassa fever, and further optimization may improve its diagnostic accuracy and utility in clinical practice.

Quantitative or real-time PCR (qRT-PCR) for the detection and quantification of LASV RNA in clinical samples provides advantages over conventional RT-PCR for the diagnosis of Lassa fever. The qRT-PCR assay provides a quantitative measurement of the amount of viral RNA in the sample, allowing for the determination of viral load and disease severity. This thus allows for earlier diagnosis and can detect disease progression and response to therapy. In general, because of the combined sensitivity and specificity of primers and probes, qRT-PCR is more sensitive and specific than conventional RT-PCR, and can detect low levels of viral RNA in patient samples (77). Unfortunately, the assay requires specialized equipment and trained personnel, which may not be available in all settings. Additionally, qRT-PCR is more expensive than conventional RT-PCR, which may limit its use in resource-limited settings. The qRT-PCR requires careful design and optimization of primers and probes, which may be time-consuming and technically challenging (77).

Sequencing for LASV characterization:

Sequencing of LASV is relatively new in the surveillance terrain for Lassa fever. The unbiased sequencing approach was one of the earlier methods used to sequence LASV and it still continues to be used (83). The unbiased sequencing approach was particularly useful when little or no information was available about the genome sequence of LASV because here, there is no bias or preference for specific regions or types of nucleic acids to be sequenced (83). It is an approach that aims to sequence all available DNA or RNA molecules present in a sample, rather than selectively amplifying or sequencing certain regions of the genome. This type of sequencing is particularly useful for identifying unknown or unexpected sequences, such as novel pathogens, and for studying genetic diversity within a population. This method was used to generate a "molecular clock" which showed LASV originated in Nigeria 1000 years ago and subsequently spread to other parts of West Africa (83).

Unbiased sequencing methods can be done with whole genome sequencing, metagenomic sequencing and RNA sequencing. Unbiased RNA sequencing (total RNAseq) has been used to characterize the evolution of LASV (83). Unfortunately, RNAseq is prone to contamination from RNA of human source or other viral sources (84). In 2019, the MinION technology (Oxford Nanopore) was used to characterize 19 LASV from patients in the 2019 outbreak with results showing the same lineages as that of the 2018 outbreak (85). Illumina technology is the leading second-generation sequencing technology used for LASV characterization. This method employs a technique called "bridge amplification", which involves amplifying DNA molecules that have adapters ligated to both ends on a solid support and generates high-quality sequence data because of the synthesis of amplified clusters (86). The Illumina unfortunately has <80% of their reference sequences captured at this 100% identity (87).

Virus isolation:

Given the challenges with the other diagnostic methods of Lassa fever, viral isolation in cell culture remains the "gold standard" for the diagnosis of Lassa fever (77). This method involves growing the virus in cell culture. The starting sample for viral isolation is patient specimens such as urine or semen, and inoculating it into a cell culture. The most commonly used cell lines for virus isolation are Vero E6 and L929 cells (69,88). These cells are grown in a laboratory and are used to create a monolayer, a single layer of cells, in a tissue culture flask or well. The patient's sample is then added to the monolayer and incubated at 37°C (67). If the LASV is present in the patient's sample, it will infect the cells in the monolayer and begin replicating. After a few days, the virus-infected cells will show typical cytopathic effects such as syncytial (cell to cell fusion) and/or cell death, that are unique to LASV, which can be identified by trained technicians (89).

While virus isolation is considered the "gold standard" for confirming Lassa fever diagnosis, virus isolation is one of the most technically demanding and dangerous methods (76,89). LASV is considered a biosecurity level 4 agent, the highest level of biosecurity, requiring a high level of biosafety measures to handle. Therefore, virus isolation should be carried out only in specialized laboratories that have the necessary facilities and training to safely handle the virus. Virus isolation is not usually carried out as a first-line diagnostic test due to its technical complexity

and risk, but usually used as a confirmatory test for patients who have tested positive for Lassa fever using other methods such as PCR or ELISA.

Lassa fever vaccines

Currently, there is no licensed vaccine for Lassa fever, and treatment is primarily supportive care. However, there are at least 35 different vaccine candidates in various stages of development (11,90,91). Vaccines that have been shown to be safe and effective in preclinical studies such as the recombinant vesicular stomatitis virus (rVSV) vector-based, virus-like particle (VLP) based, and DNA based vaccines, have also been shown to induce protective immunity in animal models. However, animal models may not translate to humans. Recombinant Lassa vaccines in general aim to stimulate the host immune system to produce protective immunity against the virus. They are generally based on nucleoprotein (NP), glycoprotein (GP), Z protein, L polymerase, and Z matrix proteins of the virus (8,92).

Nucleoprotein-based recombinant Lassa virus vaccines utilize the NP protein of the virus as the main antigenic component (10,93). NP is an abundant protein in the virus and is known to elicit both humoral and cellular immune responses (94). Various recombinant NP-based vaccines have been developed and tested in preclinical studies (92, 95). These vaccines are typically delivered as DNA or protein subunit vaccines, with the NP protein expressed from a bacterial, yeast, or insect cell system. Preclinical studies have shown that NP-based vaccines are able to induce both cellular and humoral immune responses against LASV (95). The cellular immune response is mediated by $CD8^+$ T cells that recognize NP peptides presented on the surface of infected cells, leading to their elimination. The humoral response involves the production of neutralizing antibodies that recognize the NP protein and prevent its interaction with host cells, thus inhibiting viral replication. However, NP-based vaccines have been found to provide incomplete protection against LASV infection in animal models (95). This is because NP is not the sole antigenic component of LASV, and other viral proteins, such as GP, are also known to elicit strong immune responses. Therefore, current efforts are focused on the development of vaccines that include both NP and GP proteins to provide broad-spectrum protection against LASV

The GP is the major surface protein of LASV and mediates virus entry into host cells to initiate infection (95,96). Several recombinant Lassa fever vaccines have been developed based on the GP protein (92,95), as protein subunit vaccines, DNA vaccines, viral vector vaccines, and virus-like particle vaccines. One major approach is use of virus-like particles (VLPs) that present the GP protein on their surface. VLPs are self-assembling structures that mimic the virus but lack the genetic material necessary for replication. They can be produced by expressing the GP protein in a cell line and allowing it to self-assemble into VLPs. The resulting particles can then be purified and used as a vaccine. Another approach is to use DNA vaccines that express the GP protein (96). In this case, the gene encoding the GP protein is inserted into a plasmid vector and delivered to the cells. Once inside the cells, the plasmid is transcribed into RNA and translated into protein, which is then presented to the immune system (95). DNA vaccines have the advantage of being relatively easy to produce and store, but their efficacy in humans has not yet been established. A third approach is to use viral vectors to deliver the GP protein to cells. Viral vectors are modified viruses that can infect cells and express foreign genes, such as the gene encoding the GP protein. The most commonly used viral vectors for Lassa vaccines are adenoviruses and vesicular stomatitis viruses (VSVs) (92,96). Adenoviruses are non-replicating and have been used in several clinical trials for Lassa vaccines. VSVs are also non-replicating but have the advantage of inducing strong cellular immune responses.

The Z protein is another potential target used for LASV vaccine development (92,94). The Z protein is a multifunctional protein that plays a crucial role in the replication cycle of the virus through involvement in assembly and release of viral particles (11), and inhibiting the host immune response. It has been shown that the Z protein can induce a potent immune response and confer protection against LASV infection in animal models. Several studies have investigated the use of Z protein-based vaccines for LASV. One approach has been to use recombinant DNA technology to express the Z protein in a viral vector such as the vaccinia virus or the vesicular stomatitis virus (VSV). One promising specific vaccine candidate is the rVSV vector-based vaccine, which has been shown to be safe and effective in preclinical studies and is currently in phase 1 clinical trials (8,72,91).

The rVSV vector used in this vaccine contains the genetic material of LASV, which is able to induce a protective immune response in animal models and confer protection against LASV infection. Another approach has been to use virus-like particles (VLPs) expressing the Z protein. Studies have shown that VLPs expressing the Z protein can induce a potent immune response and protect against LASV infection in animal models. A third type of vaccine candidates are the DNA based vac-

cines, which are composed of plasmids containing the genetic material of the virus. These plasmids can be delivered *in vivo* to the patient through different methods, such as electroporation or intramuscular injection (72). When three doses were given intradermally, it was shown to be safe and effective in preclinical studies and currently in phase 1 clinical trials. DNA/plasmid-based vaccines have been shown to induce a lasting immunity and to cross-protect against other arenaviruses, such as Lujo and lymphocytic choriomeningitis virus (9). Table 2 shows the current vaccine candidates, category and phases of trial.

Table 2: Vaccine types, phase of trial, manufacturer, and category

Vaccine Type	Phase	Manufacturer	Category	Reference
EBS-LASV, a dual attenuated rVSV vectored	Phase 1	Emergent BioSolutions	Viral vector	97
Baculovirus-expressed Lassa virus nucleoprotein vaccine	Phase 2	NewLink Genetics	Protein subunit	98
Baculovirus-expressed Lassa virus nucleoprotein vaccine	Preclinical	CEPI (Coalition for Epidemic Preparedness Innovations)	Protein subunit	99,100
ChAdOx1-vectored Lassa virus vaccine	Phase 1	University of Oxford	Viral vector	101
ML29 L-AttV, rLCMV (IGR/S-S)	Preclinical	The Scripps Research Institute, USA	Live attenuated	102
Viral genome rearrangement for the development of live-attenuated arenavirus vaccines	Preclinical	University of Rochester; The Scripps Research Institute	Live attenuated	103
Single cycle infectious viruses as live attenuated arenavirus vaccines		University of Rochester; The Scripps Research Institute	Live attenuated	103
DNA vaccine expressing Lassa virus nucleoprotein and glycoprotein precursor	Preclinical	University of Texas Medical Branch	DNA vaccine	104
DNA vaccine expressing Lassa virus nucleoprotein and glycoprotein precursor	Preclinical	Thomas Jefferson University	DNA vaccine	105
VSVDG/LASVGPC (VSV vector)	Preclinical	International Aids Vaccines Initiative; Public Health Agency of Canada	Viral vector	106
Inactivated Lassa virus vaccine	Phase 2	Themis Bioscience	Inactivated virus	107
Inactivated Lassa virus vaccine	Phase 1	Sinovac Biotech	Inactivated virus	108,109
Influenza-vectored Lassa virus vaccine	Preclinical	University of Geneva	Viral vector	72
Lassa virus replicon particle (VRP) vaccine	Preclinical	University of Texas Medical Branch	RNA vaccine	110
Lassa virus-like particle (VLP) vaccine	Preclinical	GeoVax Labs	VLP vaccine	111
Lassa virus-like particle (VLP) vaccine	Preclinical	Themis Bioscience	VLP vaccine	112
Lassa virus-like particle (VLP) vaccine with codon optimized glycoprotein gene	Preclinical	Thomas Jefferson University	VLP vaccine	113
Alphavirus replicon encoding LASV genes	Preclinical	Medigen, Inc.; University of Louisville	VLP vaccine	114
Measles virus-vectored Lassa virus vaccine	Preclinical	Institute Pasteur & Themis Bioscience	Viral vector	115
Lassa GPCclamp	Preclinical	The University of Queensland; Australian Government – National Health and Medical Research Council (NHMRC)	DNA vaccine	116

Lassa Polyhedrin-encased glycoprotein vaccine	Preclinical	University of Cambridge; Imperial College London	VLP vaccine	117
MVA-vectored Lassa virus vaccine	Preclinical	Oxford University	Viral vector	108
RABV inactivated rabies virus with Lassa Virus coGPC (LASSARAB)	Preclinical	Thomas Jefferson University	Viral vector	8
MV-LASV	Phase 1	Themis Bioscience and CEPI	Viral vector	118
RepliVAX Lassa virus vaccine	Phase 1	SIGA Technologies	Viral vector	119
Self-assembling protein nanoparticles presenting Lassa virus GP epitopes	Preclinical	Medicago	Protein subunit	120
Sendai virus-vectored Lassa virus vaccine	Preclinical	Georgia State University	Viral vector	121
RABV-Lassa virus vaccine candidate GPC	Preclinical	National Institute of Allergy and Infectious Diseases (NIAID), National Institute of Health (NIH)	Viral vector	8
YF 17D GPC	Preclinical	Texas Biomedical Research Institute; University of Louisville; Leiden University Medical Center	Viral vector	122
ML29 virus – reassortant encodes major immunogenic proteins from LASV and RNA polymerase and Z protein from MOPV	Preclinical	Medigen, Inc.	Viral vector	123
MOPEVAC (Modified Mopeia virus expressing antigens of pathogenic arenaviruses)	Preclinical	Institut Pasteur	Viral vector	124
Live attenuated rLCMV/CD	Preclinical	The Scripps Research Institute	Viral vector	125
GPC441-449 subunit	Preclinical	Emergent BioSolutions, University of Vermont, California and The Scripps Research Institute	DNA vaccine	126
HLA-A02 and 10 HLA-A03-restricted epitopes	Preclinical	The University of Vermont College of Medicine; University of California; Pharmexa-Epimmune	DNA	126
LASV VLP	Preclinical	Tulane University Health Sciences Center; Autoimmune Technologies, LLC	Protein subunit	127

Despite the promise of these vaccine candidates, it should be noted that they are still in early stages of development and it is crucial to perform more studies to evaluate the safety and efficacy of these vaccines in humans. Additionally, some of these vaccines may not prove to be effective for certain population subgroups and may require more than one dose to achieve immunity. In addition, the protective efficacy of Lassa fever vaccines may vary depending on the type of vaccine, the population being vaccinated, and the individual's immune response to the vaccine. Factors such as the genetic variability of the virus, age, and overall health of the person being vaccinated can also affect the efficacy of the vaccine (77,128). It is also worth noting that a single vaccine may not provide enough protection, multiple vaccinations may be needed to reach the desired level of protection, and also the protection could be short-lived and booster shots would be needed. Until these vaccines are fully evaluated and licensed, their protective efficacy remains uncertain.

Conclusion:

In conclusion, Lassa fever continues to be a significant cause of outbreaks. The evolutionary history of Lassa fever is complex and multifactorial, including host-virus interactions, human population density, and land use changes. Recent molecular epidemiological studies have provided insight into the evolutionary history, origin and dispersal of LASV, revealing a complex evolutionary history with multiple introductions into human populations. The ecological and genetic factors that drive the evolution of LASV are still not fully understood, but it is believed that the virus may have adapted to its human

hosts through genetic changes in the viral genome.

Diagnosis of Lassa fever has evolved from basic serological tests to more advanced methods such as qRT-PCR for surveillance and sequencing for more in-depth characterization. Next generation sequencing techniques are increasingly being used to characterize outbreak strains from West Africa. While no vaccines are in clinical use, recombinant vesicular stomatitis virus (rVSV) vector-based, virus-like particle (VLP), and DNA based vaccines have shown to induce protective immunity in animal models and some of these have progressed to phase 2 clinical trials.

The control and prevention of Lassa fever are crucial for the health and well-being of the affected populations. Implementing effective control measures such as rodent con trol, improved sanitation, and early detection and isolation of infected individuals are crucial for reducing the transmission of LASV. Additionally, ongoing research into the genetic and ecological factors that drive LASV evolution, are essential for reducing the transmission and impact of Lassa fever. Lassa fever is not only a public health problem in West Africa, but also a significant economic burden on the affected communities. Therefore, it is crucial to continue investing in research and control measures to better understand and combat Lassa fever, in order to protect the health and livelihoods of the people in the affected areas.

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NM, BM and KM searched for articles, and jointly wrote the initial draft. AB conceptualised the research, contributed additional information and reviewed the article.

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References:

1. Günther, S., and Lenz, O. Lassa virus. Crit Rev Clin Lab Sci. 2004; 41 (4): 339–390. doi: 10.1080/10408360490497456
2. Isiguzo, G. C., and Iroezindu, M. O. Epidemiology and Management of Lassa Fever in the West African Sub-Region: Overcoming the Socio cultural Challenges. In: Tangwa, G. B., et al. (eds). Socio-cultural Dimensions of Emerging Infectious Diseases in Africa. Springer Nature Switzerland. 2019: 41- 55. doi: 10.1007/978-3-030-17474-3_4
3. McLay, L., Liang, Y., and Ly, H. Comparative analysis of disease pathogenesis and molecular mechanisms of New World and Old-World arenavirus infections. J Gen Virol. 2014; 95 (Pt 1): 1-15. doi: 10.1099/vir.0.057000-0
4. Yun, N. E., and Walker, D. H. Pathogenesis of Lassa fever. Viruses. 2012; 4 (10): 2031 – 2048. doi: 10.3390/v4102031
5. Okogbenin, S., Okoeguale, J., Akpede, et al. Retrospective cohort study of Lassa fever in pregnancy, southern nigeria. Emerg Infect Dis, 2019; 25 (8): 1495–1500. doi: 10.3201/EID2508.181299
6. Demby, A. H., Chamberlain, J., Brown, D. W. G., et al. Early diagnosis of Lassa fever by reverse transcription-PCR. J Clin Microbiol. 1994; 32 (12): 2898–2903. doi: 10.1128/jcm.32.12.2898-2903.1994
7. Lukashevich, I. S., and Pushko, P. Vaccine platforms to control Lassa fever. Expert Rev Vaccines. 2016; 15 (9): 1135–1150. doi: 10.1080/14760584.2016.1184575
8. Abreu-Mota, T., Hagen, K. R., Cooper, K., et al. Non-neutralizing antibodies elicited by recombinant Lassa–Rabies vaccine are critical for protection against Lassa fever. Nat Commun. 2018; 9 (1): 4223. doi: 10.1038/S41467-018-06741-W
9. Marzi, A., Feldmann, F., Geisbert, T. W., et al. Vesicular stomatitis virus-based vaccines against Lassa and Ebola viruses. 2015; 21 (2): 305–307. doi: 10.3201/eid2102.141649
10. Fisher-Hoch, S. P., and McCormick, and J. B. Lassa fever vaccine: A review. 2004; 3(2): 103-111. doi: 10.1586/14760584.3.2.189
11. Isaac, A. B., Karolina, W., Temitope, A. A., et al. Prospects Of Lassa Fever Candidate Vaccines. Afr J Infect Dis. 2022; 16 (2 Suppl): 46-58. doi: 10.21010/AJID.V16I2S.6
12. Buckley, S. M., and Casals, J. Lassa fever, a new virus disease of man from West Africa. 3. Isolation and characterization of the virus. Am J Trop Med Hyg. 1970; 19 (4): 680–691. doi: 10.4269/AJTMH.1970.19.680
13. Townsend Peterson, A., Moses, L. M., and Bausch, D. G. Mapping transmission risk of Lassa fever in West Africa: The importance of quality control, sampling bias, and error weighting. PLoS One. 2014; 9 (8): e100711 doi: 10.1371/journal.pone.0100711
14. Radoshitzky, S. R., Bào, Y., Buchmeier, M. J., et al. Past, present, and future of arenavirus taxonomy. Arch Virol. 2015; 160(7): 1851-1874 doi: 10.1007/s00705-015-2418-y
15. Auperin, D. D., and McCormick, J. B. Nucleotide sequence of the Lassa virus (Josiah strain) S genome RNA and amino acid sequence comparison of the N and GPC proteins to other arenaviruses. Virology. 1989; 168 (2): 421-425 doi: 10.1016/0042-6822(89)90287-0
16. Clegg, J. C. S., Wilson, S. M., and Oram, J. D. Nucleotide sequence of the S RNA of Lassa virus (Nigerian strain) and comparative analysis of arenavirus gene products. *Virus Res*, 1991; 18 (2–3): 151–164. doi: 10.1016/0168-1702(91)90015-N
17. Ter Meulen, J., Koulemou, K., Wittekindt, T., et al. Detection of Lassa virus anti-nucleoprotein immunoglobulin G (IgG) and IgM antibodies by a simple recombinant immunoblot assay for field use. J Clin Microbiol. 1998; 36(11): 3143–3148. doi: 10.1128/JCM.36.11.3143-3148
18. Günther, S., Emmerich, P., Laue, T., et al. Imported Lassa fever in Germany: molecular characterization of a new Lassa virus strain. Emerg Infect Dis. 2000; 6 (5): 466. doi: 10.3201/EID0605.000504
19. Bowen, M. D., Rollin, P. E., Ksiazek, T. G., et al. Genetic Diversity among Lassa Virus Strains. J Virol. 2000; 74 (15): 6992–7004. doi: 10.1128/JVI.74.15.6992-7004.2000
20. Whitmer, S. L. M., Strecker, T., Cadar, D., et al. New lineage of Lassa virus, Togo, 2016. Emerg Infect Dis. 2018; 24 (3): 599–602. doi: 10.3201/EID2403.171905

21. Kafetzopoulou, L. E., Pullan, S. T., Lemey, P., et al. Metagenomic sequencing at the epicenter of the Nigeria 2018 Lassa fever outbreak. Science. 2019; 363 (6422): 74–77. doi: 10.1126/science.aau9343
22. Lecompte, E., Fichet-Calvet, E., Daffis, S., et al. *Mastomys natalensis* and Lassa fever, West Africa. Emerg Infect Dis. 2006; 12 (12): 1971–1974. doi: 10.3201/eid1212.060812
23. Olayemi, A., Cadar, D., Magassouba, N., Obadare, A., et al. New Hosts of The Lassa Virus. Sci Rep. 2016: 6: 25280 doi: 10.1038/SREP25280
24. Keenlyside, R. A., McCormick, J. B., Webb, P. A., Smith, E., Elliott, L., and Johnson, K. M. Case-control study of *Mastomys natalensis* and humans in Lassa virus-infected households in Sierra Leone. Am J Trop Med Hyg. 1983; 32 (4): 829–837. doi: 10.4269/AJTMH.1983.32.829
25. Adebayo, D., Nwobi, E. A., Vincent, T., and Gonzalez, J. Response Preparedness to Viral Hemorrhagic Fever in Nigeria: Risk Perception, Attitude towards Lassa Fever. Epidemiology (Sunnyvale). 2015; 5(3): 199 doi: 10.4172/2161-1165.1000199
26. Bonwitt, J., Kandeh, M., Dawson, M., Ansumana, R., Sahr, F., Kelly, A. H., and Brown, H. Participation of women and children in hunting activities in Sierra Leone and implications for control of zoonotic infections. PLoS Negl Trop Dis. 2017; 11 (7): e0005699. doi: 10.1371/JOURNAL.PNTD.0005699
27. At home with Mastomys and Rattus: preview and related info. Mendeley. (n.d.). https://www.mendeley.com/catalogue/dd23ca34-acfb-3f81-aac7-673f6143a0b9/2023. (Accessed March 2, 2023)
28. Goeijenbier, M., Wagenaar, J., Goris, M., et al. Rodent-borne hemorrhagic fevers: Under-recognized, widely spread and preventable-epidemiology, diagnostics and treatment. Crit Rev Microbiol. doi: 2013; 10.3109/1040841X.2012.686481
29. Richmond, J. K., and Baglole, D. J. Lassa fever: Epidemiology, clinical features, and social consequences. BMJ. 2003; 327(7426): 1271-1275. doi: 10.1136/bmj.327.7426.1271.Erratum in BMJ. 2004; 328(7431): 96
30. Abdulraheem, I. S. Public health importance of Lassa fever epidemiology, clinical features and current management review of literature. Afr J. Clin Exper Microbiol. 2002; 3 (1): 33–37. doi: 2002; 10.4314/ajcem.v3i1.7349
31. Greenky, D., Knust, B., and Dziuban, E. J. What Pediatricians Should Know About Lassa Virus. JAMA Pediatr. 2018; 172 (5): 407. doi: 10.1001/JAMAPEDIATRICS.2017.5223
32. CDC. Lassa Fever Factsheet. In Lassa Fever. 2019
33. McCormick, J. B., Webb, P. A., Krebs, J. W., Johnson, K. M., and Smith, E. S. A Prospective Study of the Epidemiology and Ecology of Lassa Fever. J Infect Dis. 1987; 155(3):437-444 doi: 10.1093/infdis/155.3.437
34. Lukashevich, I. S., Clegg, J. C. S., and Sidibe, K. Lassa virus activity in Guinea: Distribution of human antiviral antibody defined using enzyme-linked immunosorbent assay with recombinant antigen. J Med Virol. 1993; 40 (3): 210–217. doi: 10.1002/JMV.1890400308
35. Tomori, O., Fabiyi, A., Sorungbe, A., Smith, A., and McCormick, J. B. Viral hemorrhagic fever antibodies in Nigerian populations. 1998; 38 (2): 407–410. doi: 10.4269/ajtmh.1988.38.407
36. Amorosa, V., MacNeil, A., McConnell, R., et al. Imported Lassa fever, Pennsylvania, USA 2010. Emerg Infect Dis. 2010; 16 (10): 1598–1600. doi: 10.3201/EID1610.100774
37. Johnson, K. M., and Monath, T. P. Imported Lassa Fever — Reexamining the Algorithms. NEJM; 1990; 323 (16): 1139–1141. doi: 10.1056/NEJM199010183231611
38. Grahn, A., Bråve, A., Lagging, M., et al. Imported case of Lassa fever in Sweden with encephalopathy and sensorineural hearing deficit. Open Forum Infect Dis. 2016; 3(4): ofw198. doi: 10.1093/OFID/OFW198
39. Okoro, O. A., Bamgboye, E., Dan-Nwafor, C., et al. Descriptive epidemiology of Lassa fever in Nigeria, 2012-2017. Pan Afr Med J. 2020; 37: 15. doi: 10.11604/pamj.2020.37.15.21160
40. Liao, B. S., Byl, F. M., and Adour, K. K. Audiometric comparison of Lassa fever hearing loss and idiopathic sudden hearing loss: Evidence for viral cause. Otolaryngol Head Neck Surg. 1992. 106(3): 226-229 doi: 10.1177/019459989210600303
41. Lehmann, C., Kochanek, M., Abdulla, D., et al. Control measures following a case of imported Lassa fever from Togo, North Rhine Westphalia, Germany, 2016. 2017; 22 (39):17-00088 doi:10.2807/1560-7917.ES.2017.22.39.17-00088
42. Akpede, G. O., Asogun, D. A., Okogbenin, S. A., and Okokhere, P. O. Lassa fever outbreaks in Nigeria. Expert Rev Anti Infect Ther. 2018; 16 (9): 663–666. doi: 10.1080/14787210.2018.1512856
43. Maxmen, A. Deadly Lassa-fever outbreak tests Nigeria's revamped health agency. Nature. 2018; 555 (7697): 421–422. doi: 10.1038/D41586-018-03171-Y
44. Mofolorunsho, K. C. Outbreak of Lassa fever in Nigeria: measures for prevention and control. 2016; 23: 210. doi: 10.11604/pamj.2016.23.210.8923
45. Fatiregun, A., and Isere, E. Epidemic preparedness and management: A guide on Lassa fever outbreak preparedness plan. 2017; 58 (1): 1. doi: 10.4103/0300-1652.218414
46. Aloke, C., Obasi, N. A., Aja, P. M., et al. Combating Lassa Fever in West African Sub-Region: Progress, Challenges, and Future Perspectives. Viruses, 2023; 15 (1): 146. doi: 10.3390/v15010146
47. Atkin, S., Anaraki, S., Gothard, P., et al. The first case of Lassa fever imported from Mali to the United Kingdom, February 2009. Euro Surveill. 2009; 14(10): pii=19145 doi: 10.2807/ese.14.10.19145-en
48. Tarn, W. Y., Cheng, Y., Ko, S. H., and Huang, L. M. Antisense oligonucleotide-based therapy of viral infections. Pharmaceutics. 2021; 13(12): 2015. doi: 10.3390/pharmaceutics13122015
49. Rojek, J. M., and Kunz, S. Cell entry by human pathogenic arenaviruses. Cellular Microbiol. 2008; 10(4): 828-835 doi: 10.1111/j.1462-5822.2007.01113.x
50. Brosh-Nissimov, T. Lassa fever: another threat from West Africa. Disaster Mil Med. 2016; 2: 8 doi: 10.1186/s40696-016-0018-3
51. Azeez-Akande, O. Review of Lassa fever, an emerging old world haemorrhagic viral disease in sub-Saharan Africa. 2016; 17(4): 282 doi: 10.4314/ajcem.v17i4.9
52. Ogbu, O., Ajuluchukwu, E., and Uneke, C. J. Lassa fever in West African sub-region: An overview. J Vector Borne Dis. 2007; 44 (1): 1–11.
53. McCormick, J. B., King, I. J., Webb, P. A., et al. Case-Control Study of the Clinical Diagnosis and Course of Lassa Fever. 1987; 155 (3): 445–455. doi: 10.1093/INFDIS/155.3.445
54. Cummins, D., McCormick, J. B., Bennett, D., Samba, J. A., Farrar, B., Machin, S. J., and Fisher-Hoch, S. P. Acute Sensorineural Deafness in Lassa Fever. 1990; 264 (16): 2093–2096. doi: 10.1001/JAMA.1990.03450160063030
55. Frame, J. D., Baldwin, J. M., Gocke, D. J., and Troup, J. M. Lassa fever, a new virus disease of man from West Africa. I. Clinical description and pathological findings. Am J Trop Med Hyg. 1970; 19 (4): 670–676. doi: 10.4269/AJTMH.1970.19.670

56. Edington, G. M., and White, H. A. The pathology of Lassa fever. A tribute to the late Dr. J.M. Troup. Trans R Soc Trop Med Hyg. 1972; 66 (3): 381–389. doi: 10.1016/0035-9203(72)90268-4
57. Hotta, H. Viral hemorrhagic fever--Ebola hemorrhagic fever, Marburg disease and Lassa fever. Rinsho Byori, 1998; 46 (7): 651–655.
58. Salami, K. A., Mandi, H. E., Imbault, N., et al. The promise, problems, and pitfalls of including pregnant women in clinical trials of Lassa fever vaccine: a qualitative assessment of sub-Saharan Africa investigators' perception. Pan Afr Med J. 2022; 41: 242 doi: 10.11604/pamj.2022.41.242.33863
59. Monson, M. H., Frame, J. D., Jahrling, P. B., et al. Endemic Lassa fever in Liberia. I. Clinical and epidemiological aspects at Curran Lutheran Hospital, Zorzor, Liberia. Trans R Soc Trop Med Hyg, 1984; 78 (4) : 549–553 doi:10.1016/0035-9203(84)90082-8
60. Asogun, D. A., Günther, S., Akpede, G. O., et al. Lassa Fever: Epidemiology, Clinical Features, Diagnosis, Management and Prevention. Infect Dis Clin North Am. 2019; 33 (4): 933–951. doi: 10.1016/J.IDC.2019.08.002
61. Bonwitt, J., Kelly, A., 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): 234-237. doi: 10.1007/s10393-016-1098-8
62. Marennikova, S. S., Malceva, N. N., and Habahpaseva, N. A. ELISA--a simple test for detecting and differentiating antibodies to closely related orthopoxviruses. Bull World Health Organ. 1981; 59 (3): 365–369.
63. Jahrling, P. B., Niklasson, B. S., and McCormick, J. B. Early diagnosis of human Lassa fever by ELISA detection of antigen and antibody. 1985; 325 (8423), 250–252. doi: 10.1016/S0140-6736(85)91029-3
64. Bausch, D. G., Demby, A. H., Coulibaly, M., et al. Human Disease and Clinical Observations. Vector Borne Zoonotic Dis. 2001; 1(4): 269-281. doi: 10.1089/15303660160025903
65. Akpogheneta, O., Dicks, S., Grant, D., et al. Boosting understanding of Lassa fever virus epidemiology: Field testing a novel assay to identify past Lassa fever virus infection in blood and oral fluids of survivors and unexposed controls in Sierra Leone. PLoS Negl Trop Dis. 2021; 15(3): e0009255 doi: 10.1371/journal.pntd.0009255
66. Ibekwe, T., Nwegbu, M., Okokhere, P., et al. The sensitivity and specificity of Lassa virus IgM by ELISA as screening tool at early phase of Lassa fever infection. 2012; 53 (4): 196. doi: 10.4103/0300-1652.107552
67. Bausch, D. G., Rollin, P. E., Demby, A. H., et al. Diagnosis and clinical virology of Lassa fever as evaluated by enzyme- linked immunosorbent assay, indirect fluorescent-antibody test, and virus isolation. J Clin Microbiol. 2000; 38 (7): 2670–2677. doi: 10.1128/JCM.38.7.2670-2677.2000
68. Takah, N. F., Brangel, P., Shrestha, P., et al. Sensitivity and specificity of diagnostic tests for Lassa fever: A systematic review. BMC Infect Dis. 2019; 19 (1): 1–11. doi: 10.1186/S12879-019-4242-6/FIGURES/2
69. Raabe, V., and Koehler, J. Laboratory Diagnosis of Lassa Fever. J Clin Microbiol. 2017; 55 (6): 1629. doi: 10.1128/JCM.00170-17
70. Kenmoe, S., Tchatchouang, S., Ebogo-Belobo, J. T., et al. Systematic review and meta-analysis of the epidemiology of Lassa virus in humans, rodents and other mammals in Sub-Saharan Africa. PLoS Negl Trop Dis. 2020; 14 (8): 1–29. doi: 10.1371/journal.pntd.0008589
71. Hartnett, J. N., Boisen, M. L., Oottamasathien, D., et al. Current and emerging strategies for the diagnosis, prevention and treatment of Lassa fever. Future Virol. 2015; 10(5) : 559–584. doi: 10.2217/FVL.15.41
72. Salami, K., Gouglas, D., Schmaljohn, C., et al. A review of Lassa fever vaccine candidates. Curr Opin Virol. 2019; 37: 105–111 doi: 10.1016/j.coviro.2019.07.006
73. Drosten, C., Göttig, S., Schilling, S., et al. Rapid detection and quantification of RNA of Ebola and Marburg viruses, Lassa virus, Crimean-Congo hemorrhagic fever virus, Rift Valley fever virus, dengue virus, and yellow fever virus by real-time reverse transcription-PCR. J Clin Microbiol. 2002; 40 (7): 2323–2330. doi: 10.1128/JCM.40.7.2323-2330.2002
74. Niedrig, M., Meyer, H., Panning, M., et al. Follow-up on diagnostic proficiency of laboratories equipped to perform orthopoxvirus detection and quantification by PCR: the second international external quality assurance study. J Clin Microbiol. 2006; 44 (4): 1283–1287. doi: 10.1128/JCM.44.4.1283-1287.2006
75. Ölschläger, S., Lelke, M., Emmerich, P., et al. Improved detection of Lassa virus by reverse transcription-PCR targeting the 5' region of S RNA. J Clin Microbiol. 2010; 48 (6): 2009–2013. doi: 10.1128/JCM.02351-09
76. Panning, M., Asper, M., Kramme, S., et al. Rapid detection and differentiation of human pathogenic Orthopoxviruses by a fluorescence resonance energy transfer real-time PCR assay. Clin Chem. 2004; 50 (4), 702–708. doi: 10.1373/clinchem.2003.026781
77. Dedkov, V. G., Magassouba, N. 'Faly, Safonova, M. V., et al. Development and Evaluation of a One-Step Quantitative RT-PCR Assay for Detection of Lassa Virus. J Virol Methods. 2019; 271: 113674. doi: 10.1016/J.JVIROMET.2019.113674
78. Pemba, C. M., Kurosaki, Y., Yoshikawa, R., et al. Development of an RT-LAMP assay for the detection of Lassa viruses in southeast and south-central Nigeria. J Virol Methods. 2019; 269: 30–37. doi: 10.1016/J.JVIROMET.2019.04.010
79. Fukuma, A., Kurosaki, Y., Morikawa, Y., et al. Rapid detection of Lassa virus by reverse transcription-loop-mediated isothermal amplification. Microbiol Immunol. 2011; 55 (1): 44–50. doi: 10.1111/j.1348-0421.2010.00286.x
80. Happi, A. N., Happi, C. T., and Schoepp, R. J. Lassa Fever Diagnostics: Past, Present, and Future. Curr Opin Virol. 2019; 37: 132. doi: 10.1016/J.COVIRO.2019.08.002
81. Mazzola, L. T., and Kelly-Cirino, C. Diagnostics for Lassa fever virus: a genetically diverse pathogen found in low-resource settings. BMJ Glob Health. 2019; 4 (Suppl 2): e001116. doi: 10.1136/BMJGH-2018-001116
82. Oliveira, B. B., Veigas, B., and Baptista, P. V. Isothermal Amplification of Nucleic Acids: The Race for the Next "Gold Standard". Front Sens. 2021; 2: 752600. doi: 10.3389/FSENS.2021.752600
83. Andersen, K., Shapiro, B., and Matranga, C., et al. Clinical sequencing uncovers origins and evolution of Lassa virus. Cell. 2015; 162: 738–750. doi: 10.1016/j.cell.2015.07.020
84. Matranga, C. B., Gladden-Young, A., Qu, J., Winnicki, S., et al. Unbiased deep sequencing of RNA viruses from clinical samples. J. Vis Exp. 2016; 113: 54117. doi: 10.3791/54117
85. Lassa virus sequencing in Nigeria - Lassa virus / Lassa virus Nigeria 2019 - Virological. (n.d.). https://virological.org/t/2019-lassa-virus-sequencing-in-nigeria/287. (Accessed March 6, 2023)
86. Slatko, B. E., Gardner, A. F., and Ausubel, F. M. Overview of Next Generation Sequencing Technologies. Curr Protoc Mol Biol. 2018; 122 (1): e59. doi: 10.1002/CPMB.59
87. Stefan, C. P., Hall, A. T., Graham, A. S., and

Minogue, T. D. Comparison of Illumina and Oxford Nanopore Sequencing Technologies for Pathogen Detection from Clinical Matrices Using Molecular Inversion Probes. 2022; 24 (4): 395–405. doi: 10.1016/J.JMOLDX.2021.12.005

88. Clegg, J. C. S., and Oram, J. D. Molecular cloning of Lassa virus RNA: Nucleotide sequence and expression of the nucleocapsid protein gene. Virology. 1985; 144(2): 363-372 doi: 10.1016/0042-6822(85)90278-8
89. Dolskiy, A. A., Grishchenko, I. V., and Yudkin, D. V. Cell Cultures for Virology: Usability, Advantages, and Prospects. Int J Mol Sci. 2020; 21 (21): 1–23. doi: 10.3390/IJMS21217978
90. Lingas, G., Rosenke, K., Safronetz, D., et al. Lassa viral dynamics in non-human primates treated with favipiravir or ribavirin. PLoS Comput Biol. 2021; 17 (1): e1008535 doi: 10.1371/JOURNAL.PCBI.1008535
91. Mire, C. E., Geisbert, J. B., Marzi, A., et al. Vesicular Stomatitis Virus-Based Vaccines Protect Nonhuman Primates against Bundibugyo ebolavirus. PLoS Negl Trop Dis. 2013; 7 (12): e2600. doi: 10.1371/JOURNAL.PNTD.0002600
92. Grant-Klein, R. J., Altamura, L. A., and Schmaljohn, C. S. Progress in recombinant DNA-derived vaccines for Lassa virus and filoviruses. Virus Res. 2011; 162 (1–2): 148–161. doi: 10.1016/J.VIRUSRES.2011.09.005
93. Fisher-Hoch, S. P., and McCormick, J. B. Toward a human Lassa fever vaccine. Rev Med Virol. 2001; 11 (5) : 331–341. doi: 10.1002/rmv.329
94. Mateo, M., Reynard, S., Carnec, X., Journeaux, A., et al. Vaccines inducing immunity to Lassa virus glycoprotein and nucleoprotein protect macaques after a single shot. Sci Transl Med. 2019; 11 (512): eaaw3163 doi: 10.1126/SCITRANSLMED.AAW3163
95. Fisher-Hoch, S. P., and McCormick, J. B. Lassa fever vaccine. Expert Rev Vaccines. 2004; 3 (2): 189–197. doi: 10.1586/14760584.3.2.189
96. Cashman, K. A., Broderick, K. E., Wilkinson, E. R., et al. Enhanced Efficacy of a Codon-Optimized DNA Vaccine Encoding the Glycoprotein Precursor Gene of Lassa Virus in a Guinea Pig Disease Model When Delivered by Dermal Electroporation. Vaccines (Basel). 2013; 1(3): 262–277. doi: 10.3390/vaccines1030262
97. Emergent BioSolutions Initiates Phase 1 Study Evaluating Lassa Virus Vaccine Candidate | BioSpace. (n.d.). https://www.biospace.com/article/releases/emergent-biosolutions-initiates-phase-1-study-evaluating-lassa-virus-vaccine-candidate/. (Accessed March 12, 2023)
98. Hummel, K. B., Martin, M. L., and Auperin, D. D. Baculovirus expression of the glycoprotein gene of Lassa virus and characterization of the recombinant protein. 1992; 25 (1–2): 79–90. doi: 10.1016/0168-1702(92)90101-e
99. Plotkin, S. A. Vaccines for epidemic infections and the role of CEPI. Hum Vaccin Immunother. 2017; 13 (12): 2755–2762. doi: 10.1080/21645515.2017.1306615
100. CEPI partners with the International AIDS vaccine initiative to advance Lassa fever vaccine development. (n.d.).
101. Ewer, K., Sebastian, S., Spencer, A. J., et al. Chimpanzee adenoviral vectors as vaccines for outbreak pathogens. 2017; 13 (12), 3020–3032. doi: 10.1080/21645515.2017.1383575
102. Zapata, J. C., Poonia, B., Bryant, J., et al. An attenuated Lassa vaccine in SIV-infected rhesus macaques does not persist or cause arenavirus disease but does elicit Lassa virus-specific immunity. Virol J. 2013; 10 (1) : 1–11. doi: 10.1186/1743-422X-10-52/FIGURES/6
103. Cai, Y., Ye, C., Cheng, B., et al. A Lassa Fever Live-Attenuated Vaccine Based on Codon Deoptimization of the Viral Glycoprotein Gene. MBio. 2020; 11 (1). e00039-20 doi: 10.1128/MBIO.00039-20
104. Jiang, J., Banglore, P., Cashman, K. A., et al. Immunogenicity of a protective intradermal DNA vaccine against Lassa virus in cynomolgus macaques. Hum Vaccin Immunother. 2019; 15 (9): 2066–2074. doi: 10.1080/21645515.2019.1616499
105. Salvato, M., and Lukashevich, I. Vaccines against Lassa Fever. Marcel Dekker, Inc. 2009
106. Russell, C. J., Jones, B. G., Sealy, R. E., et al. A Sendai virus recombinant vaccine expressing a gene for truncated human metapneumovirus (hMPV) fusion protein protects cotton rats from hMPV challenge. Virology, 2017; 509: 60–66. doi: 10.1016/J.VIROL.2017.05.021
107. McCormick, J. B., Mitchell, S. W., Kiley, M. P., et al. Inactivated Lassa virus elicits a non-protective immune response in rhesus monkeys. 1992; 37 (1): 1–7. doi: 10.1002/jmv.1890370102
108. Accelerating the licensure of Lassa vaccines: Generating robust evidence on vaccine efficacy and safety. (n.d.). https://www.who.int/newsroom/events/detail/2022/10/25/default-calendar/save-the-date---accelerating-the-licensure-of-lassa-vaccines--generating-robust-evidence-on-vaccine-efficacy-and-safety. (Accessed March 12, 2023)
109. Cross, R. W., Woolsey, C., Prasad, A. N., et al. A recombinant VSV-vectored vaccine rapidly protects nonhuman primates against heterologous lethal Lassa fever. Cell Rep. 2022; 40(3): 111094. doi: 10.1016/j.celrep.2022.111094
110. Kainulainen, M. H., Spengler, J. R., Welch, S. R., et al. Use of a Scalable Replicon-Particle Vaccine to Protect Against Lethal Lassa Virus Infection in the Guinea Pig Model. 2018; 217 (12): 1957–1966. doi: 10.1093/INFDIS/JIY123
111. Salvato, M. S., Domi, A., Guzmán-Cardozo, C., et al. A single dose of modified vaccinia Ankara expressing Lassa virus-like particles protects mice from lethal intra-cerebral virus challenge. 2019; 8(3). 133. doi: 10.3390/PATHOGENS8030133
112. A Trial to Evaluate the Optimal Dose of MV-LASV (V182-001) - Full Text View - ClinicalTrials.gov. https://clinicaltrials.gov/ct2/show/NCT04055454. (Accessed 12 March 12, 2023)
113. Voysey, M., Clemens, S. A. C., Madhi, S. A., et al. Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK. 2021; 397 (10269): 99–111. doi: 10.1016/S0140-6736(20)32661-1
114. Pushko, P., Geisbert, J., Parker, M., et al. Individual and bivalent vaccines based on alphavirus replicons protect guinea pigs against infection with Lassa and Ebola viruses. 2001; 75 (23): 11677–11685. doi: 10.1128/jvi.75.23.11677-11685.2001
115. Ramsauer, K., Schwameis, M., Firbas, C., et al. Immunogenicity, safety, and tolerability of a recombinant measles-virus-based chikungunya vaccine: A randomised, double-blind, placebo-controlled, active-comparator, first-in-man trial. Lancet Infect Dis. 2015; 15 (5): 519–527. doi: 10.1016/S1473-3099(15)70043-5
116. Study shows clamp technology promising for future vaccine development - UQ News - The University of Queensland, Australia. (n.d.). https://www.uq.edu.au/news/article/2021/04/study-shows-clamp-technology-promising-future-vaccine-development. (Accessed March 12, 2023)
117. Pennington, H. N., and Lee, J. Lassa virus glycoprotein complex review: insights into its unique fusion machinery. Biosci Rep. 2022; 42 (2): 20211930. doi: 10.1042/BSR20211930
118. Themis Bioscience and CEPI trial Lassa fever vaccine. (n.d.). https://www.clinicaltrialsarena.com/news/themi

s-lassa-fever-vaccine-trial/. (Accessed March 12, 2023)
119. A Clinical Trial to Evaluate the Safety and Immunogenicity of rVSVΔG-LASV-GPC Vaccine in Adults in Good General Heath - Full Text View -ClinicalTrials.gov. (n.d.). https://clinicaltrials.gov/ct2/show/NCT04794218. (Accessed March 12, 2023)
120. Brouwer, P., Antanasijevic, A., Ronk, A. J., et al. Lassa virus glycoprotein nanoparticles elicit a neutralizing antibody that defines a new site of vulnerability. bioRxiv. 2022 (preprint) doi: 10.1101/2022.03.28.486091
121. Mateo, M., Reynard, S., Pietrosemoli, N., et al. Rapid protection induced by a single-shot Lassa vaccine in male cynomolgus monkeys. 2023; 14 (1): 1–18. doi: 10.1038/s41467-023-37050-6
122. Jiang, X., Dalebout, T. J., Bredenbeek, P. J., et al. Yellow fever 17D-vectored vaccines expressing Lassa virus GP1 and GP2 glycoproteins provide protection against fatal disease in guinea pigs. Vaccine. 2011; 29 (6): 1248–1257. doi: 10.1016/J.VACCINE.2010.11.079
123. Lukashevich, I. S. Advanced Vaccine Candidates for Lassa Fever. Viruses. 2013; 4 (11): 2514–2557. doi: 10.3390/v4112514
124. Carnec, X., Mateo, M., Page, A., et al. A Vaccine Platform against Arenaviruses Based on a Recombinant Hyper-attenuated Mopeia Virus Expressing Heterologous Glycoproteins. J Virol. 2018 ; 92 (12): e02230-17 doi:10.1128/JVI.02230-17
125. Cheng, B. Y. H., Ortiz-Riaño, E., Nogales, A., et al. Development of Live-Attenuated Arenavirus Vaccines Based on Codon Deoptimization. J Virol. 2015; 89 (7): 3523–3533. doi: 10.1128/JVI.03401-14
126. Botten, J., Whitton, J. L., Barrowman, P., et al. A Multivalent Vaccination Strategy for the Prevention of Old-World Arenavirus Infection in Humans. J Virol. 2010; 84 (19): 9947–9956. doi:10.1128/JVI.00672-10
127. Branco, L. M., Grove, J. N., Geske, F. J., et al. Lassa virus-like particles displaying all major immunological determinants as a vaccine candidate for Lassa hemorrhagic fever. Virol J. 2010; 7. doi: 10.1186/1743-422X-7-279
128. Demby, A. H., Inapogui, A., Kargbo, K., et al. Lassa fever in Guinea: II. Distribution and prevalence of Lassa virus infection in small mammals. Vector Borne Zoonotic Dis. 2001; 1(4): 283–297. doi: 10.1089/15303660160025912