Journal of Clinical Virology 54 (2012) 349–351

Contents lists available at SciVerse ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Case report

Molecular evidence of St. Louis encephalitis virus infection in patients in Buenos Aires, Argentina

Laura E. Valinotto[a,b,*], Paola R. Barrero[a,b], Mariana Viegas[a,b], María C. Alvarez López[a], Alicia S. Mistchenko[a,c]

[a] Hospital de Niños R. Gutiérrez, Laboratorio de Virología, Gallo 1330, 1425 Buenos Aires, Argentina
[b] Consejo Nacional de Investigaciones Científicas y Técnicas, Buenos Aires, Argentina
[c] Comisión de Investigaciones Científicas de la Provincia de Buenos Aires, Buenos Aires, Argentina

ARTICLE INFO

Article history:
Received 30 November 2011
Received in revised form 23 March 2012
Accepted 17 April 2012

Keywords:
St. Louis encephalitis virus
Molecular typing
Patients

SUMMARY

We report two cases of St. Louis encephalitis where the virus was detected in patients' sera directly by molecular techniques allowing subsequent typing. Phylogenetic analysis of both samples showed that NS5 sequences clustered with viruses previously classified as genotype III.

1. Why these cases are important

St. Louis encephalitis virus (SLEV) is an arthropod-borne, single-stranded positive RNA member of the *Flaviviridae* family, and belongs to the Japanese encephalitis virus serocomplex.[1] Although humans are considered dead-end hosts, infections occasionally occur, causing mainly a febrile illness that can be fatal if neurological complications arise.[2]

Currently, rapid serologic tests such as IgM-capture enzyme-linked immunosorbent assay and IgM immunofluorescent assay are preferred for diagnosis, but results must be carefully analysed, since false positives can arise due to cross-reaction between antibodies against different flaviviruses and time-consuming confirmatory plaque-reduction neutralisation tests are often needed.

Here, we report two SLE cases that occurred in Buenos Aires city affecting patients with different health and social backgrounds. In both cases, viral RNA was detected directly in patients' sera, thus allowing the subsequent molecular typing.

2. Case description

Case 1: In March 2007, a 56-year-old kidney transplant recipient from Buenos Aires city with no recent travel history presented with symptoms of ataxia, asthenia, fever, migraine and paralysis of the left leg. Serum sample was taken on the seventh day after the onset of the symptoms and submitted for Alpha and Flavivirus screening. Viral RNA was obtained by using PureLink Viral RNA/DNA kit (Invitrogen, Carlsbad, CA, USA) and used as a template for a duplex reverse transcription-polymerase chain reaction (RT-PCR) for detection followed by a nested PCR for identification of Alpha and Flaviviruses by amplification of a NS5 gene fragment.[3] Duplex RT-PCR resulted positive for Flavivirus and nested PCR positive for SLEV.

Case 2: On 15 January 2010, a 35-year-old previously healthy woman from a slum area in Buenos Aires, with no recent travel history, had a 4 day-evolution period of fever, headache, retro-orbital pain, myalgia, arthralgia, nausea and vomiting. Plasma and serum samples were submitted for Dengue virus (DENV) diagnosis. NS1 Dengue antigen detection (Platelia, BioRad, Marnes-la-Coquette, France) and Dengue RT-PCR[4] were performed, both with negative results. Additionally, anti-DENV IgM capture and IgG (DxSelect, Focus Diagnostics, Cypress, CA, USA) were tested and showed negative results. A second sample was sent on January 28, in which anti-DENV IgM and IgG were positive. First and second samples were sent to the National Reference Laboratory (INEVH, Pergamino, Buenos Aires) for plaque reduction neutralising tests, that showed seroconversion for SLEV. In light of these findings, first serum

Abbreviations: SLEV, St. Louise encephalitis virus; DENV, Dengue virus.

* Corresponding author at: Hospital de Niños Dr. R. Gutiérrez, Laboratorio de Virología, Gallo 1330, 1425 Buenos Aires, Argentina. Tel.: +54 11 4964 3118; fax: +54 11 4964 4320.
E-mail address: valinottohnrg@gmail.com (L.E. Valinotto).

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http://dx.doi.org/10.1016/j.jcv.2012.04.005

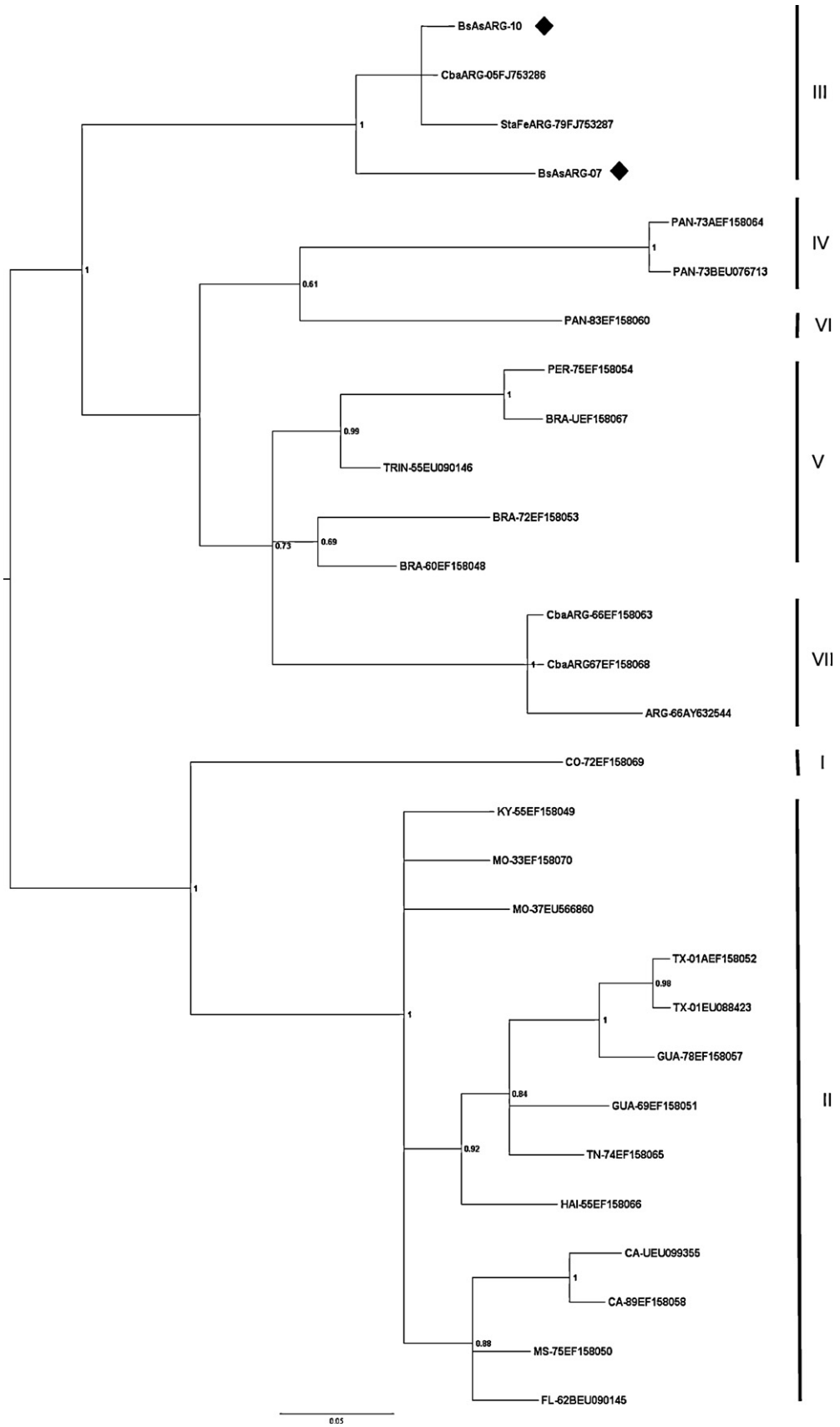

Fig. 1. SLEV NS5-based phylogenetic tree. Phylogeny was constructed from sequences available in GenBank using the NS5 gene with MrBayes software (v3.1.2; 600,000 ngen, 100 samplefreq, 4 nchains, 250 burnin). Lineages I–VII were defined by Kramer.[9] Isolates were named according to location and year of isolation. CA, California; TX, Texas; MEX, Mexico; CO, Colorado; MS, Mississippi; KY, Kentucky; MO, Missouri; TN, Tennessee; FL, Florida; BRA, Brazil; GUA, Guatemala; TRIN, Trinidad; PAN, Panama; PER, Peru and ARG, Argentina. Solid diamonds indicate viral sequences from Buenos Aires reported in this work. Only posterior probabilities above 0.6 are shown. The tree was mid-point rooted.

sample was retrospectively tested for SLEV and resulted positive by duplex RT-PCR detection and nested PCR identification.[3]

As in both cases viral isolation and envelope protein (E) gene amplification attempts were unsuccessful, the NS5 amplified fragments resulted from the nested duplex RT-PCR were purified and sequenced using an automated capillary sequencer MegaBACE 1000 in case 1 or ABI 3500 and Roche GS-FLX 454 pyrosequencing platform in case 2.

NS5 sequences from different SLEV strains that were previously classified into different genotypes based on E sequence were downloaded from GenBank and aligned with fragments (576 nt.) of the two sequences from SLEV strains detected in patients in Buenos Aires, Argentina (GenBank accession no. JQ003909 and JQ003910) with ClustalX v2.1[5] and manually edited by BioEdit v7.0.1.[6] A Bayesian phylogenetic analysis was performed with MrBayes software v3.1.2[7] (settings: 600,000 ngen, 100 samplefreq, 4 nchains, 250 burnin), using the General Time Reversible (GTR + I + Γ4) nucleotide substitution model calculated from the alignment with jModeltest.[8] Phylogenetic analysis of both samples showed that NS5 sequences clustered with viruses previously classified as SLEV genotype III (Fig. 1). As a result, Buenos Aires strains infecting humans in 2007 and 2010 clustered with previously reported SLEV genotype III sequences isolated from *Cx. quinquefasciatus* in Córdoba (2005) and Santa Fe (1979) Argentina (Fig. 1) with 98% and 100% identity with CbaARG-05 strain (accession no. FJ753286.1) for patient one and two, respectively. Other phylogenetic inferences such as distance, maximum parsimony and maximum likelihood were performed and showed similar topologies strongly supported by high bootstrap values (data not shown). The derived trees were plotted using FigTree software v1.3.1.[10]

3. Other similar and contrasting cases in the literature

In 2005, the first outbreak in Argentina was reported in Córdoba Province, with 47 cases, including nine deaths.[11] All these cases were only confirmed by serological evidence, without detection or isolation of the virus. Buenos Aires city, located 700 km southeast of Córdoba, suffered an SLEV outbreak in 2010 with 13 serologically confirmed cases.[12]

4. Discussion

In this study, we demonstrate that SLEV molecular detection and typing is possible in patients, leading to unambiguous results. It should be emphasised that viral RNA detection was possible, although the patients had very different backgrounds. Case 1 was sporadic, occurred 3 years before the SLEV outbreak in Buenos Aires, and affected an immunosuppressed 56-year-old man. Case 2 occurred during the outbreak, and affected a young and previously healthy woman.

This is the first report of SLEV molecular typing and sequencing directly from clinical samples in Argentina accomplished by new molecular technologies after ruling out dengue suspicion. Our results indicate that strains detected in patients from Buenos Aires in 2007 and 2010 were similar to the strains previously classified as genotype III isolated from *Cx. quinquefasciatus* during 2005 in Córdoba province.[13] Together with the reported outbreak during 2010 in Buenos Aires,[12] SLEV might be circulating in Buenos Aires city since its re-emergence in Cordoba province in 2005.[11] This information might help both to elucidate the urban transmission cycles and to identify local reservoirs to prevent further spread of the virus. However, it is essential to increase awareness among physicians to improve viral recovery and diagnosis at early stages in patients with neurological compromise, thus intensifying the molecular surveillance of SLEV circulation to determine if it is endemic in our area.

Funding

This work was partially supported by Agencia Nacional de Promoción de Ciencia y Técnica, Ministerio de Ciencia, Tecnología e Innovación Productiva de la Nación, Argentina (grant PICT 1624/07).

Competing interests

None declared.

Ethical approval

Not required.

Acknowledgements

We thank the National Reference Laboratory (INEVH, Pergamino, Buenos Aires) for performing plaque reduction neutralising tests and the Instituto de Agrobiotecnología Rosario (INDEAR, Rosario, Argentina) for performing Next Generation Sequencing.

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