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# Increased Prevalence of Human Herpesvirus Type 8 (HHV-8) Genome Among Blood Donors From North-Western Argentina

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The prevalence of HHV-8 infection varies widely in South American populations, displaying geographical variations in its distribution. The heterogeneous genetic contributions provided by the transatlantic parental populations that modified the Native American genomes may explain this epidemiological observation. Aiming to determine the prevalence of HHV-8 genome among healthy South American blood donors and its potential association with genetic ancestry, 772 individuals were screened by a highly sensitive PCR protocol and ancestry was assessed in 414 samples. HHV-8 DNA was significantly more prevalent among Northwestern Argentines than among those from the metropolitan region ($P = 0.001$) and Bolivians ($P = 0.0008$), but no differences were found when compared with Peruvians and Paraguayans. Although significant differences were observed in the ancestry components of the studied populations, no association was found in the genetic admixture between HHV-8 [+] and HHV-8 [−] samples from the same place. These results support the hypothesis of the existence of geographical factors related to HHV-8 prevalence which could be explained by the presence of specific risk factors, cultural characteristics or behaviors, probably related to contaminated saliva and/or sexual transmission. The presence of HHV-8 in South American blood units available for transfusion and an increased risk of infection in some provinces of North-western Argentina represent a hazard for immunosuppressed recipients. ***J. Med. Virol. 89:518–527, 2017***.



**KEY WORDS:** human herpesvirus type 8 (Kaposi's sarcoma-associated herpesvirus); epidemiology; ancestry; South American blood donors; North-Western Argentina

## INTRODUCTION

The human herpesvirus type 8 (HHV-8), also known as Kaposi's sarcoma-associated herpesvirus (KSHV) was discovered in 1994 [Chang et al., 1994]. This gamma-herpesvirus is considered to be the etiological agent of four clinico-epidemiological forms (classic, endemic, iatrogenic, and epidemic) of Kaposi sarcoma (KS), as well as multicentric Castleman disease and primary effusion lymphoma (PEL), occurring mainly, but not exclusively in HIV-infected patients [Edelman, 2005].

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Abundant epidemiological and serological studies indicate that HHV-8 transmission can occur through sexual and non-sexual routes, such as saliva, transfusion of contaminated blood, nasal secretions, and transplanted organs [Vitale et al., 2000; Challine et al., 2001; Hladik et al., 2006].

Worldwide prevalence of HHV-8 infection varies widely depending on the geographical region. High HHV-8 seroprevalence rates are found in endemic regions such as central and southern Africa (>50%); whereas the Mediterranean, East European, and Caribbean countries show intermediate rates (5–20%). Instead, HHV-8 appears to be infrequent in the general population of northern Europe, Asia, and North America (0–5%) [Moore, 2000].

In South America, HHV-8 infection depicts striking geographical and ethnic-associated distribution patterns following a north-south gradient [Souza et al., 2010]. Native Americans from the Amazon region of Brazil and Ecuador show the highest worldwide prevalence of HHV-8 antibodies (>70%), followed by the Amerindian populations residing between the Amazon forest and the savannah of central Brazil (50–83%), the Ayoreo tribe from Bolivia and Paraguay (18–45%), and Native Americans living in southern Brazil who exhibit the lowest seroprevalence rates (0–14%) [Souza et al., 2010]. Moreover, it was reported that HHV-8 seroprevalence is 10-fold higher in Amerindians living in the same geographical area and in similar conditions than non-Amerindian populations, suggesting that these singular differences could be related, in part, to host genetic factors of the Amerindian populations [de Souza et al., 2007]. Unfortunately, as far as we know, there is no information regarding HHV-8 prevalence in isolated Native American tribes living in Argentina.

The genetic background of the South American extant population is the result of three major genetic admixture events involving Native Americans, Europeans, and West Sub-Saharan Africans contributions. The first admixture episode included Native Americans and Western Europeans and started after the arrival of the Spanish conquerors in early 16th century. The second event involved Western Sub-Saharan Africans introduced by the European as slaves. Finally, particularly in Argentina, a third major admixture period involved a large number of Europeans immigrants—who mostly came from Italy and Spain, followed by France, Poland, Russia, and Germany—and took place between 1856 and 1930 [Corach et al., 2010]. Although the South American population is highly diverse and admixed, there is no data regarding the association between the striking differences observed in HHV-8 prevalence rates and the gene pool of admixed populations.

Hence, the aim of this study was to determine the prevalence of HHV-8 viral genome among healthy South American blood donors and its association with genetic ancestry.

## SUBJECTS AND METHODS

### Study Population

Along the period 2012–2014, 3 ml of EDTA blood were obtained from 772 biological unrelated blood donors after signing an informed consent statement upon enrollment. All volunteers passed the standard donor medical and behavioral screening examination and were non-reactive to all other infectious agents routinely tested in blood donors (hepatitis B and C viruses, HIV, Human T-lymphotropic viruses I and II, *Treponema pallidum*, *Brucella* ssp., and *Trypanosoma cruzi*). Samples were collected by the DNA and blood bank at the Italian Hospital of Buenos Aires, and blood banks at the "Pablo Soria" Hospital in San Salvador de Jujuy city and the "Sardá" Maternity Hospital in Buenos Aires city.

Volunteers were grouped, according to their self-declared place of birth, as: (i) Argentines from the metropolitan area (Buenos Aires city) (n = 200) and North-western region (Jujuy, Tucumán, and Salta provinces) (n = 211); and (ii) immigrants who recently arrived to Buenos Aires city from other Latin American countries (n = 361). These recruited immigrants were 187 Bolivians (mostly from Potosí, La Paz, and Cochabamba cities), 98 Paraguayans and 76 Peruvians who were predominantly born in Asunción and Lima cities, respectively. None of the Argentines but only 40% of the Bolivians, 8% of the Peruvians, and 3% of the Paraguayans admitted to being an active member of a native community. The demographics of the recruited subjects are shown in Table I.

This study was approved by the Ethics Committee on Research from the Italian Hospital of Buenos Aires (CEPI N°2235) and conducted according to the Declaration of Helsinki.

### Detection of HHV-8 DNA by PCR

DNA was extracted from 2 ml of whole blood by using FlexiGene DNA Kit (QIAGEN, GmbH, Hilden, Germany) and dissolved in 200 μl of hydration buffer according to the manufacturer's instructions. To test the integrity of DNA and exclude the possibility of PCR inhibitors, PCR amplification of human inosine triphosphate pyrophosphatase (ITPA) gene was performed in all samples [Kudo et al., 2009]. The presence of HHV-8 DNA was determined in all samples by a previously described nested PCR protocol for partial amplification of the conserved ORF-26. The first amplification round was performed with previously described primers [Lock et al., 1997]. The reaction volume was set at 25 μl, and the PCR mixture consisted of 400–1,000 ng of total DNA, 1 μl each of 10 uM primers, 1 μl of 10 mM dNTPs, 1.25 U of GoTaq® polymerase (Promega, Madison, WI), and 5 μl of 5X GoTaq® reaction buffer. The PCR cycle conditions included an initial denaturation step at 95°C for 6 min and 39 amplification cycles consisting of denaturation at 94°C for 30 sec, annealing at

TABLE I. Demographics and Prevalence of HHV-8 DNA Among the 772 Recruited Volunteers

| | Total samples | | | HHV-8 [+] samples | | HHV-8 [−] samples | |
|---|---|---|---|---|---|---|---|
| Groups | Gender (m:f) | Age (mean ± SD) | Prevalence of HHV-8 (%) | Gender (m:f) | Age (mean ± SD) | Gender (m:f) | Age (mean ± SD) |
| MET | 103:97 | 36.0 ± 11.4 | 7/200 (3.5) | 3:4 | 30.8 ± 11.1 | 100:93 | 36.2 ± 11.4 |
| NWA | 111:100 | 32.2 ± 11.3 | 26/211 (12.3) | 20:6 | 32.9 ± 9.2 | 91:94 | 33.3 ± 11.5 |
| BOL | 52:135 | 28.1 ± 6.5 | 6/187 (3.2) | 4:2 | 30.2 ± 8.7 | 48:133 | 28.0 ± 6.5 |
| PER | 31:45 | 28.9 ± 7.2 | 5/76 (6.6) | 3:2 | 33.6 ± 7.4 | 28:43 | 30.5 ± 7.7 |
| PAR | 37:61 | 28.9 ± 7.2 | 7/98 (7.1) | 2:5 | 30.6 ± 8.3 | 35:56 | 28.8 ± 6.9 |
| Total population | 334:438 | 31.9 ± 10.0 | 51/772 (6.6) | 32:19 | 32.0 ± 8.9 | 302:419 | 31.9 ± 10.1 |

SD, standard deviation; MET, metropolitan Argentines; NWA, North-western Argentines; BOL, Bolivians; PER, Peruvians; PAR, Paraguayans.

60°C for 30 sec, and elongation at 72°C for 30 sec. Reactions also included a final elongation step of 5 min at 72°C. The second amplification round was performed with previously described primers [Chang et al., 1994]. The cycling conditions and the PCR reaction mixture were the same as the ones used for the first round, but 1 μl of the product of the first round was included in the mixture and the annealing temperature was 65°C. Appropriated precautions and procedures were strictly followed to avoid cross-contamination in every step of DNA amplification and detection [Kwok and Higuchi, 1989]. PCR products were visualized after electrophoresis in 2% ethidium bromide-stained agarose gels.

All PCR-amplified fragments were bi-directionally sequenced by Big-Dye Termination chemistry system (Applied Biosystem, Life Technologies Corp., CA). The sequencing chromatograms were analyzed using the BioEdit Sequence Alignment Editor version 7.1.3.0 [Hall, 1999] and compared with the original HHV-8 sequence from the Gene Bank library (Accession no. U75698.1).

Furthermore, to confirm the presence of HHV-8 viral genome and exclude the possibility of false positives in the screening PCR, all those samples with an amplified ORF-26 were individually subjected to a PCR protocol for the partial amplification of ORF-72 [Pan et al., 2001].

Recombinant plasmids containing 300 and 139 bp amplicons of HHV-8 ORF-26 and ORF-72, respectively, were prepared in order to determine the sensitivity of both PCR protocols. The purified amplicons were ligated to the pDrive cloning vector (QIAGEN) and used to transform *Escherichia coli* DH5α competent cells. Recombinant plasmids were sequenced using Big-Dye Termination chemistry system (Applied Biosystem) and compared with HHV-8 sequences retrieved from GenBank database. The DNA concentrations of pure preparations of ORF-26 and ORF-72 recombinant plasmids were determined by fluorometry (Quantus™ Fluorometer, Promega, Madison, WI), as follows: 19 μg/ml, corresponding to $4.24 \times 10^{12}$ copies/ml of ORF-26 and 12 μg/ml corresponding to $2.79 \times 10^{12}$ copies/ml of ORF-72 recombinant plasmid. Serial dilutions of these plasmids solutions were amplified using the PCR protocols (three replicates), and the analytical sensitivity of these techniques was considered to be the last dilution exhibiting an amplicon band.

### Molecular Evaluation of Ancestry

In order to characterize the ancestry of the studied populations, both maternal and paternal lineages were analyzed by haplogroups in mitochondrial DNA (mtDNA) (haplogroups A2, B2, C, and D1) and Y chromosome (haplogroups E1b1b, G2a, I, J2,R1b1b2, Q1a3a) using real-time PCR followed by High Resolution Melting analysis (HRMA) as previously described [Zuccarelli et al., 2011]. This analysis included 413 randomly selected samples from the 772 recruited blood donors from Argentina and other Latin American countries (107 from Buenos Aires metropolitan area, 100 from North-western Argentina, 100 from Bolivia, 52 from Peru, and 54 from Paraguay).

In order to determine the existence of a potential association between HHV-8 prevalence rates and the genetic ancestry of the population, both maternal and paternal haplogroups were analyzed in all HHV-8 [+] samples (n = 51) and in 362 randomly selected HHV-8 [−] subjects.

Furthermore, a total of 101 samples—including all HHV-8 [+] samples (n = 51) and 50 randomly selected HHV-8 [−] samples keeping the proportions obtained for each studied population—were additionally subjected to autosomal ancestry typing by carrying out two multiplex minisequencing reactions using SNaPshot (Thermo Fisher, Palo Alto, CA) (12 amplicons each) according to a previously described protocol [Lao et al., 2010].

The following SNPs were used: rs1876482, rs2179967, rs1048610, rs1371048, rs1478785, rs1369290, rs952718, rs1405467, rs1344870, rs1391681, rs1461227, rs1907702, rs2052760, rs714857, rs721352, rs722869, rs926774, rs1448484, rs1667751, rs1858465, rs1465648, rs16891982, rs1808089, and rs3843776. This set of markers was ascertained from a pool of 62 preselected markers in order to obtain the maximum

ancestry information previously described [Lao et al., 2006]. Furthermore, the parental population used in this study was obtained from the Genome Diversity Project-Centre des etudes de polymorphisms humane (CEPH) and was previously analyzed by Lao et al. [2006].

### Statistical Analysis

Chi-square and Fisher's exact tests were used for proportions comparison. Odds Ratio and 95% confidence intervals were calculated by R-program (www.r-project.org). Multivariate logistic regression analyses were used to determine the independent factors associated with the prevalence of HHV-8 DNA. Gender, age, place of birth and mtDNA ancestry were considered as variables. Analyses were performed with the R-program (www.r-project.org). A *P*-value of less than 0.05 was considered as statistically significant.

Admixture proportions were established by using STRUCTURE v 2.3.4 software [Pritchard et al., 2000]. Ten iterations for parental populations (k) from two to four were set and 10.000 burning followed by 20.000 Markov Chain Monte Carlo simulations were performed for each round. Boolean flag and start at pop info were set for parental populations. Admixture model, independent allelic frequencies and POPINFO parameter were chosen. The most likely value for the number of populations (K) was determined using STRUCTURE HARVESTER program that enables to implement the Evanno method [Earl and Von Holdt, 2012] varying K from a minimum of 2 to a maximum of 5 and always performing 10 runs for each value of K. It was obtained a value of $k = 3$ that best fit the dataset and it was selected for all further analysis. Data analysis was refined using CLUMPP software [Jakobsson and Rosenberg, 2007] and a bar plot was obtained with the help of DISRUPT software [Rosemberg, 2004]. Multi-dimensional scaling (MDS) plots were obtained using Microsoft Excell 2007 starting from a matrix identical by state calculated by R-program (www.r-project.org).

## RESULTS

### Prevalence of HHV-8 DNA

The analytical sensitivity of ORF-26 nested PCR was 33.1 copies/ml (100% rate). This viral genomic region was amplified in 6.6% (51/772) of the samples (Table I). The sequences obtained from the amplified DNA demonstrated 97.3–99.3% homology with nucleotide positions 987–1,220 in the original HHV-8 sequence (Gen Bank, accession no.U75698.1). Additionally, to confirm the presence of HHV-8 viral genome and exclude the possibility of false positives, all these ORF-26 positive samples were successfully amplified by a nested PCR protocol for ORF-72 with an analytical sensitivity of 279 copies/ml (100% rate).

The presence of HHV-8 DNA was significantly more prevalent among blood donors from North-Western Argentina (12.3%) than among those from the metropolitan area (3.5%) (OR 3.8, 95% CI 1.6–10.8 [$P = 0.001$]) and Bolivians (3.2%) (OR 4.2, 95% CI 1.6–12.8 [$P = 0.0008$]). However, no significant differences were found when compared with Peruvians (6.6%) and Paraguayans (7.1%) ($P = 0.2$ in both cases) (Table I).

There were significant differences in the viral prevalence between men and women in North-western Argentina, where 77% of the infected individuals were men (OR 3.4, 95% CI 1.3–8.9 [$P = 0.01$]). This statistical significant difference was not observed among the other studied populations (Table I).

Furthermore, when the viral prevalence of each group was analyzed regarding age, there were no significant differences observed (data not shown).

Multivariate analysis confirmed the absence of association between the presence of HHV-8 DNA and age. However, male gender and place of birth in North-western Argentina exhibited statistically significant associations with HHV-8 DNA prevalence (OR 0.91, 95% CI 0.83–0.97 [$P = 0.0091$] and OR 1.3, 95% CI 1.15–1.4 [$P = 1.01 \times 10^{-6}$], respectively).

### Genetic Ancestry of the Studied Populations

Significant differences were detected in the ancestry component of the studied populations, as expected since the region had different human population history [Sans, 2000]. The prevalence of Native American maternal (A2, B2, C, and D1) and paternal haplogroups (Q1a3a) was the lowest in the metropolitan area of Buenos Aires when compared with North-western Argentines, Bolivians, Peruvians, and Paraguayans (Fig. 1).

Furthermore, the major ancestry component as revealed by STRUCTURE analysis was: 83.3% European for metropolitan Argentines, 85.7% Native American for Bolivians, 81.2% for Peruvians, and 71.7% for North-western Argentines. The minor component (<4.6%) for these four populations was the West African ancestry. However, the ancestry components of Paraguayans were: 46.2% European, 40% Native American, and 13% West Sub-Saharan African ancestry. Most of the metropolitan Argentines samples clustered with or closer to Europeans while North-western Argentines, Paraguayans, Peruvians, and Bolivians cluster closer to Native Americans, as observed in the MDS plot (Supplementary Fig. S1).

### HHV-8 DNA Prevalence and Genetic Ancestry

The association between Native American and Non-native American maternal and paternal lineages in both HHV-8 [+] and HHV-8 [−] samples from Paraguayans, metropolitan and North-western Argentines was considered to be not statistically significant ($P > 0.05$) (Fig. 2). However, the

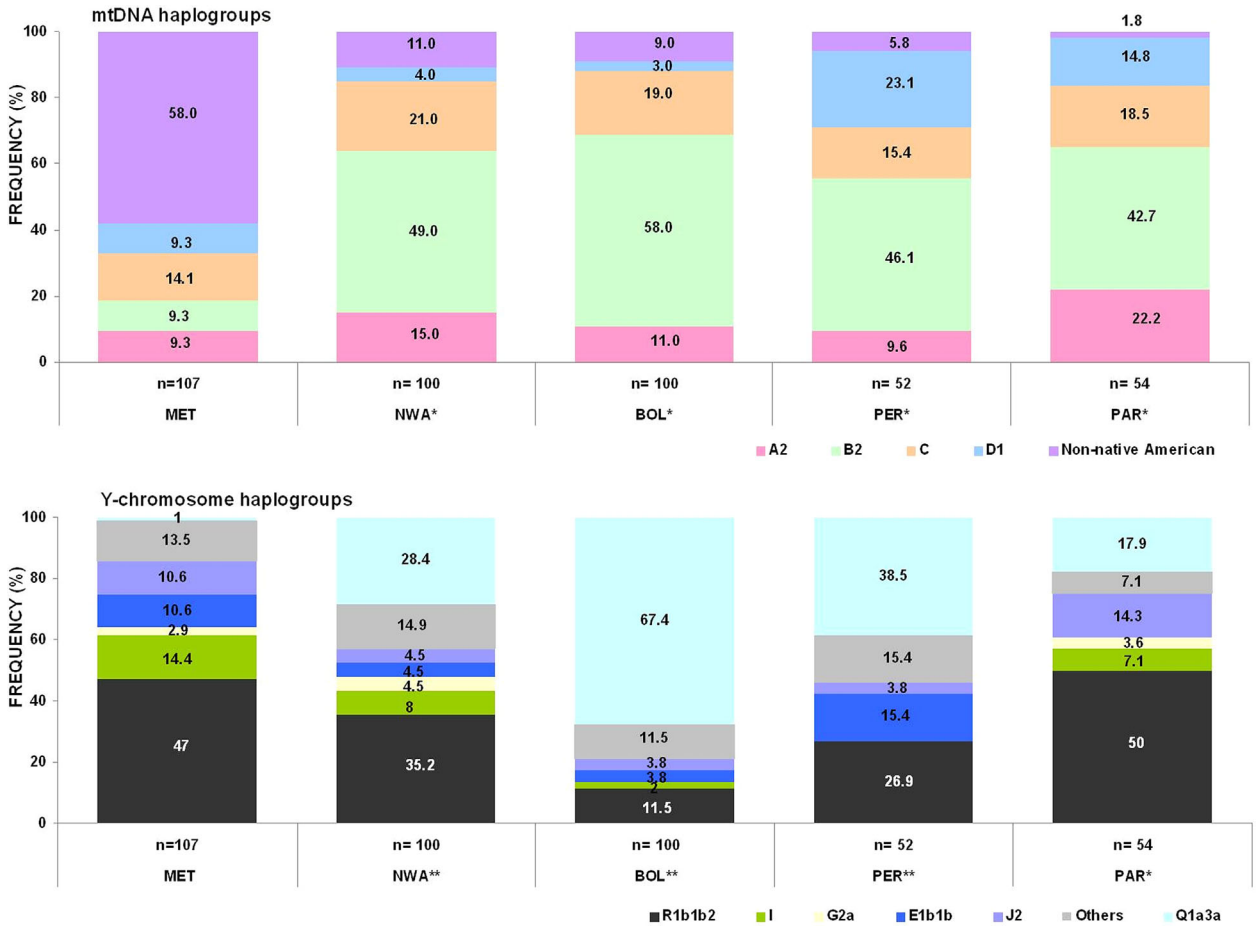


Fig. 1. Prevalence of mitochondrial DNA (mtDNA) and Y-chromosome haplogroups among metropolitan and North-western Argentines, Bolivians, Peruvians, and Paraguayans. The Native American haplogroups in mtDNA are A2, B2, C, and D1 (upper). The Native American haplogroup in Y-chromosome is Q1a3a (lower). Samples were grouped as metropolitan Argentines (MET), North-western Argentines (NWA), Bolivians (BOL), Peruvians (PER), and Paraguayans (PAR). $^{*}P < 0.0001$, $^{**}P < 0.002$ when comparing metropolitan Argentines with the other groups.

prevalence of Native American mtDNA haplogroup A2 was significantly higher among the HHV-8 [+] group when compared with the HHV-8 [−] samples in the Bolivian (3/6 vs. 8/94; $P < 0.02$) and Peruvian (3/5 vs. 2/48; $P = 0.004$) populations.

In order to study the admixture process of South American populations, autosomal ancestry informative markers typing was carried out in 51 HHV-8 [+] and 50 HHV-8 [−] samples. The STRUCTURE analysis—using European, Native American, and African samples as parental populations—revealed that there were no statistical significant differences in the genetic admixture between HHV-8 [+] and HHV-8 [−] samples of the studied populations ($P = 0.6$) (Table II). Moreover, no differences were observed in the ancestry components between HHV-8 [+] and HHV-8 [−] samples from the same place of birth (Supplementary Fig. S2).

Multivariate analysis confirmed the absence of association between HHV-8 DNA presence and genetic ancestry.

## DISCUSSION

HHV-8 has been postulated as a new infectious agent for screening in blood units [Perez et al., 2010]. However, most epidemiological studies in South America have focused on groups with an increased risk of acquiring the infection [Biggar et al., 2000; Kazanji et al., 2005] and only a few have dealt with the viral prevalence among blood donors [Perez et al., 2004; Mohanna et al., 2007]. To our knowledge, this is the first study focused on determining a potential association between the prevalence of HHV-8 DNA among South American blood donors and the genetic ancestry of the population.

Although other studies from Latin American countries analyzed the prevalence of HHV-8 DNA, they performed PCR only on samples obtained from HHV-8 seropositive individuals [Perez et al., 2010; Levi et al., 2011]. In this study, a highly sensitive HHV-8 nested PCR protocol was used to screen blood donors who had not been previously subjected to serological tests.

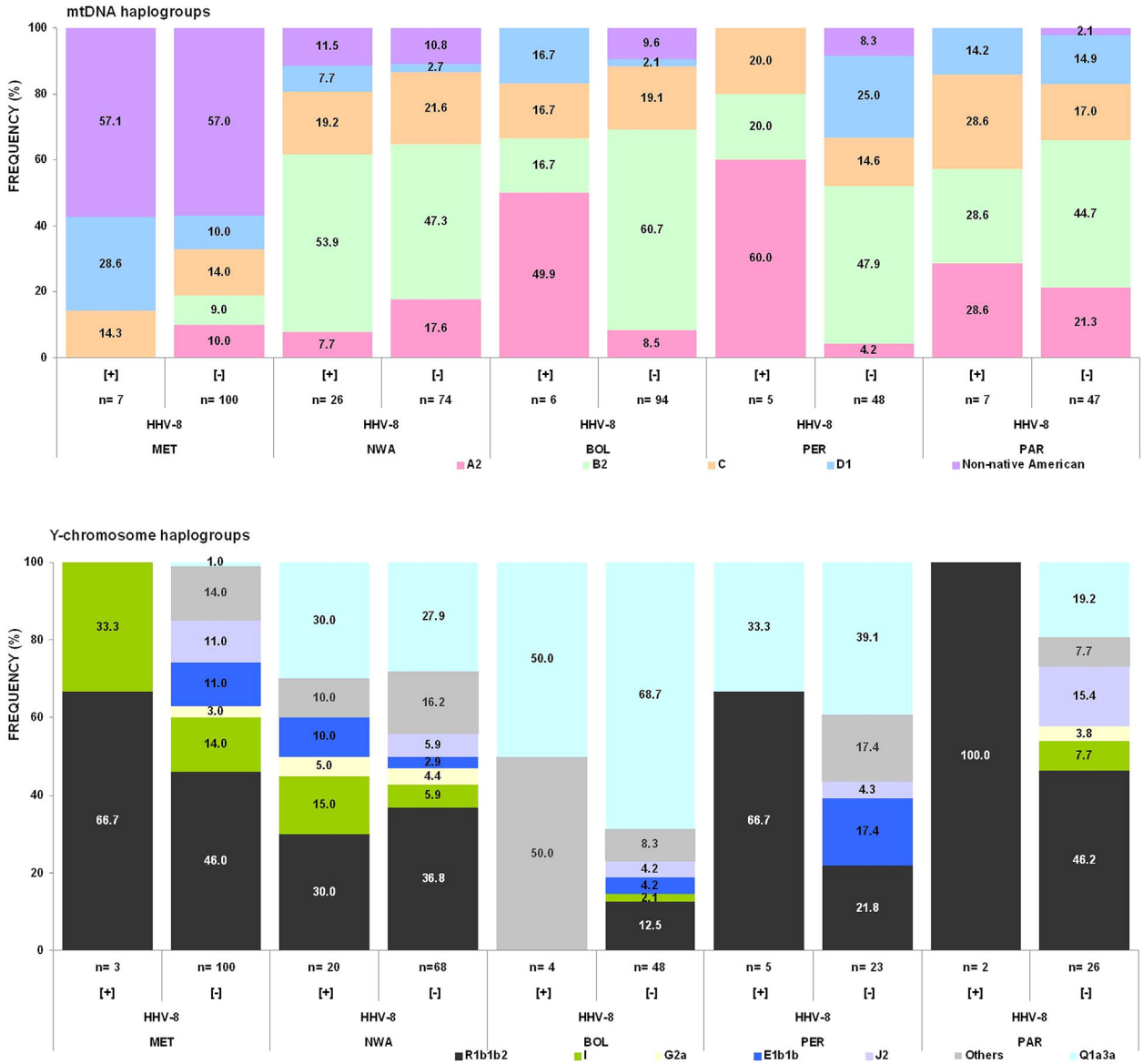


Fig. 2. Prevalence of Native American and Non-native American maternal and paternal lineages among HHV-8 [+] and [−] samples. The Native American haplogroups in mtDNA are A2, B2, C, and D1 (upper). The Native American haplogroup in Y chromosome is Q1a3a (lower). Samples were grouped as metropolitan Argentines (MET), North-western Argentines (NWA), Paraguayans (PAR), Peruvians (PER), and Bolivians (BOL), and also by the presence or absence of HHV-8 (HHV-8 [+] and [−], respectively). $^{*}P < 0.02$, $^{**}P = 0.004$ when comparing haplogroup A2 in the Bolivian and Peruvian samples, respectively.

Until now, there is no gold standard HHV-8 detection technique due to several technical limitations [Souza et al., 2010]. First, since the discovery of this virus [Chang et al., 1994] it was found that almost all patients with KS have HHV-8 antibodies, usually in high titers [Gao et al., 1996]. However, in inter-laboratory comparisons, using standard panels of samples from asymptomatic individuals, detection of HHV-8 infection turned out to be much more problematic [Biggar et al., 2003] as there were different laboratory algorithms, cutoffs, and assays that were used [Gao et al., 1996; Casper et al., 2002]. Secondly, it is known that depending on the serological technique used, cross reaction may occurs leading to the occurrence of false positives, and thus, confounding the data interpretation [Corchero et al., 2001]. Thirdly, because of the lack of a clearly defined acceptable standard test to detect the seropositivity of HHV-8, it is possible that samples exhibiting low titres of HHV-8 specific antibodies may have been documented as false negative [Biggar et al., 2003; Perez et al., 2006; Kakavand-Ghalehnoei et al., 2016]. Therefore, the detection of HHV-8 DNA among healthy and asymptomatic blood donors using highly

TABLE II. Proportions of Native American, European, and African Ancestry in HHV-8 [+] and HHV 8 [−] Samples Based on STRUCTURE Analysis

| Individuals | European (%) | Native American (%) | West Sub-Saharan African (%) | Number of individuals |
|---|---|---|---|---|
| HHV-8 [+] | 31.2 | 64.4 | 4.3 | 51 |
| HHV-8 [−] | 33.5 | 60.3 | 6.3 | 50 |

sensitive PCR protocols could be used in combination with serological assays as the infection can be identified in the early stages before the host mounts a detectable humoral response, as proposed by Cook et al. [2002].

The comparison of the results obtained in this study with previous reports from Latin America [Perez et al., 2010; Levi et al., 2011] is limited due to the use of different detection techniques and the characteristics of the studied population. In those studies, the presence of HHV-8 DNA could only be detected in some or none of the seropositive samples. These discrepant results in the HHV-8 DNA prevalence could be explained by two reasons. First, due to the fact that HHV-8 is a circulating virus not integrated into the human genome with reported low viral loads in blood donors [Tedeschi et al., 2001; Kaye, 2014], low concentration of DNA input in the PCR mix could limit the possibility of viral DNA detection, even by high sensitive PCR techniques. While Levi et al. [2011] purified DNA from 200 μl of whole blood obtained from HHV-8 seropositive samples and the total DNA input in the PCR was 5–800 ng, in the results presented herein, DNA extractions were carried out from 2 ml of whole blood and the total DNA input in the PCR was 400–1,000 ng. Lastly, and most importantly, the PCR protocols employed in the above-mentioned studies exhibited dissimilarities to the ones carried out by this report. Levi et al. used a well calibrated 40-cycle real-time PCR protocol with an analytical sensitivity of 1,000 copies/ml. In this study, we carried out an ORF-26 screening PCR which included two successive 39-cycle rounds (Nested PCR) with a slight modification from the original previously published protocol [Lock et al., 1997] and a sensitivity of 33.1 copies/ml. Unfortunately, there is no data regarding the nested PCR protocol used by Perez et al. [2010] nor its analytical sensitivity to make any comparisons.

Moreover, all the ORF-26 positive samples in this study were tested by a nested PCR protocol for ORF-72, a different viral genomic region commonly used for HHV-8 detection. Despite the lower sensitivity of this protocol compared to ORF-26 PCR, all samples could be successfully amplified by both methods indicating that the concentration of HHV-8 DNA was sufficient enough to be amplified and visualized in an agarose gel.

The inability to obtain serum samples of all individuals recruited in this study could resulted in a possible underestimation of the prevalence of infection in the analyzed populations due to the fact that seropositive individuals with negative detection of HHV-8 DNA have been documented [Perez et al., 2010]. Our study could have missed such cases, and therefore, future serological testing in these regions would be useful to support our findings.

In this study, the higher prevalence of HHV-8 DNA in North-western Argentina exhibited statistically significant differences to the Argentines from the metropolitan area and Bolivians. However, it is possible that the small sample size of the immigrants and the lower prevalence of infection in the remaining regions could be responsible for the lack of significant differences between the other groups.

The prevalence rate observed in the metropolitan area of Buenos Aires (3.5%) is in agreement with previously reported data in blood donors (HHV-8 prevalence detected by serological techniques) from the same geographical region of Argentina (4%) [Perez et al., 2004]. Indeed, similar results have been reported in a study involving HIV [−] heterosexuals without history of drug abuse (5.7%) from the central area of Argentina [Sosa et al., 2001]. With regard to the HHV-8 epidemiology among Peruvians, Bolivians, and Paraguayans, the rates presented herein are lower than those previously reported: 56% in Peruvians blood donors from the city of Huaral [Mohanna et al., 2007] and 18–45% in self-declared natives from the Ayoreo and Guarani tribes in Bolivia and Paraguay [Souza et al., 2010]. These discrepancies could be probably explained by the different geographical origin within each country and the characteristics of the studied populations, due to the fact that the Peruvians, Bolivians, and Paraguayans recruited in our study were predominantly born in big cities and most of them were not self-declared members of native communities.

With regard to age distribution, HHV-8 seroprevalence rates showed no differences [Perez et al., 2004] or—in some studies—they were higher in the older age group [Huang et al., 2000; Hudnall et al., 2003]. In a Peruvian study, no significant differences were found among age groups; however, two high seroprevalence peaks were observed at the 18–24 and 30–34 years old age group [Mohanna et al., 2007], suggesting that—in this case—sexual transmission plays an important role in viral transmission. The results herein show no age differences, which may be related to the absence of enough statistical power of this study to demonstrate these differences if they exist, which will probably require an increased

sample size (particularly in the immigrant groups) to analyze age related differences.

Most studies showed that HHV-8 seroprevalence was almost equally distributed between men and women [Plancoulaine et al., 2000; Perez et al., 2004]. In this study, significant differences were only observed in the HHV-8 DNA prevalence between men and women in North-western Argentina. Unfortunately, the male:female ratio in the Bolivian group was not equally distributed (52 male:135 female) which could have resulted in the lack of statistical significant association between gender in this region (Table I). However, it is possible that these findings on gender differences in HHV-8 DNA prevalence among North-western Argentines were due to the larger number of infected cases in this area, and it was not observed in the other groups because of the smaller sample size and lower prevalence of infection.

The reported differences in the HHV-8 prevalence in South America [Perez et al., 2004; Mohanna et al., 2007; Souza et al., 2010] could be explained, in part, by the analysis of the complex genetic ancestry picture depicted in South American populations, which exhibit tri-hybrid ancestral roots and five centuries of admixture [Suarez-Kurtz, 2010]. To our knowledge, no previous study has analyzed the genetic ancestry background of South American populations with regards to HHV-8 prevalence.

The population diversity in South America should be described by molecular approaches [Suarez-Kurtz, 2010] in order to avoid drawing false conclusions [Gómez et al., 2008; Sala and Corach, 2014; Calderón et al., 2015]. In admixed populations, it is essential to analyze matri and patrilineage as well as bi-parentally transmitted markers of the genome because both can reveal different geographic ancestry components [Kayser et al., 2006, 2008]. In the results presented herein, the genetic ancestry backgrounds of the Argentines and Bolivians analyzed in this study were similar to those previously described; for the reason that the European ancestry component prevailed in metropolitan Argentina whereas the Native American component was the most prevalent in the North-western region and Bolivia [Corach et al., 2010; Avena et al., 2012; Vullo et al., 2015] (Supplementary Fig. S2). Unfortunately, there is no literature to date to compare with the genetic ancestry background of Peruvians and Paraguayans obtained in this study.

With regards to the potential association of HHV-8 prevalence with the genetic ancestry of the population, only one of the three populations with higher proportion of Native American ancestry (North-western Argentines) show significant differences in the viral prevalence when compared with metropolitan Argentines, a population with high European ancestry; but also with Bolivians, a population with high Native American ancestry (Table I). Moreover, when the distribution of matri and patrilineage as well as bi-parentally transmitted markers of the genome were compared regarding the presence or absence of HHV-8 DNA in Paraguayans, metropolitan and North-western Argentines, no significant associations were observed (Fig. 2 and Supplementary Fig. S2). Interestingly, a higher prevalence of Native American mtDNA haplogroup A2 was observed among those Bolivian and Peruvian blood donors who were positive for HHV-8 DNA. However, the low number of samples emphasizes the need of further studies to confirm this finding.

It is known that HHV-8 infection is endemic in African populations [Edelman, 2005] and African slavery could have been a route of introduction of HHV-8 in South American populations. However, in our study, the proportion of African ancestry in the HHV-8 [+] Paraguayan population was lower than among the HHV-8 [−] group (Supplementary Fig. S2). Unfortunately, the low African ancestry component in the remaining populations limited the analysis of this potential association. Therefore, the analysis of a higher number of samples is urgently needed to clarify these results.

This study revealed the lack of association between genetic ancestry and HHV-8 DNA prevalence by analysis of matri and patrilineage as well was bi-parentally transmitted markers of the genome. Furthermore, statistical significant differences in HHV-8 DNA prevalence were found in two geographically neighboring regions with similar genetic ancestry background (North-western Argentina and Bolivia). Both results support the hypothesis of the existence of geographical factors—such as environmental or cultural characteristics—related to HHV-8 prevalence.

In Brazil, reported HHV-8 seroprevalence rates exhibit striking geographic variations in their distribution, suggesting the possibility of an endemic pocket in the Amazon region—with 77% of positivity for HHV-8 antibodies—when compared to central and southern Brazil [Souza et al., 2010]. Our results also showed geographic differences in HHV-8 DNA prevalence between neighboring regions of the continent which could suggest the presence of an endemic pocket in North-western Argentina, probably explained by the presence of specific risk factors, cultural characteristics or behaviors in the male population of this area and related to contaminated saliva and/or sexual transmission. However, due to the to the small number of Bolivians, Peruvians, and Paraguayans samples as well as HHV-8 DNA positive cases detected in these areas, more studies are needed to confirm this hypothesis.

It is important to mention that all HHV-8 DNA [+] samples detected in this study were non-reactive to all other infectious agents tested in blood banks and consequently these blood units were available for transfusion. As a consequence, special caution should be taken with immunosuppressed recipient patients who are prone to acquire infections and develop the HHV-8 associated diseases, as previously suggested [Perez et al., 2004].

The variation in the distribution of HHV-8 in South America remains intriguing and may be associated with specific risk factors or behaviors but not with the genetic ancestry. Another possible explanation could be related to the main HHV-8 strain circulating in the different regions and its particular virus-host interactions. Therefore, more epidemiological and molecular studies should be carried out to determine possible regional differences and detect new related risk factors.

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