Chapter 9

A Reemerging Lassa Virus: Aspects of Its Structure, Replication, Pathogenicity and Diagnosis

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Abstract

Lassa virus is a linear, bisegmented, single-stranded RNA virus, which belong to the *Arenaviridae* family that causes viral hemorrhagic fever transmitted by rats. The virus is endemic in West African countries, which may be due to its zoonotic nature. Lassa virus infection occurs through contact with the vector *Mastomys natalensis* or infected humans and can lead to wide symptoms from a mild infection to Lassa fever and to a severe fatal viral hemorrhagic fever, which include delayed cellular immunity resulting to fulminant viremia. The virus replicates through a strategy known as the Ambisense, where two RNA strands code for genes in both the sense and antisense direction that is rapid and demonstrate temporal control in replication. Different diagnostic tests for the virus are available, which range from viral culture to serological and molecular diagnostic tests. There is an urgent need to develop drugs and vaccines against the virus because the World Health Organization (WHO) has identified Lassa virus as one of the viruses that is likely to cause a future epidemic, although a research is ongoing to evaluate Lassa virus vaccine immunogenicity in the CBA/J-ML29 mouse model. This chapter gives an overview on the structure, replication cycle, pathogenesis, and diagnosis of the virus.

Keywords: Lassa virus, Lassa fever, replication, pathogenesis, diagnosis

1. Introduction

Lassa virus (LASV) is first described in the 1950s [1] but not identified until 1969 in Jos, Nigeria [2, 3]. The virus causes Lassa fever that is hemorrhagic in nature, which is severe and fatal. It affects 2–3 million people annually [4, 5] and has been known to be endemic in Benin Republic in 2014, Ghana in 2011, Guinea, Liberia, and Mali in 2009, Sierra Leone, and Nigeria [3, 4, 6], but probably exists in other West African countries as well [4].

It is a reemerging virus with a select agent, which requires Biosafety Level 4-equivalent containment [7]. It is endemic in West African countries including Sierra Leone, the Republic of Guinea, Nigeria, and Liberia, where cases of the infection is between 300,000 and 500,000 yearly resulting in 5000 deaths annually [4, 8, 9]. About 80% infected with the virus are asymptomatic and 1 in 5 infection results in severe disease, where the virus affects several organs such as the liver, spleen, and kidneys [10]. The virus is harbored by the multimammate rats of the genus *Mastomys* and transmitted to Mans through primary aerosols of the rat's urine, close contact with urine, feces, saliva, or ingestion of contaminated foods of the rat [11]. LASV is also spread through contaminated hospital equipment but interestingly, it cannot be contracted by humans to humans only via bodily fluids contacts [12]. Findings have reported the presence of the virus in seminal fluids up to 3 months after infection of the virus. Research to show that Lassa virus can be gotten via sexual intercourse has not been reported but there are speculations that LASV might possibly be used for bioterrorism, so it is now being studied at greater lengths [13, 14].

Due to the variability of the clinical course of the disease, detection of the disease in affected patients has been challenging. When presence of the virus is confirmed in a locality, quick isolation of infected patients, good infection prevention and control practices, and rigorous contact tracing can help halt epidemicity [15].

2. Aspect of Lassa virus structure

Lassa fever virus (LASV) is a member of the family Arenaviridae ("*Arena*" means "*Sand*" in Latin root) [16, 17]. The virus is single-stranded, bisegmented, ambisense RNA virus. Arenaviruses are classified as segmented negative-sense RNA (nsRNA) viruses and are phylogenetically closely related to other segmented nsRNA viruses belonging to the family *Bunyaviridae* and *Orthomyxoviridae* [9, 18]. The three virus families share similar characteristics of the intracellular replication cycle. It is round, oval, or pleomorphic, 110–130 nm in diameter, and has a protective envelop. LASV differ from 10 to 19 kilobases and contains two RNA species, which are the big and small units [11, 19]. The two genes at different point do not cross exist in every unit [20, 21]. The genetic material that encodes nucleoprotein is 1710 nucleotides in length and the protein has 569 amino acids and that of glycoprotein is 1473 nucleotides long. The viral agent has four lineages, which differ in strain by 27% in respect to their nucleotides and 15% in respect to amino acids [22]. In the virus, the 3′ half of the genetic material has a negative polarity and the 5′ half is of a positive polarity that makes few virus proteins to encode in virus-complementary subgenomic mRNA species, while other viral proteins are encoded in virus-sense subgenomic mRNA sequences. The replication strategy (Ambisense) of the virus is somewhat seldom among viruses and thus distributed among groups of the Arenaviridae [15, 18].

The virus envelope is gotten when new particles bud off through the plasma membrane of the host cell and it carries club-shaped surface projections that are about 10 nm long [4, 19]. Sandy-appearing granules that resemble ribosomes are found within the unstructured interior

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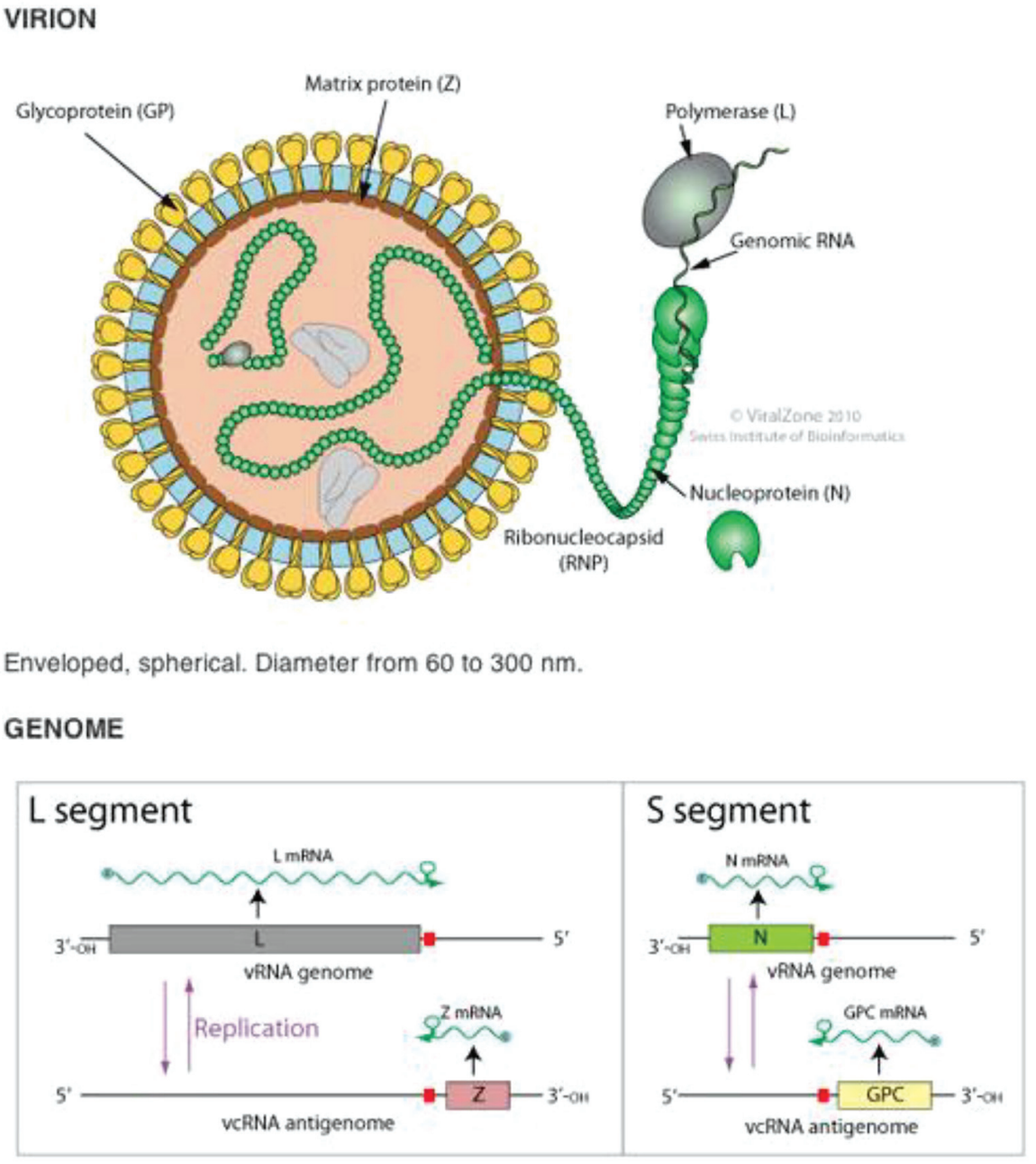

Figure 1. The Virion and genome having the L and S segments [15, 17, 21, 23].

of new viruses. However, these RNAs do not seem to have a required function in virus replication cycle and new work by the Viral Hemorrhagic Fever Consortium [23] sheds suspicion on whether these sandy granules are indeed host ribosomes (**Figure 1**).

3. How Lassa virus replicates

Lassa virus adopts a replication strategy known as "*Ambisense*," which is very rapid and demonstrates the replication of temporal control [24]. The initial stage of transcription of mRNA

copies of the negative sense gene, which makes sufficient deposition of virus proteins for the next stage of the replication process. Subsequently, L and NP proteins are translated from the mRNA. Copies of the viral complementary RNA (vcRNA) are made from the positive sense gene. Negative-sense progeny are produced by templates of RNA copies while mRNA is synthesized from it. The mRNAs produced from vcRNA are later translated to synthesize the Z and GP proteins. The temporal controls enhance the production of proteins spikes lastly, and therefore, delay the recognition by host immune system [25].

The virus enters into the host cell by means of the cell-surface receptor (alpha-dystroglycan alpha-DG), which is a versatile receptor for proteins of the extracellular matrix [26]. Receptor recognition is based on a specific sugar modification of alpha-DG by a group of glycosyltransferases known as the LARGE proteins. Specific variants of the genes encoding these proteins appear to be under positive selection in West Africa, where the virus is endemic [26]. Enveloped viruses makes use of clathrin-coated pits mostly to enter cells and bind to their receptors in a pH dependent way while Lymphocytic choriomeningitis and Lassa virus makes use of the endocytotic pathway independent of caveolin, clathrin, dynamin, and actin. The viruses are quickly delivered once within the cell to endosomes via vesicular trafficking albeit, which is extensively independent of the tiny GTPases Rab5 and Rab7. pH-dependent membrane binding happens on contact with the endosome, which is mediated by the enveloped glycoprotein, and at the acidic pH, the endosome fuses the lysosome protein LAMP1 that yields in membrane bind and endosome escape [19].

NP is the most dominant viral protein in virions and infected cells, which consists the main structural component of the viral ribonucleoprotein (RNP), which plays an important role in the RNA synthesis of the virus. Subsequently, the function required to assist replication of the virus, at least two viral proteins (NP and Z), have been proposed to modulate the cell response of the host to infection. Experimental data has showed that NP has a role in virus-induced inhibition of type I IFN signaling [27]. This role has been mapped to the C-terminal domain of NP with a folding that camouflages the DEDDH family of 3′-5′ exoribonucleases. The little RING finger protein Z is the arenavirus counterpart of the matrix (M) protein of other negative sense RNA viruses. The Z protein of lymphocytic choriomeningitis virus (LCMV) interacts with the promyelocytic leukemia (PML) protein as well as the eukaryotic translation initiation factor 4E (eIF4E) in infected cells and has been observed to function in the noncytopathic nature of LCMV infection and repression of cap-dependent translation [22, 28, 29]. Functional assays reported the exonuclease activity of LASV NP that has been proposed as important for its type I IFN counteracting functions [29].

The replication cycle of Lassa virus is similar to the Old World arenaviruses. It was reported that virus internalization is limited upon cholesterol depletion. Dystroglycan, which is later cleaved into alpha-dystroglycan and beta-dystroglycan is initially expressed in most cells to mature tissues, which provides molecular bridge between the actin-based cytoskeleton and extracellular matrix (ECM) [26, 30]. After the viral agent enters the cell by alpha-dystroglycan-mediated endocytosis, low-pH domain enhances pH-dependent membrane bind and releases ribonucleoprotein of the virus (RNP) complex into the cytoplasm. The enzymatic machinery for RNA synthesis in arenaviruses is housed within a single L polymerase protein. This 250–450 kDa

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protein utilizes viral RNA templates that consist of genomic RNA encapsidated by the viral nucleocapsid protein NP and comprises viral ribonucleoprotein [21, 30]. L polymerase of arenaviruses contains the SDD motif feature of all RNA-dependent RNA polymerases (RdRp) [10]. When infected, the viral agent RNP is inserted into the cytoplasm of the host cell, the L polymerase associated with the viral RNP starts transcription from the genome promoter located at the 3′-end of each genomic RNA segment, L and S. The 5′ and 3′ terminal 19 nt viral promoter regions of both RNA segments required for the recognition and binding by the viral polymerase exhibit a high degree of conservation among the arenaviruses. The genome segments have a high complementary 5′- and 3′-ends (19 nt) have been predicted to form panhandle structures [10, 11]. Transcription stops at the distal side of the stem-loop (SL) structure within the intergenomic region (IGR). The L polymerase adopts a replicase mode and moves across the IGR to create a full-length complementary antigenomic RNA (agRNA) that serves as a template for mRNAs synthesis of viral genes encoded in genomic orientation, GPC and Z, from the S and L segments, respectively, and for the synthesis of full-length genomic RNA (gRNA) [13, 18]. This SL structure has been discovered to stabilize the 3′-termini of the viral mRNAs [10]. The primary transcription leads in the mRNA synthesis of viral genes, which is encoded in antigenomic orientation, NP and L polymerase, from the S and L segments, respectively. The viral agent uses a cap snatching strategy to acquire the cap structures of cellular mRNAs. Cap snatching is facilitated by the endonuclease activity of the L polymerase that is co-factored by the cap binding activity of NP. Therefore, LASV synthesizes capped nonpolyadenylated mRNAs. Both gRNA and agRNA of the viral agent contain a nontemplate G residue at their 5′-ends. The proposed "prime and realign" mechanism includes the production of a $pppG_PC_{OH}$ dinucleotide primer from the CG nucleotides at positions +2 and +3 of the 3′-end genome promoter sequence, that is, then realigned such that its 3′-terminal C_{OH} is opposite the genome 3′-terminal G residue, and the realigned $pppG_PC_{OH}$ then acts as a primer for a complementary RNA strand production. The matrix protein Z is not part of the viral genome transcription and replication but shows a dose-dependent inhibitory effect on viral RNA production. This inhibitory effect of Z has been proposed for Old and New World arenaviruses [27].

4. An aspect of Lassa virus pathogenicity

The Lassa virus is well-known to cause Lassa fever [31]. Its symptoms include flu-like illness characterized by fever, general body weakness, cough, tonsillitis, headache, and gastrointestinal disorders. Hemorrhagic manifestations are other features of Lassa fever, which include vascular permeability [10].

The virus pathogenesis is still unclear, but it has been shown that the virus chiefly target the antigen-presenting cells (mainly dendritic cells) and endothelial cells [32]. Lassa virus infects most tissues in the human body when gained entry. It starts with the mucosa, intestine, lungs, and urinary system, and then moves to the vascular system. There are findings that the viral agent can prevent a host's innate immune system by NP activity [33]. Usually, when a microbe penetrates a host, the innate defense system detects the pathogen-associated molecular patterns (PAMPs) and aggravates the response of the immune system. One of the

mechanisms identifies double-stranded RNA that is only produced by negative-sense viral agents [34]. In the cytoplasm, dsRNA receptors, such as melanoma differentiation-associated gene 5 (MDA-5) and retinoic acid-inducible gene I (RIG-I), detects dsRNAs and facilitates signaling pathways that results in the translocation of interferon regulatory factor 3 (IRF-3) and other transcription factors to the nuclear material [9]. Translocated transcription factors enhance expression of interferons **α** and **β**, and secreted interferons facilitate antiviral responses including adaptive immunity. NP encoded in the viral agent is important in the replication and transcription of the virus, but it also stops host innate IFN response by inhibiting translocation of IRF-3. NP of the virus is reported to have an exonuclease activity to only dsRNAs [12]. Double-stranded RNA exonuclease activity of the NP leads to counteract IFN responses by digesting the PAMP that leads to the evasion of host immune responses.

The recent understanding of the pathogenesis of the viral fever does not involve the chain of functions that take place during development of the disease state and leads to mortality of severed ill patients [35]. The high death and truly dramatic course of the disease state, the pathological findings do not give the bench that would explain the mechanism of disease progression and the cause of mortality by the viral agent [5, 8]. Development of the cellular immune response failure, which would control dissemination of LASV is indicated by high serum titers of the virus, together with dispersed replication in tissues and lack of neutralizing antibodies that could lead to the fatal Lassa fever development [6, 36]. Patients check physically after fever onset usually depicts facial oedema, bilateral conjunctival hemorrhages, purulent pharyngitis, and abdominal disorders [5]. Pathological changes physically may include pulmonary oedema, ascites, pleural effusions, and hemorrhagic signs in the gastrointestinal mucosa while examination under the microscope reveals splenic necrosis, hepatocellular necrosis, adrenocortical necrosis and apoptosis, mild mononuclear interstitial myocarditis without myocardial fiber necrosis, alveolar oedema with capillary blockage and mild interstitial pneumonitis, lymph nodal sinus histiocytosis with mitoses, gastrointestinal mucosal petechiae, renal tubular injury, lymph nodal sinus histiocytosis with mitoses, and interstitial nephri. More often, lesions of Lassa fever in man happen in the hepatic cells [5, 8]. There are four major characteristic hepatitis of LASV, which is derived:

i. Focal cytoplasmic degeneration of hepatocytes related to phagocytosed apoptotic fragments.

ii. Distribution of multifocal hepatocellular necrosis randomly.

iii. Monocytic reaction to necrotic hepatocytes.

iv. Hepatocellular mitoses.

The physical impacts do not happen uniformly in all cases, rather in some instances can be observed simultaneously.

The virus fever is not associated with coagulation dysfunction, for example, decrease in the coagulation factors and disseminated intravascular coagulation (DIC) have been revealed in infected subjects. More so, moderate thrombocytopenia with importantly damaged functionality of thrombocytes is reported in severe Lassa fever subjects [7, 36]. One significant mechanism involved in the pathogenesis of Lassa fever is infection-triggered induction of uncontrolled cytokine expression, which looks like what is seen in sepsis [9]. In this subject

that died from hemorrhagic shock and multi-organ failure, the proinflammatory cytokines, tumor necrosis factor α (TNF-α), and interferon γ (IFN-γ) rises to extremely high level just before death. In a related study, no increase of both cytokine levels was reported in the checked fatal cases of the virus fever, and it is suggestive that the levels of IFN-γ and TNF-α are either elevated only in a fraction of patients or during a limited period that would involve frequent sampling for assay [12, 34].

Virus-induced immunosuppression may be involved in a severe Lassa fever pathogenesis where the LASV infection fails to trigger macrophages (MP) and monocyte-derived dendritic cells (DC) of human. Human-infected DC with the naturally nonpathogenic mopeia virus, induces stronger CD4 and CD8 T-cell responses when compared with those infected with LASV [5, 8]. Infected DC fail to secrete proinflammatory cytokines, do not upregulate costimulatory molecules, such as CD40, CD80, and CD86, and poorly induce proliferation of T cells. Downregulation of immune responses due to infection by LASV has been depicted in vitro, and it is also in consonance with findings of clinical reports demonstrating that the virus fever fatal outcome relates with low levels interleukin (IL) 8 and IFN inducible protein 10 (IP-10) in the system [14].

5. Aspect of Lassa virus diagnosis

Different diagnostic tests in the laboratory are carried out in order to check the presence of an infection and assess its course and complications. The unavailability of lab tests can compromise diagnosis confidence. The most disturbing factor is the presence of febrile illnesses in Africa that mimics the Lassa fever, such as typhoid fever especially for manifestations of nonspecific Lassa fever [31]. In illness with abdominal pain, in countries where the virus is epidemic, Lassa fever may be misdiagnosed as intussusception and appendicitis that leads to delay in treatments with the antiviral drug (ribavirin) [37]. In West Africa, where the virus is most prevalent, it is difficult for laboratory scientists to diagnose due to the absence of the right equipment to perform the tests [4].

The Federal Drug Administration (FDA) has not approved any widely validated laboratory test for the virus, but there are diagnostic tests, which have been able to provide definitive evidence of the presence of the virus [4, 14]. These tests include viral cultures, polymerase chain reaction (PCR) where the virus can be uncovered using reverse transcription PCR after first reverse transcribing the RNA of the virus into DNA, Enzyme Linked Immunosorbent Assay (ELISA) test, immunofluorescence test, and plaque neutralization. But, immunofluorescence tests give less definitive presence evidence of the viral presence. Other laboratory reports in the virus fever include thrombocytopenia, lymphopenia, and elevated aspartate aminotransferase levels in the blood. The viral agent can occasionally be present in cerebrospinal fluid [38, 39].

The following diagnostic methods are briefly discussed below.

5.1. Viral culturing

Viral isolation in cell culture remains the "gold standard" for the diagnosis of Lassa fever given the challenges in diagnosing the virus due to mutations [38], although RT-PCR and

immunoassays are commonly used assays for a clinically actionable diagnosis in recent time [7, 40]. Viral culturing is carried out by the inoculation of suspected samples containing the virus in Vero E6 cells in incubator at 37°C. A positive result may yield a cell cytopathic effect (CPE) [41], while, a second method of detection, such as viral antigen detection, RT-PCR, or electron microscopy, should be used as confirmatory for the presence of the virus. There is always viremia presence at the time of presentation to medical care and reduces after 6 days of illness in subjects, who survived the infection and there may be persistence presence until death in fatal cases [42]. The viral agent can also be cultured from throat swabs, blood, urine, and cerebrospinal fluid specimens from subjects [4]. The detection of virus in urine and throat swabs can be inconsistent in subjects with serum viremia. Positivity of the viral culture may exists in organ specimens such as spleen, liver, lung, heart, kidney, and placenta at post mortem in fatal infections cases [4].

Culturing of viruses allows for the identification, which is genetically independent of variabilities between types and further typing of the viral agent, if desired is achieved [38]. This technique also guarantee the quantification of viremia that might provide further viral typing data, as viremia with 10^3 50% tissue culture infective doses ($TCID_{50}$)/ml has a fatality odds ratio of 3.7 as regards to viremia with less than 10^3 $TCID_{50}$/ml. The method is neither fast, taking at least several days to yield results, and it is not widely available due to the need for BSL-4 precautions to handle live viral samples that limits its utility for the early diagnosis of the viral infection [31].

5.2. Rapid immunogenic tests

Rapid immunogenic tests are attractive alternatives to the technical requirements and high specificity of the PCR methods especially in LASV endemic areas [14, 37]. The antibody/antigen binding is usually less specific than primer/probe hybridization, leaving for greater flexibility in identifying diverse viral agents. Detection of antigens relies on specific antibodies usage against Lassa virus components to detect viral antigens in blood samples [14]. Nonspecific Lassa virus antigens with polyclonal antibodies are detected with initial assays, whereas more current ELISAs target the Lassa virus nucleoprotein antigen [31, 38]. A diagnosis based on the detection of the relatively conserved Lassa nucleoprotein antigen could reduce the differences in test efficacy between genetically diverse virus types and comparison to DNA-based techniques [43]. Antigenemia-increased levels have been identified in fatal cases of the fever when compared with nonfatal cases. The short time of antigenemia enhances the detection of the virus antigen more specific to acute infection with the virus when compared to detection with antibody tests. The antigen of the virus nucleoprotein can be detected in subjects with the virus in the first week of illness and wanes during the second week in temporal association with the increase in detectable immunoglobulins [42]. Antigen detection tests could identify Lassa fever earlier during illness than antibody tests, as antibodies often may not be detectable until the second week of illness [43, 44]. The virus antigen levels might become undetectable despite viremia persistence. It should be noted that, negative antigen test during an acute illness does not mean that the patient is free from the fever [44].

A lot of IgM and antigen capture ELISA methods have been invented with inactivated viral agent; but the protocol is restricted to BSL-4 capable machine [41]. Recombinant antigens

usage allows improved access and assay development. A lateral passage test for the virus nucleoprotein (ReLASV) is another type of diagnostic test, which may be used for point-of-care diagnosis [45]. Following initial development and testing efforts, this test received the CE mark in 2013, although approval by the US FDA has not been granted for the test. According to the product insert, the assay generates results in 15–25 minutes and has 85 and 99% sensitivity and specificity, respectively using confirmed Lassa virus-positive blood specimens [4].

In real sense, a diagnostic test would not only identify Lassa virus infection but would also check for other pathogens with similar clinical symptoms endemic in West Africa at the same time [39]. A transitioning Lassa and Ebola virus antigen- and IgM-based ELISAs onto a MAGPIX system has been described that uses individually labeled magnetic beads to identify multiple targets in a single test. This test has lower limits of detection for Lassa virus nucleoprotein and IgM than conventional ELISAs. Further development of multiplex MAGPIX assays, including testing for Lassa virus antigen and common endemic infections such as malarial infection, will assist with the diagnosis and clinical management of suspected cases of Lassa fever, especially in cases of coinfection with other pathogens where multiple therapeutic modalities may be indicated [38]. Patients on medical care dalliance following onset of the disease could affect negatively on the virus identification in a nucleoprotein detection test, and detection by viral-specific IgM might be more appropriate and reliable [37, 42]. IgG of the virus levels may increase later than IgM levels, with a mean time to detection of 25.6 days after symptom onset, although positive IgG titers have occasionally been detected presence in subjects with acute fever within the first few days of sickness onset [38]. IgM of the virus usually becomes noticeable and detectable in the second week of infections onset, although it could be detectable within 4 days of onset of illness in some subjects. Lack of an antibody response has been found in some fatal cases of Lassa fever.

5.3. Polymerase chain reaction (PCR) methods

Real-time RT-PCR is a commonly used diagnostic technique for infectious agents due to the high specificity and sensitivity and has become a gold standard clinically for Lassa fever identification [37, 46, 47]. Automated coupled specimen processing and 96-well plate thermocyclers, large samples can be evaluated fast and cheaply. The methods could detect viral agent for early illness and a longer time when compared to culturing of virus and might be carried out on samples that are inactivated chemically [4, 48]. Cycle threshold figures usage with quantitative rt-PCR can help with estimates of viremia using the right positive-control equipment for generation of standard curve [49]. Based on the virus strain and primers used, the 95% probability limit of detection estimates with RT-PCR vary from 1237 to 4290 RNA copies/ml [49]. With highly diverse viral agent such as Lassa virus, molecular diversity can be a problem for such assay, as even a single nucleotide variant in one of the primers can have a significant negative effect on the sensitivity of the assay depending on the location of the nucleotide variant [50–52].

The availability of additional sample testing and sequencing data has made mismatches identified using established assays, necessitating assay redesign to enhance performance. Multiplex panels to simultaneously detect a multitude of viruses that could produce hemorrhagic fever syndromes, including Lassa and Ebola viruses, using RT-PCR alone or in synergy with either enzyme hybridization or ligase detection reactions have also been produced [53, 54].

6. Conclusion

The Lassa virus is one of several viruses that are likely to cause a future epidemic as reported by World Health Organization [4, 43, 44]. The appropriate diagnosis of Lassa fever will likely involve a combination of a clinically compatible observation along with serological and molecular diagnostic assays [5]. There is a need for urgent research and development of new diagnostic tests, vaccines, and drugs [7]. Future directions of research for diagnostics in the viral infection are improvement of assays to enhance detection of different viral strains genetically, validation of assays to depict variations in viral lineages and regions, creation of point-of-care detection and field validation, and expansion of multiplex assays content to differentiate the virus fever from other fevers with alike clinical features. Research on the virus vaccine immunogenicity in the CBA/J-ML29 mouse subjects is ongoing. Until now, no licensed vaccine for humans against the virus exists [45].

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