

vaccine effectiveness is 92% for persons who received 1 dose and 95% for those who received 2 doses (9). Assuming that vaccine effectiveness is lower shifts the curve (Figure) to the left and would result in a lower estimate of vaccination coverage. Second, different numbers of persons who received 1 and 2 doses complicate the identification of overall vaccine effectiveness. Third, vaccination status is unknown for some measles case-patients. The proportion of nonvaccinated persons among those case-patients might be higher than that among those known to be vaccinated, also leading to a lower estimate of vaccination coverage. Finally, nonvaccinated persons might be clustered together, and their risk for infection could be higher than that for the general population (10). This scenario would imply that the estimated vaccination coverage does not reflect the general population but instead corresponds to a clustered subpopulation among whom vaccination rates are lower. The effects of these complexities warrant further investigation. However, as the examples demonstrate, a model ignoring those effects is in good agreement with empirical data.

Our analysis suggests that the number of vaccinated measles case-patients should be closely followed through surveillance programs. A continuous decrease in the proportion of measles case-patients who had been vaccinated over the years could indicate a decrease in vaccination rates. Conversely, an increase in the proportion of measles case-patients who had been vaccinated would demonstrate the effectiveness of ongoing efforts to increase vaccination rates and could serve as a benchmark toward measles elimination.

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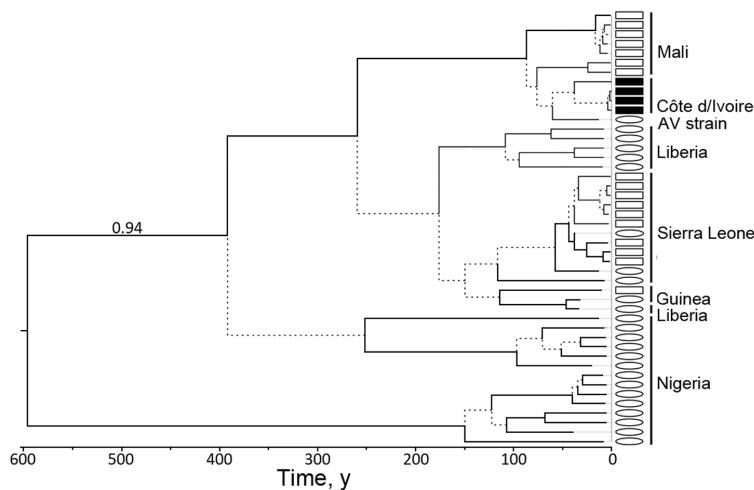
## Lassa Virus in Multimammate Rats, Côte d'Ivoire, 2013

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**To the Editor:** Lassa fever is a zoonosis caused by Lassa virus (LASV; family Arenaviridae, genus Lassavirus). The primary reservoir of LASV is the multimammate rat (*Mastomys natalensis*), which is found throughout sub-Saharan Africa. LASV outbreaks among humans occur only in West Africa in 2 noncontiguous areas: 1 in Guinea, Liberia, and Sierra Leone; and 1 in Nigeria. Rare cases and evidence of exposure of humans have been documented in neighboring countries (i.e., Benin, Burkina Faso, Côte d'Ivoire, Ghana, Mali, and Togo) (1). LASV RNA has been detected in only 4 patients: 1 in Germany who had traveled in Burkina Faso, Côte d'Ivoire, and Ghana (2); 1 in the United Kingdom who had returned from Mali (3); and 2 in



**Figure.** Bayesian chronogram of Lassa virus (LASV) sequences determined on the basis of a fragment of the large genomic segment. Branches receiving posterior probability values  $<0.95$  and bootstrap values  $<50$  (poorly supported) are dashed. LASV sequences of human origin are indicated by ovals, and those of multimammate rats are indicated by squares. Sequences reported in this study are indicated by black squares. This tree was built under the assumption of a molecular clock and is therefore rooted. The numerical value on the tree's most basal branch is the root posterior probability of this branch; it supports the notion that LASV sequences from Nigeria and other countries are not reciprocally monophyletic. GenBank accession nos. of sequences used for phylogenetic analyses are shown in online Technical Appendix Table 2 (<http://wwwnc.cdc.gov/EID/article/21/8/15-0312-Techapp.pdf>). AV strain indicates the strain from a German patient.

Ghana, for whom no viral sequence was available because detection was performed by reverse transcription PCR only (4). In the region in Mali where the patient from the United Kingdom was infected, identical LASV sequences were found in multimammate rats (5). The sequence of the strain identified from the patient in Germany, who was designated AV, is the closest known relative of the clade formed by sequences from Mali (5). However, LASV was not found in its natural host in any of the countries visited by patient AV (6,7).

For a study investigating zoonotic pathogens in rural habitats, we caught small mammals in 3 ecologic zones of Côte d'Ivoire: 1) dry bushland in northern Côte d'Ivoire, around Korhogo (2); semiarid bushland in central Côte d'Ivoire, around Bouake; and rainforest in southwestern Côte d'Ivoire, near the Taï National Park (3) (online Technical Appendix Figure, <http://wwwnc.cdc.gov/EID/article/21/8/15-0312-Techapp.pdf>). Traps were installed within and around 15 villages and enabled the capture of 27 eulipotyphlans and 254 rodents during August–October 2013. Animals were assigned at the genus level in the field on the basis of morphology. For 88% of them, assignment could later be refined to the species level by sequencing a fragment of the mitochondrial cytochrome *b* gene. A total of 14 animal species representing 8 genera were detected. All host sequences were deposited in Dryad (<http://www.datadryad.org/>; online Technical Appendix Table 1). Multimammate rats were the dominant commensals at all sampling locations, comprising 64.5% of the overall sample (online Technical Appendix Figure).

Tissue samples were collected from all animals according to standard protocols. Total nucleic acids were extracted from lung samples and tested for the presence of LASV RNA by using a real-time PCR system amplifying a 400-bp fragment of the large genomic segment (8) (online Technical

Appendix). LASV RNA was detected in 4 of 18 specimens of *M. natalensis* captured in Gbalôhò, near Korhogo (online Technical Appendix Figure). This site is much farther north in Côte d'Ivoire than previously examined sites (6). The 4 PCR-positive animals were 3 males and 1 female that were all captured indoors, 3 in the same house. PCR products were sequenced according to the Sanger method (GenBank accession nos. LN823982–LN823985). According to phylogenetic analyses performed in maximum likelihood and Bayesian frameworks (online Technical Appendix), LASV sequences identified in multimammate rats from Côte d'Ivoire formed a robust clade with sequences from the human AV strain and the LASV infecting multimammate rats in southern Mali (bootstrap 97, posterior probability 1.00; Figure). This phylogenetic placement opens up the possibility that patient AV was infected during her travel through Côte d'Ivoire, possibly in or near the city of Korhogo. Tip date calibration of Bayesian analyses showed that the most recent common ancestor of all LASV sequences from Côte d'Ivoire and Mali circulated  $\approx 90$  years ago (Figure; online Technical Appendix Table 2).

Further studies will be needed to investigate the geographic distribution of LASV in Côte d'Ivoire and the frequency of human infections. The current lack of diagnosed cases in the area may be caused by underreporting. Sensitization campaigns are needed to increase awareness of the risk for LASV infection among the local population and to improve detection of cases by health workers.

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## ***Rickettsia felis* Infection among Humans, Bangladesh, 2012–2013**

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**To the Editor:** *Rickettsia felis*, which belongs to the spotted fever group of rickettsiae, causes febrile illness in humans. The main vector of this bacterium is the cat flea (*Ctenocephalides felis*). Since publication of reports of *R. felis* as a putative pathogen of humans in the United States in 1994, *R. felis* infection in humans worldwide has been increasingly described, especially in the Americas, Europe, Africa, and eastern Asia (1,2). *R. felis* infection is common among febrile patients (≈15%) in tropical Africa (3) and among apparently healthy persons in eastern coastal provinces of China (4). However, little is known about prevalence of *R. felis* infection of humans in southern Asia, although 3 serologically diagnosed cases in Sri Lanka have been described (5) and *R. felis* has been detected in rodent fleas in Afghanistan (6). Hence, we conducted a cross-sectional study in Bangladesh to explore the presence of rickettsial pathogens among patients with fever of unknown origin.

Study participants were 150 patients at Mymensingh Medical College (MMC) hospital in Mymensingh, north-central Bangladesh, from July 2012 through January 2014, and 30 healthy control participants from the staff at the same college. Selected patients met the following criteria: 1) fever (axillary temperature >37.5°C) for >15 days that did not respond to common antimicrobial drug therapy; 2) any additional clinical features including headache, rash, lymphadenopathy, myalgia, and eschars on skin; and 3) titer according to the Weil-Felix test (antibodies against any of 3 *Proteus* antigens) of >1:80. Patients with evident cause of fever (e.g., malaria diagnosed by blood smear or immunochromatography) were excluded from the study. This research was approved by the college institutional review board, and informed consent was obtained from patients (or guardians) and healthy controls before their entry into the study.

Venous blood samples were aseptically collected from the patients, and DNA was extracted by conventional method by using proteinase K and sodium dodecyl sulfate. Nested PCR selective for the 17-kDa antigen gene was used to screen for rickettsiae according to the method described previously (7); ≈100 ng of DNA in a 50-mL reaction mixture was used. For each PCR, a negative control (water) was included and utmost care was taken to avoid contamination. Among the 150 samples tested, results were positive with a 232-bp amplified product for 69 (46%) and negative for all controls.

PCR products from 20 samples were randomly selected for sequence analysis. All nucleotide sequences from

# Lassa Virus in Multimammate Rats, Côte d'Ivoire, 2013

## Technical Appendix

### Experimental Procedures and Results

#### Sampling

Small mammals were captured during August–October 2013 in 15 villages in northern, central, and southwestern Côte d'Ivoire (Technical Appendix Figure). Animals were trapped with Sherman traps (Pet Factory, Hülse, Germany). Following methods of Fichet-Calvet et al. (1), we placed 50 traps inside houses (2 trap set in each room) and 40 traps in the cultivations and forests surrounding every village for 3 consecutive nights (1). Traps were baited daily with a mixture of peanuts, dry fish, and wheat flour.

All animals were anesthetized and euthanized with ether, according to animal welfare guidelines. A predefined set of morphologic features was recorded for each animal (sex, age category, weight, length of head and body, length of tail, and length of hind foot). At this stage, a preliminary assignment to the genus level was performed. Whole blood and tissue samples were taken in the following order: liver, spleen, lung, kidney, and intestine; intestine was sampled last to minimize risk of fecal contamination of the other organs. All samples were immediately preserved in liquid nitrogen, transported on dry ice, and stored at  $-80^{\circ}\text{C}$  upon arrival at the Robert Koch Institute (Berlin, Germany). Handling and necropsies of the animals were performed only by trained workers who applied appropriate safety measures.

#### Molecular Biology

Total nucleic acids were extracted from lung samples by using a NucleoSpin kit (Macherey-Nagel, Düren, Germany). Reverse-transcription PCR was performed by using the SuperScript II Reverse transcription kit (Invitrogen, Carlsbad, CA, USA) and random hexamer primers (Bioline, London, UK).

Five microliter complementary DNA (cDNA) samples were tested for the presence of Lassa virus genetic material by using a PCR assay targeting a ca. 400 bp fragment of the RNA polymerase (L) gene (2). PCR reactions (total volume 25  $\mu$ L) were prepared to contain 4 U Platinum *Taq* (Invitrogen), 1X PCR buffer, 1.5 mM MgCl<sub>2</sub>, 200  $\mu$ M dNTPs, 0.3  $\mu$ M forward primers (LVL3359A, LVL3359D and LVL3359G), and 0.6  $\mu$ M reverse primers (LVL3754A and LVL3754D). Cycling conditions were as follows: 95°C for 2 minutes; 45 cycles at 95°C for 20 seconds, 55°C for 45 seconds, and 72°C for 60 seconds; and final extension at 72°C for 10 minutes.

One microliter nucleic acid extract was also used to amplify a ca. 800 bp fragment of the mitochondrial cytochrome *b* gene of the host (3). PCR reactions (total volume 25 $\mu$ L) were prepared to contain 2 U Platinum *Taq* (Invitrogen), 1X PCR buffer, 4 mM MgCl<sub>2</sub>, 200  $\mu$ M dNTPs, and 0.2  $\mu$ M of each primer (L14724 and H15506). Cycling conditions were as follows: 95°C for 5 minutes; 40 cycles at 95°C for 30 seconds, 52°C for 30 seconds, and 72°C for 45 seconds; and final extension at 72°C for 10 minutes.

All PCR products were sequenced on both strands according to Sanger's method. Sequence identity was confirmed with BLAST (National Center for Biotechnology Information, Bethesda, MD, USA) (4). BLAST results were further used to assign host sequences to species in combination with genus-level morphologic identification and occurrence data derived from the International Union for Conservation of Nature Red List database (<http://www.iucnredlist.org/>; Technical Appendix Table 1).

## **Isolation of Virus from Positive Samples Not Attempted Thus Far**

### **Lassa Virus Phylogenetic Analyses**

A dataset of the sequences generated in this study, selected reference sequences derived from infected humans, and all sequences derived from multimammate rats were reduced to unique haplotypes by using ALTER (5). The haplotyped dataset comprised 53 sequences, including 46 with known dates of collection. Further analyses were performed on the full haplotyped dataset (D<sub>53</sub>), and the same dataset reduced to sequences with known dates of collection (D<sub>46</sub>). Technical Appendix Table 2 describes the sequences comprised in D<sub>53</sub> (GenBank accession numbers, host, and place and date of sample collection). We used

jModelTest v2.1.4 (6) and the Bayesian information criterion to select the model of nucleotide substitution that best fit the datasets (HKY+G). Maximum likelihood trees were inferred by using PhyML v3.0 (7), as implemented on the PhyML webserver (8). Tree search started from a BioNJ tree and used the BEST algorithm; branch robustness was assessed by nonparametric bootstrapping (250 pseudo-replicates). Bayesian analyses were performed by using BEAST v1.8.0 (9). For both datasets, analyses were performed under an uncorrelated relaxed molecular clock (lognormal) and 2 different demographic models (constant population size and SkyGrid). For any analysis, 2 Bayesian Markov chain Monte Carlo analyses were run; convergence was checked visually within and between individual runs by using Tracer v1.6 (<http://tree.bio.ed.ac.uk/software/tracer/>), and appropriate sampling of the posterior was documented (effective sample sizes >200 for all model parameters).

The relaxed clocks were calibrated as follows: for  $D_{53}$ , a strong prior was placed on the root age (normal distribution of real mean 750 years, standard deviation 130 years; 95% highest-posterior density: 500–100 years) on the basis of a published estimate of Lassa virus ancestry (10); for  $D_{46}$ , tip dates were used to inform the evolutionary rate. Both calibrations ended with very similar substitution rate estimates (Table 3). Control experiments sampling only from the prior showed that divergence dates were informed by sequences; however, these results should be considered with care as no real clock-likeness signal could be detected from the maximum likelihood tree when it was analyzed with Path-O-Gen v1.4 (<http://tree.bio.ed.ac.uk/software/pathogen/>).

The output of the Bayesian Markov chain Monte Carlo runs was combined by using LogCombiner v1.8.0 (distributed with BEAST). The resulting tree file was analyzed with RootAnnotator (11), which itself used TreeAnnotator v1.8.0 (distributed with BEAST); this analysis enabled plotting of root posterior probabilities along with other values of interest. The final tree was plotted by using FigTree v1.4 (<http://tree.bio.ed.ac.uk/software/figtree/>) and further edited with Inkscape (<https://inkscape.org/en/>).

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**Technical Appendix Table 1.** Small mammal species determination of the 248 animals that could be assigned to the species level (i.e., 88% of the sampling)

Order	Species	Bouaké	Korhogo	Täi	Total (%)
Eulipotyphla	<i>Crociodura buettikoferi</i>			2	2 (0.8)
	<i>Crociodura juvenetae</i>	1			1 (0.4)
	<i>Crociodura olivieri</i>	9	4	1	14 (5.6)
	<i>Crociodura theresae</i>	1			1 (0.4)
Rodentia	<i>Lophuromys sikapusi</i>		2		2 (0.8)
	<i>Mastomys natalensis</i>	54	65	41	160 (64.5)
	<i>Mus mattheyi</i>	1			1 (0.4)
	<i>Mus minutoides</i>	9	1	16	26 (10.5)
	<i>Mus setulosus</i>	9		1	10 (4.0)
	<i>Praomys daltoni</i>	1			1 (0.4)
	<i>Praomys rostratus</i>		4	6	10 (4.0)
	<i>Rattus rattus</i>	1		13	14 (5.6)
	<i>Taterillus gracilis</i>		2		2 (0.8)
	<i>Uranomys ruddi</i>	4			4 (1.6)
Total (%)		90 (36.3)	78 (31.5)	80 (32.3)	

**Technical Appendix Table 2.** Sequences used for phylogenetic analyses of Lassa fever viruses in multimammate rats, Côte d'Ivoire, 2013

GenBank accession no.	Host	Place of collection	Date of collection*
AY179171	<i>Homo sapiens</i>	Germany	2000
AY628200	<i>Homo sapiens</i>	Guinea	1981
AY628204	<i>Homo sapiens</i>	Liberia	?
AY693638	<i>Homo sapiens</i>	Liberia	1980
AY870334	<i>Homo sapiens</i>	Liberia	2004
GU979509	<i>Homo sapiens</i>	Liberia	2005
GU979510	<i>Homo sapiens</i>	Liberia	2005
GU979511	<i>Homo sapiens</i>	Liberia	2005
GU979512	<i>Homo sapiens</i>	Liberia	2005
AY179175S1	<i>Homo sapiens</i>	Nigeria	2000
AY693637	<i>Homo sapiens</i>	Nigeria	2004
AY693639	<i>Homo sapiens</i>	Nigeria	1993
AY693640	<i>Homo sapiens</i>	Nigeria	1974
GU481057	<i>Homo sapiens</i>	Nigeria	2005
GU481062	<i>Homo sapiens</i>	Nigeria	2007
GU481065	<i>Homo sapiens</i>	Nigeria	2008
GU481067	<i>Homo sapiens</i>	Nigeria	2008
GU481069	<i>Homo sapiens</i>	Nigeria	2008
GU481071	<i>Homo sapiens</i>	Nigeria	2008
GU481073	<i>Homo sapiens</i>	Nigeria	2008
GU481075	<i>Homo sapiens</i>	Nigeria	2008
GU481077	<i>Homo sapiens</i>	Nigeria	2008
GU481079	<i>Homo sapiens</i>	Nigeria	2008
AY179172	<i>Homo sapiens</i>	Sierra Leone	2000
AY363905	<i>Homo sapiens</i>	Sierra Leone	?
AY363906	<i>Homo sapiens</i>	Sierra Leone	?
AY363907	<i>Homo sapiens</i>	Sierra Leone	?
AY363908	<i>Homo sapiens</i>	Sierra Leone	?
AY363909	<i>Homo sapiens</i>	Sierra Leone	?
AY363910	<i>Homo sapiens</i>	Sierra Leone	?
GU979507	<i>Homo sapiens</i>	Sierra Leone	2006
U63094	<i>Homo sapiens</i>	Sierra Leone	1976
LN823982	<i>Mastomys natalensis</i>	Cote d'Ivoire	2013
LN823983	<i>Mastomys natalensis</i>	Cote d'Ivoire	2013
LN823984	<i>Mastomys natalensis</i>	Cote d'Ivoire	2013
LN823985	<i>Mastomys natalensis</i>	Cote d'Ivoire	2013
GU979513	<i>Mastomys natalensis</i>	Guinea	2003
GU573541	<i>Mastomys natalensis</i>	Mali	2009
GU573545	<i>Mastomys natalensis</i>	Mali	2009
GU573546	<i>Mastomys natalensis</i>	Mali	2009
KF478760	<i>Mastomys natalensis</i>	Mali	2012
KF478761	<i>Mastomys natalensis</i>	Mali	2012
KF478763	<i>Mastomys natalensis</i>	Mali	2012
KF478764	<i>Mastomys natalensis</i>	Mali	2012
KM822110	<i>Mastomys natalensis</i>	Sierra Leone	2010

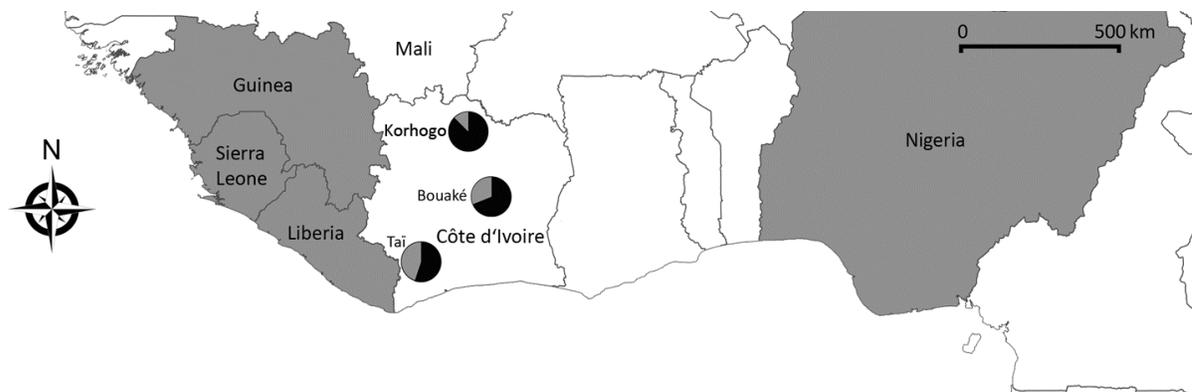
GenBank accession no.	Host	Place of collection	Date of collection*
KM822112	<i>Mastomys natalensis</i>	Sierra Leone	2010
KM822114	<i>Mastomys natalensis</i>	Sierra Leone	2009
KM822116	<i>Mastomys natalensis</i>	Sierra Leone	2012
KM822119	<i>Mastomys natalensis</i>	Sierra Leone	2012
KM822121	<i>Mastomys natalensis</i>	Sierra Leone	2012
KM822123	<i>Mastomys natalensis</i>	Sierra Leone	2012
KM822125	<i>Mastomys natalensis</i>	Sierra Leone	2012
KM822129	<i>Mastomys natalensis</i>	Sierra Leone	2011

\*?, no precise date of collection provided in the original publication.

**Technical Appendix Table 3.** Substitution rate, divergence dates, and root position across models used in a study of Lassa fever viruses in multimammate rats, Côte d'Ivoire, 2013\*

Calibration	Demography	Median rate [95% HPD](subst.site <sup>-1</sup> .year <sup>-1</sup> )	Divergence date [95% HPD]		Root posterior probability (MCC tree root/Nigeria vs. rest)
			Human CI + rat CI	Mali CI	
Root	Constant size	7.6 <sup>-4</sup> [3.9 <sup>-4</sup> -1.3 <sup>-3</sup> ]	56 [18–104]	89 [32–162]	0.887/0.074
	Skygrid	6.7 <sup>-4</sup> [3.3 <sup>-4</sup> -1.2 <sup>-3</sup> ]	64 [25–122]	102 [38–185]	0.890/0.074
Tips	Constant size	8.1 <sup>-4</sup> [5.1 <sup>-4</sup> -1.1 <sup>-3</sup> ]	60 [36–92]	87 [53–132]	0.943/0.041
	Skygrid	7.9 <sup>-4</sup> [4.9 <sup>-4</sup> -1.1 <sup>-3</sup> ]	63 [37–98]	90 [54–138]	0.929/0.047

\*CI, Côte d'Ivoire; HPD, highest posterior density; MCC, maximum clade credibility.



**Technical Appendix Figure.** Map of West Africa showing regions endemic for Lassa fever (LASV) and sampling sites for study of LASV in multimammate rats, Côte d'Ivoire, 2013. Pie charts in map represent sampling sites in Côte d'Ivoire (Global Positioning System coordinates: 30 P 211825 1045219, Korhogo; 30 N 276206 849368, Bouaké; 29 N 670895 649275, T'ai). Within the charts, samples from *Mastomys* sp. rats are indicated in black; those from other rodents are in gray. On the map, countries in which LASV is endemic are shaded in gray.