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Lassa virus circulating in Liberia: a retrospective genomic characterisation



Michael R Wiley*, Lawrence Fakoli*, Andrew G Letizia*, Stephen R Welch*, Jason T Ladner*, Karla Prieto, Daniel Reyes, Nicole Espy, Joseph A Chitty, Catherine B Pratt, Nicholas Di Paola, Fahn Taweh, Desmond Williams, Jon Saindon, William G Davis, Ketan Patel, Mitchell Holland, Daniel Negrón, Ute Ströher, Stuart T Nichol, Shanmuga Sozhamannan, Pierre E Rollin, John Dogba, Tolbert Nyenswah, Fatorma Bolay, César G Albariño†, Mosoka Fallah‡, Gustavo Palacios†

Summary

Background An alarming rise in reported Lassa fever cases continues in west Africa. Liberia has the largest reported per capita incidence of Lassa fever cases in the region, but genomic information on the circulating strains is scarce. The aim of this study was to substantially increase the available pool of data to help foster the generation of targeted diagnostics and therapeutics.

Methods Clinical serum samples collected from 17 positive Lassa fever cases originating from Liberia (16 cases) and Guinea (one case) within the past decade were processed at the Liberian Institute for Biomedical Research using a targeted-enrichment sequencing approach, producing 17 near-complete genomes. An additional 17 Lassa virus sequences (two from Guinea, seven from Liberia, four from Nigeria, and four from Sierra Leone) were generated from viral stocks at the US Centers for Disease Control and Prevention (Atlanta, GA) from samples originating from the Mano River Union (Guinea, Liberia, and Sierra Leone) region and Nigeria. Sequences were compared with existing Lassa virus genomes and published Lassa virus assays.

Findings The 23 new Liberian Lassa virus genomes grouped within two clades (IV.A and IV.B) and were genetically divergent from those circulating elsewhere in west Africa. A time-calibrated phylogeographic analysis incorporating the new genomes suggests Liberia was the entry point of Lassa virus into the Mano River Union region and estimates the introduction to have occurred between 300–350 years ago. A high level of diversity exists between the Liberian Lassa virus genomes. Nucleotide percent difference between Liberian Lassa virus genomes ranged up to 27% in the L segment and 18% in the S segment. The commonly used Lassa Josiah-MGB assay was up to 25% divergent across the target sites when aligned to the Liberian Lassa virus genomes.

Interpretation The large amount of novel genomic diversity of Lassa virus observed in the Liberian cases emphasises the need to match deployed diagnostic capabilities with locally circulating strains and underscores the importance of evaluating cross-lineage protection in the development of vaccines and therapeutics.

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Introduction

Lassa fever is a haemorrhagic disease caused by Lassa virus, a member of the Mammarenavirus genus in the family Arenaviridae. Lassa virus was first discovered in Nigeria in 1969¹ and later detected in Liberia in 1972.² Historical evidence and molecular dating indicate that Lassa virus has circulated in west Africa for hundreds of years.³ The majority of the confirmed cases are from the Mano River Union region (Guinea, Liberia, and Sierra Leone) and Nigeria, but there is evidence of Lassa virus in Benin, Burkina Faso, Ghana, Côte d'Ivoire, Mali, and Togo.⁴

An estimated 300 000 infections of Lassa fever occur annually, resulting in approximately 5000 deaths.⁴ For approximately 80% of these cases, symptoms are mild and undiagnosed.⁴ In the other 20% of infections, common symptoms include back and chest pain, facial

swelling, haemorrhaging, respiratory distress, and vomiting.⁴ The estimated fatality rate for all Lassa fever infections is 1%, with a 15–20% case fatality rate in patients who are admitted to hospital.⁴ Because of the lack of standardised surveillance programmes, these numbers are all estimates and may not be an accurate representation of the true burden of Lassa fever in west Africa. Standardisation of surveillance has been complicated by the non-specific symptoms of Lassa fever, the high level of nucleotide (20–25%) and amino acid (8–12%) diversity within Lassa virus, and an overall lack of access to care for a large percentage of the population in endemic areas, resulting in a majority of clinical cases going undiagnosed.^{4–6}

To address the lack of effective diagnostics, public and private groups have started addressing the gaps. Immunoassays have been developed to detect anti-Lassa

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Research in context

Evidence before this study

Genomic sequencing of epidemics is becoming a powerful tool to investigate outbreaks, reservoir jumps, and transmission. Additionally, genomic characterisation enables identification of possible target erosion in diagnostic assays, informs countermeasures development including vaccines, and informs molecular epidemiology tracing of outbreaks. We searched PubMed without language restrictions in March, 2019, for articles using the terms "Lassa," "LASV" or "Lassa virus" with the terms "genomic epidemiology," "diagnostic," "epidemic or endemic" or "Liberia". Although numerous previous studies were discovered, only 59 articles were identified when using both "Lassa" and "Liberia" terms in the search and most of these only mentioned Lassa virus in the introduction, while the main topic of the paper was on another pathogen.

Added value of this study

We obtained 23 near-complete Lassa virus genomes from Liberia. This represents a ten-fold increase compared with the

two coding-complete genomes currently found on GenBank. The new genomes span both temporally (1972–2017) and spatially (Bong, Nimba, Montserrado, and Lofa counties) across Liberia. Our data indicate that the Liberian Lassa virus strains are distinct from those circulating elsewhere and that improved molecular diagnostics are needed to detect Lassa fever more effectively in Liberia.

Implications of all the available evidence

The large genomic diversity of Lassa virus emphasises the need to match deployed diagnostics capabilities with locally circulating strains and underlines the importance of evaluating cross-lineage protection in the development of vaccines and therapeutics. The data also highlight the need to increase Lassa fever surveillance using appropriate diagnostics, and to implement strategies that can cope with high levels of diversity.

virus IgM, IgG, and Lassa virus antigen, and these immunoassays have been converted into rapid diagnostic tests that are pan-Lassa, cheap, and transportable.^{6,7} However, diagnostic tests labelled "for research use only" are the only ones that have been made commercially available to all countries. Typically, immunoassays are not as sensitive as PCR-based tests, and severe infections can suppress the production of IgM and IgG and cause false-negative results.⁷ A recent study in Sierra Leone did show greater sensitivity of immunoassays based on Lassa virus antigen compared with the PCR-based tests that were being used.⁸ Molecular diagnostic tests are considered the most sensitive method, but can produce false-negative results due to the high degree of genetic diversity. There are commercially available assays from three different vendors that have the CE IVD determination from EU regulators. An appropriate diagnostic algorithm will require a combination approach of serological and molecular diagnostics to capture the wide range of Lassa virus diversity that can be present in a given country. Reviews of currently available serological and molecular based diagnostics have been published.^{6,7}

In 2018, Nigeria had an increase in Lassa fever cases associated with a case fatality rate of approximately 25%, causing concern in the global public health community.^{9,10} Genomic analysis of the circulating strains compared with historical data indicated that the rise in cases was not caused by a new circulating variant or sustained human-to-human transmission, but that it was consistent with transmission from the rodent reservoir.^{9,10} During the same period, Liberia also had a seasonal outbreak of Lassa fever. Despite the long history of Lassa virus in Liberia,² a history of exported cases,^{11,12} and the high per capita rate of reported cases (table 1), little is known about the viral

genetic diversity circulating in this country. We report 17 Lassa virus genome sequences from clinical cases (one from Guinea and 16 from Liberia) and 17 from viral stocks (two from Guinea, seven from Liberia, four from Nigeria, and four from Sierra Leone). We used these genomes to infer the evolutionary history of Lassa virus in Liberia and more broadly in west Africa, and investigated the appropriateness of current sequence-based diagnostic tools for detecting Lassa virus lineages from Liberia.

Methods

Sample processing and sequencing

De-identified human serum samples obtained from patients determined to be positive for Lassa fever by the Liberian National Reference Laboratory were processed for sequencing at the Liberian Institute for Biomedical Research. Accompanying metadata were provided by the National Public Health Institute of Liberia (ethics statement is provided in the appendix, p 3). Samples in Liberia were sequenced on an Illumina MiSeq at the Liberian Institute for Biomedical Research using an enrichment-based method as previously described¹³ with modifications to enrich Lassa virus. Archival isolates at the US Center for Disease Control and Prevention (CDC) Viral Special Pathogens branch in Atlanta, GA, were sequenced using the TruSeq Stranded mRNA kit on an Illumina MiniSeq. Additional details on sequencing methods can be found in the appendix (p 3). The consensus genome sequences were deposited in GenBank (appendix p 6).

Phylogenetics and molecular clock analysis

We generated Large (L) and Small (S) segment-level coding sequence alignments (concatenated open reading frames)

See Online for appendix

	2014	2015	2016	2017	2018
Liberia					
Reported cases	10	3	96	81	191
Confirmed cases	1	0	14	30	21
Confirmation rate	10.0%	0	14.6%	37.0%	11.0%
Population size (million)	4.39	4.50	4.61	4.73	4.85
Reported per 100 000	0.23	0.07	2.08	1.71	3.94
Confirmed per 100 000	0.02	0.00	0.30	0.63	0.43
Nigeria					
Reported cases	929	430	901	1022	3276
Confirmed cases	110	25	101	308	588
Confirmation rate	11.8%	5.8%	11.2%	30.1%	17.9%
Population size (million)	176.46	181.18	185.99	190.87	195.88
Reported per 100 000	0.53	0.24	0.48	0.54	1.67
Confirmed per 100 000	0.06	0.01	0.05	0.16	0.30

2014 and 2015 had known under-reporting in Liberia due to the Ebola virus disease outbreak. Case numbers for Liberia were obtained from the Liberian Ministry of Health (personal communication) and the Liberia IDSR Epidemiology Bulletin. Case numbers for Nigeria obtained from Nigeria Centre for Disease Control weekly Lassa update reports. Population data were obtained from Worldometers on Feb 14–19, 2019.

Table 1: Lassa fever cases in Liberia and Nigeria, 2014–18

For the **Liberia IDSR Epidemiology Bulletin** see <https://nationalphil.org>

For more on **Worldometers** see <http://www.worldometers.info/world-population>

in Geneious (version 11.1.4; Biomatters, Auckland, New Zealand) using publicly available sequences covering at least 80% of the protein-coding portions of either the L or S genome segment. Maximum-likelihood phylogenies were generated using RAxML Next Generation with the GTR+G4 model and 100 bootstrap replicates.¹⁴ Root-to-tip distances using the same dataset were generated with TempEst (v1.5.1)¹⁵ using the best-fitting root option. Bayesian phylogenies for each of the segment-level alignments were generated in BEAST (v1.8.4).¹⁶ Detailed methods for the analysis and model comparisons are provided in the appendix (pp 3–4, p 7).

Lassa virus PCR assay evaluation

Lassa virus specific PCR assays were evaluated using both in vitro and in silico methods. The in vitro analysis was done using the two assays deployed in Liberia, the Nikisins¹⁷ and Trombley Josiah-MGB.¹⁸ The in silico analysis was done with the PCR Signature Erosion Tool (version 1)¹⁹ using 16 published Lassa virus assays, including the Nikisins and Trombley Josiah-MGB. A final in silico analysis was done by aligning the PCR primers and probes to all the available Lassa virus sequences and measuring the number of mismatches (appendix p 8, pp 12–21, p 24). A more detailed description of the assay evaluation can be found in the appendix (p 4).

Role of the funding source

The sponsor of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Serum samples positive for Lassa virus were provided by the National Public Health Institute of Liberia and were processed and sequenced at the Liberian Institute for Biomedical Research. We sequenced six samples from 2018 (one was an imported case from Guinea) and 11 archival samples collected between 2011 and 2017 (table 2). Most samples originated from known Lassa fever hotspots in Bong and Nimba counties. Cases from Montserrado county probably represent imported cases from endemic counties—for instance, the patient of sample LIB-LF17-001 was a resident of Ganta in Nimba county who became febrile in Montserrado. Coding-complete genomes were obtained for 12 samples; the remaining five had gaps in coverage in the L protein (appendix p 6). We generated 17 coding-complete sequences from viral stocks of historical reference samples (two from Guinea, seven from Liberia, four from Nigeria, and four from Sierra Leone), from the Centers for Disease Control Viral Special Pathogens Branch (CDC-VSPB) as well. Of the seven samples originating from Liberia, three were imported into the USA with clear epidemiological links to Liberia.^{11,12} Pairwise comparisons of the new coding-complete Liberian genomes showed nucleotide diversity that ranged up to 27% in the L segment and 18% in the S segment.

Segment-level phylogenetic analysis, including publicly available Lassa virus genomes, indicated that all of our Liberian genomes belong to lineage IV (figure 1).^{20,21} The only exception was the L segment from isolate 807978-P28, which falls outside of the diversity previously characterised for lineage IV (appendix p 22). The Liberian Lassa virus genomes are split between two major clades within lineage IV (labelled IV.A and IV.B in the

	Country	County or prefecture	District of residence	Health-care facility	Specimen type	Collection date
LIB-LF11-003	Liberia	Bong	Suakoko	Phebe Hospital	Serum	Feb 20, 2011
LIB-LF11-011	Liberia	Bong	Jorkoquleleh	Phebe Hospital	Serum	April 14, 2011
LIB-LF13-006	Liberia	Nimba	Seclepea Mah	Seclepea Health Center	Serum	April 29, 2013
LIB-LF13-007	Liberia	Nimba	Seclepea Mah	Seclepea Health Center	Serum	April 29, 2013
LIB-LF16-004	Liberia	Bong	Jorkoquleleh	Phebe Hospital	Serum	Feb 26, 2016
LIB-LF16-016	Liberia	Nimba	Garr Bain	Ganta United Methodist Hospital	Serum	April 13, 2016
LIB-LF17-001	Liberia	Montserrado*	Ganta	Ganta United Methodist Hospital	Serum	Jan 9, 2017
LIB-LF17-007	Liberia	Nimba	Seclepea Mah	Ganta United Methodist Hospital	Serum	Jan 9, 2017
LIB-LF17-013	Liberia	Bong	Jorkoquleleh	Phebe Hospital	Serum	Feb 13, 2017
LIB-LF17-019	Liberia	Bong	Jorkoquleleh	Phebe Hospital	Serum	May 4, 2017
LIB-LF17-020	Liberia	Nimba	Seclepea Mah	Phebe Hospital	Serum	May 2, 2017
LIB-LF18-001	Liberia	Bong	NA	NA	Serum	Jan 10, 2018
LIB-LF18-002	Guinea	Yomou	NA	Diécké	Serum	Jan 10, 2018
LIB-LF18-037	Liberia	Nimba	NA	NA	Serum	Feb 13, 2018
LIB-LF18-040	Liberia	Montserrado	NA	NA	Serum	March 2, 2018
LIB-LF18-041	Liberia	Montserrado	NA	NA	Serum	March 3, 2018
LIB-LF18-042	Liberia	Nimba	NA	NA	Serum	March 1, 2018

Samples were provided by the National Public Health Institute of Liberia and were processed and sequenced at the Liberian Institute for Biomedical Research. Country, county, and prefecture indicate where the patient first presented with symptoms and district of residence indicates where the patient lives. NA=not available.
*Patient was in Montserrado county when becoming febrile but suspected to have become infected in Nimba county.

Table 2: Clinical serum samples positive for Lassa virus collected from Liberia and Guinea

L and S genome segment trees of figures 1 and 2). The genome segments from the 2018 imported case from Guinea grouped into clade IV.A which, until now, only contained partial S genome segments of Liberian origin (appendix p 23). Clade IV.B had been observed in Guinea, Liberia, and Sierra Leone and contains the two previously available coding-complete genomes from Liberia. We estimated that these two clades diverged approximately 300–350 years ago (L: median 1669, 95% highest posterior density [HPD] interval 1616–1717; S: median 1720, 95% HPD interval 1674–1758) by fitting a molecular clock to the data. Notably, the Liberian isolate 807978-P28 appears to be a reassortant, with an S segment from clade IV.A and an L segment that does not fall within lineage IV or V but belongs to a previously unobserved phylogenetic lineage (appendix pp 22–23). A more detailed analysis below the lineage level is provided in the appendix (pp 4–5).

Our molecular clock analysis of lineages IV and V estimated substitution rates (L: 8.4×10^{-4} substitutions per site per year, 95% HPD interval 7.6×10^{-4} to 9.2×10^{-4} ; S: 8.2×10^{-4} , 95% HPD interval 7.2×10^{-4} to 9.1×10^{-4}) that are consistent with previous studies^{3,21} that included all five Lassa virus lineages. Our estimates for the time of most recent common ancestors (tMRCA) for the large clade from Sierra Leone in lineage IV and lineage V (figure 2A and 2B) are in line with previous estimates of about 150 years ago.^{3,21} However, new genomes from Liberia and Guinea have pushed back estimates for the introduction of Lassa virus into the Mano River Union from about 220 to 300–350 years ago (figure 2A and 2B).³ Both S-based and L-based segment estimates were highly

consistent. Our analysis indicated that with the current data, Liberia was most likely the entry point for Lassa virus to the Mano River Union (posterior probability $\geq 94\%$), and that the virus was later introduced, at least four times, into Guinea (0–40, 40–60, 50–130, and 90–280 years ago) and at least once into Sierra Leone (about 150 years ago; figure 2A and 2B). Root-to-tip distances were also generated using the identical dataset and a positive correlation between sampling date and root-to-tip distance is observed, indicative of molecular clock signal in the data (figure 2C and 2D).

Liberia was identified as the most likely location for the MRCA of lineages IV and V, although the low posterior support indicates ambiguity in this assignment with the current dataset. Although the S and L segment trees were highly consistent, they differed substantially in their tMRCA estimates for the ancestor of lineages IV and V. This is likely due to the L segment of isolate 807978-P28, which, as mentioned previously, is consistent with a reassortment event and does not belong to either lineage IV or V. Evidence for an additional sequence outside of lineage IV and V is present in the S genome segment phylogeny after inclusion of the partial genomes (appendix p 23). These results suggest that the genetic diversity of Lassa virus remains undersampled. Thus, these tMRCAs will likely need further revision with the addition of more sequence data.

The Liberian National Reference Laboratory in Charlesville uses two quantitative real-time PCRs (rtPCRs) assays to detect Lassa virus: the Lassa Josiah-MGB assay (which was designed to detect the lineage IV and targets the glycoprotein precursor [GPC] gene on the S genome

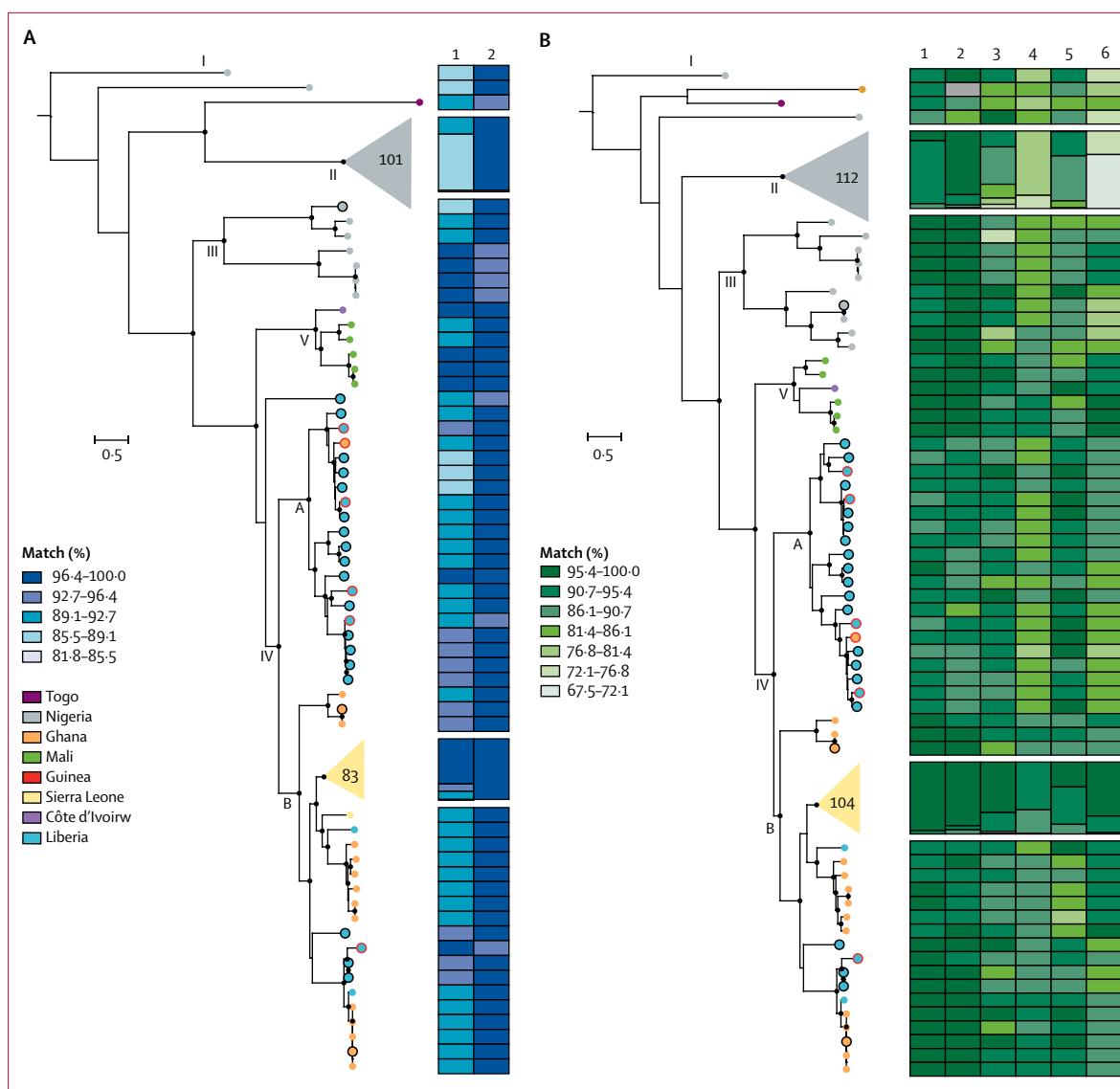


Figure 1: Maximum-likelihood trees for Lassa virus L and S genome segments sequenced from human infections and reservoir hosts in west Africa
 (A) Maximum-likelihood tree for L genome segment. (B) Maximum-likelihood tree for S genome segment. Nodes are coloured based on the country of origin. Sequences generated from the 2018 cases are outlined in red (see table 2 and appendix p 6 for sample metadata and sequence completeness), other sequences from this study are outlined in black. For the collapsed clades, the number of contained sequences is specified. Scale bars indicate number of nucleotide substitutions per site. Roman numerals are used to label previously defined Lassa virus lineages^{20,21} (see appendix pp 22–23 for subtrees of lineage IV and V which include partial sequences). The heatmaps to the right of the phylogenetic trees in panels A and B represent the percent identity of the assay target site as presented in the appendix (pp 12–21). The numbers above the heatmap in panel A represent the following assays: 1 Nikisins²² and 2 Vieth.¹⁷ The numbers above the heatmap in panel B represent the following assays: 1 Bowen,²⁰ 2 Coulibaly,²³ 3 Demby,²⁴ 4 Leski,²⁵ 5 Olschläger,²⁶ and 6 Safronetz²⁷ (see appendix p 8 for assay sequences and the gene targets).

segment)¹⁸ and the La-V assay (designated Nikisins, which was designed to be a pan-Lassa assay and targets the RNA-dependent RNA polymerase [L] gene on the L genome segment).¹⁷ Samples analysed at the Liberian National Reference Laboratory revealed a large number of discrepancies between the results of the two assays; the Nikisins assay often produced positive results for samples for which the Lassa Josiah-MGB was negative. We aligned the primers and probes from the two assays to the newly sequenced Lassa virus genomes and observed a large

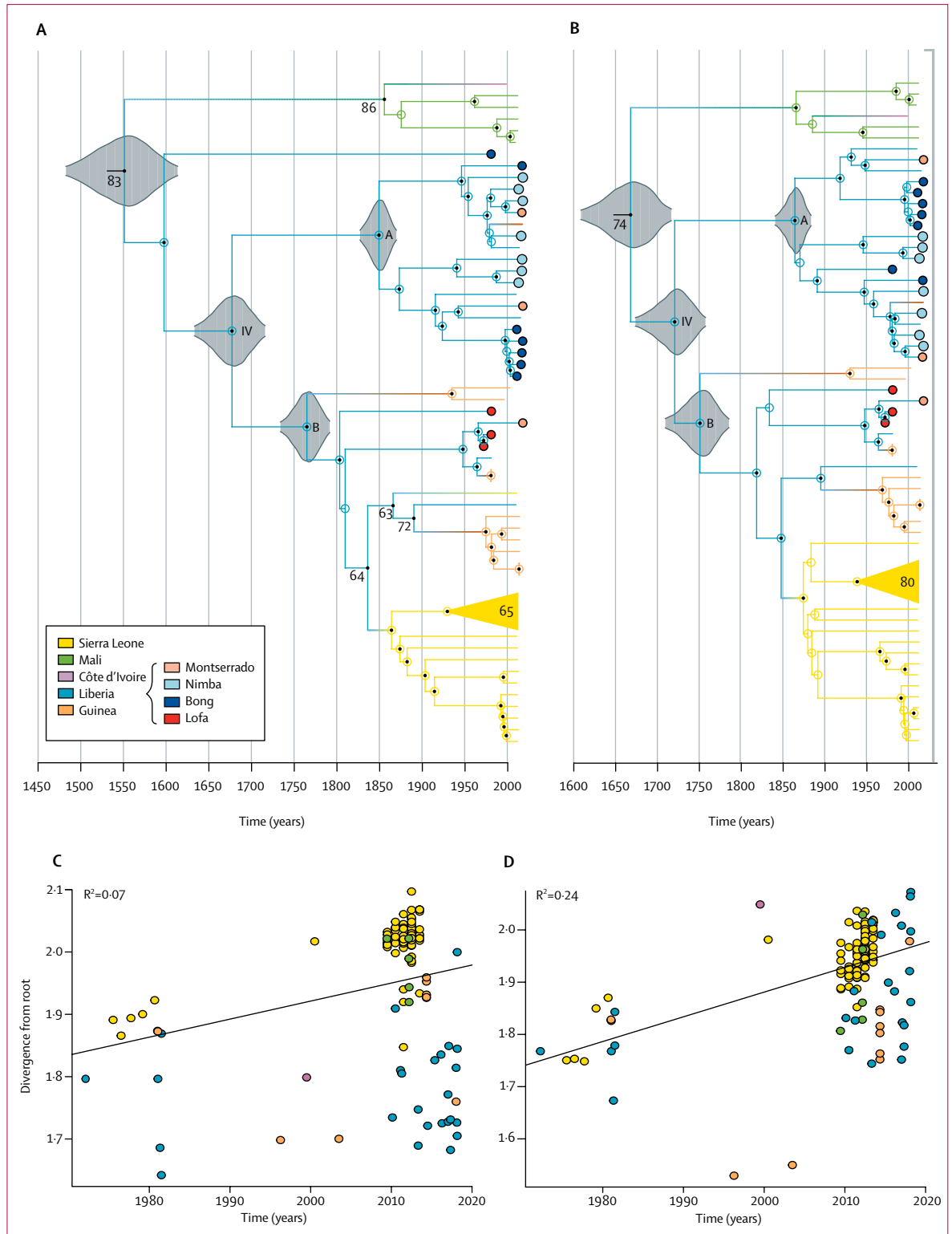
number of mismatches in the target sites of the Lassa Josiah-MGB assay (75.0–98.4% identity across target sites, as seen in appendix p 24). Fewer mismatches were present in the targeted regions of the Nikisins assay (94.6–100% identity across target sites), mainly due to well-placed ambiguous bases to account for Lassa virus diversity.

To determine how these mismatches would affect the ability to detect Lassa virus using the Nikisins or Lassa Josiah-MGB assays, we did *in vitro* tests using 16 viral stocks that originated from west Africa (appendix p 9).

Four ten-fold dilutions of each viral stock were tested to see if either assay could detect all the dilutions. The Nikisins assay was able to detect all the Liberian isolates at every dilution; however, the Josiah-MGB assay was only able to detect two of the seven Liberian isolates at all four dilutions. A correlation was observed between the

Figure 2: Temporal maximum clade credibility trees for Lassa virus L and S genome segments sequenced from human infections and reservoir hosts in west Africa

(A) Temporal maximum clade credibility tree for L genome segment. (B) Temporal maximum clade credibility tree for S genome segment. Only lineages IV and V were included in this analysis. Small black circles indicate clades with posterior probabilities of at least 90%. Branches are coloured according to the inferred ancestral location of each lineage. Coloured rings indicate nodes with ancestral state posterior probabilities of at least 90%; nodes below this threshold are labelled with their respective probabilities. The grey violin plots represent the 95% highest posterior density intervals for the time of most recent common ancestor (tMRCA) for clades IV.A, IV.B, lineage IV, and lineages IV and V together (tree root). Liberian samples with known county of origin are indicated with filled circles and coloured according to county of origin. Since the L genome segment of sample 807978-P28 fell outside of the diversity previously described for lineages IV it was not used in the tMRCA analysis for this lineage. (C) Root-to-tip distances for the L genome segment. (D) Root-to-tip distances for the S genome segment. Colours indicate country of origin and the line represents a simple linear regression (see appendix p 7 for the BEAST model comparison for the L and S genome segments).



number of mismatches in the target region and ability to detect all dilutions.

Because of the discrepancies observed between the Lassa Josiah-MGB assay and the Nikisins assay, we expanded our evaluation to other published Lassa virus rtPCR assays using *in silico* methods. The goal was to quickly evaluate alternatives for detection of Lassa virus in Liberia and other endemic regions in Africa. We evaluated 16 published assays with the PCR Signature Erosion Tool (appendix p 10) by calculating the percent identity across the target regions and inferring the minimal number of assays needed to cover all the diversity in the L and S segment (appendix p 11). We listed the percent identities of the two L segment assays and the top six S segment assays along with accession numbers (appendix pp 12–22) and presented the data as a heatmap in figure 1 to coordinate with the tips of the phylogenetic tree. Among the two published L segment assays tested, the Nikisins assay showed the highest percent identity across the target site of the analysed Lassa virus genomes, with a range of 94·6–100% and an average of 98·6% (figure 1; appendix pp 12–22). Between the six S segment assays tested, the Bowen²⁰ and Coulibaly²³ assays had the highest average identity at 95·2% for Bowen and 96·1% for Coulibaly (appendix pp 12–22). When focusing on just genomes of Liberian origin, the Ölschläger assay²⁶ had the highest average identity for an S segment assay at 94·2%.

Discussion

Liberian Lassa virus genomes are substantially under-represented in genomic databases by comparison with other Mano River Union countries and Nigeria. As of January, 2019, only two coding-complete Lassa virus genomes, 36 partial S genome segments (27 GPC at 60–80% coverage and nine nucleoprotein at 40% coverage), and six partial L genome segments (6–12% coverage of L gene) of Liberian origin were available on GenBank. The short sequence length limits the type of downstream analyses that can be done compared with coding-complete genomes. To address the scarcity of genetic information, we obtained coding-complete viral genomes from confirmed cases during the 2018 Lassa fever season and from a collection of archival samples. In total, we were able to generate 34 near-complete genomes for the L and S segments, 23 of these coming from samples of Liberian origin (figure 1; table 2; appendix p 6).

These newly sequenced genomes show a large amount of diversity is present among circulating strains in Liberia. The genomes were grouped into two distinct and well-supported clades, which we designated IV.A and IV.B. When geographical information was available, we found that the Liberian genomes grouped according to location rather than collection dates, which is consistent with previous observations in Guinea, Nigeria, and Sierra Leone.^{3,9,10,20,25,28} Most newly sequenced samples grouped in clade IV.A, which until now had only been represented in GenBank by partial genomes (figure 1; appendix pp 22–23).

The Liberian genomes from clade IV.A originate from Bong, Montserrado, and Nimba counties, and could not be resolved further than county-level based on the available metadata. Clade IV.A could be split further into three well supported subclades that include a grouping of all three counties, a group of Bong and Montserrado counties, and a group of just Nimba county. The imported 2018 case from Guinea also grouped in clade IV.A, specifically in the subclade that included representatives from Bong, Montserrado, and Nimba. More details on the movement of Lassa fever between Liberia and Guinea can be found in the appendix (p 5).

Clade IV.B has been previously observed in Guinea, Liberia, and Sierra Leone, and both of the previously available coding-complete genomes from Liberia belong to this clade. The majority of Liberian genomes that belong to this clade are older (originating from 1972–81) and were isolated from patients diagnosed at Curran Lutheran Hospital in Zorzor, Liberia, in Lofa County.^{29,30} One of the 2018 samples, LF18040, falls within a well-supported subclade of IV.B, which had not been detected for the past 37 years (appendix pp 22–23), showing that this clade continues to circulate.

Even with additional sequencing data, it appears further sampling is still needed to capture all the Lassa virus diversity circulating in Liberia. Multiple indicators of this include the L and S genome segments of the previously reported G1200 strain (KM821797 and KM821798), which group only with sequences from Guinea and Sierra Leone, and the L segment of strain 807978-P28 and the partial S segment from the previously reported Lib05-4094 strain (GU830819), which both fall outside of lineage IV and V (appendix pp 22–23). The available metadata indicate that 807978-P28 is from Bong country, but for G1200 and Lib05-4094 only the country of origin is provided, making it difficult to determine the region of the country in which this diversity is present. Additional Lassa virus surveillance and sequencing of confirmed cases is required to ensure that the complete viral diversity in Liberia has been covered.

To expand successful surveillance activities of Lassa fever in Liberia, deployed diagnostics need to detect all of the known genomic diversity. Reliance solely on sequence-based assays can cause limitations as shown in a 2015 international quality assessment study.¹⁷ In this study, Nikisins and colleagues¹⁷ observed 11 out of 24 international reference laboratories had at least one false-negative result and four of these laboratories did not identify any of the samples originating from Liberia.¹⁷ One solution would be to switch to multiple pan-Lassa assays that target the L and S segment (eg, Altona RealStar Nikisins assays), as done during the 2018 Lassa fever outbreak in Nigeria to help eliminate false-negative results due to potential target erosion.¹⁰ Another solution would be to incorporate immunoassays into the testing algorithms as has been shown in field testing in Sierra Leone.⁸ The rapid diagnostic antigen test was able to detect samples missed by the

For more on the **Altona kits** see <https://www.altona-diagnostics.com/en/products/reagents-140/reagents/realstar-real-time-pcr-reagents/realstar-lassavirus-rt-pcr-kit-ruo.html>

rtPCR assay and new pan-Lassa rapid diagnostic tests could provide an even broader detection capacity.

The dramatic increase of Lassa fever cases in west Africa, including Liberia and Nigeria, highlights the growing risk Lassa virus poses to regional and global health security. The overwhelming amount of observed Lassa virus diversity underscores the requirement that medical countermeasures and diagnostic assays developed against commonly used Lassa virus laboratory strains are re-evaluated locally to ensure effectiveness at preventing, detecting, and treating all the circulating strains. Our data identify concerns in the current molecular diagnostic assays being used in Liberia and highlight the need to evaluate more recently developed pan-Lassa rtPCR assays and rapid diagnostic tests in Liberia, to develop a more comprehensive testing algorithm. Our results are probably not unique to Liberia and emphasise that these problems might not be completely resolved if we focus too much on any given country or region.

Contributors

MRW, AGL, CGA and GP conceptualized the study. MRW, JTL, NE, ND, JAC, HM, DN and SS analysed the data. MRW, LF, SW, KaPr, DR, WGD, and KePa generated the data. FT, JD, TN and MF provided study resources. MRW, AGL, JTL, CBP, SS, PER, CGA and GP wrote and edited the manuscript. DW, JS, US, STN, TN, FB, CGA, MF, and GP supervised the study.

Declaration of interests

We declare no competing interests.

Data sharing

Lassa virus consensus genomes are available on GenBank under accession numbers MG812630-MG812685 and MH215278-MH215289.

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