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Evidence of Lymphocytic Choriomeningitis Virus (LCMV) in Domestic Mice in Gabon: Risk of Emergence of LCMV Encephalitis in Central Africa

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Lymphocytic choriomeningitis virus (LCMV) can cause acute fatal disease on all continents but was never detected in Africa. We report the first detection of LCMV RNA in a common European house mouse (*Mus musculus domesticus*) in Africa. Phylogenetic analyses show a close relationship with North American strains. These findings suggest that there is a risk of the appearance of LCMV acute encephalitis cases. This is a perfect example of virus dissemination by its natural host that may have dramatic public health consequences.

The lymphocytic choriomeningitis virus (LCMV) is a member of the *Arenaviridae* family found on the American, Asian, and European continents. It is the only member of this family causing disease in humans in Europe. While other viruses of the same family cause human severe hemorrhagic fever in Africa (Lassa virus) and South America (Junin virus, Machupo virus. . .), clinical manifestations of human LCMV infection can range from mild febrile illness to severe meningitis, encephalitis, and disseminated disease (1, 2). Human infection occurs through direct contact with infected rodents or by inhalation of infectious rodent excreta or secretions. LCMV belongs to the Old-World arenavirus serocomplex within the *Arenaviridae* family. Its genome contains two negative-sense single-stranded RNA segments: the small (S) and large (L) segments. The S segment encodes the nucleocapsid protein (NP) and the glycoprotein precursor (GPC). The L segment encodes the viral RNA-dependent RNA polymerase (L) and the small zinc finger-like protein (Z). The main natural reservoir of LCMV is the common house mouse, *Mus musculus*, but other rodent species can be alternative reservoirs (3, 4). LCMV has been shown to circulate among both its natural host and humans on the European, Asian, and American continents. In Africa, only antibodies against the virus have been found in rodents (5). Here we report the first detection of the LCMV genome in its natural host, the house mouse, *M. musculus domesticus*, in Africa.

From March 2012 to July 2014, we trapped a total of 797 rodents around and inside human dwellings in four Gabonese towns: Libreville, Makokou, Franceville, and Léconi (Fig. 1). One hundred eighty-eight (25%) of the mice captured belonged to *M. musculus*, a species native to Asia. This high incidence of the presence of this non-African rodent species indicates that this species tends to progressively replace, or at least push away from housing, the local species.

In a real-time quantitative reverse transcription-PCR assay targeting the GPC gene (the detailed protocol is available on request), 26 animals (17 from Libreville and 9 from Makokou) tested positive. All of them belonged to *M. musculus*, yielding an LCMV prevalence of about 13% in this species. One should stress that no specimen from local species tested positive. The introduction of

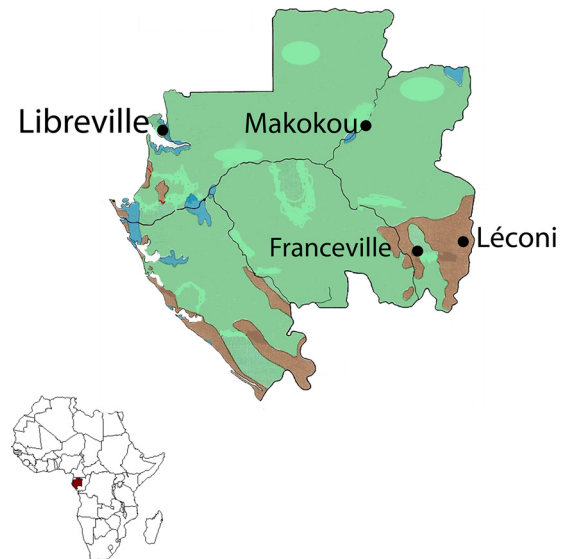


FIG 1 Localizations of capture sites in Gabon. Four cities have been chosen because they present different ecosystems, a forest ecosystem (Makokou), a savanna ecosystem (Léconi), both forest and savanna ecosystems (Franceville), and an urban environment (Libreville). The plain map of Africa is from Cartes & Cliparts, and the map of Gabon is from Imago Mundi Encyclopédie gratuite en ligne.

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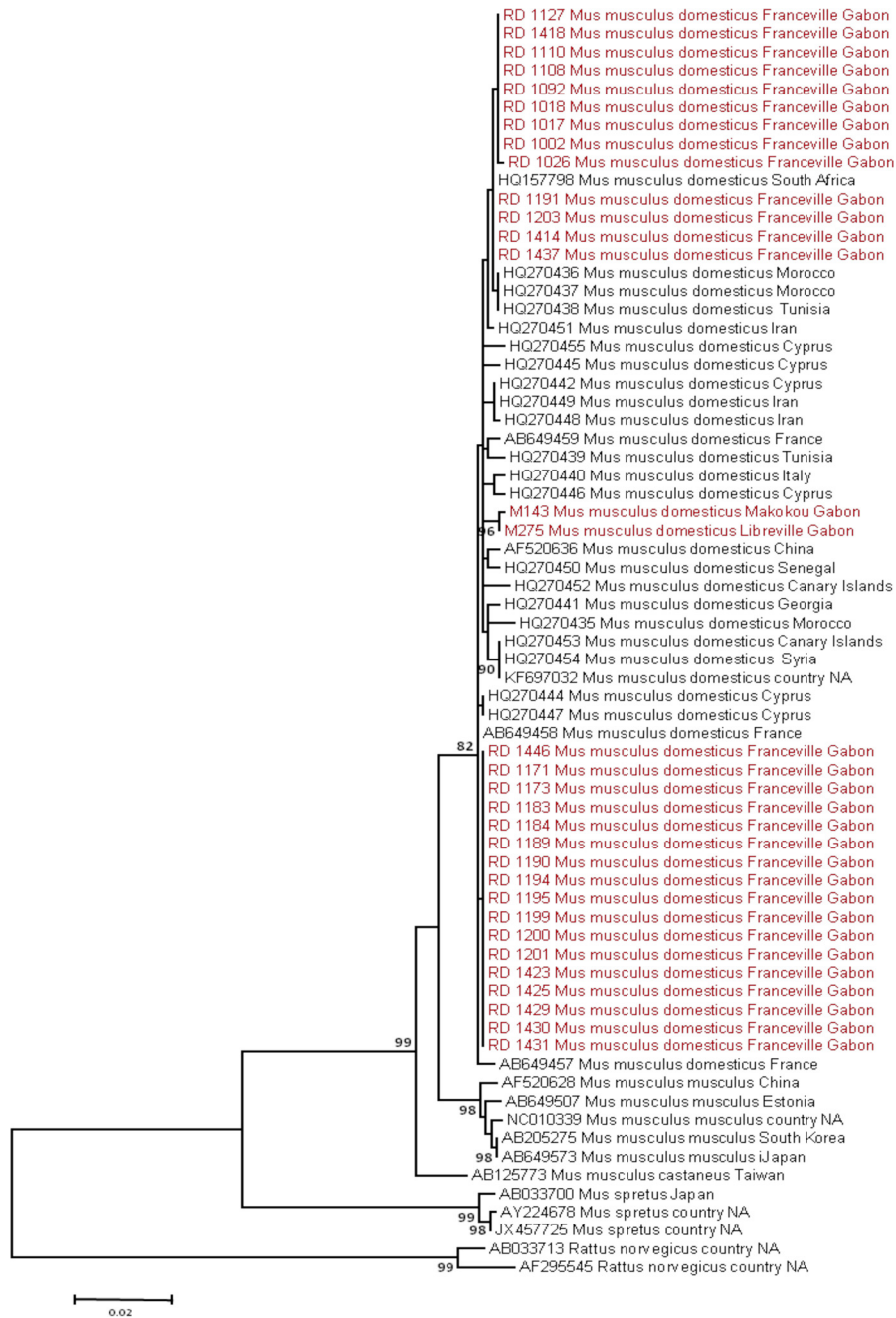


FIG 2 Phylogenetic relationships among Gabonese *M. musculus* and other *Mus* species based on a partial sequence (949 bp) of the cytochrome *b* gene. Bootstrap values of >80% are indicated. The GenBank accession number is indicated at the beginning of each sequence represented in the tree, and the sample's country of origin is also indicated. NA, not available. The scale bar indicates the number of nucleotide substitutions per site.

TABLE 1 Comparison of the S segment of LCMV strain Makokou with those of other LCMV strains present in GenBank

Strain ^a	Size (nt) of:					
	Full length	5' UTR	GP	IGR	NP	3' UTR
Makokou, Gabon, 2012	3,374	76	1,497	64	1,677	60
Pasteur, France, 2006	3,376	77	1,497	64	1,677	61
Douglas, New York, USA, 1947	3,375	77	1,497	64	1,677	60
Traub, USA, 1936	3,358	59	1,497	64	1,677	61
WE (ngs), Nagasaki, Japan, 2011	3,375	77	1,497	64	1,677	60
WE (UBS 57135), New York, USA, 1935	3,376	77	1,497	64	1,677	60

^a The name, country of origin, and year of isolation each strain are indicated.

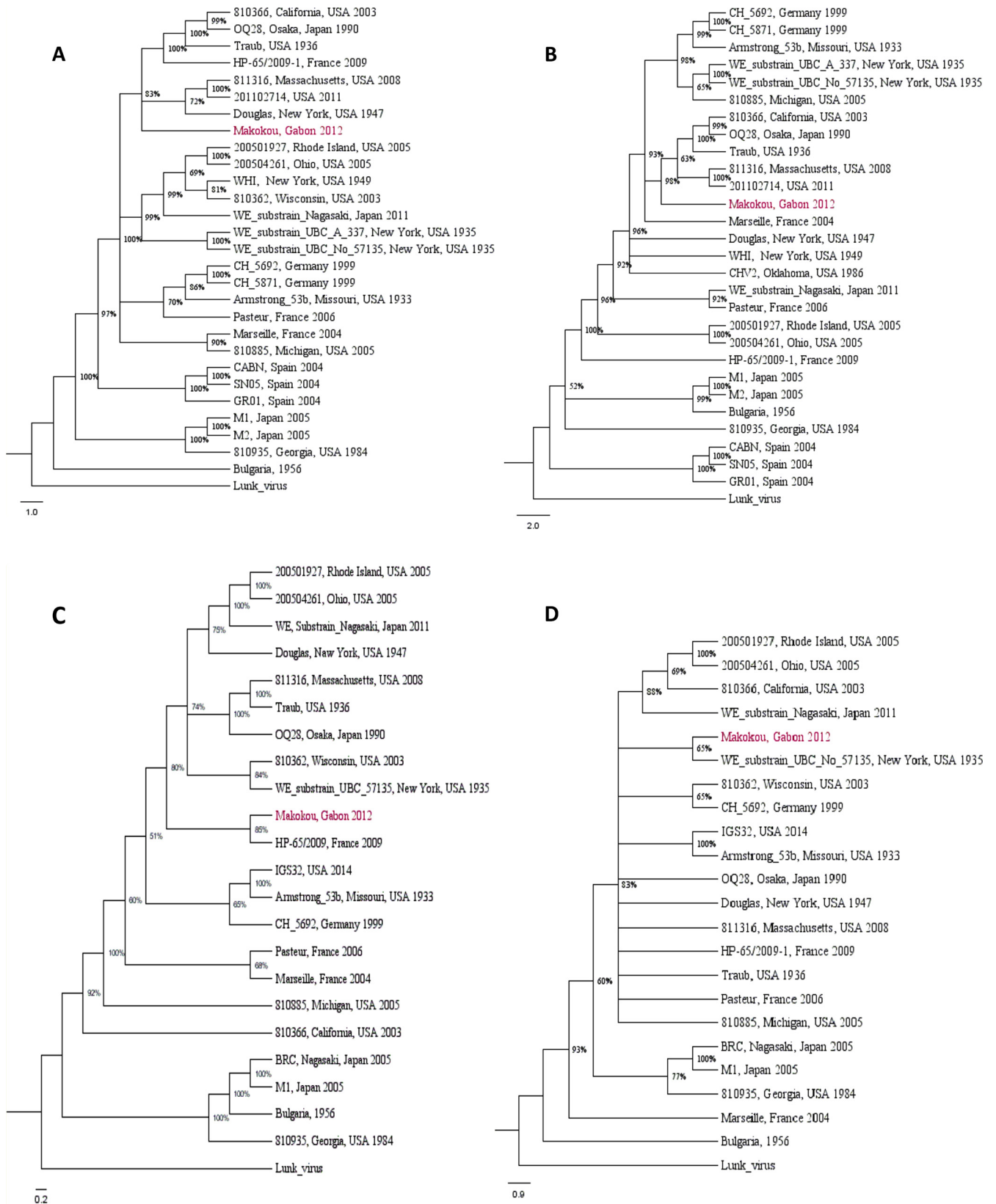


FIG 3 Phylogenetic comparison of the LCMV Makokou strain with other LCMV strains. Lunk virus was used as the outgroup. Analyses of the complete nucleotide sequences of the full-length NP (A) and GP (B) genes and partial sequences of the L (C, 6,599 nt) and Z (D, 191 nt) genes were performed. The name, year, and country of origin of each strain are indicated. At the nodes are bootstrap values based on 1,000 replications. The scale bar indicates the number of nucleotide substitutions per site.

M. musculus on the African continent is thus associated with the introduction of LCMV, for which it is a natural reservoir.

Because three *M. musculus* subspecies can carry LCMV (6–8), we needed to determine the subspecies harboring the virus in Gabon. Genomic characterization of two LCMV-positive mice from Libreville and Makokou and 30 LCMV-negative mice from Franceville (the detailed protocol is available on request) was done by using a partial sequence of the mitochondrial cytochrome *b* gene (9). A phylogenetic tree revealed that all of the mice belonged to the subspecies *M. musculus domesticus* (Fig. 2).

Genomic characterization of full-length S segment (GenBank accession number [KM523323](#)) and partial L (6,599 nucleotides [nt]; GenBank accession number [KM882857](#)) and Z (191 nt) sequences from the liver and spleen of one animal from Makokou was done (the detailed protocol is available on request). The S segment of this new strain, named LCMV Makokou, is 3,374 nt long. The comparison of this genomic sequence with those of other LCMV strains for which the S genome is available revealed that the lengths of their intergenic noncoding regions (IGRs) and coding regions are highly consistent while the lengths of the 5' and 3' untranslated regions (UTRs) differed among all of the strains (Table 1). The NP and GP gene sequences diverge from those of other known LCMV strains, respectively, by 4 to 10% and 3 to 20% at the amino acid level and by 15 to 28% and 16 to 37% at the nucleotide level (see Table S1 in the supplemental material).

The complete NP and GP sequences and the partial L (6,599 nt) and Z (191 nt) sequences have been aligned with other LCMV sequences by using the ClustalW algorithm with the MEGA4 software package (10). Phylogenetic analyses with NP and GP genes place the Makokou strain in groups in which the majority of the strains derive from humans and have caused severe clinical infections in the United States (multiorgan dysfunction after transplantation, congenital infection, meningitis, or encephalitis) (11) (Fig. 3). In the same way, Z gene analysis places the Makokou strain with a strain derived from the pathogenic American WE strain, while the analysis with the L gene places this strain with strain HP-65/2009-1 from *M. musculus* from France.

To get more information about virus strains that could circulate in Gabon, we sequenced partial NP (445 nt) and L (480 nt) genes from 18 samples from the two trapping sites (15 from Libreville and 3 from Makokou). Phylogenetic analysis placed all of the Gabonese strains in the same cluster (data not shown), indicating that only one viral strain was introduced from America into Gabon together with its natural host.

This study showed for the first time the presence of LCMV in an African country within its natural host, *M. musculus domesticus*. The virus was introduced into Gabon together with *M. musculus domesticus* and tends to spread across Gabon as domestic mice advance and colonize new areas. Transmission of LCMV to local rodent species must now be monitored, as it may cause dramatic health damage in these local species.

LCMV strains exhibit high genetic diversity and have been divided into four different lineages (11). Half of the strains, included in the same cluster as the Makokou strain (Fig. 3A and B), belong to the first lineage associated with severe human disease, and these strains have been directly associated with *M. musculus* mice (11). Several cases of direct LCMV transmission from rodents to humans, resulting in severe disease, have been documented in France, Spain, and the United States (12–15). Together with the high LCMV prevalence among house mice (13%), these findings

point to the possible emergence of LCMV in humans in Gabon. Indeed, similar conditions have been found in the United States, where up to 5% of the population is infected, possibly owing to the large population of infected house mice (16). So, in addition to the search for toxoplasmosis, malaria, HIV, and herpesviruses, there is now an urgent need to systematically perform LCMV diagnosis in cases of unexplained meningitis and encephalitis. Health facilities must therefore be prepared to deal with a case of LCMV in the near future.

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N.N. and J.B.M. performed the molecular biology experiments. N.B. and C.B. performed the bioinformatic analysis and high-throughput sequencing, respectively. N.N., V.R., and J.B.M. organized and performed the capture of mice. P.D. and F.R. performed the mouse genetic characterization. N.N., N.B., G.D.M., B.S.S., J.F., and E.M.L. analyzed the data. J.F., B.S.S., and E.M.L. conceived and designed the experiments. N.N., N.B., and E.M.L. wrote the manuscript. All of us approved the final version of the manuscript.

REFERENCES

- Barton LL, Mets MB, Beauchamp CL. 2002. Lymphocytic choriomeningitis virus: emerging fetal teratogen. *Am J Obstet Gynecol* 187:1715–1716. <http://dx.doi.org/10.1067/mob.2002.126297>.
- Barton LL, Peters CJ, Ksiazek TG. 1995. Lymphocytic choriomeningitis virus: an unrecognized teratogenic pathogen. *Emerg Infect Dis* 1:152–153. <http://dx.doi.org/10.3201/eid0104.950410>.
- Ledesma J, Fedele CG, Carro F, Lledo L, Sanchez-Secco MP, Tenorio A, Soriguer RC, Saz JV, Dominguez G, Rosas MF, Barandika JF, Gegundez MI. 2009. Independent lineage of lymphocytic choriomeningitis virus in wood mice (*Apodemus sylvaticus*), Spain. *Emerg Infect Dis* 15:1677–1680. <http://dx.doi.org/10.3201/eid1510.090563>.
- Tagliapietra V, Rosa R, Hauffe HC, Laakkonen J, Voutilainen L, Vapalahti O, Vaeheri A, Henttonen H, Rizzoli A. 2009. Spatial and temporal dynamics of lymphocytic choriomeningitis virus in wild rodents, northern Italy. *Emerg Infect Dis* 15:1019–1025. <http://dx.doi.org/10.3201/eid1507.081524>.
- el Karamany RM, Imam IZ. 1991. Antibodies to lymphocytic choriomeningitis virus in wild rodent sera in Egypt. *J Hyg Epidemiol Microbiol Immunol* 35:97–103.
- Morita C, Suzuki H, Tanikawa T, Yabe T, Eda J, Hirukawa H, Ueno H, Chenchittikal M. 1994. Origin of lymphocytic-choriomeningitis-virus-bearing mice in Japan. *Zentralbl Veterinarmed B* 41:291–293.
- Salazar-Bravo J, Ruedas LA, Yates TL. 2002. Mammalian reservoirs of arenaviruses. *Curr Top Microbiol Immunol* 262:25–63. http://dx.doi.org/10.1007/978-3-642-56029-3_2.
- Traub E. 1936. An epidemic in a mouse colony due to the virus of acute

- lymphocytic choriomeningitis. *J Exp Med* 63:533–546. <http://dx.doi.org/10.1084/jem.63.4.533>.
9. Jaarola M, Searle JB. 2002. Phylogeography of field voles (*Microtus agrestis*) in Eurasia inferred from mitochondrial DNA sequences. *Mol Ecol* 11:2613–2621. <http://dx.doi.org/10.1046/j.1365-294X.2002.01639.x>.
 10. Tamura K, Dudley J, Nei M, Kumar S. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol Biol Evol* 24:1596–1599. <http://dx.doi.org/10.1093/molbev/msm092>.
 11. Albariño CG, Palacios G, Khristova ML, Erickson BR, Carroll SA, Comer JA, Hui J, Briese T, St George K, Ksiazek TG, Lipkin WI, Nichol ST. 2010. High diversity and ancient common ancestry of lymphocytic choriomeningitis virus. *Emerg Infect Dis* 16:1093–1100. <http://dx.doi.org/10.3201/eid1607.091902>.
 12. Amman BR, Pavlin BI, Albariño CG, Comer JA, Erickson BR, Oliver JB, Sealy TK, Vincent MJ, Nichol ST, Paddock CD, Tumpey AJ, Wagoner KD, Glauer RD, Smith KA, Winpisinger KA, Parsely MS, Wyrick P, Hannafin CH, Bandy U, Zaki S, Rollin PE, Ksiazek TG. 2007. Pet rodents and fatal lymphocytic choriomeningitis in transplant patients. *Emerg Infect Dis* 13:719–725. <http://dx.doi.org/10.3201/eid1305.061269>.
 13. Emonet S, Retornaz K, Gonzalez JP, de Lamballerie X, Charrel RN. 2007. Mouse-to-human transmission of variant lymphocytic choriomeningitis virus. *Emerg Infect Dis* 13:472–475. <http://dx.doi.org/10.3201/eid1303.061141>.
 14. Knust B, Macneil A, Wong SJ, Backenson PB, Gibbons A, Rollin PE, Nichol ST. 2011. Exposure to lymphocytic choriomeningitis virus, New York, USA. *Emerg Infect Dis* 17:1324–1325. <http://dx.doi.org/10.3201/eid1707.101349>.
 15. Pérez-Ruiz M, Navarro-Mari JM, Sanchez-Seco MP, Gegundez MI, Palacios G, Savji N, Lipkin WI, Fedele G, de Ory-Manchon F. 2012. Lymphocytic choriomeningitis virus-associated meningitis, southern Spain. *Emerg Infect Dis* 18:855–858. <http://dx.doi.org/10.3201/eid1805.111646>.
 16. Bonthius DJ. 2012. Lymphocytic choriomeningitis virus: an underrecognized cause of neurologic disease in the fetus, child, and adult. *Semin Pediatr Neurol* 19:89–95. <http://dx.doi.org/10.1016/j.spen.2012.02.002>.