

At the time the bacteria was speciated by 16S rRNA gene sequencing, the patient's infection had already resolved. The clinical record does not document any additional antimicrobial treatments she may have received from other clinical teams, including the infectious disease, transplant, and nephrology departments. This organism appeared in 3 consecutive respiratory specimens collected when the patient's symptoms worsened and raised concerns among the attending clinical teams of potential infection with an innately drug-resistant species. However, we cannot definitively rule out the potential for colonization because a combination of factors likely led to clinical improvement in the patient. The organism was not detected in any subsequent bronchoscopies.

The genus *Acetobacter* encompasses a group of acetic acid-producing organisms that can survive at low pH, largely occupy environmental niches, are used industrially to produce acetic acid products, and are not generally thought to be human pathogens (1). Analysis of the medical literature revealed 2 other documented clinical cases of *A. indonesiensis* infection among humans (2,3). The first case involved a patient with cystic fibrosis who had undergone a recent lung transplant (2). Similar to our case-patient, the patient had undergone bilateral lung transplants and *A. indonesiensis* pneumonia subsequently developed in both after a long course of broad-spectrum antimicrobial drugs. The second case involved a child with metachromatic leukodystrophy who was found to have *A. indonesiensis* bacteremia after extensive nursing care and invasive devices, including a port catheter thought to be the source of the infection (3). As with the patient we report, the patient in that report had been treated with a 2-week course of piperacillin/tazobactam, although her initial diagnosis was bacteremia rather than pneumonia.

The case of *A. indonesiensis* human infection we report and both previous cases we found in the literature involved chronically ill patients with complex medical conditions who were exposed to a long course of broad-spectrum antimicrobial drugs. Although the source of the infecting organism in all 3 cases could not be definitively determined, the similarities between the cases raise the possibility that *A. indonesiensis* may represent a novel and emerging opportunistic and highly drug-resistant pathogen. Furthermore, the use of specific genotypic techniques such as 16S rRNA sequencing may aid in the identification of environmental organisms that are not identified by using traditional microbiological techniques.

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New Lineage of Lassa Virus, Togo, 2016

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We describe a strain of Lassa virus representing a putative new lineage that was isolated from a cluster of human infections with an epidemiologic link to Togo. This finding extends the known range of Lassa virus to Togo.

Lassa virus is endemic to the West Africa countries of Guinea, Sierra Leone, Liberia, Mali, Côte d'Ivoire, and Nigeria (1–3). The virus causes Lassa fever, a hemorrhagic disease with a case-fatality rate $\approx 30\%$ in the current hospital setting in West Africa. So far, 4 lineages of Lassa virus are firmly established: lineages I, II, and III circulate in Nigeria, and lineage IV circulates in Guinea, Sierra Leone, Liberia, Mali, and Côte d'Ivoire (1–3). Recently, strains from Mali and Côte d'Ivoire were proposed to represent a separate lineage V (4). The newly discovered Lassa virus strain Kako from *Hylomyscus pamfi* rodents trapped in Nigeria is designated lineage VI for the purpose of this article (5).

Lassa virus has not been previously detected in humans or rodents in Togo; therefore, the virus was not considered endemic to this country. We describe a strain of Lassa virus representing a new lineage that was isolated from a cluster of human infections with an epidemiologic link to Togo (online Technical Appendix, <https://wwwnc.cdc.gov/EID/article/24/3/17-1905-Techapp1.pdf>) (6, 7). The clinical courses of the 3 case-patients and medical and public health interventions are described elsewhere (8–10).

The Lassa virus infections in the index case-patient, secondary case-patient 1, and secondary case-patient 2 were confirmed by laboratory investigations at Bernhard Nocht Institute (Hamburg, Germany); Centers for Disease Control and Prevention (Atlanta, GA, USA); and Philipps University (Marburg, Germany), respectively. The viruses from all 3 patients were isolated in Vero E6 cell culture in the respective Biosafety Level 4 laboratories. Full-length virus sequences were generated directly from clinical specimens, from the isolates, or both using next-generation

sequencing technology in combination with Sanger sequencing (sequences deposited into GenBank under accession nos. KU961971, KU961972, LT601601, LT601602, MF990886–MF990889) (online Technical Appendix). The sequence from the index case-patient was submitted to GenBank on March 23, 2016, and immediately made publicly available to support the laboratory and public health response in Togo and the other affected countries.

The virtually identical viruses from the 3 patients confirmed the transmission chains suggested by the epidemiologic data. Only the virus from secondary case-patient 2 showed differences in coding regions—a deletion of 3 nt and a nucleotide exchange in the polymerase (L) gene—from the viruses in the other 2 case-patients. These differences were confirmed by sequencing the virus in the clinical specimens. Differences among the 3 strains in the highly structured intergenic regions might represent artifacts created by the difficulty in sequencing these regions.

The phylogeny was inferred using BEAST2 (<https://www.beast2.org/>) with nucleotide sequences of full-length nucleoprotein (NP), glycoprotein precursor (GPC), and L and Z genes of the Togo strain in conjunction with representative sequences of Lassa virus and other Old World arenaviruses. The most stable reconstruction was obtained for the L gene with the Togo strain being placed in sister relationship with lineage II (all branches with posterior support values ≥ 0.97) (Figure). In the NP- and GPC-based phylogenies, the Togo strain clusters with lineages I and VI (Pinneo and Kako strains); however, the branching order is not well supported (posterior values 0.51–0.86) (Figure). The phylogeny based on the small Z gene further supports a relationship of the Togo strain with lineages I, II, and VI (online Technical Appendix Figure 1). The ambiguous position of the Togo strain relative to lineages I, II, and VI is consistent with a recombination analysis showing that most of the L gene sequence is related to lineage II, and NP and GPC comprise sequence stretches mainly related to lineages I and VI (online Technical Appendix Figure 2). This mosaic structure might be the result of recombination, reassortment, or both or might have evolved by chance.

The long branch (i.e., large phylogenetic distance) separating the Togo strain from known lineages suggests that it represents a new lineage. Because Lassa virus lineages were originally established on the basis of uncorrected sequence distances (1), we used the same method here. The frequency distribution of pairwise amino acid distances in GPC, NP, and L between the Togo strain and all other Lassa virus strains perfectly overlaps with the distribution of distances between Lassa virus lineages I, II, III, IV, and VI indicating that the Togo strain is a separate lineage (online Technical Appendix Table 1). However, we noted that the distance between the proposed lineage V and lineage IV rather corresponds to intralinear distances, and therefore, we considered lineage

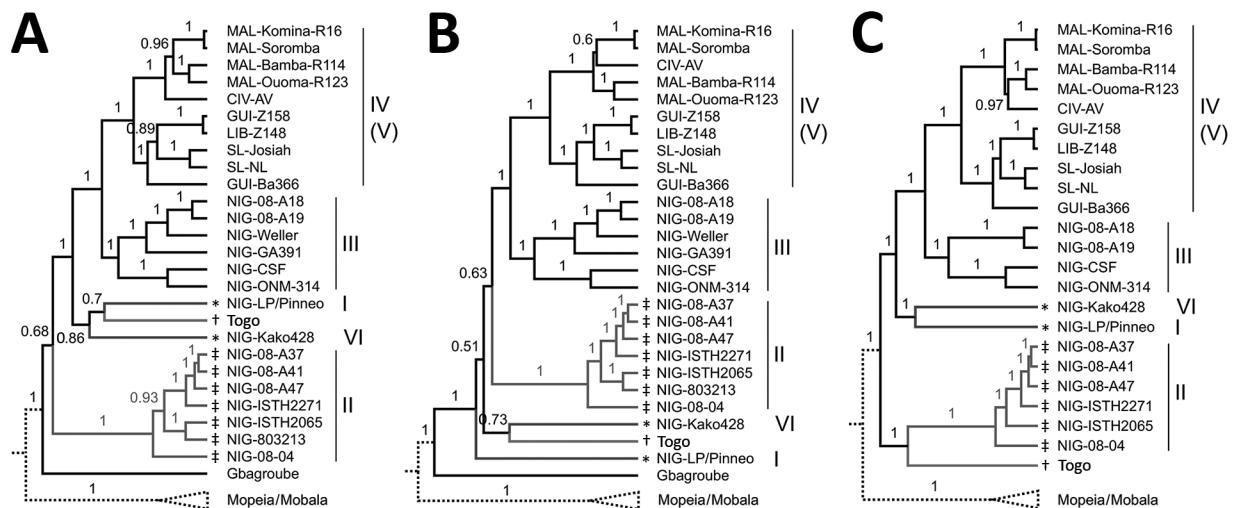


Figure 3. Phylogeny of the Lassa virus strain from Togo, 2016. Phylogenetic trees were inferred by using BEAST2 (<https://www.beast2.org/>) for full-length glycoprotein precursor (A), nucleoprotein (B), and polymerase (C) genes. The analysis included representative Lassa virus strains and other Old World arenaviruses (online Technical Appendix, <https://wwwnc.cdc.gov/EID/article/24/3/17-1905-Techapp1.pdf>). Posterior support values are shown at the branches. Lassa virus lineages are indicated by roman numbers on the right. The branch for Mopeia and Mobala virus is shown schematically and the branches for the remaining Old World arenaviruses have been removed for clarity of presentation. The branches for the Togo strain and most closely related Lassa virus lineages are labeled (†, Togo; ‡, lineage II; *, lineages I and VI). The origins of the Lassa virus strains are abbreviated as follows: CIV, Côte d'Ivoire; GUI, Guinea; LIB, Liberia; MAL, Mali; NIG, Nigeria; SL, Sierra Leone. A color version of this figure is available online (<https://wwwnc.cdc.gov/EID/article/24/3/17-1905-F1.htm>).

V a subclade of lineage IV in our analysis (online Technical Appendix Table 1). We propose that formal recognition of Lassa virus lineages should be decided by the International Committee on Taxonomy of Viruses.

In conclusion, sequencing Lassa virus from a cluster of imported infections, with the index case-patient originating from Togo, reveals a new lineage of Lassa virus in West Africa. It seems to be related to lineage II or lineages I/VI, which are all circulating in Nigeria.

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Evidence for Previously Unidentified Sexual Transmission of Protozoan Parasites

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Knowing the mode of transmission of a disease can affect its control and prevention. Here, we identify 5 protozoan parasites with demonstrated presence in seminal fluid, only 1 of which has been identified as a sexually transmitted disease among humans.

A recent publication by Salam and Horby (1) identified at least 27 viruses present in human semen, some potentially transmissible through sexual contact. *Trichomonas vaginalis* is a protozoan parasite recognized as sexually transmissible among humans (2). Similar to that which

occurs with viruses, parasites could reach seminal fluid by passing from the bloodstream to the male genital tract or by directly infecting reproductive organs. In this context, more parasitic infections might also be transmitted sexually. Considering that parasitic diseases represent one of the most common infections worldwide, mainly in developing countries, sexual transmission of parasitic diseases could represent a major global problem in terms of public health.

To investigate whether parasites could enlarge the broad list of potential sexually transmitted infections (STIs), we conducted an online search on November 3, 2017, by using PubMed (<https://www.ncbi.nlm.nih.gov/pubmed/>), EMBASE (<https://www.elsevier.com/solutions/embase-biomedical-research>), and the Cochrane Library (<http://www.cochranelibrary.com/>) with no language restrictions. We used the terms “parasites OR parasitic disease” and “semen OR seminal plasma.” We also made a manual search of the references of selected reports. Two reviewers independently screened the 512 returned results of titles, abstracts, and full text in selected articles.

Our search resulted in 5 parasite species with demonstrated presence in seminal fluid of humans: *Entamoeba histolytica* (3), *Schistosoma haematobium* (4), *Trichomonas vaginalis* (2), *Trypanosoma cruzi* (5), and *Toxoplasma gondii*; the latter has been documented as sexually transmitted among animals, but not humans (6) (Table). *E. histolytica* is a worldwide anaerobic protozoan; its prevalence increases disproportionately in areas of poor sanitation in low-income countries. *E. histolytica* has been identified in the testicles, epididymis, and seminal fluid (3,7), can reportedly cause infertility as a result of reproductive organ damage (8), and is transmitted by sexual contact (both oral-anal and oral-genital sexual practices) (7).

Urogenital schistosomiasis caused by *S. haematobium* infection affects male and female children and adults mainly in Africa, the Middle East, and Corsica, France. After the larval *S. haematobium* cercariae penetrate intact skin from contaminated fresh water, they migrate and mature into adult worms, predominantly in the venous plexus of the bladder. These worms can then travel to the seminal vesicles and prostate, causing local pathology (9). *S. haematobium* eggs have been found in up to 43% of 44 semen samples and in 33.3% of cervix biopsies obtained from 36 women from endemic area populations (4,10); nevertheless, sexual transmission has not been reported.

T. vaginalis protozoa are the most common nonviral STI in the world, and incidence is increasing (11). The genital tract of humans is the natural habitat for this parasite, which can cause urogenital tract infection. *T. vaginalis* has been identified in seminal fluid and has been related to decreased sperm quality (2,8).

Chagas disease is caused by *T. cruzi* protozoa and affects nearly 6 million persons in Latin America countries.

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Technical Appendix

Patients

On February 25, 2016, a healthcare professional who worked in Sansanné-Mango, Savanes Region in northern Togo, was air-evacuated with a diagnosis of severe malaria to the University Hospital Cologne (Cologne, Germany). He died within a few hours after hospital admission. Lassa fever was diagnosed postmortem after the suspicion of hemorrhagic fever had been raised on evaluation of histopathologic findings of the liver. Secondary case-patient 1 was a healthcare professional who treated the index case-patient in Togo. Lassa fever was clinically diagnosed in Togo, and the patient was air-evacuated to Atlanta, Georgia, USA, on March 12, 2016, for medical treatment at Emory University Hospital. Secondary case-patient 2 was a mortician in Germany who prepared the body of the index case-patient for repatriation. He was treated at Frankfurt University Hospital (Frankfurt, Germany). Both secondary case-patients survived.

Methods

Lassa Virus Sequencing in Hamburg, Germany

The sequence of Lassa virus strain Togo from the index case-patient was determined directly from clinical specimens using next-generation sequencing combined with Sanger sequencing technology. Briefly, the serum sample was filtered through a 0.45- μm filter (Merck Millipore, Darmstadt, Germany) to remove cell debris and bacteria and treated with a mixture of DNase and RNase to digest unprotected nucleic acids, including host DNA/RNA. RNA was extracted and converted to double-strand cDNA. Library preparation was performed using Nextera XT DNA Library Preparation Kit (Illumina, San Diego, CA, USA). Sequencing was performed on the Illumina MiSeq platform with 250 base-paired ends and dual barcoding for each library. The paired-end reads generated by MiSeq were trimmed and de novo assembled using Geneious 9 (Biomatters, Auckland, New Zealand). Reads and contigs >100 bp were

subjected to mapping using Lassa virus sequences from GenBank. Complete genome assembly coverage for S segment was obtained except for the intergenic regions. Gaps were filled by Sanger sequencing. The genome sequences were deposited at GenBank under accession nos. KU961971.1 and KU961972.1.

Lassa Virus Sequencing in Atlanta, Georgia, USA

Unbiased next-generation sequencing was performed using Illumina TruSeq stranded total RNA using RNA extracted from blood from secondary case-patient 1 (drawn on March 12, 2016) and a viral isolate generated from the same material. RNA was extracted from 100 μ L of blood or tissue culture supernatant using MagMAX Pathogen RNA/DNA isolation kit (Thermo Fisher Scientific, Waltham, MA, USA) and BeadRetriever (Thermo Fisher Scientific). Samples were sequenced by Omega Bioservices, Inc. (Norcross, GA, USA), on an Illumina MiSeq using version 2, 2 \times 150 cycle kits. Consensus sequences from the viral isolate were generated using Geneious 9.1.3 (Biomatters, Auckland, New Zealand) by mapping reads to KU961971 and KU961972 with 5-fold iterative remapping. Low-quality reads/bases were filtered to Pred scores of 15, and duplicate reads were removed. We observed 100% genome coverage using a 3-fold cutoff for the L and S segments. Consensus sequences from the clinical specimen were generated using Geneious 9.1.3 by mapping reads to KU961971 and KU961972 with 5-fold iterative remapping. Consensus genomes were generated with no read trimming; duplicate removal and cutoffs were set at 3-fold coverage. We observed 90% coverage for the L segment and 96% coverage for the S segment. Using Sanger sequencing, we increased coverage to 100% for the S segment and 95% for the L segment. The genome sequences were deposited at GenBank under accession nos. MF990886 and MF990888 for the virus in blood; and MF990887 and MF990889 for the viral isolate.

Lassa Virus sequencing in Marburg/Giessen, Germany

Vero E6 cells were inoculated with serum of secondary case-patient 2. Genome sequences were determined from viruses after the first passage. Cell culture supernatant was collected, cellular debris were removed by 2 \times centrifugation at 2,500 rpm for 10 min at 4°C and subjected to ultracentrifugation through a 20%-sucrose cushion. Pelleted virions were resuspended in RLT buffer (Qiagen, Hilden, Germany) and RNA was extracted using the RNeasy mini kit (Qiagen) according to the manufacturer's manual. rRNA was depleted using the Ribo-Zero Gold Kit (Epicentre, Madison, WI, USA). A cDNA Library was constructed

according to Illumina's TruSeq total RNA protocol as described earlier (1). Library quality control was performed with DNA HS Assay on a 2100 Bioanalyzer (Agilent, Santa Clara, CA, USA). Normalized and pooled libraries were then sequenced on an Illumina MiSeq using v3 chemistry and paired-end sequencing (2 × 301 cycles). After adaptor clipping and quality filtering, reads were mapped against Lassa virus L and S segment references (GenBank accession nos. KU961971.1 and KU961972.1, respectively). The median coverage rates for L and S segments were 145 and 185, respectively. Genome regions with poor coverage, as well as variations from the sequences of the index case-patient and secondary case-patient 1, were verified by Sanger sequencing of clinical samples. The genome sequences were deposited at the European Nucleotide Archive under accession nos. LT601601 and LT601602 (2).

Phylogenetic Analysis

Glycoprotein precursor (GPC), nucleoprotein (NP), polymerase (L), and Z nucleotide sequences of Lassa virus strain Togo (GenBank accession nos. KU961971.1, KU961972.1) were aligned with sequences of Lassa virus strains Josiah (GenBank accession nos. J04324, U63094, NC_004297), NL (AY179173, AY179172), Z148 (AY628205, AY628204), Z158 (AY628201, AY628200), Ba366 (GU830839, GU979513), Komina-R16 (KF478767, KF478760), Soromba (KF478765, KF478762), Bamba-R114 (KF478766, KF478761), Ouoma-R123 (KF478768, KF478764), AV (AF246121, AY179171), NIG08-A18 (GU481070, GU481071), NIG08-A19 (GU481072, GU481073), Weller (AY628206), GA391 (X52400), ONM-314 (KT992423, KT992433), CSF (AF333969, AY179174), NIG08-A37 (GU481074, GU481075), NIG08-A41 (GU481076, GU481077), NIG08-A47 (GU481078, GU481079), ISTH2271-NIG-2012 (KM821986, KM821985), ISTH2065-NIG-2012 (KM821974, KM821973), 803213 (AF181854), NIG08-04 (GU481068, GU481069), LP/Pinneo (AF181853, KM822127), Kako 428 (KT992425, KT992435); and the Old World arenaviruses Gbagroube CIV608 (GU830848), Mopeia AN21366 (M33879), Mopeia AN20410 (AY772170, AY772169), Mopeia Mozambique (DQ328874, DQ328875), Morogoro 3017 (EU914103, EU914104), Mobala Acar3080 (AY342390, DQ328876), Gairo 27421 (KJ855308, KJ855307), Menekre CIV1227 (GU830862), Ippy DakAnB188d (DQ328877, DQ328878), Merino-Walk (GU078660, NC023763), Lujo (FJ952384, FJ952385); and lymphocytic choriomeningitis virus strains WE (M22138, AF004519), Armstrong (M20869, J04331, AY847351), CH-5692 (AF325214, DQ868484), CH-5871 (AF325215), Marseille 12 (DQ286931, DQ286932), MX (EU195888, EU195889,

AJ131281), M1 (AB261991), UBC aggressive (EU480450), Traub (DQ868487, DQ868488), Pasteur (DQ868485, DQ868486), Dandenong (EU136038, EU136039), SN05 (FJ895884), GR01 (FJ895883), CABN (FJ895882), and Y (DQ118959).

Phylogenies were inferred with BEAST2 program (3) using the general time-reversible (for GPC, NP, and L) or the Hasegawa–Kishino–Yano (for Z) model of sequence evolution with gamma distribution of among-site nucleotide substitution rate, and 10^7 steps with sampling every 10^3 rd step. An initial evaluation revealed minimal rate variation among branches in NP and L gene (median ucl.d.stdev 0.13–0.15); therefore, these phylogenies were inferred with the strict clock model. We used the relaxed log-normal clock model for GPC and Z, as the rate variation was higher (median ucl.d.stdev 0.24 and 0.28, respectively). Uncorrected pairwise amino acid distances were calculated in MacVector (MacVector, Apex, NC, USA). Analysis of potential recombination or reassortment was conducted with RDP4 (<http://web.cbio.uct.ac.za/~darren/rdp.html>) (4). The aligned GPC, NP, and L nucleotide sequences were concatemerized in the order “L–GPC–reverse complement of NP” according to the gene arrangement in L and S segments. A full exploratory recombination scan (RDP, GENECONV, Bootscan, MaxChi, Chimaera, SiScan, 3Seq) revealed a potential recombination event in GPC of Lassa strain Togo involving NIG08-A41 as major and LP/Pinneo as minor parent (RDP uncorrected $p = 2.3 \times 10^{-5}$, corrected for multiple tests $p = 0.21$). A manual Bootscan analysis was performed for Lassa strain Togo as potential recombinant and NIG08-A41, Kako 428, and LP/Pinneo as parents using a window size of 500 nt with a step size of 10, UPGMA (unweighted pair group method with arithmetic mean) tree, 100 bootstrap replicates, and F84 model.

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Technical Appendix Table. Uncorrected pairwise amino acid distances among Old World arenaviruses*

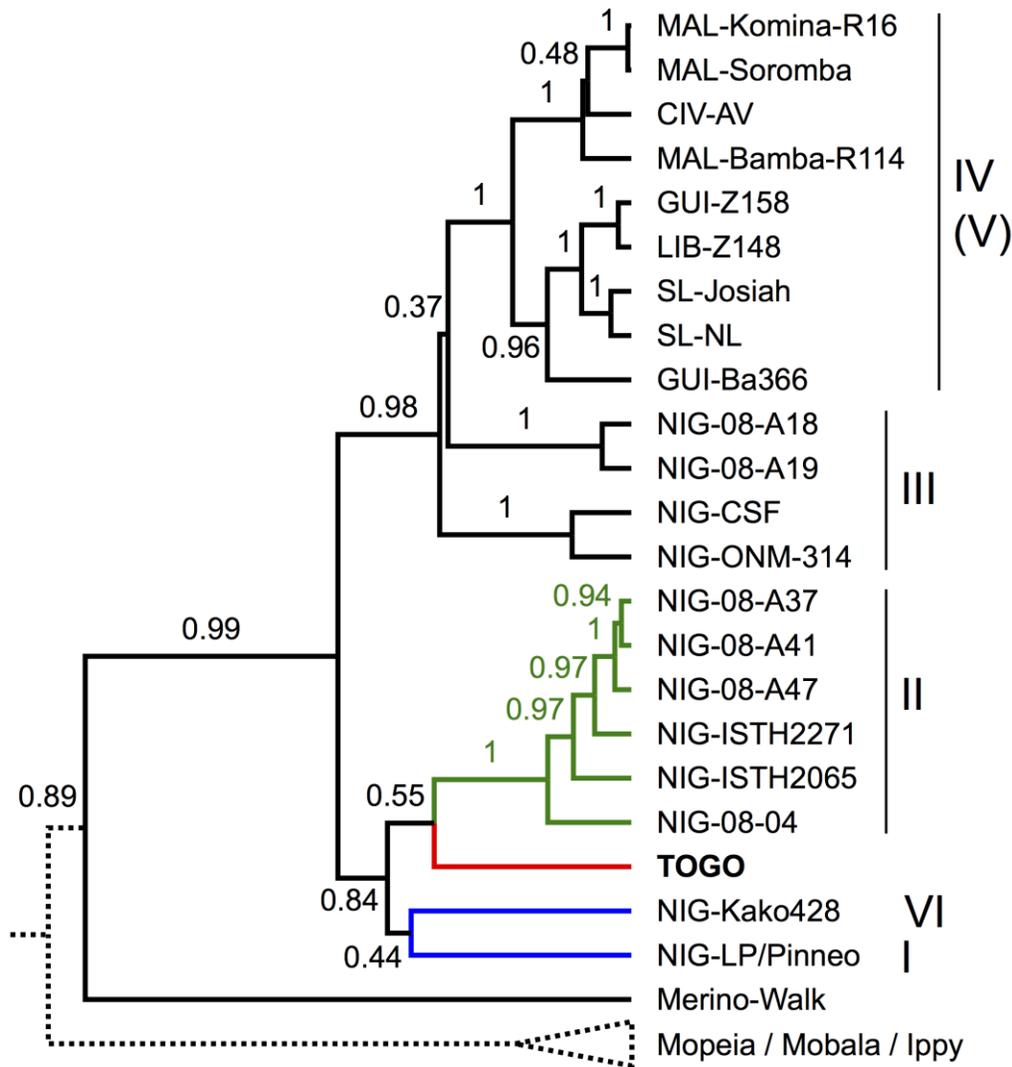
Gene/strains and species compared	Percentiles of the frequency distribution of pairwise amino acid distances, %†				
	0	25	50	75	100
GPC					
Lassa virus intralineage‡	0.6	3.1	4.3	5.5	8.2
Lassa virus interlineage§	5.1	6.5	7.1	8.1	11.4
Togo vs. other Lassa virus strains	4.9	6.1	6.5	7.3	10.6
Lassa strains vs. other Old World arenavirus species	9.8	21.8	24.2	29.6	43.5
NP					
Lassa virus intralineage‡	0.5	3.3	5.3	6.3	9.6
Lassa virus interlineage§	7.0	8.8	9.7	10.4	14.6
Togo vs. other Lassa virus strains	7.7	8.9	9.2	9.6	11.6
Lassa strains vs. other Old World arenavirus species	14.1	25.5	27.0	32.0	38.6
L					
Lassa virus intralineage‡	0.9	8.4	13.1	15.8	18.8
Lassa virus interlineage§	19.8	21.3	24.6	25.2	26.0
Togo vs. other Lassa virus strains	22.4	24.8	25.2	25.7	26.9
Lassa strains vs. other Old World arenavirus species	41.2	42.0	43.7	49.0	54.7

*GPC, glycoprotein precursor; L, polymerase; NP, nucleoprotein.

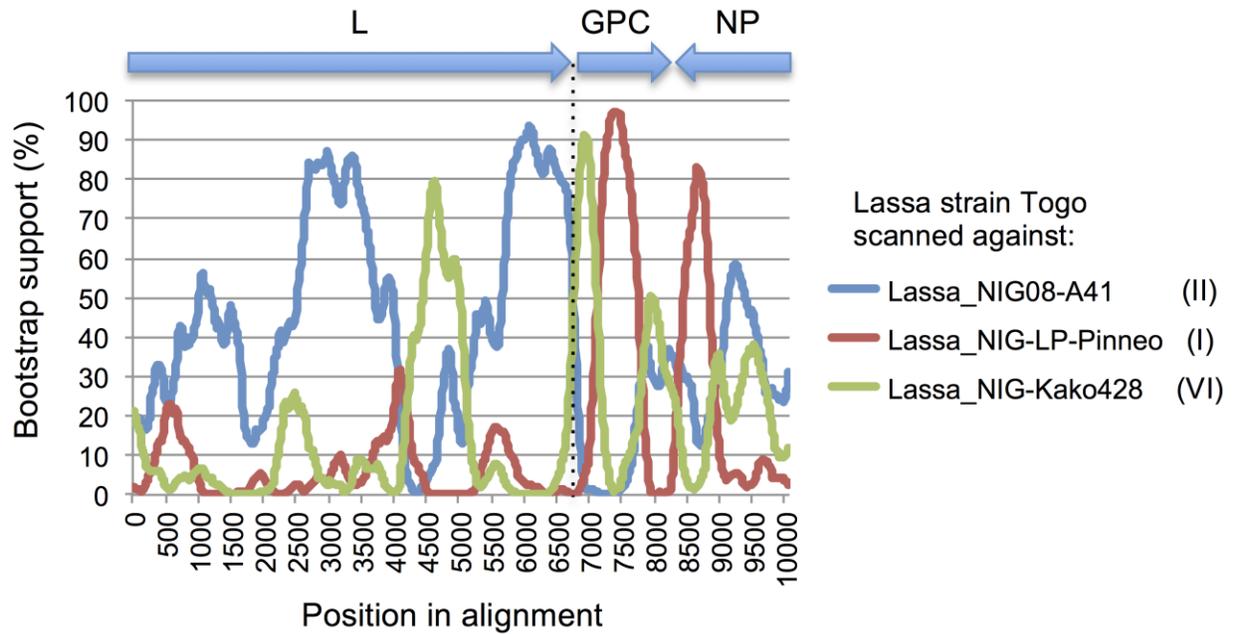
†Lassa virus lineages were previously established on the basis of uncorrected amino acid distances. Interlineage distances ranged from 5.7% to 7.4% in GPC and 9.5% to 12% in NP (5).

‡The uncorrected pairwise amino acid distances between the previously proposed lineage V (6) (strains AV, Komina-R16, Soromba, Bamba-R114, and Ouoma-R123) and lineage IV (strains Josiah, NL, Z148, Z158, and Ba366) ranged from 4.1% to 7.1% in GPC, 6.0% to 7.4% in NP, and 15.1% to 16.5% in L. Because these ranges fall within the distribution of intralineage distances, the proposed lineage V was considered here a subclade of lineage IV rather than a separate lineage. The corresponding distances were included in the intralineage analysis.

§The uncorrected pairwise amino acid distances between Kako virus (7) and other Lassa virus strains ranged from 8.6% to 11.4% in GPC, 11.8% to 14.6% in NP, and 23.8% to 25.7% in L. Because these ranges fall within the distribution of interlineage distances, Kako virus was considered here a separate lineage (tentatively designated VI) of Lassa virus and included in the interlineage analysis.



Technical Appendix Figure 1. Phylogeny of the Lassa virus strain from Togo using Z gene sequences. The phylogenetic tree was inferred using BEAST2 program (6). Posterior support values are shown at the branches. Lassa virus lineages are indicated by roman numbers on the right. The branch for Mopeia, Mobala, and Ippy viruses is shown schematically and the branches for the remaining Old World arenaviruses have been removed for clarity of presentation. The origin of the Lassa virus strains is abbreviated as follows: CIV, Côte d'Ivoire; GUI, Guinea; LIB, Liberia; MAL, Mali; NIG, Nigeria; SL, Sierra Leone.



Technical Appendix Figure 2. Analysis of potential recombination and/or reassortment in evolution of Lassa virus strain Togo. A manual Bootscan analysis was performed in RDP4 for Lassa virus strain Togo as potential recombinant and NIG08-A41 (lineage II), Kako 428 (lineage VI), and LP/Pinneo (lineage I) as potential parents. The border between L and S segment is shown by a vertical dotted line. GPC, glycoprotein precursor; L, polymerase; NP, nucleoprotein.