

Review Article

Outbreaks and laboratory investigations of Lassa fever in Nigeria: How prepared are we?

Abstract

Lassa is a highly contagious and devastating re-emerging vector-borne disease transmitted primarily by *Mastomys natalensis*. Lassa fever virus (LFV) and its vector are largely conserved to West African communities hence they experience frequent sporadic outbreaks. Lassa are usually associated with high mortality rates as the cases are usually presented late to healthcare centers. The transmission rate of LFV is usually very high in affected areas in Nigeria partly due to inadequate standard pathogen containment which is necessary for medical care of infected individuals and laboratory investigations of their specimens, consequently placing healthcare providers at higher risk of contracting LFV. More so, many physicians find it difficult to diagnose Lassa until complications set in because of the similarity of presentation with other more common febrile illnesses such as malaria, typhoid and fulminant hepatitis. Understanding its virology, ecology, transmission pattern and immunology is crucial in embarking appropriate diagnostic laboratory protocols and control strategies.

Keywords: Lassa; *Mastomys natalensis*; Outbreaks; laboratory investigations; Nigeria.

20 1.0 Introduction

21 Lassa is an acute viral zoonotic illness caused by Lassa fever virus, an arenavirus known
22 to be responsible for a severe haemorrhagic fever characterized by fever, muscle aches, sore
23 throat, nausea, vomiting, and chest and abdominal pain [1]. The virus exhibits persistent,
24 asymptomatic infection, with profuse urinary virus excretion in *Mastomys natalensis*, the
25 ubiquitous and highly commensal rodent host [2]. Lassa fever is endemic in West Africa and has
26 been reported from Sierra Leone, Guinea, Liberia, and Nigeria [3]. Some studies indicate that
27 300,000 to 500,000 cases of Lassa fever and 5000 deaths occur yearly across West Africa [1].

28 In spite of the great progress made in recent years in the understanding of the life cycle of
29 arenaviruses, including Lassa virus and the new insights gained in the pathogenesis and
30 molecular epidemiology of Lassa fever, as well as the development of the state-of-the-art
31 technologies for diagnosing this life-threatening disease [3], the emergence of this highly
32 virulent and contagious virus in many districts and states of endemic countries of the West
33 African sub-region and the increasing sporadic cases of Lassa fever outside the endemic regions
34 may be as a result of huge inter-border traffic and international travels. This necessitates that
35 healthcare providers have comprehensive information about the virus and the disease it causes
36 [5]. Furthermore, Lassa virus has been associated with nosocomial outbreaks with high mortality
37 [1, 5], however, the enlightenment campaign for the prevention of Lassa fever and diagnostic
38 facilities are either lacking or rudimentary in most countries where Lassa fever is endemic.
39 Compared to HIV/AIDS, Lassa fever is more infectious to close associates and it rapidly kills in
40 dozens. However, Lassa fever does not get the global attention it deserves. Hence, early
41 identification of infected individuals is important for prompt implementation of appropriate
42 barrier medical guidelines [5].

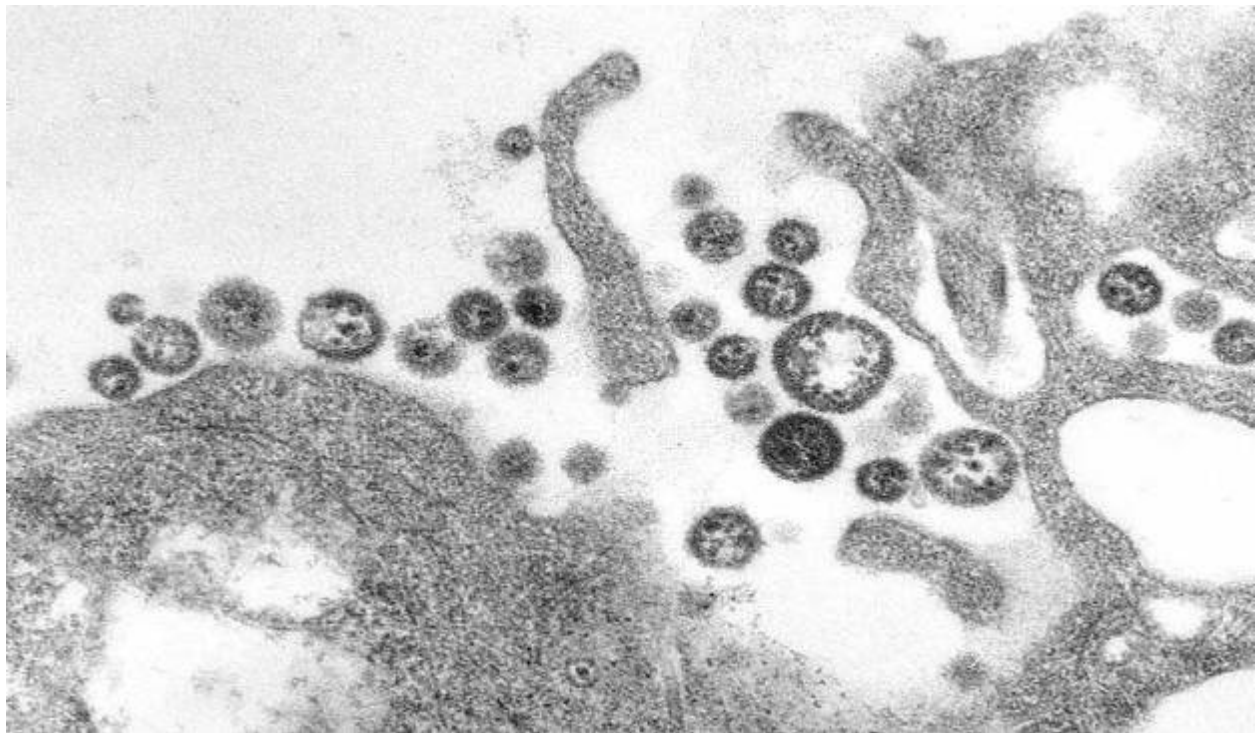
1.2 Basic Virology of Lassa

Lassa virus (LASV) is an enveloped, single-stranded, bisegmented RNA virus belonging to the Arenaviridae family. Like other arenaviruses, Lassa virus lacks a conventional negative-strand coding arrangement and the isolates of the virus differ in their genetic, serologic, and pathogenic characteristics [6]. Lassa virus is spherical in shape and measures between 70 and 150 nm in diameter. It has a smooth surface envelope with T-shaped spikes measuring 7–10 nm and built with glycoprotein. The envelope encloses the genome which has helical nucleocapsid measuring between 400 and 1300 nm in length [7]. Often the interior contains electron dense granule identified as the host cell ribosome from where the name “arena” was derived meaning sand [8]. Lassa virus can be inactivated in ultraviolet, gamma irradiation, heating from 56–100°C and pH range between 5.5 and 8.5. Chemical agents like 0.5% sodium hypochlorite, 0.5% phenol and 10% formalin are good inactivants against the virus [8].

The arenavirus genome consists of a small (s) and a large (l) RNA fragment, sizes 3.4 and 7 kb, respectively and the sRNA encodes the viral glycoprotein precursor protein (GPC) and the nucleoprotein (NP), while the RNA encodes the viral polymerase and a small, zinc-binding (Z) protein [9]. New methods for full-length sRNA amplification are facilitating research efforts on the identification and molecular analysis of new arenaviruses or arenavirus strains [9].

The sequencing of Lassa virus sRNA has enabled the identification and molecular characterization of four Lassa virus strains. These include: the strain Josiah, originating from Sierra Leone, the strain Nigeria and strain LP, both from Nigeria and the strain AV imported into Germany by a traveler who had visited Ghana, Côte D’Ivoire, and Burkina Faso. sRNA Sequence findings of Lassa virus indicated a considerable genetic variation among the strains of

65 the virus, however, phylogenetically, strain AV appears to be the most closely related to strain
66 Josiah from Sierra Leone [9].



67

68 Figure: Electron Micrograph of Lassa fever virus particle Source: CDC. 2014

69

70 **1.3 The immunology of Lassa fever**

71 Design of an effective vaccine and diagnostic laboratory assays require some understanding of
72 the immunology of the disease against which the vaccine must protect and reliability of test
73 results. Clearly, this rodent virus is handled quite differently by the immune systems of rodents
74 and primates. The immunological response in primates to arenavirus infection is complex. In
75 general, the evidence we have indicates that Lassa and LCM viruses depend primarily on
76 cytotoxic T-cell responses for virus clearance, while neutralising antibodies are associated with
77 clearance of viraemia due to the South American arenaviruses [10]. There appears to be a brisk

B-cell response to Lassa virus with a classic primary IgG and IgM antibody response early in the illness. Development of antibody does not, however, coincide with virus clearance, and high viraemia and high IgG and IgM titres often coexist in both humans and primates. Indeed virus may persist in the serum and urine of humans for several weeks after infection, and possibly in occult sites, such as renal tissue, for longer periods. Lassa virus specific cytotoxic T cell responses can be measured in patients recovered from Lassa fever [11].

Neutralizing antibodies to Lassa virus cannot be measured in the serum of patients at the beginning of convalescence; in most people, and in experimentally infected monkeys, they are never detectable in a classical plaque-reduction neutralization assay. In a minority of patients some low-titre serum neutralizing activity may be observed several months after resolution of the disease, but only using a fixed-serum, varying virus dilution assay (log neutralization assay) [12].

Passive protection from Lassa virus has been demonstrated in animals given selected antiserum but only at the time of or soon after inoculation with virus. While there have been anecdotal reports of the clinical effectiveness of Lassa immune plasma, controlled clinical trials with human convalescent plasma containing high titred antibodies have shown no protective effect [12]. Thus the clearance of Lassa virus appears to be independent of antibody formation, and presumably depends on the CMI response. In Lassa fever infections, the presence of antibody to neither glycoprotein nor nucleoprotein at the time of hospital admission is associated with survival, or even attenuation of disease. Indeed there is some correlation between death and early detection of IgM antibody [13].

These consistent observations contrast sharply with those made in Argentinian haemorrhagic fever (AHF), caused by the South American arenavirus, Junin virus. Here

neutralising antibodies can be detected easily, and immune therapy has been shown to be very effective in this disease, and to correlate with the level of neutralising antibody. Nevertheless there is evidence from vaccine studies in Argentina that patients who do not produce measurable neutralizing antibodies following vaccination do mount a Junin-virus specific lymphocytic proliferative response [8]. The precise nature of protective immune responses in arenaviruses, however, remains to be elucidated using the more sophisticated assays now available, which for reasons of biosafety and inaccessibility of patients and specimens have not yet been applied to arenaviruses.

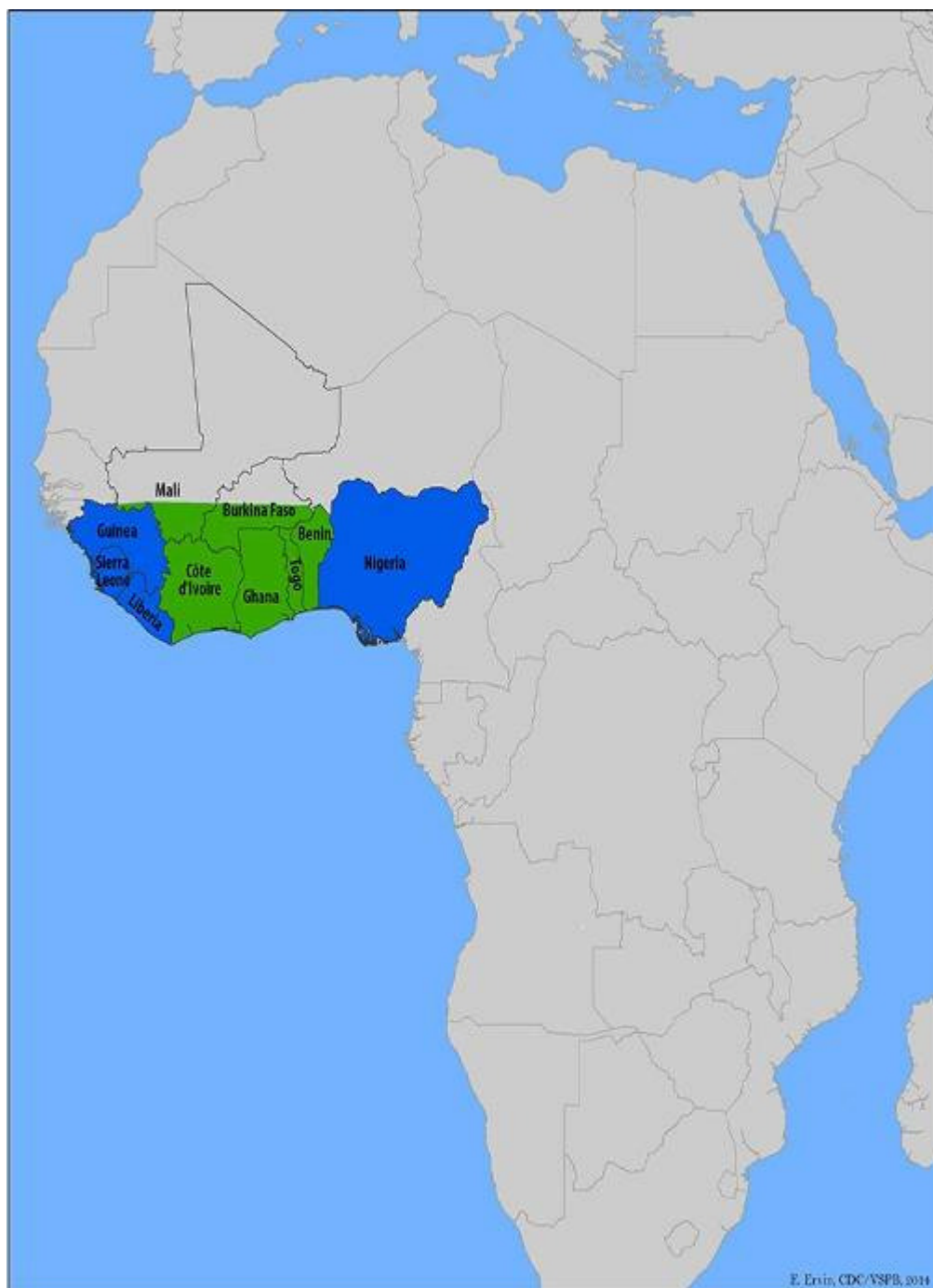
1.3 Epidemiology of Lassa fever

LASV was first isolated in 1969 from a missionary nurse who worked in a clinic in a small town, Lassa, in northeastern Nigeria [14]. The nurse presumably acquired infection from an obstetrical patient residing in Lassa. She died approximately one week after the onset of symptoms. Subsequently, two more nurses that attended the first patient contracted the disease, which was later named Lassa fever and caused the death of one of them. Infectious virus was isolated from all three cases [15].

Initially, several countries of West Africa were identified to be endemic for LASV, namely Sierra Leone [16], Guinea [17], Liberia [18] and Nigeria [19]. However, a serological survey among patients admitted with a history of fever and missionaries that had experienced a febrile illness showed that LFV was also present in Ivory Coast, Mali, and Central African Republic [14]. The notion that LFV was endemic in larger areas of West Africa was further supported by the results of investigation of an imported case of Lassa fever in Germany in 2000.

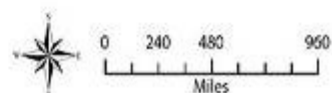
122 During the incubation period, the index patient traveled through several countries, namely
123 Ghana, Ivory Coast, and Burkina Faso that were not considered to be endemic at that time. Later,
124 cases of Lassa fever have been reported from Burkina Faso, Ivory Coast, Ghana, Senegal,
125 Gambia, and Mali [20].

126 According to estimations, LFV is responsible for 100,000-300,000 infections and
127 approximately 5,000 deaths annually [21]. However, there is high degree of seroprevalence of
128 LASV-specific antibodies in the general population residing in the endemic regions, although
129 highly variable depending on the geographical location (from 1.8% to 55%). indicates that most
130 infections are mild or possibly even asymptomatic and do not result in hospitalization. This is
131 also supported by the findings indicating a high incidence of LASV-specific seroconversion,
132 from 5% to 20% of the nonimmune population per year [22]. Nosocomial outbreaks are
133 associated with higher mortality rates ranging from 36% to 65% [23]. However, serosurveillance
134 studies in hospitals dealing with suspected Lassa fever cases showed that the hospital staff that
135 routinely practiced basic hygiene measures had no higher risk of infection than the local
136 population. Infection with LASV presumably occurs through contact with body fluids or excreta,
137 or inhalation of aerosols produced by infected animals. LASV is stable in aerosol [24], and
138 animal-to-animal transmission via the airborne route has been demonstrated in the laboratory
139 setting [25]. Hunting of peridomestic rodents and consumption of their meat is another important
140 route of LASV transmission to humans residing in endemic areas of West Africa [13, 26].



LASSA FEVER DISTRIBUTION MAP

- Countries reporting endemic disease and substantial outbreaks of Lassa fever
- Countries reporting few cases, periodic isolation of virus, or serologic evidence of Lassa virus infection
- Lassa fever status unknown



141

142 Figure 2. Lassa fever distribution map

Source: CDC, 2014

143 1.3.1 Host of transmission

144 The multimammate mouse, *Mastomys natalensis*, was originally identified as the primary host
145 species for LASV. However, due to the poor understanding of the taxonomy of the genus, it is
146 uncertain which species and particular subspecies serve as a reservoir for the virus [27]. The
147 studies addressing the importance of *M. natalensis* for the circulation of LASV in nature
148 demonstrated that newborn animals inoculated intraperitoneally develop persistent asymptomatic
149 infection [28].

150 Significant infectious virus titers were detected in many organs, tissues, and fluids
151 including lymph node, liver, spleen, lung, blood, and brain up to 74 days after inoculation.
152 Moreover, LASV was isolated from the urine and throat swabs of infected animals. No
153 significant histopathological alterations were observed in these animals. Interestingly, adult *M.*
154 *natalensis* infected with LASV also developed a disseminated infection that lasted up to 30 days.
155 Some animals cleared the virus from some organs, but there was persistence in other organs up
156 to 103 days when the study was terminated. The only consistent histopathological finding
157 observed in adult animals was a moderate chronic meningoencephalitis [28]. These data
158 demonstrate that *M. natalensis* has an optimal pattern of infection and virus shedding for the
159 maintenance of LASV in nature.



160

161 Figure 3: *Mastomys natalensis*; the rodent that transmit Lassa fever virus Source: CDC, 2014

162 1.3.2 Recent Lassa outbreak in Nigeria

163 Lassa has been occurring in Nigeria as sporadic outbreaks from the year it was identified in
 164 1969. some outbreaks of the disease have been reported in various parts of Nigeria
 165 including Ekpoma, Aboh-Mbaise, Onitsha, Jos, Ebonyi, Taraba, Nasarawa, Yobe, Rivers,
 166 Ondo, and Edo states. Despite the epidemic nature of LF in Nigeria and other African
 167 countries, the details of outbreaks and subsequent responses to contain it have not been
 168 well documented in these places, and it is difficult to learn from these experiences to
 169 improve the management of future outbreaks. Available Nigerian reports have focused
 170 mainly on a nosocomial outbreak that occurred almost two decades ago or more recently

171 on laboratory diagnosis of blood samples of suspected cases sent to a national reference
172 laboratory. These outbreaks became heightened in the last decade. In the first quarter of 2012,
173 623 suspected cases, including 70 deaths were recorded from 19 of the 36 States of Nigeria.
174 Laboratory analysis undertaken at the Irrua Specialist Teaching Hospital, Irrua Edo State,
175 Nigeria confirmed the presence of Lassa virus infection in 108. Three doctors and four nurses
176 were reported to be among the fatalities [5].

177 On 4th January 2014, a patient was admitted on with symptoms of severe fever with
178 bleeding, which compelled the medical personnel to send his blood specimen to the Lassa fever
179 diagnostic Centre at Irrua in Edo state where it was confirmed positive and the first case in 2014. On
180 25th October, 2014, Nigeria recorded another 10 confirmed outbreak of Lassa at Oyo state (CDC,
181 2014). In 2015, Nigerian center for disease control reported 21 cases, 4 confirmed cases and 1
182 death of Lassa between 1st January to 8th March, 2015. Out of these 21 cases, Bauchi state had
183 the most recent 3 confirmed outbreaks and 1 death [29].

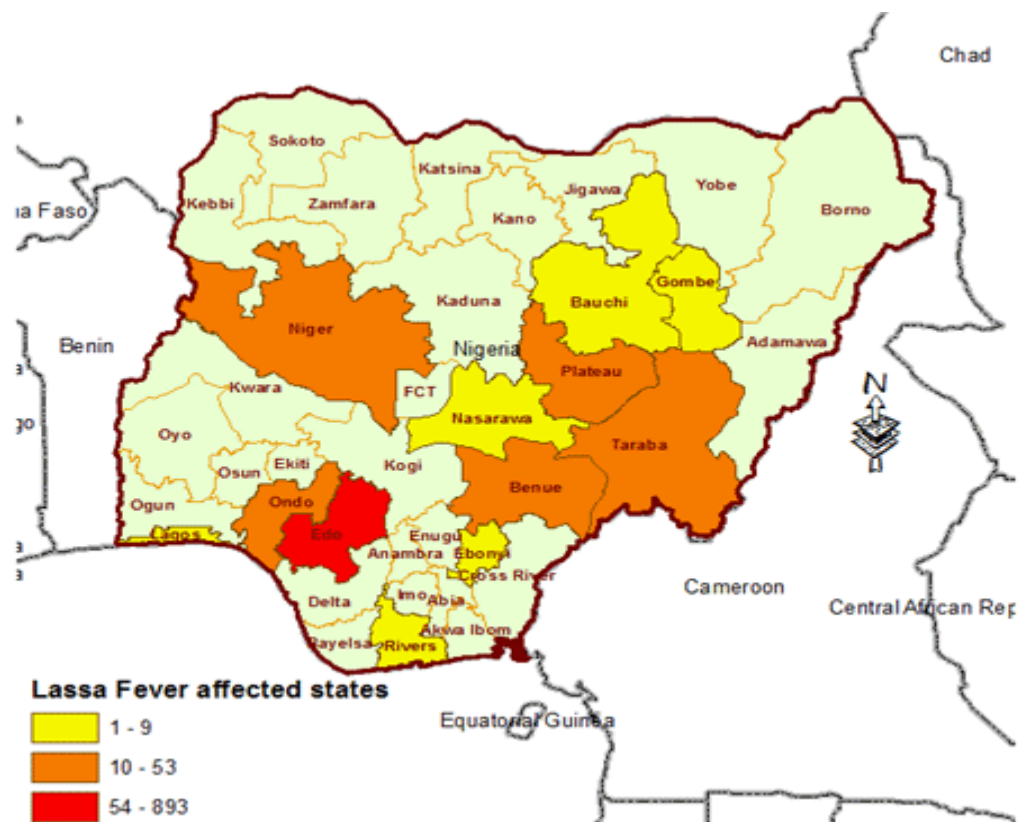


Figure 4. Lassa fever affected states, 2012

Source Nigerian

Federal ministry (www.fmoh.gov.ng)

1.4 Diagnosis of Lassa fever

The signs and symptoms of Lassa fever may be difficult to distinguish from diseases that are common in the tropics such as severe malaria, typhoid fever, yellow fever and other viral haemorrhagic fevers, but diagnosis can be assisted with simple laboratory support but definitive diagnosis requires testing that is available only in highly specialized laboratories. As the symptoms of Lassa fever are so varied and nonspecific, clinical diagnosis is often difficult especially early in the course of infection. Hence, to make accurate diagnosis of Lassa fever,

clinical manifestation, epidemiological data, and result of laboratory findings should be taken into consideration.

1.4.1 Laboratory investigations

Lassa fever is diagnosed by detection of Lassa antigen, antibodies, or virus isolation techniques. In the laboratory, the virus can be isolated using laboratory animals such as albino mice, guinea pigs, Vero cell or African green monkeys. Albino mice inoculated intracerebrally die between 3 and 5 days. Lassa fever virus causes conspicuous cytopathic effect on confluent monolayer of Vero cell culture within 96 hours. The antigens to be used for viral isolation can be obtained from the patients' blood, urine, pleural fluid, throat swab and in case of death, pathological materials from liver, kidney, spleen and heart [30].

The virus can be seen under electron microscope using specimens obtained from infected persons. Although virus isolation remains the most sensitive, it is still uniquely a research tool. The classical method to detect Lassa virus is inoculation of Vero cells with serum, cerebrospinal fluid (CSF), throat washing, pleural fluid or urine of the patient. Specimen for laboratory analysis should be collected as soon as possible from the patient suspected of having the infection. Lassa virus is infectious by aerosol and the human and rodent specimens should be processed with appropriate precautions in biosafety level-4 laboratories. The specific diagnosis is readily made by the isolation and identification of the virus. This is usually done by the inoculation of blood from the patient into Vero cell cultures [32].

Virus antigen can be detected by enzyme linked immunosorbent assays (ELISA) using Lassa virus-specific antibodies. These tests are easy to handle and rapid, and can be performed with inactivated specimens, which is advantageous in the field if sophisticated equipment is not

218 available. Results should be mentioned as soon as they are ready to help in monitoring the
219 prognosis of the disease.

220 The indirect fluorescent-antibody (IFA) test has traditionally been employed in the
221 laboratory diagnosis of acute Lassa virus infection [32]. Although the interpretation of IFA
222 results is complicated by the presence of IFA during both acute and convalescent stages of
223 infection and by the subjective nature of the assay, the appearance of IFA antibody early in the
224 course of Lassa infection may be useful in identifying patients with poor prognosis. However,
225 due to lack of specificity in populations in non-endemic areas, the technique has been largely
226 replaced by ELISA for Lassa virus antigen and Lassa virus-specific immunoglobulin M (IgM)
227 and G (IgG) antibodies (Niklasson et al., 1984). A thorough evaluation of the Lassa virus ELISA
228 on field-collected samples to assess its true sensitivity and specificity was performed in Sierra
229 Leone and Guinea in West Africa. In the study, isolation of virus as detected by
230 immunofluorescent staining for viral antigen along with a positive reverse transcription-PCR
231 (RT-PCR) test on the isolate was employed as the “gold standard” test of Lassa virus infection.
232 The results showed that the combined ELISA Ag/IgM assay was highly sensitive and specific for
233 the diagnosis of Lassa fever and the antigen detection assay offered a particular advantage in
234 providing early diagnosis as well as prognostic information [31].

235 From this research, the technique appeared to be a better diagnostic tool for Lassa virus
236 infection compared to other serological techniques. Although the RT-PCR assays are very
237 sensitive, their applicability in the West African countries where Lassa fever is endemic is
238 limited by issues of strain variation, cross contamination, lack of qualified personnel, inadequate
239 facilities and expenses [33]. Another valuable diagnostic tool is the rapid diagnostic immunoblot
240 assay (RDIA) for Lassa fever. Unfortunately, its usefulness is limited by its low capacity to

241 provide prognostic information and also its low sensitivity. In any case, biosafety level-4
242 containment is required for analyzing samples suspected of Lassa.



243
244 Figure 5. Laboratory professional working in BSCL 4 laboratory Sources: CDC,
245 2014

246 Differential diagnosis: Lassa haemorrhagic fever must be differentiated from other febrile
247 diseases like Ebola (Marburg) haemorrhagic fever, malaria, diphtheria, viral hepatitis, legionella,
248 dengue, yellow fever, Congo-haemorrhagic fever, etc. Lassa fever virus has a peculiar natural
249 reservoir rodent host (*M. natalensis*). It is very imperative that clinical assessment be combined
250 with specific laboratory diagnosis to adequately identify the Lassa fever virus in order to
251 commence early treatment which is paramount to the survival of infected individuals [34].

252 Most Healthcare facilities in Nigeria and host of many African nations rarely have
253 standby BSCL-4 Laboratories and quarantine camps, which are necessary for investigating
254 highly contagious pathogens. This consequently led to increased transmission rate among
255 healthcare care workers and even relatives of infected individuals.

Whenever Lassa outbreak is suspected in any part of Nigeria, samples are collected and sent to a reference laboratory at Irrua specialist hospital in south-southern part of the country for definitive diagnosis. This center is several hundred kilometers from extreme northern and western states of Nigeria. Surprisingly recent outbreaks occurred in these zones. Most times, patients don't get prompt laboratory testing and eventually die before being diagnosed. More so, because most health workers have inadequate knowledge and understanding of the viral infection. There is increased incidence of healthcare workers contracting the infection during the course of rendering patient care.

1.5 Prevention and control

1.5.1 The individual

The affected person should be admitted to a special center for the treatment of Lassa fever.

Where this is not possible, the patient should be barrier-nursed. Health care providers and close associates of the patient should wear protective clothing, masks and gloves. Excrements from affected persons should be properly disposed

1.5.2 The community

Legislation is needed to prevent widowhood rites, traditional autopsies, bush burning and unhygienic preparation of 'garri' and other staple foods. Animal husbandry and fisheries should be encouraged in order to provide alternative sources of first-class proteins for rat eaters. Regular and sustainable environmental sanitation is needed to prevent rat breeding.

The public should be made aware of the mode of contact of Lassa fever and its high case fatality rate using print and electronic media. Community involvement and participation is

277 necessary to provide sustainable Lassa fever control. Food vendors should be educated on the
278 need to prevent food contamination with Lassa fever virus. Grains, flours and left-over foods
279 should be adequately covered to prevent contamination by rats. Rodenticides should be used for
280 the destruction of rats in homes, and development of Lassa fever vaccine should be facilitated.
281 Regular seminars should be held for health-care providers on early diagnosis and treatment of
282 Lassa fever, while diagnostic kits should be made available in district hospitals.

283 Affected people should be referred early to the special center in order to prevent or limit
284 disability, while those with disabilities should be rehabilitated functionally, socially and
285 psychologically so that they can be gainfully employed.

286 1.5.3 Vaccine

287 Although the prevention of human contact with the *Mastomys* rodents is an essential factor in the
288 control of Lassa fever, widespread prevention of such contact is presently impractical in the
289 endemic regions of West Africa, so provision of a vaccine for community and hospital use is an
290 imperative public health need because vaccination is the most viable control measure [35].

291 A previous report had indicated that a vaccinia virus expressing the Lassa virus
292 glycoprotein protected four non-human primates against lethal challenge with Lassa virus. This
293 was followed by the finding that single administration of a vaccine expressing the full-length
294 Lassa virus glycoprotein affords protection against Lassa fever in primates, with or without
295 expression of the nucleoprotein. The challenge, however, is to overcome the scientific, political
296 and economic obstacles in producing a human use vaccine candidate. It is well established that
297 the G-protein confers protection but its duration is unknown and if the N-protein is also included

298 there may be a better duration of protection but it is unclear whether the N-protein as a vaccine
299 may possibly enhance the infection [36].

300 Adequate funding and applications of new vaccine technologies give hope that there may
301 soon be a vaccine in clinical trials, however, the difficulty of conducting trials in endemic areas
302 of the West African sub-region and lack of political stability remain serious problems.

303 **1.6 Challenges associated with Lassa eradication in Nigeria**

304 Poor education of people in high-risk areas about ways to decrease rodent populations in their
305 homes, inadequate provision of rapid diagnostics, barrier medical preactice and quarantine
306 camps which has led to poor disease control in Nigeria. Presently we have only one Lassa fever
307 diagnostic center situated at Irrua Specialist Teaching Hospital (ISTH), Edo State. This is partly
308 because of the unavailability of laboratory containment facilities, laboratory reagents and
309 equipment. More so, another issue worthy of note is the inadequate re-training of medical
310 laboratory professionals on technical-know-how of recent laboratory techniques such molecular
311 assays.

312 **1.7 Conclusion and recommendations**

313 It is unequivocally established that Lassa fever is a very important vector borne disease
314 that has assumed epidemiological proportion in West Africa where it records high endemicity.
315 The public health implication of this cannot be overstated. Apart from possible periodic
316 outbreaks of Lassa fever epidemic within the region, the unprecedented increase in inter-border
317 traffic and international travels elevates the chances of introducing the virus to other regions
318 within and outside the African continent.

The scarcity of resources available for healthcare delivery system and the political instability that characterize the West African countries would continue to impede efforts for the control of both emerging and currently ravaging infectious diseases in the region. However, adequate education of health care providers and other public health personnel as well as the establishment of well-equipped infectious disease laboratories and research centers would aid in the prompt diagnosis and treatment of highly infectious diseases like Lassa fever would help in averting possible outbreaks. Furthermore, ribavirin should be made available in hospitals and health centers in the endemic areas particularly in rural communities. This would help to control the disease.

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