

Sequence Note

Documentation of Subtype C HIV Type 1 Strains in Argentina, Paraguay, and Uruguay

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ABSTRACT

HIV subtypes B, F, and BF recombinants have been previously reported in South America. This report describes the presence of HIV-1 subtype C infection in the countries of Argentina, Uruguay, and Paraguay dating back to at least 1999. Surveillance for uncommon non-B/non-F subtype viruses circulating in South America has been conducted in samples obtained from nine countries. Peripheral blood mononuclear cells (PBMC), dried filter paper (FP), and fresh blood (FB) samples were collected from HIV-positive patients from Ecuador, Colombia, Venezuela, Peru, Chile, Bolivia, Argentina, Uruguay, and Paraguay. From a total of 2962 HIV seropositive samples examined during a 9-year period (1995–2003), only 11 (0.4%) were found to be infected with non-B/non-F HIV variants. Eight of these 11 strains were determined to be subtype C by heteroduplex mobility assay (HMA). Five of these 8 strains were further characterized by sequencing and phylogenetic analysis of the protease (Pro) and reverse transcriptase (RT) region of the genome and two were sequenced full length. One of the strains was found to be a unique BC recombinant. The spread of a third subtype of HIV, subtype C, should raise the question of its potential future role in the HIV epidemic in this region.

THE DISTRIBUTION OF HIV-1 SUBTYPES around the world varies according to region. Subtype B has been found to predominate in Europe, America, and Thailand and subtype C is predominant in countries of southern Africa, India, China, and Russia.^{1–7} More recently, C subtypes have been identified in Brazil (1994)⁸ and concern exists that they may spread to other countries in this region. These data suggest that C subtypes of HIV are present in other countries of South America, and suggest that they either spread from Brazil, or were introduced from elsewhere.

We have conducted numerous studies in nine countries (Ecuador, Columbia, Venezuela, Peru, Chile, Bolivia, Paraguay, Uruguay, and Argentina) of South America among risk groups of men who have sex with men (MSM), female commercial sex workers (FCSW), patients with AIDS and tuberculosis (Tb), and pregnant women (ANC) and their partners during the years of 1995–2003.⁹ The objectives of these studies were to determine the prevalence of human immunodeficiency virus type-1 (HIV-1) infection and to map the genetic diversity distribution of HIV-1 strain subtypes.

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We extracted viral DNA from blood, peripheral blood mononuclear cells (PBMC) using a QIAamp kit10 or on FTA paper11 (Whatman BioScience, Newton Center, MA). We carried out nested polymerase chain reaction (PCR) on genomic DNA using HIV-1 Env subtyped by the C2-V4 region of envelope through the heteroduplex mobility assay (HMA) using ED3/ED14 as outer and ED31/ED33 as inner primers.12 Five strains were PCR amplified in the protease/RT region of the genome using a nested strategy and then sequenced, and one of those was subjected to full genome amplification and sequencing.13 Sequences were aligned and phylogenetically analyzed.14,15 Bootscanning was used to identify recombinants.16 From a total of 2962 HIV-1-positive samples, we did genotypic analysis on 1629 (55%). A total of 1357 (83.3%) were subtype B, 233 (14.3%) were subtype F, 27 (1.7%) were BF recombinants, 1 (0.06%) was A or E, 1 (0.06%) was a BC recombinant, and 7 (0.4%) were subtype C. We also found two recombinants (0.1%) that were CRF02_AG.17

The first four recorded subtype C strains were detected in Argentina in 1999–2002, and four additional strains were also identified in Paraguay and Uruguay (Table 1). Five of these eight subtype C strains were analyzed phylogenetically in the protease/RT region (Fig. 1A). Four were subtype C and one was a BC recombinant. The BC recombinant contained a very small segment of subtype C on a B background. The sample was from a male commercial sex worker (MCSW) from Rivera, Uruguay. Two samples, one each from Argentina and Uruguay, were sequenced over a nearly whole genome and were found to consist of subtype C throughout (example: Fig. 1B). One case each was identified in the capital cities of Argentina (Buenos Aires) and Paraguay (Asunción), and six others were found in smaller cities and rural areas in these countries (Fig. 2).

The presence of subtype C in Uruguay and Paraguay may be explained by the proximity of these countries to Brazil where this subtype was first identified in 1994.8 The predominance of subtype B HIV-1 viruses in South American countries may change because of frequent international travel from areas endemic for other subtypes of HIV. However, the possible implications on the dynamics of the HIV-1 epidemic will require additional follow-up. Our findings suggest that subtype C may

have been introduced in these three countries near cities that provide points of entry by immigrants and tourists, a phenomenon that has been described in the initiation of local epidemics or in the increase of HIV-1 diversity worldwide.18

The phylogenetic determination of the genetic subtype of HIV-1 is of little value for routine clinical care of individual patients, however, it provides a powerful tool for the monitoring and surveillance of the dynamics and circulation of the different genetic forms of HIV-1. Our report clearly describes the geographic spread of C subtype strains of HIV-1. This raises concern regarding its further spread among at-risk groups in the region.

SEQUENCE DATA

GenBank accession numbers for TRA3011 and ARG4006, nearly full lengths, are AY563169 and AY563170, respectively. The accession numbers for the partial *pol* sequences are AY563171–AY563175.

ACKNOWLEDGMENTS

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TABLE 1. CHARACTERISTICS OF PATIENTS INFECTED WITH SUBTYPE C HIV-1 STRAINS IN ARGENTINA, URUGUAY, AND PARAGUAY^a

Study group	Year	Code	Country (city)	Age	Sex	HMA	ProRT/FL
ANC	1999	ARG 0125	Argentina (Buenos Aires)	24	F	C	ND/ND
FCSW	2001	ARG 4005	Argentina (La Plata)	26	F	C	C/ND
FCSW	2002	ARG 4009	Argentina (Salta)	38	F	C	C/ND
MSM	2001	ARG 4006	Argentina (Mar del Plata)	39	M	C	C/C
MCSW	2001	TRA 3011	Uruguay (Chuy) ^b	25	M	C	C/C
MCSW	2002	TRA 3026	Uruguay (Rivera)	20	M	C	BC/ND
MSM	2002	PSM 0059	Paraguay (Asunción)	20	M	C	ND/ND
AIDS	2002	PSP 0117	Paraguay (Lambare) ^b	56	M	C	ND/ND

^aHMA, heteroduplex mobility assay; ProRT/FL, phylogenetic results of protease/reverse transcriptase region/nearly full-length sequencing; ND, not done; ANC, pregnant female; MSM, men who have sex with men; FCSW, female commercial sex worker; AIDS, patients with AIDS and tuberculosis coinfection.

^bThe nationality for TRA 3011 is Brazil and for PSP 0117 is Argentina.

A

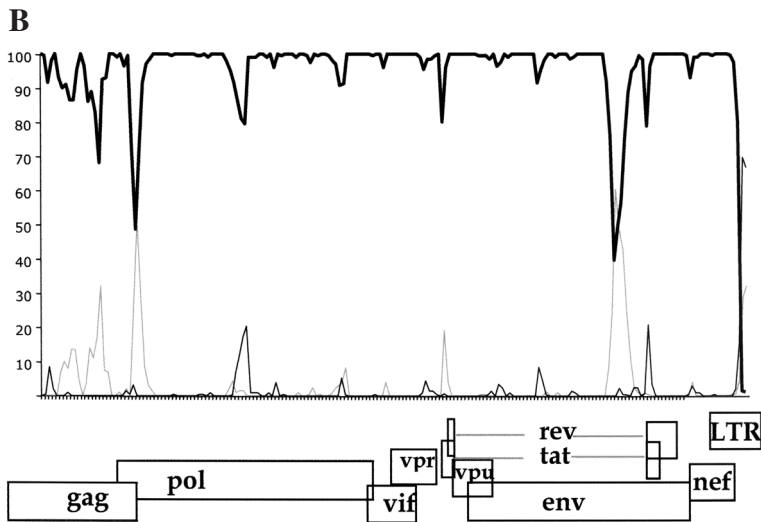
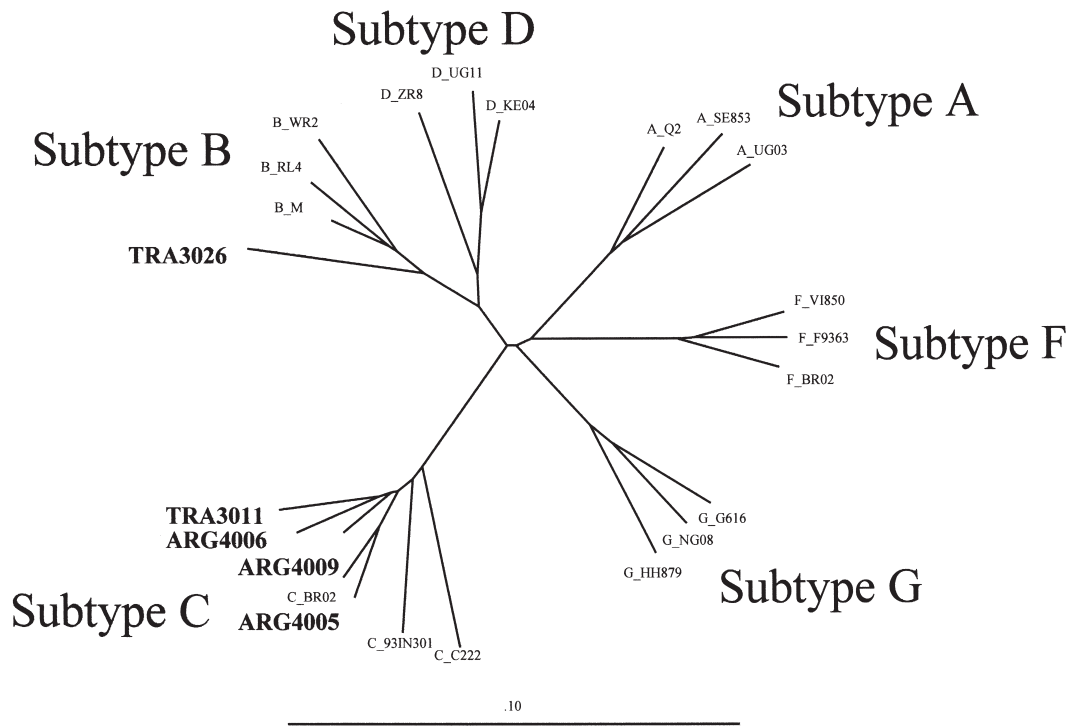


FIG. 1. (A) Phylogenetic analysis of the protease/RT region of the *pol* gene for five strains from South America that were subtype C by *env* HMA. Phylogenetic analysis was performed using neighbor-joining with the Kimura two-parameter method of distance estimation. Scale bar measures 10% genetic distance. (B) Bootsacan analysis of Arg 4006. A bootsacan was performed comparing Arg 4006 to subtype C (thick black line, BR025), subtype B (gray line, MN), and subtype F (thin black line, BR020) across the genome. Diagram of the genes of HIV-1 across the bottom shows the location of different genes.

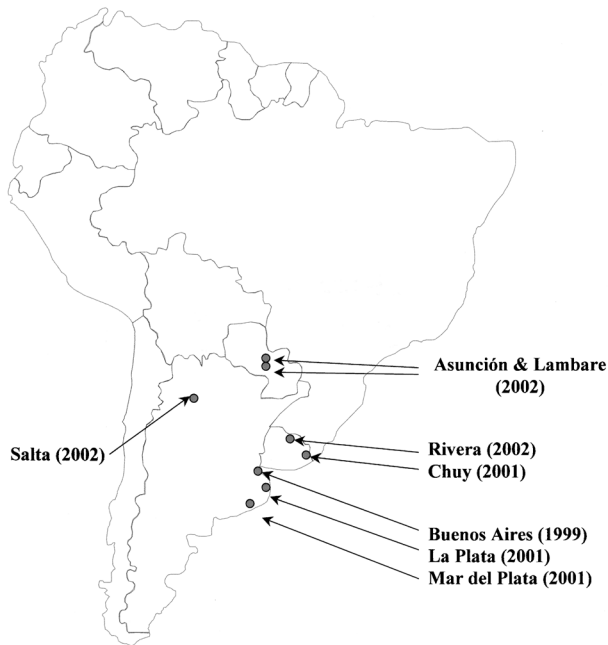


FIG. 2. Distribution of subtype C strains by city (and year) during the period of 1999–2002.

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