

First report of phleboviruses in phlebotomine sandfly communities from northern Colombia

Primer registro de flebovirus en comunidades de flebotomíneos del norte de Colombia

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Abstract

The genus *Phlebovirus* is considered a medically important group of viruses due to the pathogenic behavior of some species. In the Americas, several species of this genus have been identified, most of which are found in Panamá. However, the lack of knowledge about the circulation of phleboviruses in Colombia led us to ask several questions, including the distribution of this pathogen in the Americas, especially in Caribbean coastal areas where ideal conditions for phlebovirus vectors exist. Here, we report the first detection of the genus *Phlebovirus* in phlebotomine sandfly communities from the Caribbean coast of Colombia. Sandflies were collected and pooled for ribonucleic acid (RNA) extraction and retrotranscription, all pools were tested for 18S ribosomal RNA and screened for phleboviruses by nested reverse transcription-polymerase chain reaction (RT-PCR). Positive products for the RNA-dependent RNA polymerase (RdRp) detection were sequenced. Four pools of 58 were positive for viruses belonging to the Punta Toro complex, Aguacate complex, Old-World viruses, and one related to sloth phleboviruses. This is the first evidence of phleboviruses in the Caribbean region of Colombia, especially of species related to the Punta Toro and Aguacate complex.

Keywords: Aguacate complex, La Piche, *Phlebovirus*, Phlebotominae, Punta Toro complex

Resumen

El género *Phlebovirus* se considera un grupo de virus de importancia médica debido al comportamiento patogénico de algunas especies. En América se han identificado varias especies de este género, la mayoría en Panamá, sin embargo, el desconocimiento sobre la circulación en Colombia nos hace plantearnos varias interrogantes, entre ellas la distribución de este patógeno en la zona, especialmente en la costa Caribe donde existen las condiciones ideales para estos vectores. En este trabajo reportamos la primera detección del género *Phlebovirus* en comunidades de flebotomíneos de la costa Caribe de Colombia. Los insectos se recolectaron y agruparon para la extracción y transcripción reversa (RT-PCR) de ácido ribonucleico (ARN), todos los grupos se analizaron para detectar ARN ribosomal 18S y virus del género *Phlebovirus* mediante RT-PCR anidada. Se secuenciaron los productos de PCR positivos de ARN polimerasa dependiente de ARN (RdRp). Cuatro grupos de 58 resultaron positivos a virus pertenecientes al complejo Punta Toro,

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complejo Aguacate, virus del Viejo Mundo y un último relacionado con flebovirus de Perezosos. Esta es la primera evidencia de flebovirus en la región Caribe de Colombia, especialmente de especies relacionadas con el complejo Punta Toro y Aguacate.

Palabras clave: complejo Aguacate, complejo Punta Toro, La Piche, *Phlebovirus*, Phlebotominae

Phlebovirus (Hareavirales: Phenuiviridae) is known as a group of viral entities with a tri-segment genome, some of which are responsible for several human diseases. The clinical manifestations of phleboviral infections may include non-specific symptoms like fever, headache, myalgia, fatigue, and retro-orbital pain. In severe cases, the central nervous system may also be involved with symptoms such as depression, meningitis, meningoencephalitis, and even death. Phleboviruses are mainly transmitted by phlebotomine sandflies, ticks, and mosquitoes (Ayhan & Charrel, 2019).

The genus *Phlebovirus* (most of which are currently named with a binomial name), comprises 67 viral entities, 58 of which have been detected or isolated from phlebotomine sandflies. Due to their relevance in public health, *Phlebovirus* (*P.*) *toscanaense*, *P. siciliaense*, *P. napoliense*, and *P. riftense* reported from Europe, Eurasia, and Africa, are the best-known phleboviruses. Meanwhile, in the Americas, sandfly-borne phleboviral diseases in humans so far are caused by *P. aguacateense*, *P. chagresense*, *P. cocleense*, and *P. toroense* in Panama, and there are reports of *P. alenquerense* and *P. candiruense* causing self-limiting fever in humans from Brazil (Lambert & Hughes, 2021). On the other hand, in Colombia, poor knowledge about isolation records of *P. leticiaense* (Amazonian region); *P. buenaventuraense* (Pacific region); *P. aguacateense* and *P. duraniaense* from the Andean region during the 1980s (Tesh et al., 1989) was obtained from phlebotomine sandfly communities.

In Colombia, phlebotomine sandflies are represented by at least 154 species, 33 of which are recorded in the Caribbean region (Bejarano & Estrada, 2016; Velasquez-Londono et al., 2022). In this region, Los Montes de Maria, located in the departments of Bolívar, Córdoba and Sucre (Rueda-Concha et al., 2022) is the most important mixed foci of visceral and cutaneous leishmaniasis where *Lutzomyia evansi*, an anthropophilic sandfly species has been involved in *Leishmania infantum* transmission. Also, there is a recurrent pattern of anthropophilic behavior in other

phlebotomine species from the region even in those sandfly species not involved in current *Leishmania* transmission (Paternina et al., 2016).

Despite many common geographical and ecological features between Panama and Colombia, there are no recent reports of phlebovirus detection from phlebotomine sandflies, nor records of phleboviral diseases in Colombia. However, it is suspected that phleboviral diseases may be overlooked because of the high incidence of common tropical fevers such as dengue, Zika, and chikungunya (Mattar & González, 2017). To support the knowledge about these viruses, we provide the first report of *Phlebovirus* circulation among phlebotomine sandfly communities from areas of transmission of *Leishmania* spp. in the Caribbean region of Colombia.

Phlebotomine sandflies were caught between February and November 2021 in forest patches from three Departments in northern Colombia selected by convenience: Bolívar Department, Córdoba Department and Sucre Department (Figure 1). Locations from Sucre: a periurban residential neighborhood of Sincelejo city (Capitol, urban population ~ 265,000 inhabitants) located at 9°15'51.6"N, 75°25'27.4" W, a rural area of Ovejas city (rural population ~ 15,000 inhabitants) located at 9°31'28.1"N, 75°13'37.0"W, and a rural area of Toluviejo city (rural population ~ 13,000 inhabitants) located at 9°31'03.6"N, 75°25'11.3"W; Bolívar Department: a periurban area San Juan de Nepomuceno city (urban population ~ 26,000 inhabitants) located at 9°57'45.5"N, 75°05'26.9"W; Córdoba Department: a rural area from Montería (Capitol, rural population ~ 110,000 inhabitants) located at 8°40'51.7"N, 75°54'28.1"W and a periurban area from Purísima de la Concepción (urban population ~ 7,000 inhabitants) in the coordinates 9°19'40.7"N, 75°39'28.7"W.

Phlebotomine sampling was performed in each locality using a Shannon trap between 18:00 and 22:00 with a sampling effort of four hours per person, and four Centers for Disease Control and

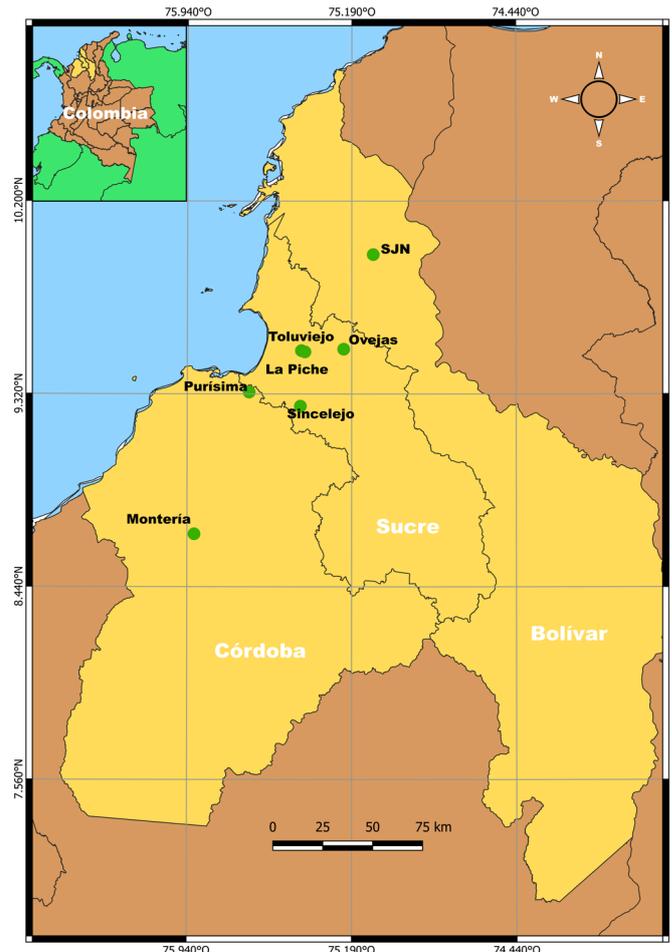


Figure 1. Geographical distribution of sampling points across the three departments in the Caribbean region of Colombia. San Juan Nepomuceno (S.J.N.).

Prevention (CDC) light traps between 18:00 and 06:00 with a sampling effort of 12 hours per trap. Live sandflies were transported to the laboratory and were grouped (up to 120 individuals per pool) according to location and collection date. The entire group of phlebotomine sandflies was tested without a morphological identification in order to increase the chance of detecting viral ribonucleic acid (RNA) (Dachraoui et al., 2023).

To get an approximate sandfly species composition for each pool, a subsample of sandflies was used for identification by comparing their morphological features (morphotyping) to taxonomic keys for American phlebotomine sandflies (Galati, 2021). This identification followed the traditional taxonomic scheme of three New World genera, *i.e.*, *Lutzomyia*, *Brumptomyia*, and *Warileyia* (Young & Duncan, 1994). This identification process requires a chemical clearance of anatomical structures of the insects by using lactophenol (mix of lactic acid and phenol, 1:1 proportion) that is extremely harmful to nucleic acids and other biomolecules. Therefore,

those insects used for taxonomic identification were not available for molecular analysis.

Phlebotomine sandfly pools were subjected to RNA extraction using an RNA Miniprep Monarch kit of New England Biolabs (NEB), following manufacturer instructions. RNA was stored at $-80\text{ }^{\circ}\text{C}$ until further molecular tests. Total complementary DNA (cDNA) was obtained from three microliters of total RNA solution and 200 U of reverse transcriptase of Moloney Murine Leukemia Virus (M-MuLV) and subsequently submitted to 18S ribosomal RNA amplification using primers 18S417/18S920c (Hoffmann et al., 2004).

A ~244-bp phleboviral RNA-dependent RNA polymerase (RdRp) gene fragment was targeted for molecular detection using a generic nested polymerase chain reaction (nPCR) (Sanchez-Seco et al., 2003). *Uukuvirus lihanense* was used as a positive control. The polymerase chain reaction (PCR) products of the RdRp gene were sequenced and then analyzed for molecular identification

using Basic Local Alignment Search Tool for standard nucleotide (BLASTn) online software and Maximum likelihood (ML) tree in MEGA XI software to identify viral sequences according to clustering pattern and genetic distances.

In total, 5954 phlebotomine sandflies were caught, 5776 of which were grouped into 58 analysis pools for RNA extraction, insect 18S ribosomal RNA amplification, and *Phlebovirus* detection. A total of 178 morphotyped specimens (~3%) were identified as belonging to 13 phlebotomine sandfly species (Table 1). According to our sampling, San Juan de Nepomuceno had the highest number of sandfly species and Montería had the lowest. In all localities, anthropophagic and medically relevant sandfly species such as *Lutzomyia evansi* (known as the dominant species and regional vector of *Leishmania infantum*), *Lutzomyia gomezi*, *Lutzomyia micropyga*, *Lutzomyia shannoni* and *Lutzomyia rangeliana* (Paternina et al., 2016) were predominate.

All pools were positive for sandfly 18S quality control PCR, four of which also were positive for *Phlebovirus* with the expected size (Figure 2), which corresponds to a minimum infection rate (MIR) of 1.4 in sandflies of Toluvejo municipality (18 pools, three positive) and a MIR of 1.25 in

sandflies from Sincelejo municipality (10 pools, one positive).

The sequences obtained from amplicons were named after the place of origin as LaPicheV49, LaPicheV53, and LaPicheV57 for positive samples from Toluvejo (rural area) collected in October and SanNicolasV for the pool from Sincelejo (periurban area) collected in April. Sequences were deposited in GenBank Database under accession codes ON093906-ON093909. Protein BLAST (BLASTp) analysis of amino acid sequences translated, showed that LaPicheV49 is related to *P. aguacateense* (HM566138, Coverage: 97%, Identity: 85%), LaPicheV53 to *P. toroense* (KP272043, Coverage: 94%, Identity: 76%), LaPicheV57 to *P. penshurtense* (MN163121, Coverage: 100%, Identity: 77%), SanNicolasV to two Old World viruses found in sandflies, *i.e.*, an unclassified *Phlebovirus* from sandflies of Cyprus (KM111521; coverage, 99%; identity, 68%) and to *P. siciliaense* (AB905361, Coverage: 94%, Identity: 68%).

The BLAST analysis results were supported by the ML (Figure 3) trees, both of which have the same topology in the location of the sequences obtained. These results show with high confidence the clustering of the LaPicheV57 sequence with viruses

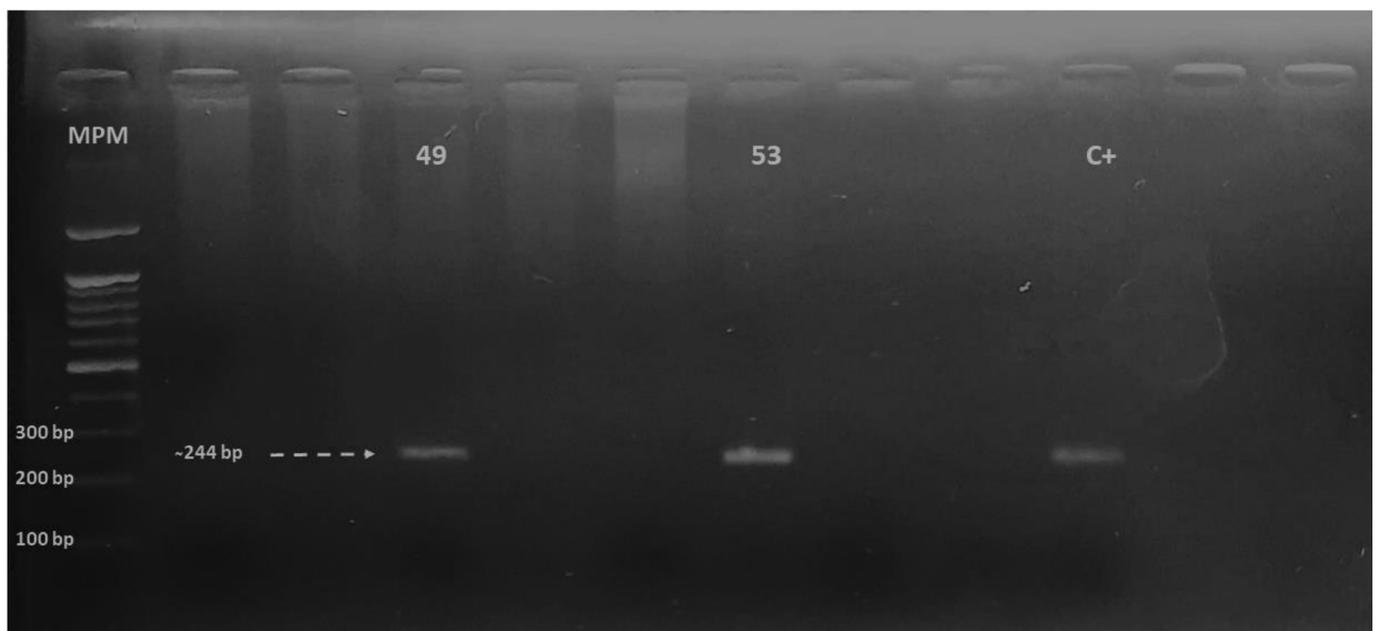


Figure 2. Agarose gel electrophoresis with two positive samples. 49: sample LaPicheV49; 53 sample LaPicheV53, bands of approximately 244 bp.

isolated from sloths such as *P. penshurtense* and *P. anhangense* (de Oliveira Filho et al., 2020) despite the considerable divergence (11.39%). LaPicheV49 belongs to Aguacate complex and their low distance values (3%) show that it is probably a local variant of *P. aguacateense*. LaPicheV53 belongs to the Punta Toro complex and could be a variant of a *P. toroense* strain, previously named Capira virus (CAPV) because they are closely related (3.7%). Finally, the clustering of SanNicolasV with medically important Old World phleboviruses is striking but presents high genetic distances (33%) from known viruses reported in genetic databases. In consideration of the aminoacidic distances threshold for species delimitation in this genus according to the International Committee on Taxonomy of Viruses (ICTV) (5%), we believe that LaPicheV57 and SanNicolasV may represent a new viral species.

In Colombia, previous studies show phleboviruses were restricted to phlebotomine sandflies from the Andean, Pacific, and Amazonian regions (Tesh et al., 1989). Meanwhile, in the Caribbean region of Colombia there is only one report of arboviruses

in sandflies, the insect-specific Piura virus from *Lutzomyia evansi* (Contreras-Gutiérrez et al., 2016). Although previous to this report there was no evidence of *Phlebovirus* species circulation in the Caribbean region of Colombia. Mattar and González (2017) expressed strong concerns about the possibility that *P. toroense* is present in the country, and phleboviral infections causing febrile syndrome among humans might be overlooked and misdiagnosed as dengue or other common viral infections, similar to the Panamanian scenario.

In addition, it is important to highlight the presence of *Lutzomyia panamensis* in Toluviéjo, since this species has been found with *Phlebovirus almendrasense*, *Phlebovirus gloriaense* and *Phlebovirus penablancaense* in Panama (Marklewitz et al., 2019; Tesh, 1988).

Concerning the 201 bp nucleotide fragment, represented by 67 amino acids used for the molecular identification of the viruses, it was useful due to the similarity of the results obtained when comparing strains of the same or different species with the complete RdRp protein. Because

Table 1. Phlebotomine sandfly community composition from northern Colombia

	Sincelejo	Ovejas	Toluviéjo	Montería	Purísima	SJN
Sandflies tested	798	461	2145	267	1660	445
Pools (<i>Phlebovirus</i> +)	10 (1)	6 (0)	18 (3)	3 (0)	14 (0)	7 (0)
Sandflies for taxonomy	27	29	51	30	21	20
Species composition of sandfly communities						
<i>Lutzomyia olmeca bicolor</i>	-	-	4	-	-	-
<i>Brumptomyia mesai</i>	-	-	3	-	-	2
<i>Lutzomyia dubitans</i>	5	4	2	-	-	2
<i>Lutzomyia. gomezi</i> *	-	6	-	24	6	2
<i>Lutzomyia c. cayennensis</i>	7	2	-	-	-	1
<i>Lutzomyia micropyga</i> *	-	2	-	-	-	2
<i>Lutzomyia trinidadensis</i>	1	-	-	-	-	5
<i>Lutzomyia venezuelensis</i>	-	-	-	-	1	2
<i>Lutzomyia evansi</i> *	7	10	8	6	9	3
<i>Lutzomyia rangelliana</i>	3	-	-	-	-	-
<i>Lutzomyia shannoni</i> *	-	1	-	-	1	-
<i>Lutzomyia carpenteri</i>	-	-	1	-	-	1
<i>Lutzomyia panamensis</i> *	4	4	33	-	4	-
	27	29	51	30	21	18

Asterisk (*) denotes sandfly species with proven anthropophagic behavior. San Juan de Nepomuceno (SJN).

the detected viruses are related to viral groups with some relevance in veterinary and medical health, we believe that the epidemiological scenarios suggested by Mattar and Gonzalez (2017) are highly possible since some members of the Aguacate complex are capable of infecting primate cells and are highly pathogenic for mice in laboratory tests and some Punta Toro Complex agents can cause human febrile syndromes similar to dengue (Gundacker et al., 2017; Palacios et al., 2011; Tesh et al., 1989).

Therefore, it is important to conclude that the viruses of the genus *Phlebovirus* are present on the Caribbean coast of Colombia, some of which are related to viruses implicated in febrile diseases. This geographic area has several sandfly species that would support the transmission of the virus to humans due to their anthropophagic behavior,

such as *Lu. evansi* the species most abundant which has been reported in previous studies (Lambrano et al., 2012; Paternina et al., 2016; Pérez-Doria et al., 2008). Our results represent the first detection of these viruses in Colombia since the Robert Tesh group works during 1980's and sheds light on the epidemiology of arbovirus transmitted by sandflies in northern Colombia, although further research on vector identification and isolation of the virus to characterize the viruses are required to cover most aspects of transmission cycle.

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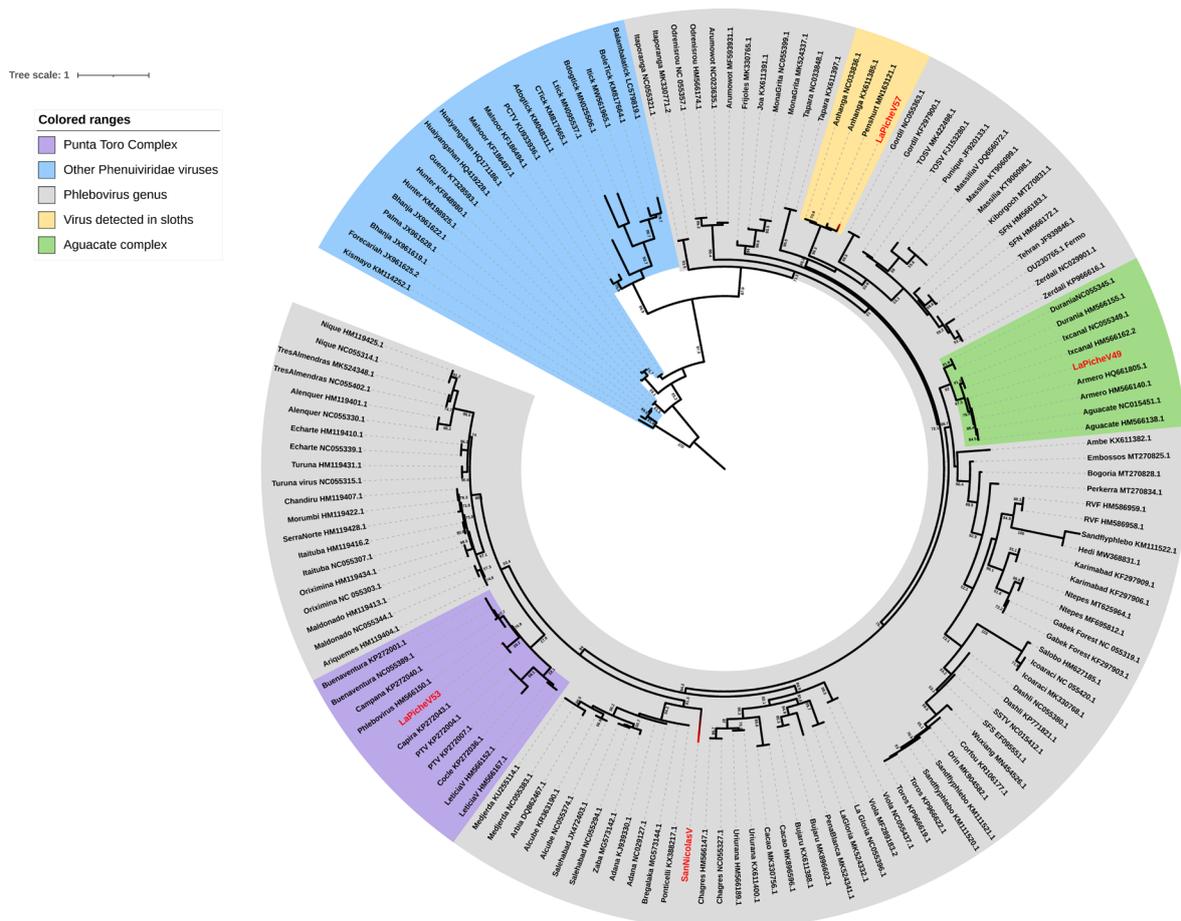


Figure 3. Maximum likelihood tree of known *Phlebovirus* species strains from northern Colombia samples using the partial sequence of RdRp gene. The analysis was performed by the ML method and LG+G model with 1000 bootstrap replicates on aminoacidic sequences. Names in red are the sequences obtained in this work. Changping Tick Virus (CTick); Pacific coast tick virus (PCTV); *Uukuvirus dermacentoris* (Adogtick); *Uukuvirus lihanense* (Ltick); Brown dog tick virus (Bdogtick); Iftin tick virus (Itick); *P. siciliaense* (SSTV); *P. riftense* (RVF); *P. ntepesense* (Ntepes); Sandfly fever Naples virus (SFN); *P. toscanaense* (TOSV); *P. toroense* (PTV).

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CONFLICT OF INTEREST

The authors declare no competing interests.

AUTHOR CONTRIBUTION

Luis Romero Ricardo participated as the principal author of the investigation in the methodology, analysis in software, and writing the original draft. Luis Paternina Tuirán participated in designing methods, writing – review & editing. Eduar Bejarano Martínez supported the methodology, Writing – review & editing.

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