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SCIENTIFIC ARTICLE
Animal Infectious Diseases

Molecular analysis and environmental description of vesicular stomatitis New Jersey viruses isolated in Venezuela from 2009 to 2017

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ABSTRACT

Vesicular stomatitis virus is the causative agent of the vesicular stomatitis disease, which mainly affects cattle, swine, and horses causing significant economic losses. Many other animal species, including humans, can be also affected by the disease. Up to now, the epidemiology of vesicular stomatitis disease is not well understood. Previous epidemiological studies in Central America described the existence of an association between the presence of vesicular stomatitis New Jersey virus antibodies and environmental risk factors such as mean annual rainfall, temperature, and elevation. Additionally, molecular epidemiology studies revealed that the phylogenetic relationship between vesicular stomatitis New Jersey virus isolates is more related to ecological factors than temporal factors. We have performed a genetic analysis of vesicular stomatitis virus samples isolated from animals in different geographical regions in Venezuela. We sequenced the hypervariable region of the phosphoprotein gene of 51 samples collected over eight years. The virus was found in a wide range of environmental conditions, at high and low altitudes and with variable levels of precipitation and temperatures. In contrast with previous reports, our phylogenetic analysis showed that viruses from different ecological regions did not present significant genetic differences.

Keywords: vesicular disease surveillance; sequence analysis; molecular epidemiology.

INTRODUCTION

Vesicular stomatitis virus (VSV) is the causative agent of the vesicular stomatitis disease, which mainly affects cattle, swine, and horses with clinical disease. Many other animal species, including humans, can be also affected by the disease (Hanson, 1981). The virus belongs to the *Vesiculovirus* genus of the Rhabdoviridae family, with two major serotypes recognized: New Jersey (VSNJV) and Indiana (ICTV, 2018). The incidence of the disease can vary widely among affected herds, and up to 10–15% of the animals may show clinical signs (WOAH, 2022). Clinically affected animals present vesicles and erosions,

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affecting the epithelial tissues of the mouth, nostrils, teats, and feet (WOAH, 2022). Those similar lesions are used as indicators for the surveillance of foot and mouth disease (FMD). Both diseases are clinically indistinguishable, so the diagnosis and epidemiological surveillance of vesicular diseases involve integrating information from both the field and the laboratory (Cottral, 1972).

Despite the clinical similarity with FMD, there is not a complete knowledge of the pathogenesis and epidemiology of vesicular stomatitis. Epidemiological studies in Costa Rica described the existence of an association between the presence of VSNJV antibodies and environmental risk factors such as mean annual rainfall, temperature, and elevation (Atwill et al., 1993). Additionally, molecular epidemiology studies revealed that the phylogenetic relationship between VSNJV isolates is more related to ecological factors than temporal factors (Rodriguez et al., 1996). These and other reports have shown that specific genetic lineages exist in different geographical areas (Rodriguez et al., 2000; Velazquez-Salinas et al., 2014). Moreover, six distinct clades have been reported analyzing samples from countries of South, Central, and North Americas (Pauszek; Rodriguez, 2012). Although the VSV has been extensively studied at the molecular level, particularly samples from Central and North America, many unknowns remain regarding its epidemiology.

In Venezuela, any event with signs of vesicular disease in ruminants and/or pigs is of mandatory immediate notification to the animal health authorities. As in other countries of the region, the Venezuelan vesicular disease surveillance system is mainly aimed at detecting FMD, with vesicular stomatitis only as part of the process of differential diagnosis for FMD. A low sensitivity of the surveillance program can cause a delay in FMD detection, and subsequently in the response, worsening the impact due to losses in livestock production and international trade.

The first case of vesicular stomatitis in Venezuela was identified in 1941 in the state of Barinas, affecting cattle, swine, and horses (Hanson et al., 1968; Obregon; Montoya, 2010). Since then, the disease has been identified every year, mostly at the end of the rainy season (Conde, 2020). The majority of samples with positive VSV results are of the New Jersey type, and just on a few occasions, the Indiana one has been identified in Venezuela (Obregon; Montoya, 2010; Conde, 2020). So far, no molecular studies have been reported presenting VSV isolates from Venezuela together with the environmental factors related to those isolates.

Here we present the results of collaborative work between Instituto Nacional de Investigaciones Agrícolas/Centro Nacional de Investigaciones Agropecuarias and Instituto Nacional de Salud Agricola Integral from Venezuela, Laboratório Federal de Defesa Agropecuária/Minas Gerais - Ministério da Agricultura e Agropecuária from Brazil, and the Pan American Center for Food-and-Mouth Disease and Veterinary Public Health/Pan American Health Organization - World Health Organization. The goals of the study were to molecularly analyze samples of VSNJV collected in Venezuela from 2009 to 2017 and to characterize the outbreak areas through the analysis of variables related to environmental factors.

MATERIAL AND METHODS

Viruses

A total of 78 original epithelial samples from Venezuela or its passage in cell culture were received at the Pan American Center for Food-and-Mouth Disease and Veterinary Public Health Foot-and-Mouth Disease Virus (FMDV)/VSV Reference Laboratory (Table 1). Samples were collected by the Venezuelan vesicular disease national surveillance program, from clinical vesicular cases that occurred in 17 states of Venezuela in the period 2009–2017. Samples were analyzed in origin by typing enzyme-linked immunosorbent assay (ELISA) for differential diagnosis FMD/VSV and tested positive for VSVNJ according to information from the FMD National Reference laboratory Centro Nacional de Investigaciones Agropecuarias/Instituto Nacional de Investigaciones Agrícolas, from Venezuela. The information regarding the location (i.e., second administrative division level) and the species affected was available for those samples.

Table 1. Identification and geographical origin of viruses included in the study.

Sample ID	Location			Collected date mm/dd/year	GenBank accession number
	Municipality	State	Parroquia		
21905	Guanare	Portuguesa	Capital Guanare	07/21/2009	N/A
21908	Ospino	Portuguesa	Capital Ospino	08/12/2009	OR187407
21909	Araure	Portuguesa	Rio Acarigua	08/14/2009	N/A
21911	S Planas	Lara	Buría	08/22/2009	OR187408

continue...

Table 1. Continuation...

Sample ID	Location			Collected date mm/dd/year	GenBank accession number
	Municipality	State	Parroquia		
21912	Zamora	Aragua	Zamora	08/25/2009	OR187409
21917	Guanare	Portuguesa	Capital Guanare	09/15/2009	N/A
21918	Guanare	Portuguesa	Capital Guanare	09/15/2009	OR187410
21919	Guanare	Portuguesa	Capital Guanare	09/15/2009	OR187411
21922	Obispo	Barinas	El Real	09/15/2009	N/A
21932	Buchivacoa	Falcon	Guajiro	10/17/2009	OR187412
21933	Buchivacoa	Falcon	Guajiro	10/17/2009	OR187413
21942 (s)	Sucre	Merida	Pueblo Nvo del Sur	01/06/2010	OR187414
21943 (s)	A Pinto Salinas	Merida	Santa Cruz de Mora	01/06/2010	OR187415
21944	Tulio F Cordero	Merida	M C Palacios y Blanco	02/02/2010	OR187416
21966	Maturín	Monagas	Jusepin	07/27/2010	N/A
21979	Veroes	Yaracuy	Capital Veroes	09/24/2010	OR187418
21961	Yagua	Carabobo	Guacara	10/09/2010	OR187417
21985	M Monjes	Yaracuy	Yumare	10/22/2010	OR187419
21992	Ribero	Sucre	Cariaco	10/28/2010	N/A
21993	Ribero	Sucre	Cariaco	11/11/2010	OR187420
21997	Sucre	Zulia	Gibraltar	11/19/2010	OR187421
21998	Baralt	Zulia	Pueblo Nuevo	12/22/2010	OR187422
22001	Palmasola	Falcon	Palmasola	01/06/2011	OR187427
22003	Colon	Zulia	San Carlos del Zulia	01/07/2011	OR187432
22006	M Monje	Yaracuy	Yumare	01/19/2011	OR187433
22013	Juan J Mora	Carabobo	Urama	02/01/2011	OR187424
22030	G de Hevia	Táchira	José Antonio Páez	07/27/2011	N/A
22031	J Losada	Zulia	José Ramón Yepez	08/02/2011	OR187434
22034	Ospino	Portuguesa	Capital La Aparición de Ospino	08/13/2011	OR187435
22036	San R Onoto	Portuguesa	Santa Fe	09/13/2011	N/A
22039	Cabimas	Zulia	Aristides Calvani	10/22/2011	OR187437
22042	San R Onoto	Portuguesa	Thermo Morles	01/04/2012	OR187438
22043	Guanare	Portuguesa	Guanare	01/17/2012	OR187439
22056	S Marquina	Merida	Tabay	08/13/2012	OR187440
22061	S R Onoto	Portuguesa	Santa Fe	10/25/2012	OR187429
22069B	Torres	Lara	Las Mercedes	10/21/2012	OR187442
22071	J Losada	Zulia	San José	12/06/2012	OR187443
22086	Escuque	Trujillo	Escuque	08/14/2013	OR187444
22088	Zea	Merida	Caño El Tigre	09/19/2013	OR187428
22092	Jose F Ribas	Merida	Tucupido	01/22/2013	N/A
22093 (e)	Girardot	Aragua	Las Delicias (Las Cocuizas)	01/22/2013	OR187423
22099	Andres Bello	Merida	La Azulita	11/28/2013	N/A
22101 (s)	Andres Bello	Táchira	Cordero	12/12/2013	N/A
22106	Alberto Adriani	Merida	Gabriel Picón González	03/13/2014	N/A
22120	Lima Blanco	Cojedes	La Aguadita	06/25/2014	OR187441
22124 (s)	F Miranda	Guárico	El Calvario	08/22/2014	OR187445
22128	JG Monagas	Anzoátegui	Zuata	08/29/2014	N/A
22129	Acevedo	Miranda	Caucagua	08/29/2014	OR187430

continue...

Table 1. Continuation...

Sample ID	Location			Collected date mm/dd/year	GenBank accession number
	Municipality	State	Parroquia		
22132	Ospino	Portuguesa	Capital La Aparición de Ospino	09/03/2014	OR187431
22136 (s)	Santa Ana	Anzoátegui	Santa Ana	10/08/2014	OR187436
22146	Rojas	Barinas	Palacios Fajardo	10/15/2014	OR187446
22148	San R de Onoto	Portuguesa	Thermo Morles	10/20/2014	OR187447
22149	Baralt	Zulia	Libertador	10/27/2014	OR187449
22152	Esteller	Portuguesa	Uveral	11/05/2014	OR187448
22153	Ricaurte	Cojedes	Libertad de Cojedes	11/07/2014	OR187425
22155	Ospino	Portuguesa	Aparición	11/13/2014	OR187450
22158	A R Costa	Táchira	Las Mesas	11/27/2014	OR187451
22161	J C Salas	Zulia	Capital Julio Cesar Salas	12/03/2014	OR187429
22164	Miranda	Zulia	San Antonio	12/15/2014	N/A
22165	R de Perija	Zulia	El Rosario	12/15/2014	N/A
22168	Mo Iturriza	Falcon	Tocuyo de la Costa	01/06/2015	OR187452
22173 B	Aragua	Anzoátegui	Aragua de Barcelona	07/28/2015	OR187453
22178	S Planas	Lara	Sarare	11/23/2015	N/A
22176 B	Guacara	Carabobo	Yagua	10/20/2015	OR187454
22179 (s)	S Planas	Lara	Sarare	12/18/2015	OR187455
22180	Libertador	Merida	Milla	12/18/2015	OR187456
22183	Aricagua	Merida	Aricagua	04/20/2016	N/A
22184	C Quintero	Merida	Santo Domingo	05/18/2016	N/A
22187	M Carmelo	Trujillo	Santa María de Horcón	07/04/2016	N/A
22188	San Carlos	Cojedes	San Carlos de Austria	07/04/2016	N/A
22189	San Carlos	Cojedes	San Carlos de Austria	07/29/2016	N/A
22190	San Carlos	Cojedes	San Carlos de Austria	09/08/2016	N/A
22192	G de Hevia	Táchira	José Antonio Páez	09/14/2016	N/A
22196	Pedraza	Barinas	Páez	11/24/2016	N/A
22197	Baralt	Zulia	Marcelino Briceño	11/21/2016	N/A
22198	Baralt	Zulia	Marcelino Briceño	11/21/2016	N/A
22194	Piritu	Falcon	Piritú	09/19/2016	OR187457
22203	A Adriani	Merida	Presidente Páez	02/22/2017	N/A

s: swine origin; e: equine origin. All other samples are from cattle.
Source: Elaborated by the authors.

RNA extraction and reverse transcription polymerase chain reaction

RNA was extracted using TRIzol LS reagent (Thermo, United States of America). After extraction, RNA was submitted to reverse transcription polymerase chain reaction (RT-PCR) using primers described by Rodriguez et al. (1993) – VSV.NJ.P-102 (5'GAGAGGATAAATATCTCC3') and VSV.NJ.P-744 (5'GGGCATACTGAAGAATA3') – to amplify a 642 nucleotide segment of the hypervariable region phosphoprotein gene. Reverse transcription and amplification were performed in one step using One-Step RT-PCR Kit (Qiagen, Germany) and primers in a final concentration of 1, and 5 μM in a 25-μL reaction. Thermocycler (Proflex, Thermo, United States of America) was programmed for the following protocol: 50°C for 30 minutes, 95°C for 15 minutes, and 40 cycles of 94°C for 1 minute, 50°C for 1 minute and 72°C for 1 minute, and a final extension step of 72°C for 5 minutes. During all the procedures, three controls were used: an extraction control to access contamination during RNA extraction, a blank control for PCR, and a positive control for PCR. Reverse

transcription-quantitative polymerase chain reaction (RT-qPCR) for FMD polymerase was also run in all samples to rule out the presence of the FMD virus following the methodology described elsewhere (Callahan et al., 2002).

Nucleotide sequence determination and phylogenetic analysis

After cDNA amplification, the samples were submitted to sequencing using Big Dye v.3.1 (Thermo, United States of America) and the equipment Genetic Analyser 3500 (Thermo, United States of America). Samples were edited using BioEdit (Hall, 1999). Phylogenetic trees were reconstructed using Tamura 3 parameters with gamma distribution nucleotide substitution model and maximum likelihood model for tree reconstruction with 1,000 bootstrap replicas in Mega 7.0 (Kumar et al., 2016). Sequences obtained from GenBank were used for comparison.

Exploration of spatial distribution and environmental factors

The spatial distribution of outbreaks where samples were collected was defined using the Geographical Information System (ArcGIS, version 10.6). The outbreaks that occurred in Venezuela between 2009 and 2017 were mapped by *parroquia* (third subnational administrative level).

Information related to altitude, mean monthly precipitation, and mean monthly temperature between 2000 and 2018 was obtained from WorldClim, developed by Harris et al. (2020), on Climatic Research Unit Time-Series (CRU-TS 4.06) downscaled with WorldClim 2.1 (Fick; Hijmans, 2017).

Seven vegetation categories represented in Venezuelan territory (deserts and xeric shrublands, flooded grasslands and savannas, mangroves, montane grasslands and shrublands, tropical and subtropical dry broadleaf forests, tropical and subtropical grasslands, savannas and shrublands, and tropical and subtropical moist broadleaf forests). Data from ecoregions and vegetation were obtained from the World Wide Fund for Nature (WWF) developed by Olson et al. (2001). Twenty-five terrestrial ecoregions represented in Venezuelan territory were extracted from The Nature Conservancy (TNC) developed by Olson; Dinerstein (2002): Amazon-Orinoco-Southern Caribbean mangroves, Apure-Villavicencio dry forests, Araya And Paria xeric scrub, Catatumbo moist forests, Cordillera de Mérida Páramo, Cordillera la Costa Montane forests, Cordillera Oriental Montane forests, Guajira-Barranquilla xeric scrub, Guianan Highlands moist forests, Guianan moist forests, Guianan piedmont, and lowland moist forests, Guianan savanna, Japurá-Solimoes-Negro moist forests, La Costa xeric shrublands, Lara-Falcón dry forests, Llanos, Maracaibo dry forests, Negro-Branco moist forests, Northern Andean Páramo, Orinoco Delta swamp forests, Orinoco wetlands, Pantepui, Paraguana xeric scrub, Rio Negro Campinarana, Venezuelan Andes montane forests (Table 2).

Starting from the climatic variables at the *parroquia* level, the clustering of all samples was performed using principal component analysis followed by hierarchical clustering (Lê et al., 2008; Husson et al., 2010; Husson, 2011). Subsequently, through graphical representations of the distributions of means and standard deviations of the samples per cluster, the behavior of the groups to climatic variables was examined.

Using the genetic groups as covariable, the climatic variables were evaluated through exploratory analysis, and later, all samples were classified using principal components analysis, followed by hierarchical clustering (Lê et al., 2008; Husson et al., 2010; Husson, 2011), to detect whether outbreaks would have any association related to environmental determinants.

RESULTS

Reverse transcription polymerase chain reaction and phylogenetic analysis

A cDNA segment 642 nt length comprising the hypervariable region of the phosphoprotein gene of VSVNJ was amplified from all samples in Table 1. Among the total amount of samples (78), 51 gave a cDNA product of quality enough to be sequenced. A phylogenetic tree was built with the obtained sequences. From the topology of the constructed tree, it was observed that sequences aligned closely from each other (Fig. 1). Eight sequences from Colombia, Costa Rica, Ecuador, and Honduras aligned close to the Venezuelan samples (Fig. 1). Also, a group of sequences from Colombia and Ecuador obtained from GenBank aligned close to the Venezuelan samples, in a separate group (Fig. 1 – the GenBank code of sequences included for comparison are available from authors upon request).

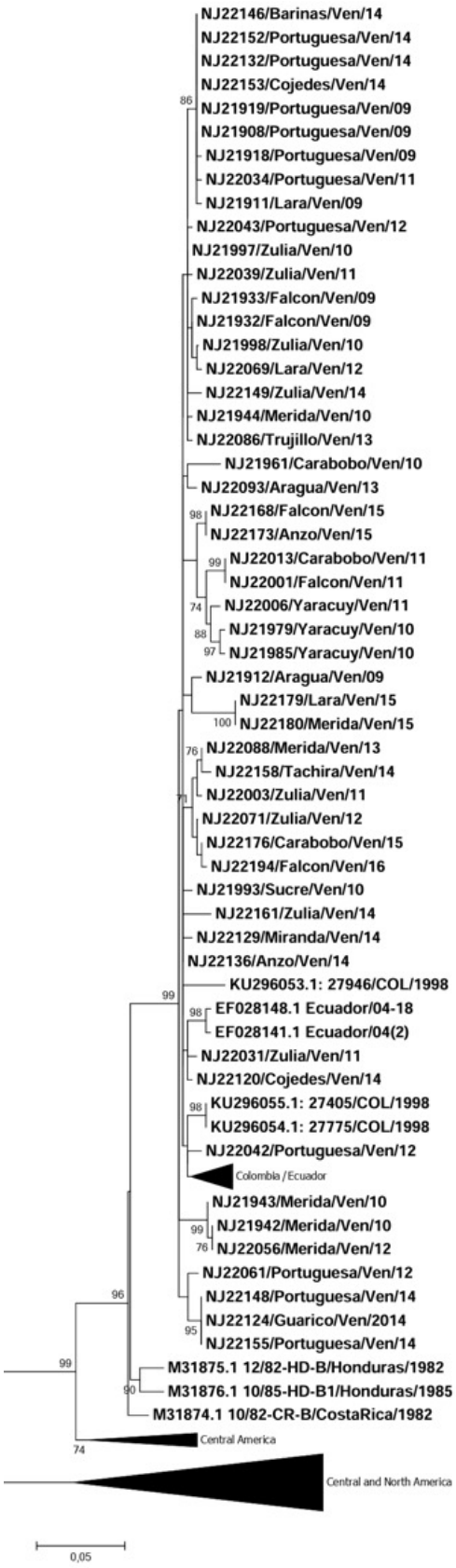

Figure 1. Maximum likelihood phylogenetic tree containing 51 sequences from vesicular stomatitis virus samples collected in Venezuela from 2009 to 2017 and their relationship with sample sequences retrieved from GenBank. Black triangles contain sequences from Colombia and Ecuador, countries from Central America, and countries from Central and North America, respectively. Reference for the sequences are available from authors upon request.
Source: Elaborated by the authors.

Spatial distribution and environmental factors

Samples analyzed were collected in vesicular stomatitis outbreaks distributed in 17 states, 53 municipalities (second administrative division), and 62 *parroquias* (third administrative division). Figure 2 shows the spatial distribution of those vesicular stomatitis outbreaks from 2009 to 2017. It was observed that the outbreaks are distributed throughout the northern part of the country, without a well-defined distribution pattern.

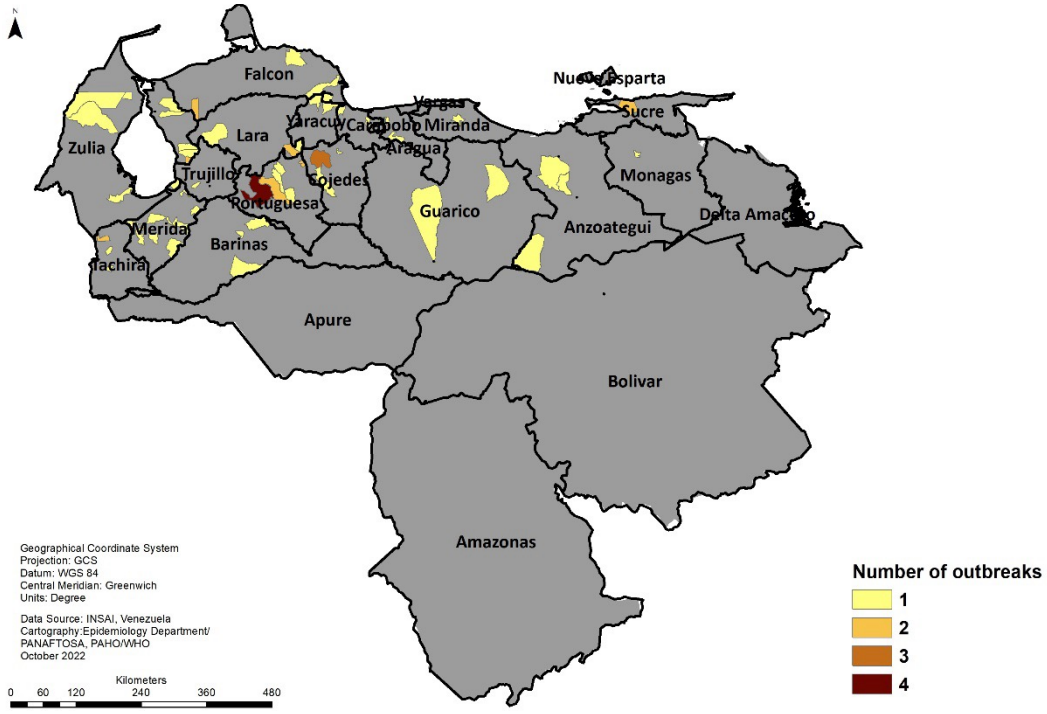

Figure 2. Spatial distribution of those vesicular stomatitis outbreaks from 2009 to 2017 per parroquia.
Source: Elaborated by the authors.

The altitude of affected municipalities varies widely, from sea level (Sucre, and surroundings of Lake Maracay in Zulia and Trujillo) up to mountain level (Andean area up to 1,000–3,400 m of altitude in Mérida, Zulia, Táchira, and Trujillo). The mean monthly temperature between 2000–2018 varies from 8°C in subtropical high areas to 28°C in tropical low areas. The mean monthly precipitation registered varies between 66 and 180 mm. Samples analyzed came from 11 of the 25 terrestrial ecoregions described in Venezuela, corresponding to Apure-Villavicencio dry forests (NT0201), Araya and Paria xeric scrub (NT1301), Catatumbo moist forests (NT0108), Cordillera de Mérida Páramo (NT1005), Cordillera la Costa montane forests (NT0117), Guajira-Barranquilla xeric scrub (NT1308), La Costa xeric shrublands (NT1309), Lara-Falcón dry forests (NT0219), Llanos (NT0709), Maracaibo dry forests (NT0222), and Venezuelan Andes montane forests (NT0175). These ecoregions represent five different types of vegetation: deserts and xeric shrublands (13), montane grasslands and shrublands (10), tropical and subtropical dry broadleaf forests (2), tropical and subtropical moist broadleaf forests (1), and tropical and subtropical grasslands, savannas and shrublands (7). Description of environmental determinants per Parroquia were VS outbreaks occurred are available from authors upon request.

The hierarchical clusters analysis resulted in the composition of three groups, with altitude, inversely proportional and highly correlated with temperature, and precipitation being the two axes that best divide the cases found (Fig. 3). Cluster 1 represents cases that occur at significantly higher altitudes and lower temperatures than clusters 2 and 3. Clusters 2 and 3, in turn, are defined mainly by their rainfall volume difference: the first being drier and the second rainier. The spatial distribution of the three clusters can be seen in Fig. 4.

Thus, the cases evaluated are composed of three major weather patterns: cluster #1 (n = 9) presented high altitudes with low temperatures and precipitation (2,259 m, 15,2°C and 92.2 mm/m^2, respectively); cluster #2 (n = 43) presented low altitude and precipitation with high temperatures (231 m, 94.6 mm/m^2, 26,1°C), and cluster #3 (n = 10) presented low altitude and high precipitation and temperature (175 m, 138 mm/m^2, 27°C).

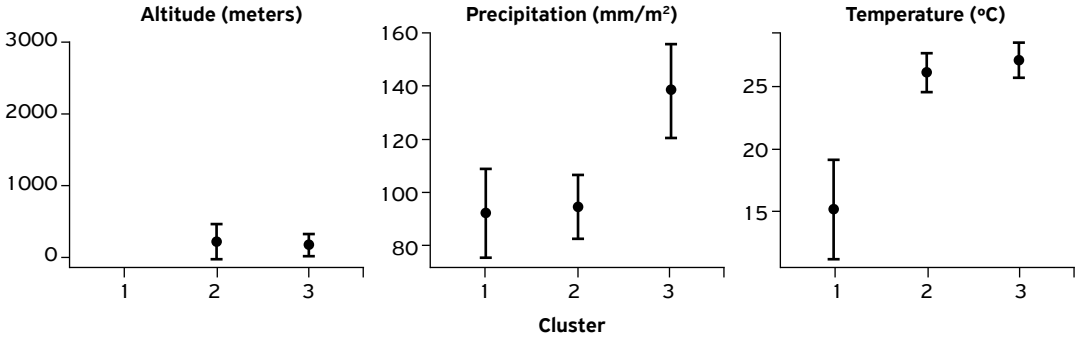

Figure 3. Mean and standard deviation of altitude, precipitation, and temperature through hierarchical groups. The graphs display the means of the samples and approximately one standard deviation for the climatic variables within each cluster. Altitude distinguishes cluster 1 from the other ones, while precipitation sets apart clusters 2 and 3. Through this analysis, a clear separation among the groups can be observed, highlighting that the disease manifests across distinct climatic patterns found in Venezuela.
Source: Elaborated by the authors.

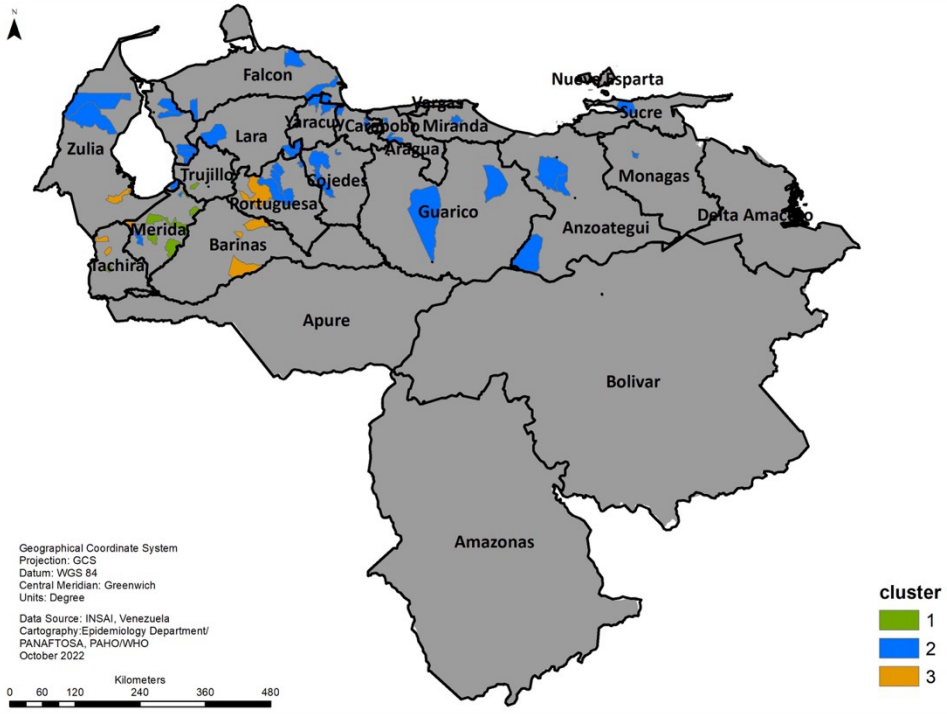

Figure 4. The spatial distribution of clusters 1, 2, and 3, is described in Fig. 3.
Source: Elaborated by the authors.

DISCUSSION AND CONCLUSIONS

The present study focused on VSVNJ isolates obtained, as part of the routine vesicular disease surveillance activity, in the official Veterinary Diagnostic Laboratory in Venezuela. The isolates originated in outbreaks that occurred in the country during the period from 2009 to 2017. Only samples that tested VSVNJ positive by typing ELISA were sent to PANAFTOSA for molecular characterization. The topology of the phylogenetic tree obtained shows that sequences align close to each other, suggesting that, despite the time elapsed between outbreaks and the different ecological regions where samples came from, the virus remains genetically stable in the field. This finding is in contrast with the occurrence of virus selection by ecological factors reported previously on VSNJV isolates from countries in Central America (Rodriguez et al., 1996; Rodriguez et al., 2000; Velazquez-Salinas et al., 2014). The limited field data available in the present study do not allow authors to elaborate an explanation for differences observed with previous reports.

We found the VSVNJ in a wide range of environmental conditions, at high and low altitudes and with variable levels of precipitation and temperatures, being impossible to define an ecological pattern favoring the occurrence of clinical

disease or driving the genetic evolution of the virus. Furthermore, as the analysis was limited to positive VSNJV samples identified by typing ELISA, we did not attempt to extrapolate any conclusion regarding epidemiological characterization. This study did not intend to establish an associative quantitative gradient of occurrence of vesicular stomatitis according to the ecological factor, but to describe the environmental conditions where it occurred.

Venezuela is engaged in the Hemispheric Plan for FMD Eradication. In this context, the veterinary services maintain a vesicular disease surveillance system to detect and investigate events compatible with vesicular diseases in the country. The identification, control, and eradication of FMD is the main target of the system. However, as VSV is present in the country and its clinical manifestation in animals is similar to FMD, its occurrence is detected and recorded by the same surveillance system. The report of VSV outbreaks with laboratory diagnosis is indicative that the vesicular disease surveillance system from Venezuela can detect vesicular events. However, it should be acknowledged as a limitation of the possibility of bias due to underreporting.

To our knowledge, this is the first VSVNJ molecular study in Venezuela with an analysis of environmental factors associated with the outbreak areas. Further embracing studies would contribute to gaining insights into the epidemiology of the disease in the country and its impact on national livestock.

AUTHORS' CONTRIBUTIONS

Conceptualization: Sánchez-Vázquez, M.J.; Allende, R.M.; **Resources:** Álvarez Rivera, A.M.; Alcázar Guerra, W.J.; **Methodology:** Sánchez-Vázquez, M.J.; **Investigation:** Álvarez Rivera, A.M.; Lima, D.M.; Nascimento, M.L.; **Data curation:** Álvarez Rivera, A.M.; Alcázar Guerra, W.J.; Fonseca Junior, A.A.; Almeida, I.G.; Pituco, E.M.; **Formal analysis:** Buzanovsky, L.P.; Fonseca Junior, A.A.; Almeida, I.G.; **Visualization:** Buzanovsky, L.P.; Fonseca Junior, A.A.; Almeida, I.G.; Lima, D.M.; **Supervision:** Sánchez-Vázquez, M.J.; Pituco, E.M.; **Writing – original draft:** Allende, R.M.; **Writing – review and editing:** Sánchez-Vázquez, M.J.; Allende, R.M.

AVAILABILITY OF DATA AND MATERIAL

All the data will be available from authors upon request.

FUNDING

Not applicable.

CONFLICTS OF INTEREST

Nothing to declare.

ETHICAL APPROVAL

Not applicable.

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