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GENOME SEQUENCES

Vesicular Stomatitis Virus Isolated from a Bovine Brain Sample in Costa Rica

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ABSTRACT The genome of Vesicular stomatitis (New Jersey) virus was obtained by high-throughput sequencing after the nucleic acid was extracted from the supernatant of Vero E6 cells inoculated with a sample of a bovine brain with nervous signs. The sample was negative for rabies by direct Immunofluorescence and bovine spongiform encephalopathy by histopathology.

Vesicular stomatitis virus (VSV) belongs to the *Vesiculovirus* genus with two serotypes New Jersey VSV-NJ and Indiana VSV-IN in the *Rhabdoviridae* family (1).

VSV affects cattle, horses, swine, and llamas. In cattle, VSV causes fever, vesicles in the mouth, tongue, nose, teat, coronary band, and foot lesions that end in painful ulcerations. In humans, VSV produces influenza-like signs (2). Experimentally, VSV infection can cause severe encephalitis via intranasal administration in mice (3) and nonhuman primates (4). Moreover, a case of severe encephalitis caused by an Indiana strain has been reported in a Panama child (5).

Here, we reported the genome of a VSV isolated from a brain of a bovine with encephalitis symptoms in Costa Rica.

The bovine brain sample (D3619-09) was taken on November 04, 2009 from a 1.5 years old Brahman male located on the north pacific coast, Fig. 1A. The sample tested negative for bovine spongiform encephalopathy by histopathology (6) and rabies by direct immunofluorescence (7). The sample was stored at −80°C with other animal brains as part of an encephalitis project. The sample was thawed in 2020 and approximately 25 mg of tissue was mechanically macerated and homogenized in 1 mL of DMEM and centrifuged at 10,000 × *g* at 4°C. The supernatant (200 μL) was inoculated in Vero-E6 cells, and a cytopathic effect was observed after 48 h. The cell supernatant (200 μL) was extracted with TRIzol LS (Invitrogen, Carlsbad, USA) following the manufacturer's methodology (8).

RNA quality and quantity were measured using a NanoDrop (Thermofisher, Waltham, USA) and a Quantus fluorometer (Promega Wisconsin, USA). Random primers were used for the retrotranscription phase, and cDNA synthesis and library preparation using Nextera XT were done as previously described (9), using a Miseq platform. Run quality was assessed using a sequence analysis viewer (Illumina, San Diego, USA). Reads pretrimming and posttrimming quality was assessed using FastQC v0.11.5 (10), and the genome was assembled and processed using the Genome Detective tool 1.126 v (11). A total of 1,172,046 raw reads were obtained with read lengths of 35 to 151 nt. After trimming and quality control 1,002,023 reads remained with read lengths of 50 to 136 nt. A single contig of11.016 nucleotides (nt) was obtained, using the curated NCBI reference sequence NC_024473.1 (Vesicular stomatitis New Jersey virus isolate NJ1184HDB) as reference. The depth of coverage ranged from 4,000× to 40,000× with an average of 10,827.3×, and a

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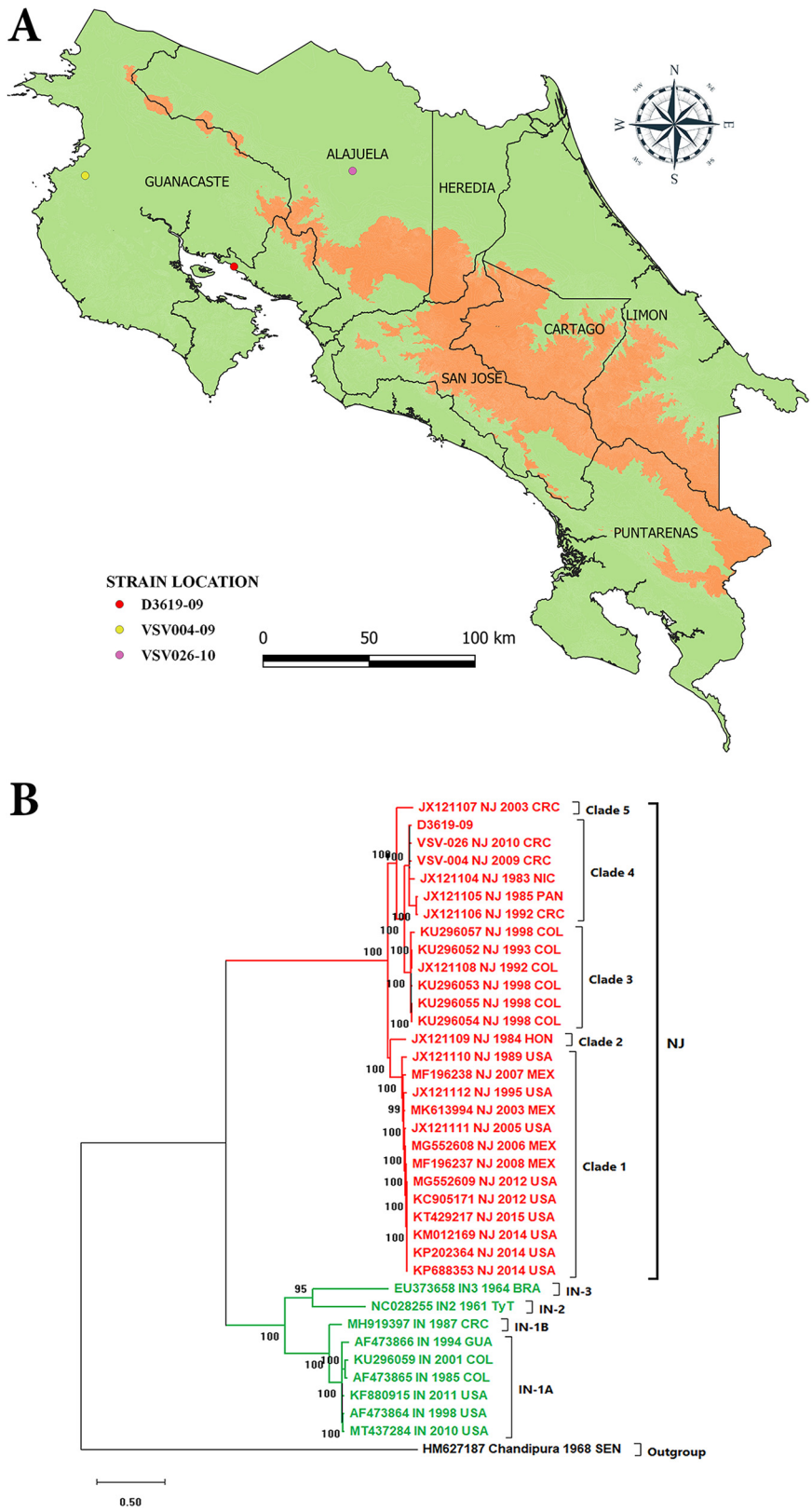

FIG 1 (A) presents the location of the VSV Costa Rica cases (20), and (B) shows the phylogenetic tree of VSV genotypes. INV sequences are shown in green and, NJV in red divided into 5 genotypes (21).

TABLE 1 Positive selection by gene, branch, and site was obtained with three different methods

Method	FEL[a] 28 NJ sequences D3619-09		Mutations D3619-09 vrs VSV-004-026 JX121104 NIC 1983
Gene/amino acids	PP[b]	PN[c]	
Nucleoprotein 422	0	278	
D3619-09	0	0	
Matrix 229	0	148	
D3916-09	0	0	
Phosphoprotein 274	1 (235)	104	201 L(S)[d] VSV-004-026 JX121104
D3619-09	2[b]	0	243 I (V)[d] VSV-004-026 JX121104
Glycoprotein 517	0	317	209 G (E)[d] VSV-004-026 JX121104
D3619-09	0	0	
Polyprotein 2109	0	1334	50 T (M)[d] VSV-004-026 JX121104
D3619-09	2[b]	0	918 T(A)[d] VSV-004-026 JX121104
			1596 Q (P)[d] VSV-004-026 JX121104
			1920 I (V) VSV-004-026 JX121104

[a] The BUSTED and ABSREL analysis did not show evidence of positive selection while FEL analysis showed positive selection in the P and L genes of the D3916-09.
[b] *PP*, pervasive positive/diversifying selection, the number refers to amino acid location into the gene, 243 I (V), 201 L (STK) phosphoprotein, 1596 Q (P) 1920 I (V) polyprotein. The letters represent the amino acid name. I (isoleucine) was present in the D3619-09 sequence while the V (valine) was present in the sequences VSV-004-026 and JX121104, S (serine), G (glycine), E (glutamic acid), T (threonine), M (methionine), A (alanine), Q (glutamine), and P (proline).
[c] PN, pervasive negative/purifying selection, the numbers indicate the total of amino acids with PN selection into the respective genes.
[d] These mutations were unique and only present in D3619-09 compared with the 28 NJ sequences analyzed.

GC content of 40.06%, the sequence was identified by genome Detective as VSV-NJ. The final version of the publicly released genome (GenBank accession no. OM909025) was annotated JX121104 as a reference sequence in Bankit.

Three sequences isolated from bovines in Costa Rica (ON805823 VSV-004 and ON805824 VSV-026, and the D3619-09) were aligned using Bioedit (12), with 34 VSV sequences downloaded from GenBank. A maximum-likelihood tree with 1000 bootstraps was then built-in MEGA X (13).

In Fig. 1B, the phylogenetic analysis shows where the D3619-09 sequence clustered within VSV-NJ clade 4, which comprises viruses from southern Central America and Panama (14), sharing this cluster with sequences. A BLAST showed nucleotide percent identities of 97.5% and 97.6% with the sequences VSNJV-026 and VSNJV-004, respectively, and 96.10% identity with the genome JX121104 isolated from a porcine case in Nicaragua in 1983 (14, 15).

Only 29 sequences (VSV-NJ) were tested for selection analysis in DataMonkey (16), using a branch-site unrestricted statistical test for episodic diversification (BUSTED) (17), fixed effects likelihood (FEL) (18), and adaptive branch-site random effects likelihood (aBSREL) (19).

There were 18 amino acid mutations between D3619-09 and VSV-004, VSNJV-026, and JX121104. Interestingly, six of them were found only in the sequence D3619-09 compared with all the 28 NJ sequences (labeled with a *d* Table 1), located in the genes: phosphoprotein (2), glycoprotein (1), and polyprotein (3). Two mutations found in the phosphoprotein (P) and two in the polyprotein (L) were identified by FEL as positive selection mutations Table 1.

Future studies could clarify the effect of these mutations on animal models or cell culture over the pathogenesis of this virus.

Data availability. Data was made available at https://www.ncbi.nlm.nih.gov/nuccore/OM909025. SRA accession number for the reads used to assemble the genome (PRJNA867315).

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