Barrita Virus, a Novel Virus of the Patois Serogroup (Genus *Orthobunyavirus*; Family *Peribunyaviridae*)

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Abstract. During ecological investigations for arboviruses conducted in coastal Chiapas, Mexico, in 2007, isolate MP1078 was obtained from a pool of *Psorophora varipes* mosquitoes. Based on antigenic characterization, this isolate was classified as a strain of Patois virus (PATV) (*Orthobunyavirus* genus, *Peribunyaviridae* family). Recently, we conducted nearly complete genome sequencing of this isolate to gain further insight into its genetic relationship with other members of the Patois serogroup. Based on the genetic characterization, we determined that MP1078 contains S, M, and L genome segments that are genetically distinct from other viruses within the Patois serogroup. Serological analyses confirmed the taxonomic classification of MP1078 as a new virus and species within the Patois serogroup, and we propose the name Barrita virus (BITV).

The PATV was originally isolated from mosquitoes collected in Panama in 1961,¹ but it was also frequently isolated along the tropical Gulf coast of southeastern Mexico during ecological investigations in the 1960s.^{2,3} Zegla virus, another member of the Patois serogroup, was also frequently isolated from the same area as part of these ecological investigations.^{2,3} Given the frequent isolation of PATV, studies were conducted at the time to investigate human exposure to these viruses. These studies revealed the presence of hemagglutination inhibition and neutralizing antibodies to PATV in residents at the study site and in terrestrial wild mammals, including cotton rats, opossums, and raccoons.³ However, despite the serological evidence of human exposure to PATV, evidence of human disease caused by PATV and related viruses remains uncertain.

Ecological investigations conducted in South, Central, and North America have continued to frequently yield the isolation of viruses belonging to the Patois serogroup.^{4,5} In 2007, as part of ecological investigations for arbovirus in coastal Chiapas, Mexico, the isolation of virus strain MP1078 was described.⁶ This isolate was made from a pool of four *Psorophora varipes* mosquitoes collected in June 2007 in a mangrove habitat near the village of Isla Morelos in the municipality of Pijijiapan. As part of a systematic ecological investigation into the natural transmission cycle of Venezuelan equine encephalitis virus subtype IE (VEEV-IE) in coastal Chiapas, mosquitoes and small mammals were collected at various times over the course of one calendar year. During that year, no VEEV-IE isolates were made, but two orthobunyaviruses, Nepuyo and Patois-like MP1078, were isolated from mosquito pools.

The mosquitoes were collected from a hamster-baited trap, identified morphologically, pooled, and stored in liquid nitrogen for approximately 2 weeks before being shipped to Galveston, Texas, for processing at BSL-3 at University of Texas Medical Branch (UTMB). Mosquito pools were homogenized with a mini-bead-beater, clarified by centrifugation, and the

supernatant was applied to Vero cells. After MP1078 caused primary and secondary cytopathic effects on Vero cells, the etiologic agent was identified as Patois-like virus by complement fixation assay.⁶

Recently, we conducted sequencing analyses of viruses belonging to the Patois serogroup viruses and obtained nearly complete genome sequencing information of the prototype viruses.⁷ In an attempt to obtain additional sequence information for other Patois virus (PATV) strains isolated from field investigations and to provide additional evidence that MP1078 is in fact a strain of PATV, we conducted full genome sequencing and phylogenetic analyses of this isolate to determine its relationship to PATV and other members of the Patois serogroup. The isolate MP1078 was obtained from the World Reference Center for Emerging Viruses and Arboviruses and propagated in African green monkey kidney (Vero) cells. On the appearance of cytopathic effects, the cell culture supernatant was harvested and clarified by centrifugation, and viral RNA was extracted as previously described.⁸ The viral RNA was then fragmented, and a cDNA library was prepared and sequenced on a HiSeq 1000 instrument. Reads were quality-filtered and assembled using the de novo assembly strategy implemented in ABySS software.⁹ The longest contigs were selected and reads were mapped back to the contigs using bowtie2 software,¹⁰ and then visualized with the Integrated Genomics Viewer¹¹ to verify that the assembled contigs were correct. Additional details are available on request. Using this approach, we were able to obtain nearly complete S, M, and L segment sequences of MP1078, which cover all open reading frames. To determine the genetic relationship of MP1078 to PATV and other Patois group viruses, we carried out multiple sequencing alignments on both nucleotide and amino acid sequences of S, M, and L RNA segments using the MAFFT software.¹² Phylogenetic trees were constructed by the maximum likelihood (ML) method and inferred using IQ-TREE version 1.4.3 software under the best-fit model based on Bayesian information criterion determined by ModelFinder with 1,000 replicates.^{13,14} Our phylogenetic analyses revealed that MP1078 is in fact closely related to other

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FIGURE 1. Maximum likelihood phylogenetic relationship of Patois serogroup viruses with other orthobunyaviruses. (A) S segment based on TMP2+F+G4 nucleotide substitution model. (B) M segment based on GTR+F+I+G4 nucleotide substitution model. (C) L segment based on TIM2+F+I+G4 nucleotide substitution model. Phylogenies are midpoint rooted and with proportional branches for clarity of presentation. The scale bar indicates the evolutionary distance in number of substitutions per amino acid substitutions/site, and the principal bootstrap support levels were indicated. The arrows indicate the position of the Patois-like MP1078 strain (Barrita virus).

viruses within the Patois serogroup (Figure 1). Although relationships derived from each genomic segment differed slightly, bootstrap values were not high enough to determine if these differences reflected reassortment. However, the S, M, and L RNA segments presented high genetic divergence (S segment = 10–23% nucleotides, 5–22% amino acid difference; M segment = 20–31% nucleotide, 8–29% amino acid difference; and L segment = 17–24% nucleotide, 4.5–16% amino acid difference) when compared with the sequences of other Patois serogroup viruses (Figure 2). In the past, the International Committee on Taxonomy of Viruses approved the classification of new species based on distinct biological characteristics of the respective virus. In recent years, the ICTV has refined its classification criteria for virus species

demarcation.^{15–17} Specifically, ICTV has adopted sequence comparisons using pairwise sequence similarity and phylogenetic relationships as one of the primary criteria to define and distinguish virus taxa.¹⁶ Currently, the ICTV states that species within the genus *Orthobunyavirus* can be defined by less than 96% identity in the complete amino acid sequence of the L protein (https://talk.ictvonline.org/ictv-reports/ictv_online_report/negative-sense-ma-viruses/bunyavirales/w/peribunyaviridae/1238/genus-orthobunyavirus). Because MP1078 fulfill this criterion, it can be considered genetically distinct from other members within the Patois serogroup.

To gain further insights into the relationship of MP1078 to other Patois group viruses, we carried out one-way antigenic cross-neutralization analyses using antisera raised against

FIGURE 2. P-distance analysis of Barrita virus within the members of Patois serogroup. Distance based on the S segment (left panel), M segment (middle panel), and L segment (right panel) are shown. Distances in the nucleotide level are showed in purple and the amino acids level in green. Barrita and Zegla viruses share 95.5% amino acid sequence identity in the L protein (depicted as 96% in the figure). This figure appears in color at www.ajtmh.org.

TABLE 1
Neutralization of MP1078 by Patois serogroup virus antisera

Virus	Mouse immune ascitic fluid		
	Patois	Shark River	Zegla
MP1078 (Barrita)	< 20	< 20	640
Patois	160	< 20	< 20
Shark River	40	640	< 20
Zegla	20	< 20	10,240

Neutralization titers are expressed as the dilution of mouse immune ascitic fluid antiserum inhibiting 80% of plaque-forming units.

the different Patois group viruses. Notably, we observed that these antisera displayed a minimum 4-fold difference in neutralization titers among these viruses, indicating that MP1078 is antigenically distinct from other Patois serogroup viruses (Table 1).

In summary, in this study, we have provided genetic and antigenic support for the classification of MP1078 as a new virus and species in the Patois serogroup. Thus, based on the current ICTV criteria for species demarcation, we propose the name Barrita virus (BITV) for this newly identified virus within the Patois serogroup. Further investigation into the public health impact of this virus is warranted.

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