Invited review/chapter- Infectious Disease Clinics of North America

Chapter 6

Title:

Lassa Fever – Epidemiology, Clinical Features, Diagnosis, Management and Prevention.

Running title: Lassa Fever

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KEYPOINTS

- Lassa fever (LF) is an acute zoonotic disease of humans endemic to West Africa, caused by the Lassa virus (LASV), an enveloped, single-stranded RNA arenavirus.
- First discovered in 1969 in Nigeria, Lassa fever outbreaks continue in West Africa with upto 500,000 cases of LF annually with 10,000 deaths. Case fatality rates in hospitalised patients is upto 50%.
- Primary infection of humans occurs from contact with LASV-infected rodents. Secondary person to person transmission occurs and can be prevented by instituting strict infection control measures.
- The incubation period ranges from 2–21 days. Initial presentation of LF is difficult to distinguish from other febrile illnesses.
- LF presents with a wide spectrum of clinical manifestations from the asymptomatic, mild, moderate to severe fulminant haemorrhagic disease.
- Treatment involves supportive care with appropriate fluid and electrolyte balance, oxygenation, organ support and specific antiviral treatment with Ribavirin or Favipiravir. Vaccines are under development.

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SUMMARY

Fifty years after its first discovery in 1969 in Nigeria, Lassa fever outbreaks continue in West Africa. Annually, an estimated 300,000 to 500,000 cases of LF occur in west Africa with upto 5 to 10,000 deaths. Travel associated LF cases outside West Africa have been recorded in the USA, Canada, United Kingdom, Netherlands, Israel, Sweden and Germany. Primary infection of humans occurs from contact with Lassa virus (LASV)-infected rodents and exposure to their excreta (urine or faeces) or blood or meat. Secondary person to person transmission in humans has been recorded in people living in the rural communities, and within hospitals where proper infection prevention and control practices are inadequate. Sexual transmission occurring months after recovery from acute disease can occur. The incubation period of LF ranges from 2–21 days. Symptoms of Lassa fever are difficult to distinguish from malaria, typhoid, dengue, yellow fever and other viral haemorrhagic fevers. LF presents with a wide spectrum of clinical manifestations from the asymptomatic, mild to severe fulminant disease. Upto 80% of LF cases have non-specific symptoms and may remain undiagnosed. In severe cases multi-organ failure, with disseminated intravascular coagulation, and bleeding from mucosa of all organs occurs. Neurological involvement leads to fits, tremors, gait disturbance, disorientation and loss of consciousness and sensorineural deafness, and abortion. Treatment with Ribavirin can improve treatment. Whilst the overall mortality is between 1% to 15%, the mortality in hospitalised patients is much higher up to 70%. Lassa Fever should be considered in the differential diagnosis of anyone returning from travel to West Africa. There is an urgent need for rapid field-friendly diagnostics and preventive vaccine.

INTRODUCTION

Lassa fever (LF), is an acute zoonotic disease of humans that is mainly endemic to West African countries of Guinea, Liberia, Nigeria, and Sierra Leone.[1-3] Other countries such as Mali, Benin, Togo, Cote d'Ivoire, Burkina Faso, and Ghana have reported sporadic LF cases.[1,5] LF is caused by the Lassa fever virus (LSAV), one of several viral causes of 'haemorrhagic fever' which can result in severe life-threatening systemic illness, characterized by disseminated intravascular coagulation, widespread mucosal bleeding, multi-organ failure and shock requiring advanced life support.[6] Fifty years after its first discovery in 1969 in Nigeria, LF outbreaks have continued in West Africa and LF is now on the WHO Blueprint list of priority pathogens under its Research and Development blue print for action.[7,8] Travel associated LF cases been recorded in several countries outside West Africa including the USA, Canada, United Kingdom, Netherlands, Israel, Sweden and Germany, creating much media hype.

HISTORICAL

Lassa fever first attracted global attention when missionary nurses in Nigeria developed a mysterious febrile illness in 1969.[9-12] They are thought to have acquired LASV infection while working in the mission station in the town of Lassa in the State of Borno, north-eastern Nigeria. The nurses were evacuated to ECWA Hospital in Jos for further treatment. Two of three missionary nurses at ECWA Hospital died, and a doctor who performed an autopsy on one of the nurses[11-13] also fell ill and subsequently died. The third missionary nurse was flown to the United States where she was diagnosed with Lassa fever and survived. The Lassa fever virus itself was first isolated from the nurse at the Yale Arbovirus Unit, Yale School of Medicine in 1970. [11,12] The chain of Lassa virus transmission was traced back to the missionary nurses.

EPIDEMIOLOGY

Causative agent - Lassa virus (LASV):

Lassa virus (LASV) is an enveloped, single-stranded, bi-partite Ribonucleic acid (RNA) virus which belongs to the family Arenaviridae.[11,14] 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 encodes 4 proteins: the nucleoprotein (NP) and glycoprotein precursor (GPC) on the Small (S) segment, and the RNA-dependent RNA-polymerase (L) and matrix RING Zinc-finger protein (Z) on the Large (L) segment.[14,15] The LASV has a high level of nucleotide diversity between strains which is correlated to clustering of strains around geographic locations. This has led to recognition of 6 major LASV clades or lineages: I–III in Nigeria; clade IV covering the countries of Sierra Leone, Guinea, and Liberia; and clade V in southern Mali, and a more recent clade VI, originating from Togo.[16] An important feature is the ability of these strains to evolve over time. A new lineage has recently been discovered in the recent Nigerian LF outbreak and if confirmed will make a total of 7 lineages.[17,18] The high genetic variability of Lassa virus is relevant for the design of diagnostic molecular assays as well as for the development of a universal LF vaccine that can be used in different geographical settings irrespective of circulating strain.[18-22] It may also have a bearing on the clinical presentation and severity of infection.[4,16, 23-27]

***Geographical distribution*:**

Lassa fever is endemic to West Africa (**Fig.1**) with cases being reported from Nigeria, Benin, Liberia, Sierra Leone, Guinea, Mali, Senegal and Ghana.[1-4] However, the true geographical prevalence, incidence and distribution of LF has been difficult to ascertain due to a large proportion of cases being asymptomatic; the protean and non-specific wide spectrum of clinical presentations; paucity of effective surveillance systems; lack of specific point of care diagnostic tests for LF, human migration, civil unrest, deforestation, among other.[4,23-28] Exportation of travel associated Lassa fever cases outside West Africa to the USA, Canada, United Kingdom, Netherlands, Israel, and Germany by aid workers, missionaries, foreign military personnel is well documented.[29,30]

***Animal reservoir*:**

The animal reservoir for LASV is considered to be the "multimammate rat" *Mastomys natalensis* (**Fig.2a, 2b).** This is a rodent of the genus *Mastomys* which is ubiquitous in West Africa and breeds prolifically.[30-35] The rats are infected *in utero* and remain infected for the rest of their life. Rats infected with LSAV do not become ill, but they shed the virus in their urine and faeces. Whilst *M. natalensis* was considered to be the natural reservoir of LASV, but other rodent reservoirs (*M. erythroleucus* and *Hylomyscus pamfi*) discovered recently could also affect distribution of LASV and LF cases over time. [34,35]

***Mode of transmission of LASV to humans*:**

Primary infection of humans occurs from direct or indirect contact with LASV-infected rodents.[1,35,36] Persons at 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.[27,37-41] The Mastomys rodents invade homes of humans during the dry season in search of food. The source of LSAV infection for humans is exposure to urine, faeces, blood or meat from LSAV-infected Mastomys rodents. Direct contact with these materials, through touching soiled objects, eating contaminated food, or exposure to open cuts or sores, can lead to infection.[39-47] Infection is thought to occur from direct inoculation of mucous membranes or from inhalation of aerosols produced when rodents urinate or defecate. The relative frequency of these modes of transmission remains unknown.

Secondary person- to- person spread in among humans has been recorded in people living in the community in overcrowded dwellings, families in the context of providing care to a sick person and in communities in the context of burial practices. Healthcare workers are at increased risk of LASV infection. Nosocomial transmission of LASV occurs within hospitals among and between patients and healthcare workers because of poor adherence to infection prevention and control practices.[46-54] LSAV can transmit through direct contact with the blood, urine, faeces, or other bodily secretions or via accidental inoculation with sharp needles and contact with contaminated equipment.[50,51] Large healthcare facility LF outbreaks are fuelled by transmission where barrier nursing and infection control practices are inadequate.[52] Staff and other patients on maternity wards are at increased risk since Lassa fever is an important cause of spontaneous abortion and the virus is present in the blood and placenta of aborted foetuses.[56]

There have been reports of sexual transmission occurring months after recovery from acute disease.[51] Aerosol transmission between humans in natural settings has not been proven but artificial production of infectious aerosols has.[36] The 1970 LF outbreak in Nigeria[10,12] was attributed to airborne transmission from a female patient with severe pulmonary disease although definitive evidence of airborne transmission from subsequent outbreaks has not been forthcoming. Disease outbreaks appear to occur commonly through multiple independent reservoir-to-human transmissions.[17,22] The period of infectivity of patients with LF is dependent on the clinical state, with the highest infectivity periods being in late in the course of severe disease in the haemorrhagic phase.[48 52,59,60]

Age, gender and susceptibility:

Lassa fever can affect all age groups and both genders.[4,49,47] Paediatric Lassa fever is known to occur more commonly in male children for yet unknown reasons. Presenting as an acute febrile illness, the case fatality rate may approach 30% in children with generalised oedema, abdominal distension and bleeding. Genetic and immunological studies are ongoing in to provide better understanding of the pathogenesis and underlying protective mechanisms operating in LF.[57-60]

Environmental and seasonal factors and risk of transmission:

Mastomys rats live in savannah and forests of west Africa, and breed frequently, producing large numbers of offspring.[32] They rapidly colonize human homes, huts, sheds and food storage areas. Since they live in and around humans and scavenge on leftover human food items or poorly stored food, direct contact transmission is common, resulting in the relatively efficient spread from LASV infected rats to humans. The seroprevalence of LASV antibodies among people living in houses correlates with households with large numbers of rats, due to close contact with contaminated surfaces, utensils and foodstuffs [38, 39, 40,41,45, 53,61] human LASV infection may also occur when rodents are trapped and prepared for cooking and consumption, a common practice in some parts of West Africa.[38,45]

Factors which may affect the increase in LASV transmission and spill-over into human populations include seasonal changes, urbanization, environmental sanitation, deforestation and occurrence of disasters with involuntary migration.[37-39, ,61-64] This re-enforces the need for a One-Human-Environmental-Animal Health (ONE HEALTH) approach for surveillance, control, early detection of spill-over into human populations, and rapid emergency public response during outbreaks.[64,65]

LASSA FEVER OUTBREAKS (2016-2019)

Since 2016, there has been an increase in the number of reported LF cases from West Africa, especially in Nigeria, Benin and Togo.[16-20,66-68] Whilst this increase seems unlikely to be due to the emergence of a new LASV variant other factors may be playing a significant role: increased human-rodent interactions, improved case recognition, increasing awareness and availability of diagnostics and therapy, increase in surveillance, changing demographics, other environmental changes or a combination of these factors.[16,17,22] Nigeria has experienced several outbreaks with large numbers of LF cases in 2018 and 2019.[4,10,54,68] There have been LF cases reported from Benin (54 cases and 28 deaths), Togo (2 cases), Liberia (7 cases, 3 deaths) and Sierra Leone (2 cases).[1] Cases have also been reported outside West Africa, exported by travellers to Sweden[69] and Germany.[70] The case in Germany resulted in limited secondary transmission when twelve days after having been exposed to the corpse of a Lassa fever case imported from Togo, a symptomatic undertaker tested positive for LASV RNA.[70]

Out of the countries in West Africa that have reported LF outbreaks, Nigeria by far has had the largest LF disease burden[68] with 23 states reporting LF cases[54] (**Fig.3**). In Edo State, a recent spatial mapping and analysis of outbreaks supports earlier reports that some communities in the often-crowded university town of Ekpoma have geographical hotspots of LF cases.[66,67] Hotspot identification is important in planning of an effective control programs because it can reveal common environmental factor(s) causing the dense clustering of the disease in particular geographical areas.[23,71,72]

Ongoing outbreak of LF in Nigeria:

The Nigeria Centre for Disease Control (NCDC) reported an unusually large increase in Lassa fever cases in 2018, with a total of 3498 suspected cases from 1 Jan to 31 Dec 2018.[22,28,54] Of these, 633 cases were confirmed positive by laboratory testing. Public health officials were concerned that the Lassa fever outbreak in Nigeria in 2018 might be driven by previously unknown factors, or a new or more virulent Lassa virus strain. From 1st January – 24th March 2019, a total of 1,924 suspected cases were reported from 73 local government areas involving 21 States (**Fig.3**), with each state having recorded at least one confirmed LF case. Out of these, 495 were confirmed positive, with 117 deaths giving a Case Fatality Rate of 22.9% for confirmed LF cases.[68] An important challenge is to define the diversity across LSAV lineages and strains and the ability of these strains to evolve over time. Global public health authorities are concerned that the 2018-2019 LF outbreak in Nigeria might be driven by previously unknown factors, or a new or more virulent LSAV strain. Real-time analysis of 36 LASV genomes from the 2018 Nigeria LF outbreak[17] revealed that LSAV genomes appear to be drawn from a diverse range of viruses previously observed in Nigeria rather than from a single dominant strain. The extensive diversity and phylogenetic due to intermingling with previous LSAV strains suggest independent zoonotic transmission events with humans becoming infected through contact with rodent faeces or urine rather than human to human transmission.

CLINICAL FEATURES

Incubation period, Symptoms and Signs:

The incubation period of LF ranges from 2-21 days.[1,2,4] Signs and symptoms of Lassa fever manifest upto 3 weeks after primary LSAV infection. The infection to disease ratio is not known. A wide spectrum of clinical manifestations occur in patients with LF, ranging from the asymptomatic, through mild, moderate to the severe and fulminant disease (**Table 1**).[4,6,9,52] Upto to 80% of LASV human infections cause mild illness and thus LF may remain undetected and undiagnosed in the community. The onset of the disease is usually gradual, starting with non-specific symptoms of fever, general weakness, malaise and headache. After a few days, symptoms worsen and sore throat, muscle pain, chest pain, nausea, vomiting, diarrhoea, cough, arthralgia, and pain in the abdomen and back may follow. In up to one fifth of infected individuals, the disease may progress to more serious symptoms. In severe cases there may be facial swelling, petechiae and bruising, respiratory distress, hepatitis, renal failure, bleeding from the mucosa of the mouth, nose, vagina or gastrointestinal tract, fits, tremors, gait disturbance, disorientation and loss of consciousness.[4,6,9,52] Bleeding is a feature of about 30% of LF patients (**Fig.4**).[9,67,52] LF can present as an acute abdomen and should be considered as a differential diagnosis of 'febrile surgical acute abdomen' and acute appendicitis in in children in West Africa.[73,74]

Clinical Complications:

Severe cases of LF manifest bleeding from mucosal surfaces (conjunctiva, mouth and gut), disseminated intravascular coagulation, pleural or pericardial effusion, spontaneous abortion. renal failure, multi-organ failure, hypovolaemic sepsis-like shock, encephalitis, encephalopathy and bilateral or unilateral eighth-nerve deafness.[4,9,75-77] The specific pathogenesis and molecular pathways that underlie these features remain poorly understood.[24,59]

A common long-term sequelae of Lassa fever is deafness from sensorineural hearing loss.[77-80] Auditory nerve spiral ganglion degeneration and damage to cochlear hair cells and immune-mediated systemic vasculitis have been suggested as underlying causes.[80] Various degrees of deafness occur in approximately one-third of infections, and in many cases hearing loss is permanent. Severity of the disease does not appear to affect this complication and deafness has been reported in mild as well as in severe cases. Other long-term neurological complications include seizures, gait disturbances, tremors and encephalitis.

Lass Fever occurring in pregnancy can cause severe disease, high maternal death rates in the third trimester, and spontaneous abortion with an estimated 95% mortality in foetuses [56,81,82]

Mortality, risk and clinical predictors of management outcomes:

Whilst the overall mortality of LF in the community is low with only 1% of all LASV infections result in death, approximately 15% to 50% of hospitalized patients die within 14 days of onset of disease.[82,83] Pregnant women, children under 5 years, and individuals with HIV or other immunosuppressive conditions have an increased risk of death. Complications associated with poor management outcomes in hospitalised patients include acute kidney injury, liver failure, encephalopathy, seizures, reduced consciousness, disseminated intravascular coagulation with mucosal bleeding, septic shock progressing to multi-organ failure.[79,82,83] Studies of LF outbreaks in Nigeria show that patients with ≥1 feature of severe illness such as acute kidney injury, encephalopathy, shock, DIC and bleeding are associated with increased case fatality rates (**Table 1**). [20,23,75,84]

LABORATORY DIAGNOSIS

Early identification of patients with LF is crucial for maximizing the benefit of available antiviral therapy, and for instituting infection control measures. Identifying the causative microbial cause of an acute febrile illness in sub-Saharan Africa can be challenging diagnostically.[72] Since the symptoms of LF are non-specific, LF is difficult to distinguish from other common endemic microbial causes of fever such as malaria, shigellosis, typhoid fever and other viral haemorrhagic fevers such as Ebola virus disease and yellow fever, both of which are also endemic to West Africa.

Specimen collection:

Making an accurate and specific definitive diagnosis of LSAV requires tests that are available for use only in high containment laboratories.[85] Since LSAV can spread from person-to-person virus spread via bodily fluids, laboratory staff should be aware of the risk of LSAV infection when processing potentially infectious patient specimens. Poor sample handling poses a safety hazard. The WHO has issued step-by-step guidance on how to safely collect blood and other clinical samples from patients suspected to be infected with LSAV[86] and how to transport the patient samples to diagnostic reference laboratories.[87] Laboratory specimens may be hazardous and must be handled with extreme care. Ideally, every laboratory specimen for diagnosis of Lassa fever should be tested in a BSL-3 or 4 laboratory and should be treated as a highly infectious specimen. Lassa fever and other VHFs are category 4 pathogens. In outbreak situations, rapid deployment of mobile biosafety level-3 laboratories have been successfully used in the field.[88,89]

Lassa virus diagnostic tests:

A range of LASV diagnostic tests are available from cell culture, immunofluorescence assay, complement fixation tests, Enyzyme-Linked Immunosorbant Assays (ELISA) for LASV antigens and IgM antibodies, Polymerase chain reaction (PCR) with several assayd and tragets, lateral flow assays and other in house rapid tests developed by research groups.[90] Definitive testing for LASV can only be done at reference laboratories, through virus isolation by cell culture. The virus itself may be cultured in 7 to 10 days, but this procedure should only be done in a high containment laboratory (BSL-4). Active infections can also be diagnosed by LASV-specific PCR, and LASV-specific IgG or IgM antibody response or LASV antigens shed during replication.[90-92]

LASV RNA is detected using a nucleic acid amplification test, which can include techniques such as PCR, loop-mediated isothermal amplification (LAMP) and strand displacement assays. However, Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) is the gold standard for making a definitive diagnosis of LASV infection in the early stages of the LF disease. [89,90, 93, 94]

Detection of LASV antibodies and antigens can be used to complement diagnosis. These can be detected by indirect immunofluorescence assay test (IFA or IIFT), western blot (WB), rapid diagnostic test (RDT) formats or by Enzyme-linked immunosorbent serologic assays (ELISA), which detect IgM and IgG antibodies. Many laboratories use in-house LASV assays.[91] For making post-mortem diagnosis, immunohistochemistry is performed on formalin-fixed tissue specimens.

Diagnostic tests under evaluation:

A rapid immunoassay for the LASV subtypes found in Sierra Leone and a similar test that is designed to detect all strains is being assessed in Nigeria.[93,94] Emerging technologies, such as CRISPR-based specific high-sensitivity enzymatic reporter unlocking, may soon provide multiplexed and portable nucleic acid detection platform for testing for new LASV strains.[95] There remains an urgent need for field friendly, cheap, accurate and rapid diagnostic tests for outbreak investigation and patient management.[96]

Specimen type and LASV detection:

LASV can be present in several body fluid or tissues such as blood, urine, pleural fluid, semen, cerebrospinal fluid, throat swabs and sputum. Acute LASV infections detected in the CSF can be negative in blood[76] and LASV can persist in the central nervous system, urine and semen long after viral clearance in the blood.[97]

MANAGEMENT OF PATIENTS

Supportive care:

Supportive care is important and appropriate fluid and electrolyte balance, oxygenation, and blood pressure control must be maintained.[1,2,4,98] To maintain renal function dialyses is necessary (**Fig. 5**). Secondary bacterial infections should be treated with antibiotics. The treatment of patients with suspected or confirmed LSAV infections during outbreaks in dedicated LF treatment wards with facilities for enhanced supportive care including dialysis and respiratory support could reduce nosocomial case fatality and transmission rates.

***Specific anti-viral therapy*:**

Specific antiviral therapy with the antiviral agent Ribavirin can improve treatment outcome if given early in the course of illness.[98,99] However, whilst Ribavirin has been extensively used for treatment and as post-exposure prophylaxis, treatment of LF with ribavirin has been evaluated in only a single non-randomized clinical[100] and in retrospective analyses of field studies.[83] Animal and/or human studies of the efficacy of ribavirin against the multiple LASV lineages and at various stages of LF disease progression are needed, as well as assessment of different administration routes and dosing regimens.[7]

Newer therapies:

Favipiravir is another broad-spectrum RNA inhibitor that has broad-spectrum activity against RNA viruses and has been shown to decrease LASV viremia in animal models.[101] Monoclonal antibodies specific for LASV neutralization cloned from West African LF survivors[102] appear to bind to individual or combined Lassa GP protein subunits, which can potently neutralise all four LASV lineages—an early start to immunotherapeutic development. Human monoclonal antibody therapy appears to protect non-human primates against advanced Lassa fever.[103]

PREVENTION

Avoiding or reducing contact with rats:

Avoiding contact with *Mastomys* rodents can reduce the risk of primary transmission of LASV to humans.[104] Placing food away in rodent-proof containers and keeping the home and surroundings clean, as well as trapping in and around homes can help reduce rodent populations and contact with their droppings or urine. Further, educating people in high-risk areas about ways to decrease rodent populations in their homes will reduce risk of LASV infection.

Preventing nosocomial spread:

Strict adherence to standard infection prevention and control precautions is mandatory for prevention of human LSAV infection spread in health-care settings especially when caring for patients with fever of undetermined origin and suspected viral haemorrhagic fevers. These include basic hand hygiene, respiratory hygiene, use of personal protective equipment (to block splashes or other contact with infected materials), safe injection practices and safe burial practices. Health-care workers caring for patients with suspected or confirmed Lassa fever should take measures to prevent contact with the patient's blood and body fluids and contaminated surfaces or materials such as clothing and bedding. Laboratory workers should be trained to handle and process biological samples and process these in suitably equipped laboratories under maximum biological containment conditions.

When caring for patients with confirmed or suspected LF, transmission of LSAV in healthcare facilities through person-to-person contact or nosocomial routes can be avoided by following strict infection control procedures and use of VHF isolation precautions and barrier nursing methods).[105] All healthcare facilities caring for suspected or confirmed LF cases should use generic precautions include wearing protective clothing, such as masks, gloves, gowns, and goggles; using infection control measures, such as complete equipment sterilization; and isolating infected patients from contact with unprotected persons until the disease has run its course.

Vaccines:

The recurrent and increasing epidemic of Lassa fever has had major socio-economic consequences on West Africa countries making the development of effective medical counter-measures urgent. One of these measures is development of effective vaccines against LF.[106] Currently there are no effective LF vaccines. In 2017, the WHO released a Target Product Profile for LASV vaccine development, and in 2018, the US Food and Drug Administration added LF to its priority list of infections for development of preventive measures. Several vaccines are under development, including LASSARAB and an inactivated recombinant LASV. The recombinant VSV-LASV-GPC vaccine is among one of the leading candidates developed thus far and is targeted for accelerated development by The Coalition for Epidemic Preparedness Innovations (CEPI)[107] who are supporting the development of Lassa vaccine candidates.

CONCLUSIONS

Whilst malaria, typhoid fever, and many other tropical infections are much more common, the diagnosis of Lassa fever should be considered in febrile patients returning from West Africa, especially if they have had exposures in rural areas or hospitals in countries where Lassa fever is known to be endemic. Health-care workers should have a high index of clinical suspicion of the possibility of Lassa in returning travelers to Europe or USA with fever. When a patient suspected to have Lassa fever is seen, the health worker or attending physician should immediately contact local and national public health authorities for advice and to arrange for laboratory testing. There is a great need for point of-care diagnostics for detecting LF cases to enable timely isolation and treatment and for defining outbreaks more accurately.

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References

1. WHO 2019. Lassa fever. https://www.who.int/emergencies/diseases/lassa-fever/en/

2. CDC 2019. Lassa fever. https://www.cdc.gov/vhf/lassa/index.html

3. Owolabi JB, Mamah CM, Okoro CC, Iheanacho CA. Re-Emerging Human Viral Hemorrhagic Fevers: A Review. American Journal of Infectious Diseases and Microbiology. 2016 Sep 22;4(4):79-90.

4. Akpede GO, Asogun DA, Okogbenin SA, Okokhere PO. Lassa fever outbreaks in Nigeria. Expert Rev Anti Infect Ther. 2018 Sep;16(9):663-666

5. Mylne AQ, Pigott DM, Longbottom J, Mapping the zoonotic niche of Lassa fever in Africa. Trans R Soc Trop Med Hyg 2015 Aug;109(8):483-92

6. Ippolito G, Feldmann H, Lanini S, Vairo F, DiCaro A, Capoblanchi MR, Nicastri N. Viral hemorrhagic fevers: advancing the level of treatment. BMC Medicine 2012:10:31:1-8

7. WHO (2016). Blueprint for action to prevent epidemics. May 2016 WHO, 2017. R&D Blueprint for action to prevent epidemics. Available from: http://www. who. int/ blueprint/ en/ [accessed 26 Oct2018].

8. WHO (2018). List of Blueprint priority diseases. 2018 annual review. https://www.who.int/blueprint/priority-diseases/en/

9. Frame JD, Baldwin JM, Jr, Gocke DJ, et al. : 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):670–6. 10.4269/ajtmh.1970.19.670

10. Troup JM, White HA, Fom AL, Carey DE. An outbreak of Lassa fever on the Jos plateau, Nigeria, in January-February 1970. A preliminary report. Am J Trop Med Hyg. 1970 Jul;19(4):695-6

11. Buckley SM, Casals J. Lassa fever, a new virus disease of man from West Africa.III. Isolation and Characterization of the virus. *Amer J Trop Med Hyg*. 1970; 19: 670-676.

12. Carey DE, Kemp GE, White HA, Pinneo L, Addy RF, Fom AL, Stroh G, Casals J, Henderson BE. Lassa fever. Epidemiological aspects of the 1970 epidemic, Jos, Nigeria. Trans R Soc Trop Med Hyg. 1972;66(3):402-8

13. Bond N, Schieffelin JS, Moses LM, Bennett AJ, Bausch DG. A historical look at the first reported cases of Lassa fever: IgG antibodies 40 years after acute infection. The American journal of tropical medicine and hygiene. 2013 Feb 6;88(2):241-4.

14. Radoshitzky SR, Bào Y, Buchmeier MJ, Charrel RN, Clawson AN, Clegg CS, et al. Past, present, and future of arenavirus taxonomy. Arch Virol. 2015 Jul;160(7):1851-74.

15. Andersen KG, Shapiro J, Matranga CB, Sealfon R, Lin AE, Moses LM, et al. Clinical sequence uncovers origins and evolution of Lassa virus. Cell 2015; 162: 736-750

16. Whitmer SLM, Strecker T, Cadar D, Dienes HP, Faber K, Patel K, et al. New Lineage of Lassa Virus, Togo, 2016. Emerg Infect Dis. 2018 Mar; 24(3):599-602

17. Kafetzopoulou LE, Pullan ST, Lemey P, Suchard MA, Ehichioya DU, Pahlmann M, et al. Metagenomic sequencing at the epicenter of the Nigeria 2018 Lassa fever outbreak. Science. 2019 Jan 4;363(6422):74-77

18. Lukashevich IS. The search for animal models for Lassa fever vaccine development. Expert Rev Vaccines 2013 Jan;12(1):71–86

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

20. Ehichioya DU, Hass M, Becker-Ziaja B, Ehimuan J, Asogun DA, Fichet-Calvet E, et al. Current molecular epidemiology of Lassa virus in Nigeria. J Clin Microbiol. 2011 Mar;49(3):1157-61.

21. Hallam HJ, Hallam S, Rodriguez SE, et al. Baseline mapping of Lassa fever virology, epidemiology and vaccine research and development. NPJ Vaccines 2018 Mar;3:11. 1-8.

22. Siddle KJ, Eromon P, Barnes KG, Mehta S, Oguzie JU, Odia I, et al. Genomic Analysis of Lassa Virus during an Increase in Cases in Nigeria in 2018. N Engl J Med. 2018 Nov 1;379(18):1745-1753

23. Gibb, R., Moses, L. M., Redding, D. W. & Jones, K. E. Understanding the cryptic nature of Lassa fever in West Africa. *Pathogens and Global Health* (2017). doi:10.1080/20477724.2017.1369643

24. Yun NE, Walker DH. Pathogenesis of Lassa fever. Viruses. 2012; 4(10):2031–48. Epub 2012/10/09. https://doi.org/10.3390/v4102031 PMID: 23202452; PubMed Central PMCID: PMCPMC3497040.

25. Safronetz D, Sogoba N, Lopez JE, Maiga O, Dahlstrom E, Zivcec M, et al. Geographic distribution and genetic characterization of Lassa virus in sub-Saharan Mali. PLoS Negl Trop Dis. 2013 Dec 5;7(12):e2582

26. Sogoba, N., Feldmann, H. & Safronetz, D. Lassa Fever in West Africa: Evidence for an Expanded Region of Endemicity. *Zoonoses and Public Health* (2012). doi:10.1111/j.1863-2378.2012.01469.x

27. Adebayo D, Nwobi EA, Vincent T, Gonzalez JP. Response Preparedness to Viral Hemorrhagic Fever in Nigeria: Risk Perception, Attitude towards Lassa Fever. Epidemiology (sunnyvale). 2015;5(199):2161-1165

28. Roberts L. Nigeria hit by unprecedented Lassa fever outbreak. Science 2018 Mar 16;359 (6381):1201-1202

29. Kofman A, Choi MJ, Rollin PE. Lassa Fever in Travelers from West Africa, 1969-2016. Emerg Infect Dis. 2019 Feb;25(2):245-248

30. 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 May 21;9(5):e0003793.

31. Lecompte, E. *et al*. Mastomys natalensis and Lassa fever, West Africa. *Emerg. Infect. Dis.* (2006). doi:10.3201/eid1212.060812

32. Monath TP, Newhouse VF, Kemp GE, Setzer HW, Cacciapuoti A. Lassa virus isolation from *Mastomys natalensis* rodents during an epidemic in Sierra Leone. Science. 1974 Jul 19;185(4147):263-5

33. Olayemi A, Obadare A, Oyeyiola A, Igbokwe J, Fasogbon A, Igbahenah F, et al. Arenavirus Diversity and Phylogeography of Mastomys natalensis Rodents, Nigeria. Emerg Infect Dis. 2016 Apr;22(4):694-7.

34. Coulibaly-N'Golo D, Allali B, Kouassi SK, Fichet-Calvet E, Becker-Ziaja B, et al. (2011) Novel Arenavirus Sequences in Hylomyscus sp. and Mus (Nannomys) setulosus from Co ˆte d'Ivoire: Implications for Evolution of Arenaviruses in Africa. PLoS ONE 6(6): e20893. doi:10.1371/journal.pone.0020893

35. Olayemi A, Cadar D, Magassouba N, Obadare A, Kourouma F, Oyeyiola A, et al. New Hosts of The Lassa Virus. Sci Rep. 2016 May 3;6:25280.

36. CDC, 2014. Transmission: Lassa fever. Available at https://www.cdc.gov. Accessed 17th Mar, 2019

37. Bonwitt J, Kandeh M, Dawson M, Ansumana R, Sahr F, Kelly AH, 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 Jul 27;11(7):e0005699

38. Bonwitt J, Kelly AH, Ansumana R, Agbla S, Sahr F, Saez AM,et al.. Rat-atouille: A Mixed Method Study to Characterize Rodent Hunting and Consumption in the Context of Lassa Fever. Ecohealth. 2016 Jun;13(2):234-47.

39. Bonwitt J, Sáez AM, Lamin J, Ansumana R, Dawson M, Buanie J, et al. At Home with Mastomys and Rattus: Human-Rodent Interactions and Potential for Primary Transmission of Lassa Virus in Domestic Spaces. Am J Trop Med Hyg. 2017 Apr;96(4):935-943

40. McCormick JB, 1987. Epidemiology and control of Lassa fever. Curr Top Microbiol Immunol 134: 69–78.

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

42. Akhuemokhan OC, Ewah-Odiase RO, Akpede N, Ehimuan J, Adomeh DI, Odia I, et al. Prevalence of Lassa Virus Disease (LVD) in Nigerian children with fever or fever and convulsions in an endemic area. PLoS Negl Trop Dis. 2017 Jul 3;11(7):e0005711

43. Bello OO, Akinajo OR, Odubamowo KH, Oluwasola TA. Lassa Fever in Pregnancy: Report of 2 Cases Seen at the University College Hospital, Ibadan. Case reports in obstetrics and gynecology. 2016 Mar 9;2016.

44. Lupi O, Diniz C, de Carvalho Serra F, de Lemos ER. 20 Arenaviruses. Mucocutaneous Manifestations of Viral Diseases: An Illustrated Guide to Diagnosis and Management. 2016 Apr 19:400.

45. Ter Meulen J, Lukashevich I, Sidibe K, Inapoqui A, Marx M, Dorlemann A, 1996. Hunting of peridomestic rodents and consumption of their meat as possible risk factors for rodent-to-human transmission of Lassa virus in the Republic of Guinea. Am J Trop Med Hyg 1996; 55: 661–666.

46. Brosh-Nissimov T. Lassa fever: another threat from West Africa. Disaster and Military Medicine. 2016 Apr 30;2(1):8.

47. Asogun DA, Adomeh DI, Ehimuna J, Odia I, Hass M, Gabriel M 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(9):e1839

48. Bajani MD, Tomori O, Rollin PE, Harry TO, Bukbuk ND, Wilson L, Childs JE, Peters CJ, Ksiazek TG. A survey for antibodies to Lassa virus among health workers in Nigeria. Trans R Soc Trop Med Hyg. 1997 Jul-Aug;91(4):379-81

49. 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. Emerg Infect Dis. 2015 Apr;21(4):609-18

50. Simonsen L, Kane A, Lloyd J, Zaffran M, Kane M. Unsafe injections in the developing world and transmission of bloodborne pathogens: a review. Bull World Health Organ. 1999;77 (10):789-800.

51. Arias A, Watson SJ, Asogun D, Tobin EA, Lu J, Phan MVT, et al. Rapid outbreak sequencing of Ebola virus in Sierra Leone identifies transmission chains linked to sporadic cases. Virus Evol. 2016 Jun 22;2(1):vew016. doi: 10.1093/ve/vew016. eCollection 2016 Jan

52. Okokhere P, Colubri A, Azubike C, Iruolagbe C, Osazuwa O, Tabrizi S, 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 Jun;18(6):684-695.

53. Isere EE, Fatiregun AA, Ilesanmi O, Ijarotimi I, Egube B, Adejugbagbe A, Famokun GA. Lessons Learnt from Epidemiological Investigation of Lassa Fever Outbreak in a Southwest State of Nigeria December 2015 to April 2016. PLoS Curr. 2018 Jun 29;10. pii: ecurrents.outbreaks.bc4396a6650d0ed1985d731583bf5ded. doi: 10.1371/currents.outbreaks.bc4396a6650d0ed1985d731583bf5ded

54. Nigeria Center for Disease Control (2019). An update of Lassa fever outbreak in Nigeria. Available at: https://ncdc.gov.ng/diseases/sitreps/?cat=5&name=An update of Lassa fever outbreak in Nigeria. (Accessed: 30th March 2019)

55. 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 of poor medical practice. BMJ. 1995 Sep 30;311(7009):857-9

56. 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 Sep 3;297(6648):584-7

57. Andersen KG, Shapiro J, Matranga CB, Sealfon R, Lin AE, Moses LM, et al. Clinical sequence uncovers origins and evolution of Lassa virus. Cell 2015; 162: 736-750

58. Andersen KG, Shylakhter I, Tabrizi S, Grossman SH, Happi CT, Sabeti PC. Genome-wide scans provide evidence for positive selection of genes implicated in Lassa fever. Phil Trans Roy Soc Biol 2012; 367: 868-77; doi:10.1098/rstb.2011.0299

59. Paessler S, Walker DH. Pathogenesis of the viral hemorrhagic fevers. Ann Rev Pathol Mech Dis 2013; 8: 411-40

60. Shao J, Liang Y, Ly H. Human hemorrhagic fever causing arenaviruses: Molecular mechanisms contributing to virus virulence and disease pathogenesis. Pathogens 2015; 4: 283-306; doi:10.3390/pathogens4020283

61. Ehizibolo, DO, Ehizibolo, PO, Ehizibolo, EE, Sugun, MY, Idachaba, S.E. The control of neglected zoonotic diseases in Nigeria through animal intervention. African J. Biomed. Res. (2011).

62. Brown H, Kelly AH, Marí Sáez A, Fichet-Calvet E, Ansumana R, Bonwitt J, et al. Extending the "social": anthropological contributions to the study of viral haemorrhagic fevers. PLoS Negl Trop Dis. 2015 Apr 16;9(4):e0003651.

63. Redding, D. W., Moses, L. M., Cunningham, A. A., Wood, J. & Jones, K. E. Environmental-mechanistic modelling of the impact of global change on human zoonotic disease emergence: a case study of Lassa fever. *Methods Ecol. Evol.* (2016). doi:10.1111/2041-210X.12549

64. Tambo, E., Adetunde, O. T. & Olalubi, O. A. Re-emerging Lassa fever outbreaks in Nigeria: Re-enforcing 'One Health' community surveillance and emergency response practice. *Infectious Diseases of Poverty* (2018). doi:10.1186/s40249-018-0421-8

65. Zumla A, Dar O, Kock R, Muturi M, Ntoumi F, Kaleebu P, et al. Taking forward a 'One Health' approach for turning the tide against the Middle East respiratory syndrome coronavirus and other zoonotic pathogens with epidemic potential. Int J Infect Dis. 2016 Jun;47:5-9.

66. Ilori EA, Frank C, Dan-Nwafor CC, Ipadeola O, Krings A, Ukponu W et al. Increase in Lassa Fever Cases in Nigeria, January-March 2018. Emerg Infect Dis. 2019 May 17;25(5). doi: 10.3201/eid2505.181247

67. Akpede GO, Asogun DA, Okogbenin SA, Dawodu SO, Momoh MO, Dongo AE, et al. Caseload and case fatality of Lassa fever in Nigeria, 2001-2018: A specialist center's experience and its implications. Frontiers Public Health 2019-in press.

68. Nigeria Center for Disease Control (2019). An update of Lassa fever outbreak in Nigeria. Available at: https://ncdc.gov.ng/diseases/sitreps/?cat=5&name=An update of Lassa fever outbreak in Nigeria. (Accessed: 27th March 2019).

69. Grahn A, Bråve A, Lagging M, Dotevall L, Ekqvist D, Hammarström H, et al. Imported Case of Lassa Fever in Sweden With Encephalopathy and Sensorineural Hearing Deficit. Open Forum Infect Dis. 2016 Sep 20;3(4):198. eCollection 2016 Oct

70. Ehlkes L, George M, Samosny G, Burckhardt F, Vogt M, Bent S, et al. Management of a Lassa fever outbreak, Rhineland-Palatinate, Germany, 2016. Euro Surveill. 2017 Sep;22(39). doi: 10.2807/1560-7917.ES.2017.22.39.16-00728.

71. Mylne AQ, Pigott DM, Longbottom J, Shearer F, Duda KA, Messina JP, et al. Mapping the zoonotic niche of Lassa fever in Africa. Trans R Soc Trop Med Hyg. 2015 Aug;109(8):483-92

72. Schoepp, RJ, Rossi, CA, Khan, SH, Goba, A, Fair, JN. Undiagnosed acute viral febrile illnesses, Sierra Leone. Emerg. Infect. Dis. (2014). doi:10.3201/eid2007.131265.

73. Akpede GO, Adetunji AE, Udefiagbon EO, Eluehike SO, Odike AI, Ewah-Odiase RO, et al. Acute Abdomen in Pediatric Patients With Lassa Fever: Prevalence and Response to Nonoperative Management.J Pediatric Infect Dis Soc. 2018 Sep 28. doi: 10.1093/jpids/piy093. [Epub ahead of print]

74. Dongo AE, Kesieme EB, Iyamu CE, Okokhere PO, Akhuemokhan OC, Akpede GO. Lassa fever presenting as acute abdomen: a case series. Virol J. 2013 Apr 19;10:123. doi: 10.1186/1743-422X-10-123.

75. Akpede GO, Asogun DA, Okogbenin SA, Dawodu SO, Momoh MO, Dongo AE, et al. Caseload and case fatality of Lassa fever in Nigeria, 2001-2018: A specialist center's experience and its implications. Frontiers Public Health 2019-in press.

76. Okokhere PO, Bankole IA, Iruolagbe CO, et al. Aseptic meningitis caused by Lassa virus: case series report. Case Rep Neurol Med 2016; 2016: 4.

77. 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 Feb 22;12(2):e0006187

78. Ibekwe TS, Okokhere PO, Asogun D, Blackie FF, Nwegbu MM, Wahab KW, Omilabu SA, Akpede GO. Early-onset sensorineural hearing loss in Lassa fever. Eur Arch Otorhinolaryngol. 2011 Feb;268(2):197-201. doi: 10.1007/s00405-010-1370-4. Epub 2010 Sep 1.

79. Okokhere PO, Ibekwe TS, Akpede GO. Sensorineural hearing loss in Lassa fever: two case reports.J Med Case Rep. 2009 Jan 29;3:36. doi: 10.1186/1752-1947-3-36.

80. Cashman KA, Wilkinson ER, Zeng X, Cardile AP, Facemire PR, Bell TM, et al. Immune-Mediated Systemic Vasculitis as the Proposed Cause of Sudden-Onset Sensorineural Hearing Loss following Lassa Virus Exposure in Cynomolgus Macaques.MBio. 2018 Oct 30;9(5). pii: e01896-18.

81. Walls B. Lassa fever and pregnancy. Midwives Chron. 1985 May;1168(98):136-8

82. Dahmane A, van Griensven J, Van Herp M, Van den Bergh R, Nzomukunda Y, Prior J et al. 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 Mar;108(3):126–32

83. Shaffer JG, Grant DS, Schieffelin JS, Boisen ML, Goba A, Hartnett JN, et al Lassa fever in post-conflict sierra leone. PLoS Negl Trop Dis. 2014 Mar 20;8(3):e2748.

84. Okogbenin SA, Eigbefoh JO, Omorogbe F, Okogbo F, Okonta PI, Ohihoin AG. Eclampsia in Irrua Specialist Teaching Hospital: a five-year review. Niger J Clin Pract. 2010 Jun;13(2):149-53

85. Asogun DA, Adomeh DI, Ehimuna J, 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(9):e1839

86. WHO 2018. How to safely collect blood samples by phlebotomy from patients suspected to be infected with Lassa https://www.who.int/emergencies/diseases/lassa-fever/collection-of-blood-samples-for-lassa.pdf?ua=1 -accessed March 22nd 2019.

87. WHO 2018. How to safely ship human blood samples from Lassa cases within a country by road, rail and sea. https://www.who.int/emergencies/diseases/lassa-fever/shipment-of-blood-samples-lassa.pdf?ua=1

88. Zhang Y, Gong Y, Wang C, Liu W, Wang Z, Xia Z, et al. Rapid deployment of a mobile biosafety level-3 laboratory in Sierra Leone during the 2014 Ebola virus epidemic. PLoS Negl Trop Dis. 2017 May 15;11(5):e0005622

89. Wölfel R, Stoecker K, Fleischmann E, Gramsamer B, Wagner M, Molkenthin P, et al. Mobile diagnostics in outbreak response, not only for Ebola: a blueprint for a modular and robust field laboratory.Euro Surveill. 2015;20(44). doi: 10.2807/1560-7917.ES.2015.20.44.30055

90. Raabe V, Koehler J. Laboratory Diagnosis of Lassa Fever. J Clin Microbiol. 2017 Jun;55(6):1629-1637.

91. Gabriel M, Adomeh DI, Ehimuan J, Oyakhilome J, Omomoh EO, Ighodalo Y, et al Development and evaluation of antibody-capture immunoassays for detection of Lassa virus nucleoprotein-specific immunoglobulin M and G. PLoS Negl Trop Dis. 2018 Mar 29;12(3):e0006361

92. Mazzola LT, Kelly-Cirino C. Diagnostics for Nipah virus: a zoonotic pathogen endemic to Southeast Asia.BMJ Glob Health. 2019 Feb 1;4(Suppl 2):e001118

93. Boisen ML, Hartnett JN, Shaffer JG, Goba A, Momoh M, Sandi JD, et al. Field validation of recombinant antigen immunoassays for diagnosis of Lassa fever.Sci Rep. 2018 Apr 12;8(1):5939. doi: 10.1038/s41598-018-24246-w.

94. Emperador DM, Yimer SA, Mazzola LT, Norheim G, Kelly-Cirino C. Diagnostic applications for Lassa fever in limited-resource settings. BMJ Glob Health. 2019 Feb 7;4(Suppl 2):e001119

95. Gootenberg JS, Abudayyeh OO, Kellner MJ,Joung J, Collins JJ, Zhang F. Multiplexed andportable nucleic acid detection platform withCas13, Cas12a, and Csm6. Science 2018;360: 439–44.

96. Dhillon RS, Srikrishna D, Garry RF. Early detection of Lassa fever: the need for point-of-care diagnostics. Lancet Infect Dis 2018 Jun;18(6):601-602

97. Günther S, Weisner B, Roth A, Grewing T, Asper M, Drosten C, et al. Lassa fever encephalopathy: Lassa virus in cerebrospinal fluid but not in serum. J Infect Dis. 2001 Aug 1;184(3):345-9.

98. McCormick JB, Fisher-Hoch SP. Lassa fever. Curr Top Microbiol Immunol. 2002;262:75-109

99. Rusnak JM. Experience with ribavirin for treatment and post-exposure prophylaxis of hemorrhagic fever viruses: Crimean Congo hemorrhagic fever, Lass fever, and Hantaviruses. Applied Biosafety 2011; 16: 67-87.

100. McCormick JB, King IJ, Webb PA, Scribner CL, Craven RB, Johnson KM, Elliott LH, Belmont-Williams R. Lassa fever. Effective therapy with ribavirin. N Engl J Med. 1986 Jan 2;314(1):20-6

101. Raabe VN, Kann G, Ribner BS, et al. Favipiravir and ribavirin treatment of epidemiologically linked cases of Lassa fever. Clin Infect Dis 2017; 65:855–9.

102. Robinson JE, Hastie KM, Cross RW, Yenni RE, Elliott DH, Rouelle JA, et al. Most neutralizing human monoclonal antibodies target novel epitopes requiring both Lassa virus glycoprotein subunits. Nat Commun. 2016 May 10;7:11544. doi: 10.1038/ncomms11544

103. Mire CE, Cross RW, Geisbert JB, Borisevich V, Agans KN, Deer DJ *et al*. Human-monoclonal antibody therapy protects nonhuman primates against advanced Lassa fever. *Nat Med* 2017; 23:1146–9.

104. Bonwitt J, Kandeh M, Dawson M, Ansumana R, Sahr F, Kelly AH, 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 Jul 27;11(7):e0005699

105. Center for Disease Control and Prevention. Viral Haemorrhagic Fevers: Infection Control of Viral Haemorrhagic Fevers in African Health Care Setting. January 8, 2014. Page available at https://www.cdc.gov/vhf/abroad/vhf-manual.html. Accessed on Mar 17th, 2019

106. Lukashevich IS, Paessler S, de la Torre JC. Lassa virus diversity and feasibility for universal prophylactic vaccine.F1000Res. 2019 Jan 31;8. pii: F1000 Faculty Rev-134

107. Plotkin SA. Vaccines for epidemic infections and the role of CEPI. Hum Vaccin Immunother. 2017 Dec 2;13(12):2755-2762

108. Warner BM, Safronetz D, Stein DR. Drug Design, Development and Therapy. 2018:12 2519–2527

LEGENDS TO FIGURES AND TABLES

Figure 1: Map of Africa – geographical distribution of Lassa Fever

Figure 2:

2a: The multimammate rat *Mastomys natalensis* *(courtesy of Prof Danny Asogun)*

2b: Ventral surface of *Mastomys natalensis* showing two rows of mammary glands *(Courtesy of Prof George Akpede)*

Figure 3: Geographical distribution of Lassa Fever cases in Nigeria (2018-2019)

Figure 4: Severely ill Nigerian child with Lassa Fever with facial oedema, spontaneously bleeding and acute kidney injury *(courtesy of George Akpede)*

Figure 5: Lassa fever patient undergoing renal dialysis at Lassa isolation ward, Irrua Lassa Fever Hospital, Irrua, Nigeria *(Courtesy of Prof Danny Asogun)*

Figure 1: Map of Africa – geographical distribution of Lassa Fever

Figure 2a:

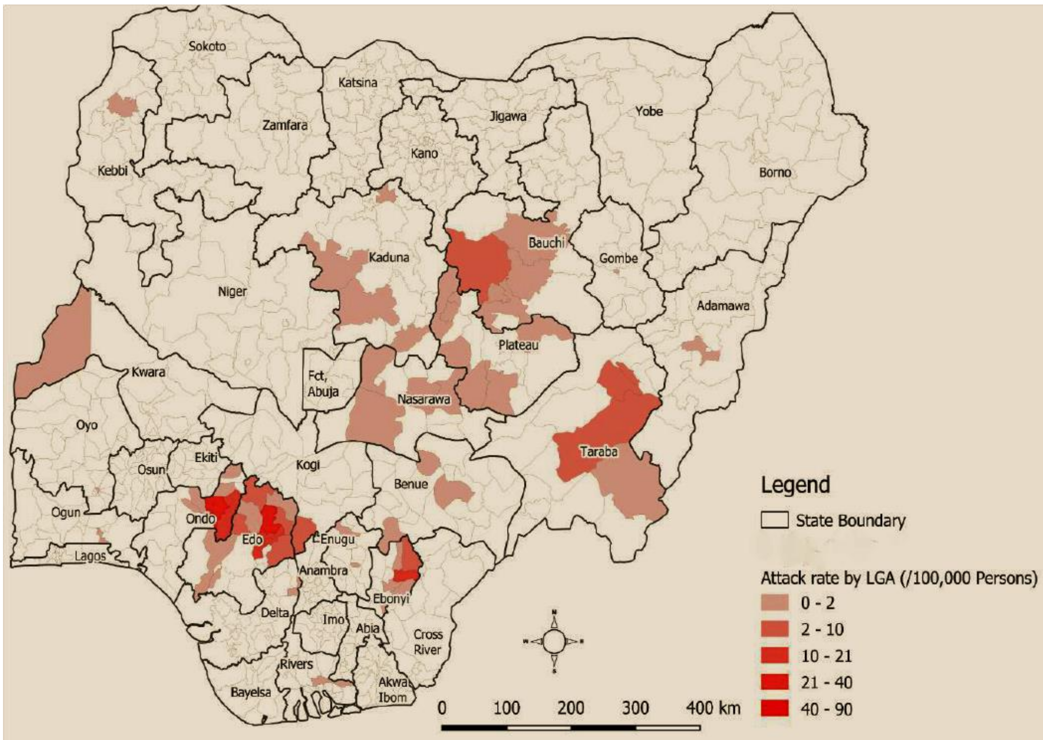
The multimammate rat *Mastomys natalensis* *(courtesy of Prof Danny Asogun)*

Figure 2b.

Ventral surface of *Mastomys natalensis* showing two rows of mammary glands *(Courtesy of Prof George Akpede)*

Figure 3: Geographical distribution of Lassa Fever cases in Nigeria (2018-2019)

(can use either of the two figures -whichever reproduces clearly or please re-draw)



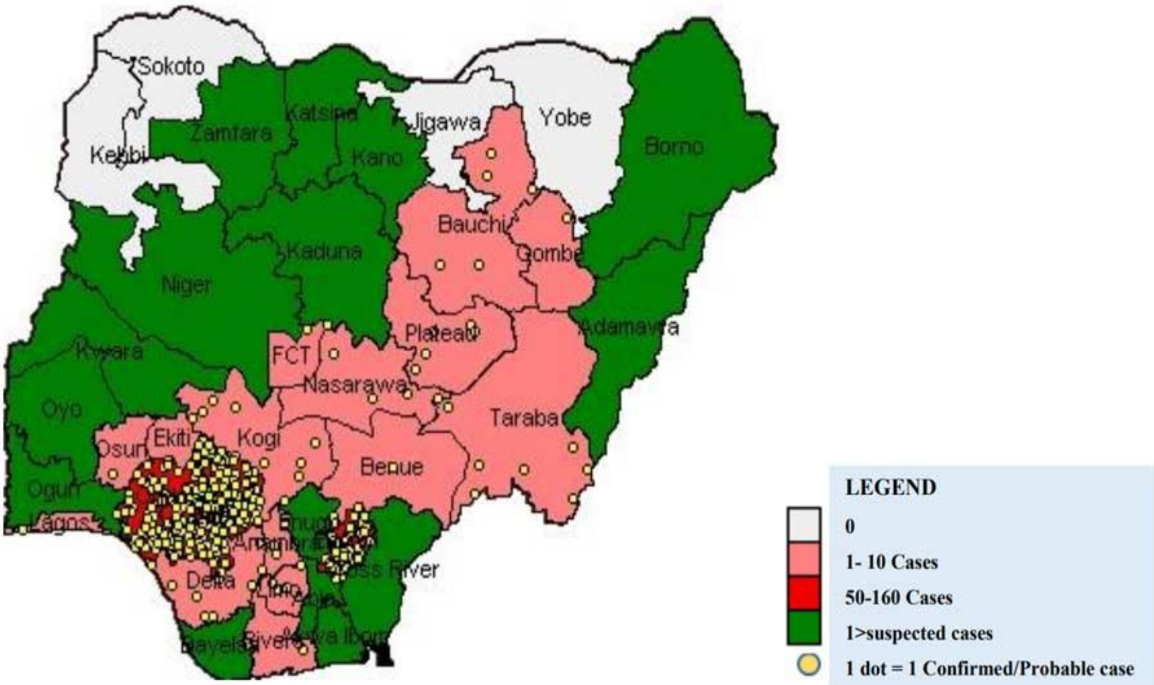


Figure 4:

Severely ill Nigerian child with Lassa Fever with facial oedema, spontaneously bleeding and acute kidney injury
(courtesy of George Akpede)

Figure 5:

Lassa fever patient undergoing renal dialysis at Lassa isolation ward, Irrua Lassa Fever Hospital, Irrua, Nigeria ***(Courtesy of Prof Danny Asogun)***

Table 1

Clinical signs, symptoms, complications and prognostic indicators

SYMPTOMS	**SIGNS**
Fever, malaise, headache, weakness Sore throat, Muscle aches, Joint pain, Back pain, Chest pain, Cough, Breathlessness Loss of appetite, Nausea, Vomiting, Diarrhoea, Acute abdominal pain Red eyes, Yellow eyes, Swelling of eyes and legs, Difficulty in urination Bruising and Bleeding (Skin, mouth, conjunctiva, rectal) Tinnitus, vertigo, hearing loss Hand tremors, Unsteady walking, confusion, disorientation, fits, loss of consciousness	Febrile, Tachycardia, Bradycardia, Low pulse pressure, Arrythmia Dehydration, Mouth ulcers, Pharyngitis, Conjunctival injection, sub-conjunctival haemorrhage, Skin and mucosal petechiae/ecchymosis, Jaundice, Oedema, Ascites Lymphadenopathy, Splenomegaly, Hepatomegaly Lung crackles, rhonchi, bronchial breathing Pericardial Effusion, Pleural Effusion Sensorineural hearing loss (8^{th} cranial nerve) Signs of Encephalitis, Unsteady gait, drowsiness, coma
COMPLICATIONS	**POOR PROGNOSTIC INDICATORS**
Acute Renal failure Liver failure Multi-organ Failure Widespread bleeding Disseminated intravascular coagulation Shock -hypovolaemic and sepsis Encephalitis Foetal loss (spontaneous abortion) Deafness due to 8th nerve sensorineural loss Death	High viral load and viraemia Grossly abnormal Liver function tests (High AST levels) Renal failure (high urea and creatinine) Severe bleeding Encephalitis Third trimester pregnancy Generalised oedema

Table 2

Case studies of Lassa Fever at Irrua Specialist Teaching Hospital, Irrua, Nigeria

	Adults		Children	Pregnant women
Study Reference	Asogun *et al*[20]	Okokhere *et al*[23]	Akpede *et al*[75]	Okogbenin *et al*[84] *
Year of study	2009 - 2010	2011 - 2015	2009 - 2017	2009 - 2018
No. of patients *	198	284	57	30
Deaths -No. (%)	61 (30.8)	68 (24.0)	16 (28.1)	11 (36.7)
Factors associated with death (**Odds ratio [95% CI])				
Bleeding	Yes (6.2 [2.11,18.2])	Yes (1.9 [1.1,3.4])	Yes (17.68 [4.38,71.31])	Yes (not applicable@)
Shock	ND	No	Yes (30.8 [3.39, 285.4])	ND
Acute kidney injury	Yes** (ND)	Yes (15 [8, 28])	Yes (29.57 [3.17, 275.7])	Yes (31.5 [2.98, 333.2)
Encephalopathy+	No (2.86 [0.78,10.58])	Yes (15 [7, 34])	Yes (15.6 [4.21, 72.75)	Yes (31.5 [2.98, 333.2])

ND = no data

*The same factors were associated with both maternal death and fetal loss;

@9/11 patients with versus 0/19 without extra-vaginal bleeding died.

**Data not available on the numbers with acute kidney injury but both the mean blood urea nitrogen ($p<0.001$) and mean serum creatinine ($p<0.001$) were significantly higher among those who died compared with those that survived.

+Defined by the presence of coma and/or seizures.