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Isolation of Madre de Dios Virus (*Orthobunyavirus*; *Bunyaviridae*), an *Oropouche Virus* Species Reassortant, from a Monkey in Venezuela

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Abstract. *Oropouche virus* (OROV), genus *Orthobunyavirus*, family *Bunyaviridae*, is an important cause of human illness in tropical South America. Herein, we report the isolation, complete genome sequence, genetic characterization, and phylogenetic analysis of an OROV species reassortant, Madre de Dios virus (MDDV), obtained from a sick monkey (*Cebus olivaceus* Schomburgk) collected in a forest near Atapirire, a small rural village located in Anzoategui State, Venezuela. MDDV is one of a growing number of naturally occurring OROV species reassortants isolated in South America and was known previously only from southern Peru.

INTRODUCTION

Viruses in the family *Bunyaviridae* are currently classified into five genera: *Orthobunyavirus*, *Hantavirus*, *Nairovirus*, *Phlebovirus*, and *Tospovirus*.[1] The *Orthobunyavirus* genus includes a number of zoonotic pathogens transmitted by arthropod vectors like culicoides midges, mosquitoes, phlebotomine sandflies, and ticks; it is the largest of the five genera within the family.[2] Orthobunyaviruses are enveloped viruses with negative sense RNA genomes consisting of three segments: small (S), medium (M), and large (L). The S segment encodes the nucleocapsid and a small nonstructural protein, NSs. The M segment encodes the glycoproteins Gn and Gc, whereas the L segment encodes the viral polymerase.[3]

Oropouche virus (OROV) is a species within the Simbu serogroup of the *Orthobunyavirus* genus, and it causes a febrile illness in humans characterized by headache, dizziness, weakness, myalgia, and arthralgia.[4–6] OROV was first isolated from a pool of *Coquillettidia venezuelensis* mosquitoes collected in Trinidad,[7] and later from *Aedes* (*Ochlerotatus*) *serratus* and *Culex quinquefasciatus* mosquitoes,[8] and frequently from the midge *Culicoides paraensis* in Brazil.[7,9,10]

The virus has also been isolated from several wild mammals including a three-toed sloth, *Bradypus tridactylus*[11] and the marmoset, *Callithrix penicillata* (Nunes and others.[12] Because of the clinical similarity of OROV infection to other endemic arboviral illness diseases such as dengue, Mayaro, chikungunya, and Zika fevers, and the paucity of cases confirmed by laboratory tests, the true public health burden of OROV infection in South American remains unclear.

Genetic reassortment among orthobunyaviruses of the same serogroup occurs frequently in nature and has led to the emergence of new viruses, occasionally with increased pathogenicity.[13] On the basis of genetic and antigenic analyses, several reassortant viruses involving OROV and other unknown Simbu serogroup viruses have been described. These include Iquitos virus (IQTV), Madre de Dios virus (MDDV), and Perdões virus (PDEV), which contain the S and L segments of OROV, and the M segments of other still unrecognized Simbu serogroup viruses.[14–16]

Herein, we report the complete genome sequence of an isolate of an OROV reassortant obtained from a moribund monkey (*Cebus olivaceus* Schomburgk) collected in Venezuela during an epizootic of monkey deaths in 2010. This is the first report of the isolation, complete genome sequence, and evidence of an OROV reassortment event in Venezuela. We also discuss the importance of this evolutionary mechanism in terms of the epidemiology of OROV.

MATERIALS AND METHODS

Study site and epizootic background. Atapirire village is located in the southeastern part of Venezuela, within Anzoategui State, Miranda municipality. Atapirire lies 51 km south of El Tigre city and 96 km north of the largest city in the region, Ciudad Bolivar, Capitol of Bolivar State, which borders Brazil (Figure 1). Atapirire is a small rural village with a population of ~800, surrounded by gallery and secondary forests in close proximity to the Orinoco river. The region is part of the biogeographic Central Llanos (lowland plains or savannas) of Venezuela. The llanos are part of the Orinoco river basin; the river separates this biogeographic region from the Guiana Shield and Amazonian regions in southern Venezuela, adjacent to the borders with Colombia and Brazil (Figure 1).

In August of 2010, an epizootic of illness among sylvatic monkeys near Atapirire was reported to the Central Office of Environmental Health (COEH), and a field commission was sent to investigate. Given the difficult access to the forests around Atapirire and the delay in outbreak notification, carcasses of many dead monkeys were observed upon arrival of the field team. Two moribund monkeys were found: a white-faced monkey (*C. olivaceus*) and red howler monkey

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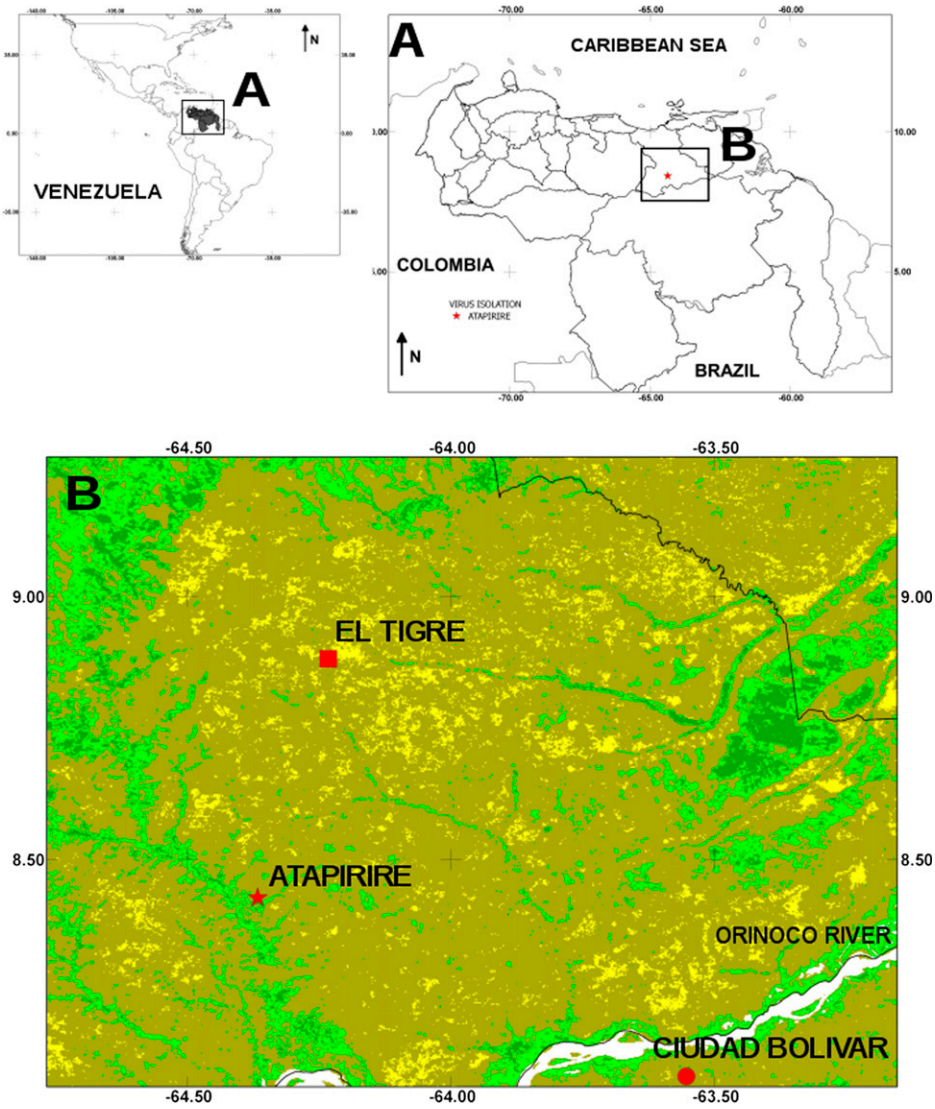

FIGURE 1. Geographic location of the epizootic and source of INHRR 17a-10 isolate. (**A**) Venezuela location in the geographical context of South America. (**B**) Political map of Venezuela showing location of the outbreak, Atapirire village, Municipality of Miranda, Anzoategui State, C. MODIS image of central plains (Llanos) showing forest patches los Llanos surrounding the urban-rural village, the main cities near Atapirire and the Orinoco river (MODIS VCF. 2010, percent tree cover, Townsend and others, 2011). Bright green color = tree cover > 50%. This figure appears in color at www.ajtmh.org.

(*Alouatta seniculus Linnaeus*). COEH personnel are authorized by the Venezuelan Ministry of Health to collect samples and euthanize sick monkeys in situations like this, as part of the national yellow fever surveillance program. According to protocol, the monkeys were euthanized to obtain tissue samples, which were sent immediately to the National Institute of Hygiene Rafael Rangel (INHRR) in Caracas.

Virus isolation and identification. At the INHRR, tissue samples from both sick monkeys were first tested by reverse transcription polymerase chain reaction for yellow fever virus (YFV) and yielded a negative result. Tissue homogenates were also inoculated into flask cultures of Vero cells. A 20% tissue homogenate (w/v) of lung and liver samples of each animal was prepared in minimum essential medium supplemented with 2% fetal bovine serum and antibiotics (200 mg of streptomycin and 200 U/mL of penicillin). A total of 100 μL of the filtered homogenate was inoculated into flasks of confluent African green monkey kidney (Vero E6) cells, as previously described.[15,17] Vero cell cultures were maintained at 37°C, and were examined daily for 7 days for evidence of viral cytopathic effects (CPEs). CPE was observed in a culture of a lung homogenate from the *C. olivaceus*, identified as INHRR 17a-10. Spot slides of the infected Vero cells were subsequently prepared, and an indirect immunofluorescence assay was performed using polyclonal broadly reacting antibodies against alphaviruses, flaviviruses, and bunyaviruses provided by the Centers for Disease Control and Prevention, Fort Collins, CO (Supplemental Table 1). In 2010, there also were concurrent outbreaks of YFV infection in monkeys, and Mayaro virus (MAYV) infection in humans in other regions of Venezuela.[18] After confirming that the etiologic agent from monkey INHRR 17a-10 was in fact a bunyavirus and not YFV or

MAYV, OROV-specific polyclonal mouse immune ascitic fluids were used to identify the agent.

RNA extraction, genome sequencing, and assembling. Total RNA was isolated from the supernatant of infected Vero-E6 cells using the Qiamp Viral RNA minikit (Qiagen, Valencia, CA), according to the manufacturer's instructions. The RNA was used for genome sequencing utilizing the ion semiconduction method implemented in the Ion Torrent PGM device,[19] as previously described.[20] Genome assembly was carried out using a de novo assembler strategy implemented in MIRA software v.4.9.2 (Bastien Chevreux, San Francisco, CA).[21] Final contigs (assembled with at least five reads) were inspected for quality and used to reconstruct the RNA segments. Terminal noncoding sequences (3′ and 5′ noncoding region [NCR]) for the virus isolate INHRR 17a-10 were obtained using both the 3′ and 5′ rapid amplification of cDNA ends method, as previously described,[22] using a specific set of primers (Supplemental Table 2). Complete sequences for the S, M, and LRNAs of INHRR 17a-10 were deposited in the GenBank database (from KJ866389-R1 to KJ866391-R1).

Genome characterization. The genome size, open reading frame (ORF) descriptions, 5′ and 3′ terminal NCRs, as well as conserved motifs, glycosylation sites, cysteine residues, and cleavage sites were determined using the Geneious v R7,[23] InterProScan (http://www.ebi.ac.uk/Tools/pfa/iprscan5/), and NetNGlyc v.1.0 Server (http://www.cbs.dtu.dk/services/NetNGlyc/). RNA secondary structure (termini cyclization) was predicted, using the RNA fold structure application available on Geneious v.R7 software.[23]

Genetic variability and phylogenetic analyses. Genetic variability was determined using the complete ORF sequences for the S, M, and LRNA segments of virus isolate INHRR 17a-10 and other viruses belonging to the Simbu group (Supplemental Table 3) using the multiple sequencing alignment approach implemented in Geneiuos vR7 software,[23] which estimates the evolutionary divergence between sequences (number of amino acid or nucleotide substitutions per site between sequences). Analyses were conducted using the JTT matrix-based model. The rate of variation among sites was modeled with a gamma distribution (shape parameter = 1). The genetic relationships were also assessed by estimating the genetic divergences and plotting the results as a box-plot graphic using the R core team software (http://www.R-project.org). Cutoff values were established according to Williamson.[24]

Phylogenetic reconstructions were made, using the complete coding sequences for the N, NSs, Gn, Gc, and polymerase genes. Initially, the appropriate DNA substitution model was determined by the RAxML v.8 software (Alexandros Stamatakis, Lausanne, Switzerland), and was used to construct the phylogenetic relationship with complete ORF sequences available for other selected orthobunyaviruses belonging to the Simbu serogroup. *Bunyamwera virus* (BUNV) was used as the outgroup[25] (Supplemental Table 3). Both maximum likelihood (ML) and Bayesian methods were performed. For ML, trees were constructed using the RAxML v.8 software, whereas Bayesian analyses were implemented in BEAUTi and the BEAST programs (Alexei J. Drummond, Marc A. Suchard, Dong Xie, and Andrew Rambaut, Oxford University, Oxford, United Kingdom).[26,27] Phylogenetic trees were selected according to the highest probabilistic values and visualized using Figtree (Andrew Rambaut, University of Edinburgh, Edinburgh, United Kingdom).[28]

Analysis of genetic reassortment. Evidence of genome reassortment was evaluated using the complete coding sequences for the N, M polyprotein, and polymerase gene of the INHRR 17a-10 isolate together with selected members of the Simbu serogroup. Bootscan analysis was performed with Simplot software.[29] Concatenated coding regions (S, M, and LRNAs) of INHRR 17a-10 virus were compared with concatenated homologous sequences of closely related viruses within the Simbu serogroup. The analysis was conducted in a screenshot window of 200 nucleotides (nt) and along the genomes (X-axis); values for phylogenetic permutation trees or PPTV (percentage of phylogenetic similarity in a given genomic position) was expressed in percentages along the Y-axis and evaluated as high (PPTV > 90%), medium (50% > PPTV > 90%), and low (PPTV < 50) values. Genome reassortment was considered when PPTV was equal or higher than 90% across the entire genome segment (genome coverage above 95%).

RESULTS

Nucleotide sequence and protein prediction. Complete genome sequences were obtained for all three RNA segments of the INHRR 17a-10 isolate. The SRNA was 949 nt (220 × coverage), the MRNA was 4,395 nt (105 × coverage), and LRNA was 6,850 nt in length (95 × coverage). After the ORF predictions, six coding regions were observed: N and NSs (S RNA segment), M polyprotein (MRNA), and L polyprotein (LRNA). The evaluation of the gene products depicted six proteins: three structural (N, Gn, and Gc) and three nonstructural (NSs, NSm, and L). Table 1 shows the genomic organization and predicted ORFs for INHRR 17a-10 in comparison to other Simbu viruses.

Cysteine residues, glycosylation, and M polyprotein cleavage sites. A total of 106 cysteine residues was found for N ($N = 1$), NSs ($N = 0$), Gn ($N = 17$), NSm ($N = 8$), Gc ($N = 40$), and L ($N = 36$) proteins. Furthermore, three N-glycosylation sites (Gn = 2; NSm = 0; Gc = 1) were observed, as well as M polyprotein cleavage sites were assessed for the INHRR 17a-10 isolate.

5′–3′ Terminal sequences and predicted RNA folding structures. Terminal sequences of S, M, and LRNAs for the isolate INHRR 17a-10 showed highly conserved nucleotide sequences as shown in Table 1. RNA folding structures revealed evidence of sequence complementarity (Supplemental Figure 1).

Conserved motifs. As observed with other orthobunyavirus polymerases, conserved motifs designated as I, II, III, and IV were observed.[30] Furthermore, the PreA (KGQKTAKDRE IFLGEFEAKMCLYLVERIAK), A (GLKIEINADMSKWS AQDV), B (TVEIKRNWLQGNLNYTSSYLHSC), C (EAL VNSMVHSDDNQT), D (GNQANMKKTYLT), and E (IKE FVSLFNIHGEPFSIYG) domains were also assessed for the virus INHRR 17a-10. Our analyses involving the M segment polyprotein indicated the presence of a Zinc finger motif within the Gn protein. Within the NSm protein, the domains I to VI were observed. There was high conservation noted in domain I, in comparison to BUNV in domain I, and only moderate conservation for domains II, IV, and V. Low conservation was observed in domain III for INHRR 17a-10 virus as TNKCGTCICGC. At the Gc protein level, the highly conserved transmembrane region (amino acid positions 1,060 and

TABLE 1

Genome organization of INHRR 17a-10 isolate in comparison to other Simbu viruses according to strain, 5′ and 3′ NCR, RNA segments, genes, and protein sizes

			Genome regions (nt/aa)							
RNA segment	Virus	Strain		5′–NNNNNNNN	N	NSs	M polyprotein	L polyprotein		3′-NNNNNNN	Total lenght
SRNA	INHRR 17a-10	TVP 19255	44	AGTAGTGTACT-CCAC…	696 (231aa)	279 (92aa)	NA	NA	209	…TGGGAGCACACTACT	949
	MIS 0397	TVP 19261	44	AGTAGTGTACT-CCAC…	696 (231aa)	279 (92aa)	NA	NA	203	…TGGGAGCACACTACT	943
	PDEV	BeAn790177	44	AGTAGTGTACT-CCAC…	696 (231aa)	279 (92aa)	NA	NA	207	…TGGGAGCACACTACT	947
	OROV	BEAN 19991	44	AGTAGTGTACT-CCAC…	696 (231aa)	279 (92aa)	NA	NA	218	…TGGGAGCACACTACT	958
	JTBV	BeAn 423380	44	AGTAGTGTACT-CCAC…	696 (231aa)	279 (92aa)	NA	NA	200	…TGGGAGCACACTACT	940
	SIMV	SA Ar 53	33	AGTAGTGTACT-CCAC…	702 (233aa)	276 (91aa)	NA	NA	134	…TGGGAGCACACTACT	860
	AKAV	OBE-1	33	AGTAGTGTACT-CCAC…	702 (233 aa)	276 (91aa)	NA	NA	123	…TGGGAGCACACTACT	858
										Mean	922
MRNA	INHRR 17a-10	TVP 19255	30	AGTAGTGTACTACCA…	NA	NA	4.272 (1.423aa)	NA	108	…TGGTAGCACACTACT	4.395
	MIS 0397	TVP 19261	30	AGTAGTGTACTACCA…	NA	NA	4.257 (1.418aa)	NA	92	…TGGTAGCACACTACT	4.379
	PDEV	BeAn790177	23	AGTAGTGTACTACCA…	NA	NA	4257 (1.418aa)	NA	138	…TGGTAGCACACTACT	4.418
	OROV	BEAN 19991	31	AGTAGTGTACTACCA…	NA	NA	4.263 (1.420aa)	NA	91	…TGGTAGCACACTACT	4.385
	JTBV	BeAn 423380	23	AGTAGTGTACTCCCA…	NA	NA	4.266 (1.421aa)	NA	114	…TGGTAGCACACTACT	4.403
	SIMV	SA Ar 53	23	AGTAGTGAACTACCA…	NA	NA	4.230 (1.409aa)	NA	154	…TGGTAGCACACTACT	4.407
	AKAV	OBE-1	22	AGTAGTGAACTACCA…	NA	NA	4.206 (1.401aa)	NA	81	…TGGTAGAACACTACT	4.309
										Mean	4385
LRNA	INHRR 17a-10	TVP 19255	44	AGTAGTGTACTCCTA…	NA	NA	NA	6.756 (2.251 aa)	50	…TAGGAGCACACTACT	6.850
	MIS 0397	TVP 19261	43	AGTAGTGTACTCCTA…	NA	NA	NA	6.759 (2.252 aa)	50	…TAGGAGCACACTACT	6.852
	PDEV	BeAn790177	43	AGTAGTGTACTCCTA…	NA	NA	NA	6.759 (2.252 aa)	50	…TAGGAGCACACTACT	6.852
	OROV	BEAN 19991	43	AGTAGTGTACTCCTA…	NA	NA	NA	6.759 (2.252 aa)	50	…TAGGAGCACACTACT	6.852
	JTBV	BeAn 423380	42	AGTAGTGTACTCCTA…	NA	NA	NA	6.759 (2.252 aa)	47	…TAGGAGCACACTACT	6.848
	SIMV	SA Ar 53	28	AGTAGTGTACCCCTA…	NA	NA	NA	6.762 (2.253 aa)	71	…TAGGGGCACACTACT	6.861
	AKAV	OBE-1	42	AGTAGTGTACTCCTA…	NA	NA	NA	6.759 (2.252 aa)	47	…TAGGAGCACACTACT	6.848
										Mean	6.852

aa = amino acid; AKAV = Akabane virus; JTBV = Jatobal virus; NA = not applied; NCR = noncoding region; nt = nucleotide; OROV = *Oropouche virus*; PDEV = Perdões virus; SIMV = Simbu virus.
SRNA, MRNA, and LRNA are small, medium, and large segments of RNA, respectively.

1,086) was described, as well as the fusion peptide motif WGCEExGCLAxxxGCV(F/Y)GSCQD. For the N gene, amino acid residues involved in the bunyavirus ribonucleoprotein packaging process (P113, G138, Y86 e I231), RNA synthesis (F18, F145, L161, Y186, L82, K80, Y186, W194, M195, F226), and virus RNA ligation to viral proteins (R41, R95 e K51) were observed.

Analysis of evolutionary divergence. A matrix of nucleotide and amino acid similarities, based on the complete ORFs for INHRR 17a-10 and selected members of the Simbu serogroup, revealed different degrees of genetic relatedness according to the RNA segment studied. For the S and LRNA segments, low means of evolutionary divergences were determined: ranging from 0.07 to 0.1 nucleotide substitutions/site, and from 0 to 0.03 amino acid substitutions/site, respectively. In the case of MRNA, the mean evolutionary divergence was estimated as 0.7 nucleotide substitutions/site and 0.65 amino acid substitutions/site. Minimal evolutionary divergences were observed with OROV isolates, and with MDDV isolate FMD1303 when comparing the MRNAs (Table 2).

Calculations were carried out based on the nucleotide and amino acid distances for the three RNA segments of the isolate INHRR 17a-10, and other selected members of the genus *Orthobunyavirus*, using pairwise distances; Simbu virus intragroup and intergroup distances are depicted in Supplemental Figure 2.

Phylogenetic analysis. Regardless of the RNA segment analyzed, our phylogenetic reconstructions grouped the INHRR 17a-10 virus together with other members of the Simbu group, and depicted two major groups (I and II) corresponding to the main genetic clades of the Simbu virus group, genus *Orthobunyavirus*. For the SRNA (N gene), the INHRR 17a-10 isolate fell into the group I with Bayesian posterior probability (BPP) equal to 93, and more specifically within the subclade I-a (BPP = 92) together with MDDV (BPP = 100), and closely related to different OROV, PDEV, and IQTV SRNAs (BPP = 99) (Figure 2A). A more specific analysis using 56 OROV, PPDV, and IQTV N genes available in the GenBank database, revealed that IHNRR 17a-10 is closely related to MDDV (Figure 2B). Analyses using complete M polyprotein also included the INHRR 17a-10 virus in group I (BBP = 100), clustering together with MDDV (BBP = 100) within the subgroup I-b, and separately from subgroups I-a (IQTV and MIS 0397) and I-c (OROV isolates) (Figure 2C). In case of L polyprotein, as observed in N gene analysis, the INHRR 17a-10 isolate showed a strong relationship to OROV with BPP equal to 100 (Figure 2D).

Genetic reassortment. Analyses of reassortments (segment exchange), using the entire ORFs of INHRR 17a-10 and selected Simbu members demonstrated high phylogenetic permutation tree values (PPTV) for the SRNA (PPTV > 96% with OROV SRNA), and MDDV MRNA (PPTV > 95%), and moderate values (PPTV > 80%) for the LRNA (with OROV LRNA), suggesting that the INHH-17a-10 isolate is a reassortant virus involving OROV and MDDV (Supplemental Figure 3).

DISCUSSION

The Brazilian Amazon is one of the world's richest ecosystems in terms of biodiversity; approximately 5,000 species of vertebrates, 50,000 species of insects, and 10 to 15 million plants and innumerable microorganisms (viruses, bacteria, and fungi) coexist.[4,31–34] OROV is one of the most important arboviruses affecting humans in the Amazon region. More than 1,500,000 people have been infected with OROV in the Amazon region of Brazil, since it was first described in Brazil in 1961.[6,11,35–38] Since the original detection and description of OROV in Trinidad in 1955,[7] more than 30 outbreaks of Oropouche fever have been reported from Brazil, Peru, and Panama.[6,35–38]

Molecular studies, using sequence information for the genomic segments of OROV and other Simbu viruses, have provided a better understanding of the molecular epidemiology and evolution of viruses included in the OROV species and the Simbu virus serogroup.[15,16,39,40]

Our recovery of MDDV from a sick monkey is the first evidence that this virus occurs in Venezuela. Sick and dying monkeys in South America are usually associated with YFV infections.[41] However, in the present case, the monkey was infected with an OROV reassortant. It is unknown whether INHRR 17a-10 virus was the cause of the severe illness in the animal, or if it was responsible for the other monkey deaths observed in the vicinity.

The INHRR 17a-10 virus has similar genomic organization and genetic characteristics to other orthobunyaviruses, including three genome segments with compatible size, functional ORFs (N, NSs, M polyprotein, and L polyprotein), structural (N, Gn, and Gc) and nonstructural proteins (NSs, NSm, and polymerase), conserved motifs, protein cleavage sites, cysteine residues, and glycosylation sites (Table 1).

RNA fold analysis has been used to determine the RNA curvature, structural stabilization, and prediction of complementary 5′–3′ terminal genomic regions over a given energy level for other RNA viruses.[42–44] In the case of INHRR 17a-10, the RNA structures generated for the three segments (S, M, L) showed similar folding structures with a high complementarity level at the 3′–5′ ends. Together with RACE 5′ and 3′ termini recovering strategies, it indicates that the genome segments were complete (Supplemental Figure 1A). Regarding size heterogeneity, differences in 3′ termini were noted. We analyzed the 3′ ends of distinct OROV strains in comparison to INHRR 17a-10 virus (Supplemental Figure 1B), and observed that size heterogeneity is present among OROV SRNAs; however, these differences appear not to be related to geographic location, as observed previously among YFV strains.[45] Further studies are necessary to evaluate if the 3′ NCR heterogeneity could be related to virus establishment, selection, and adaptive mechanisms to a given host.

Previous work involving the genetic characterization and phylogenetic analysis of Simbu serogroup viruses have provided a better understanding of molecular and evolutionary aspects of this group of viruses.[30,46,47] Reassortment is a common mechanism described for orthobunyaviruses of the Bunyamwera, Wyeomyia, and Simbu serogroups.[15,47–49] In the case of INHRR 17a-10 virus, we used extensive and robust analyses for testing the possibility of genetic reassortment of the isolate, including matrix of evolutionary distance, phylogenetic reconstructions, and Simplot method. Phylogenetic analyses indicated distinct origins for the S, M, and LRNA segments; the S and LRNAs were related to OROV, whereas the MRNA was related to MDDV (Figure 1). A more in-depth analysis using 95 sequences for the N gene of distinct OROV strains isolated in Brazil, Trinidad, Panamá, and

TABLE 2

Matrix of nucleotide and amino acidic evolutionary distances among INHRR 17a-10 isolate and other Simbu viruses (within the black frame box)

Genome segment		Viruses	Pairwise distance (Nucleotide) / Pairwise distance (amino acid) 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
SRNA	1	KP026181_Oropouche_TRVL9760	–	0.000	0.000	0.000	0.000	0.000	0.000	0.069	0.385	0.496	0.498	0.490	0.421	0.506	0.497	0.489	0.494	0.494	0.960	1.670
	2	KP052852_Oropouche_BeAn19991	0.015	–	0.000	0.000	0.000	0.000	0.000	0.069	0.385	0.496	0.498	0.490	0.421	0.506	0.497	0.489	0.494	0.494	0.960	1.670
	3	MIS-0397	0.063	0.049	–	0.000	0.000	0.000	0.000	0.069	0.385	0.496	0.498	0.490	0.421	0.506	0.497	0.489	0.494	0.494	0.960	1.670
	4	KF697144_Iquitos_IQT9924	0.063	0.049	0.000	–	0.000	0.000	0.000	0.069	0.385	0.496	0.498	0.490	0.421	0.506	0.497	0.489	0.494	0.494	0.960	1.670
	5	KP691626_Perdoes_BeAn_789726	0.049	0.039	0.037	0.037	–	0.000	0.000	0.069	0.385	0.496	0.498	0.490	0.421	0.506	0.497	0.489	0.494	0.494	0.960	1.670
	6	TVP_19255_INHRR 17a-10	0.094	0.090	0.095	0.095	0.088	–	0.000	0.069	0.385	0.496	0.498	0.490	0.421	0.506	0.497	0.489	0.494	0.494	0.960	1.670
	7	KF697146_Madre_de_Dios_FMD1303	0.094	0.090	0.095	0.095	0.088	0.000	–	0.069	0.385	0.496	0.498	0.490	0.421	0.506	0.497	0.489	0.494	0.494	0.960	1.670
	8	JQ675601_Jatobal_strain_BeAN_423380	0.202	0.197	0.212	0.212	0.212	0.198	0.198	–	0.408	0.501	0.504	0.497	0.422	0.516	0.494	0.486	0.490	0.490	1.014	1.616
	9	HE795089_Aino	0.561	0.541	0.544	0.544	0.563	0.524	0.524	0.535	–	0.592	0.596	0.588	0.616	0.648	0.694	0.700	0.700	0.700	1.031	1.860
	10	HE800143_Shuni	0.542	0.542	0.536	0.536	0.569	0.496	0.496	0.526	0.066	–	0.004	0.009	0.203	0.219	0.251	0.250	0.255	0.255	0.918	1.602
	11	HE795095_Peaton	0.561	0.560	0.586	0.586	0.601	0.555	0.555	0.526	0.102	0.095	–	0.004	0.209	0.225	0.245	0.244	0.249	0.249	0.913	1.605
	12	HE795092_Douglas	0.499	0.481	0.507	0.507	0.520	0.486	0.486	0.463	0.299	0.298	0.311	–	0.203	0.219	0.239	0.238	0.244	0.244	0.904	1.624
	13	HE795104_Sathuperi	0.556	0.551	0.548	0.548	0.573	0.526	0.526	0.486	0.302	0.314	0.314	0.081	–	0.225	0.233	0.239	0.238	0.238	1.040	1.716
	14	HE795107_Shamonda	0.517	0.511	0.524	0.524	0.528	0.502	0.502	0.478	0.336	0.316	0.316	0.080	0.081	–	0.248	0.252	0.257	0.257	0.963	1.786
	15	JX853181_Schmallenberg	0.507	0.502	0.514	0.514	0.518	0.502	0.502	0.469	0.330	0.316	0.322	0.090	0.079	0.024	–	0.004	0.009	0.009	0.996	1.702
	16	NC_018477_Simbu	0.513	0.522	0.510	0.510	0.518	0.507	0.507	0.401	0.306	0.315	0.322	0.259	0.285	0.297	0.297	–	0.004	0.004	0.994	1.698
	17	NC_009896_Akabane	0.528	0.509	0.488	0.488	0.514	0.509	0.509	0.491	0.326	0.337	0.338	0.301	0.293	0.320	0.324	0.296	–	0.000	0.999	1.696
	18	JX983192_Oya	0.447	0.451	0.477	0.477	0.470	0.473	0.473	0.457	0.602	0.545	0.568	0.632	0.688	0.671	0.689	0.563	0.602	–	0.999	1.696
	19	HM627177_Leanyer	0.939	0.938	0.978	0.978	0.918	0.949	0.949	0.875	0.878	0.819	0.819	0.837	0.836	0.851	0.822	0.905	0.904	1.054	–	1.632
	20	NC_001927_Bunyamwera	1.576	1.524	1.555	1.555	1.631	1.668	1.668	1.620	1.410	1.538	1.611	1.627	1.590	1.790	1.788	1.577	1.754	1.778	1.580	–

(continued)

TABLE 2
Continued

Genome segment		Viruses	Pairwise distance (Nucleotide) / Pairwise distance (amino acid) 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
MRNA	1	KP026181_Oropouche_TRVL9760	–	0.010	0.658	0.658	1.177	0.666	0.666	1.183	2.049	2.077	2.344	2.193	2.150	2.352	2.174	1.896	2.541	1.036	2.060	2.519
	2	KP052852_Oropouche_BeAn19991	0.022	–	0.657	0.657	1.180	0.668	0.668	1.180	2.053	2.090	2.347	2.204	2.161	2.362	2.185	1.902	2.540	1.041	2.064	2.505
	3	MIS-0397	0.764	0.763	–	0.000	1.142	0.171	0.171	1.129	1.994	1.983	2.337	2.196	2.149	2.377	2.177	1.944	2.415	1.033	2.102	2.372
	4	KF697144_Iquitos_IQT9924	0.764	0.763	0.000	–	1.142	0.171	0.171	1.129	1.994	1.983	0.764	0.763	0.000	–	1.142	0.171	0.171	1.129	1.994	1.983
	5	KP691626_Perdoes_BeAn_789726	1.077	1.075	1.120	1.120	–	1.188	1.188	0.304	2.148	2.103	2.279	2.189	2.178	2.334	2.160	1.958	2.389	1.065	2.073	2.480
	6	TVP_19255_INHRR 17a-10	0.791	0.791	0.336	0.336	1.059	–	0.000	1.165	1.974	1.996	2.385	2.190	2.145	2.398	2.160	1.938	2.412	1.033	2.152	2.367
	7	KF697146_Madre_de_Dios_FMD1303	0.791	0.791	0.336	0.336	1.059	0.003	–	1.165	1.974	1.996	2.385	2.190	2.145	2.398	2.160	1.938	2.412	1.033	2.152	2.367
	8	JQ675601_Jatobal_strain_BeAN_423380	1.047	1.055	1.100	1.100	0.474	1.056	1.056	–	2.077	2.007	2.346	2.208	2.191	2.439	2.152	1.934	2.398	1.084	2.054	2.396
	9	HE795089_Aino	1.685	1.644	1.659	1.659	1.731	1.646	1.646	1.459	–	0.315	2.062	0.823	0.822	2.110	0.830	1.038	2.090	2.161	2.559	2.750
	10	HE800143_Shuni	1.607	1.628	1.494	1.494	1.572	1.651	1.651	1.415	0.477	–	2.151	0.854	0.846	2.077	0.847	1.062	2.014	2.178	2.551	2.672
	11	HE795095_Peaton	1.727	1.737	1.803	1.803	1.597	1.794	1.794	1.684	1.767	1.675	–	2.002	2.019	1.275	2.048	2.030	1.122	2.328	2.702	2.912
	12	HE795092_Douglas	1.817	1.814	1.729	1.729	1.710	1.794	1.794	1.701	0.859	0.854	1.611	–	0.086	2.049	0.122	1.068	2.053	2.128	2.578	2.757
	13	HE795104_Sathuperi	1.760	1.756	1.758	1.758	1.723	1.741	1.741	1.659	0.879	0.879	1.681	0.187	–	2.040	0.103	1.066	2.083	2.083	2.533	2.716
	14	HE795107_Shamonda	1.790	1.786	1.885	1.885	2.007	1.963	1.963	1.818	1.838	1.702	1.220	1.668	1.675	–	2.050	1.996	1.211	2.469	2.690	2.847
	15	JX853181_Schmallenberg	1.704	1.720	1.715	1.715	1.728	1.694	1.694	1.644	0.884	0.886	1.722	0.265	0.257	1.701	–	1.044	2.092	2.079	2.589	2.745
	16	NC_018477_Simbu	1.546	1.530	1.715	1.715	1.430	1.606	1.606	1.453	0.962	0.967	1.714	1.049	1.036	1.610	1.051	–	2.062	1.940	2.450	2.630
	17	NC_009896_Akabane	1.863	1.853	1.917	1.917	1.908	1.920	1.920	1.826	1.896	1.722	1.148	1.738	1.632	1.220	1.753	1.895	–	2.449	2.643	2.926
	18	JX983192_Oya	1.061	1.060	1.000	1.000	1.066	1.047	1.047	0.997	1.704	1.597	1.830	1.663	1.673	1.932	1.641	1.557	1.885	–	1.976	2.440
	19	HM627177_Leanyer	1.729	1.735	1.785	1.785	1.639	1.937	1.937	1.630	2.035	1.972	1.948	1.923	1.948	2.205	1.977	1.881	2.137	1.687	–	2.669
	20	NC_001927_Bunyamwera	1.848	1.815	1.815	1.815	1.935	1.910	1.910	1.812	2.057	1.847	2.079	2.179	2.012	2.121	2.093	1.808	2.060	1.826	2.052	–

(continued)

TABLE 2
Continued

Genome segment		Viruses	Pairwise distance (Nucleotide) / Pairwise distance (amino acid) 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
LRNA	1	KP026181_Oropouche_TRVL9760	–	0.0084	0.0151	0.0151	0.0164	0.0544	0.0532	0.1519	0.7766	0.7647	0.7766	0.8072	0.7998	0.8071	0.8052	0.7775	0.7731	0.5276	0.7644	1.1467
	2	KP052852_Oropouche_BeAn19991	0.0228	–	0.0156	0.0156	0.0178	0.0535	0.0523	0.1488	0.7737	0.7618	0.7742	0.8000	0.7932	0.8024	0.8005	0.7723	0.7704	0.5241	0.7599	1.1398
	3	MIS-0397	0.1158	0.1168	–	0.0000	0.0075	0.0511	0.0489	0.1488	0.7693	0.7586	0.7709	0.8061	0.7933	0.8018	0.8019	0.7704	0.7680	0.5201	0.7549	1.1412
	4	KF697144_Iquitos_IQT9924	0.1158	0.1168	0.0000	–	0.0075	0.0511	0.0489	0.1488	0.7693	0.7586	0.7709	0.8061	0.7933	0.8018	0.8019	0.7704	0.7680	0.5201	0.7549	1.1412
	5	KP691626_Perdoes_BeAn_789726	0.1212	0.1252	0.0548	0.0548	–	0.0530	0.0518	0.1486	0.7724	0.7629	0.7747	0.8045	0.7949	0.8001	0.7995	0.7704	0.7695	0.5209	0.7583	1.1419
	6	TVP_19255_INHRR 17a-10	0.2274	0.2245	0.2327	0.2327	0.2443	–	0.0018	0.1490	0.7887	0.7793	0.7836	0.7994	0.7976	0.8057	0.8028	0.7686	0.7779	0.5308	0.7649	1.1559
	7	KF697146_Madre_de_Dios_FMD1303	0.2274	0.2245	0.2327	0.2327	0.2443	0.0000	–	0.1472	0.7837	0.7743	0.7786	0.7943	0.7925	0.8006	0.7978	0.7637	0.7730	0.5271	0.7610	1.1483
	8	JQ675601_Jatobal_strain_BeAN_423380	0.3744	0.3650	0.3703	0.3703	0.3816	0.3743	0.3743	–	0.7809	0.7751	0.7619	0.7947	0.7904	0.7962	0.7883	0.7664	0.7960	0.5279	0.7761	1.1493
	9	HE795089_Aino	0.8489	0.8407	0.8263	0.8263	0.8399	0.8289	0.8289	0.8197	–	0.0459	0.1232	0.4880	0.4885	0.4864	0.4814	0.4339	0.4942	0.8162	0.9124	1.2299
	10	HE800143_Shuni	0.8239	0.8265	0.8402	0.8402	0.8417	0.8459	0.8459	0.8244	0.2030	–	0.1204	0.4881	0.4873	0.4909	0.4851	0.4350	0.4869	0.8063	0.9171	1.2387
	11	HE795095_Peaton	0.8374	0.8347	0.8207	0.8207	0.8331	0.8450	0.8450	0.8331	0.3232	0.3243	–	0.4900	0.4909	0.4902	0.4823	0.4366	0.4865	0.8280	0.9451	1.2474
	12	HE795092_Douglas	0.8657	0.8552	0.8710	0.8710	0.8819	0.8596	0.8596	0.8780	0.6483	0.6244	0.6433	–	0.0373	0.0558	0.0512	0.4517	0.4453	0.8512	0.9236	1.2442
	13	HE795104_Sathuperi	0.8918	0.8801	0.8838	0.8838	0.8914	0.8745	0.8745	0.8761	0.6413	0.6377	0.6317	0.1692	–	0.0420	0.0412	0.4466	0.4337	0.8433	0.9101	1.2280
	14	HE795107_Shamonda	0.8914	0.8875	0.8646	0.8646	0.8797	0.8928	0.8928	0.8898	0.6562	0.6491	0.6471	0.2144	0.2086	–	0.0157	0.4541	0.4363	0.8440	0.9179	1.2257
	15	JX853181_Schmallenberg	0.8719	0.8635	0.8756	0.8756	0.8778	0.8726	0.8726	0.8746	0.6258	0.6412	0.6098	0.2127	0.2168	0.0824	–	0.4519	0.4359	0.8429	0.9173	1.2174
	16	NC_018477_Simbu	0.8691	0.8618	0.8486	0.8486	0.8572	0.8603	0.8603	0.8674	0.5780	0.5948	0.6002	0.5961	0.5971	0.6059	0.5936	–	0.4393	0.7958	0.9018	1.2326
	17	NC_009896_Akabane	0.8654	0.8536	0.8658	0.8658	0.8577	0.8511	0.8511	0.8945	0.6309	0.6577	0.6436	0.6157	0.6013	0.6082	0.5970	0.6001	–	0.8092	0.9311	1.1822
	18	JX983192_Oya	0.6833	0.6797	0.6823	0.6823	0.6914	0.6631	0.6631	0.6359	0.8666	0.8466	0.9053	0.8699	0.8896	0.8863	0.9045	0.8495	0.8790	–	0.8133	1.2055
	19	HM627177_Leanyer	0.8031	0.8070	0.7946	0.7946	0.7914	0.8110	0.8110	0.8217	0.9361	0.9522	0.9711	0.9170	0.8973	0.9223	0.9114	0.9088	0.9266	0.8683	–	1.2306
	20	NC_001927_Bunyamwera	1.1130	1.1012	1.1171	1.1171	1.1044	1.1339	1.1339	1.1277	1.1025	1.1396	1.1202	1.1231	1.1627	1.1639	1.1314	1.1626	1.1103	1.1441	1.1174	–

SRNA, MRNA, and LRNA are small, medium, and large segments of RNA, respectively.
Nucleotide and amino acid genetic distances are highlighted in light blue color. Grey boxes represent no genetic distance between the same virus isolate.

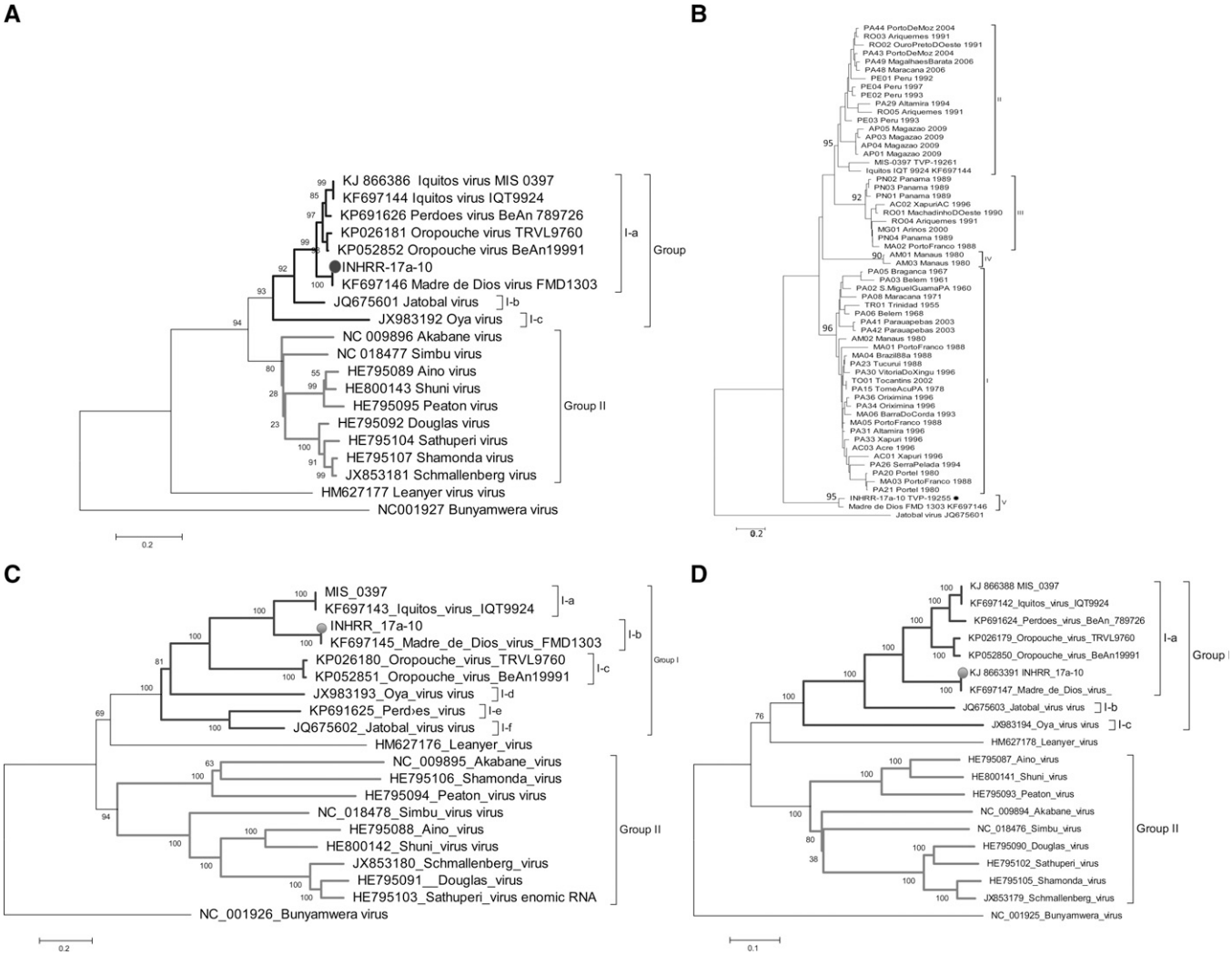

FIGURE 2. Phylogenetic analysis using the entire (**A**, **B**) N, M polyprotein (**C**) and polymerase gene (**D**) coding regions of the INHRR 17a-10 virus and selected orthobunyaviruses using the Bayesian inference. Values over each tree node represent the Bayesian posterior probabilities expressed in percentage. Main phylogenetic groups (I and II) are highlighted within collared (blue and red) brackets. Subgroups are indicated as I-a to I-f. The INHRR 17a-10 isolate is indicated with a blue circle.

Peru, indicated that INHRR 17-10a, although related to OROV, has a distinct SRNA segment from other available OROV sequences, clustering separately and together with MDDV (Figure 1B).

OROV is an important human pathogen, and it appears to be involved in different processes of genetic reassortment as a parental virus, contributing to the emergence of new human pathogens such as IQTV in Peru, PDEV in Brazil, and MDDV in Peru and Venezuela.[14–16] Interestingly, PDEV was isolated from a dead marmoset (*C. penicillata*) collected in Minas Gerais State, in western Brazil in 2013.[14] This is the same geographic area and host from which OROV was previously isolated in 2005.[37]

Matrices of evolutionary distances (Table 2), as well as box plots (Supplemental Figure 2), and Simplot (Supplemental Figure 3) analyses in our study indicate that INHHR 17a-10 represents a strain of MDDV, a previously characterized reassortant virus[16] involving OROV and a still unknown Simbu member as parental viruses.

Systematic virus surveillance programs are essential for the evaluation of the true prevalence of a specific viral agent in a given geographic area. Surveillance programs have contributed substantially to virus discovery and detection of emerging pathogens in the Amazon region of Brazil and in other South American countries.[4,6,37,38] Evidence of OROV circulation is periodically reported within endemic areas, especially in Brazil[39] and sporadically in areas considered to be endemic or enzootic.[37,50] The isolation of an OROV reassortant in Venezuela, in a biogeographic area completely distinct from the usual Amazonian endemic transmission region, indicates that the virus is present in other ecologic zones and this finding is of epidemiological importance.

The data presented here constitute the first isolation, molecular description, complete genome sequencing, genetic characterization, and evolutionary analyses of MDDV in Venezuela. MDDV has been associated with human illness in Peru. Further surveillance and molecular epidemiologic studies of OROV and OROV reassortants in tropical

America are needed to allow us to better understand the bunyavirus biodiversity and its impact on human and animal health in the region.

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SUPPLEMENTAL TABLE 1

Specific primer sets used for recovering the 5′ and 3′ noncoding terminal sequences for INHRR 17a-10 isolate

Virus	Genome segment	Primer	Sequence 5′→3′	MT (oC)	Product (base pairs)
INHRR 17a-10		5′ RACE FPS1	TACGTAAGACATCTTTGGCC	55	207
		5′ RACE FPS2	TCTAACAACACCAGCATTGA	55	164
	SRNA	5′ RACE FPS3	TCTAGCTTCAAATGCCACAT	55	131
		3′ RACE RPS1	CGTGAAGAGATAGTTGCTGT	55	329
		3′ RACE RPS2	GAGATTTCTTGCGCCAATTC	55	244
		5′ RACE FPM1	CTCTTATCAAGTTCCCTCCG	55	138
		5′ RACE FPM2	AAGCACCTATCACCAATCTG	55	116
	MRNA	5′ RACE FPM3	GGATAATGGATGTGATGCCA	55	90
		3′ RACE RPM1	TGGTACCAATCTTCAGGTTG	55	189
		3′ RACE RPM2	GGAGTATACCACAGAGCAAA	55	139
		5′ RACE FPM1	CAAGGACTTCAGCACAGATA	55	234
		5′ RACE FPM2	TCTGTACTCGAGATTTGCAG	55	194
	LRNA	5′ RACE FPM3	TCCCGAGAAAAGTAGTTGTG	55	163
		3′ RACE RPM1	TGAGGATTTCCAAACGAACA	55	192
		3′ RACE RPM2	AAGCACACAGTGTAGCATTA	55	154

SRNA, MRNA, and LRNA are small, medium, and large segments of RNA, respectively.

SUPPLEMENTAL TABLE 2

Polyclonal antibodies used for serologic identification of virus isolates

Virus/group	Antibody	Dilution
A	Broad Group A/Mouse HIAF	1:80
B	Broad Group B/SLE Proteina Específica E, MAB6B6C-1	1:80
C	Arbovirus Group Ascitic Fluid Group C-1	1:60
OROV	Oropouche, TRVL9760-Pool B/HI Mouse AF	1:60
YFV	Monoclonal Yellow Fever HIMAF 2E10	1:60

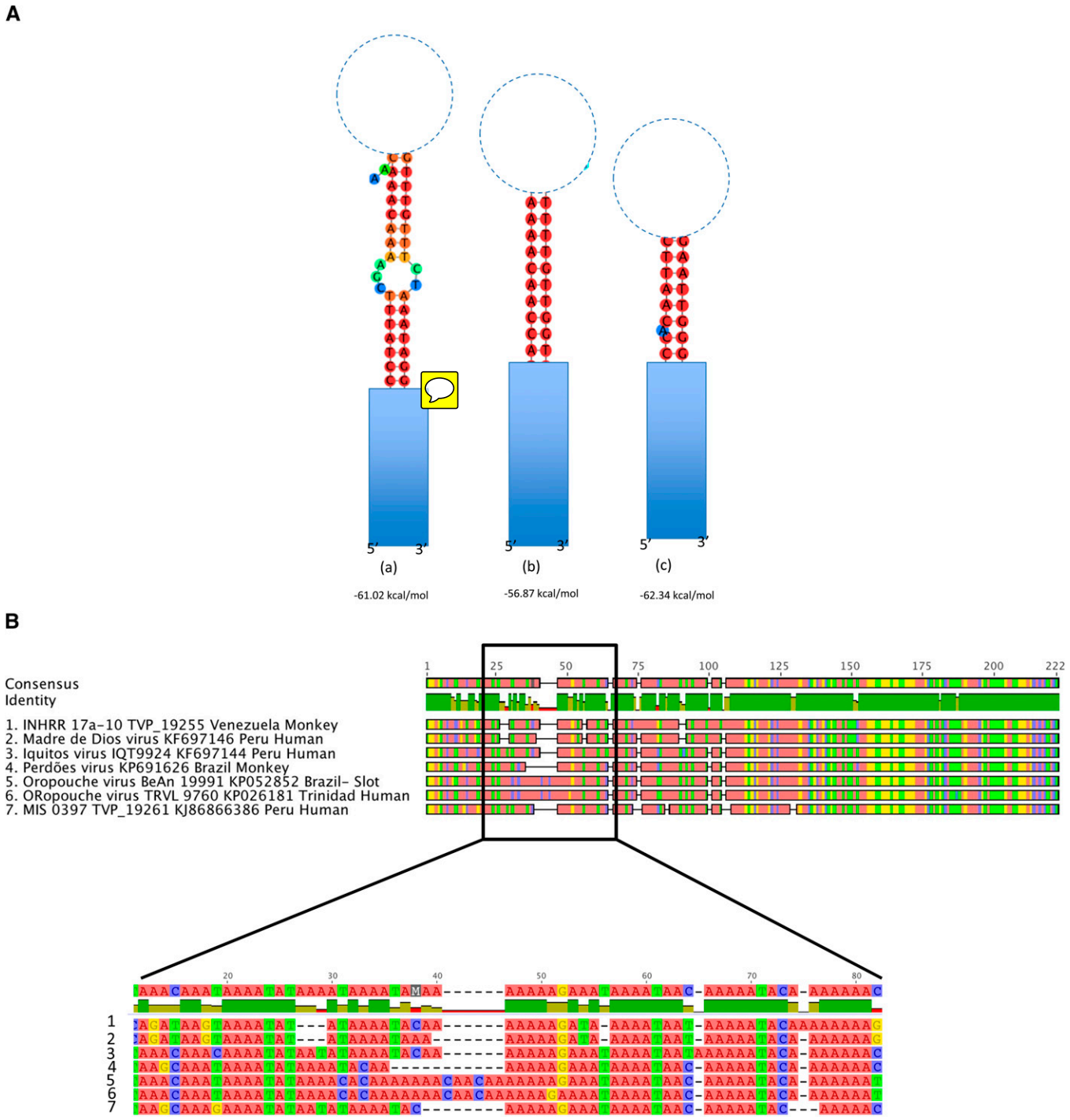
AF = ascitic fluid; HIAF = hyperimmune ascitic fluid; HIMAF = hyperimmune mouse ascitic fluid; OROV = *Oropouche virus*; YFV = yellow fever virus.

SUPPLEMENTAL TABLE 3

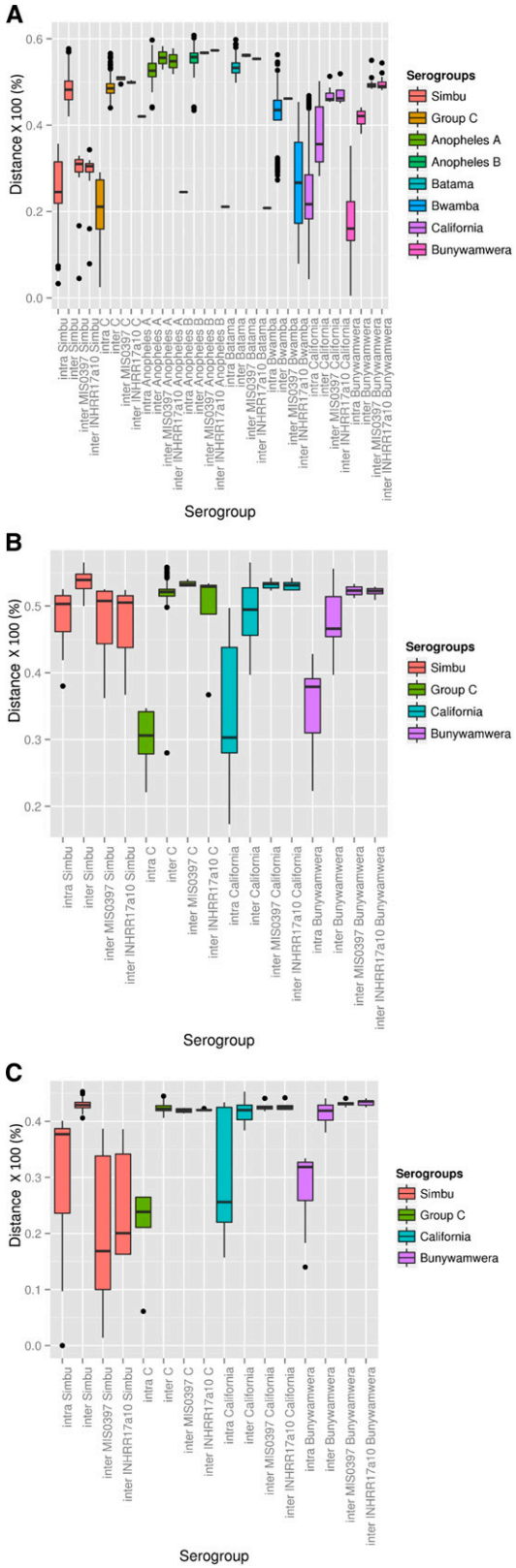
Viruses used for multiple sequencing alignment and phylogenetic analyses according to strain, host association, and place of isolation

Virus	Legend	Isolate	GenBank access number	Place of isolation
INHRR 17a-10	NA	TVP-19255	S: KJ866389; M: KJ866390; L= KJ866391	Venezuela
Schmallenberg virus	NA	BH80/11–4	S: JX853181; M: JX853180; L: JX853179	Germany
Shamoda virus	NA	Ib An 5550	S: HE795107; M: HE795106; L: HE795105	Nigeria
Sathuperi virus	NA	IG10310	S: HE795104; M: HE795103; L: HE795102	India
Douglas virus		93–6	S: HE795092; M: HE795091; L: HE795090	Australia
Akabane virus	NA	OBE-1	NC009896; M: NC009895; L: NC009894	Japan
Shuni virus	NA	Ib An 10107	S: HE800143; M: HE800142; L: HE800141	Nigeria
Aino virus	NA	38K	S: HE795089; M: HE795088; L: HE795087	Japan
Peaton virus	NA	CSIRO 110	S: HE795095; M: HE795094; L: HE795093	Australia
Simbu virus	NA	SA Ar 53	S: NC018477; M: NC018478; L: NC018476	South Africa
Iquitos virus	NA	MIS0397	S: KJ866386; M: KJ866387; L: KJ866388	Peru
	NA	IQT9924	S: KF697144; M: KF697143; L: KF697142	
Perdões virus	NA	BeAn789726	S: KP691626; M: KP691625; L: KP691624	Brazil
Madre de Dios	NA	FMD1303	KF697146; M: KF697145; L: KF697147	Peru
Jatobal virus	NA	BeAn 423380	S: JQ675601; M: JQ675602; L: JQ675603	Brazil
Oya virus	NA	SC0806	S: JX983192; M: JX983193; L: JX983194	China
Leanyer virus	NA	AusN16701	S: HM627177; M: HM627176; L: HM627178	Australia
Oropouche virus	TR01	TRVL 9760	S: KP026181; M: KP026180; L: KP026179	Trinidad and Tobago
	PA02	BeAn 19991	S: KP052852; M: KP052851; L: KP052850	Brazil
	PA03	H 29086	S: HM470108	Brazil
	PA 05	H 121293	S: HM470110	Brazil
	PA06	AR 136921	S: HQ830443	Brazil
	PA08	AN 208402	S:AY993910	Brazil
	PA15	H 355186	S: HQ830446	Brazil
	PA20	H 384192	S: HQ830450	Brazil
	PA21	H 384193	S: HQ830451	Brazil
	AM01	H 389865	S: HQ830453	Brazil
	AM02	H 390233	S: AF154536	Brazil
	AM03	H 390242	S: HQ830454	Brazil
	MA01	AR 473358	S: AF164539	Brazil
	MA02	H 472433	S: HQ830455	Brazil
	MA03	H 472435	S: HQ830456	Brazil
	MA04	H 472200	S: AF154537	Brazil
	MA05	H 472204	S: AF164538	Brazil
	PA23	H 475248	S: AF164540	Brazil
	PN01	GML 444477	S: AF164555	Brazil
	PN02	GML 444911	S: AF164556	Brazil
	PN03	GML 445252	S: AF164557	Brazil
	PN04	GML 450093	S: AF164558	Brazil
	RO01	H 498913	S: HQ830457	Brazil
	RO02	H 505442	S: AF164542	Brazil
	RO03	H 505663	S: AF164543	Brazil
	RO04	H 505764	S: HQ830458	Brazil
	RO05	H 505768	S: HQ830459	Brazil
	PE01	IQT 1690	S: AF164549	Brazil
	MA06	H 521086	S: AY704559	Brazil
	PE02	MD O 23	S: AF164550	Brazil
	PE03	DE I209	S: AF164551	Brazil
	PA26	H 532422	S: HQ830462	Brazil
	PA29	H 541140	S: HM470126	Brazil
	PA30	H 541863	S: AF164544	Brazil
	PA31	H 544552	S: AF164546	Brazil
	AC01	H 543091	S: HQ830465	Brazil
	AC02	H 543100	S: HQ830466	Brazil
	AC03	H 543087	S: AF164547	Brazil
	PA33	H 543618	S: AF164548	Brazil
	PA34	H 543629	S: HQ830467	Brazil
	PA36	H 543639	S: HQ830469	Brazil
	MG01	AN 622998	S: AY117135	Brazil
	TO01	H 622544	S: EF467368	Brazil
	PA41	H 669314	S: EF467370	Brazil
	PA42	H 669315	S: EF467369	Brazil
	PA43	H 682426	S: EF467371	Brazil
	PA44	H 682431	S: EF467372	Brazil
	PA48	H 707157	S: HQ830476	Brazil
	PA49	H 707287	S: HM470137	Brazil
	AP01	H 758687	S: HQ830478	Brazil
	AP03	H 759525	S: HQ830480	Brazil
	AP04	H 759541	S: HQ830481	Brazil
	AP05	H 759531	S: HQ830482	Brazil

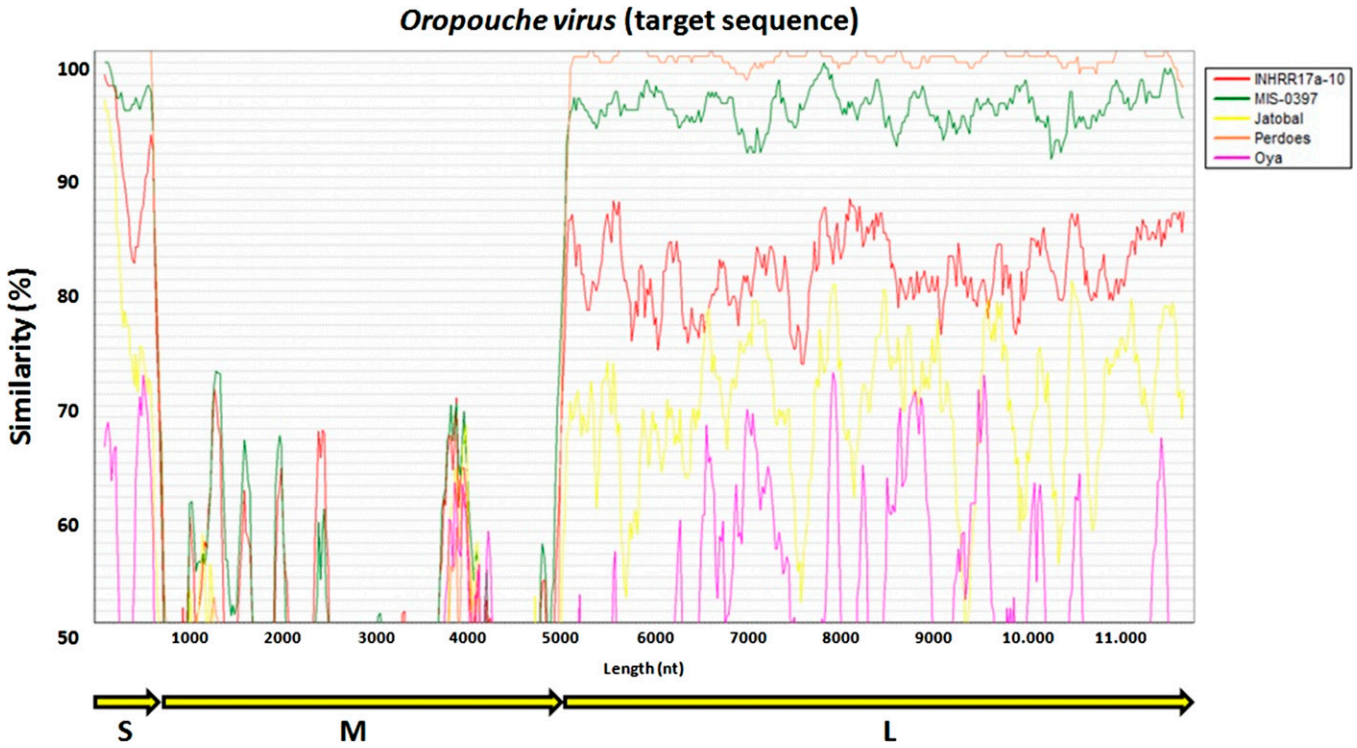
NA = not applicable.



SUPPLEMENTAL FIGURE 1. (**A**) Predicted RNA secondary structure for the (a) LRN1A, (b) MRNA and (c) SRNA genome segments of INHRR 17a-10 isolate showing the terminal base complementarity between 5′ and 3′ noncoding regions (NCRs). Numbers below each structure represent the energy expressed in Kcal/mol required for structure stabilization. Light blue boxes are indicating the 11 highly conserved and complementary nucleotides. (**B**) Multiple alignment using the INHRR 17a-10 3′ NCR in comparison to homologue sequences of other OROV and OROV-like isolates.



SUPPLEMENTAL FIGURE 2. Box plot calculations for the INHRR 17a-10 isolate and determination of inclusion in the Simbu virus group using complete coding nucleotide sequences for (**A**) SRNA, (**B**) MRNA, and (**C**) LRNA.



SUPPLEMENTAL FIGURE 3. Symplot analysis for genome reassortment determination among INHRR 17a-10 isolate and selected Simbu viruses.