

Maguari Virus Associated with Human Disease

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Despite the lack of evidence for symptomatic human infection with Maguari virus (MAGV), its close relation to Cache Valley virus (CVV), which does infect humans, remains a concern. We sequenced the complete genome of a MAGV-like isolate (OBS6657) obtained from a febrile patient in Pucallpa, Ucayali, Peru, in 1998. To facilitate its classification, we generated additional full-length sequences for the MAGV prototype strain, 3 additional MAGV-like isolates, and the closely related CVV (7 strains), Tlacotalpan (1 strain), Playas (3 strains), and Fort Sherman (1 strain) viruses. The OBS6657 isolate is similar to the MAGV prototype, whereas 2 of the other MAGV-like isolates are located on a distinct branch and most likely warrant classification as a separate virus species and 1 is, in fact, a misclassified CVV strain. Our findings provide clear evidence that MAGV can cause human disease.

Maguari virus (MAGV) was first isolated from a mixed pool of mosquitoes collected in Utinga forest in Brazil in 1957 and has since been isolated from a variety of mosquito species (including *Aedes* spp., *Anopheles* spp., *Culex* spp., *Wyeomyia* spp., and *Psorophora* spp.) in Ecuador, Brazil, Trinidad and Tobago, Colombia, Argentina, and French Guiana (1). MAGV also has been isolated from horses in Guyana and Colombia (2,3) and from sentinel mice in Brazil (1), with further serologic evidence for infection with MAGV-like viruses in cattle, water buffalo, sheep, and birds (1). However, there had been no clear evidence of symptomatic human infection with MAGV.

In 2015, an orthobunyavirus isolate from a patient with febrile illness that had been tentatively classified as MAGV on the basis of serologic reactivity by indirect immunofluorescence test was shown instead to be a new Caraparu (Group C) reassortant, for which the name Itaya virus has been proposed (4). However, serologic evidence

of infection with MAGV-like viruses in humans has been reported in Argentina, Brazil, Peru, Colombia, and French Guiana (1,5,6). Furthermore, MAGV is considered a subtype of Cache Valley virus (CVV), which sometimes causes symptomatic human infection (7–9), reinforcing lingering concerns about the possible pathogenicity of MAGV for humans.

Given that cross-reactivity among bunyaviruses when using serologic techniques is not uncommon, their identification has increasingly involved incorporating genetic approaches to characterize these virus isolates. Such serologic cross-reactivity appears particularly to be an issue with MAGV, which was originally reported as CVV, and also shows cross-reactivity to other closely related viruses (1). Therefore, to clarify the genetic relationship between MAGV or MAGV-like viruses and the closely related CVV, as well as their association with acute human disease, we determined the complete genome sequences for all available MAGV isolates and conducted a comprehensive sequencing analysis of several full-length reference sequences for CVV strains and available strains of the closely related Tlacotalpan virus (TLAV), Playas virus (PLAV), and Fort Sherman virus (FSV) to facilitate the reliable classification of these and related virus isolates.

Materials and Methods

Viruses

We obtained 4 isolates identified as MAGV (including the prototype strain BeAr7272), spanning 1957–1998 and originating from 3 of the 7 countries from which the virus has been isolated (Colombia, Brazil, and Argentina) (Figure 1). Isolates were obtained from the World Reference Center for Emerging Viruses and Arboviruses (WRCEVA) or the Division of Vector-Borne Diseases, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention (DVBD-CDC, Atlanta, GA, USA) (online Technical Appendix Table 1, <https://wwwnc.cdc.gov/EID/article/23/8/16-1254-Techapp1.pdf>). In addition, we obtained 7 isolates of CVV spanning 1956–2003 and originating from diverse locations throughout the United States and Mexico, as well as 1 isolate of Tlacotalpan virus (Mexico),

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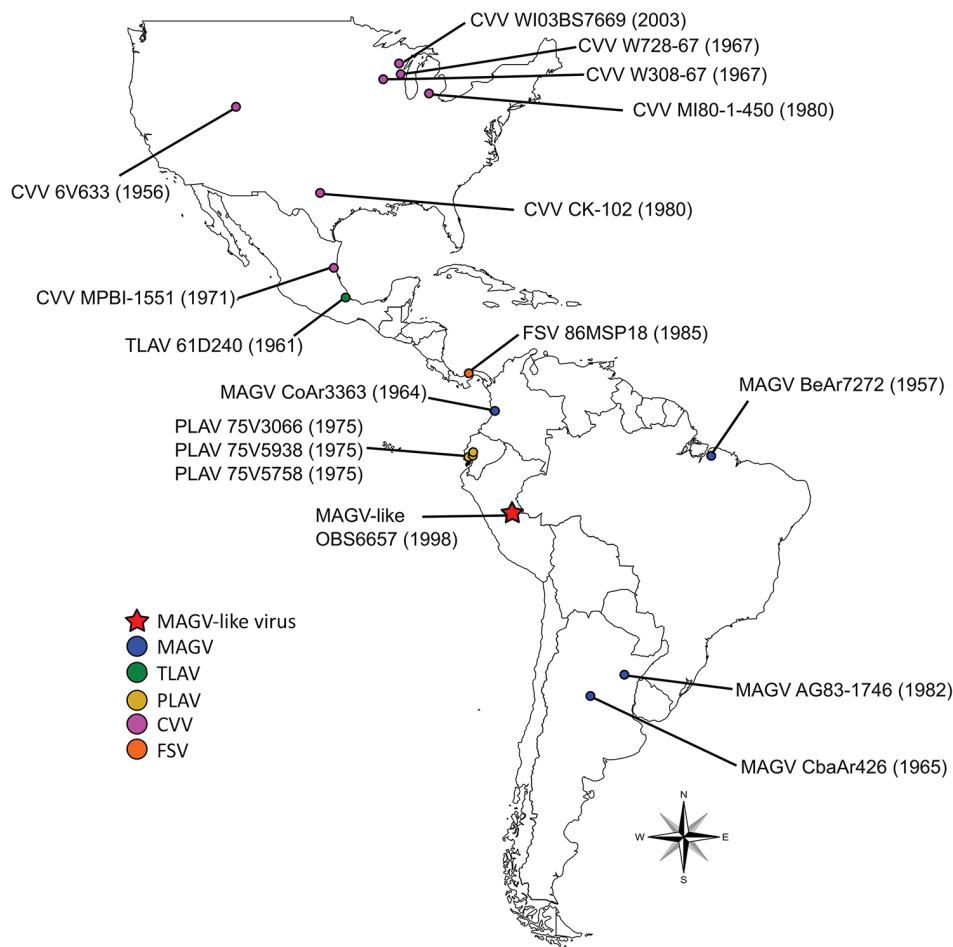


Figure 1. Geographic locations from which virus strains used in study of human infection with MAGV were isolated. Star indicates the location of the MAGV-like isolate OBS6657; circles indicate source locations for other isolates used in this study. CVV, Cache Valley virus; FSV, Fort Sherman virus; MAGV, Maguari virus; PLAV, Playas virus; TLAV, Tlacotalpan virus.

3 isolates of Playas virus (Ecuador), and 1 isolate of FSV (Panama) (Figure 1) from either WRCEVA or DVBD-CDC. An additional MAGV-like virus strain, isolated from a patient exhibiting fever, headache, myalgia, and chills in Pucallpa, Ucayali, Peru, in 1998 (strain OBS6657), was obtained from WRCEVA. This isolate was collected under the terms of a human use protocol (NMRCD.2000.0006), which, along with the consent procedure, were approved by the Naval Medical Research Center Institutional Review Board in compliance with all US and Peruvian federal regulations governing the protection of human subjects.

Sequencing

We extracted viral RNA using the QIAamp viral RNA mini kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions with reverse transcription PCR (RT-PCR) reactions performed using the Superscript III reverse transcription kit (Life Technologies, Carlsbad, CA, USA) and the iProof High-Fidelity PCR Kit (Bio-Rad Laboratories, Hercules, CA, USA). We obtained preliminary virus genome sequences of the MAGV BeAr7272 and

AG83-1746 strains, as well as PLAV (strain 75V3066), TLAV (strain 61D240), and FSV (strain 86MSP18), using the 454 FLX pyrosequencing technology platform (454 Life Sciences [Roche], Branford, CT, USA). We constructed libraries using previously described methods (10) and assembled and analyzed genomes using various publically available algorithms. Based on the preliminary 454-determined sequences, we designed primer sets to enable confirmation by Sanger sequencing, including analysis of additional strains of MAGV and PLAV. CVV strains were similarly sequenced by using primers based on complete sequences of the MNZ-92011 strain (GenBank accession nos. KC436108 [small (S) segment], KC436107 [medium (M) segment], KC436106 [large (L) segment]). The sequences of the noncoding regions, including the conserved genome termini, were amplified by using 3' and 5' rapid amplification of cDNA ends based on ligation-anchored PCR, as previously described (11-13). We deposited complete genome sequences determined in this study in GenBank (online Technical Appendix Table 1); primer sequences are available on request.

Phylogenetic and Sequence Analyses

Phylogenetic and sequence divergence analyses were conducted on the open reading frames (ORFs) and their corresponding amino acid sequences. These analyses incorporated the sequences for MAGV, CVV, TLAV, PLAV, and FSV determined in this study and additional representative orthobunyavirus sequences available in GenBank (online Technical Appendix Table 2).

Sequences for each ORF or corresponding amino acid sequence were aligned using the MUSCLE algorithm, and the evolutionary history for each tree construction was inferred using the neighbor-joining (14) and maximum-likelihood (15) methods, as implemented in MEGA5 (16). For neighbor-joining analysis of amino acid sequences, we used a Poisson model (17) and uniform rates; for analysis of nucleotides, uniform rates also were specified. We computed evolutionary distances using the maximum composite likelihood method (18). For maximum-likelihood analysis of amino acids the Jones, Taylor, and Thornton model (JTT) (19), gamma-distributed (+ G) was used for all 3 segments. Alternatively, for both the amino acid and nucleotide analyses, we used the best fit model for each segment, based on the calculated Bayesian information criterion, as determined using the Model Selection Tool implemented in MEGA5 (16) (i.e., JTT + G [N amino acid]; Le-Gascuel [LG] model [20] + G plus invariant sites [+ I] [LG + G + I; GPC amino acid], LG + G, + I plus frequencies [LG + G + I + F; L amino acid] or Tamura 3-parameter [21] + G [T92 + G, N nucleotide]; general time reversible [22] + G + I [GTR + G + I; GPC and L nucleotide]). Evaluation of statistical support for the neighbor-joining and maximum-likelihood tree topologies was based on bootstrap resampling (23); values were calculated on the basis of 1,000 replicates, and values >60 are indicated. We conducted an additional analysis based on Bayesian inference using Mr Bayes version 3 (24,25), as implemented in TOPALi version 2 (26) with the best fit model for each segment selected on the basis of the calculated Bayesian information criterion, using the integrated model selection tool in TOPALi (i.e., JTT + G [N amino acid]; JTT + G + I [GPC and L amino acid] or GTR + G [N amino acid]; GTR + G + I [GPC and L amino acid]). Posterior probability values >0.60 were indicated. The use of these different methods and models did not produce substantially different tree topologies with respect to the virus lineages under study (Figure 2; online Technical Appendix Figures 1, 2).

We calculated sequence divergence values on the basis of nucleotide and amino acid alignments constructed with ClustalW as implemented in MegAlign (LaserGene 12; DNASTAR, Madison, WI, USA). Furthermore, an additional recombination analysis was performed on concatenated (S, M, and L) genome sequences of all CVV, MAGV, PLAV, TLAV, and FSV isolates where full-length sequences were available. Initial alignment was performed by using the MUSCLE algorithm, as implemented in

MEGA5 (16), and these data were then further analyzed by using the RDP (27), GENECONV (28), BOOTSCAN/RECSCAN (29), MAXCHI (30), CHIMAERA (31), SISCAN (32), and 3SEQ (33) programs, as implemented in RDP4 Beta 4.83 (34).

Results and Discussion

Analysis of MAGV and MAGV-Like Sequences

In our phylogenetic analysis, the MAGV isolates we examined form 2 distinct clades: 1 contains the prototype strain BeAr7272 and “PLAV” (strain 75V5758), the other comprises the AG83-1746 strain and the CbaAr426 strain (Figure 2). Both of these atypical “MAGV” isolates (AG83-1746 and CbaAr426) were collected in Argentina and were isolated 17 years apart, indicating that this virus most likely continues to stably circulate in that region. MAGV (CoAr3363) was found to be an incorrectly classified isolate of CVV and thus is not considered further in this section. Of particular interest to the aims of this study was the MAGV-like isolate designated OBS6657, which was previously identified by complement fixation as a MAGV-like virus, after isolation from a symptomatic patient in Peru. Phylogenetic analysis indicates that this virus is closely related to the prototype MAGV isolate BeAr7272, with which it shares a clade.

Our divergence analysis comparing the 2 groups formed by the isolates originally identified as MAGV showed 95.7% (N), 84.3%–85.2% (GPC), and 90.1%–90.7% (L) amino acid divergence. The extremely low level of N protein variability seems to be a characteristic of these and related virus groups, and the L protein divergence values are barely above the 10%-aa divergence sometimes suggested as a cutoff for speciation. However, the glycoprotein, which is the most variable of the viral protein products, differs substantially from all other related virus groups and also between these 2 groups. Furthermore, the consistent evidence for separate evolutionary histories inferred by the phylogenies for all 3 proteins indicates that these viruses most likely differ sufficiently to warrant reclassification of the AG83-1746 strain and CbaAr426 strains as a distinct virus species. In accordance with the prevailing naming conventions for bunyaviruses, which dictate naming based on the location of initial isolation, we use “Córdoba virus” (CODV) and “CODV lineage” herein to refer to viruses belonging to this group and to distinguish them from the canonical MAGV strains.

In contrast to these various misidentified viruses, the OBS6657 isolate clearly grouped with the prototype MAGV strain BeAr7272 and showed 100% (N), 97.1% (GPC), and 99.0% (L) amino acid identity, respectively, across each of the three major gene products, despite being isolated >40 years later. Given that this virus was isolated from a patient exhibiting clear evidence of an acute febrile infection, our data provide strong evidence that MAGV infection can

cause clinical disease in humans. In light of this evidence, and particularly when considered together with serologic

data that indicate frequent infection with MAGV-like viruses (2%–64% positive serum samples) (1,5,6) in South America,

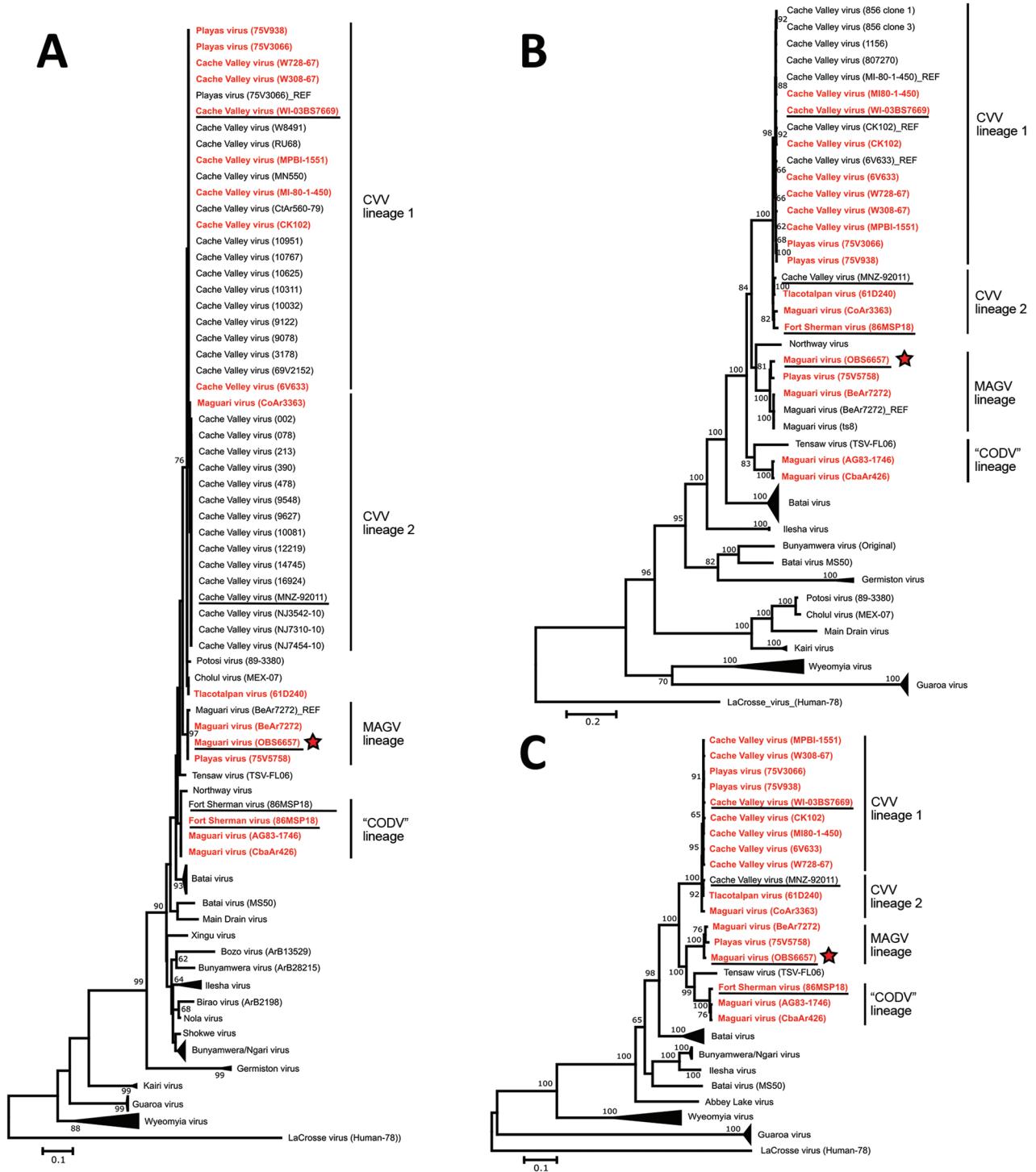


Figure 2. Phylogenetic relationship of MAGV-like isolate OBS6657 to other MAGV and CVV isolates and reference orthobunyaviruses. Maximum-likelihood trees (Jones, Taylor, and Thornton model, gamma-distributed) were constructed on the basis of the amino acid sequences of the nucleoprotein (A), glycoprotein (B), and polymerase (C). Bootstrap values based on 1,000 replicates are indicated for values >60. Sequences generated in this study are shown in red bold. Human isolates within the CVV, MAGV, and Córdoba virus clades are underlined, and the OBS6657 isolate is indicated with a red star. Scale bars indicate nucleotide substitutions per site. CVV, Cache Valley virus; CODV, Córdoba virus; MAGV, Maguari virus.

it appears likely that the failure to recognize MAGV as a causative agent of febrile infection in humans is due to a lack of systematic surveillance and diagnostic testing rather than a lack of pathogenic potential. Furthermore, the inclusion of PLAV (75V5758) in this group suggests that, even if human virulence were restricted to this group, the affected region might cover a large portion of Central and South America, including Brazil (strain BeAr7272), Peru (strain OBS6657), and Ecuador (PLAV strain 75V5758).

Analysis of CVV Sequences

To obtain sufficient data to provide a reliable framework for analyzing the MAGV isolates in this study, and in light of their recently demonstrated potential as human pathogens, we further conducted full-genome sequence analysis for several CVV strains and for the closely related PLAV and TLAV, and for the human pathogenic FSV, which also has been described to be closely related to CVV (35). Of the 7 CVV isolates that we fully sequenced, all appear to belong to the recently described lineage 1 of CVV, consistent with the suggestions that older strains from the United States, such as those used in this study, belong to this lineage and have only recently been supplanted in some areas by lineage 2 viruses (36). In addition, TLAV (strain 61D240) and 2 of the 3 strains of PLAV virus analyzed (strains 75V3066 and 75V5938) clearly grouped within the CVV clade for all 3 segments (Figure 2). The other PLAV strain (strain 75V5758) belonged to the canonical MAGV lineage, as noted earlier. The need to reclassify these virus strains as isolates of CVV is further supported by divergence analysis (online Technical Appendix Figure 3, panels A–C), which shows that each of these viruses shows >90% aa identity to all known CVV isolates for all 3 major proteins, including the highly variable glycoprotein. Given the surprising nature of this result, we further confirmed this finding using multiple virus passages and stocks provided from 2 different collections (WRCEVA and DVBD-CDC); however, the results remained consistent (data not shown). Overall, our data provide unequivocal support, on the basis of full-genome sequencing data for all 3 segments of multiple virus strains, for previous observations from S-segment data from single strains of each of TLAV and PLAV (36) that had suggested these strains might indeed have been misclassified as new virus species upon discovery. Similarly, we also identified 1 isolate previously classified as MAGV (strain CoAr3363) as a misidentified CVV lineage 2 virus (Figure 2; online Technical Appendix Figure 3, panels A–C).

We also included FSV in our analysis. This isolate was collected in 1985 at Fort Sherman, a former US Army base in Panama, from a patient exhibiting fever (101°F), malaise, muscle aches, and sore throat. The isolate cross-reacted very closely with CVV, MAGV, and PLAV virus in complement

fixation but only with CVV and PLAV by plaque-reduction neutralization test (37). During the original isolation, FSV was suggested to be a subtype of CVV (35). However, sequencing of the S segment has shown it to be only moderately related to CVV, although statistical support for the branching arrangement was poor (38). On the basis of our full-length analysis of all 3 gene segments, we can now clarify and reconcile these apparently disparate findings, by identifying FSV as a reassortant between CVV lineage 2 and “CODV” (CODV_S/CVV_M/CODV_L). Taken together, the phylogenetic arrangement (Figure 2) and divergence analysis (online Technical Appendix Figure 3, panels A–C), as well as a recombination/reassortment analysis using concatenated genomes (online Technical Appendix Figure 3, panel D), indicate that CODV (CbaAr426) is the closest relative of the S and L segment donor, and the strain CoAr3363 (previously misclassified as MAGV) from CVV lineage 2 is the closest to the M segment donor. This observation would appear to explain the close antigenic relationship of FSV to both CVV and MAGV by complement fixation but not plaque-reduction neutralization test, as well as the lack of sequence relationship between the S segments of these viruses.

One important consequence of the genetic reassignment of not only TLAV and PLAV, but also of MAGV (CoAr3363) to the CVV group, and identification of FSV as a previously unrecognized CVV reassortant, is to substantially expand the known geographic range of CVV. On the basis of these data, the range of CVV must now be considered to include not only the United States and northern and central Mexico but probably all of Central America and the northern areas of South America. Furthermore, although both PLAV strains appear to belong to CVV lineage 1, TLAV belongs to CVV lineage 2, as does the CVV donor of the FSV M segment. This finding suggests that both lineages exist in Central America and that lineage 2 has been circulating in that region since at least the 1960s, when the first of these viruses were isolated. This supposition is also supported by the assignment of the MAGV CoAr3363 strain, which was isolated in 1964, to lineage 2. Taken together, these findings suggest that although these CVV lineage 2 strains have only recently emerged in the eastern United States, they might in fact have been circulating in South America for decades and have only recently been introduced into the United States from these regions.

Technical Considerations in the Genetic and Serologic Analysis of Nucleoprotein Sequences

Our analysis of the ORF and amino acid data identified several distinct clades representing CVV (lineages 1 and 2) and 2 separate clades of what had been identified as MAGV strains (Figure 2). These clades were all well-supported for the GPC and L ORFs. However, although the members of the identified groups also were suggested to remain consistent

when data for the nucleoprotein were analyzed, suggesting a lack of reassortment in these viruses, the branching was only poorly supported. This observation most likely resulted from an overall low level of total variation between these closely related groups, which is exacerbated when shorter datasets, such as that for N, are used. It is possible (and maybe even likely) that this issue will continue to present a growing problem for orthobunyavirus group analyses based on N as an increasing number of sequences for related virus groups continue to become available.

The extent of this issue is further highlighted by our divergence analysis (online Technical Appendix Figure 3, panels A–C), which shows extremely high sequence identity (93.6%–100% aa) among members of these groups, even when the analysis includes more distantly related and clearly geographically distinct viruses, such as Batai virus. These high levels of conservation may then also suggest a basis for the historical difficulties in serologic virus identification that seem to be increasingly identified during retrospective virus identification efforts, including this one. Surprisingly, a recent report showed that a virus isolate originally reported as MAGV was, in fact, a reassortant of Caraparu, a group C virus (4), which is genetically only distantly related to the Bunyamwera serogroup viruses. This finding suggests that the accurate serologic identification of orthobunyaviruses is difficult. This observed lack of overall variability in the N proteins of these viruses seems to suggest that the serologic identification of Bunyamwera group viruses, and perhaps also other groups, by complement fixation might be particularly difficult, if not impossible, and needs to be approached carefully. Furthermore, given the apparent extent of this problem and the widespread availability and modest costs associated with RT-PCR and Sanger sequencing, it may be prudent to recommend routinely incorporating at least partial genetic characterization as a part of the identification process for all new bunyavirus isolates. Indeed, given the high level of conservation observed within the N gene of related orthobunyavirus groups, it would make an ideal target for the development of broadly cross-reactive RT-PCR primer sets for diagnostic applications.

In summary, we identified MAGV as the causative agent of a human febrile infection in Peru and showed that the virus associated with this infection is highly similar to the prototype MAGV isolate, suggesting that other viruses of this lineage also might have pathogenic potential in humans. In addition, our in-depth analysis of the closely related CVV group and other similar viruses showed that PLAV and TLAV do not exist as distinct virus species but are misclassified strains of other existing groups and that the human pathogenic FSV is a previously unrecognized reassortant between CVV, itself a known human pathogen, and a new lineage whose members had previously been

identified as MAGV but are genetically distinct from the lineage occupied by the prototypical MAGV isolates. On the basis of its location of origin, we suggest the name “Córdoba virus” for this new lineage. Based on the reclassification of these virus isolates, it is clear that the endemic region for CVV is much larger than previously recognized and that CVV and MAGV most likely are responsible for unrecognized febrile infections throughout North, Central, and South America. We hope that a better understanding of the genetic relationships between bunyavirus groups, particularly in relation to their relevance for human infection, coupled with an increased availability of reference genome sequence information, such as provided by this study, will help enable future surveillance and diagnostic efforts and increase awareness of the importance of orthobunyaviruses as human pathogens.

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Dr. Groseth is the group leader for the Arenavirus Biology Workgroup, Friedrich-Loeffler-Institut. Her research focuses on virus evolution, virulence acquisition, and the identification of virulence determinants.

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Maguari Virus Associated with Human Disease

Technical Appendix

Technical Appendix Table 1. Source and sequence information for virus strains sequenced in this study

Virus/strain	Host	Year	Location	GenBank accession no.
Maguari virus				
BeAr 7272 prototype	Mixed mosquito pool*	1957	Utinga forest, Pará, Brazil	S: KX100103 M: KX100104 L: KX100105
CoAr 3363	<i>Aedes scapularis</i>	1964	Buenaventura, Valle del Cauca, Colombia	S: KX100106 M: KX100107 L: KX100108
CbaAr 426	<i>Ae. albifasciatus</i>	1965	Córdoba, Argentina	S: KX100109 M: KX100110 L: KX100111
AG83–1746	<i>Psorophora varinervis</i>	1982	Calchaquí Forest, Santa Fe, Argentina	S: KX100112 M: KX100113 L: KX100114
Maguari-like virus: OBS 6657	Human	1998	Pucallpa, Ucayali, Peru	S: KX100115 M: KX100116 L: KX100117
Tlacotalpan virus: 61D240	<i>Mansonia titillans</i>	1961	Tlacotalpan, Veracruz, Mexico	S: KX100118 M: KX100119 L: KX100120
Playas virus				
75V3066	<i>Ae. taeniorhynchus</i>	1975	Playas, Ecuador	S: KX100121 M: KX100122 L: KX100123
75V5938	<i>Aedeomyia (ochler) taeniorhynchus</i>	1975	Guayaquil, Ecuador	S: KX100124 M: KX100125 L: KX100126
75V5758	<i>Aedeomyia (ochler) taeniorhynchus</i>	1975	La Florida, Ecuador	S: KX100127 M: KX100128 L: KX100129
Fort Sherman virus: 86MSP18	Human	1985	Fort Sherman, Panama	S: KX100130 M: KX100131 L: KX100132
Cache Valley virus				
6V633 prototype	<i>Culiseta inornata</i>	1956	Cache Valley (near Wellsville), Utah, USA	S: KX100133 M: KX100134 L: KX100135
W728–67	<i>Ae. communis</i>	1967	Mazomanie, Wisconsin, USA	S: KX100136 M: KX100137 L: KX100138
W308–67	<i>Ae. trivittatus</i>	1967	Wyalusing, Wisconsin, USA	S: KX100139 M: KX100140 L: KX100141
MPB1–1551	<i>Psorophora confinnis</i>	1971	Palo Blanco, Tamaulipas, Mexico	S: KX100142 M: KX100143 L: KX100144
CK-102	Sheep (sentinel)	1980	San Angelo, Texas, USA	S: KX100145 M: KX100146 L: KX100147
MI80–1-450	Horse	1980	Cass County, Michigan, USA	S: KX100148 M: KX100149 L: KX100150
WI-03BS7669	Human	2003	Wisconsin, USA	S: KX100151

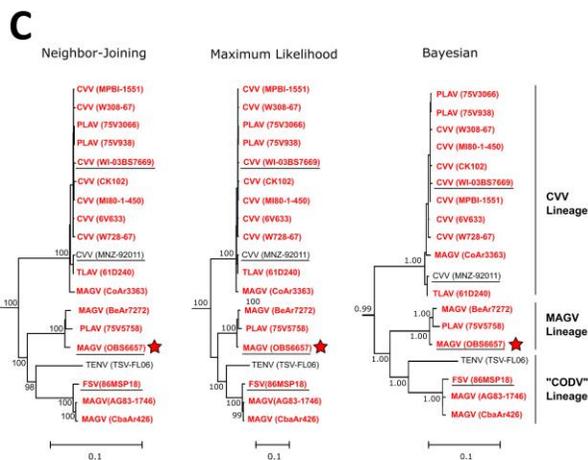
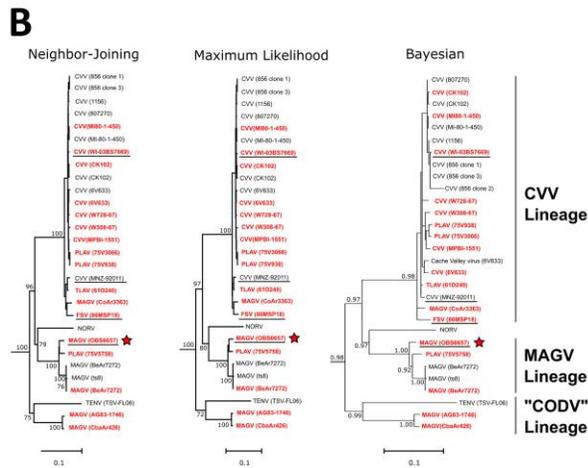
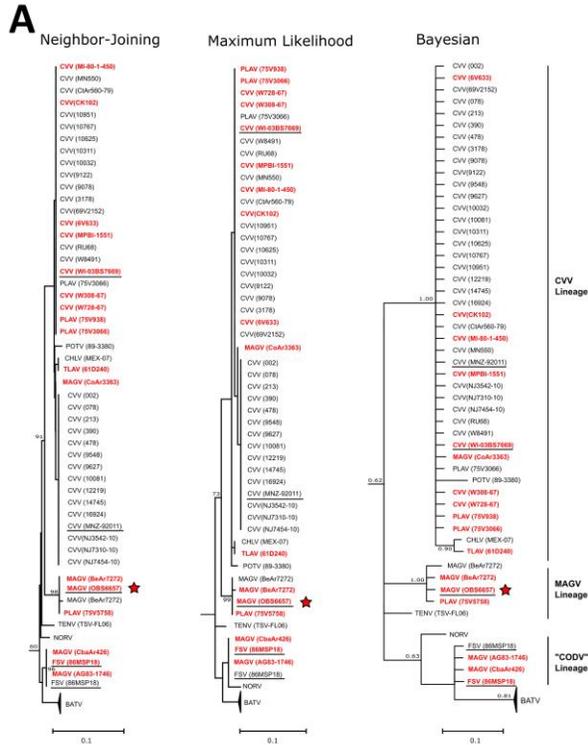
Virus/strain	Host	Year	Location	GenBank accession no.
				M: KX100152 L: KX100153

*Contained *Aedes scapularis*, *Ae serratus*, *Ae sexlineatus*, *Mansonia* spp. and *Psorophora ferox*.

Technical Appendix Table 2. GenBank accession numbers for reference orthobunyavirus sequences used in the phylogenetic analyses

Group/virus	Strain	GenBank accession nos.		
		S segment	M segment	L segment
Bunyamwera				
Abbey Lake virus	Cu20-XJ	KJ710424	KJ710423	KJ710425
Batai virus	MM2222	JX846595	JX846596	JX846597
	8627-11	FJ436802	FJ436799	
	804922	FJ436805		
	804986	FJ436803	FJ436798	
	804988	FJ436800	FJ436804	
	IG 20217 (Chittoor)	JX846598	JX846599	JX846600
	Italy-2009	KC168046	KC168047	KC168048
	NM-12	KJ187040	KJ187039	KJ187038
	ON-1-E-94	AB257761	AB257764	
	ON-7-B-01	AB257762	AB257765	
	UgMP6830	JX846601	JX846602	JX846603
	XQ-B	KJ398936		
	MS50	JX846604	JX846605	JX846606
	Calovo 134	KJ542624	KJ542625	KJ542626
	Calovo 184		DQ334335	
	Calovo 8020	KJ542630	KJ542631	KJ542632
	Calovo 8040	KJ542633	KJ542634	KJ542635
	Calovo JAn MS3	KJ542627	KJ542628	KJ542629
Birao virus	ArB2198	AM711131		
Bozo virus	ArB13529	AM711132		
Bunyamwera virus	Original	NC_001927	NC_001926	NC_001925
	ArB28215	AM711130		
	ArB29051	AM709778		
Cache Valley virus	MNZ-92011	KC436108	KC436107	KC436106
	002	GU018033		
	6V633		AF082576	
	69V2152	KP835919		
	078	GU018034		
	213	GU018035		
	390	GU018036		
	478	GU018037		
	3178	KP835920		
	9078	KP835921		
	9122	KP835922		
	9548	KP835930		
	9627	KP835923		
	10032	KP835931		
	10081	KP835932		
	10311	KP835933		
	10625	KP835924		
	10767	KP835934		
	10951	KP835935		
	12219	KP835936		
	14745	KP835928		
	16924	KP835929		
	CK-102		AF186242	
	CtAr560-79	KP835925		
	MI80-1-450		AF186241	
	MN550	KP835926		
	NJ3542-10	KF296339		
	NJ7310-10	KF296340		
	NJ7454-10	KF296341		
	RU68	KP835927		
	W8491	KP835937		
Cholul virus	MEX-07	EU879062	JN808310	
Fort Sherman virus	86MSP18	EU564829		

Group/virus	Strain	GenBank accession nos.		
		S segment	M segment	L segment
Germiston virus	SAAr1050	M19420	M21951	
Ilesha virus	8e	KC608151	KC608150	KC608149
	R5964	AY729651	KF234074	KF234075
	KO/2		AY859372	
	ArB16282	AM709780		
	HB80P125	AM709779		
Kairi virus	MEX-07	EU879063	GQ118699	
	TRVL8900	X73467	EU004186	
Maguari virus	BeAr7272	D13783	AY286443	
Main Drain virus	BFS5015	X73469	EU004187	
Mboke virus	DakArY357	AY593727		
M'Poko virus	ArB365	AM711133		
Nola virus	ArB2882	AM711134		
Ngari virus	9800521	JX857325	JX857326	JX857327
	9800535	JX857328	JX857329	JX857330
	Adrar	KJ716848	KJ716849	KJ716850
	DaKArD28542	JX857316	JX857317	JX857318
	GSA-TS7-5170	KM507341	KM514677	KM507336
	SUD HKV66	JX857319	JX857320	JX857321
	SUD HKV141	JX857322	JX857323	JX857324
	ISL-TS2-5242	KM507342	KM514678	KM507334
	TND-S1-19801	KM507343	KM514679	KM507335
Northway virus	0234	X73470	EU004188	
Playas virus	75V3066	KP83593		
Potosi virus	89-3380	AY729652	EU004189	
Shokwe virus	SAAr4042	EU564831		
Tensaw virus	TSV-FL06	FJ943507	FJ943506	FJ943509
Wyeomyia/Anhembi				
Anhembi virus	SPAr2984	JN572064	JN572063	JN572062
Cachoeira Porteira virus	BeAr328208	JN968592	JN968591	JN968590
Iaco virus	BeAr314206	JN572067	JN572066	JN572065
Macaua virus	BeAr306329	JN572070	JN572069	JN572068
Sororoca virus	BeAr32149	JN572073	JN572072	JN572071
Taiassui virus	BeAr671	JN572076	JN572075	JN572074
Tucunduba virus	BeAr278	JN572079	JN572078	JN572077
Wyeomyia virus	Original	JN572082	JN572081	JN572080
	TRVL8349	JN801033	JN801034	JN801035
	Darien	JN801036	JN801037	JN801038
Guaroa virus				
	ASA1165	KM245552	KM245553	KM245554
	BeH22063	KM245522	KM245523	KM245524
	CoH352111	KM245519	KM245520	KM245521
	FPI01900	KM245546	KM245547	KM245548
	FSJ2035	KM245549	KM245550	KM245551
	FVB0546	KM245528	KM245529	KM245530
	FVB0840	KM245537	KM245538	KM245539
	FVB0849	KM245540	KM245541	KM245542
	FVB2032	KM245543	KM245544	KM245545
	IQD8537	KM245525	KM245526	KM245527
	MIS0239	KM245534	KM245535	KM245536
	OBT5637	KM245531	KM245532	KM245533
California encephalitis (outgroup):	Human-78	NC_004110	NC_004109	NC_004108
LaCrosse virus				



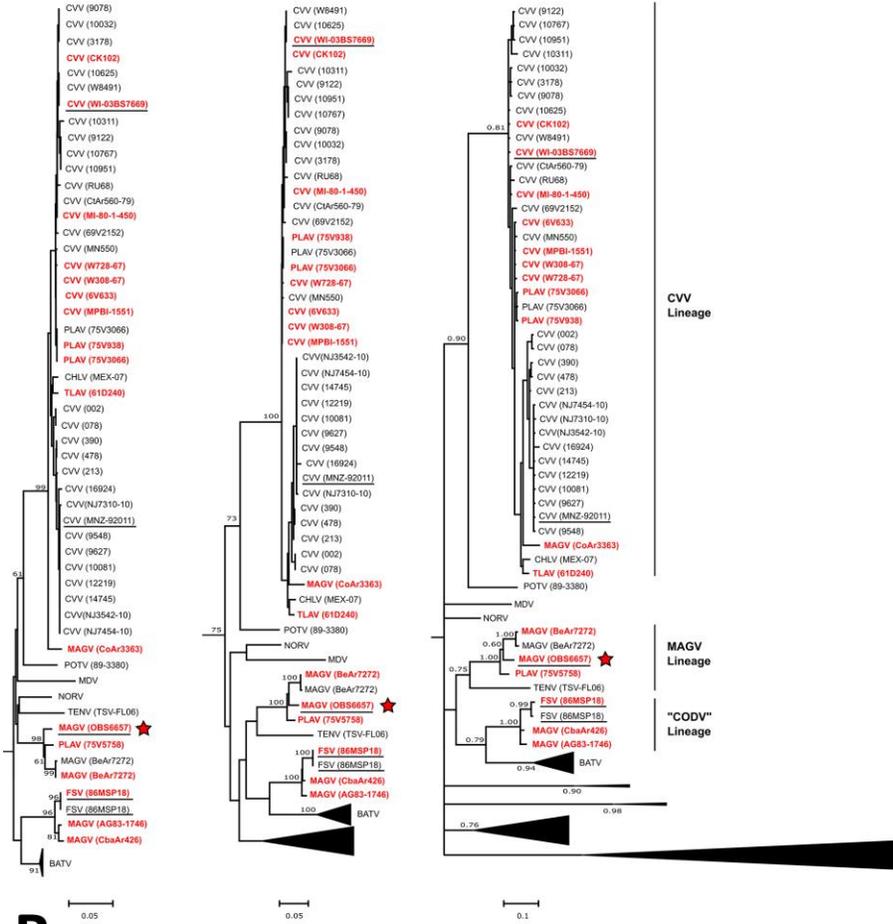
Technical Appendix Figure 1. Comparison of phylogenetic analyses of (A) nucleoprotein, (B) glycoprotein, and (C) polymerase amino acid sequences generated using various phylogenetic methods and models. Neighbor-joining trees for all 3 segments were generated using the Poisson model with uniform rates. Maximum-likelihood trees were constructed based on the best fitting model for each dataset (i.e., N = JTT+G; GPC = LG+G+I; L = LG+G+I+F). Bootstrap values for both methods were based on 1,000 replicates and are indicated if >60. Analysis by Bayesian inference also used the best fitting model for each dataset (i.e., N = JTT+G; GPC and L = JTT+I+G) and shows posterior probability values >0.60. For clarity, only the portion of the tree containing the sequences generated in this study (i.e., CVV, MAGV, and CODV lineages) are shown, and measures of statistical support for branching within the CVV lineage are omitted. Sequences generated in this study are shown in red and bold. The OBS6657 isolate is indicated with a red star and human-derived isolates are underlined. BATV, Batai virus; CVV, Cache Valley virus; CHLV, Cholul virus; CODV, Córdoba virus; FSV, Fort Sherman virus; MAGV, Maguari virus; MDV, Main Drain virus; NORV, Northway virus; PLAV, Playas virus; POTV, Potosi virus; TLAV, Tlacotalpan virus; TENV, Tensaw virus.

A

Neighbor-Joining

Maximum Likelihood

Bayesian

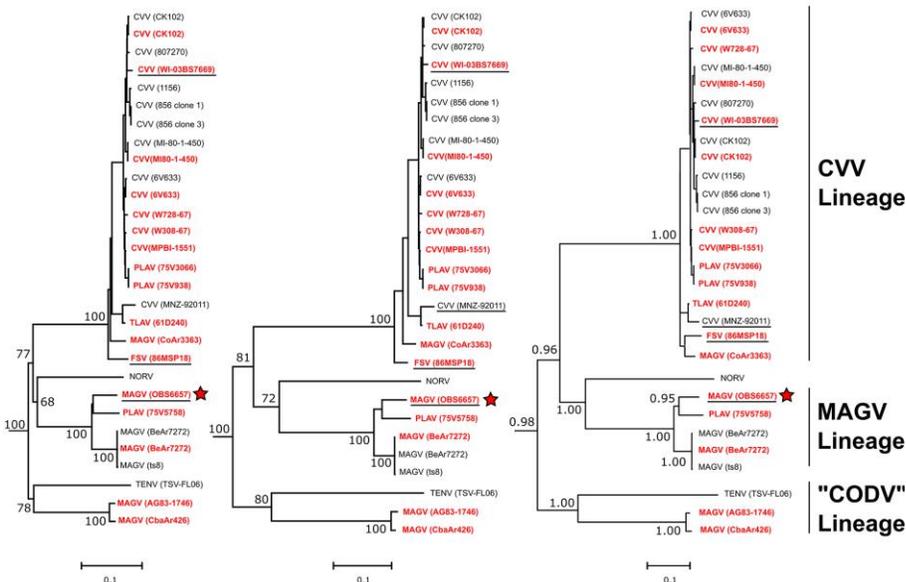


B

Neighbor-Joining

Maximum Likelihood

Bayesian



Technical Appendix Figure 2. Comparison of phylogenetic analyses of nucleoprotein (A), glycoprotein (B), and polymerase (C) open reading frame nucleotide sequences generated using various phylogenetic methods and models. Neighbor-joining trees for all 3 segments were generated using uniform rates. Maximum-likelihood trees were constructed based on the best fitting model for each dataset (i.e. N = T92+G; GPC and L= GTR+G+I). Bootstrap values for both of these methods were based on 1,000 replicates and are indicated if >60. Analysis by Bayesian inference also used the best fitting model for each dataset (i.e., N = GTR+G; GPC and L = GTR+G+I) and shows posterior probability values >0.60. For clarity, only the portion of the tree containing the sequences generated in this study (i.e., CVV, MAGV, and CODV lineages) are shown, and measures of statistical support for branching within the CVV lineage are omitted. Sequences generated in this study are shown in red and are bold. The OBS6657 isolate is indicated with a red star, and human-derived isolates are underlined. BATV, Batai virus; CVV, Cache Valley virus; CHLV, Cholul virus; CODV, Córdoba virus; FSV, Fort Sherman virus; MAGV, Maguari virus; MDV, Main Drain virus; NORV, Northway virus, PLAV, Playas virus; POTV, Potosi virus; TLAV, Tlacotalpan virus; TENV, Tensaw virus.

Technical Appendix Figure 3. Divergence analyses for nucleoprotein (A), glycoprotein (B), and polymerase (C) sequences. Nucleotide (top) and amino acid (bottom) sequences of MAGVs (blue) and CVVs (purple) and for other closely related reference sequences (gray). Amino acid identities among members of the same group are highlighted in dark shades of the corresponding colors. Although in the analysis of the nucleoprotein sequence all pairings showed identity values >90%, in the polymerase sequence analysis pairing outside of a clade that showed identity values >90% are highlighted in red. D) Recombination analysis. Concatenated full-length genomes for all members of the CVV, MAGV, and CODV lineages with available data were generated and analyzed for evidence of recombination using RDP4 Beta 4.83 (1). A single recombination event (equivalent to reassortment in the segmented virus) was identified and corresponded to the M segment junctions in the concatemer. The major parent (i.e., S and L segment donor) was identified as being most closely related to CODV strain CbaAr426 (shown in green), and the minor parent (i.e., M segment donor) was most closely related to CVV strain CoAr3363 (shown in blue). BATV, Batai virus; CVV, Cache Valley virus; CODV, Córdoba virus; FSV, Fort Sherman virus; MAGV, Maguari virus; NORV, Northway virus; PLAV, Playas virus; TLAV, Tlacotalpan virus; TENV, Tensaw virus.

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