Lymphocytic choriomeningitis virus (LCMV) was detected in 2 patients with acute meningitis in southern Spain within a 3-year period. Although the prevalence of LCMV infection was low (2 [1.3%] of 159 meningitis patients), it represents 2.9% of all pathogens detected. LCMV is a noteworthy agent of neurologic illness in immunocompetent persons.

**The Study**

The study period was January 2008–June 2010. The population included patients at Hospital Virgen de las Nieves (Granada, Spain) who had suspected AME. Routine virologic investigation included reverse transcription PCR (RT-PCR) of cerebrospinal fluid (CSF) samples for detection of HEV (Xpert EV system, Cepheid, Sunnyvale, CA, USA), TOSV (7), and mumps virus (8), and PCR of herpes simplex virus (HSV) and varicella-zoster virus (LC VHS 1/2 Qual and LC VZV systems, respectively; Roche Diagnostics, Mannheim, Germany). Negative samples were subsequently tested for Epstein-Barr virus, cytomegalovirus, human herpes 6 virus (9), flavivirus (10), arenavirus (2), adenovirus (11), and phlebovirus (12). Specific LCMV RT-PCR was conducted by using a previously described protocol (2). Finally, CSF specimens from PCR-negative case-patients were inoculated in Vero and MRC-5 cell lines for viral culture.

CSF and acute-phase serum samples were also tested for IgG and/or IgM against TOSV (EIA Enzywell Toscana virus IgG/IgM, Diesse, Siena, Italy), West Nile virus (ELISA IgG and IgM-capture ELISA; Focus Diagnostics, Cypress, CA, USA), and LCMV by indirect fluorescent assay (IFA) with further confirmation by Western blot (2).

We studied 159 CSF samples by using PCRs for the presence of HEV, HSV, varicella-zoster virus, TOSV, and mumps virus, yielding 68 positive cases. The remaining viruses were further investigated in the 91 negative samples. A viral agent was detected in 70 (44%) cases: HEV accounted for 44 (63%) of positive cases, followed by varicella-zoster virus in 11 (16%), HSV-1 in 8 (11%), TOSV in 4 (6%), LCMV in 2 (3%), and HSV-2 in 1 (1%).

Case-patient 1, a 21-year-old woman, came to the hospital’s emergency unit in April 2008 exhibiting headache, chills, fever (38.9°C), confusion, nausea, vomiting, and slight nuchal rigidity. Aseptic meningitis was suspected, and samples of CSF and serum were obtained. Laboratory results were 415 leukocytes/mm³ (100% mononuclear cells), 43 mg/100 dL glucose level (reference 35–65 mg/dL), and 128 mg/dL protein level (reference 7–45 mg/dL). No viral agent was detected in CSF and serum samples. CSF and serum samples were subsequently tested for Epstein-Barr virus, cytomegalovirus, human herpes 6 virus (LC VHS 1/2 Qual and LC VZV systems, respectively; Roche Diagnostics, Mannheim, Germany). A viral agent was detected in 70 (44%) cases: HEV accounted for 44 (63%) of positive cases, followed by varicella-zoster virus in 11 (16%), HSV-1 in 8 (11%), TOSV in 4 (6%), LCMV in 2 (3%), and HSV-2 in 1 (1%).

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isolate (EEB-7) was used for genetic characterization. To sequence the genome, degenerate and specific primers were designed on the basis of an alignment of all complete LCMV large (L), glycoprotein complex and nucleocapsid protein sequences (Table). Terminal sequences were generated by using a universal arenavirus primer, targeting the conserved viral termini (5′-CGC ACM GDG GAT CCT AGG C-3′), combined with 4 specific primers positioned near the ends of each segment. Amplification products were size-fractionated by electrophoresis in 1% agarose gels, purified (MinElute, QIAGEN, Valencia, CA, USA), and sequenced in both directions on an ABI PRISM 3700 DNA analyzer (PE Applied Biosystems, Foster City, CA, USA). To determine the evolutionary history of isolate EEB-7, we performed Bayesian phylogenetic analysis of all available complete genome sequences of LCMV using BEAST, BEAUti, and Tracer analysis software packages (13). The analysis showed that EEB-7 (GenBank accession nos. JN872494–5) belonged to LCMV lineage I (Figures 1, 2). Clinical diagnosis was acute meningitis and the patient was discharged from hospital on day 9.

Case-patient 2, a 39-year-old man, sought treatment in May 2010 with headache, nausea, vomiting, increased perspiration, and a temperature of 37.5°C. Aseptic meningitis was suspected, and CSF and serum samples were collected. CSF analysis demonstrated 1,715 leukocytes/mm3 (95% mononuclear cells), normal glucose level (68 mg/dL), and elevated protein levels (240 mg/dL). Results of a cranial computed tomographic scan were normal. Further virologic investigation detected LCMV RNA in the CSF and an IgG antibody titer of 640 by IFA and IgM antibodies against LCMV in the investigation detected LCMV RNA in the CSF and an IgG antibody titer of 640 by IFA and IgM antibodies against LCMV in the cell culture supernatant because no cytopathic effect was observed.

Isolates from both LCMV case-patients belonged to the classical lineage I, which has been detected elsewhere in Europe. Lineage I is usually associated with human disease (as are lineages II and III) and is linked to the common house mouse as its reservoir (14). Lineage IV viruses were previously detected in Spanish wood mice (2) and have not been associated with human disease.

Although no further epidemiologic studies could be conducted to search for infected reservoirs, the common genetic lineage and the fact that both case-patients resided near the ends of each segment. Amplification products were size-fractionated by electrophoresis in 1% agarose gels, purified (MinElute, QIAGEN, Valencia, CA, USA), and sequenced in both directions on an ABI PRISM 3700 DNA analyzer (PE Applied Biosystems, Foster City, CA, USA). To determine the evolutionary history of isolate EEB-7, we performed Bayesian phylogenetic analysis of all available complete genome sequences of LCMV using BEAST, BEAUti, and Tracer analysis software packages (13). The analysis showed that EEB-7 (GenBank accession nos. JN872494–5) belonged to LCMV lineage I (Figures 1, 2). Clinical diagnosis was acute meningitis and the patient was discharged from hospital on day 9.

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References

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Figure 2. Phylogenetic tree showing genetic lymphocytic choriomeningitis virus sequences relationship within the small segment coding for the glycoprotein complex (A) and nucleocapsid proteins (B). The name of the strain is followed by GenBank accession number, country, and year of detection. Clusters grouped in brackets depict the lymphocytic choriomeningitis virus lineage. Scale bar indicates nucleotide substitutions per site.

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