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Molecular Epidemiological Analysis of Dengue Fever in Bolivia from 1998 to 2008

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Abstract

Dengue fever was first recognized in Bolivia in 1931. However, very limited information was available to date regarding the genetic characterization and epidemiology of Bolivian dengue virus strains. Here, we performed genetic characterization of the full-length envelope gene of 64 Bolivian isolates from 1998 to 2008 and investigated their origin and evolution to determine whether strains circulated simultaneously or alternatively, and whether or not multiple introductions of distinct viral variants had occurred during the period studied. We determined that, during the last decade, closely related viruses circulated during several consecutive years (5, 6, and 6 years for DENV-1, DENV-2, and DENV-3, respectively) and the co-circulation of two or even three serotypes was observed. Emergence of new variants (distinct from those identified during the previous episodes) was identified in the case of DENV-1 (2007 outbreak) and DENV-2 (2001 outbreak). In all cases, it is likely that the viruses originated from neighboring countries.

Keywords

Dengue; Epidemiology; Genetics

OVER THE PAST 50 YEARS a variety of arboviral human diseases have been reported in Bolivia, including yellow fever (Bevier et al. 1953), Mayaro fever (Taylor et al. 2005), and dengue fever (Gianella et al. 1998, Van der Stuyft et al. 1998, Kochel et al. 2008), although many other arboviruses potentially circulate and cause disease in this country. Dengue fever was first recognized in Bolivia in 1931 (Garret 1993). However, the first documented Bolivian case of dengue fever was notified in 1987. The virus was isolated at the Instituto Fiocruz, Rio de Janeiro, Brazil (Anonymous 1995) and was believed to belong to dengue virus serotype 1 (DENV-1). This serotype is believed to have circulated until 2003. It then

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apparently disappeared and was not identified again until 2008. Dengue virus serotype 2 (DENV-2) was first detected in 1997 (Gianella et al. 1998, Van der Stuyft et al. 1998, Foster et al. 2004), and evidence of its circulation in Bolivia has been reported every year to 2007. Dengue virus serotype 3 (DENV-3) was first detected in 2002 (Kochel et al. 2008) and has subsequently been identified every year. It is now recognized that cases of dengue fever arise predominantly in Bolivia between November and June and are referred to as the “annual epidemic” (in this paper, they are reported as E1998–E2007; see Fig. 1). Most dengue fever cases are reported in the eastern region of Bolivia—in particular, in the Santa-Cruz region. The western region is dominated by the Andes Cordillera, where the altitude is too high for sustained arbovirus circulation. Co-circulation of DENV-2 and DENV-3 was reported during E2003, E2004, E2005, and E2006. Co-circulation of DENV-1, DENV-2, and DENV-3 was reported only during E2002. However, before the current study, very limited information was available regarding the genetic characterization of DENV strains. Here, we carried out the genetic characterization of isolates from 1998 to 2008 stored in the Centro Nacional de Enfermedades Tropicales (CENETROP) Microbiology Diagnostics Department (Santa Cruz, Bolivia). Our objective was to investigate the origin of viral strains and, more specifically, to determine whether or not multiple introductions of distinct viral variants had occurred between 1998 and 2008.

Sixty-four selected DENV isolates (16 DENV-1, 23 DENV-2, and 25 DENV-3) isolated at CENETROP between 1998 and 2008 were studied (Table 1). Viruses were propagated using C6/36-HT cells (Roche et al. 2000) in EMEM (Eagle’s Minimum Essential Medium) at 34°C; 200 μ L of supernatant medium was collected at day 5 postinfection, and viral RNA was extracted using the QIAamp viral RNA minikit (Qiagen, Dusseldorf, Germany) according to the manufacturer’s recommendations. Reverse transcription was performed using random hexamers and the TaqMan reverse transcription reagents kit (Applied Biosystems, Foster City, CA). The complete viral envelope gene was then amplified using the Triplmaster PCR system kit (Eppendorf, Hamburg, Germany), standard PCR amplification conditions, and a series of serotype-specific primers (DENV-1: E1F [AACAAGARCYGA RACRTGGATGTC] and E4R [YARTTCATTTGATATTTGYT TCCACAT]; DENV-2: DEN2-env-S3 [ACACCATAGGRAC GACRYATTT] and DEN2-env-R3 [CCRCTGCCACAYTTY AGTTCT]; DENV-3: DEN3-E2-S [GARAARGTAGARACA TGGGC] and DEN3-E2-R [TCNGCYTGRAATTTGTATTG CTC]). Amplicons were directly sequenced using the amplification primers. Viral nucleotide sequences of each dengue serotype were aligned using ClustalX (Thompson et al. 1997) together with relevant sequences retrieved from GenBank. Phylogenetic analyses were performed using three different methods. First, analyses were conducted within MEGA version 3.1 (Kumar et al. 2004) using either the uncorrected *p*-distance or the Kimura 2-parameter algorithm for distance calculation, and Neighbor-Joining (NJ) and 500 bootstrap replicates for construction of unrooted trees (see Fig. 2). Maximum likelihood (ML) analyses were also carried out. The model of nucleotide substitution and parameter values were selected via Modeltest (Posada and Crandall 1998). ML phylogenetic trees were then estimated in GARLI (Zwickl 2006) using the GTR + G(gamma) + I model of nucleotide substitution. The substitution matrix, base composition, gamma distribution of among-site rate variation (G), and the proportion of invariant sites (I) are available from the corresponding author on request. A bootstrap resampling analysis was conducted using 1000 replicate NJ trees based on the ML substitution matrix described above, in PAUP (Swofford 2000). Phylogenetic analyses were also performed via Bayesian analysis in MrBayes v3.1.2 (Huelsenbeck and Ronquist 2001) with model parameters again specified according to Modeltest, one million generations and a burnin of 10%. Stationary was assessed using Tracer v1.4.1 (part of the BEAST package; Drummond and Rambaut 2007). All methods produced similar topologies at the genotype level with one exception—namely, DENV-1 2007 isolates. In this case, the ML reconstruction is shown in addition to the NJ trees

presented in Figure 2. Other detailed phylogenetic reconstructions are available upon request to the corresponding author.

In the case of DENV-1, isolates from E1998, E2000, E2001, and E2007 were studied. No previously characterized sequences from Bolivian DENV-1 were available in public databases. Based on E gene analysis, all Bolivian viruses belonged to the “America/Africa” genotype V (see Fig. 2A). Isolates from E1998, E2000, and E2001 were very similar (uncorrected p -distance in the E gene <0.010) and shared a common phylogenetic ancestor. These observations demonstrate that the E1998–E2001 viruses circulated persistently during this period with no introductions of new viral variants. Moreover, on the basis of sequence homology and phylogenetic data, the most probable origin of this Bolivian DENV-1 is South America, because the E gene sequence is highly similar to viruses that circulated previously in neighboring countries such as Brazil and Argentina. However, the virus that has circulated more recently in Bolivia (E2007) is more distantly related to viruses from the E1998–E2001 period than it is to the 2006–2007 isolates from Puerto Rico or Venezuela (uncorrected p -distance in the E gene 0.016–0.025 vs. 0.005–0.013) (Fig. 2A), suggesting that it may have been introduced more recently from one of these countries. Isolate 1868/08 is located apart from other Bolivian isolates based on NJ analysis, but clusters with other E2007 Bolivian isolates based on ML and Bayesian analyses. The possibility that emergence of this new variant might be explained by immune selection seems unlikely because the E1998–E2001 DENV-1 strains would be expected to provide complete immune cross protection against the DENV-1 E2007 variant.

In the case of DENV-2, isolates from E2001, E2002, E2004, and E2006 were studied. When compared with two E gene sequences available in GenBank and previously produced from Bolivian strains isolated in 1997 (Foster et al. 2004), all Bolivian viruses belonged to the “Asia/America” genotype (see Fig. 2B). Isolates from E2001–E2006 are very similar (maximum uncorrected p -distance in the E gene <0.008) and share a recent common phylogenetic ancestor. This suggests that viruses belonging to a unique and recent DENV-2 lineage circulated during the E2001–E2006 period. However, the virus that circulated in Bolivia in 1997 is more distantly related (uncorrected p -distance ~ 0.025), and more similar to Brazilian strains (uncorrected p -distance in the E gene 0.003–0.009), indicating that viruses from 1997 and from the E2001–E2006 period originate from two separate introductions. The precise origin of the viruses is difficult to establish but probably in neighboring South/Central American countries or the Caribbean (Fig. 2B), as suggested by all phylogenetic analyses performed. In addition, viruses from the E2001–E2006 period share considerable identity with isolates from Paraguay in 2001 and 2005, suggesting sustained circulation of DENV-2 between the two bordering countries.

In the case of DENV-3, isolates from E2002, E2003, E2004, and E2006 were studied. When compared with two E gene sequences available in GenBank and previously produced from Bolivian strains isolated in 2003 (Kochel et al. 2008), all these Bolivian viruses belonged to genotype III (Fig. 2C) with uncorrected p -distances in the E gene <0.010 and a common recent phylogenetic ancestor, regardless of the method used for phylogenetic reconstruction. Viruses belonging to this lineage, presumably originating from South America (Brazil, Venezuela) or the Caribbean, circulated in Bolivia during the E2002–E2006 period.

In conclusion, our study of the molecular epidemiology of dengue fever in Bolivia during the last decade reveals that closely related viruses (i.e., viruses with a common recent ancestor) circulated during several consecutive years (5, 6, and 6 years for DENV-1, DENV-2, and DENV-3, respectively). Emergence of new variants (distinct from those identified during the previous episodes) was identified in the case of DENV-1 (E2007) and DENV-2 (E2001). In all cases, it is likely that the viruses originated from neighboring

countries. The determinants of the emergence and disappearance of variants remain poorly understood in Bolivia in the absence of comprehensive epidemiological and entomological studies. The co-circulation of two or even three serotypes was observed. Future studies may reveal whether these distinct viral populations can be associated with different epidemiological (temporal or geographical distribution, clinical presentation, and previous immunity) or ecological characteristics such as vector subpopulations.

References

- Anonymous. Dengue y dengue hemorragico en las Americas: Guia para su prevencion y control. Vol. 548. Organizacion Panamericana de la Salud. Publicacion Cientifica; Washington, DC: 1995.
- Bevier G, Torres-Munoz N, Doria-Medina J. Yellow fever in Bolivia, its history and epidemiology. *Am J Trop Med Hyg.* 1953; 2:464–482. [PubMed: 13040681]
- Drummond AJ, Rambaut A. BEAST: bayesian evolutionary analysis by sampling trees. *BMC Evol Biol.* 2007; 7:214. [PubMed: 17996036]
- Foster JE, Bennett SN, Carrington CV, Vaughan H, et al. Phylogeography and molecular evolution of dengue 2 in the Caribbean basin, 1981–2000. *Virology.* 2004; 324:48–59. [PubMed: 15183052]
- Garret, J., editor. Historia de la medicina en Santa Cruz. Bolivia; Sirena: 1993.
- Gianella A, Pirard M, Holzman A, Boelaert M, et al. Epidemic outbreak of dengue virus 2/Jamaica genotype in Bolivia. *Salud Publica Mex.* 1998; 40:469–473. [PubMed: 9927881]
- Huelsenbeck JP, Ronquist F. MRBAYES: bayesian inference of phylogeny. *Bioinformatics.* 2001; 17:754–755. [PubMed: 11524383]
- Kochel T, Aguilar P, Felices V, Comach G, et al. Molecular epidemiology of dengue virus type 3 in Northern South America: 2000–2005. *Infect Genet Evol.* 2008; 8:682–688. [PubMed: 18674640]
- Kumar S, Tamura K, Nei M. Integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform.* 2004; 5:150–163. [PubMed: 15260895]
- Posada D, Crandall KA. Modeltest: testing the model of DNA substitution. *Bioinformatics.* 1998; 14:817–818. [PubMed: 9918953]
- Roche RR, Alvarez M, Guzmán MG, Morier L, et al. Comparison of rapid centrifugation assay with conventional tissue culture method for isolation of dengue 2 virus in C6/36-HT cells. *J Clin Microbiol.* 2000; 38:3508–3510. [PubMed: 10970418]
- Swofford, DL. PAUP*. Phylogenetic Analysis Using Parsimony (*and other methods). 4th ed. Sinauer Associates; Sunderland, MA: 2000.
- Taylor SF, Patel PR, Herold TJ. Recurrent arthralgias in a patient with previous Mayaro fever infection. *South Med J.* 2005; 98:484–48. [PubMed: 15898531]
- Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, et al. The ClustalW windows interface: flexible strategies for multiple sequence alignment aided by quality tools. *Nucleic Acids Res.* 1997; 25:4876–4882. [PubMed: 9396791]
- Van der Stuyft P, Gianella A, Pirard M, Holzman A, et al. Dengue serotype 2 subtype III ('Jamaica') epidemic in Santa Cruz, Bolivia. *Trop Med Int Health.* 1998; 3:857–858. [PubMed: 9855395]
- Zwickl, DJ. Ph.D. dissertation. The University of Texas at Austin; Austin: 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion.

FIG. 1.

Dengue IgM-positive sera and dengue virus isolation, CENETROP, Santa-Cruz, 1998–2008.

(A) Dengue IgM-positive sera. Detection of specific IgM to dengue virus was performed using an immuno-capture assay. The antigen was produced from a pool of supernatants of DENV-1 (strain Hawaii), DENV-2 (strain new Guinea C), DENV-3 (strain H87), and DENV-4 (strain H241) strains propagated on C6/36-HT cells. (B) Isolation and characterization of dengue strains. Isolates were obtained after inoculation of human sera on C6/36-HT cells in EMEM (Eagle's Minimum Essential Medium) at 34°C. Identification of serotype was performed using specific monoclonal antibodies (DENV-1: D2-IFI-3; DENV-2: 3H5-1-21; DENV-3: D6-8A1-12; DENV-4: 1H10-6-7; all from CDC, San Juan, Puerto Rico) and an immunofluorescence assay. (C) Isolates included in the current study.

X-axis: The period studied (1998 outbreak to 2007 outbreak). Y-axis: (A) the number of sera

with IgM antibody to DENV identified monthly by the CENETROP microbiology department (Santa Cruz, Bolivia). **(B)** the number of DENV isolates made by the CENETROP laboratory and their serotype. **(C)** the DENV isolates analysed in the current study.

FIG. 2.

Phylogenetic analysis in the Envelope gene (nucleotide sequences). All trees presented were obtained using the Kimura 2-parameter algorithm for distance calculation, NJ, and 500 bootstrap replicates (values greater than 50% are indicated on major nodes branches). Horizontal bars are proportional to genetic distances. Identical topologies were obtained using the *p*-distance algorithm and ML methodologies, with the exception of DENV-1 2007 in the ML analyses, results of which are shown in addition to the NJ tree. (A) DENV-1 sequences. Phylogenetic tree reconstructed using DENV-1 E gene sequences (nt 772–2617 referring to the numbering of strain Indonesia_A88_1988; GenBank accession number AB074761). The topology of 2007 Bolivian isolates in ML reconstructions is slightly different and shown on the right of the main figure. (B) DENV-2 sequences. Phylogenetic tree reconstructed using DENV-2 E gene sequences (nt 863–2411 referring to the numbering

of strain Puerto-Rico_BID/V678_1998; GenBank accession number EU482735). To improve legibility, the reconstruction for the “Asia/America” genotype is presented after magnification. (C) DENV-3 sequences. Phylogenetic tree reconstructed using DENV-3 E gene sequences (nt 817–2478 referring to the numbering of strain Thailand_C0331/94_1994; GenBank accession number AY876494). To improve legibility, the reconstruction for genotype III is presented after magnification.

Table 1**Main Characteristics of DENV Isolates Studied**

Serotypes	Isolate name	Date	Department	Province
DENV-1	Bolivia DEN1 JR-16/99 E1998	03/1999	Santa Cruz de la Sierra	Ciudad
	Bolivia DEN1 JR-22/99 E1998	03/1999	Santa Cruz	Tornos
	Bolivia DEN1 JR38/99 E1998	03/1999	Santa Cruz	Tornos
	Bolivia DEN1 119/02 E2001	02/2002	Santa Cruz	Ichilos
	Bolivia DEN1 163/02 E2001	03/2002	Santa Cruz de la Sierra	Ciudad
	Bolivia DEN1 221/02 E2001	03/2002	Santa Cruz	Obispo
	Bolivia DEN1 273/02 E2001	03/2002	Santa Cruz de la Sierra	Ciudad
	Bolivia DEN1 289/02 E2001	03/2002	Santa Cruz	Ichilos
	Bolivia DEN1 25/99 E1998 ^a	03/1999	Santa Cruz	Torno
	Bolivia DEN1 71/01 E2000	03/2001	Santa Cruz de la Sierra	Ciudad
	Bolivia DEN1 1868/08 E2007	03/2008	Santa Cruz de la Sierra	Ciudad
	Bolivia DEN1 1877/08 E2007	03/2008	Santa Cruz	Florida
	Bolivia DEN1 2369/08 E2007	04/2008	Santa Cruz de la Sierra	Ciudad
	Bolivia DEN1 2370/08 E2007	04/2008	Santa Cruz de la Sierra	Ciudad
	Bolivia DEN1 2575/08 E2007	05/2008	Santa Cruz de la Sierra	Tornos
	Bolivia DEN1 391/02 E2001	04/2002	Tarija	
DENV-2	Bolivia DEN2 293/02 E2001	03/2002	Santa Cruz	Ichilo
	Bolivia DEN2 297/02 E2001	03/2002	Santa Cruz	Ichilo
	Bolivia DEN2 376/02 E2001	03/2002	Santa Cruz	Ichilo
	Bolivia DEN2 4/03 E2002	01/2003	Santa Cruz de la Sierra	Ciudad
	Bolivia DEN2 36/02 E2002	12/2002	Santa Cruz	Ichilo
	Bolivia DEN2 133/03 E2002	01/2003	Santa Cruz	Sara
	Bolivia DEN2 147/03 E2002 ^a	01/2003	Santa Cruz	Ichilo
	Bolivia DEN2 159/03 E2002	01/2003	Santa Cruz	Ichilo
	Bolivia DEN2 166/03 E2002	01/2003	Santa Cruz	Obispo Santistevan
	Bolivia DEN2 354/03 E2002	01/2003	Santa Cruz	Andres Ibanez
	Bolivia DEN2 404/03 E2002	01/2003	Santa Cruz	Andres Ibanez
	Bolivia DEN2 641/03 E2002 ^a	01/2003	Santa Cruz	Andres Ibanez

Serotypes	Isolate name	Date	Department	Province
DENV-3	Bolivia DEN2 4093/03 E2002	04/2003	Santa Cruz	Andres Ibanez
	Bolivia DEN2 4749/03 E2002	06/2003	Santa Cruz	Obispo Santistevan
	Bolivia DEN2 126/05 E2004	01/2005	La Paz	Palos Blancos
	Bolivia DEN2 270/05 E2004	01/2005	La Paz	Sud-Yungas
	Bolivia DEN2 760/05 E2004	03/2005	La Paz	Caranavi
	Bolivia DEN2 269/06 E2006	01/2007	Cochabamba	Carrasco
	Bolivia DEN2 318/07 E2006	01/2007	Santa Cruz	Andres Ibanez
	Bolivia DEN2 663/07 E2006	01/2007	Santa Cruz	Andres Ibanez
	Bolivia DEN2 2007/07 E2006	02/2007	Santa Cruz	Obispo Santistevan
	Bolivia DEN2 2106/07 E2006	02/2007	Santa Cruz	Andres Ibanez
	Bolivia DEN2 3118/07 E2006	03/2007	Santa Cruz	Obispo Santistevan
	Bolivia DEN3 751/03 E2002	01/2003	Santa Cruz	Andres Ibanez
	Bolivia DEN3 1465/03 E2002	02/2003	Santa Cruz	Andres Ibanez
	Bolivia DEN3 1593/03 E2002	02/2003	Santa Cruz	Andres Ibanez
	Bolivia DEN3 1726/03 E2002	02/2003	Santa Cruz	Andres Ibanez
	Bolivia DEN3 4445/03 E2002	04/2003	Tarija	Padcaya
	Bolivia DEN3 608/04 E2003	02/2004	Santa Cruz	Andres Ibanez
	Bolivia DEN3 704/04 E2003	03/2004	Santa Cruz	Andres Ibanez
	Bolivia DEN3 786/04 E2003	03/2004	Santa Cruz	Andres Ibanez
	Bolivia DEN3 805/04 E2003	03/2004	Santa Cruz	Andres Ibanez
	Bolivia DEN3 867/04 E2003	03/2004	Santa Cruz	Andres Ibanez
	Bolivia DEN3 869/04 E2003	03/2004	Santa Cruz	Andres Ibanez
	Bolivia DEN3 892/04 E2003	02/2004	Tarija	Bermejo
	Bolivia DEN3 991/04 E2003	03/2004	Santa Cruz	Andres Ibanez
	Bolivia DEN3 1023/04 E2003	03/2004	Santa Cruz	Andres Ibanez
	Bolivia DEN3 1035/04 E2003	03/2004	Santa Cruz	Andres Ibanez
	Bolivia DEN3 1951/04 E2003	05/2004	Santa Cruz	Andres Ibanez
DENV-2	Bolivia DEN3 67/05 E2004	01/2005	Beni	Riberalta
	Bolivia DEN3 84/05 E2006	01/2005	Beni	Riberalta
	Bolivia DEN3 334/07 E2006	01/2007	Santa Cruz	Andres Ibanez
	Bolivia DEN3 377/07 E2006	01/2007	Santa Cruz	Andres Ibanez

Serotypes	Isolate name	Date	Department	Province
	Bolivia DEN3 2019/07 E2006	02/2007	Santa Cruz	Obispo Santistevan
	Bolivia DEN3 2184/07 E2006	02/2007	Santa Cruz	Andres Ibanez
	Bolivia DEN3 2201/07 E2006	02/2007	Santa Cruz	Andres Ibanez
	Bolivia DEN3 2302/07 E2006	02/2007	Santa Cruz	Andres Ibanez
	Bolivia DEN3 2322/07 E2006	02/2007	Santa Cruz	Andres Ibanez

^aPersons deceased after DENV infection.