ISSN 2348-0319 International Journal of Innovative and Applied Research [2022]
(Volume 10, Issue 08)
01-11

Journal home page: http://www.journalijiar.com

INTERNATIONAL JOURNAL
OF INNOVATIVE AND APPLIED RESEARCH

RESEARCH ARTICLE

LASSA FEVER: CAUSES, PREVENTION, DIAGNOSIS AND MANAGMENT

Emmanuel Ifeanyi Obeagu[1], Matthew Chibunna Igwe[2], Ziana Kalyankolo[3], Chimaobi Chukwuemeka Nwankpa[4], Byamungu Pahari Kagenderezo[5] and Getrude Uzoma Obeagu[6]

1. Department of Medical Laboratory Science, Kampala International University, Western Campus, Uganda.
2. Department of Medical Laboratory Science,Enugu State University and Science and Technology, Enugu, Enugu State, Nigeria.
3. Department of Electrical, Telecommunications and Computer Engineering, Kampala International University, Western Campus, Uganda.
4. Babcock University Teaching Hospital, Ilishan-Remo, Ogun State, Nigeria.
5. Department of General Surgery/ Faculty of Clinical Medicine and Dentistry, Kampala International University, Western Campus, Uganda.
6. Department of Nursing Science, Ebonyi State University, Abakaliki, Ebonyi State, Nigeria.

Manuscript Info

Manuscript History
Received: 25 Jul 2022
Final Accepted: 20 Aug 2022
Published: 31Aug 2022

Keywords:
Lassa Fever, Prevention, Clinical Manifestations, Diagnosis, Management

Abstract

Acute viral hemorrhagic fever, known as Lassa, was first identified in 1969 in the town of Lassa, Borno State, Nigeria. Lassa is located in the valley of the Yeseram River near the southern end of Lake Chad. Three weeks after being infected with Lassa virus, patients feel sick. Pathogenesis is associated with immunosuppression, uncontrolled viral replication and host response, and infection does not cause lytic damage. Lassa viruses disable the host's immune system in several ways. The typical endosomal trafficking pathway essential for innate immune system recognition is bypassed. The most useful way for diagnosis is polymerase chain reaction (PCR) from blood. Sensitivity was reported as 79 % on the first day of hospitalization, increasing to 100 % on the third day. One of the most effective approaches to contain the spread of Lassa fever in endemic areas is to improve community cleanliness.

***Corresponding Author:-** **Emmanuel Ifeanyi Obeagu**

Introduction:-

Acute viral hemorrhagic fever, known as Lassa, was first identified in 1969 in the town of Lassa, Borno State, Nigeria. Lassa is located in the valley of the Yeseram River near the southern end of Lake Chad (Frame et al., 2017; Hassan et al., 2022). The disease is endemic in West African countries, with 300,000–500,000 cases and approximately 5000 deaths per year (Ogbuetet al., 2007). Nigeria, Liberia, Sierra Leone, Guinea and the Central African Republic all have outbreaks of the disease (Ogbuet al., 2007). Human infections have also been reported in Mali, Senegal and the Democratic Republic of the Congo (Atkins et al., 2009). Infected travelers are exporting cases to other countries. The cause is the Lassa virus, her RNA virus of the Arenaviridae family. Found throughout West Africa and living closely with humans, the African soft fur rat (Mastomysnatalensis) is the natural host of Lassa disease. Mastomys sheds virus in the urine, and human food contamination is a possible mechanism of transmission. The virus is also spread from person to person through contact with bodily fluids of infected individuals in health care settings, causing nosocomial epidemics with a case fatality rate (CFR) of up to 65% (Fisher-Hoch et al., 2012). However, the majority of Lassa virus cases show only mild symptoms or no symptoms at all. 1 in 5 will become seriously ill (Fisher-Hoch et al., 2012). The classification of Lassa virus as a category A pathogen by the National

Institute of Allergy and Infectious Diseases limits access to laboratory testing. Biosafety Level 4 (BSL-4) precautions are recommended for handling potentially infectious specimens.

Three weeks after being infected with Lassa virus, patients feel sick (CDC, 2016). Early signs and symptoms of Lassa fever are vague and include:

Fever, pain, headache, sore throat, cough, chest or abdominal discomfort and vomiting (WHO, 2016). In most cases, symptoms are severe. However, about 20% of cases develop abnormal bleeding, generalized edema, dyspnea, hypotension, proteinuria, transaminitis, deafness, encephalopathy, or hypotension (Frame, 2012). Despite the fact that Lassa mortality is generally low (Richard, 2003). In hospitalized patients, 15-20% (McCormick et al., 2012). Outbreaks and increased mortality in pregnant women, especially in the third trimester, have been reported (Price et al., 2005).

LASSA FEVER

Lassa virus, an enveloped RNA virus of the Arenaviridae family, causes Lassa fever, also known as Lassa hemorrhagic fever. This is an acute and often fatal viral hemorrhagic fever (Lehmann et al., 2017). The disease was identified in 1969 after the death of two of his missionary nurses in the town of Lassa, Borno State, Nigeria, in the Yesselam River Valley near the southern tip of Lake Chad (Frame et al., 2017). It is endemic in West African countries, with 300,000 to 500,000 cases and approximately 5,000 deaths each year (Ogbuet al., 2007). Lassa fever is endemic in Nigeria and some parts of the Mano River Union, particularly Sierra Leone, Guinea and Liberia. Further cases have been reported in Ghana, Mali and Benin.

The single-stranded RNA virus is a zoonotic, or animal-borne, member of the Arenaviridae virus family. The virus spreads to people when they come into touch with food or household objects that have been contaminated by a zoonotic reservoir, typically an infected multimammate rat (Lehmann et al., 2017). There is proof that exposure to aerosolized vector excreta can also cause human infection (Mylneet al., 2015).

Historyof Lassa Fever

The virus that causes Lassa fever was not discovered in 1969 in the small town of Lassa, in the Nigerian state of Borno, until the death a week later of a missionary nurse suspected of being infected by a community obstetrician. . This is despite reports of symptoms similar to Lassa fever reported in Sierra Leone in the 1950s (Ogbuet al., 2007). Her one of the missionary sisters who cared for her two other victims of the first victim later died of a viral infection (Olayiwola and Bakarey, 2017). During the 1970s and 1980s, a number of in-depth studies on the ecological and clinical impact of Lassa fever virus were conducted in West Africa, particularly Sierra Leone and Nigeria (Sogobaet al., 2012). In 2009, south of Mali, Mali reported its first case of Lassa fever after a British man who was believed to have been working with a fever for 10 days died in the town of Soromba (Sogobaet al., 2012). . By contrast, Ghana's first case of Lassa fever was discovered in 2011, when a man in the Ashanti region was thought to have contracted the disease after eating rotten rats he had shot. (Sogobaet al., 2012). Imported cases have been reported so far in the United States, United Kingdom, Sweden, Germany, and the Netherlands (Asogunet al., 2012).

LASSA Virus

According to the Arenavirus family, single-stranded RNA viruses known as Lassa viruses silently and persistently infect rodents. Each member of this family consists of two Ambisense RNA segments and a nucleoprotein (NP) surrounded by a lipid envelope and a glycoprotein (GP). Its name “arena = sand” comes from the unique granular particles that electron microscopy reveals to be host ribosomes inside the virus (Yun and Walker, 2012).

Because of its unusual and unconventional pathophysiology, genetics, and serology, Lassa virus is distinguished from other members of the arena virus family. Furthermore, the genetic diversity of Lassa viruses makes vaccine development difficult. Lassa viruses have a smooth surface envelope with Ts-like shaped spikes and internal glycoproteins. The Lassa virus nucleocapsid, which is 70–150 nm in diameter and 400–1300 nm long, houses the viral genome and is located on the surface envelope of the virus (Olayiwola and Bakarey, 2017). Reports from Guinea, Liberia, and Sierra Leone indicate that there are at least four lineages of Lassa virus, three of which have been detected in Nigeria (Sakaet al., 2017). Additionally, a fifth lineage of Lassa viruses has been identified in Mali and Ivory Coast (Lehmann et al., 2017).

Epidemiology Of Lassa Virus

With a sero-prevalence of 7% to over 20%, the disease is endemic in Sierra Leone, Liberia and Nigeria (Yun and Walker, 2012). Other countries with confirmed cases or positive sero-status are Côte d'Ivoire, Guinea, Central African Republic, Mali, Senegal and Congo (CDC, 2015). An estimated 100,000-300,000 cases occur each year, 5,000 of which are fatal (Yun and Walker, 2012). In fact, the 2014 outbreak of Lassa fever in Liberia was due to the high activity of EVD, which confused the differential diagnosis of suspected cases. These loci are also unique to other hemorrhagic fever viruses, including Ebola (WHO, 2014). Clinical manifestations are nonspecific and the incubation period can be very long. Despite its low prevalence, with only about 27 cases reported to date, Lassa fever remains a potential importation for travelers from endemic countries (Bausch et al., 2010). The United States reported his last two cases in 2014 and 2015 (CDC, 2015). Of the 24 cases with data, a third of him were in health workers and five were in aid workers or peacekeepers. Almost all of these cases have spent significant time in endemic areas. Seven of them died (CDC, 2015). Some patients required medical evacuation from Africa, while others traveled on their own, sometimes following the onset of illness.

The past decade has seen an increase in the frequency of suspected and confirmed Lassa fever infections (Shaffer et al., 2014). These increases may be attributed to the shift from learning about Lassa fever from books and research articles to actual case reports in disease surveillance reports (Gibb et al., 2017). In recent years, cases of Lassa fever have increased more frequently in areas where the virus is endemic. This can be explained by increased surveillance for Lassa fever in West Africa (Gibb et al., 2017). The increase in Lassa fever infections in Nigeria from 430 suspected cases in 2015 to 900, 700 and 1081 in 2016, 2017 and 2018, respectively, indicates that the number of suspected cases of these diseases has increased is a good example of

Reservoir Host Of Lassa Virus

The multi-mammal rat (Mastomysnatalensis), one of the most common rodents in sub-Saharan Africa, serves as the primary host for Lassa virus. Multimothered rats can reproduce all year round, but produce fewer offspring during the rainy season. This may be because flooding is more likely at this time of year (Mylne, 2015). Polymammalian rats serve as animal hosts for Lassa virus, but the virus does not cause disease in rats. (Peterson et al., 2014). Reports indicate that Lassa virus transmission in multimammalian rats occurs both horizontally and vertically, with horizontal transmission being more common than vertical transmission (Gibb et al., 2017).

According to newly available research, both the African wood mouse Hylomyscuspamfi and the Guinean polymammalian mouse Mastomyserythroleucus may serve as reservoir species for Lassa virus (Olayemiet al., 2016). Multiple mammary rats are more common in rural areas than in woodlands and urban areas, and their presence is a reliable predictor of the risk of human infection with Lassa virus (Gibb et al., 2017).

Mode Of Transmission Of Lassa Virus

When polymammal rats become infected with Lassa virus, they may shed virus in their faeces and urine for the rest of their lives (Saka et al., 2017). The likelihood of Lassavirus spreading from an animal host to a human host is increased by a series of complex human-environmental interactions (Gibb et al., 2017). For example, tropical West Africa, which has the highest number of Lassa fever hospitalizations during the dry season, has a seasonal pattern associated with the risk of contracting the virus (Iacano et al., 2016). Polymammary rats have a favorable breeding environment during the dry season, as less precipitation reduces the likelihood of flooding. Zoonotic spillovers are thought to occur when humans come into contact with rodent faeces and urine through inhalation of aerosols from contaminated food or dried faeces and urine (Gibb et al., 2017). The virus can spread from person to person, especially in rural health care facilities, where health care workers may come into contact with the blood, throat secretions, or urine of infected individuals (Sakaet al., 2017).). Transmission via sexual contact has also occurred, as Lassa virus can persist in semen for up to 3 months after infection (Sakaet al., 2017). There is no evidence that healthy people can become infected with Lassa virus through aerosol contact with infected individuals (WHO, 2017). People most at risk of contracting Lassa virus are those who live in rural areas where polymammary mice are common. When caring for infected individuals, health professionals are at increased risk of infecting themselves, especially if infection prevention and control procedures to avoid nosocomial transmission of Lassavirus are not followed (WHO, 2017).

Pathogenesis Of Lassa Virus

Lassaviruses have broad cell tropism and primarily use the cytoskeletal-associated peptide α-dystroglycan as a receptor. The main cell types in which viruses propagate after infection are macrophages, dendritic cells, and endothelial cells. Pathogenesis is associated with immunosuppression, uncontrolled viral replication and host response, and infection does not cause lytic damage. Lassa viruses disable the host's immune system in several ways. The typical endosomal trafficking pathway essential for innate immune system recognition is bypassed. Viral NP directly suppresses interferon production and infected immune cells do not secrete other pro-inflammatory cytokines such as TNF-α, IL-6 and IL-1β. As a result, unlike other hemorrhagic viral fevers, Lassa fever does not experience a 'cytokine storm (McLayet al., 2015).

Increased capillary permeability is the most important pathogenic alteration, along with the development of edema and hypovolemic shock. Hepatitis with splenic and adrenal necrosis and liver necrosis are other alterations (McLayet al., 2015).

Uncertainty exists regarding the immune response to Lassa virus. A major component of immunity is cellular, with prominent T cell responses seen in survivors (Yun and Walker, 2012). Although specific antibodies are produced early in the disease, neutralizing antibodies do not emerge with low titers and avidity for weeks or months, suggesting that the immune response is likely less important. (Sereginet al., 2015).

Life Cycle Of Lassa Virus

Lassa mammary arenaviruses have a life cycle comparable to Old World arenaviruses. Lassa mammary arena viruses enter cells by receptor-mediated endocytosis. The exact endocytic mechanism used is unknown, but the cell entrance is at least vulnerable to cholesterol starvation. According to one report, low cholesterol levels limit viral internalization. α-Dystroglycan is a highly conserved and widespread cell surface receptor for extracellular matrix proteins and receptors used for cell entry. Most cells express dystroglycan, which is later cleaved into beta- and alpha-dystroglycans and serves as a molecular bridge between the ECM and the actin-based cytoskeleton of mature tissues. The low-pH environment triggers pH-dependent membrane fusion, releasing the RNP complex (the viral ribonucleoprotein) into the cytoplasm when the virus enters the cell via α-dystroglycan-driven endocytosis. Viral RNA is thawed and transcription and replication are initiated in the cytoplasm. Upon initiation of replication, both the S and L RNA genomes generate S and L antigenome RNAs, which in turn generate S and L genome RNAs. Both genomic and antigenomic RNA are required for transcription and translation. The GP and NP (viral nucleocapsid proteins) proteins are encoded by S RNAs and the Z and L proteins are encoded by L RNAs. The viral RNA-dependent RNA polymerase is most likely expressed by the L protein. (Drostenet al., 2003).

L-polymerase binds to viral RNPs and initiates transcription of genomic RNA when the virus infects a cell. The viral promoter region of 19 bases at the 5' and 3' ends is required for the viral polymerase to recognize and bind both RNA segments. The genomic S and L RNAs encoding the NP and L proteins, respectively, are first translated into mRNA by primary transcription. Within the intergenomic region, transcription terminates at a stem-loop (SL) structure. The endonuclease activity of L-polymerase and the cap-binding activity of nucleoproteins are used by arenaviruses to steal cap structures from cellular mRNAs. The viral genes GPC and Z are encoded in the genomic orientation and translated by antigenome RNA from the S and L segments, respectively. In addition, replication templates are made from antigenomic RNA (Yun and Walker, 2012). GPC undergoes post-translational changes in the endoplasmic reticulum after translation. GP1 and GP2 are formed from GPC late in the secretory pathway. This cleavage has been suggested to be triggered by the cellular protease SKI-1/S1P. The cleaved lycoprotein is incorporated into the virion envelope as the virus buds and emerges from the cell membrane.

Clinical Manifestation Of Lassa Virus

The incubation period usually lasts 7-10 days, but has been known to last 3-21 days (Seregin et al., 2015). Most people (80%) have moderate or asymptomatic infections, but 20% have severe illness requiring hospitalization. It is estimated that he may die in 1-2% of cases. According to (Ogbu et al., 2007), up to 50% of his outbreaks report nosocomial case fatality in Africa, which ranges from 15 to 20 D44 (Branco et al., 2011).

A nonspecific flu-like illness with fever and malaise indicates an insidious onset of the disease. Patients complain of headache, sore throat, muscle pain, abdominal discomfort, retrosternal chest pain, cough, diarrhea, and vomiting after 1 to 3 days. Hypotension, exudative pharyngitis, lymphadenopathy, conjunctivitis, and a rash of macular

papules were all noted on physical examination. Severe Lassa fever usually presents in the second week with neurologic symptoms, including confusion and convulsions, hypovolemic shock, facial and pulmonary edema, pleural effusion, ascites, and renal failure. Bleeding occurs in only 17% of cases and when it occurs, it is a mild mucosal hemorrhage and is not associated with shock. Fatal cases lead to coma, shock, and death by the third week, while survivors begin to recover within 8–10 days (Yun and Walker, 2012). Age >18 years, neurological symptoms, pharyngitis, vomiting, aspartate transaminase (AST) levels >110 IU, bleeding episodes, and plasma viral loads >103.6 TCID50 (50% tissue culture infectious dose) were all factors and Mortality risk (Sereginet al., 2015).

Differential diagnosis of travelers returning from Africa is complicated by the clinical manifestations of Lassa fever, which are very similar to other common and endemic African diseases such as malaria, typhoid fever, rickettsiosis and influenza.

Clinicians in Israel and other countries should be aware of the latest information regarding Lassa fever and other communicable disease activity. Knowledge of disease activity in the country of origin may help diagnose travelers with mild infections. The following symptoms have been found to be indicators of Lassa fever:

Fever, retrosternal chest discomfort, pharyngitis, proteinuria (Brancoet al., 2011). The first three of these symptoms had a positive predictive value of 80% for the diagnosis of Lassa fever (Brancoet al., 2011).

Elevated transaminases, proteinuria, leukopenia, anemia, and mild thrombocytopenia are among the laboratory findings, along with significant problems with platelet function. Most coagulation tests are normal (Brancoet al., 2011)

Symptoms Of Lassa Fever

The many symptoms of Lassa fever make it difficult for local doctors to make a proper diagnosis. This is especially true for those unfamiliar with the disease (WHO, 2017). Lassa virus remains dormant in infected individuals for 6-21 days before developing moderate symptoms such as low-grade fever, headache, malaise and general weakness. Most Lassa virus infections are asymptomatic and approximately 80% of cases do not progress beyond mild disease (Sogobaet al., 2012). Up to 20% of infected people develop bleeding, persistent vomiting, hypotension, edema around the neck and face, difficulty breathing, and hypovolemic shock 4 to 7 days after the onset of moderate symptoms more severe symptoms (Sogoba et al., 2012). Approximately 25% of Lassa fever cases result in some degree of hearing loss, as normal hearing gradually returns 30 to 90 days after recovery from viral infection. Those who recover also endure some hair loss and stumbles. In more severe cases, bleeding from the mouth, nose, vagina, gastrointestinal tract, and lung fluid may also occur. People infected with Lassa fever may have excessive levels of protein in their urine (WHO, 2017).People develop more serious symptoms such as seizures, tremors, disorientation and coma. Death often results from organ failure within 10–14 days of gestation (WHO, 2017). More than 80% of all cases of Lassa fever during pregnancy, especially in the third trimester of pregnancy, die in him (Sogobaet al., 2012).

Laboratory Diagnosis

The most useful way for diagnosis is polymerase chain reaction (PCR) from blood. Sensitivity was reported as 79 % on the first day of hospitalization, increasing to 100 % on the third day (Sereginet al., 2015). Genetic strain variation might rarely lead to false negative results (Panning et al., 2010), and laboratory contamination to false positive ones (Asogunet al., 2013).

Several methods have been discovered that aid in the diagnosis of Lassa fever and they are:

Antigen And Antibody Detection Assays (ELISA)

It also takes up Lassa antigens, IgM and IgG antibodies. Antigen- and antibody-based tests are an attractive alternative to the high specificity and technical requirements of PCR assays. This is due to the great diversity of the Lassa virus genome and the humble laboratory environment in which Lassa fever is endemic. Compared to primer/probe hybridization, antibody/antigen binding is generally less specific, allowing more flexibility in detecting different infections. Specific antibodies to Lassa virus components are used to detect viral antigens in blood samples. Use of several serological techniques such as B. Direct detection of NP antigens and specific IgG and IgM antibodies against NP (nucleoprotein) and GP (glycoprotein). The combined sensitivity and specificity of the NP and IgM ELISA are 88% and 90%, respectively. IgG persists for decades, whereas IgM persists for months or years

(Bond et al., 2013). Patients with Lassa fever have detectable Lassa virus nucleoprotein antigen in the first week of illness, followed by a temporally correlated decrease in detectable immunoglobulin increase in the second week (Bausch et al. ., 2000). Fatal cases of Lassa fever were found to have higher levels of antigenemia than non-fatal cases (Brancoet al., 2011).

Viral Culture

The diagnostic challenge of Lassa virus diversity has made RT-PCR and immunoassays widely used assays for clinically viable diagnosis, whereas virus isolation in cell culture has been used for Lassa fever. It remains the "gold standard" of diagnostics. In patients who eventually recover from infection, viremia is often present at the time medical care is initiated, decreases after 6 days of illness, and may persist until death in fatal cases. Yes (Johnson et al., 1998). Culturing is performed by inoculating her Vero E6 cells at 37 °C with a specimen sample suspected of containing the virus (Bausch et al., 2001). However, his second detection method, which includes RT-PCR, viral antigen detection, and electron microscopy, should be used to determine the true identity of the virus. A positive result can lead to cytopathic effects in cells (Drostenet al., 2003). Lassa virus can be cultured from blood, CSF, urine, and other patient samples, although virus identification differs between throat swabs and urine from patients with serum viremia (Bausch et al., 2001). Viral culture allows independent detection of genetic differences between strains and, if desired, further characterization of the virus. Viral cultures can also measure viremia and provide detailed data on viral characteristics. Viral culture is neither rapid (often several days for results) nor widely available, as the handling of live virus specimens requires BSL-4 (Biosafety Level 4) procedures. However, it has limited usefulness for early detection of Lassa infection.

Nucleic Acid Detection Method (PCR)

In the early stages of this disease, reverse transcription-polymerase chain reaction (RT-PCR) is used. Due to the high specificity and sensitivity of the method, real-time RT-PCR is widely used as a diagnostic strategy for infectious diseases and has established itself as the gold standard for Lassa fever diagnosis. Combined with automated sample handling and a thermal cycler built on a 96-well plate, large numbers of samples can be interrogated quickly and inexpensively. Compared to viral cultures, PCR assays have the potential to identify the presence of virus earlier in the disease process over a longer period of time (Trappieret al., 1997). It can also be used with chemically inactivated samples. Generating a standard curve using carefully selected positive controls and using cycle thresholds in quantitative RT-PCR helps to obtain rough estimates of viremia (Drostenet al., 2002).

RNA can be used as a template for reverse transcription PCR. Additional steps are required for RNA identification and amplification. Reverse transcriptase is used to convert RNA into complementary DNA (cDNA). Successful RT-PCR requires high quality and purity of RNA template. Making a DNA/RNA hybrid is the first step in RT-PCR. RNase H, a hallmark of reverse transcriptase, degrades the RNA component of the hybrid. The DNA-dependent DNA polymerase activity of reverse transcriptase then converts the single-stranded DNA molecules into cDNA. The amplification process can be affected by the efficiency of the first strand reaction. From here, the cDNA is amplified using standard PCR methods. The ability to convert RNA to cDNA by RT-PCR has several advantages. Handling of RNA is difficult due to its single-stranded nature and extreme instability.

The use of RT-PCR for the detection of RNA transcript has revolutionized the study of gene expression in the following important ways:

1. It has made it theoretically possible to detect the transcripts of practically any gene (Deepak et al., 2007).
2. It has enabled sample amplification and eliminated the need for abundant starting material required when using northern blot analysis (Bustinet al., 2002).
3. It has provided tolerance for RNA degradation as long as the RNA spanning the primer is intact (Bustinet al., 2002).
4. As a result of percutaneous inoculations and contact with mucosal surfaces, clinical samples from Lassa fever patients pose a serious risk to laboratory workers. This requires a high standard of safety for the collecting and processing of samples, as well as the use of personal protective equipment by lab staff. The gold standard for obtaining Lassa virus is to isolate it in animal and cell cultures, however doing so requires extremely strict biosafety measures (Biosafety level 4) (Bond et al., 2013).

Qiagen Extraction

The QIAamp Viral RNA Mini Kits provide the fastest and easiest way to purify viral RNA for reliable use in amplification technologies. Plasma (treated with anticoagulants other than heparin), serum, and other cell-free

bodily fluids can all be used to purify viral RNA. Samples may be frozen or fresh, however they must not be thawed more than once if they are frozen. Plasma samples should not be repeatedly frozen and thawed as this will diminish virus titers and reduce sensitivity. When samples are subjected to numerous freeze-thaw cycles, cryoprecipitates build up. When employing the vacuum procedure, this could result in the QIAamp membrane becoming clogged. (QIAamp Viral RNA Mini Handbook, 2020)

The Procedure

For the production of viral RNA for general usage, QIAamp Viral RNA Mini Kits represent a proven technology. The kit is ideal for processing numerous samples simultaneously because it combines the selective binding properties of a silica-based membrane with the speed of microspin or vacuum technology. It is possible to fully automate QIAamp Viral RNA spin processes on QIAcube Connect. To render RNases inactive and ensure that intact viral RNA is isolated, the material is first lysed under extremely denaturing conditions. After making necessary adjustments to the buffering conditions, the sample is placed into the QIAamp Mini spin column in order to achieve the best possible RNA binding to the QIAamp membrane.When the RNA attaches to the membrane, contaminants are effectively removed in two phases with the help of two distinct wash buffers. A specific RNase-free buffer is used to elute high-quality RNA that is then suitable for usage right away or secure storage. Nucleases, proteins, and other impurities and inhibitors are not present in the pure RNA. Without using alcohol precipitation or phenol/chloroform extraction, the unique QIAamp membrane ensures an incredibly high recovery of pure, undamaged RNA in just 20 minutes. (QIAamp Viral RNA Mini Handbook, 2020).

Adsorption to the QIAamp membrane

Before loading the sample onto the QIAamp Mini column, the buffering parameters of the lysate must be modified to give the viral RNA with the best possible binding conditions. Viral RNA is adsorbed onto the QIAamp silica membrane either by vacuum or during two quick centrifugation processes. Protein and other impurities, which can impede subsequent enzymatic reactions, are not retained on the QIAamp membrane as a result of the salt and pH conditions in the lysate. It will be necessary to put the lysate onto the QIAamp Mini column in stages if the initial sample volume is greater than 140 µl (QIAamp Viral RNA Mini Handbook, 2020).

Removal of residual contaminants

Viral RNA, bound to the QIAamp membrane, is washed free of contaminants during 2 short centrifugation or vacuum steps. The use of 2 different wash buffers, AW1 and AW2, significantly improves the purity of the eluted RNA.

Elution with Buffer AVE

Buffer AVE is RNase-free water that contains 0.04% sodium azide to prevent microbial growth and subsequent contamination with RNases. Sodium azide affects spectrophotometric absorbance readings between 220 and 280 nm but has no effect on downstream applications, such as RT-PCR. To determine the purity of the eluted RNA, elution with RNase-free water instead of Buffer AVE is recommended.

The Sample volumes

QIAamp Mini columns can bind RNA greater than 200 nucleotides in length. Actual yield will depend on sample size, sample storage and virus titer. The procedure is optimized for use with 140µl samples, but samples up to 280µl can be used. Small samples should be adjusted to 140 µl with phosphate-buffered saline (PBS) before loading, and samples with a low viral titer should be concentrated to 140 µl before processing. For samples larger than 140µl, the amount of lysis buffer and other reagents added to the sample before loading must be increased proportionally, but the amounts of Buffers AW1 and AW2 used in the wash steps usually do not need to be increased. If the initial sample volume is increased, application of the lysed sample to the QIAamp Mini column will require multiple loading steps. There is no danger of overloading the QIAamp Mini column, and the quality of the purified RNA will be unaffected.(QIAamp Viral RNA Mini Handbook, 2020)

Lysis of the sample

To inactivate RNases and guarantee the separation of intact viral RNA, the sample is first lysed under the intensely denaturing conditions offered by Buffer AVL. In low-titer samples in particular, the addition of carrier RNA to buffer AVL increases the binding of viral RNA to the QIAamp membrane and prevents potential viral RNA destruction brought on by any lingering RNase activity (QIAamp Viral RNA Mini Handbook, 2020).

Carrier RNA

Carrier RNA has two functions. First and foremost, it improves the binding of viral nucleic acids to the QIAamp Mini membrane, especially when not enough target molecules are present in the sample. Inclusion of large amounts of carrier RNA reduces the likelihood of viral RNA degradation, even if the RNase molecules escape denaturation by the chaotropic salts and detergents in Buffer AVL. Buffer AVL may recover less viral RNA if no carrier RNA is included. Different amplification techniques have varying degrees of efficiency, depending on the total amount of nucleic acids involved in the reaction. Both viral nucleic acid and carrier RNA are present in the eluate produced by this kit, but carrier RNA is much more prevalent than viral nucleic acid. Therefore, the additional calculation of eluate for downstream amplification should depend on the amount of carrier RNA added. Adjusting the amount of carrier RNA added to Buffer AVL is critical to achieving the highest levels of sensitivity in amplification reactions (QIAamp Viral RNA Mini Handbook, 2020).

Addition of internal controls

An internal control may need to be added to the purification process when using the QIAamp Viral RNA Mini procedures in conjunction with commercially available amplification systems. The carrier RNA should be added to the lysis buffer along with internal control RNA or DNA. Smaller molecules are not recovered as well after purification, thus internal control molecules should be longer than 200 nucleotides.

Determination ofyield

It is challenging to quantify viral RNA yields photometrically since they are often less than 1 g in biological samples. The majority of the RNA will be the carrier RNA (5.6 g per 140 l sample). For the measurement of viral RNA production, quantitative RT-PCR is advised.

Altona Kit

The RealStar® Lassa Virus RT-PCR Kit 2.0 contains 2 different RT-PCR assays with 48 reactions each. It contains two different Positive Controls: one for the GPC gene specific amplification and detection system and one for the L gene specific amplification and detection system.

GPC Master A (1 volume): 107.5	L Master A (1 volume): 107.5
GPC Master B (3 Volume): 322.5	L Master B (3 volume): 322.5

The IC(Internal Control) is used as a RT-PCR inhibition control, but not as a control for the samples preparation. The master mix is set up according to the following pipetting scheme:

Number of Reactions	1	12
Master A	5ml	60ml
Master B	15ml	180ml
IC (Internal Control)	1ml	12ml
Volume of Master Mix	21ml	252ml

If the IC is used as a control for the sample preparation procedure and as a RT-PCR inhibition control, add the IC during the nucleic acid extraction procedure. Irrespective of the nucleic acid extraction used, the IC must not be added directly to the specimen. The IC should always be added to the lysis buffer mixture and the volume of IC which has to be added must always depend on the elution volume. It represents 10% of the elution volume. For instance, per sample must be added into the specimen/lysis buffer mixture.

The master mix will follow the following procedures if the IC was added into the specimen.

1. 20ul of the Master G reagent will be pipetted into the G labeled tubes(0.1ml tube)
2. 20ul of the Master L reagent will be pipetted into the L labeled tubes(0.1ml tube)
3. 1ul of Internal control will be added into the tubes
4. 10ul of the sample (extracted RNA) from the elution tube will be added into the 0.1ml tube
5. 10ul of positive control will be added to the positive tubes of both the G tube and L tube
6. Nuclease Free Water will be added to the negative control tubes, this serve as negative control for the diagnosis.
7. The above will be mixed and transported to the PCR room and the thermocycler will be used.

Treatment Of Lassa Virus

The cornerstone of clinical management of Lassa fever, as with other severe hemorrhagic fevers, is supportive care. The primary goal is volume resuscitation that causes 3rd degree intervals, diarrhea and vomiting. Volume overload is also avoided due to the increased risk of pulmonary edema. Electrolyte balance and respiratory support are other goals.

Convalescent plasma has been useful in some animal studies, but failed in clinical trials, probably due to the lack of neutralizing antibodies. A guanosine analogue with broad antiviral activity called ribavirin has good Lassa virus activity. While standard-dose intravenous treatment produces plasma concentrations significantly above the minimum inhibition threshold (MIC), oral treatment produces borderline concentrations that are less than adequate in vivo due to side effects and 50% bioavailability. Inhibition of Lassa virus is suspected (Bausch et al., 2000). Parenteral ribavirin has been shown in animal studies to protect and promote survival in nonhuman primates, even when administered 5 days after infection (Bausch et al., 2019).

The main side effect of ribavirin is dose-dependent hemolysis, which affects 20% of patients and often results in slightly decreased hematocrit (Bond et al., 2013). Taking oral medications comes with a variety of additional side effects, including: Tachycardia, thrombocytosis, elevated lipase levels, mood swings, insomnia, metallic taste, dry mouth, muscle pain, fatigue, headache, jaundice, rash. However, no deaths have been confirmed with ribavirin therapy (Bausch et al., 2000). Because the prognosis of Lassa fever in pregnant women is severe, the risks of ribavirin must be weighed against its benefits. Ribavirin is teratogenic and embryotoxic to rats and is contraindicated during pregnancy and lactation.

Prevention Of Lassa Virus

One of the most effective approaches to contain the spread of Lassa fever in endemic areas is to improve community cleanliness (WHO, 2017). The CDC claims that avoiding all contact with multi-mammal rats, especially in areas where the virus is prevalent, can prevent people from contracting Lassa virus (CDC, 2015). We also recommend storing food in rat-proof containers and keeping the surroundings clean to prevent rats from building nests in your home. This is hoped to reduce the chances of Lassa virus infecting humans. Additionally, Lassa virus can be transmitted to humans when catching, trapping, or cleaning infected mice, so it is important that humans do not eat rodents. Capturing multimammary rats may help reduce the number of potential hosts for Lassavirus, but given the widespread distribution of multimammary rats in West Africa, Not realistic (CDC, 2015).

For those who have Lassa virus. According to the CDC (CDC, 2015), we recommend taking precautions to stop the spread of infection. Create conditions of biological containment, wear protective clothing (masks, gloves, gowns, goggles, etc.), properly disinfect equipment, and isolate sick people to prevent the virus from spreading to unprotected people Some measures can be taken, such as Residents of endemic areas, especially rural areas, should be educated on effective rodent population control methods to prevent the spread of Lassa fever (CDC, 2015).

Conclusion:-

Acute viral hemorrhagic fever, known as Lassa, was first identified in 1969 in the town of Lassa, Borno State, Nigeria. The most useful way for diagnosis is polymerase chain reaction (PCR) from blood. Sensitivity was reported as 79 % on the first day of hospitalization, increasing to 100 % on the third day. One of the most effective approaches to contain the spread of Lassa fever in endemic areas is to improve community cleanliness.

References:-

1. Asogun, D. A., Adomeh, D. I., Ehimuan, J., Odia, I., Hass, M., Gabriel, M. and Ehiane, P.E. (2012) Molecular diagnostics for Lassa fever at Irrua specialist teaching hospital, Nigeria: Lessons learnt from two years of laboratory operation.PLOS Neglected Tropical Diseases, **6**(9):e1839.
2. Atkin, S., Anaraki, S., Gothard, P., Walsh, A. and Brown, D. (2009). The first case of Lassa fever imported from Mali to the United Kingdom. EuroSurveillance, **14**:2–4.
3. Bausch, D. G., Hadi, C. M., Khan, S. H. and Lertora, J. J. (2010). Review of the literature and proposed guidelines for the use of oral ribavirin as postexposure prophylaxis for Lassa fever.Clinical Infectious Diseases, **51(**12):1435-1441.

4. Bausch, D. G., Rollin, P. E., Demby, A. H., Coulibaly, M., Kanu, J., Conteh, A. S., Wagoner, K. D., McMullan, L. K., Bowen, M. D., Peters, C. J. andKsiazek, T. G. (2000). Diagnosis and clinical virology of Lassa fever as evaluated by enzyme linked immunosorbent assay, indirect fluorescent-antibody test, and virus isolation. Journal Clinical Microbiology, **38**:2670–2677
5. Bond, N., Schieffelin, J. S., Moses, L. M., Bennetta, A. J. and Bausch, D. G. (2013). A historical look at the first reported cases of lassa fever: IgG antibodies 40 years after acute infection. American Journal of Tropical Medicine and Hygiene, **88**(2):241–4.
6. Branco, L. M., Boisen, M. L., Andersen, K. G., Grove, J. N., Moses, L. M. and Muncy, I. J. (2011). Lassa hemorrhagic fever in a late term pregnancy from northern sierra leone with a positive maternal outcome: case report.Journal of Virology, **8**(1):404.
7. Branco, L. M., Grove, J. N., Boisen, M. L., Shaffer, J. G., Goba, A., Fullah, M., Momoh, M., Grant, D. S. and Garry, R. F. (2011). Emerging trends in Lassa fever: redefining the role of immunoglobulin M and inflammation in diagnosing acute infection. Virology Journal, **8**:478.
8. Bustin, S. A. (2002). "Quantification of mRNA using real-time reverse transcription PCR (RT-PCR): trends and problems". Journal on Molecular Endocrinology, **29** (1):23–39.
9. Centers for Disease Control and Prevention (CDC). (2016). Lassa fever. Centers for Disease Control and Prevention, Atlanta.
10. Centers for Disease Control and Prevention (CDC). (2015). Lassa Fever. Centers for Disease Control and Prevention, Atlanta, Georgia.
11. Deepak, S. Kottapalli, K., and Rakwal, R. (2007). "Real-Time PCR: Revolutionizing Detection and Expression Analysis of Genes", **8** (4):234–51.
12. Drosten, C., Gottig, S., Schilling, S., Asper, M., Panning, M., Schmitz, H. and Gunther, S. (2002). Rapid detection and quantification of RNA of Ebola and Marburg viruses, Lassa virus, Crimean-Congo hemorrhagic fever virus, Rift Valley fever virus, dengue virus, and yellow fever virus by real-time reverse transcription-PCR. Journal Clinical Microbiology,**40**:2323–2330.
13. Drosten, C., Kummerer, B. M., Schmitz, H. and Gunther, S. (2003). Molecular diagnostics of viral hemorrhagic fevers. Antiviral Resistance, **57**:61–87.
14. Fisher-Hoch, S. P., Tomori, O., Nasidi, A., Perez-Oronoz, G. I. and Fakile, Y. (2012). Review of cases of nosocomial Lassa fever in Nigeria: the high price of poor medical practice, **311**:857–859.
15. Frame, J. D., Baldwin, J. M., Gocke, D. J. and Troup, J. M. (2017). "Lassa fever, a new virus disease of man from West Africa. International clinical description and pathological findings. American Journal Tropical Medicine Hygiene, **19**(4):670-6.
16. Fukushi, S., Tani, H., Yoshikawa, T., Saijo, M. and Morikawa, S. (2012). Serological assays based on recombinant viral proteins for the diagnosis of arenavirus hemorrhagic fever Viruses, **4:**2097–2114.
17. Gibb, R., Moses, L. M., Redding, D. W. and Jones, K. E. (2017). Understanding the cryptic nature of Lassa fever in West Africa. Pathogens and Global Health, **111**(6):276-288.
18. Iacono, G. L., Cunningham, A. A., Fichet-Calvet, E., Garry, R. F., Grant, D. S., Leach, M. and Webb, C. T. (2016) A unified framework for the infection dynamics of zoonotic spillover and spread. PLOS Neglected Tropical Diseases, **10**(9):e0004957.
19. Johnson, K. M., McCormick, J. B., Webb, P. A., Smith, E. S., Elliott, L. H. and King, I. J. (1987). Clinical virology of Lassa fever in hospitalized patients. Journal of Infectious Disease, **155:**456–464.
20. Lehmann, C., Kochanek, M., Abdulla, D., Becker, S., Böll, B., Bunte, A. and Feldt, T. (2017) Control measures following a case of imported Lassa fever from Togo, North Rhine Westphalia, Germany, 2016. EuroSurveillance, **22**(39):17-88.
21. McCormick, J. B., King, I. J., Webb, P. A., Johnson, K. M., O'Sullivan, R., Smith, E. S., Trippel, S. and Tong, T. C. (2012). A case-control study of the clinical diagnosis and course of Lassa fever. Journal of Infectious Disease, **155**:445–455.
22. McLay, L., Liang, Y. and Ly, H. (2014). Comparative analysis of disease pathogenesis and molecular mechanisms of new world and old world arenavirusinfections.Journal of General Virology, **95**:1–15.
23. Mylne, A. Q., Pigott, D. M., Longbottom, J., Shearer, F., Duda, K. A., Messina, J. P. and Hay, S.I. (2015) Mapping the zoonotic niche of Lassa fever in Africa. Transaction of the Royal Society of Tropical Medicine and Hygiene,**109**(8):483-492.
24. Hassan,A.O., Onyeaghala, E.O. Etafo, J., Gbenga-Ayeni, B.O. and Obeagu, E.I. (2022). COMPARATIVE ANALYSIS OF THE IMPACT OF TWO VARIATIONS OF QIAGEN EXTRACTION PROCEDURE ON PCR AMPLIFICATION IN LASSA FEVER DIAGNOSIS IN OWO. Madonna University Journal of Medicine and Health Sciences. **2** (3): 85-101

25. Ogbu, O., Ajuluchukwu, E. and Uneke, C. J. (2007). 'Lassa fever in West African sub-region: an overview.' Journal of vector borne disease,**44**(1):1-11
26. Olayemi, A., Cadar, D., Magassouba, N. F., Obadare, A., Kourouma, F., Oyeyiola, A. and Jérôme, H. (2016). New hosts of the Lassa virus.Scientific Reports,**6**(1):1-6
27. Olayiwola, J.O. and Bakarey, A. S. (2017) Epidemiological trends of Lassa Fever Outbreaks and Insights for Future Control in Nigeria. International Journal of Tropical Disease Health,**24**(4):1-14.
28. Panning, M., Emmerich, P., Ölschläger, S., Bojenko, S., Koivogui, L. and Marx, A. (2010). Laboratory diagnosis of Lassa fever, Liberia.Emerging Infectious Diseases, **16**(6):1041–3.
29. Peterson, A. T., Moses, L. M. and Bausch, D.G. (2014) Mapping transmission risk of Lassa fever in West Africa: The importance of quality control, sampling bias, and error weighting. PLoS One, **9**(8):e100711.
30. Price, M. E., Fisher-Hoch, S. P., Craven, R.B. and McCormick, J. B. (2005). A prospective study of maternal and fetal outcome in acute Lassa fever infection during pregnancy, **297**:584–587.
31. Saka, M. J., Gubio, A. B., Yennan, S. K., Saka, A. O. and Oyemakinde, A. (2017).Lassa fever epidemic in Nigeriaoutbreak investigation, risk factors and empirical analysis from 2012 Journal ofCommunity Public Health Nurse.,**3**(2):170.
32. Seregin, A., Yun, N. and Paessler, S (2015). Lymphocytic choriomeningitis, lassa fever, and the South American hemorrhagic fevers. Mandell, Douglas and Bennett's principles and practice of infectious diseases. 8th edition. Philadelphia: Elsevier Saunders; p.2031–7.
33. Shaffer, J. G., Grant, D. S., Schieffelin, J. S., Boisen, M. L., Goba, A., Hartnett, J.N. and Momoh, M. (2014) Lassa fever in post-conflict Sierra Leone. PLoSNeglected Tropical Diseases, **8**(3):e2748.
34. Sogoba, N., Feldmann, H. and Safronetz, D. (2012). Lassa fever in West Africa: Evidence for an expanded region of endemicity. Zoonoses Public Health, **59**(s2):43-47.
35. Trappier, S. G., Conaty, A. L., Farrar, B. B., Auperin, D. D., McCormick, J. B. and FisherHoch, S. P. (1993). Evaluation of the polymerase chain reaction for diagnosis of Lassa virus infection.American Journal of Tropical Medical Hygiene, **49**:214–221.
36. World Health Organization (2014) .Overview of reported outbreaks in WHO African Region, 24. Outbreak. **4**(1):1–8.
37. World Health Organization (2016). Lassa fever. World Health Organization, Geneva.
38. World Health Organization. (2017). Lassa Fever. World Health Organization, Geneva.
39. Yun, N. E. and Walker, D.H. (2012) Pathogenesis of Lassa fever. Viruses, **4**(12):2031–48.