New Lineage of Lassa Virus, Togo, 2016

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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.

### About the Author

Dr. Basu is a Research Fellow at Brigham and Women’s Hospital, Boston, Massachusetts. His primary research interests include the development and application of novel mass spectrometry platforms in the clinical and research space.

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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 ≈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 intralineage distances, and therefore, we considered lineage...
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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Figure. 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-Ttechapp1.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).
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 and 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.