Genomic Diversity and Spatiotemporal Distributions of Lassa Virus Outbreaks in Nigeria

CURRENT STATUS: POSTED

Abdulwahid Abaukaka Yahaya
Tehran University of Medical Sciences

Yazdan Asgari
Tehran University of Medical Sciences

yasgari@tums.ac.ir Corresponding Author
ORCiD: https://orcid.org/0000-0001-6993-6956

DOI: 10.21203/rs.2.16266/v2

SUBJECT AREAS
Epigenetics & Genomics

KEYWORDS
Genomic, Lassa Virus, Phylogeography, Spatiotemporal and Nigeria
Abstract
Abstract Background Lassa virus (LASV) is a single-negative strand RNA Arenavirus (genus Mammarenavirus), oriented in both negative and positive senses. Due to the increase in the fatality rate of deadly disease LASV caused (Lassa fever), widespread LASV in Nigeria has been a subject of interest. Following the upsurge of LASV endemicity in 2012, another marked incidence recorded in Nigeria, 2018, with 394 confirmed cases in 19 states, and an estimated 25% cases led to death. This study aimed at acquiring the genetic variation of LASV ancestral evolution with the evolution of new strains in different lineage and its geographical distributions within a specific time of outbreaks through Bayesian inference, using genomic sequence across affected states in Nigeria. Results From the result, we were able to establish the relationship of Lassa mamarenavirus and other arenaviruses by classifying them into distinct monophyletic groups, i.e., the old world arenaviruses, new world arenaviruses, and Reptarenaviruses. Corresponding promoter sites for genetic expression of the viral genome were analyzed based on Transcription Starting Site (TSS), the S_Segment (MK291249.1) is about 2917–2947 bp and L_Segment (MH157036.1), is about 1863–1894 bp long. LASV sequences obtained from different parts of Nigeria were genetically related. Benue, Imo, and Bauchi states represent the host etiology of the LASV. Spread across other neighboring states were based on genetic pedigree dated to previous outbreaks as at year 2008 to 2012. Phylogeography of recent transmission from the year 2017 to 2019 indicates vectors were fast spreading LASV from Ondo states to Delta, Edo, and Kogi states, while spread across northeastern states suggests a vector origin from Bauchi state. Conclusions The study outlined the path of transmission based on the genetic homology of the sampled LASV sequences in affected geographical locations. We suggest the federal government should initiate a vector surveillance program to curtails further spread of LASV, especially states bordering with northwestern states and north-central Nigeria.

Background
Based on clinical investigation, Lassa virus (LASV) was endemic in several countries of West Africa, Nigeria, Sierra Leone, Guinea, and Liberia[1]. Cases of LASV also reported in Senegal, Gambia, Mali, Burkina Faso, Ivory Coast, and Ghana [1-3]. LASV is an Arenavirus with segmented negative-strand
RNA, oriented in both negative and positive senses (ambisense gene coding) on the two RNA segments[4]. LASV genome comprised of two genetic segments, L (7.3kb) and S (3.4 kb), encode four proteins: Z protein, L protein, nucleoprotein, and glycoprotein [5]. The RNA polymerase L protein (200 kDa) and a RING finger Z protein (11 kDa) are coded in the L segment, while the S segment code for nucleoprotein (64 kDa) and two cleaved glycoproteins GPC1 (42 kDa) and GPC2 (38 kDa)[6].

Currently, 35 species including Lassa mammarenavirus are carried by mammalian host and they are categorized into two main groups, Old World and New World viruses[7]. This zoonotic disease which pose a greater threat to public health further classified into virus subgroups based on phylogenetic, serological, and geographical characteristics; the Old World viruses, comprises of LASV and Lujo virus (LUJV), while the New World viruses includes Machupo and Chapare virus from Bolivia, Junín virus, Sabiá virus and Guanarito virus found in Argentina, Brazil and Venezuela, respectively[8]. Genomic studies of LASV can enhance the acquisition of data-driven distribution and determinant of the Lassa fever disease by defining genetic variations and viral-specific lineage [9].

Out of all LASV proteins that exist in the virions and infected host cells, nucleoprotein has the largest number of polypeptide encapsidated genomic RNA to prevent it from degradation in infected host cells [10]. LASV nucleoprotein made up a peptide having 569 amino acids residue [11] and consists of a separate amine group (N-terminal) and carboxylic group (C-terminal) domains [12]. The N-terminal domain proposed a cap-binding cellular structure through deep binding cavity for the synthesis of viral mRNA[13], while the C-terminal domain revealed a binding site functions as exoribonuclease, leading to suppression of type I IFN production by interfering with IFN regulatory factor 3 (IRF-3) activation [12, 14, 15]. This pathway plays an important role in transcription and replication of Lassa virus and immunosuppression of infected host [11, 16]

LASV glycoprotein (GP) is a trimeric single polypeptide chain, glycosylated cotranslationally, cleaved subunit GP-1 and GP-2 by enzyme peptidase in the endoplasmic reticulum (ER) which play an important role in viral-host infection [17, 18] Glycoprotein facilitates the entry of the virion into the host cell through receptor binding and fusion to the cell membrane as the only unique protein of the capsid that protect humoral immunity [17]. Before the GP-1 attachment to the host cells, there is a
low immune response that poses a major problem to the host immune system as the virus spike in response to protective antibodies. This low response is due to the extensive protection by N-linked glycan, a phenomenon that is similar to other infectious diseases caused by viruses such as human immunodeficiency virus and hepatitis C virus [19]. Unlike GP-1, the fusion of GP-2 facilitates by internal fusion loop (I-FP) that triggers the transmembrane fusion as a result of acidic pH [20]. It has observed, absence of peptidase and subtilase, subtilisin kexin isozyme-1 (SKI-1)/site 1 protease (S1P) that facilitates biochemical activity of glycoprotein cleavage attracts the rational for LASV vaccine and therapeutic production due to its infectivity as the essential surface functionality of the virion [21] The L and Z proteins are encoded by L segment of RNA, unlike S segment that encodes nucleoprotein and glycoprotein [22]. L protein is majorly made up of RNA polymerase that is dependent at C domain and related to the viral nucleocapsid [23], and N domain functions as transcription terminus for the virion through enzymatic processes of endonuclease [24].

The causative agent of virulent acute hemorrhagic fever, Lassa fever, was first discovered in Borno state, Nigeria, 1960 [5]. It has an incubation period of 6-21 days and characterized by symptoms such as fever, general weakness, and malaise. It was often associated with headache, sore throat, muscle pain, chest pain, nausea, vomiting, diarrhea, and cough, a few days after infection, followed by abdominal pain. Severe cases usually result in the puffy face, pleural effusion, bleeding from a different body orifice, gastrointestinal tract, and hypotension followed by death after 14 days of fatal cases [25]. The widespread of LASV in Nigeria has been a subject of interest due to the increase in this deadly disease. According to the report by Agbonlahor et al., it was narrated that the year 2012 recorded the widest spread and higher incidence of Lassa hemorrhagic fever among different states in Nigeria. Edo, Delta, Ondo, Rivers, Ebonyi, Kano, Yobe, Benue, Kaduna, Kogi, Bauchi, Adamawa, Abia, Anambra, Imo States and the Federal Capital Territory, Abuja, were affected states [26]. Following the surge of LASV endemicity in 2012, another marked incidence recorded in Nigeria, 2018, with 394 confirmed cases in 19 states, and an estimated 25% cases led to death [9]. Increased in Lassa fever widespread in Nigeria has posed a serious threat to public health, with the recent outbreaks from January to February 2019. A total of 324 confirmed cases with 72 death cases were
reported by Nigeria Centre for Disease Control (NCDC), while two separate cases reported in the fifth week with confirmed cases of 68 and week 6, 37 cases, and ten death cases were also reported [27].

This analysis aimed at acquiring the genomic variation of Lassa virus ancestral evolution with the evolvement of new strains in different lineage and its geographic distributions through Bayesian inference, using genomic sequence across affected states in Nigeria and endemic countries in West Africa.

Results

**Homologous classification and genomic sequence flow of LASV in West Africa.**

Evolutionary relationship of Lassa mamarenavirus and other arenaviruses has revealed the distribution of different viruses across the globe. Basically, the phylogenetic analysis helped to classified the viruses into 3 distinct monophyletic groups, i.e. the old world arenaviruses, which were Lassa mammarenavirus, Mopeia virus AN20410, Morogoro mammarenavirus, Luna mammarenavirus, Mobala mammarenavirus, Ippy mammarenavirus, Merino Walk mammarenavirus, Lymphocytic choriomeningitis mammarenavirus and Lujo mammarenavirus, mainly from African countries, while the new world is Allpahuayo mammarenavirus, Flexal mammarenavirus, Cali mammarenavirus, Pirital mammarenavirus, Brazilian mammarenavirus, Paraguayan mammarenavirus, Cupixi mammarenavirus, Whitewater Arroyo mammarenavirus, Machupo mammarenavirus, Bear Canyon mammarenavirus, Argentinian mammarenavirus, Chapare mammarenavirus, Tacaribe mammarenavirus, Oliveros mammarenavirus, Guanarito mammarenavirus, Latino mammarenavirus, and Tamiami mammarenavirus mostly found in North and south America [28]. The other group was Reptarenaviruses, viruses have a host origin of reptiles, e.g., Snake, and they are, University of Giessen virus, Golden Gate virus, ROUT virus, University of Helsinki virus, and CAS virus as shown in Figure 1.

GenBank and virus pathogen resource database (ViRP) recorded a total of 1,903 genomic sequences for LASV and 1,796 sample sequences from Africa. 347 are multimammate mouse (Mastomys natalensis) origin, 1,374 are from the human host, and 75 sequences are an unknown source. The distribution of the sequences across endemic West African countries is shown in Figure 2.
Transcription Starting Site (Promoter) and TATA Box in LASV Genome

Table 1, shows promoter sites of two Lassa mammarenavirus segments, S and L variants of the genome. The homologous gene MK291249.1 and MH157036.1 have the highest alignment scores with different strains isolated at different locations in Nigeria. The S segments started from position 2917 to 2947 and L segments from 1863 to 1894 as predicted by promHG promoter prediction tool.

Table 1: Promoter site and TATA Box in LASV Genome.

| S/N | Segment   | Homologous Gene | Predicted Promoter Site + TATA Box               |
|-----|-----------|-----------------|--------------------------------------------------|
| 1.  | S_Segment | MK291249.1      | ATATAAACACCTGAGCTTAGTGGCCTTTCTG                  |
| 2.  | L_Segment | MH157036.1      | ATATAACGTCTCAAGAATGAATGATGTGGC                  |

Effective LASV siRNA

Table 2 shows the list of 10 Lassa mammarenavirus siRNA designed by BLOCK-iT RNAi Designer based on a statistical analysis of valid siRNA and branded algorithm. The regions of each designed Oligos represented as an open reading frame (ORF) and 5′ untranslated region (5′ UTR), the leader RNA, followed by the GC content of each siRNA designed. BLAST results of the highly species-specific gene for the designed RNAi DNA sequences shown in the last left column.

Table 2: LASV siRNA and targeted genes in humans.
| S/N | S_Segment (MK291249.1) | L_Segment (MH157036.1) |
|-----|-----------------------|------------------------|
|     | Sequence(DNA) | Region | GC% | Sequence(DNA) | Region | GC% |
| 1.  | GCTACAAACTC TAGAGCTA  | 5’UTR  | 42.11 | CCATTGAACTC TTTGTCTTT | ORF  | 36.85 |
| 2.  | GCTAACACTGC GGACTGACTA  | 5’UTR  | 52.64 | CCACACACCCCA GATGCTAT | ORF  | 47.37 |
| 3.  | GCAAGCGAGACA ACATGATA  | 5’UTR  | 42.11 | GCTAAGTGCCT CAGAATTA | ORF  | 36.85 |
| 4.  | GCATATGGCAT GATCTTTTTT  | ORF  | 36.85 | GCACACACTTC CTTACTTA | ORF  | 36.85 |
| 5.  | CCATGAGAATA TTTGGCAT  | ORF  | 36.85 | GCATAACACTT TGAGCATT | ORF  | 36.85 |
| 6.  | GCATAAGCT GCAGCTTT  | ORF  | 47.37 | GCACCTTACAA CCTGGATAT | ORF  | 47.37 |
| 7.  | CCTACAACTC CGTCTCTTT  | ORF  | 47.37 | GCAAGGAACCT ATCACCAG | ORF  | 47.37 |
| 8.  | GCTGCTGTGTA CTCAAATT  | ORF  | 42.11 | GCTTGTCAGTT AGAACATT | ORF  | 36.85 |
| 9.  | GCAGGTCATCT GAGGTCA  | ORF  | 52.64 | CCAACAGACTC CAAATCAT | ORF  | 42.11 |
| 10. | GCATTAACGC TGACATT  | ORF  | 42.11 | GCTAACTTCTG TCTTGATA | ORF  | 36.85 |

**Phylogenies and discrete phylogeography**

Based on comprehensive genomic sequence evaluation, it recorded from GenBank, a total estimation of LASV sequences from affected 16 states in Nigeria between the years 2008 to 2018 to be 735.

Estimated sequences were grouped into two regions of Nigeria (North and South); the Northern states were Bauchi=33, Benue=3, Kaduna=3, Kogi=22, Nassarawa=28, Plateau=17, and Taraba=11, while southern states include Anambra=9, Delta=18, Ebonyi=107, Edo=241, Ekiti=4, Enugu=43, Imo=10, Ondo=182, and Rivers=4, as shown in Figure 3.

Figure 4 shows the circular phylogenetic tree of the LASV S-segment genome inferred by Maximum-Likelihood phylogeny with General Time Reversible (GTR) substitution model and Gamma Distributed Invariant (G+I) rates among sites in MEGA-X software and visualized with iTOL online tool. LASV
lineage I to VI indicated with different color ranges, and newly 75 sequenced segments represented in red color.

From the MCC phylogenetic tree in Figure 5, it can infer the domestic relationship of LASV in different states represented by the tree branches and nodes. The strains from Bauchi, Benue, Plateau, Nasarawa, and Kaduna from the same clades show a distinct evolutionary relationship, comparing the nodes. Most of the sampled strains in the southern part of the country were shown to evolve from two major monophyletic groups; Ebonyi, Edo, Delta, and Anambra, which form the first clade and second clade, Ondo Kogi and Ekiti from the second one. The strains from Plateau and Rivers were seen to distribute across different clades genetically.

MCC phylogenies of LASV (LASVsSgp1) among the affected states in Nigeria. Descendant nodes and branches colored according to the most probable location. A year before recent analysis showed by the scale bar at the bottom of the tree.

With the above MCC tree, the spread of LASV in Nigeria can be understood using the annotated tree to determine the spatiotemporal distribution on Google Earth using a KML file generated with SPREAD, as shown in Figure 6.

Discussion

The arenaviruses are majorly transmitted by rodent hosts and some species by reptiles, causing different human pathogenic diseases. The single-stranded ambient RNA virus classified into old world arenavirus, new world arenavirus, and reptarenavirus based on the phylogenetic classification in Figure 1, they predominantly found in Africa, South America and other parts of the world. The old and new world arenaviruses are taxonomically grouped into one genus, the Mamarenavirus, while the reptile-host viruses from the outer group of homologous classification are Reptarenavirus. The human pathogenic infections of old world arenavirus include severe Lassa fever and mild Lymphocytic choriomeningitis caused by Lassa mammarenavirus and Lymphocytic choriomeningitis mammarenavirus respectively; no human infection has recoded for other viruses in the group.

Importantly, several reasserting studies using the Mopeia virus for the development of attenuated LASV vaccines reported by Johnson et al., [29] and Moshkoff et al., [30]. Among the new world
arenaviruses, the genomic sequence distribution across different West African endemic countries showed in Figure 2. It recorded, Nigeria has the highest sampled sequence in GenBank, followed by Sierra Leone, Liberia, Guinea, and Togo. This data only recorded for countries where there was a substantial outbreak, while countries where there were few cases, periodic isolation, and serological pieces of evidence, are not included. Among the new world arenaviruses, *Brazilian mammarenavirus*, *Machupo mammarenavirus*, *Argentinian mammarenavirus*, and *Guanarito mammarenavirus* found in Brazil, Bolivia, Argentina, and Venezuela respectively were transmitted by rodent host and known to cause hemorrhagic fever in human [35, 38]. The predicted small interfering RNA revealed the potential of the down-regulation activities of some human genes through specific base pairing. Müller and Günther reported siRNA targeting the upper stream of the S and L segment are capable of downregulating reporter gene expressing LASV mRNA construct and replicon [31]. Importantly, LASV genetic makeup has characterized by the genotypic differences of ancestral evolution within the varying time of outbreaks. Similar to the study is the report of Kafetzopoulou et al. [32], which highlighted the lineage into six groups (I to VI), as shown in Figure 4. Most of the sequence samples were fall within lineage II, one strain in lineage III and strain suspected to be newly emerging due to the divergence from lineage I. Based on phylogenetic analysis, LASV sequence obtained from different part of Nigeria were genetically related and grouped into distinct clades according to geographical location from the reconstructed time scale MCC tree in Figure 5. Due to the incomplete sequence reported, one of the location in the north-central part of the country (Abuja) did not cover by the analysis; this restriction was measured not to affect other states. Tracing the spread of LASV by the vector, *Mastomys natalensis* from previous to recent outbreaks, we observe the phylogeography concerning the geopolitical zones and time of isolates. It suggested, Benue, Imo, and Bauchi states represent the host etiology of the LASV, because of the root origin of the branches, time of common ancestors and dominancy of the monophyletic group, while the spread across other neighboring states was based on genetic pedigree dated to previous outbreaks as at the year 2008 to 2012. Benue state is one of the six states in the eastern Middle Belt of Nigeria. It shares boundaries with Cross River State on the south through Yala Local Government Area, Enugu and Ebonyi states.
Benue also borders with Kogi and Nassawara state on the east and north, respectively, and on the northeast by Taraba state. Ingestion of cooked rat meat is a common practice among the people of Benue state, but not raw or undercooked meat according to Olusi et al., [33]. Contrary to the previous outbreaks, phylogeography of the recent strains identified spontaneous spreads of LASV from southwest to south-south and north-central through the states that shared a common border. This finding shared a similar opinion with the recent study by Ehichioya et al., [34], confirmed that the majority of the sampled strained in lineage II evolved from Ondo state, a south-western state bordering with Kogi (north-central) and Edo (south-south).

Conclusion
The geography of recent transmission from year 2017 to 2019 indicates vectors were fast spreading LASV from Ondo states to Delta, Edo, and Kogi states, while spread across northeastern states suggests a vector origin from Bauchi state. From the result of this study, we suggests, the federal government should initiate a vector surveillance program to curtails further spread of LASV, especially states bordering with northwestern states and north-central. The study outlined the path of transmission based on the genetic homology of the sampled LASV sequences in affected geographical locations through the spatial phylogenetic reconstruction of evolutionary dynamics.

Methods
**LASV homologs and genomic sequence distribution**

To determine the homologous recombination of the LASV and other arenaviruses through DNA sequence phylogeny, we collected homologous sequences of arenaviruses from the orthology database, OrthoDB V10 [35] and viral genome database, viruSITE [36]. Following sequence collection, sequences were manually edited, aligned using multiple sequence alignment programs, ClustalW and used to construct the phylogenetic tree in MEGA X software [37]. An online tool (Interactive tree of life: iTol) was used to annotate and display the tree [38]. According to the Centers for Disease Control and Prevention (CDC), LASV was endemic in West Africa countries of Nigeria, Guinea, Sierra Leone, Liberia, Mali, Côte d’Ivoire, and new strains recently found in Togo, 2016 [3]. To determine the genomic diversity of LASV strains, a distribution test was used to measure the sequence flow among
the endemic countries and states in Nigeria. This distribution was estimated using a virus pathogen resource database, ViRP [39] and GenBank [40]. The data sequences of the LASV nucleoprotein (LASVsSgp1) gene among the affected states from Nigeria within a period of 2008 to 2019 and endemic countries of West Africa with isolation date ranging from 1969 to 2018 were analyzed. For LASV promoter sites and effective short interference RNA (siRNA) in LASV genome, PromH online promoter site prediction tool was used to predict accurate Transcription Starting Sites (TSS) with TATA box [41], while siRNA designed by BLOCK-iT™ RNAi Designer [42].

**Genomic Variation and Phylogeographic Distribution in Nigeria**

Genomic variation and phylogeographic distribution of LASV was analyzed using the S segment of the genomic sequences generated by Irrua Specialist Teaching Hospital (ISTH) in collaboration with the Institute for Lassa Fever Research and Control (ILFRC), Irrua, Edo State, published in GenBank. A total of 499 LASV sequence samples analyzed. 354 out of the sequences were real-time LASV sequences in Nigeria and other west Africa countries recorded by Kafetzopoulou et al, [32], while 70 were collected from GenBank with keyword (txid11620[Organism]) to select appropriate accession number of S segment with the collection date ranging from 2008 to 2018, while the additional 75 sequences were recently laboratory-confirmed Lassa fever patients from Edo, Ondo, Kogi, Delta, Ebonyi and Benue States with dates of onset from the end of December 2018 to March 2019 reported to virological database [43]. Maximum-likelihood phylogenetic analysis was performed on the coding sequence of the S segment to determine the lineage of recent LASV samples.

Before the BEAST setup [44], sequence data were aligned with ClustalW in MEGA-X [37] and exported in Nexus format. Using the BEAUti program, the nexus file imported; subsequently, date, site model, clock model, priors, discrete trait, and Markov chain Monte Carlo (MCMC) were set up accordingly for Maximum Clade Credibility (MCC) tree and discrete phylogeography. BEAST 2.5 tool was used to run the XML file generated from the BEAUti program for 6 hours for maximum Effective Sample Size (ESS). The Trace log and dot trees files generated were further sampled using Tracer v1.7.1 [45] and TreeAnnotator program for MCMC estimation and annotation of MCC trees at 95% highest probability density (HPD). Annotated MCC trees were constructed and visualized with FigTree v1.4.4. In
summary, spatiotemporal transmission routes of LASV in Nigeria were visualized with Google Earth Pro v7.3.2 tool after configuration of geographical locations (latitude and longitude) in SPREAD v1.0.6 software, followed by conversion of MCC trees into a keyhole markup language (KML) file. To certify the reproducibility of end result, this methodology was guided by the preceding study on norovirus in China [47], and influenza A H5N1 virus in Egypt [48].

List Of Abbreviations

**BEAST:** Bayesian Evolutionary Analysis Sampling Trees

**BP:** Base Pare

**ER:** Endoplasmic Reticulum

**ESS:** Effective Sample Size

**GP:** glycoprotein

**GTR:** General Time Reversible

**HPD:** Highest Probability Density

**IFN:** Interferon

**I-FP:** Internal Fusion Loop

**ILFRC:** Institute for Lassa Fever Research and Control

**ISTH:** Irrua Specialist Teaching Hospital

**KML:** Keyhole Markup Language

**LASV:** Lassa virus

**MCC:** Maximum Clade Credibility

**MCMC:** Markov chain Monte Carlo

**NCDC:** Nigeria Centre for Disease Control

**ORF:** Reading Frame

**S1P:** Site 1 Protease

**siRNA:** Small interfering RNA

**SKI-1:** Subtilisin Kexin Isozyme-1

**TSS:** Transcription Starting Site
**ViRP:** Virus Pathogen Resource Database

**Declarations**

**Acknowledgments**

Not applicable

**Authors’ contributions**

YAA designed the study, draft the manuscript, and generate the data. YAA and YA analyzed the data.

YA reviewed the manuscript. Both authors read and approved the final manuscript.

**Funding**

Not applicable

**Availability of Data and Materials**

Datasets generated and analyzed during the study included in the article.

**Competing interests**

Authors of this article affirm that they have no competing interests.

**Ethics approval and consent to participate**

Not applicable

**Consent for publication**

Not applicable

**References**

1. Yun NE, Walker DH: *Pathogenesis of Lassa fever*. *Viruses* 2012, 4(10):2031-2048.

2. Safronetz D, Lopez JE, Sogoba N, Traore SF, Raffel SJ, Fischer ER, Ebihara H, Branco L, Garry RF, Schwan TG et al: *Detection of Lassa virus, Mali*. *Emerging infectious diseases* 2010, 16(7):1123-1126.

3. Whitmer SLM, Strecker T, Cadar D, Dienes HP, Faber K, Patel K, Brown SM, Davis WG, Klena JD, Rollin PE et al: *New Lineage of Lassa Virus, Togo, 2016*. *Emerging infectious diseases* 2018, 24(3):599-602.

4. Gunther S, Lenz O: *Lassa virus*. *Critical reviews in clinical laboratory sciences* 2004,
5. Andersen KG, Shapiro BJ, Matranga CB, Sealfon R, Lin AE, Moses LM, Folarin OA, Goba A, Odia I, Ehiane PE et al: Clinical Sequencing Uncovers Origins and Evolution of Lassa Virus. *Cell* 2015, **162**(4):738-750.

6. Qi X, Wang W, Dong H, Liang Y, Dong C, Ly H: Expression and X-Ray Structural Determination of the Nucleoprotein of Lassa Fever Virus. *Methods in molecular biology (Clifton, N J)* 2018, **1604**:179-188.

7. Loureiro ME, D'Antuono A, Lopez N: Virus(-)Host Interactions Involved in Lassa Virus Entry and Genome Replication. *Pathogens (Basel, Switzerland)* 2019, **8**(1).

8. Ly H: Differential Immune Responses to New World and Old World Mammalian Arenaviruses. *International journal of molecular sciences* 2017, **18**(5).

9. Siddle KJ, Eromon P, Barnes KG, Mehta S, Oguzie JU, Odia I, Schaffner SF, Winnicki SM, Shah RR, Qu J et al: Genomic Analysis of Lassa Virus during an Increase in Cases in Nigeria in 2018. *The New England journal of medicine* 2018, **379**(18):1745-1753.

10. Zhang Y, Li L, Liu X, Dong S, Wang W, Huo T, Guo Y, Rao Z, Yang C: Crystal structure of Junin virus nucleoprotein. *The Journal of general virology* 2013, **94**(Pt 10):2175-2183.

11. Pattis JG, May ER: Influence of RNA Binding on the Structure and Dynamics of the Lassa Virus Nucleoprotein. *Biophysical journal* 2016, **110**(6):1246-1254.

12. Hastie KM, Liu T, Li S, King LB, Ngo N, Zandonatti MA, Woods VL, Jr., de la Torre JC, Saphire EO: Crystal structure of the Lassa virus nucleoprotein-RNA complex reveals a gating mechanism for RNA binding. *Proceedings of the National Academy of Sciences of the United States of America* 2011, **108**(48):19365-19370.
14. Brunotte L, Kerber R, Shang W, Hauer F, Hass M, Gabriel M, Lelke M, Busch C, Stark H, Svergun DI et al: Structure of the Lassa virus nucleoprotein revealed by X-ray crystallography, small-angle X-ray scattering, and electron microscopy. *The Journal of biological chemistry* 2011, 286(44):38748-38756.

15. Carnec X, Baize S, Reynard S, Diancourt L, Caro V, Tordo N, Bouloy M: Lassa virus nucleoprotein mutants generated by reverse genetics induce a robust type I interferon response in human dendritic cells and macrophages. *Journal of virology* 2011, 85(22):12093-12097.

16. Zhang Y, Chen H, Han JG: Insight into the binding modes of Lassa nucleoprotein complexed with ssRNA by molecular dynamic simulations and free energy calculations. *Journal of biomolecular structure & dynamics* 2015, 33(5):946-960.

17. Hastie KM, Saphire EO: Lassa virus glycoprotein: stopping a moving target. *Current opinion in virology* 2018, 31:52-58.

18. Schlie K, Maisa A, Lennartz F, Stroher U, Garten W, Strecker T: Characterization of Lassa virus glycoprotein oligomerization and influence of cholesterol on virus replication. *Journal of virology* 2010, 84(2):983-992.

19. Galan-Navarro C, Rincon-Restrepo M, Zimmer G, Ollmann Saphire E, Hubbell JA, Hirosue S, Swartz MA, Kunz S: Oxidation-sensitive polymersomes as vaccine nanocarriers enhance humoral responses against Lassa virus envelope glycoprotein. *Virology* 2017, 512:161-171.

20. Willard KA, Alston JT, Acciani M, Brindley MA: Identification of Residues in Lassa
Virus Glycoprotein Subunit 2 That Are Critical for Protein Function. *Pathogens* *(Basel, Switzerland)* 2018, 8(1).

21. Amanat F, Duehr J, Oestereich L, Hastie KM, Ollmann Saphire E, Krammer F: *Antibodies to the Glycoprotein GP2 Subunit Cross-React between Old and New World Arenaviruses*. *mSphere* 2018, 3(3).

22. Brunotte L, Lelke M, Hass M, Kleinsteuber K, Becker-Ziaja B, Gunther S: *Domain structure of Lassa virus L protein*. *Journal of virology* 2011, 85(1):324-333.

23. Vieth S, Torda AE, Asper M, Schmitz H, Gunther S: *Sequence analysis of L RNA of Lassa virus*. *Virology* 2004, 318(1):153-168.

24. Lehmann M, Pahlmann M, Jerome H, Busch C, Lelke M, Gunther S: *Role of the C terminus of Lassa virus L protein in viral mRNA synthesis*. *Journal of virology* 2014, 88(15):8713-8717.

25. Lassa fever [https://www.who.int/news-room/fact-sheets/detail/lassa-fever]

26. Agbonlahor DE, Erah A, Agba IM, Oviasogie FE, Ehiaghe AF, Wankasi M, Eremwanarue OA, Ehiaghe IJ, Ogbu EC, Iyen RI et al: *Prevalence of Lassa virus among rodents trapped in three South-South States of Nigeria*. *Journal of vector borne diseases* 2017, 54(2):146-150.

27. Lassa Fever - Nigeria: Emergencies preparedness, response [https://www.who.int/csr/don/14-february-2019-lassa-fever-nigeria/en/]

28. Old World/New World Arenaviruses [https://www.cdc.gov/vhf/virus-families/arenaviruses.html]

29. Johnson DM, Jokinen JD, Lukashevich IS: *Attenuated Replication of Lassa Virus Vaccine Candidate ML29 in STAT-1(-/-) Mice*. *Pathogens* *(Basel, Switzerland)* 2019, 8(1).

30. Moshkoff DA, Salvato MS, Lukashevich IS: *Molecular characterization of a*
reassortant virus derived from Lassa and Mopeia viruses. *Virus genes* 2007, 34(2):169-176.

31. Muller S, Gunther S: Broad-spectrum antiviral activity of small interfering RNA targeting the conserved RNA termini of Lassa virus. *Antimicrobial agents and chemotherapy* 2007, 51(6):2215-2218.

32. Kafetzopoulou LE, Pullan ST, Lemey P, Suchard MA, Ehichioya DU, Pahlmann M, Thielebein A, Hinzmann J, Oestereich L, Wozniak DM et al: Metagenomic sequencing at the epicenter of the Nigeria 2018 Lassa fever outbreak. *Science (New York, NY)* 2019, 363(6422):74-77.

33. T. A. Olusi et al.: Antibodies to *Toxoplasma gondii* in a rat-eating population of Benue State, Nigeria. *Annals of Tropical Medicine & Parasitology* 1994, 88(2):217-218.

34. Ehichioya DU, Dellicour S, Pahlmann M, Rieger T, Oestereich L, Becker-Ziaja B, Cadar D, Ighodalo Y, Olokor T, Omomoh E et al: Phylogeography of Lassa virus in Nigeria. *Journal of virology* 2019.

35. Kriventseva EV, Kuznetsov D, Tegenfeldt F, Manni M, Dias R, Simao FA, Zdobnov EM: OrthoDB v10: sampling the diversity of animal, plant, fungal, protist, bacterial and viral genomes for evolutionary and functional annotations of orthologs. *Nucleic acids research* 2019, 47(D1):D807-d811.

36. Stano M, Beke G, Klucar L: viruSITE-integrated database for viral genomics. *Database: the journal of biological databases and curation* 2016, 2016.

37. Kumar S, Stecher G, Li M, Knyaz C, Tamura K: MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Molecular biology and evolution* 2018, 35(6):1547-1549.

38. Letunic I, Bork P: Interactive tree of life (iTOL) v3: an online tool for the
display and annotation of phylogenetic and other trees. *Nucleic acids research* 2016, **44**(W1):W242-245.

39. Pickett BE, Sadat EL, Zhang Y, Noronha JM, Squires RB, Hunt V, Liu M, Kumar S, Zaremba S, Gu Z *et al*: ViPR: *an open bioinformatics database and analysis resource for virology research*. *Nucleic acids research* 2012, **40**(Database issue):D593-598.

40. Clark K, Karsch-Mizrachi I, Lipman DJ, Ostell J, Sayers EW: GenBank. *Nucleic acids research* 2016, **44**(D1):D67-72.

41. Solovyev VV, Shahmuradov IA: PromH: Promoters identification using orthologous genomic sequences. *Nucleic acids research* 2003, **31**(13):3540-3545.

42. BLOCK-iT™ RNAi Designer

[https://rnaidesigner.thermofisher.com/rnaiexpress/setOption.do?designOption=sirna&pid=-2159752509464319169]

43. 2019 Lassa virus sequencing in Nigeria - Final field report (75 samples)

[http://virological.org/]

44. Bouckaert R, Heled J, Kuhnert D, Vaughan T, Wu CH, Xie D, Suchard MA, Rambaut A, Drummond AJ: BEAST 2: *a software platform for Bayesian evolutionary analysis*. *PLoS computational biology* 2014, **10**(4):e1003537.

45. Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA: *Posterior Summarization in Bayesian Phylogenetics Using Tracer 1.7*. *Systematic Biology* 2018, **67**(5):901-904.

46. Bielejec F, Baele G, Vrancken B, Suchard MA, Rambaut A, Lemey P: SpreaD3: Interactive Visualization of Spatiotemporal History and Trait Evolutionary Processes. *Molecular biology and evolution* 2016, **33**(8):2167-2169.

47. Qiao N, Ren H, Liu L: Genomic diversity and phylogeography of norovirus in
China. *BMC medical genomics* 2017, **10**(Suppl 3):51.

48. Scotch M, Mei C, Makonnen YJ, Pinto J, Ali A, Vegso S, Kane M, Sarkar IN, Rabinowitz P: *Phylogeography of influenza A H5N1 clade 2.2.1.1 in Egypt.* *BMC genomics* 2013, **14**:871.

### Additional Files

#### Additional file 1:

LASV nuclear protein and 40 homologous sequences generated from ortho database and virussite databases (.txt, 18.8 MB)

#### Additional file 2:

S and L segments of LASV genomic sequence distribution among West African countries as recorded in GenBank, from 1969 to 2018 (.xls file, 28.0 KB)

#### Additional file 3:

Maximum clade credibility (MCC) phylogenies of selected LASV isolates, from states in Nigeria (.txt, 18.8 MB)

#### Additional file 4:

Genomic sequence of LASV (LASVsSgp1) from states in Nigeria (.txt, 14.1 KB)

#### Additional file 5:

LASV genomic sequence distribution among different affected states in Nigeria from 2008 to 2018 as it recorded by GenBank (.xls file, 26.5 KB)

#### Additional file 6:

Photos phylogeographic reconstruction of LASV showing spatiotemporal dispersion among different states in Nigeria, through Google Earth with KML file Generated in SPREAD (.pdf file, 307 KB)

**Figures**
Figure 1

Maximum likelihood tree of the Lassa Mammarenavirus nuclear protein, and 40 homologous sequences generated from ortho database and virus site databases. The evolutionary history was inferred by a character-based method with Jones Taylor Thornton (JTT) matrix-based substitutional model. Pairwise distances were determined by routinely applying Neighbor-join and BioNJ algorithms to the trees.
Figure 2

LASV genomic sequence distribution among West African countries (Guinea, Liberia, Nigeria, Sierra Leone, and Togo) as recorded in GenBank, from 1969 to 2018.
LASV genomic sequence distribution among different affected states in Nigeria from 2008 to 2018 as GenBank recorded it.
circular phylogenetic tree of LASV S-segment genome showing recently isolated strains in different lineage.
Figure 5

Maximum clade credibility (MCC) phylogenies of selected LASV isolates, from states in Nigeria.
Figure 6

Phylogeographic reconstruction of LASV showing spatiotemporal dispersion among different states in Nigeria, through Google Earth with KML file Generated in SPREAD.

Supplementary Files
This is a list of supplementary files associated with this preprint. Click to download.

Additional file 6.pdf
Additional file 3.txt
Additional file 1.txt
Additional file 5.xlsx
Additional file 2.xlsx
Additional file 4.txt