

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

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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 partly because cases are usually presented late to healthcare centers and its high virulence. The transmission rate of LFV is usually very high in affected areas in Nigeria due to poor pathogen containment, a necessity in medical care of infected individuals and inadequate and /or delayed laboratory confirmation of cases, consequently placing immediate communities and healthcare providers at higher risk of contracting LFV. Laboratory diagnose for Lassa are crucial because of similarities of clinical

features it presents with other **more frequent fever-causing infections** such as severe malaria, typhoid and fulminant hepatitis. Understanding its basic virology, ecology, transmission pattern and immunology are crucial in embarking on appropriate infection control strategies and diagnostic protocols. In view of these, we sort to present this mini-review to summarize the pertinent literatures for healthcare providers especially in endemic locations, suggest the need to use the information from epidemiologic and laboratory findings to incorporate international best practices into community health and clinical practice in Nigeria.

Keywords: Lassa; *Mastomys natalensis*; Outbreaks; Containment; Laboratory investigations; Nigeria.

1.0 Background

Lassa is an acute viral and zoonotic disease caused by Lassa fever virus (LFV), **a member of the arenaviridae family particularly responsible for a severe haemorrhagic fever characterized by febrile illnesses, myalgia, sore throat, nausea, emesis, chest and abdominal pains** [1]. The virus exhibits persistent and asymptomatic infection with profuse viruria in *Mastomys natalensis*, the ubiquitous and highly commensal rodent host [2]. In 2014, the US Center for Disease Control and prevention (CDC) reported that four West African countries (Sierra Leone, Guinea, Liberia and Nigeria) are endemic to Lassa (figure 1). It has been estimated that 300,000 to 500,000 cases of Lassa fever and 5000 deaths occur yearly across West Africa [3]. However, there is no specific data in Nigeria.

LFV has been implicated with nosocomial outbreaks with high mortality in affected areas [4], however, the enlightenment campaign for the prevention of Lassa fever and diagnostic facilities are either lacking or rudimentary in most countries where Lassa fever is endemic. Compared to Human immunodeficiency virus, Lassa is more contagious to close associates and it rapidly kills. However, Lassa fever does not get the global attention it deserves. Hence, appropriate barrier medical guidelines and early identification of infected individuals through definitive laboratory diagnosis are important for prompt implementation of appropriate control measures [5].

In spite of the outstanding progress made in recent time in understanding the replication pattern of LFV and the breakthroughs in the pathogenesis and molecular epidemiology of Lassa, as well as the development of the state-of-the-art technologies for diagnosing this highly virulent disease [6], the emergence of this highly virulent and contagious virus in many districts and states in endemic West African countries and the frequent sporadic cases of Lassa outside the endemic regions may be as a result of inter-border traffic and international travels. This necessitates that healthcare workers have comprehensive knowledge about LFV and the disease it causes [5, 6].

In view of these, we sort to present this mini-review to summarize the pertinent literature for Lassa healthcare providers in our healthcare facilities, suggest the need to use the information from epidemiologic and laboratory investigations to incorporate international best practices into community health and medical practice guidelines.

2.0 Review and Discussions

2.1 Basic Virology

Lassa fever virus (LFV) is an enveloped, single-stranded, bisegmented RNA virus that belongs to the Arenaviridae family. LFV lacks a conventional negative-strand coding arrangement and the isolates of the LFV differ in their genetic, serologic, and pathogenic characteristics [7]. LFV 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 [7]. The envelope encloses the genome which has helical nucleocapsid measuring between 400 and 1300 nm in length [8]. Often the interior contains electron dense granule identified as the host cell ribosome from where the name “arena” was derived meaning sand (figure 2) [9]. LFV can be inactivated in ultraviolet and gamma irradiations, heating from 56–100°C and pH range between 5.5 and 8.5. Chemical agents such as 0.5% sodium hypochlorite, 0.5% phenol and 10% formalin are good inactivating against the virus [10].

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 lRNA encodes the viral polymerase and a small, zinc-binding (Z) protein [10]. Recent molecular techniques for full-length sRNA amplification have facilitated research efforts on the identification and molecular analysis of novel arenaviruses and their various strains.

The sequencing of Lassa virus sRNA has enabled the identification and molecular characterization of four Lassa virus strains [11], 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 [8, 12, 13, 14]. sRNA Sequencing of LFV indicated a considerable genetic variation among the strains of the virus,

however, phylogenetically, strain AV appears to be the most closely related to strain Josiah from Sierra Leone [14]

There has been no licensed vaccine or immunotherapy available for prevention or treatment of Lassa. The severity of Lassa, its ability to be transmitted through aerosol droplets [15], and the lack of a vaccine or curative drug led to its classification as the United States National Institutes of Allergy and Infectious Diseases (NIAID) Category A pathogen and biosafety level-4 (BSL-4) agent [16]. The antiviral drug ribavirin has been demonstrated to reduce fatality by 50%, but only if it is administered within 6 days after the onset of clinical symptoms [17].

2.2 Immunology of Lassa fever

Selection of appropriate diagnostic laboratory tests require understanding the immunology of the disease against which test results must be reliable. Clearly, LFV is handled differently by the immune systems of *mastomys* and human hosts.

The immunological response in primates to Lassa infection is complex. In general, evidence indicated that LFV depends primarily on cytotoxic T-cell responses for virus clearance [16, 18]. Neutralizing antibodies to LFV cannot be measured in the serum of patients at the beginning of acute infections in most people and in experimentally infected monkeys, they are never detectable in a classical plaque-reduction neutralization assay and ELISA, therefore, a “window period” for serological Lassa IgM may hamper early diagnosis [19].

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 [20]. 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 [20]. It was found that Lassa patients who were antigen positive were more likely to die than suspected cases who were only IgM positive [16]. The metabolic and immunological parameters in antigen positive Lassa patients demonstrated a strong correlation between survival and low levels of some cytokines, CD40L, blood urea nitrogen and Liver transaminases [16]. Despite presenting to the hospital with symptoms consistent with Lassa, the profiles of antigen negative IgM positive individuals were similar to those of normal donors and nonfatal (NF) Lassa cases studies. These suggest that IgM status cannot necessarily be considered a diagnostic marker of acute Lassa in suspected cases in endemic areas of West Africa [16, 20].

2.3 Ecology and host of transmission

Mastomys natalensis (Figure 3) was identified as the primary host for LFV. The ecology of Lassa was discovered to involve enzootic cycle of LFV in commensal populations of this single murine species. Apart from rodents of the genus *Mastomys*, the main reservoir of LFV, other rodent species have been involved as well. A report demonstrated a total of eight Lassa virus strains isolated from the tissues and blood of rodents identified in the field. They were three different species: *M. natalensis*, *Rattus rattus*, and *Mus minutoides* [21].

LFV transmission may cross borders from the rodent cycle to man by several routes. Secondary human-to- humans spread may occur within domiciliary groups, and persons infected within the community who develop clinical Lassa hemorrhagic disease may introduce LFV into a healthcare centers and begin another cycle of nosocomial infections [22].

The distribution of *Mastomys* in West Africa is highly variable and in some areas, 50% of domestic rodents may be *Mastomys* [23]. Since the rodents do not move far from their nest,

and because Lassa virus is transmitted vertically in rodents, infection in local populations of rodents tends to cluster. Thus, human infections tend also to be focal with periodic familial or village clusters with secondary cases due to person-to-person spread. These outbreaks are seen against the background of primary infections from rodents that make up the bulk of endemic disease.

Infections peak between January and May –during the dry season – but cases are seen year round [24]. Lassa exists in all age groups and sexes, and not surprisingly given the ubiquity of the rodent host, antibody prevalence tends to increase with age. This is compatible with virus transmission to humans in and around the homes where the *Mastomys* live.

Most outbreaks are associated with poor pathogen containment through barrier nursing, inadequate disinfection and direct contact with infected blood and contaminated needles. Increasing and indiscriminate use of needles for intravenous therapy, or intramuscular injections in West African rural hospitals along with inadequate needle and syringe sterilization can lead to large-scale epidemics. These epidemics can be devastating, resulting in the deaths not only of patients but also medical staff, surgeons, nurses and other scarce trained personnel [22].

2.4 Recent Lassa outbreaks in Nigeria

Lassa has been occurring in Nigeria as sporadic outbreaks from the year it was identified in 1969. Outbreaks of Lassa have been reported in various parts of Nigeria including Edo, Ebonyi, Onitsha, Abioke, Jos, Taraba, Nasarawa, Yobe, Rivers and Ondo states. Despite the epidemic nature of Lassa in Nigeria, details of outbreaks and subsequent interventional responses to contain it have not been well documented. Unfortunately, with this nature of infection response, it is very difficult to gain experience from previous outbreaks

to improve the management of future reemergence. Most available Nigerian reports focused mainly on nosocomial outbreaks or more recently on laboratory diagnosis of blood specimens of suspected cases sent to Nigerian Lassa reference laboratory. These outbreaks became heightened in the last decade. In early 2012, 623 suspected cases, including 70 deaths were recorded from 19 of the 36 States of Nigeria. Laboratory analysis undertaken at the Lassa reference laboratory at Irrua Edo State, confirmed the presence of Lassa in 108 cases. Three medical officers and four nurses were reported to be among the fatalities [5].

As early as 4th January 2014, a patient was admitted with symptoms of severe fever and bleeding, which compelled the medical personnel to send blood specimen to the Lassa reference laboratory where it was confirmed positive and the first case in 2014. On 25th October, 2014, Nigeria recorded another 10 confirmed outbreak of Lassa at Oyo state [1]. In 2015, Nigerian center for disease control reported 21 cases, 4 confirmed cases and 1 death of Lassa between 1st January to 8th March, 2015. Out of these 21 cases, Bauchi state had the most recent with 3 confirmed cases and 1 death [25].

2.5 Diagnosis of Lassa

Lassa may be difficult to distinguish from signs and symptoms of other more common infectious diseases in tropical countries but diagnosis can be assisted with differential laboratory testing but definitive diagnosis requires investigations that are available only in highly specialized laboratories (figure 4) [26]. As Lassa fever presents with nonspecific symptoms, clinical diagnosis is often difficult especially in the early onset of infection. Hence, to make accurate diagnosis of Lassa fever, clinical manifestation, epidemiological findings, and result of laboratory testing should be taken into consideration.

2.5.1 Laboratory investigations

Diagnosis for Lassa could be made by LFV antigen detection, anti-LFV specific antibodies, viral isolation or nucleic acid-based assays. LFV has isolated using laboratory animals such as albino mice, guinea pigs and African green monkeys. Albino mice inoculated intracerebrally died between 3 and 5 days [26]. LFV causes conspicuous cytopathic effect (CPE) on confluent monolayer of Vero cell culture within 4 days. The source of antigens used for viral isolation as those obtained from the patients' specimens such as blood, urine, pleural fluid, throat swab and in case of death, histopathological materials from liver, kidney, spleen and heart in autopsy [6, 27]. The virus can be identified using electron microscope due to its characteristic 'sand shape' using processed specimens obtained from infected persons. Although virus isolation remains the most sensitive and uniquely research tool, its laborious nature and long turnaround time restricts its clinical use.

Specimen from Lassa suspected case should be collected as soon as onset of clinical signs suggestive of the infection. LFV is so infectious that it could be contracted through aerosol. Human or animal specimens should be processed with maximal precautions in BSL-4 laboratories [26, 27]. LFV antigen can be detected by the use enzyme linked immunosorbent assays (ELISA) using LFV-specific antibodies. These tests are easy to handle, rapid, and can be performed with inactivated specimens. This analysis is advantageous in field studies where sophisticated testing equipment are not available [27].

The indirect fluorescent-antibody (IFA) test has traditionally been used in the laboratory diagnosis of acute Lassa. Although the interpretation of IFA results is complicated by the presence of IFA during both acute and convalescent phases of Lassa, the appearance of IFA antibody early in the course of Lassa infection may be useful in identifying patients with poor

prognosis [10]. Due to lack of specificity in populations in non-endemic areas, IFA has been largely been phased out and replaced by ELISA for viral antigen and LFV-specific immunoglobulin M (IgM) and G (IgG) antibodies. Thorough evaluations of the LFV-ELISA on field-collected samples to assess its true sensitivity and specificity have been extensively investigated. Isolation of virus as detected by immunofluorescent staining for viral antigen along with a positive reverse transcription-PCR (RT-PCR) test on the isolate has been employed as the “gold standard” test of LFV infection [16, 28]. The results showed that the combined ELISA Ag/IgM assay was highly sensitive and specific for the diagnosis of Lassa and the antigen detection assay offered a particular advantage in providing early diagnosis as well as providing prognostic information [16, 28].

The RT-PCR assays are highly sensitive, their applicability in the West African communities where Lassa is endemic has been limited by issues of cross contamination, strain variations, inadequate of qualified laboratory scientists to execute the test, inadequate facilities and relatively expensive to conduct [29]. Another valuable diagnostic tool is the rapid diagnostic immunoblot assay (RDIA) for Lassa fever. Unfortunately, its usefulness has been shown to be limited by its low sensitivity and low capacity to provide prognostic data on patients [30]. In any case, biosafety level-4 containment is required for analyzing samples suspected of Lassa.

2.5.2 Laboratory investigations of Lassa in Nigeria

Nigeria barely has a single Lassa reference laboratory. BSL-4 centers are necessary for investigating highly contagious pathogens. In the absence of such diagnostic settings, there will most likely be increased Lassa transmission among healthcare care workers, especially within the laboratory. Whenever Lassa outbreak is suspected in any part of Nigeria, samples are

collected and sent to the 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 and frequent outbreaks occurred in these zones (i.e. northern and western Nigeria). Most time, patients don't get prompt laboratory testing and in the course of delay, die before being diagnosed. More so, because most health workers have inadequate knowledge and understanding of the viral infection. Relatively large number of healthcare workers contract Lassa during patient care.

2.6 Prevention and control of Lassa in Nigeria

2.6.1 The individual

Affected person should be admitted to a special center that care and treat Lassa cases. Where this is not possible, the patient should be on barrier nursing. Health care providers and close associates of the patient should wear protective clothing, masks and gloves. Excrements from affected persons should be adequately disposed.

2.6.2 The community

Legislation should be in place to prevent bush burning and unhygienic preparation and preservation of staple foods such as the well consumed 'garri'. Animal husbandry and fisheries should be encouraged in order to provide alternative sources of first-class proteins for rat eaters. Regrettably, most rural communities in Nigeria don't believe they could contract Lassa from consuming infectious rodents. Thus, they incessantly and adamantly consume rats as delicacies, putting them at high risk for Lassa. 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 necessary to provide sustainable Lassa fever control. Food vendors need to be regularly sensitized on the need to prevent food contamination with LFV. Grains, flours and left-over foods should be adequately covered to prevent contamination by rats. Rodenticides should be used for the destruction of rats in homes.

2.6.3 Vaccine

Prevention of human contact with the rodent vectors is an important criterion in the control of Lassa fever. Widespread prevention of such contact is largely impractical in the endemic regions of West Africa. Discovery and provision of vaccine for communities and hospital use is an imperative public health need because vaccination is the most viable and efficient control measure.

In recent time, adequate funding and applications of new vaccine technologies has led to some experimental vaccine in clinical trials, however, there will be difficulties in conducting trials in the endemic West African countries due to sociopolitical and security instabilities.

3.0 Conclusion

Lassa fever has been unequivocally established as a very important vector borne disease that has assumed a heightened epidemiological proportion in Nigeria, because of its high endemicity. The public health implications of the situation cannot be overemphasized. Apart from possible periodic outbreaks of Lassa epidemics, the unprecedented increase in inter-state traffic and international travels have elevated chances of introducing the virus to other areas within and

outside the Nigeria and African continent at large. Considering available facts, it is glaring that Nigeria is certainly underprepared for preventing reemergence of Lassa.

4.0 Recommendations

The poor availability of health care delivery systems and lack of political will that characterize Nigeria would continue to impede efforts for the control of Lassa and other emerging pathogens. However, adequate training and retraining of healthcare providers as well as the establishing well-equipped infectious disease clinics, laboratories and research centers would help in the prompt containment, diagnosis and treatment of Lassa and would help in averting possible future outbreaks. Furthermore, all hospitals and healthcare centers in the endemic areas especially those in rural communities should be provided with ribavirin. This will prove useful in minimizing mortality caused by Lassa.

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7.0 Conflict of interest

Authors declare that there is no conflict of interest associated with this manuscript.

8.0 Authors' contributions

This work was carried out in collaboration between all authors. Idris Abdullahi Nasir designed the study, wrote the protocol, the first draft manuscript and reviewed the manuscript for intellectual content. Fatima Muhammad Sani participated in the literature search, data collection and preparation of the draft manuscript. Both authors read and approved the final manuscript.

8.0 Supplementary files



Figure 2. Lassa fever distribution map

Source: CDC, 2014

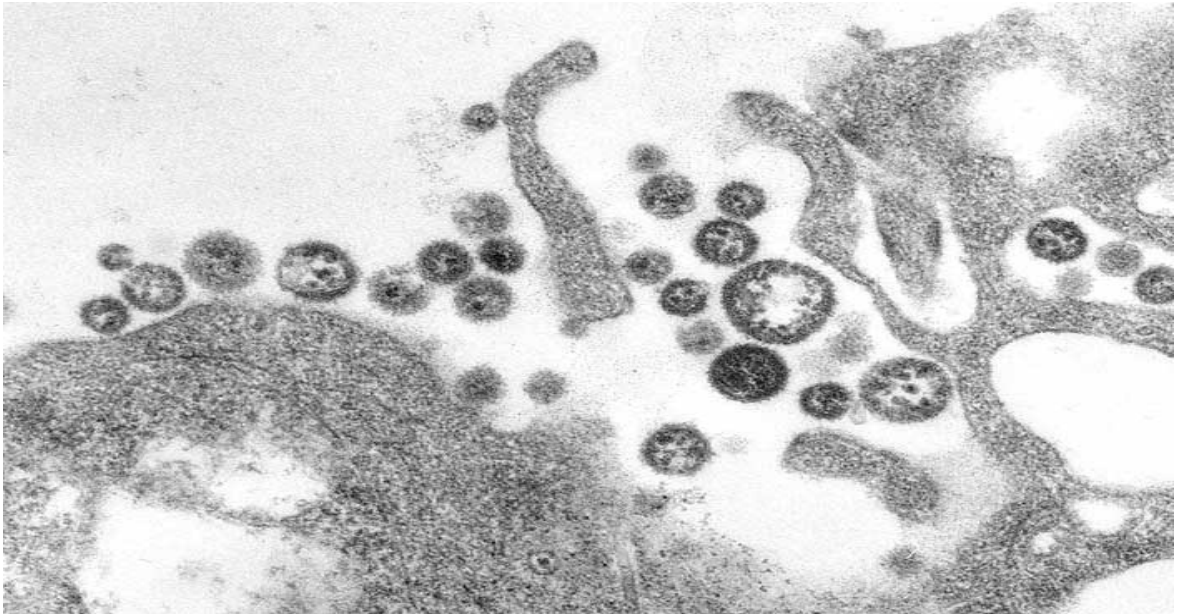


Figure 2: Electron Micrograph of Lassa fever virus particle Source: CDC, 2014



Figure 3: *Mastomys natalensis*; the rodent that transmit Lassa fever virus



Figure 4. Laboratory professional working in BSL-4 laboratory

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