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Lassa fever: With 50 years of study, hundreds of thousands of patients and an extremely high disease burden, what have we learned?

Lauren E Garnett[1,2] and James E Strong[1,2,3]

Addresses
[1] Special Pathogens, National Microbiology Laboratory, Public Health Agency of Canada, Canada
[2] Departments of Medical Microbiology and Infectious Diseases, College of Medicine, Faculty of Health Sciences, University of Manitoba, Winnipeg, Canada
[3] Departments of Paediatrics and Child Health, College of Medicine, Faculty of Health Sciences, University of Manitoba, Winnipeg, Canada

Corresponding author: Strong, James E (jim.strong@canada.ca)

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Introduction

Lassa virus (LASV), the etiological agent of Lassa Fever (LF), was first identified after the infection of two missionary nurses in Lassa, Nigeria 50 years ago in 1969 [1]. LASV remains a current public health concern today, causing an estimated 300 000 infections and 5000 deaths annually, accounting for 10–30% of adult hospitalizations in endemic areas [2]. Of the hospitalized patients, 10–20% will succumb to infection, often despite treatment with ribavirin and supportive care. Unfortunately, the numbers can be dramatically increased during outbreaks [3•]. The extensiveness of LF is often unknown due to the fact the areas impacted by outbreaks are often resource poor and underfortified with diagnostic capabilities. In addition, LF presents with generic symptoms that mirror other illnesses such as malaria, influenza, sepsis and even ebolavirus [4]. Delays in diagnostics are common and although early treatment may be effective, the highest risk populations are often the least managed. Despite this high disease burden and the WHO prioritizing LASV for accelerated research and development, LF remains underdiagnosed and understudied with no FDA approved vaccine or therapeutic [5,6].

Virion/epidemiology

The LASV genome is a bi-segmented, single-stranded RNA ambisense genome belonging to the Arenaviridae family. Their size, a large (L) 7.3 kb segment and a small (S) 3.4 kb segment, classify the two segments. The L segment encodes the viral RNA-dependent RNA polymerase and zinc-binding proteins. The S segment contains two genes that encode for three proteins including the nucleoprotein (NP) and glycoprotein precursors 1 (GP1) and 2 (GP2) [7••]. The LASV genome is packaged into a pleomorphic virion measuring approximately 50–300 nm in diameter, which is enveloped and contains a lipid bilayer membrane [8].

The sequencing of the genome has identified four genetically distinct circulating LASV lineages (clades). Three of the lineages are endemic to Nigeria while the fourth is endemic across the geographical area of West Africa (Ivory Coast, Sierra Leone, Libera, and Guinea). More recently published sequences from Mali suggest that there may also be a fifth clade [9]. Each of the clades contains multiple strains that are distinct in their genetic, serologic and possibly pathogenic characteristics [10•]. There is a high prevalence of antibodies against LASV in the endemic region population, consisting of 8–52% in Sierra Leone, 4–55% in Guinea and 21% in Nigeria; the high prevalence is likely a result of most infections being mild or asymptomatic [11,12]. Because of the genetic diversity between clades, it is unknown at present how much cross-protection is provided between different clades and suggests that prospects of a universal LASV vaccine may be doubtful [13,14].

Outside endemic areas, there have been sporadic imported cases into the USA, Europe, and Asia in addition to laboratory-acquired infections reported in the USA [15]. Since the discovery of LASV, there have been 33 imported cases to 9 countries with patients initially departing from 7 endemic West African Countries [16•].

Natural reservoir and transmission

Similar to other Arenaviruses, LASV is a rodent-borne pathogen. The peridomestic Multimammate rat (*Mastomys natalensis*) is the natural host for LASV, which is ubiquitous in equatorial Africa. These Multimammate rats are found more commonly in poorer areas, where austere home construction contributes to rat-human cohabitation [2,11]. Through phylogenetic analysis, it has been found most LASV infections are a result of multiple independent zoonotic spillover events, with an estimate of less than 20% of cases arising from human-to-human transmission [7••,17]. There is recent speculation that the large increase in Nigerian LF cases between 2017 and mid-2018 may have arisen from a new strain of

more virulent LASV. The new strain was thought to have caused increased human-to-human spread; however, analyses of the LASV genomes showed a diverse range of viruses that have been previously observed in Nigeria, likely arising from numerous rodent-to-human transmission events [18•]. The zoonotic event from the *M. natalensis* is thought to occur through direct contact or ingestion of the rodent excreta or saliva with a host's non-intact skin or mucous membranes [19]. Transmission and corresponding human disease have been found to occur mostly in March, correlating to the dry season transitioning over to the wet season; however, cases can occur year-round [11,20].

There is no associated human-to-human transmission from asymptomatic carriers; however, for patients suffering from acute infection, transmission can occur through contact with infected blood, urine, other secreted bodily fluids, or through contact with contaminated equipment such as re-used needles [15]. LASV infection is not spread through casual contact such as hugging, shaking hands, or sitting near someone [21]. The lack of casual contact spread is demonstrated by low secondary attack rates, which has been exemplified in a recent review of 33 imported LF cases originating from West Africa between 1969 and 2016. The review showcased only 2 documented secondary transmissions from imported cases, both of which were likely due to improper personal protective equipment (PPE) use [16•]. Lack of appropriate PPE has been reported elsewhere to contribute to nosocomial LF transmissions particularly for healthcare workers [21–24]. There is also the risk of sexual transmission of LASV as the virus can survive in semen for up to three months after recovery from acute illness and viral clearance from the blood [15]. Transplacental transmission may also occur and is associated with poor prognosis for both the mother and the fetus, especially during the third trimester, with a maternal mortality rate at approximately 20%, and the fetal mortality rate nearing 100% [25,26]. Like other viral hemorrhagic fevers (VHFs), the potential for aerosol transmission, especially during laboratory manipulation of high titer samples, is possible and should be considered. There is, however, little to no epidemiologic evidence supporting this as a mode of transmission [27].

Clinical presentation

Exposure to LASV, either from rodent-to-human or human-to-human transmission, is usually via the nasopharyngeal and/or oral mucosal route. It is then able to infect monocytic lineages including dendritic cells and macrophages before subsequent hematogenous spread to lymph nodes and systemic dissemination [28–30]. The incubation period for LASV is 6–21 days resulting in a spectrum of clinical presentations ranging from asymptomatic to VHF, multi-system organ failure and death. Given the large spectrum of clinical presentation, along with the inadequate surveillance in endemic regions makes the case definition of LF difficult. There is no agreed upon case definition, although some have tried [31,32•]. Khan *et al.* have outlined that the LF case definition for admission in Sierra Leone was based on a reported or documented temperature $\geq$38°C for less than three weeks with absence of local inflammation and at least two major signs (Including: Bleeding; Swollen neck or face; Conjunctivitis or subconjunctival hemorrhage; Spontaneous abortion; Petechial or hemorrhagic rash; New onset of tinnitus or altered hearing; Failure to respond within 48 h to anti-malarial therapy; Failure to respond within 48 h to antibiotic therapy), or one major plus two minor signs (including: Headache; Sore throat; Cough; Vomiting; Diarrhea; Diffuse abdominal pain/tenderness; Chest/retrosternal pain; Generalized myalgia or arthralgia; Profuse weakness; Jaundice), or at least three minor signs [31]. Critiques of this case definition include the lack of specificity given the criteria overlap many other febrile illnesses. The cause of the large discrepancy in patient symptoms, which has led to an ill-defined case definition for diagnosis, is still unknown. However, it is thought that the route, dose, as well as underlying co-infections, and genetic predisposition may play a role [31].

Although there is limited epidemiological surveillance in endemic areas it has been suggested that most infected individuals (80%) are asymptomatic or have a very mild course, which may include low grade fever, headache, and malaise. Many of these do not seek medical attention. The remaining 20% go on to more serious disease initially manifesting as pharyngitic changes with cough, nausea, vomiting, diarrhea, abdominal pain, myalgia, retrosternal and back pain [33]. Severe cases can progress to have hemorrhage, facial swelling, pulmonary edema and hypotension [19]. End stage disease can see proteinuria, renal failure, shock, neurologic manifestations including tremors, disorientation, seizures and eventual coma [19]. Although LASV is considered a hemorrhagic fever, bleeding is seen in less than 20% of LASV acute infections and is typically minor and localized to the nose, mouth, vagina and/or rectum [34•]. Studies in NHP models investigating host immunological parameters of LASV infection have shown how the immune response may affect the disease outcome. It was found that individual inflammatory markers including high levels of circulating CD80+ monocytes, timing of IFNα release, and robustness of T cell activation differ between fatal and non-fatal cases [35–37].

It usually takes patients that suffer from acute LF infection 1–3 weeks to recover [25]. However, even after recovery, patients may experience disease sequelae including polyserositis, vision distortion, vertigo, hearing loss and back pain [38]. The pathogenesis of LF sequelae is still widely unknown, although it has been suggested that LASV infection is able to persist in the smooth

muscle of the arteries causing chronic systemic vascular inflammation (arteritis) during convalescence [38].

Pathophysiology

The majority of our understanding of the pathophysiology of LASV is the result of animal models of disease. Two animal models are widely used for the study of LASV infection; an inbred strain 13 guinea pig model and the more preferred non-human primate (NHP) model [10•]. Macaques (Rhesus and Cynomolgus) have been considered the gold standard for investigating vaccine and therapeutic evaluations as well as pathogenesis studies for LF [37]. This is because the disease timing, clinical signs, gross lesions, histopathological changes, and minimal inflammatory cell infiltrate in infected organs are similar to those occurring in man [28–39].

LASV disease is believed to initiate by targeting macrophages and dendritic cells, causing myeloid cell dysregulation, with subsequent spread to the other organ systems where some combination of direct viral cytopathology and host inflammatory/vasodilatory processes occur [40–42]. The more serious end-stage of this process leads to a septic shock-like state of vascular instability and hemostatic impairment typical of other VHFs ultimately leading to multi-organ failure [43]. The systemic spread initially leads to the infection of Kupffer and parenchymal cells in the liver and adrenal gland followed by cells in a variety of other tissues including spleen, kidney, pancreas, placenta, uterus, breasts, gonads, and finally the epithelium [31,37].

Liver

The most severe lesions found in post-mortem histological studies are found in the liver showing hepatic necrosis coinciding with LASV hepatitis [44,45]. The high virus titers in the liver suggest it is one of the major target organs of virus replication and cytopathology. Although the histopathologic changes are not considered sufficient to cause death, liver damage is exemplified by a significant rise in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) [37,46]. AST levels are usually significantly greater than ALT, often at a ratio of 10:1. It has been shown that AST levels $\geq$120 international units/L at the time of presentation is associated with increased mortality risk [3•,46,47]. The occurrence of random foci of necrosis, some without associated inflammatory cells, suggests that the hepatic injury may be due to direct viral damage [44].

Renal

It has been shown that an increased risk of mortality is correlated with renal insufficiency secondary to LF with renal pathology being a common observation in post mortem examinations [3•,30,48,49]. Post-mortem renal pathology includes tubular necrosis, glomerular sclerosis, and interstitial nephritis, but there is no reported observation of ischemic pathology. Additionally, blood urea nitrogen (BUN) and creatinine (CRE) can be used to help predict fatal outcomes in patients infected with LASV, as an increase in BUN and CRE have been associated with a doubling in mortality [3•].

Nervous system

There is limited research regarding the neuropathogenicity of LASV infection; however, it has been suggested that invasion of the nervous system is important in end-stage pathogenesis [34•]. Neurological manifestations range from sensorineural hearing loss (SHL), confusion and disorientation to seizures, coma, and encephalopathy [34•,50,51]. LASV has also been isolated from the spinal fluid in a small number of cases and some have reported mild to moderate pleocytosis [51–53].

Approximately one-third of LASV patients develop unilateral or bi-lateral SHL in late stages of acute illness and into convalescence, and two-thirds of these patients are left with some degree of permanent hearing loss [40,53,54•]. The pathogenesis of the SHL is most likely caused by immunologic mechanisms. Researchers have developed a mouse model of SHL wherein LASV particles enter the inner ear through the hematogenous route or through cerebrospinal fluid and cause damage to the inner ear hair cells and auditory nerve through immunopathological mechanisms. Within this study, no apparent correlation between the severity of illness, hearing loss or subsequent recovery was found [54•,55]. The mouse models used for these experiments included Stat1$^{-/-}$ mice in order to, at least partially, recapitulate human disease. The mock infected Stat1$^{-/-}$ animals showed no observable signs of SHL including consistently normal responses to startle amplitude, no histological damage to inner ear hair cells, the auditory spiral ganglion neurons or cochlea and no infiltrate of CD3 lymphocytes [54•]. Furthermore, another study demonstrated that ANCA-positive immune-mediated systemic vasculitis may contribute to sensory-neural hearing loss in Rhesus NHP model suggests that humoral factors may be involved [55]. How much these humoral factors are disrupted in the IFN signalling-deficient mouse models or their contribution to human disease remains to be determined.

Viral induced host immune response mechanisms causing idiopathic SHL has also been suggested through a prospective study involving 222 human patients [56]. Additionally, immunogenic pathology to the inner ear has been proposed through *in vitro* analysis [57,58]. Here researchers showed that Schwann cells were targeted by LASV in the peripheral nervous system. However, the fact that inactive LASV binding to Schwann cells results in altered myelin sheath formation which can affect nerve conduction velocities and auditory functions suggests mechanisms other than direct viral cytopathology.

The central nervous system (CNS) manifestations are also seen in NHP models. Multiple strains of LASV were able to produce meningoencephalitis in the frontal lobe, cerebellum and brainstem [10•]. Another study found 7 of 9 examined monkeys had lymphocytic cuffing of blood vessels within the brain, spinal cord, and meninges. A small percentage of the NHP's also presented with inflammatory infiltrate in the spinal ganglia and choroid plexus, and on the one eye sampled mild choroidoretinitis was observed [29].

Cardiovascular/respiratory

After 4–7 days of illness, patients may develop cardiovascular and respiratory symptoms that include hypotension, interstitial pneumonia, retrosternal pain and edema in the face, neck and pulmonary system [31,59,60]. Electrocardiograms from patients with LF demonstrate abnormalities including nonspecific ST and T wave changes in over 2/3 of cases [61]. Myocarditis and pericardial effusion have been observed in some cases of LF [62–64]. Additionally, in a study of 23 hospitalized LF cases in Nigeria during January and February of 1970, 6 patients were found to have cardiac failure, which was suspected to be the cause of death in the majority [65]. Animal model data has also suggested cardiac inotropy may be negatively affected during LF infection [66].

Human cases of myocarditis associated with LF infection have been observed [62,63]. Additionally NHP studies have confirmed that LASV is myocardiotropic, resulting in pulmonary and coronary arteritis as well as perivascular myocarditis [30]. One study reported finding heart lesions with inflammatory cell infiltrates in the epicardium and myocardium in 6 of 9 NHPs infected with Josiah LASV [29]. Although there is little evidence outside of one Macaque study, left ventricular heart failure may play a role in the development of pulmonary edema found in NHP's experimentally infected with LASV [10•].

The lung also appears to be a substantial target of viral infection, as noted by high viral titers in lung tissues, although these lesions do not seem to correlate with substantial morbidity/mortality [29]. In the rhesus macaque model, virus was seen to accumulate in pulmonary macrophages and alveolar septa and the researchers speculated that the mechanism of pulmonary injury is likely the result of capillary leak. This is evidenced by pulmonary arteritis, necrosis of the alveolar capillary endothelium, associated alveolar edema, fibrin deposition and pleural effusion. Human pulmonary manifestations, including pulmonary interstitial edema, interstitial pneumonia have been reported [63]. A study using NHP's to determine differences in disease manifestations and pathogenesis between LASV isolates found that the Malian isolate, concluded to be less pathogenic, caused the most extensive pulmonary symptoms. This includes pulmonary infiltrates, diffuse pneumonia, and pulmonary edema [10•]. Another study found that interstitial pneumonia was a consistent finding, however varying in severity throughout all NHP's infected with LASV (Josiah strain) and characterized by alveolar wall thickening and varying amounts of edematous fluid [29]. Additionally, LASV infection in an inbred guinea pig model caused interstitial pneumonia along with necrotic and apoptotic cells, congestion, hemorrhage and interstitial edema [67•].

Hematology

Although there is disruption in specific hematologic cell lines and functions, bleeding dyscrasia is not a major factor in LF pathogenesis. Early in the course of infection, patients may manifest mild leukopenia with lymphopenic predominance and subsequently may go on to develop leukocytosis with neutrophilic predominance [33,46,59,68–71]. It is unknown if this is from bone marrow production of new neutrophils (bandemia) or if this represents demarginalization of non-circulating segmented neutrophils secondary to a stress response. Mild thrombocytopenia has also been observed; however, it rarely falls below 100 000/μL [72]. A few patients with severe LF may have significant bleeding diatheses secondary to platelet dysfunction, which may be due to an inhibitor of platelet aggregation as well as slightly lowered numbers [68,72]. In contrast to other VHFs, disseminated intravascular coagulation (DIC) does not significantly contribute to pathogenesis, as even in severely ill patients prothrombin and partial thromboplastic times are usually normal [73]. It has been noted that patients with LF will also show raised hematocrit levels, again suggesting hemoconcentration through increased membrane fluid losses [25].

Diagnostics

Although approximately 80% of the infected population show no observable symptoms, early detection of disease in individuals experiencing symptoms is imperative in treating and preventing disease spread. The large range of manifestations and non-pathognomonic signs and symptoms of LF can hamper the clinical diagnosis [74]. Some diagnostic challenges exist with LASV including the need for high containment facilities for diagnostics and assay development/ validation. Furthermore, diagnostic testing is often limited in endemic areas due to lack of resources and this difficulty is compounded by substantial LASV diversity amongst isolates even within an outbreak. In addition, co-infections with Malaria (*Plasmodium faviparum)* parasites are commonly seen in endemic areas as are other febrile illnesses, which can add difficulty to diagnosing LF [75].

Historically, virus culture has been considered the 'gold standard' for diagnostic testing. LASV may be cultured from blood, urine, or throat washings, but these are not routine clinical diagnostic tools, and a second confirmatory method such as qPCR or electron microscopy must

still be completed [34•,76]. Viral culture is, however, a slow method and requires an available containment level 4 laboratory limiting its utility for diagnostics [76]. More recently, a lateral flow assay using recombinant LASV (ReLASV) was developed and is the first potential rapid diagnostic test for LASV infection which has shown promise in research trials [32•].

Real-time RT-PCR has been used for LF and other VHFs due to its relatively quick turn-around time, early detection capabilities, the capacity to process a large numbers of samples in a single run, and the ability to multiplex reactions for differential diagnostic testing [77–79]. However, the high degree of sequence diversity can be problematic, even within an outbreak, as a single base mismatch may affect sensitivity [80]. While such considerations are worse in Primer-Probe based assays, more traditional, DNA-binding dye RT-PCR reactions (e.g. SYBR green) may circumvent these issues until sequences are obtained and primer-probe design is optimized. In addition, there is some cross-reactivity between LASV, LCMV, and New World arenaviruses such as Tacaribe virus [81]. Sensitivity and specificity comparisons between immunodiagnostic assays and qPCR in LASV diagnostics have been completed, and it was found that the sensitivity of qPCR was lower than that of immunoassays [32•].

The diagnosis of LF can also be established via serum enzyme-linked immunosorbent serologic assay (ELISA), which can detect IgM and IgG antibodies and LASV antigen offering early diagnostic potential as well as prognostic information [74,82]. Typically, serum IgM is detectable 10–21 days after symptom onset and serum IgG is detectable approximately 21 days after symptom onset [74,83,84]. In one study, LASV serology had sensitivity and specificity of 88% and 90%, respectively [74]. A last alternative includes a post-mortem diagnosis established via immunohistochemistry performed on formalin-fixed tissue specimens [82]

Treatment

Historically, LF patients have been treated with supportive care therapy often in combination with ribavirin [27]. The first experimental evidence of ribavirin's efficacy was provided by a rhesus macaque model, and although none of the ribavirin-treated animals died, the most beneficial effects required very early intramuscular injections (before the onset of illness) [28]. A single clinical trial from Sierra Leone using IV ribavirin showed lower case-fatality when provided in the first six days of fever onset compared with those treated seven days or more after onset [47,85]. Although oral ribavirin has been shown to improve survival rates it was shown to be less effective than the IV form [47]. In addition, some have advocated for post-exposure prophylaxes with oral ribavirin, although efficacy data and exact indications for this use are lacking [27,85].

Ribavirin is well known to cause serious side-effects and adverse events, including coronary ischemia, hemolytic anemia, and has significant teratogen and/or embryocidal effects in animal models [26,47,86–88]. Additionally, the success rate of ribavirin still relies on the availability and procurement of the drug, as well as correct and timely diagnosis [16•,47,89]. With the cost and need for IV administration empiric therapy for LF with ribavirin are more disadvantageous qualities [74].

Favipiravir, a new broad-spectrum antiviral drug with targeted specificity against RNA viruses, has recently been shown to be effective in a cynomolgus macaque model of LF disease [90]. Favipiravir may also be associated with adverse events including transient thrombocytopenia, elevated transaminases and/or lipemia. It has been used in combination with ribavirin to successfully treat two recent epidemiologically linked cases in the USA and Germany [91].

Recently, there have been improvements made on developing vaccine candidates for LASV, most of which are still in preclinical stages. One of the leading pre-clinical candidates includes the replication competent recombinant vesicular stomatitis virus, VSVΔG-LASVGPC [6,92]. The recombinant vaccine is attenuated, safe and highly immunogenic and has shown 100% protection in a lethal NHP model [93,94]. This vaccine candidate has also recently been shown to be effective against genetically and geographically distinct LASV isolates in both inbred and outbred guinea pig models [6]. Another pre-clinical candidate includes the reassortant vaccine platform, ML29, encoding the NP and GPC proteins from LASV as well as the Z and L proteins from the non-pathogenic Mopeia virus (MOPV) with additional attenuation mutations [93,95,96]. Initial guinea pig studies showed complete protection against LASV-Josiah strain and a LASV isolate from Nigeria (LASV-803213/NIG). ML29 has shown additional protection in a Marmoset model [96,97].

Additionally, there is a promising DNA candidate vaccine, INO-4500, administered through intradermal electroporation which showed 100% protection against NHPs challenged with lethal doses of Josiah strain LASV [98,99]. INO-4500 has recently moved into phase 1 clinical trials. The phase 1 randomized double-blind placebo-control trial, which is still in the recruitment stage, consists of 60 participants, evaluating outcomes of adverse events, and immunological profiles including antibody titers, neutralizing antibodies and interferon-gamma response magnitude [100•].

Conclusion

Since its discovery in 1969, LF has caused a significant public health burden still endured in endemic areas today. Transmission of the disease is most often a result of zoonotic spillover events from the reservoir host, which

results in illness in approximately 20% of individuals. Those that experience acute infection initially present with clinical manifestations that are often indistinguishable from other febrile illnesses thereby causing delayed diagnosis [4]. Such delays can result in failure to isolate patients, increasing the chance of human-to-human transmission (usually nosocomial), as well as a reduced chance of effective anti-viral treatment. A portion of patients suffering from acute illness will progress to serious disease with hepatic, pulmonary, cardiovascular, renal, hematologic and neurological symptoms. Increased research is required to understand the pathophysiology of LF, which would allow for the development of better antivirals and more targeted disease altering medications. Diagnostic methods have improved with new rapid testing; however, it is still limited in endemic areas and complicated by Lassa genetic and antigenic diversity [32•]. There is still no approved vaccine or prophylaxis for LF limiting the opportunity for control and prevent disease; however, recent research has resulted in potential vaccines showing promising results in pre-clinical and clinical studies [6,92].

Conflict of interest statement

Nothing declared.

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References

1. Hallam HJ, Hallam S, Rodriguez SE, Barrett ADT, Beasley DWC, Chua A, Ksiazek TG, Milligan GN, Sathiyamoorthy V, Reece LM: **Baseline mapping of Lassa fever virology, epidemiology and vaccine research and development review-article**. *NPJ Vaccines* 2018, **3**.

2. Safronetz D, Sogoba N, Diawara SI, Bane S, Rosenke K, Maiga O, Boisen M, Garry RF, Branco LM, Lindsay LR *et al.*: **Annual incidence of Lassa virus infection in Southern Mali**. *Am J Trop Med Hyg* 2017, **96**:944-946.

3. • Okokhere P, Colubri A, Azubike C, Iruolagbe C, Osazuwa O, Tabrizi S, Chin E, Asad S, Ediale E, Rafiu M *et al.*: **Clinical and laboratory predictors of Lassa fever outcome in a dedicated treatment facility in Nigeria: a retrospective, observational cohort study**. *Lancet Infect Dis* 2018, **18**:684-695

Okokhere *et al.* presented clinical laboratory data for 291 Lassa Fever patients in Nigeria from 2011–2015, which is the largest clinical dataset on Lassa Fever in Nigeria to date. They describe renal and hepatic indicators of injury laboratory values that can be used for prognostic information.

4. Dahmane A, van Griensven J, Van Herp M, Van den Bergh R, Nzomukunda Y, Prior J, Alders P, Jambai A, Zachariah R: **Constraints in the diagnosis and treatment of Lassa Fever and the effect on mortality in hospitalized children and women with obstetric conditions in a rural district hospital in Sierra Leone**. *Trans R Soc Trop Med Hyg* 2014, **108**:126-132.

5. Fischer WA, Wohl DA: **Moving Lassa fever research and care into the 21st century**. *J Infect Dis* 2017, **215**:1779-1781.

6. Stein DR, Warner BM, Soule G, Tierney K, Frost KL, Booth S, Safronetz D: **A recombinant vesicular stomatitis-based Lassa fever vaccine elicits rapid and long-term protection from lethal Lassa virus infection in guinea pigs**. *NPJ Vaccines* 2019, **4**:1-7.

7. •• Fullah M, Stremlau M, Vitti JJ, Andersen KG, Birren B, Fitzgerald M, Ryan EM, McCormick JB, Lander ES, Broodie N *et al.*: **Clinical sequencing uncovers origins and evolution of Lassa virus**. *Cell* 2015, **162**:738-750

Through genomic analysis on LF patient clinical samples from 2008 to 2013, *M. natalensis* field samples and laboratory isolates, Fullah *et al.* were able to identify that human LASV infections are most often a result of independent transmission from the reservoir host.

8. Cosset F-L, Marianneau P, Verney G, Gallais F, Tordo N, Pecheur E-I, ter Meulen J, Deubel V, Bartosch B: **Characterization of Lassa virus cell entry and neutralization with Lassa virus pseudoparticles**. *J Virol* 2009, **83**:3228-3237.

9. Safronetz D, Sogoba N, Lopez JE, Maiga O, Dahlstrom E, Zivcec M, Feldmann F, Haddock E, Fischer RJ, Anderson JM *et al.*: **Geographic distribution and genetic characterization of Lassa virus in Sub-Saharan Mali**. *PLoS Negl Trop Dis* 2013, **7**:4-12.

10. • Safronetz D, Strong JE, Feldmann F, Haddock E, Sogoba N, Brining D, Geisbert TW, Scott DP, Feldmann H: **A recently isolated Lassa virus from Mali demonstrates atypical clinical disease manifestations and decreased virulence in cynomolgus macaques**. *J Infect Dis* 2013, **207**:1316-1327

Safronetz *et al.* used both an inbred guinea pig model and a non-human primate model to explore a new Lassa Virus isolate from Mali in comparison to isolated from Sierra Leone and Liberia for virulence and pathogenesis. This article reports new and more detailed findings in cardiovascular and pulmonary pathogenesis of Lassa Fever in animal models that had not been previously reported.

11. Richmond JK, Baglole DJ: **Lassa fever: epidemiology, clinical features, and social consequences**. *BMJ* 2003, **327**:1271-1275.

12. McCormick JB, Webb PA, Krebs JW, Johnson KM, Smith ES: **A prospective study of the epidemiology and ecology of Lassa fever**. *J Infect Dis* 1987, **155**:437-444.

13. Leski TA, Stockelman MG, Moses LM, Park M, Stenger DA, Ansumana R, Bausch DG, Lin B: **Sequence variability and geographic distribution of Lassa virus**. *Sierra Leone* 2015, **21**.

14. Bowen MD, Rollin PE, Ksiazek TG, Hustad HL, Bausch DG, Demby AH, Bajani MD, Peters CJ, Nichol ST: **Genetic diversity among Lassa virus strains**. *J Virol* 2000, **74**:6992-7004.

15. Ogoina D: **Lassa fever: a clinical and epidemiological review**. *Niger Delta J Med Med Res* 2013, **1**:1-10.

16. • Kofman A, Choi MJ, Rollin PE: **Lassa fever in travelers from West Africa, 1969–2016**. *Emerg Infect Dis* 2019, **25**:236-239

The paper cited above examined imported cases of LF of travellers from endemic West Africa. It is highlighted that the number of secondary transmissions of Lassa Fever are extremely low and often a result of improper personal protective equipment use when dealing directly with infected bodily fluids.

17. Bhadelia N: **Understanding Lassa fever**. *Science (80-)* 2019, **363**:30.

18. • Kafetzopoulou LE, Pullan ST, Lemey P, Suchard MA, Ehichioya DU, Pahlmann M, Thielebein A, Hinzmann J, Oestereich L, Wozniak DM *et al.*: **Metagenomic sequencing at the epicenter of the Nigeria 2018 Lassa fever outbreak**. *Science (80-)* 2019, **363**:74-77

Kafetzopuoulou *et al.* used metagenomic nanopore sequencing on 120 samples from the 2018 Nigerian Lassa Virus outbreak to look if this outbreak is a result of increased human-to-human transmission. Their results follow previous conclusions that rodent zoonotic spillover is still the main source of individual infections rather than human-to-human transmission events.

19. Rosenke K, Safronetz D, Feldmann H: **Biomarkers for Lassa fever outcome?** *Ann Infect* 2018, **1**:1.

20. Bonner PC, Schmidt W-P, Belmain SR, Oshin B, Baglole D, Borchert M: **Poor housing quality increases risk of rodent infestation and Lassa fever in refugee camps of Sierra Leone**. *Am J Trop Med Hyg* 2007, **77**:169-175.

21. Centre for Disease Control and Prevention: *What You Need to Know About Lassa Fever*. 2019.

22. Fisher-Hoch SP, Tomori O, Nasidi A, Perez-Oronoz GI, Fakile Y, Hutwagner L, Mccormick JB: **Review of cases of nosocomial**

Lassa fever in Nigeria: the high price for poor medical practice. *BMJ* 1995, **311**:857-859.

23. Carey DE, Kemp GE, White HA, Pinneo L, Addy RF, Fom ALMD, Stroh G, Casals J, Henderson BE: **Lassa fever epiemiological aspects of the 1970 epidemic, Jos, Nigeria**. *Trans R Soc Trop Med Hyg* 1972, **66**:402-408.

24. Monath TP, Mertens PE, Patton R, Moser CR, Baum JJ, Pinneo L, Gary GW, Kissling RE: **A hospital epidemic of Lassa fever in Zorzor, Liberia, March-April 1972**. *Am J Trop Med Hyg* 1973, **22**:773-779.

25. Safronetz D, Robertson SJ, Prescott JB, Marzi A, Best SM, Feldmann H: **Immunobiology of Ebola and Lassa virus infections**. *Nat Rev Immunol* 2017, **17**:195-207.

26. Price ME, Fisher-hoch SP, Craven RB, Mccormick JB: **A prospective study of maternal and fetal outcome in acute Lassa fever infection during pregnancy**. *BMJ* 1988, **297**:584-587.

27. Bausch DG, Hadi CM, Khan SH, Lertora JJL: **Review of the literature and proposed guidelines for the use of oral ribavirin as postexposure prophylaxis for Lassa fever**. *Clin Infect Dis* 2010, **51**:1435-1441.

28. Jahrling PB, Hesse RA, Eddy GA, Johnson KM, Callis RT, Stephen EL: **Lassa virus infection of rhesus monkeys: pathogenesis and treatment with ribavirin**. *J Infect Dis* 1980, **141**:580-589.

29. Callis RT, Jahrling PB, DePaoli A: **Pathology of Lassa virus infection in the rhesus monkey***. *Am J Trop Med Hyg* 1982, **31**:1038-1045.

30. Walker DH, McCormick JB, Johnson KM, Webb PA, Komba-kono G, Elliot LH, Gardner JJ: **Pathologic and virologic study of fatal Lassa fever in man**. *Am J Pathol* 1982, **107**:349-356.

31. Khan SH, Goba A, Chu M, Roth C, Healing T, Marx A, Fair J, Guttieri MC, Ferro P, Imes T *et al.*: **New opportunities for field research on the pathogenesis and treatment of Lassa fever**. *Antiviral Res* 2008, **78**:103-115.

32. • Boisen ML, Hartnett JN, Shaffer JG, Goba A, Momoh M, Sandi JD, Fullah M, Nelson DKS, Bush DJ, Rowland MM *et al.*: **Field validation of recombinant antigen immunoassays for diagnosis of Lassa fever**. *Sci Rep* 2018, **8**:1-14

This article published by Boisen *et al.* describes the development and testing of a highly sensitive and specific field immunoassay for rapid diagnoses of LF. The authors explain that the development of this assay could assist in hospitals in endemic areas, in specific maternity and surgical wards, to limit transmission.

33. Frame JD: **Clinical features of Lassa fever in Liberia**. *Viruses* 1989, **11**:S783-S789.

34. • Okokhere PO, Erameh CO, Alikah F, Akhideno PE, Iruolagbe CO, Osazuwa OO, Bankole IA, Adomeh DI, Odia I, Aire CO *et al.*: **Acute Lassa virus encephalitis with Lassa virus in the cerebrospinal fluid but absent in the blood: a case report with a positive outcome**. *Case Rep Neurol* 2018, **10**:159-164

This article by Okokhere *et al.* is a case report of a patient presenting with severe central nervous system (CNS) complications and found to have positive LASV RNA in the cerebral spinal fluid. This case shows important findings including that LASV should be considered as a differential diagnosis for patients with CNS complications and that ribavirin therapy may help with CNS LASV cases.

35. Russier M, Pannetier D, Baize S: **Immune responses and Lassa virus infection**. *Viruses* 2012, **4**:2766-2785.

36. Baize S, Marianneau P, Loth P, Reynard S, Journeaux A, Chevallier M, Tordo N, Deubel V, Contamin H: **Early and strong immune responses are associated with control of viral replication and recovery in Lassa virus-infected cynomolgus monkeys**. *J Virol* 2009, **83**:5890-5903.

37. Hensley LE, Smith MA, Geisbert JB, Fritz EA, Daddario-DiCaprio KM, Larsen T, Geisbert TW: **Pathogenesis of Lassa fever in cynomolgus macaques**. *Virol J* 2011, **8**:205.

38. Liu DX, Perry DL, Evans DeWald L, Cai Y, Hagen KR, Cooper TK, Huzella LM, Hart R, Bonilla A, Bernbaum JG *et al.*: **Persistence of Lassa virus associated with severe systemic arteritis in convalescing guinea pigs (*Cavia porcellus*)**. *J Infect Dis* 2018, **3**:1-5.

39. Walker DH, Wulff H, Lange JV, Murphy FA: **Comparative pathology of Lassa virus infection in monkeys, guinea pigs, and *Mastomys natalensis***. *Bull World Health Organ* 1975, **52**:523-534.

40. Mateer EJ, Huang C, Shehu NY, Paessler S: **Lassa fever–induced sensorineural hearing loss: a neglected public health and social burden**. *PLoS Negl Trop Dis* 2018, **12**:1-11.

41. Mahanty S, Hutchinson K, Agarwal S, Mcrae M, Rollin PE, Pulendran B: **Cutting edge: impairment of dendritic cells and adaptive immunity by Ebola and Lassa viruses**. *J Immunol* 2014, **170**:2797-2801.

42. Baize S, Kaplon J, Faure C, Pannetier D, Georges-Courbot M-C, Deubel V: **Lassa virus infection of human dendritic cells and macrophages is productive but fails to activate cells**. *J Immunol* 2014, **172**:2861-2869.

43. Enria DA, Mills JN, Flick R, Bown MD, Bausch D, Shieh W, Peters CJ: **Arenavirus infection**. *Tropical Infectious Disease: Principles, Pathogens and Practice (Second Edition)*. Elsevier; 2006:734-755.

44. Carrion R, Brasky K, Mansfield K, Johnson C, Gonzales M, Ticer A, Lukashevich I, Tardif S, Patterson J: **Lassa virus infection in experimentally infected marmosets: liver pathology and immunophenotypic alterations in target tissues**. *J Virol* 2007, **81**:6482-6490.

45. Lukashevich IS, Tikhonov I, Rodas JD, Zapata JC, Yang Y, Djavani M, Salvato MS: **Arenavirus-mediated liver pathology: acute lymphocytic choriomeningitis virus infection of rhesus macaques is characterized by high-level interleukin-6 expression and hepatocyte proliferation**. *J Virol* 2003, **77**:1727-1737.

46. Schmitz H, Köhler B, Laue T, Drosten C, Veldkamp PJ, Günther S, Emmerich P, Geisen HP, Fleischer K, Beersma MFC *et al.*: **Monitoring of clinical and laboratory data in two cases of imported Lassa fever**. *Microbes Infect* 2002, **4**:43-50.

47. McCormick JB, King IJ, Webb PA, Scribner CL, Craven RB, Johnson KM, Elliot LH, Belmont-Williams R: **Lassa fever: effective therapy with ribavirin**. *N Engl J Med* 1986, **314**:20-26.

48. Asogun DA, Adomeh DI, Ehimuan J, Odia I, Hass M, Gabriel M, Ölschläger S, Becker-Ziaja B, Folarin O, Phelan E *et al.*: **Molecular diagnostics for Lassa fever at Irrua specialist teaching hospital, Nigeria: lessons learnt from two years of laboratory operation**. *PLoS Negl Trop Dis* 2012, **6**.

49. Winn WC, Walker DH: **The pathology of human Lassa fever**. *Bull World Health Organ* 1975, **52**:535-545.

50. Grahn A, Bråve A, Lagging M, Dotevall L, Ekqvist D, Hammarström H, Karlberg H, Lagerqvist N, Sansone M, Tegnell A *et al.*: **Imported Case of Lassa fever in Sweden with encephalopathy and sensorineural hearing deficit**. *Open Forum Infect Dis* 2016, **3**:ofw198.

51. Günther S, Weisner B, Roth A, Grewing T, Asper M, Drosten C, Emmerich P, Petersen J, Wilczek M, Schmitz H: **Lassa fever encephalopathy: Lassa virus in cerebrospinal fluid but not in serum**. *J Infect Dis* 2001, **184**:345-349.

52. Johnson KM, Mccormick JB, Webb PA, Smith ES, Elliot LH, King IJ: **Clinical virology of Lassa fever in hospitalized patients**. *J Infect Dis* 1987, **155**:456-464.

53. Cummins D, McCormick JB, Bennett D: **Acute sensorineural deafness in Lassa fever**. *J Am Med Assoc* 1990, **264**:2093-2096.

54. • Yun NE, Ronca S, Tamura A, Koma T, Seregin AV, Dineley KT, Miller M, Cook R, Shimizu N, Walker AG *et al.*: **Animal model of sensorineural hearing loss associated with Lassa virus infection**. *J Virol* 2016, **90**:2920-2927

The authors of the paper cited above developed the only Stat$^{-/-}$ mouse model to study the SHL associated with LASV infection. They found the mice experienced typical signs of auditory damage including dampened responses to startle amplitude, histological damage to inner ear hair cells, the auditory spiral ganglion neurons and cochlea, as well as infiltrate of CD3 lymphocytes.

55. Cashman KA, Wilkinson ER, Zeng X, Cardile AP, Facemire PR, Bell TM, Bearss JJ, Shaia CI, Schmaljohn CS: **Immune-mediated systemic vasculitis as the proposed cause of sudden-onset**

sensorineural hearing loss following Lassa virus exposure in cynomolgus macaques. *MBio* 2018, **9** pii: e01896-18.

56. Liao BS, Byl FM, Adour KK: **Audiometric comparison of Lassa fever hearing loss and idiopathic sudden hearing loss: evidence for viral cause**. *Otolaryngol Head Neck Surg* 1992, **106**.

57. Rambukkana A, Kunz S, Min J, Campbell KP, Oldstone MBA: **Targeting Schwann cells by nonlytic arenaviral infection selectively inhibits myelination**. *Proc Natl Acad Sci U S A* 2003, **100**:16071-16076.

58. Oldstone MBA, Campbell KP: **Decoding arenavirus pathogenesis: essential roles for alpha-dystroglycan-virus interactions and the immune response**. *Virology* 2011, **411**:170-179.

59. King IJ, Trippel S, Tong TC, Johnson KM, Webb PA, O'Sullivan R, McCormick JB, Smith ES: **A case-control study of the clinical diagnosis and course of Lassa fever**. *J Infect Dis* 1987, **155**:445-455.

60. Mccormick JB, King IJ, Webb PA, Johnson KM, O'Sullivan R, Smith ES, Trippel S, Tong TC: **A case-control study of the clinical diagnosis and course of Lassa fever**. *J Infect Dis* 1987, **155**:445-455.

61. Cummins D, Bennett D, Fisher-Hoch SP, Farrar B, Mccormick JB: **Electrocardiographic abnormalities in patients with Lassa fever**. *J Trop Med Hyg* 1989, **92**:350-355.

62. Frame JD, Baldwin JMJ, Gocke DJ, Troup JM: **Lassa fever, a new virus disease of man from West Africa. I. Clinical description and pathological findings**. *Am J Trop Med Hyg* 1970, **19**:670-676.

63. Hirabayashi Y, Oka S, Goto H, Shimada K, Kurata T, Fisher-Hoch SP, Mccormick JB: **An imported case of Lassa fever with late appearance of polyserositis**. *J Infect Dis* 1988, **158**:872-875.

64. Yanase O, Motomiya T, Watanabe K, Tokuyasu Y, Sakurada H, Tejima T, Hiyoshi Y, Sugiura M, Yabata Y, Kitazumi H: *Lassa Fever Associated with Effusive Constrictive Pericarditis And Bilateral Atrioventricular Annular Constriction: A Case Report*. 1990.

65. White HA: **Lassa fever A study of 23 hospital cases**. *Trans R Soc Trop Med Hyg* 1972, **66**:390-398.

66. Qian C, Jahrling PB, Peters CJ, Liu CT: **Cardiovascular and pulmonary responses to Pichinde virus infection in strain 13 guinea pigs**. *Lab Anim Sci* 1994, **44**:600-607.

67. • Bell TM, Shaia CI, Bearss JJ, Mattix ME, Koistinen KA, Honnold SP, Zeng X, Blancett CD, Donnelly GC, Shamblin JD *et al.*: **Temporal progression of lesions in guinea pigs infected with Lassa virus**. *Vet Pathol* 2017, **54**:549-562

The authors of the above paper used a strain 13 guinea pig model in a serial sacrifice study to determine the sequence and progression of LASV infection in the body using various inoculation routes. They found that LASV infection in the guinea pig model is the same regardless of inoculation route and follows closely to the disease progression in man.

68. Fisher-Hoch SP, Mccormick JB, Sasso D, Craven RB: **Hematologic dysfunction in Lassa fever**. *J Med Virol* 1988, **26**:127-135.

69. Mertens PE, Patton R, Baum JJ, Monath TP: **Clinical presentation of Lassa fever cases during the hospital epidemic at Zorzor, Liberia, March-April 1972**. *Am J Trop Med Hyg* 1973, **22**:780-784.

70. Monath TP, Maher M, Casals J, Kissling RE, Cacciapuoti A: **Lassa fever in the eastern province of Sierra Leone, 1970–1972 II. Clinical observations and virological studies on selected hospital cases**. *Am J Trop Med Hyg* 1974, **23**:1140-1149.

71. Monson MH, Frame JD, Jahrling PB, Alexander K: **Endemic Lassa fever in Liberia. I. Clinical and epidemiological aspects at Curran Lutheran Hospital, Zorzor, Liberia**. *Trans R Soc Trop Med Hyg* 1984, **78**:549-553.

72. Cummins D, Fisher-hoch SP, Walshe KJ, Mackie IJ, Mccormick JB, Bennett D, Perez G, Farrar B, Machin SJ: **A plasma inhibitor of platelet aggregation in patients with Lassa fever**. *Br J Haematol* 1989, **72**:543-548.

73. Lange JV, Mitchell SW, Mccormick JB, Walker DH, Evatt BL, Ramsey RR: **Kinetic study of platelets and fibrinogen in Lassa virus-infected monkeys and early pathologic events in Mopeia virus-infected monkeys**. *Am J Trop Med Hyg* 1985, **34**:999-1007.

74. Bausch DG, Rollin P, Demby AH, Coulibaly M, Kanu J, Conteh AS, Wagoner KD, McMullan LK, Bowen MD, Peters CJ: **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**:2670-2677.

75. Okokhere P, Asogun D, Okogbenin SA: **The effect of malaria on the outcome of Lassa Fever**. *Int J Infect Dis* 2010, **14**:e333.

76. Raabe V, Koehler J: **Laboratory diagnosis of Lassa fever**. *J Clin Microbiol* 2017, **55**:1629-1637.

77. Trombley AR, Wachter L, Garrison J, Buckley-Beason VA, Jahrling J, Hensley LE, Schoepp RJ, Norwood DA, Goba A, Fair JN *et al.*: **Short report: comprehensive panel of real-time TaqMan™ polymerase chain reaction assays for detection and absolute quantification of filoviruses, arenaviruses, and new world hantaviruses**. *Am J Trop Med Hyg* 2010, **82**:954-960.

78. Safronetz D, Lopez JE, Sogoba N, Traore' SF, Raffel SJ, Fischer ER, Ebihara H, Branco L, Garry RF, Schwan TG *et al.*: **Detection of Lassa virus, Mali**. *Emerg Infect Dis* 2010, **16**:1123-1126.

79. Nikisins S, Rieger T, Patel P, Müller R, Günther S, Niedrig M: **International external quality assessment study for molecular detection of Lassa virus**. *PLoS Negl Trop Dis* 2015, **9**:1-9.

80. Stadhouders R, Pas SD, Anber J, Voermans J, Mes THM, Schutten M: **The effect of primer-template mismatches on the detection and quantification of nucleic acids using the 5′ nuclease assay**. *J Mol Diagnostics* 2010, **12**:109-117.

81. Monath TP, Newhouse VF, Kemp GE, Setzer HW, Cacciapuoti A: **Lassa virus isolation from *Mastomys natalensis* rodents during an epidemic in Sierra Leone**. *Science (80-)* 1974, **185**:263-265.

82. Centre for Disease Control and Prevention, Diseases NC for E and ZI, Pathology D of HCP and, Branch VSP: *Lassa Fever Diagnosis*. 2014.

83. Branco LM, Grove JN, Boisen ML, Shaffer JG, Goba A, Fullah M, Momoh M, Grant DS, Garry RF: **Emerging trends in Lassa fever: redefining the role of immunoglobulin M and inflammation in diagnosing acute infection**. *Virol J* 2011, **8**:478.

84. Wulff H, Johnson KM: **Immunoglobulin M and G responses measured by immunofluorescence in patients with Lassa or Marburg virus infections**. *Bull World Health Organ* 1979, **57**:631-635.

85. Hadi CM, Goba A, Khan SH, Bangura J, Sankoh M, Koroma S, Juana B, Bah A, Coulibaly M, Bausch DG: **Ribavirin for Lassa fever postexposure prophylaxis**. *Clin Infect Dis* 2010, **16**:2009-2011.

86. Sinclair SM, Jones JK, Miller RK, Greene MF, Kwo PY, Maddrey WC: **The ribavirin pregnancy registry: an interim analysis of potential teratogenicity at the mid-point of enrollment**. *Drug Saf* 2017, **40**:1205-1218.

87. Canonico PG: **Efficacy, toxicology and clinical applications of ribavirin against virulent RNA viral infections**. *Antiviral Res* 1985, **5**:75-81.

88. Frame JD: **Clinical features of Lassa fever in Liberia**. *Rev Infect Dis* 1989, **11**:S783-S789.

89. Jeffs B: **A clinical guide to viral haemorrhagic fevers: Ebola, Marburg and Lassa**. *Trop Doc* 2006, **36**:1-4.

90. Rosenke K, Feldmann H, Westover JB, Hanley PW, Martellaro C, Feldmann F, Saturday G, Lovaglio J, Scott DP, Furuta Y *et al.*: **Use of favipiravir to treat Lassa virus infection in Macaques**. *Emerg Infect Dis* 2018, **24**:1696-1699.

91. Raabe VN, Kann G, Ribner BS, Morales A, Varkey JB, Mehta AK, Lyon GM, Vanairsdale S, Faber K, Becker S *et al.*: **Favipiravir and ribavirin treatment of epidemiologically linked cases of Lassa fever**. *Clin Infect Dis* 2017, **65**:855-859.

92. Marzi A, Feldmann F, Geisbert TW, Feldmann H, Safronetz D: **Vesicular stomatitis virus-based vaccines against Lassa and Ebola viruses**. *Emerg Infect Dis* 2015, **21**:305-307.

93. Warner BM, Safronetz D, Stein DR: **Current research for a vaccine against Lassa hemorrhagic fever virus**. *Drug Des Devel Ther* 2018, **12**:2519-2527.

94. Geisbert TW, Jones S, Fritz EA, Shurtleff AC, Geisbert JB, Liebscher R, Grolla A, Ströher U, Fernando L, Daddario KM *et al.*: **Development of a new vaccine for the prevention of lassa fever**. *PLoS Med* 2005, **2**:e183.

95. Moshkoff DA, Salvato MS, Lukashevich IS: **Molecular characterization of a reassortant virus derived from Lassa and Mopeia viruses**. *Virus Genes* 2007, **34**:169-176.

96. Lukashevich IS, Carrion RJ, Salvato MS, Mansfield K, Brasky K, Zapata JC, Cairo C, Goicochea M, Hoosien GE, Ticer A *et al.*: **Safety, immunogenicity, and efficacy of the ML29 reassortant vaccine for Lassa fever in small non-human primates**. *Vaccine* 2008, **26**:5246-5254.

97. Carrion R, Patterson JL, Johnson C, Gonzales M, Moreira CR, Ticer A, Brasky K, Hubbard GB, Moshkoff D, Zapata J *et al.*: **A ML29 reassortant virus protects guinea pigs against a distantly related Nigerian strain of Lassa virus and can provide sterilizing immunity**. *Vaccine* 2007, **25**:4093-4102.

98. Cashman KA, Broderick KE, Wilkinson ER, Shaia CI, Bell TM, Shurtleff AC, Spik KW, Badger CV, Guttieri MC, Sardesai NY *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**:262-277 http://dx.doi.org/10.3390/vaccines1030262.

99. Cashman KA, Wilkinson ER, Shaia CI, Facemire PR, Bell M, Bearss JJ, Shamblin JD, Wollen SE, Kate E, Sardesai NY *et al.*: **A DNA vaccine delivered by dermal electroporation fully protects cynomolgus macaques against Lassa fever**. *Hum Vaccin Immunother* 2017, **13**:2902-2911.

100. U.S. National Library of Medicine: *Safety, Tolerability and Immunogenicity of INO-4500 in Healthy Volunteers*. 2019

• This is the first vaccine against LASV to enter stage 1 clinical trials. It is currently in the recruiting stage for the randomized double-blind trial to determine adverse events, neutralizing antibody titers and immune response magnitude.