Review Article

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Lassa fever in West African sub-region: an overview

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Abstract

Lassa fever is an acute viral zoonotic illness caused by Lassa virus, an arenavirus known to be responsible for a severe haemorrhagic fever characterised by fever, muscle aches, sore throat, nausea, vomiting and, chest and abdominal pain. The virus exhibits persistent, asymptomatic infection with profuse urinary virus excretion in the ubiquitous rodent vector, Mastomys natalensis. Lassa fever is endemic in West Africa and has been reported from Sierra Leone, Guinea, Liberia, and Nigeria. Some studies indicate that 300,000 to 500,000 cases of Lassa fever and 5000 deaths occur yearly across West Africa. In spite of the great progress made in recent years in the understanding of the life cycle of arenaviruses, including Lassa virus, and the new insights gained in the pathogenesis and molecular epidemiology of Lassa fever, as well as the development of the state-of-the-art technologies for diagnosing this life-threatening disease.

The emergence of this highly virulent and contagious Lassa virus in many more districts and states in endemic countries of the West African sub-region and the increasing sporadic cases of Lassa fever outside the endemic regions within and outside Africa as a result of huge inter-border traffic and international trav-

Key words Infection – Lassa fever – Mastomys natalensis – West Africa

Introduction

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The emergence of this highly virulent and contagious Lassa virus in many more districts and states in endemic countries of the West African sub-region and the increasing sporadic cases of Lassa fever outside the endemic regions within and outside Africa as a result of huge inter-border traffic and international trav-
els, necessitates that health care providers should have comprehensive information about the virus and the disease it causes. Furthermore, Lassa virus has been associated with nosocomial outbreaks with high mortality\textsuperscript{10,11}, hence, early identification of infected individuals is important for prompt implementation of appropriate barrier nursing guidelines\textsuperscript{12}.

The objective of this article, therefore, is to provide a critical overview of Lassa virus infection in the West African sub-region with consideration of the origin of the virus, its properties/strains, epidemiology and clinical presentation, treatment, prevention and control as well as the current theories of its pathogenesis and efforts in vaccine development. Studies reported in English, that investigated Lassa fever with reference to West Africa were identified using the Medline Entrez-PubMed search. Combinations of key words such as \textit{Lassa fever} and \textit{West Africa} were used in the search that yielded 51 entries as at June 2006. References from selected publications were also used to identify additional relevant literature for the review. Emphasis is placed on the need for adequate training of health care workers in the sub-region regarding diagnostics of highly contagious infections, intensive care of patients under isolation, contact tracing, adequate precautionary measures in handling infectious laboratory specimens, control of the vector as well as care and disposal of infectious waste.

\section*{Historical account}

Lassa fever was first described in Sierra Leone in the 1950s but the virus responsible for the disease was not identified until 1969 when two missionary nurses died in Nigeria, West Africa, and the cause of their illness was found to be Lassa virus, named after the town in Nigeria (Lassa in the Yedseram River valley) where the first cases were isolated\textsuperscript{5,13}. Since then, a number of outbreaks of Lassa virus infection were reported in various parts of Nigeria including Jos, Onitsha, Zonkwua, Abo Mbaise, Owerri, Epkoma and Lafiya\textsuperscript{4,10,14–16}. Epidemics of Lassa fever were also documented in other West African countries including Liberia, Sierra Leone, Guinea, Mali and Senegal\textsuperscript{5–7}. A few cases of the importation of Lassa virus into other parts of the world for example by travellers were documented\textsuperscript{17–20}.

\section*{Properties/strains of Lassa virus}

Lassa virus 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\textsuperscript{21–23}. Lassa virus is spherical in shape and measures between 70 and 150 nm in diameter (Fig. 1). 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\textsuperscript{24,25}. Often the interior contains electron dense granule identified as the host cell ribosome from where the name “arena” was derived meaning sandy\textsuperscript{26}. Lassa virus can be inactivated in ultraviolet, gamma irradiation and high temperatures. Lassa virus infection can be prevented by vaccination and some antiviral agents have been shown to be effective in controlling the disease.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{Lassa_virus.png}
\caption{Lassa virus electron micrograph (Image courtesy: C.S. Goldsmith and M. Bowen, Center for Disease Control and Prevention, Atlanta, USA)}
\end{figure}
tion, 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\textsuperscript{26,27}.

The single-stranded 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\textsuperscript{28}. New methods for full-length sRNA amplification are facilitating research efforts on the identification and molecular analysis of new arenaviruses or arenavirus strains\textsuperscript{29}. The sequencing of Lassa virus sRNA has enabled the identification and molecular characterisation of four Lassa virus strains. These include: the strain Josiah, originating from Sierra Leone\textsuperscript{24}, the strain Nigeria\textsuperscript{30} and strain LP\textsuperscript{31,32}, both from Nigeria and the strain AV imported into Germany by a traveller who had visited Ghana, Côte D’Ivoire, and Burkina Faso\textsuperscript{28}. Sequencing of sRNA of Lassa virus 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\textsuperscript{28}.

**Replication of Lassa virus**

The first step in viral replication is adsorption on cell surface receptors that are found to be widely distributed and highly conserved molecules\textsuperscript{33} (Fig. 2). The glycoprotein of the spikes is responsible for the interactions with cell surface receptors\textsuperscript{34}. The next step is the penetration of the virus, then deproteinisation, and finally liberation of RNA genome into the infected host cytoplasm where both replication and transcription take place. During the process, the cell nucleus provide capped cellular mRNA for priming transcription, and the nuclear membrane provide structural support\textsuperscript{33}. It has been observed that the 5’ end of the S derived subgenomic mRNAs extend beyond the end of the genomic RNA template and the length of such an extension varies between 1 and 7 nucleotides and terminate at 5’ cap structure\textsuperscript{35}. The initiation of replication and transcription starts from the terminus of the template. As the RNA polymerase rails on the template to add new nucleotides that will form polynucleotide of the new strand, the first two slip back on the template to create nontemplated nucleus, a process peculiar to arenaviruses\textsuperscript{26}. After biosynthesis of macro-molecules, the virions are assembled through a process not yet understood. Matured virions are released through budding from the plasma membrane of acutely infected cells.

**Epidemiology**

Lassa virus is hosted by rodents in the *Mastomys natalensis* species complex and is occasionally imported into countries outside of those areas in West Africa where the disease is endemic. Infected rodents remain carriers throughout their life and do not show clinical symptoms but excrete the virus through the urine, saliva, respiratory secretion and exposed blood vessels through micro or macro trauma\textsuperscript{2}. Humans presumably become infected through contact with infected rodent excreta, urine, tissues, or blood\textsuperscript{3,27}. Transmission to man is through faecal-oral route, or respiratory tract by inhaling contaminated air containing the virus, or when infected blood touches bruised skin or by sexual intercourse. Infected persons represent serious threat to the environment. The
virus can be isolated in the blood, faeces, urine, throat swab, vomit, semen and saliva of infected persons and secretion of the virus from infected individuals can continue for 30 days or more\textsuperscript{1,10}. Outbreak of Lassa haemorrhagic fever can take place any time of the year and it is possible to transport Lassa fever from an endemic area to a non-endemic one during the incubation period\textsuperscript{27}. Person-to-person transmission of Lassa fever can also occur through contaminated medical equipment, such as reused needles or when a person comes into contact with virus in blood, tissue, secretions, or excretions of an infected individual but virus cannot be spread through casual contact (including skin-to-skin contact without exchange of body fluids)\textsuperscript{10,27}. Lassa fever occurs in all age groups and in both men and women. Persons at greatest risk are those living in rural areas where the \textit{M. natalensis} is usually found, especially in areas of poor sanitation or crowded living conditions. Health care workers are at risk if proper barrier nursing and infection control are not maintained\textsuperscript{8}.

The finding that genetic distance among Lassa virus strains correlates with geographic distance suggests that the breeding populations of \textit{Mastomys} species, the rodent hosts of Lassa virus, have exhibited little regional movement since Lassa fever was first recognised in the 1960s\textsuperscript{36}. However, the geographically restricted occurrence of the disease is not well understood as its rodent host (\textit{Mastomys} species) is prevalent in much larger areas of sub-Saharan Africa\textsuperscript{37}, therefore, research is urgently needed to determine if Lassa virus is present in \textit{Mastomys} species populations in any of the countries separating Nigeria from Guinea, Liberia, and Sierra Leone including Côte D’Ivoire, Ghana, Togo, Benin and Burkina Faso. Many epidemics have been recorded in many states of Nigeria including Taraba, Plateau, Imo, Enugu and Edo\textsuperscript{4,14–16}. It is possible that the illness may be one of the infections responsible for mysterious deaths in many parts of Nigeria and other African countries.

Approximately 15–20\% of patients hospitalised for Lassa fever die from the illness; however, approximately 80\% of human infections with Lassa virus are mild or asymptomatic, and 1\% of infections overall result in death\textsuperscript{1}. The prevalence of antibody to Lassa virus ranges from 7\% in Guinea and 15–20\% in Sierra Leone and Liberia; and to over 20\% in Nigeria\textsuperscript{38–40}. Lassa virus also causes high foetal mortality and high mortality in pregnant women\textsuperscript{41}. The mortality rate is 92\% for foetuses in early pregnancy, 75\% for foetuses in the third trimester, and 100\% in the neonatal period for full-term babies and the mortality rate for gravid women is 7\% in the first two trimesters, 30\% in the last trimester, and 50\% for pregnant women who delivered within one month. In contrast, the general mortality rate for non-pregnant women only is 13\%.\textsuperscript{41,42} High concentrations of the virus have been found in both foetal tissue and in the placenta and it is suspected that maternal T-cells cannot attack the concentrations of virus in the placenta because placental cells cannot express class I or class II MHC antigens\textsuperscript{42}.

Pathogenesis

On acquisition of the virus through contact with infected rodent urine, saliva, respiratory secretion and blood, Lassa fever infection is initiated in the victim. Lassa fever is a generalised infection with haemorrhagic dissemination of the virus to multiple organs and systems via the blood stream, lymph vessels, respiratory tract, and/or digestive tract\textsuperscript{9}. The blood vessels are always the most affected and the virus multiplies in cells of the reticuloendothelial system causing capillary lesions. The capillary permeability is increased, followed by peripheral vasoconstriction with the presence of disseminated intravascular coagulation that leads to haemorrhagic syndrome\textsuperscript{5}. Haemorrhage may be present in the intestine, liver, myocardium, lungs and the brain and are often inflamed and enlarged, and the tissues are infiltrated, lesioned and necrotic\textsuperscript{43,44}. Other observable pathological changes include black vomit with traces of blood, watery diarrhoea that gives rise to dehydration
and reduction in the volume of blood in circulation and low blood pressure, depressed lymphocyte counts and platelet function as well as moderate thrombocytopenia.

Clinical presentation

The incubation period of Lassa haemorrhagic fever is between 3 and 21 days. At onset, the infection is insidious with fever, headache, rigors, myalgia, backache and malaise. The body temperature may increase to 40°C, and often evening body temperature is higher than that of morning. Constant fever for 10 days or more is an indication of higher level of intoxication which triggers of weakness, muscle pains, abortion and mental disorder. Other signs are nausea, vomiting, abdominal and pleuritic pain, as well as pain around hepatic area. Specific features in various systems of infected person are prominent. In the oral cavity, ulcerated lesions with white or yellow exudates are present in the faucets, mandarin patches, soft plates and tonsil. Some patients experience bleeding from the gums. Other features include sore throat that can cause dysphasia, pharyngitis and watery diarrhoea that may lead to dehydration. The liver and kidney are often inflamed, enlarged and painful on palpation of their respective locations.

Capillary lesions cause haemorrhage in the stomach, small intestine, kidneys, lungs, and brain. Less than 1/3 of the patients present with bleeding; however, bleeding is a predictor of a significantly higher risk of death and in severe cases of Lassa fever, shock and vascular collapse occur, followed by death. Research suggests that the shock results from platelet and endothelial dysfunction, which cause haemorrhage and allow fluid to leak into the intravascular system.

The symptoms on cardiovascular system include pericarditis, trachycardia, bradycardia, hypertension, hypotension, thrombocytopenia, leucopenia and hyperuraemia. Also lymphadenopathy, elevated aminotransferases, decrease in protrobine level, distress of blood circulation and bleeding through the skin, lungs, gastrointestinal tract and other mucous membranes have been recorded. The neck is swollen due to inflamed lymph nodes. There are hyperemia of various intensity and on the skin are generalised maculopapular rash in some cases within the 6–9 days from the onset of fever. Common respiratory system involvement include cough, dyspnea, bronchitis, pneumonia and pleuritis while features which may be associated with the nervous system include encephalitis, meningitis, unilateral or bilateral hearing deficit or seizure.

During convalescence, although the virus may no longer be found in blood, pericarditis can occur, especially in males. The following conditions may also occur during convalescence: aseptic meningitis, encephalitis, global encephalopathy with seizures, cerebellar ataxia (uncommon), and deafness (common). Temporary or permanent deafness in one or both ears occurs in 29% of Lassa fever patients.

Diagnosis

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 specialised laboratories. As the symptoms of Lassa fever are so varied and non-specific, 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.

Laboratory investigation: 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 mon-
keys. 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 h. 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. 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 IV 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. 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 available. Results should be mentioned as soon as they are ready to help in monitoring the prognosis of the disease.

The indirect fluorescent-antibody (IFA) test has traditionally been employed in the laboratory diagnosis of acute Lassa virus infection. Although the interpretation of IFA results is complicated by the presence of IFA during both acute and convalescent stages of infection and by the subjective nature of the assay, the appearance of IFA antibody early in the course of Lassa infection may be useful in identifying patients with poor prognosis. However, due to lack of specificity in populations in non-endemic areas, the technique has been largely replaced by ELISA for Lassa virus antigen and Lassa virus-specific immunoglobulin M (IgM) and G (IgG) antibodies. A thorough evaluation of the Lassa virus ELISA on field-collected samples to assess its true sensitivity and specificity was performed in Sierra Leone and Guinea in West Africa. In the study, isolation of virus as detected by immunofluorescent staining for viral antigen along with a positive reverse transcription-PCR (RT-PCR) test on the isolate was employed as the “gold standard” test of Lassa virus infection. The results showed that the combined ELISA Ag/IgM assay was highly sensitive and specific for the diagnosis of Lassa fever and the antigen detection assay offered a particular advantage in providing early diagnosis as well as prognostic information. From this research, the technique appeared to be a better diagnostic tool for Lassa virus infection compared to other serological techniques. Although the RT-PCR assays are very sensitive, their applicability in the West African countries where Lassa fever is endemic is limited by issues of strain variation, cross contamination, lack of qualified personnel, inadequate facilities and expense. Another valuable diagnostic tool is the rapid diagnostic immunoblot assay (RDIA) for Lassa fever. Unfortunately, its usefulness is limited by its low capacity to provide prognostic information and also its low sensitivity.

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

Treatment

Ribavirin the antiviral drug is effective in the treat-
ment of Lassa fever, but only if administered early in the course of illness\textsuperscript{59}. In a study of Lassa fever in Sierra Leone, West Africa\textsuperscript{60}, it was observed that patients with a high risk of death who were treated for 10 days with intravenous ribavirin, begun within the first six days after the onset of fever, had a case-fatality rate of 5\% (1 of 20) (\textit{p} = 0.0002 by Fisher’s exact test), while patients whose treatment began seven or more days after the onset of fever had a case-fatality rate of 26\% (11 of 43) (\textit{p} = 0.01). The study confirmed the efficacy of ribavirin in the treatment of Lassa fever and that it should be used at any point in the illness, as well as for post-exposure prophylaxis.

Because of its expense, need for intravenous administration, potential toxicity, and teratogenicity, empiric therapy with ribavirin is undesirable\textsuperscript{60–62}. In a remote area of eastern Sierra Leone, West Africa, brief episodes of rigors were reported in patients receiving ribavirin\textsuperscript{61}. However, the occurrence or number of rigors in an individual patient was not associated with sex, age, weight, volume of loading dose, cumulative dose, administration of other drugs and use of intravenous lines or heparin traps. The report indicated slowing the infusion rate, generated no further episodes and concluded that epidemiologic techniques are important tools in rapid assessment of unexpected events particularly when conducting trials in remote locations.

Supportive treatment is often necessary and includes fluid replacement, blood transfusion, administration of paracetamol, phylometadione, ringer lactate, haemocoel quinine and broad spectrum antibiotics\textsuperscript{17}.

**Prevention and control**

Prevention of primary transmission of the Lassa virus from its host to humans can be achieved by avoiding contact with \textit{Mastomys} rodents, especially in the geographic regions where outbreaks occur\textsuperscript{8}. Putting food away in rodent-proof containers and keeping the home clean help to discourage rodents from entering homes. Using these rodents as a food source is not recommended. Trapping in and around homes can help reduce rodent populations. However, the wide distribution of \textit{Mastomys} in Africa makes complete control of this rodent reservoir impractical\textsuperscript{2,3,37}.

Lassa haemorrhagic fever is a highly virulent and contagious viral infection. Therefore, when caring for patients with Lassa fever, further transmission of the disease through person-to-person contact or nosocomial routes can be avoided by taking preventive precautions against contact with patient secretions by instituting strict barrier nursing\textsuperscript{10,27}. Such precautions include wearing protective clothing, such as masks, gloves, gowns and goggles; using infection control measures, such as complete equipment sterilisation and isolating infected patients from contact with unprotected persons until the disease has run its course.

Body fluids, excreta and other materials that might have been contaminated should be handled carefully and disposed properly preferably by burning. All instruments used on the patient, if not disposable must be subjected to autoclaving immediately. Absolute care should be taken when collecting pathologic materials for laboratory investigations. Also correct procedure for transporting materials suspected to contain highly virulent virus or micro-organisms must be observed\textsuperscript{27,39}. Absolute precautionary measures must be taken while carrying out bacteriological and biochemical investigations in the blood and urine samples of suspected cases and such manipulations must be done in biosafety chambers. All those who had contact directly with suspected Lassa haemorrhagic fever patients have to be traced, monitored and specimens should be collected for laboratory diagnosis. Those who test positive have to be isolated and treated as soon as possible with ribavirin.

Health education strategies for preventing infections in people living in endemic areas must be instituted
and should focus on rodent control and minimizing contact with rodent excreta. Furthermore, emphasis should be placed on measures to control virus transmission from cases that include routine use of standard precautions, isolation of suspected cases and surveillance of contacts.49

Vaccine

Although the prevention of human contact with the Mastomys rodents is an essential factor in the control of Lassa fever, widespread prevention of such contact is presently impractical in the endemic regions of West Africa, so provision of a vaccine for community and hospital use is an imperative public health need because vaccination is the most viable control measure.63 A previous report had indicated that a vaccinia virus expressing the Lassa virus glycoprotein protected four non-human primates against lethal challenge with Lassa virus.64 This was followed by the finding that single administration of a vaccine expressing the full-length Lassa virus glycoprotein affords protection against Lassa fever in primates, with or without expression of the nucleoprotein.63 The challenge, however, is to overcome the scientific, political and economic obstacles in producing a human use vaccine candidate. It is well established that the G-protein confers protection but its duration is unknown and if the N-protein is also included there may be a better duration of protection but it is unclear whether the N-protein as a vaccine may possibly enhance the infection.65 Adequate funding and applications of new vaccine technologies give hope that there may soon be a vaccine in clinical trials, however, the difficulty of conducting trials in endemic areas of the West African sub-region and lack of political stability remain serious problems.

Conclusion

From this overview, it is unequivocally established that Lassa fever is a very important vector borne disease that has assumed epidemiological proportion in West Africa where it records high endemicity. The public health implication of this cannot be overstated. Apart from possible periodic outbreaks of Lassa fever epidemic within the region, the unprecedented increase in inter-border traffic and international travels elevates the chances of introducing the virus to other regions within and outside the African continent. The scarcity of resources available for health care delivery system and the political instability that characterise 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 centres would aid in the prompt diagnosis and treatment of highly infectious diseases like Lassa fever and would help in averting possible outbreaks. Furthermore, ribavirin should be made available in hospitals and health centres in the endemic areas particularly in rural communities. This would help to control the disease.

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