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Lassa Fever, Nigeria, 2005–2008

To the Editor: Lassa fever affects ≈100,000 persons per year in West Africa (1). The disease is caused by Lassa virus, an arenavirus, and is associated with bleeding and organ failure. The case-fatality rate in hospitalized patients is 10%–20%. The reservoir of the virus is multimammate mice (*Mastomys natalensis*). Investigations in the 1970s and 1980s pointed to the existence of 3 disease-endemic zones within Nigeria: the northeastern region around Lassa, the central region around Jos, and the southern region around Onitsha (2,3). The current epidemiologic situation is less clear because no surveillance system is in place.

In 2003 and 2004, we conducted a hospital-based survey in Irrua, which demonstrated ongoing transmission of the virus in Edo State, Nigeria (4). Since then, laboratory capacity at the University of Lagos for diagnosing Lassa fever has been improved and used for small-scale passive surveillance in other parts of the country. Public health officials or hospital staff reported suspected cases. Blood

samples were sent to Lagos, or staff from Lagos collected samples on site. Confirmatory testing, sequencing, and virus isolation were performed at the Bernhard Nocht Institute for Tropical Medicine in Hamburg, Germany. Primary testing was done by reverse transcription–PCR (RT-PCR) that targeted the glycoprotein (GP) gene (5,6). An RT-PCR that targeted the large (L) gene was used as a secondary test (7), and PCR products were sequenced. Serologic testing for Lassa virus-specific immunoglobulin (Ig) G and IgM was performed by immunofluorescent antibody test using Vero cells infected with Lassa virus. Virus isolation with Vero cells was conducted in the BioSafety Level 4 laboratory in Hamburg.

From 2005 through 2008, 10 cases of Lassa fever were confirmed by virus detection (cases 3–10) or implicated by epidemiologic investigation and serologic testing (cases 1 and 2) (online Appendix Table, www.cdc.gov/EID/content/16/6/1040-appT.htm). Case-patients 1–4 were involved in a nosocomial outbreak that occurred in February 2005 at the Ebonyi State University Teaching Hospital (EBSUTH) in Abakaliki. Retrospective investigation suggests the following transmission chain. The presumed index case-patient was a male nurse living in Onitsha, who became ill on January 21, 2005, and traveled ≈200 km to EBSUTH for better medical treatment. The detection of Lassa virus-specific IgM during his convalescent phase indicates that he had Lassa fever. The second case-patient was a female nurse who had contact with the index case-patient on February 4. She was admitted on February 7 and died 6 days later. Her clinical features were compatible with Lassa fever, but laboratory confirmation is lacking because specimens were not collected. Two additional case-patients among hospital staff (case-patients 3 and 4) were seen on February 21; each had had contact with case-patient 2. Case-

patient 3 took care of case-patient 2 and slept in the same room with her for 4 days. Lassa fever was confirmed in case-patients 3 and 4 by RT-PCR as well as by IgM and IgG seroconversion in the surviving patient (case-patient 3). Case-patient 4, a pregnant nurse, had a spontaneous abortion and died on day 9 of hospitalization. Sequencing the GP and L gene PCR fragments showed that case-patients 3 and 4 were infected with the same virus strain (100% identity). In March and April 2005, blood was collected from 50 hospital staff members (including those who had had contact with the case-patients) and screened for Lassa virus-specific IgM and IgG. No positive blood samples were found, which indicated that no additional staff members were involved in the outbreak.

Case-patients 5 and 6 were admitted to EBSUTH in 2008 on January 17 and March 5, respectively. Both were medical doctors, one at a local hospital and the other at EBSUTH, and both died. Encephalopathy with generalized seizures and loss of consciousness preceded death in both cases. The source of infection is unknown, although it is likely that they became infected while they treated patients without knowing they had Lassa fever. In agreement with the epidemiology, the viruses from the 2 patients were similar, though not identical (89% and 87% identity in the GP and L genes, respectively).

Cases 7 to 10 occurred in Abuja and Jos from December 2007 through March 2008. Healthcare workers appeared not to be involved, and no molecular epidemiologic evidence indicated that transmission occurred among the 3 case-patients from Jos (94–97% and 90–94% identity in the GP and L genes, respectively).

In conjunction with our previous report (4), the cases presented here demonstrate current Lassa fever activity in the states of Edo, Ebonyi, Federal Capital Territory, and Plateau. These findings correspond to early re-

ports on Lassa fever in southern and central parts of Nigeria. That health-care workers are still at as high a risk of contracting and dying from the disease as they were 20 years ago (8) is alarming.

A key to solving this problem would be the establishment of diagnostic facilities that can provide rapid molecular testing at referral centers in the disease-endemic zones. This testing would facilitate appropriate case and contact management, including early treatment and postexposure prophylaxis with ribavirin, and eventually raise awareness that Lassa fever should be considered in every severe febrile illness in these regions.

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Laboratory Diagnosis of Lassa Fever, Liberia

To the Editor: Lassa fever is endemic in West Africa, with $\leq 300,000$ Lassa virus (LASV) infections occurring annually (1). Persons on humanitarian missions and peacekeeping forces in regions comprising Sierra Leone and Liberia are at risk for Lassa fever (2–4). Reliable laboratory diagnosis, particularly in acute cases, is crucial for triage, implementation of barrier nursing, and contact tracing, as well as for initiation of treatment with ribavirin. Reverse transcription–PCR (RT-PCR) is routinely used for confirmation of cases, but few proven assay formulations are available, and these have not been evaluated on larger cohorts of patients (5).

We summarize our experiences from testing 184 patients from Liberia with suspected cases of Lassa fever with the most widely used LASV-specific RT-PCR assay (6). Patients were suspected of having Lassa fever on clinical grounds by physicians of the United Nations peacekeeping troops and other international relief organizations. Patients included local citizens as well as members of the mentioned organizations. EDTA-plasma samples or serum specimens packed on ice were sent to our laboratory in Hamburg, Germany, by international airfreight, taking 4–7 days for shipment. Information on clinical signs and symptoms or outcome was generally not available.

Conventional RT-PCR specific for the glycoprotein precursor gene was conducted as described (7). RT-PCR results positive for LASV was seen in 35 (19%) of 184 patients. Median time between onset of symptoms and sampling was 7 days. Median time from reception of samples to final RT-PCR or culture results was 1 day and 4 days, respectively.

Appendix Table. Characteristics of Lassa fever cases, Nigeria, 2005– 2008*

Locality (hospital)	Case no.	Date of admission	Age, y/sex	Symptoms	Treatment	Outcome	HCW	GP and L gene RT-PCR	IgM IFAT†	IgG IFAT†	Virus isolation	Lassa virus strain (GenBank accession nos.)‡
Abakaliki, Ebonyi State (EBSUTH)	1	2005 Feb 4	40/M	Fever	Antimicrobial drugs, antimalaria prophylaxis	Survived	Nurse	Neg§	1:160§	>1:80§	ND	–
	2	2005 Feb 7	54/F	Fever, vomiting, diarrhea, respiratory distress, oliguria	Antimicrobial drugs, corticoid	Died Feb 13	Nurse/contact to case 1	ND	ND	ND	ND	–
	3	2005 Feb 21	35/F	Fever, severe weakness	NA	Survived	Nurse/contact to case 2	Pos Neg§	Neg 1:640§	Neg >1:5120§	Neg	Nig05-SE40 (GU481058, GU481059)
	4	2005 Feb 21	36/F	Fever, vomiting, nausea, spontaneous abortion, shock	Antimicrobial drugs, tracheotomy	Died Mar 1	Nurse/contact to case 2	Pos	Neg	Neg	Neg	Nig05-043 (GU481056, GU481057)
	5	2008 Jan 17	38/M	Fever, vomiting, diarrhea, abdominal tenderness, anuria, generalized seizure, unconsciousness	Antimicrobial drugs, antimalaria prophylaxis, dobutamine, dopamine, furosemide	Died Jan 23	Doctor	Pos	Neg	Neg	Neg	Nig08-03 (GU481066, GU481067)
	6	2008 Mar 5	38/M	Fever, vomiting, hiccups, bloody diarrhea, abdominal tenderness, generalized seizure, unconsciousness	Antimicrobial drugs, antimalaria prophylaxis, assisted ventilation	Died Mar 11	Doctor	Pos	1:20	1:20	Pos	Nig08-04 (GU481068, GU481069)
Abuja, FCT State (NHA)	7	2008 Jan 2	37/M	Fever, vomiting, diarrhea, abdominal tenderness, confusion, unconsciousness	Antimicrobial drugs, ribavirin on day of death	Died Jan 7	No	Pos	1:20	1:20	Neg	Nig08-02 (GU481063 to GU481065)
Jos, Plateau State	8	2007 Dec	19/F	NA	NA	Died	NA	Pos	Neg	Neg	Neg	Nig07-05 (GU481060 to GU481062)
	9	2008 Feb	30/M	NA	NA	Died	No	Pos	1:1250	1:80	Pos	Nig08-A18 (GU481070, GU481071)
	10	2008 Feb	28/F	NA	NA	Survived	No	Pos	1:80	1:80	Pos	Nig08-A19 (GU481072, GU481073)

*HCW, health care worker; GP, glycoprotein; L, large; Ig, immunoglobulin; RT-PCR, reverse transcription PCR; IFAT immunofluorescent antibody test; EBSUTH, Ebonyi State University Teaching Hospital; pos, positive; neg, negative; ND, not done; NA, data not available; FCT, Federal Capital Territory; NHA, National Hospital Abuja.

†Titer of IFAT (cut-off 1:20).

‡Partial GP and L gene sequences were obtained by sequencing the fragments amplified by the diagnostic RT-PCRs. Additional nucleoprotein gene sequences were generated for Nig07-05 and Nig08-02, and strains isolated in cell culture were completely sequenced (D. Ehichioya, unpub. data).

§Convalescent-phase serum sample.