SEROINCIDENCE AND PHYLOGENY OF HUMAN IMMUNODEFICIENCY VIRUS INFECTIONS IN A COHORT OF COMMERCIAL SEX WORKERS IN MONTEVIDEO, URUGUAY

JOSE VIÑOLES, MARGARITA SERRA, JOSE C. RUSSI, DORA RUCHANSKY, SERGIO SOSA-ESTANI,
SILVIA M. MONTANO, GLADYS CARRION, LINDSAY M. EYZAGUIRRE, JEAN K. CARR, JAMES G. OLSON,
CHRISTIAN T. BAUTISTA, JOSE L. SANCHEZ, AND MERCEDES WEISSENBACHER

*HIV/AIDS National Control Program, and Department of Laboratories of Public Health, Ministry of Health, Montevideo, Uruguay;
Microbiology Department, School of Medicine, University of Buenos Aires, Buenos Aires, Argentina; U.S. Naval Medical Research
Center Detachment, Lima, Peru; U.S. Military HIV Research Program, Walter Reed Army Institute of Research and Henry M.
Jackson Foundation, Rockville, Maryland*

Abstract. A cohort study involving 60 human immunodeficiency virus (HIV)–negative male transvestite commercial sex workers (CSWs) was conducted in Montevideo, Uruguay in 1999–2001. Serum samples were tested for HIV by an enzyme-linked immunosorbent assay screening with immunoblot confirmation. Six participants seroconverted for an incidence-density rate of 6.03 (95% confidence interval = 2.21–13.12) per 100 person-years. Inconsistent condom use during client sex (adjusted hazard ratio [AHR] = 6.7), during oral sex (AHR = 5.6), and at the last sexual encounter (AHR = 7.8), and use of marihuana (AHR = 5.4) were marginally associated with HIV seroconversion. Five samples were genotyped in the protease and reverse transcriptase regions; three were subtypes B and two were BF recombinants. Full genome analysis of four samples confirmed all three subtype B samples and one of the two BF recombinants. Male transvestite CSWs sustained a high rate of HIV infection. Larger prospective studies are required to better define subtypes and associated sexual and drug-related risk factors.

INTRODUCTION

The current worldwide expansion of the human immunodeficiency virus/acquired immunodeficiency syndrome epidemic is primarily driven by the sexual transmission of human immunodeficiency virus (HIV).[1] Because of high HIV infection rates and large numbers of sexual partners, commercial sex workers (CSWs) constitute a core group for the transmission of HIV and other sexually transmitted infections (STIs).[2] Cross-sectional seroepidemiologic studies have consistently reported higher HIV prevalences among female as well as male CSWs compared with rates in the general population.[3–6] Prevalences of HIV ranging between 11% and 68% have been documented among male transvestite CSWs and have been attributed to unsafe sexual behaviors and injecting drug use (IDU).[7–9]

The genetic subtypes of HIV-1 in most of South America resemble those in North America: subtype B is the predominant subtype.[5] In the countries of the Southern Cone of South America, however, BF recombinants of a wide range of subtype structures circulate.[5,10] While it is known that they have been present in the epidemic since 1986,[11] the relative proportions among recently infected persons is unknown. Knowing whether the proportion of recombinants in the epidemic is increasing over time would be extremely valuable because recombination has an impact on the prevalence and types of drug resistance.

In the baseline seroprevalence study we conducted in the capital city of Montevideo, Uruguay in 1999,[12] male transvestite CSWs were reported to have a rather high HIV prevalence (21.5%; 95% confidence interval [CI] = 16.0–27.8%); a prior IDU history and older age (≥ 26 years) were found to be associated with HIV. Despite their documented elevated HIV prevalences, no cohort-based, HIV incidence studies have been conducted in this high-risk core group. The objectives of this epidemiologic study were to determine observed HIV incidence rates and to evaluate potential risk factors associated with HIV seroconversion among initially seronegative male transvestite CSWs enrolled in the original study.

MATERIALS AND METHODS

Study design and subject enrollment. During the period March through August 1999, we performed a cross-sectional study to establish baseline HIV-1, hepatitis B virus (HBV) and hepatitis C virus (HCV) seroprevalences among 200 male transvestite CSWs in Montevideo, Uruguay. This previous study reported past exposure to HBV, HCV, and HIV in 101 (50.5%), 13 (6.5%), and 43 (21.5%) individuals, respectively.[12] All participants who were ≥ 18 years old and HIV negative at baseline were deemed to be eligible and were invited to participate in this cohort study and were followed-up until May 2001. Serologic test results were linked to the questionnaire only by a unique numeric code to preserve confidentiality and anonymity of study participants. The study protocol was reviewed and approved by the local ethical and scientific review boards, as well as by the U.S. Naval Medical Research Center's institutional review board, in compliance with all federal regulations governing the protection of human subjects.

We defined a transvestite as a subject whose biologic sex was male and who self-identified as a cross-dresser or as a male-female transgendered person, irrespective of his prior history of hormonal use or gender reassignment surgery. This definition is consistent with that applied in other studies of transgendered individuals.[13] Volunteers were recruited in streets, bathhouses, nightclubs, and brothels by trained health care personnel from the Uruguayan Ministry of Health. The HIV-negative participants who accepted to participate in this prospective study were briefed on its purpose, provided written informed consent and a blood sample (7–10 mL), and were interviewed using a standardized questionnaire format that contained information regarding sociodemographic characteristics (such as age and occupation), sexual practices (such as numbers of sexual contacts per week, workplace,

time as a CSW, frequency of condom use with clients and steady partners, sexual contact with foreigners and others), and current use of illegal drugs. All study subjects were provided with HIV and STI prevention information, which included counseling, and printed and oral information on infectious agents, as well as with condoms and lubricant gel as requested. Additional details of subject enrollment procedures have been published elsewhere.[12] Study participants were recalled after one year of the initial (baseline) study visit and followed-up approximately every six months until study closure or until lost to follow-up.

Laboratory sampling. Serum samples were subjected to screening for antibodies to HIV by laboratory-based enzyme-linked immunosorbent assay (ELISA) testing (HIV 1/2, MEIA-IMx; Abbott Laboratories, Chicago, IL) of rapid test–positive samples. Samples found to be repeatedly reactive on ELISA screening were subsequently subjected to immunoblot confirmation (New LAV Blot 1; Sanofi-Pasteur, Marnes-La-Coquette, France). Peripheral blood mononuclear cells (PBMCs) were isolated from whole heparinized blood samples by the Ficoll gradient method (lymphocyte separation medium; ICN/Cappel, Aurora, OH), and DNA was extracted from approximately 1×10^6 infected PBMCs by the QIAmp DNA extraction technique (Qiagen, Valencia, CA).

Genetic characterization of HIV-1 strains. The DNA samples were genotyped using the envelope heteroduplex mobility assay (*env* HMA) as previously described.[14,15] Briefly, after two rounds of a polymerase chain reaction (PCR) with primers ED14 and ED3 (first round) and ED31 and ED33 primers (second round), HMA electrophoresis was performed with second-round PCR products using nine reference standards in the formation of heteroduplexes.

Partial *pol* PCR amplification from PBMC DNA was performed on 37 randomly selected samples from the sample set using a nested strategy. The first-round amplification was done in a volume of 50 μL with primers Pro5F (5′-AGAAATTGCAGGGCCCCTAGGAA-3′) and RT3474R (5′-GAATCTCTCTGTTTTCTGCCAG-3′) and Amplitaq Gold *Taq* polymerase (Applied Biosystems, Foster City, CA). The second-round amplification was completed using 1 μL of the first-round product and primers Pro3F (5′-AGAICAG-AGCCAACAGCCCCACCA-3′) and ProRT (5′-TTCCCC-ACTAACTTCTGTATGTCATTGACA-3′).

This nested strategy amplified 1.1 kb of the HIV genome. The amplified product was sequenced with Big Dye terminators using an ABI 3100 automated sequencer (Applied Biosystems). The region sequenced included all of the protease region and the carboxy terminus of the reverse transcriptase (RT) protein.

Nearly full genomes were also amplified directly from the PBMC DNA of each subject using a nested PCR strategy that amplified the region from gag to the end of the R region of the 3′-long terminal repeat.[16,17] Sequences were aligned in a multiple alignment with standard subtype references and phylogenetic analyses were conducted using Neighbor-joining with Kimura's two-parameter model of distance calculation and a bootstrap computed with parsimony.[18,19] Analysis for potential recombination was done using Simplot version 3.4.[20,21]

Statistical analysis. Participants were defined as HIV seroconverters if they were tested seronegative at baseline (be-

ginning of cross-sectional study) and then were found to be HIV seropositive at some point during the 24-month follow-up period. Participants were defined as non-HIV seroconverters if they were tested seronegative throughout the whole study period. The date of seroconversion was estimated using the time of last negative test during the baseline enrollment period in 1999 and the first positive test result in the cohort study. Person-years of observation were calculated by recording the total number of surveillance days for each participant. For individuals lost to follow-up, person-time denominator data was censored at the time of the last visit. Continuous variables were compared using the Student's *t*-test or the Mann-Whitney U test. Kaplan-Meier survival analysis was applied to show and compare time to HIV seroconversion. The magnitude of associations of potential risk factors were expressed as hazard ratios. Cox proportional hazards regression analysis was performed to evaluate risk factors associated with HIV seroconversion. Ninety-five percent confidence intervals (CIs) were calculated for the incidence-density rates based on person-time denominator data by using Fisher's exact formula. All hazard ratios were adjusted for age group and workplace in a multiple Cox regression analysis; all *P* values were two-sided and a $P < 0.15$ was considered to be marginally significant. All statistical analyses were carried out using EGRET version 2.0 (Cytel Software Corporation, Cambridge, MA) and SPSS version 10.1 (SPSS Corporation, Chicago, IL).

RESULTS

Study population characteristics. Of a total of 69 male transvestite CSWs who were approached for participation in this study, 60 (87%) agreed to participate in this cohort study. At entry, the mean age of the participants was 29.6 years (range = 18–56 years), 68% worked on the street, 83% were single, widowed, or divorced, and most (97%) were born in Uruguay. The median number of different sexual partners per week was 20 (range = 1–50) and 72% had worked as a CSW for less than five years (median = 9, range = 1–39).

Incidence-density rate analysis. The 60 male transvestite CSWs enrolled contributed a total of 99.5 person-years of observation. The mean ± SD duration of follow-up was 20 ± 2.4 months with a range from 14 to 24 months. Six (10%) participants seroconverted to HIV infection during the study follow-up period. The overall incidence-density rate was estimated at 6.03 (95% CI = 2.21–13.12) per 100-person years. The median time to HIV seroconversion was 23 months and all seroconversions occurred after the 18-month follow-up timeframe. Five (83%) of the six seroconverters reported being street-based with an observed incidence-density rate of 12.2 (95% CI = 2.4–17.2) per 100-person years.

Risk factor analysis. All six seroconverters reported receiving money-for-sex. Two (33%) of them never used condoms with their steady partner, three (50%) reported using intranasal cocaine, and none gave a prior IDU history. The mean ± SD age in years of seroconverters was found to be slightly higher (but not statistically significant) than that for non-seroconverters (32.0 ± 7.6 versus 29.4 ± 8.7; $P = 0.29$). Four marginally-significant risk factors were found to be associated with HIV seroconversion by multiple Cox regression analysis after controlling for age and workplace (Table 1).

Male transvestite CSWs who reported inconsistent condom

TABLE 1
Risk factors for HIV infection among 60 CSWs in Montevideo, Uruguay, 1999–2001*

Feature	Total (n = 60)	Seroconverters (n = 6)	Multiple Cox regression analysis			
			%	AHR	95% CI	P
Age group (years)						
18–30	35	2	5.7	Ref	–	
> 30	25	4	16.0	2.09	0.32–13.79	0.444
Occupation						
Non-sex work	20	2	10.0	Ref	–	
Sex work	40	4	10.0	4.14	0.32–53.63	0.276
No. of sexual contacts per week						
0–20	32	4	12.5	Ref	–	
> 20	28	2	7.1	1.07	0.14–8.18	0.948
Workplace						
Bar or discotheque	19	1	5.3	Ref	–	
Street	41	5	12.2	3.93	0.36–42.60	0.261
Time as CSW† (years)						
0–5	15	1	6.7	Ref	–	
> 5	43	5	11.6	1.97	0.12–33.06	0.634
Always use condom with clients						
Yes	51	4	7.8	Ref	–	
No	9	2	22.2	**6.69**	**0.74–60.49**	**0.091**
Always use condom during oral sex						
Yes	41	3	7.3	Ref	–	
No	19	3	15.8	**5.63**	**0.54–58.80**	**0.149**
Always use of condom during anal sex (insertive or receptive)						
Yes	53	5	9.4	Ref	–	
No	7	1	14.3	1.85	0.17–20.88	0.617
Use of condom during last sex†						
Yes	46	3	6.5	Ref	–	
No	11	3	27.3	**7.80**	**0.55–109.81**	**0.128**
Use of drugs (any)						
No	17	1	5.9	Ref	–	
Yes	43	5	11.6	0.47	0.04–5.28	0.541
Use of marihuana						
No	50	4	8.0	Ref	–	
Yes	10	2	20.0	5.01	**0.63–39.88**	0.127
Sexual contact with foreigners						
No	27	4	14.8	Ref	–	
Yes	33	2	6.1	0.30	0.04–2.28	0.298

* HIV = human immunodeficiency virus; CSWs = commercial sex workers; AHR = adjusted hazard ratio (adjusted for age and workplace); CI = confidence interval; Ref = reference group for comparisons of AHRs. Values in **bold** represent significant values at the P < 0.15 level.
† Incomplete data on time in CSW (two subjects) and for use of condom during last sex (three subjects).

use (e.g., less than always) with clients (adjusted hazard ratio [AHR] = 6.69, P = 0.09), and those who reported not using condoms during oral sex (AHR = 5.63, P = 0.15) or at last sexual contact (AHR = 7.80, P = 0.13) were more likely to seroconvert. In addition, use of marihuana, but not of other drugs such as cocaine, was also associated with an increased risk of seroconversion (AHR = 5.01, P = 0.13). Other variables such as age, occupation, number of sexual contacts per week, workplace, time as a CSW, and sexual contact with foreigners were not found to be associated with seroconversion (Table 1).

Genetic characterization. The *env* HMA was completed on five (83.3%) of the six seroconverters' strains; three were found to be subtype B and two were subtype F. Partial *pol* sequencing of these five strains, followed by phylogenetic analysis, confirmed that three were subtype B and two were BF recombinants (Figure 1). Both *env* HMA subtype F strains were found to be BF recombinants, as has been observed consistently in this region of the world. Full-genome analysis of all of the strains identified as subtype B in partial *pol* analysis confirmed that all were non-recombinant throughout the genome. One of the BF recombinant strains was sequenced in full and found to be a mosaic of subtypes B

and F in an arrangement not yet documented elsewhere (Figure 2).

Sequence data. The nearly full genome sequences of four of the samples are Genbank Ay781125-28, and the partial *pol* sequence of OIUYTRA1116 is Ay781129.

DISCUSSION

To our knowledge, this is the first epidemiologic study to document incident HIV infection (e.g., seroconversion) and associated risk factors among HIV-infected male transvestite CSWs in Latin America. Before discussing the implications of these findings, we should point out some of the perceived study limitations. First, the non-probability (e.g., convenience) sampling method used did not allow us to be able to generalize the results of this study to populations beyond the study sample. Second, the sample size in this study was not large enough to be able to achieve sufficient study power, despite the fact that the HIV seroconversion rate of 6.03 per 100 person-years over the two-year study period was high and comparable to data obtained among groups of men who have sex with men (MSM) from other countries in the region, such
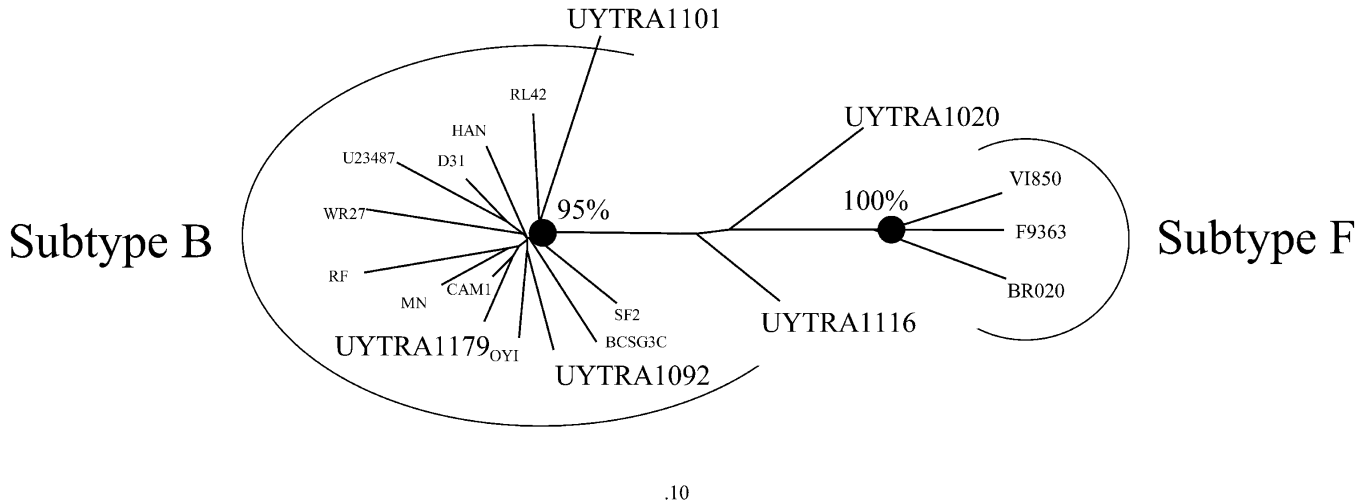
FIGURE 1. Phylogenetic analysis of partial *pol* sequences of incident cases infected with human immunodeficiency virus. The partial *pol* sequences of five of the six seroincident cases were placed in a multiple alignment with reference sequences of subtypes B and F from various parts of the world. A neighbor-joining phylogenetic analysis was done using the Kimura two-parameter method of computing genetic distance. One hundred bootstrap values were computed using the parsimony method. Reference sequences of subtypes B (RL42, HAN, D31, U23487, WR27, RF, MN, CAM1, OYI, BCSG3C, and SF2) and F (VI850, F9363, and BR020) are listed in small font size, while the samples from Uruguay are listed in the large font size. Values generated by bootstrap analysis of the phylogenetic data are indicated next to significant nodes.

as Peru and Argentina, where HIV seroconversion rates of approximately 2–6% per year have been estimated (Sanchez JL, Pando MA, unpublished data).

Available research seems to indicate that there is an elevated risk of HIV infection among male transvestite CSWs due to the myriad of high-risk sexual activities they engage in, as well the aforementioned inconsistent use of protective

measures.[7–9,22] We found suggestive evidence that inconsistent condom use during client sex (AHR = 6.7), during oral sex (AHR = 5.6), and at last sexual encounter (AHR = 7.8) increased the risk of HIV infection in this high-risk core group. The implementation of socially accepted HIV and STI counseling efforts, which includes condom use promotion, has been proven to effectively diminish high-risk, unprotected
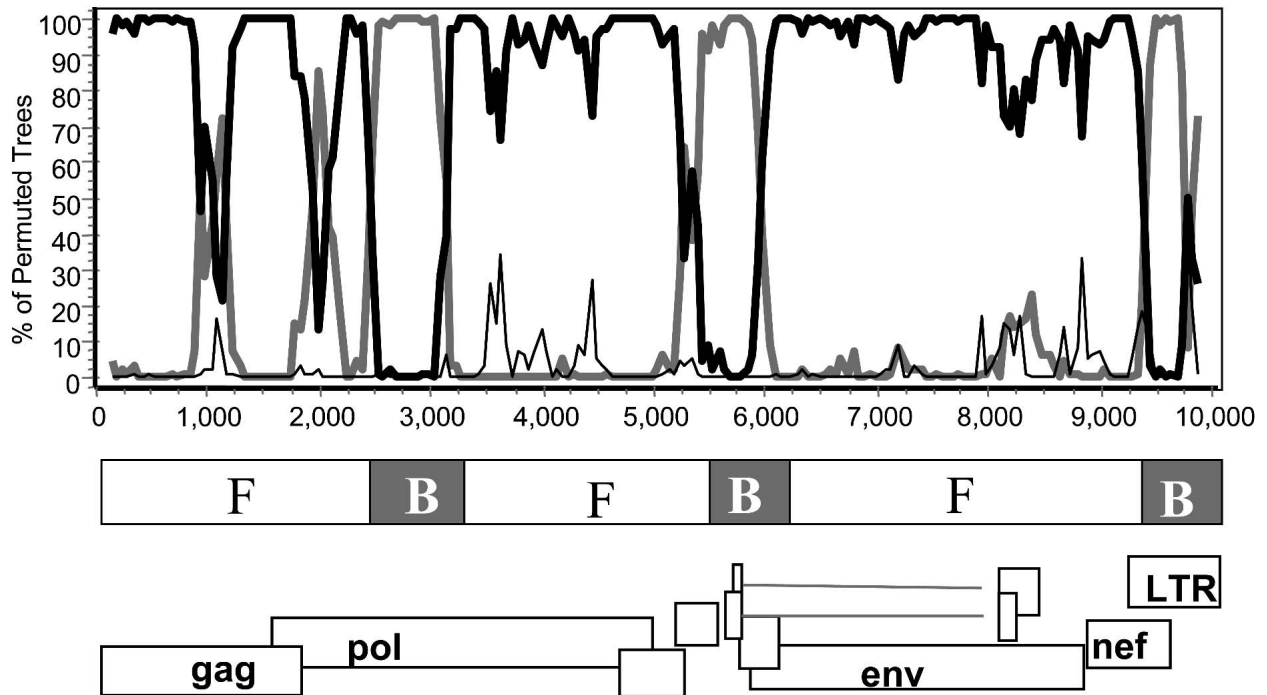

FIGURE 2. Recombinant structure of the nearly full length genome of human immunodeficiency virus (HIV) 01UYTRA1020. A bootscan analysis of 01UYTRA1020 is shown in the upper panel using standard reference subtype B strains (MN, RL42, and WR27) (**thick black line**), subtype F strains (VI850, F9363, and BR020) (**thick gray line**) and subtype C strains (C2200 and BR025) (**thin black line**). A diagram of the subtype distribution (white for F and gray for B) is shown above the diagram of the genes of HIV-1.

sexual behavior among MSM in the United States,[23] as well as among female CSWs in Thailand.[24] Similar interventional efforts appear to be needed in Uruguay at this time.

Marihuana consumption appears to have increased in recent years in Uruguay; general population sampling shows an increase from 4.2% in 1994 to 5.3% in 2001 (Viñoles J, unpublished data). It is possible that the five-fold increase in the incidence of HIV infection seen among marihuana users in this study may be related to an increase in associated high-risk behaviors, which may increase susceptibility to HIV as has been seen to occur in the past few years among injecting drug users in eastern Europe and the former Soviet Union.[22,25]

Recent increases in the incidence of HIV infections and STIs, such as hepatitis B, among populations of MSM have been noted in the United States in the past few years.[26,27] It is very likely that a similar phenomenon may be taking place in Uruguay and other regions of South America where gay and male transvestite/transgendered communities exist and may contribute to the high HIV incidence noted in this population.

The HIV subtype analysis of five of the six seroincident cases showed that both subtype B and BF recombinants were circulating in this population. The BF recombinants have been previously documented in this region of the Southern Cone of South America;[5,10,11] thus, their presence in Uruguay is not unexpected. At least one circulating recombinant form (CRF) has been identified in samples from both Argentina and Uruguay: CRF12_BF.[28] However, the incident BF recombinant fully sequenced in this analysis is not the CRF12_BF previously described by our group, nor is it similar to previously identified BF recombinants.[10,11,15]

The relatively high HIV infection rate detected in this study among male transvestite CSWs (6.03 per 100 person-years) raises serious concerns regarding the progression of this epidemic in Uruguay and clearly demonstrates the need for conduction of future, larger prospective studies to better define sex- and drug-related transmission of HIV among male transvestite CSWs in this region. An increased emphasis in the area of HIV/STI prevention and education geared specifically to male transvestite CSWs and other transgendered populations is needed now.[13] These high-risk core groups represent socially marginalized communities that are often ignored by public health authorities in the provision of prevention education and services for the control of HIV and other STIs.

Author's addresses: Jose Viñoles and Margarita Serra, HIV/AIDS National Control Program, Ministry of Health, Av. 18 de Julio 1892, 4to piso 11200, Montevideo, Uruguay. Jose C. Russi and Dora Ruchansky, Department of Laboratories of Public Health, Ministry of Health, Av. 8 de Octubre 2720 1er piso 11600, Montevideo, Uruguay, Telephone: 598-2-487-2516, Fax: 598-2-480-7014. Sergio Sosa-Estani and Mercedes Weissenbacher, Microbiology Department, School of Medicine, University of Buenos Aires, Paraguay 2155, Piso 11 (1121), Buenos Aires, Argentina, Telephone: 54-11-5950-9633 or 54-11-4508-3689, Fax: 54-11-4508-3705. Silvia M. Montano, Gladys Carrion, and James G. Olson, U.S. Naval Medical Research Center Detachment-Lima, Unit 3800, American Embassy-Lima, APO AA 34031-3800, Telephone: 51-1-561-2733, Fax: 51-1-561-3042. Lindsay M. Eyzaguirre, Jean K. Carr, Christian T. Bautista, and Jose L. Sanchez, U.S. Military HIV Research Program, Walter Reed Army Institute of Research and Henry M. Jackson Foundation, Rockville, Maryland. 1 Taft Court, Suite 250, Rockville, MD 20850, Telephone: 301-251-5077, Fax: 301-294-1898, E-mail: jsanchez@hivresearch.org.

Reprint requests: Jose L. Sanchez, U.S. Military HIV Research Program, Walter Reed Army Institute of Research, 1 Taft Court, Suite 250, Rockville, MD 20850.

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