Journal of Medical Virology 72:661–667 (2004)

Seroprevalence of Human Herpesvirus-8 in Blood Donors From Different Geographical Regions of Argentina, Brazil, and Chile

Celeste Pérez,[1]* Mónica Tous,[1] Sandra Gallego,[2] Norma Zala,[3] Oscar Rabinovich,[4] Susana Garbiero,[5] María José Martínez,[6] Andrèa Mendonça Gusmão Cunha,[7] Sofía Camino,[3] Alicia Cámara,[2] Sandra Cecília Botelho Costa,[7] Milton Larrondo,[8] Verónica Francalancia,[1] Fernando Landreau,[1] and Miguel Angel Bartomioli[5]

[1]*Departamento Virología, Instituto Nacional de Enfermedades Infecciosas-ANLIS Dr. Carlos G. Malbrán, Buenos Aires, Argentina*
[2]*Laboratorio de Virus Linfotrópicos Humanos, Instituto de Virología, Facultad de Ciencias Médicas, Universidad Nacional de Córdoba, Córdoba, Argentina*
[3]*Servicio de Hemoterapia e Inmunohematología, Hospital de Enfermedades Infecciosas Francisco J. Muñiz, Buenos Aires, Argentina*
[4]*Servicio de Hemoterapia, Hospital Británico, Buenos Aires, Argentina*
[5]*Servicio de Hemoterapia, Hospital Privado del Sur, Bahía Blanca, Argentina*
[6]*Programa Virología, I.C.B.M., Universidad de Chile, Escuela de Medicina, Santiago, Chile*
[7]*Universidade da Campinas (UNICAMP), São Paulo, Brazil*
[8]*Banco de Sangre, Universidad de Chile, Hospital Clínico J.J. Aguirre, Chile*

Human herpesvirus-8 (HHV-8) causes Kaposi's sarcoma (KS) and lymphoproliferative disorders in both HIV-infected and uninfected patients. HHV-8 has a worldwide occurrence but infection rates vary according to a combination of geographic and behavioral risks. The main transmission route seems to be sexual, nevertheless, nasal secretions, saliva, blood, and organ graft have been proposed. HHV-8 was postulated as a new infectious agent for screening in blood donors. The aim of this study was to evaluate the prevalence of antibodies against HHV-8 antigens in blood donors of South America. Serum samples from 2,470 blood donors from Argentina, Brazil, and Chile corresponding to five geographic regions were studied by indirect immunofluorescence assay (IFA). Seroprevalence rate was 3.7% (92/2,470; 95% CI 2.9–4.5) in the entire blood donor population distributed as follows: Argentina, 4.0% (Buenos Aires city, 4.3%; Bahia Blanca, 2.4%; and Córdoba, 4.0%), Campinas (Brazil), 2.8%; and Santiago de Chile, 3.0%. There was no difference ($P > 0.05$) between men and women or age related, except in Brazil where positive cases were 30–49-year-old males. The present study, which includes different geographical areas of multiple countries from South America, has not been done before. The results show similar prevalence rates among the studied zones corresponding to low-prevalence regions. South America is a large sub-continent with a wide spectrum of population and geographical characteristics, thus, more HHV-8 prevalence studies should be necessary to establish possible regional differences. ***J. Med. Virol. 72:661–667, 2004.*** © 2004 Wiley-Liss, Inc.

KEY WORDS: HHV-8; serology; epidemiology; South America

INTRODUCTION

Human herpesvirus-8 (HHV-8) also known as Kaposi's sarcoma (KS) herpesvirus is the most recently discovered HHV [Chang et al., 1994]. HHV-8 DNA has been detected in all forms of KS: classical, endemic, post-transplantation, AIDS associated [Boshoff et al., 1995; Dupin et al., 1995; Moore and Chang, 1995; Luppi et al., 2000], and also in multicentric Castleman disease as well as primary effusion lymphoma (PEL), in both HIV-infected and uninfected patients [Nador et al., 1996; Carbone et al., 1996]. HHV-8 is known to have a

*Correspondence to: Celeste Pérez, MS, Servicio Cultivo de Tejidos, Departamento Virología, Instituto Nacional de Enfermedades Infecciosas-ANLIS Dr. Carlos G. Malbrán, Av Vélez Sarsfield 563, C1282AFF Buenos Aires, Argentina.
E-mail: cperez@anlis.gov.ar

Accepted 18 November 2003
DOI 10.1002/jmv.20029
Published online in Wiley InterScience (www.interscience.wiley.com)

© 2004 WILEY-LISS, INC.

worldwide occurrence but infection rates vary according to a combination of geographic and behavioral risk factors [Cottoni et al., 1996; Kedes et al., 1996; Freitas et al., 2002]. Prevalence of HHV-8 infections in HIV negative people are high in Central and Southern Africa (>50%), intermediate in Mediterranean, Eastern European, and Caribbean countries (5–20%), and low in most of Asia, North America, and Northern Europe (0–5%) [Moore, 2000]. However, regional differences have been reported. In Asia, geographic pockets with lower or higher (1.4–46.6%) prevalences were found [Huang et al., 2000; Dilnur et al., 2001; Satoh et al., 2001]. In Europe, frequencies from 3.0 to 35% have been reported [Gambus et al., 2001; Preiser et al., 2001; Santarelli et al., 2001]. The overall HHV-8 seroprevalence in the United States is low [Gao et al., 1996; Simpson et al., 1996] but in blood donors from Texas it is substantially higher (15.0%) [Baillargeon et al., 2001]. HHV-8 is endemic in Africa (40–60%), in sub-Saharan areas, the intersection between the HHV-8 endemic and the HIV epidemic has resulted in KS becoming the most common adult malignancy in many areas [Moore, 2000]. In Central and South America, the prevalence of HHV-8 antibodies in blood donors has not been established yet. Indeed, most studies carried out to date have focused on high-risk populations [Sosa et al., 1998; Zhang et al., 1998; Biggar et al., 2000]. Epidemiological studies indicate that the main transmission route is sexual [Kedes et al., 1996; Cannon et al., 2001; Challine et al., 2001]. Nevertheless, non-sexual routes involving nasal secretions, saliva, blood, and organ graft have been proposed [Blackbourn et al., 1997; Luppi et al., 2000; Rezza et al., 2000; Vitale et al., 2000; Cannon et al., 2001; Challine et al., 2001; Sosa et al., 2001; Barozzi et al., 2003]. HHV-8 was postulated as a new infectious agent for screening in blood donors, thus, larger population-based studies would be necessary to estimate HHV-8 infection across different geographic regions of the world blood supply [Engels et al., 1999; Baillargeon et al., 2001; Hladik et al., 2003; Pellet et al., 2003]. The aim of this study was to evaluate the prevalence of antibodies, immunoglobulin G (IgG) class against HHV-8 lytic and latent antigens, in blood donors from five geographic regions of South America.

MATERIALS AND METHODS

Collection of Serological Specimens

Blood donor sera. Serum samples from 2,470 volunteer blood donors from Argentina, Brazil, and Chile corresponding to five geographic regions were studied between January 2000 and December 2002. Specimens were obtained from 1,793 men and 677 women who had passed the standard donor medical and behavioral screening. The mean age was 36 years (range 17–76 years). Sera were routinely tested for HBV, HCV, HIV, HTLV-I/II, syphilis, brucellosis, and Chagas and preserved at −20°C. All samples were sent to the Virology Department at the Instituto Nacional de Enfermedades Infecciosas-ANLIS Dr. C.G. Malbrán, Buenos Aires, Argentina, where assays for HHV-8 were carried out independently from routine tests results. Other recorded blood donor data included place of birth and current address; patient–blood donor relationship data were obtained only at the Hospital F. Muñiz from Buenos Aires city.

Blood donors from Argentina. A total of 1,859 serum samples were obtained from three different geographic regions: Buenos Aires city, Bahía Blanca city (southern city of Buenos Aires province), and Córdoba city (capital city of Córdoba province, central region of Argentina). Sera from Buenos Aires city (868) were collected at Hospital F. Muñiz and at Hospital Británico. Sera from Bahía Blanca (256) came from the blood bank of Hospital Privado del Sur. Serum samples from Córdoba (735) came from the blood bank of the Provincial Ministry of Health which comprised 14 hospitals from Córdoba city and surrounding areas.

Blood donors from Brazil. There were 319 sera collected from Campinas and surrounding cities at the Hemocentro da UNICAMP, Campinas, São Paulo.

Blood donors from Chile. Samples from Chile (300) came from the blood bank of the Hospital Clínico J.J. Aguirre, Universidad de Chile, Santiago de Chile.

Positive and negative control sera. Serum samples from 15 non-febrile children aged 3–8 years without history of KS or HIV-infected relatives, attending at Surgery and Traumatological Services from Buenos Aires city, were tested by indirect immunofluorescence assay (IFA), pooled and used as negative controls. Sera from 57 KS patients (49 male, eight female; aged 18–89 years), including 49 HIV+, three classical, four post-transplantation, and one PEL [Pérez and Rudoy, 2001], attending at Hematology and Dermatology Services from Buenos Aires hospitals were HHV-8-IFA tested. Positive samples were pooled and used as controls. All KS diagnoses were confirmed by histological examination and PCR of KS biopsy samples [Chang et al., 1994].

Cell Culture

The HHV-8 positive and Epstein–Barr Virus (EBV) negative, BCBL-1 cell line (AIDS Research and Reference Reagents Program, National Institutes of Health, Bethesda, MD) was cultured in RPMI 1640 (Hyclone, Road Logan, UT) supplemented with 10% fetal bovine serum (FBS) (Gibco, Grand Island, NJ), 2 mM L-glutamine (Sigma, St. Louis, MO), 5×10^{-5} M β-mercaptoethanol, and 10 mM HEPES (Sigma). Two B-cell lines from Burkitt lymphoma, P3HR1 (American Type Culture Collection, Rockville, MD) and Ramos (courtesy from Dr. B. Koziner, Oncolab, Buenos Aires), both HHV-8 negative cells, were cultured and assessed for use as controls. The EBV producer cell line P3HR-1 was maintained in RPMI 1640 10% FBS, 1.0 mM sodium pyruvate (Gibco), and 2 mM L-glutamine (Sigma). The EBV negative Ramos cell line was cultured in RPMI 1640 media supplemented with 2-mM L-glutamine (Hyclone) and 10% FBS. All cell lines were cultured at 37°C in 5% CO_2 atmosphere and mycoplasma tested by

PCR [Harasawa et al., 1993] and Höechst stain [Chen, 1977]. Mycoplasma free cell lines, BCBL-1 and P3HR1 were subcultured 1:4 and Ramos 1:8 once a week.

Indirect IFA

An IFA was developed using BCBL-1 cells. Lytic replicative cycle of HHV-8 in BCBL-1 cells was induced by incubating 10^6 cells/ml with 20 ng of the phorbol ester 12-*O*-tetradecanoylphorbol-13 acetate (TPA) (Sigma) [Renne et al., 1996] for 4 days. Induced cells were collected, washed three times in phosphate-buffered saline (PBS) (pH 7.4), and spotted in a density of 400–600 cells/mm^2 onto microscope multiwell slides. Smears were air dried in a laminar flow cabinet and fixed at −20°C for 20 min with cold acetone (Merck, Buenos Aires, Argentina). Slides of non-induced Ramos cells were prepared as described and used as antigens. IFA was performed as previously described [Cattani et al., 1999] using 1:40 dilutions of test samples, positive and negative controls in PBS supplemented with 1% low-fat milk. Serum samples were scored to be positive if specific fluorescence was visible on BCBL-1 cells without apparent reactivity on Ramos cells. To adsorb EBV cross-reactive antibodies, positive samples were treated with TPA induced P3HR1 cells as previously described [Moore et al., 1996] and were tested on BCBL-1 cells by IFA. Serum from a HHV-8 positive blood donor with an IgG titer for EBV-Viral Capsid Antigen (VCA) >1:1,000, was used as an adsorption control. IFA using slides coated with P3HR-1 expressing EBV-VCA (BION Enterprises, IL), was performed before and after adsorption. A significant loss of EBV antibody titer would indicate a successful adsorption.

Statistical Analysis

The results were expressed as percentages and proportions (positive/total). The prevalence of anti-HHV-8 was expressed as percentages together with 95% confidence intervals (CIs). Data were analyzed using the STATISTICA 6.0 version (StatSoft, Inc., Tulsa, OK). The relationships between categorical variables were tested by means of chi-square and Fisher exact test when necessary. Significance was defined as $P < 0.05$.

RESULTS

Seroprevalence was 3.7% (92/2,470) in the entire population. Results are summarized in Table I. Figure 1 shows geographical distribution of the prevalence rates in the studied regions. There was no difference ($P > 0.05$) between men and women in four of five regions. In Brazil, all positive cases were male. No significant differences were found between age and seropositivity ($P > 0.05$). Table II shows age distribution of HHV-8 positive cases for each blood bank. Only 9/92 (9.8%) HHV-8 positive sera were reactive to other infectious agent as is shown in Table III. Current address and place of birth data showed that more than 85% of blood bank donors from Brazil, Chile, Córdoba, Bahía Blanca, and Hospital Británico had life-long residence corresponding to their blood bank area. Meanwhile only 8% of Hospital F. Muñiz donors remained in Buenos Aires city and the rest had moved from another Argentinean provinces or neighbor countries. Regarding to patient–blood donor relationship at the Hospital F. Muñiz, 98% of donors were patient's relatives or friends.

A tenfold EBV antibody titer decrease was observed in the EBV adsorption control serum without loss of reactivity on BCBL-1 cells. None of the samples scored as HHV-8 positive was negative after P3HR-1 adsorption. Five sera were reactive against Ramos cells and were excluded. All children sera were negative (15/15) and the 57 KS serum samples were HHV-8 seropositive by IFA.

DISCUSSION

Soon after discovery of HHV-8 in KS tissue [Chang et al., 1994], it was found that almost all patients with KS have HHV-8 antibodies [Gao et al., 1996; Kedes et al., 1996; Lennette et al., 1996; Simpson et al., 1996], usually with relatively high titers [Chatlynne et al., 1998; Rabkin et al., 1998; Gambus et al., 2001]. Detection of asymptomatic HHV-8 infection has been much more problematic. Efforts to develop accurate serologic test to diagnose HHV-8 infection have been hampered by a number of factors, including an incomplete understanding of the viral proteins that may serve as potential

TABLE I. Prevalence of Human Herpesvirus-8 (HHV-8) Antibodies According to Gender and Blood Bank

Country	Blood bank	Number of seropositive sera/number tested (%)[a,b]			
		Male	Female	Total	95% CI
Argentina	Hospital Británico, Buenos Aires	4/222 (1.8)	2/96 (2.1)	6/318 (1.9)	0.4–3.4
	Hospital F. Muñiz, Buenos Aires	21/386 (5.4)	16/164 (9.8)	37/550 (6.7)[c]	4.6–8.8
	Bahía Blanca	4/156 (2.6)	2/92 (2.2)	6/248 (2.4)	0.5–4.3
	Córdoba	21/601 (3.5)	4/134 (3.0)	25/735 (3.4)	2.1–4.7
	Total of Argentina	50/1,365 (3.6)	24/486 (4.9)	74/1,851 (4.0)	3.5–6.4
Brazil	Campinas	9/233 (3.8)	0/86 (0.0)	9/319 (2.8)	1.0–4.6
Chile	Santiago de Chile	5/195 (2.6)	4/105 (3.8)	9/300 (3.0)	1.1–4.9
	Total	64/1,793 (3.6)	28/677 (4.1)	92/2,470 (3.7)	2.9–4.5

[a]All sera were tested by immunofluorescence assay (IFA), seropositivity was scored at 1:40 dilutions.
[b]No significant differences were found between men and women ($P > 0.05$) except in Brazil where all positives were males.
[c]There are significant differences ($P < 0.05$) between Hospital Muñiz and the rest of the investigated blood banks.

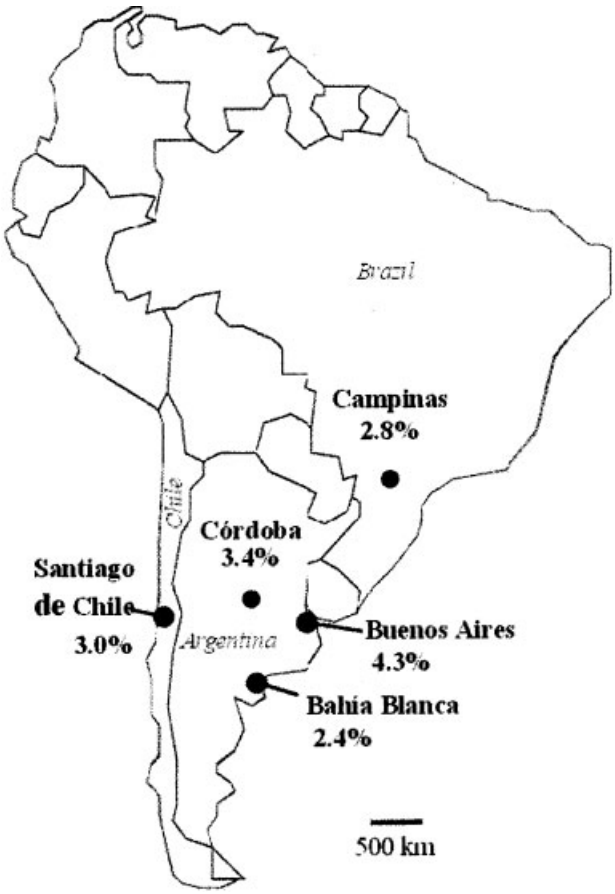

Fig. 1. Geographical distribution of human herpesvirus-8 (HHV-8) prevalence rates in five regions of South America. Argentina: Buenos Aires City, 4.3%; Bahía Blanca, 2.4%; and Córdoba, 3.4%. Brazil: Campinas, São Paulo, 2.8%. Chile: Santiago de Chile, 3.0%.

targets for serologic assays, a lack of well-characterized infected and uninfected subjects with whom to test the assay and wide geographic and demographic variations in the prevalence of HHV-8 infection. In interlaboratory comparisons, using standard panels of samples from the general population [Rabkin et al., 1998], all laboratories generally detected antibodies in KS patients, but there was much less agreement about results in asymptomatic persons. [Casper et al., 2002; Pellet et al., 2003]. Many serosurveys indicate that HHV-8 antibody prevalence has a great variability in different geographic areas worldwide. In Asia, the seropositivity rate of the general population is 1.4% [Satoh et al., 2001] in Japan, 19.2% in Taiwan [Huang et al., 2000], and 46.6% in the Xinjiang area, China [Dilnur et al., 2001]. In Europe, the seroprevalence rate in German blood donors (3.0%) is similar to that found previously in Western European countries [Preiser et al., 2001]. Meanwhile among all Italian donors, 24.1% had antibodies, but regional differences [Calabro et al., 1998; Santarelli et al., 2001] and age distribution in seroprevalence agreed with classic KS incidence [Calabro et al., 1998]. In Spain, prevalence is higher (6.5%) than in Northern Europe and the United States [Gambus et al., 2001] where a low rate was found [Lennete et al., 1996; Edelman et al., 2000]. The exception is Texas where prevalence is substantially higher (15.0%) [Baillargeon et al., 2001; Hudnall et al., 2003]. In Central and South America, the prevalence of HHV-8 antibodies in blood donors has not been established yet. Most studies carried out to date have focused on groups at theoretically increased risk of acquiring HHV-8 infection [Sosa et al., 1998; Zhang et al., 1998; Biggar et al., 2000]. Differences in reported HHV-8 seroprevalence rates may be the result, at least in part, of different laboratory algorithms [Casper et al., 2002; Hladik et al., 2003; Pellet et al., 2003], cutoffs—sera dilutions from 1:10 to 1:160—[Gao et al., 1996; Enbom et al., 2000; Baillargeon et al., 2002] and assays that were used. Available serological tests measure antibodies to latent or lytic antigens by IFA [Gao et al., 1996; Kedes et al., 1996]; against recombinant structural proteins, synthetic peptides, or whole virus by enzyme immunoassays or Western blots [Simpson et al., 1996; Pau et al., 1998; Juhasz et al., 2001]. We have utilized a well-known assay using BCBL-1 cells expressing the full complement of latent and lytic viral antigens [Blackbourn et al., 1997; Enbom et al., 2000; Casper

TABLE II. Prevalence of HHV-8 Antibodies According to Age and Blood Bank

		Number of sera positive/number tested (%)					
		Age groups (years)				Mean age (years) (range)	
Country	Blood bank	17–29	30–39	40–49	50–76	Positive cases	Total population
Argentina	Hospital Británico, Buenos Aires	0/97 (0.0)	3/93 (3.2)	2/77 (2.6)	1/51 (1.9)	42.3 (34–57)	37.1 (18–76)
	Hospital F. Muñiz, Buenos Aires	9/196 (4.6)	15/170 (8.8)	8/104 (7.7)	5/80 (6.2)	35.5 (18–59)	35.2 (18–59)
	Bahía Blanca	2/74 (2.7)	1/68 (1.5)	2/59 (3.4)	1/47(2.1)	39.3 (26–53)	37.7 (18–61)
	Córdoba	15/327 (4.6)	6/231 (2.6)	3/126 (2.3)	1/51 (1.9)	30.3 (19–39)	31.6 (17–60)
	Total of Argentina	26/694 (3.7)	25/562 (4.5)	15/366 (4.0)	8/229 (3.5)	36.9 (18–59)	35.4 (17–76)
Brazil	Campinas	0/113 (0)	5/117 (4.3)	4/61 (6.6)	0/28 (0.0)	40.5 (31–50)	37.6 (21–61)
Chile	Santiago de Chile	2/108 (1.9)	4/88 (4.5)	1/74 (1.3)	2/30 (6.6)	37.1 (24–61)	35.0 (18–63)
	Total	28/913 (3.1)	34/766 (4.4)	20/477 (4.5)	10/255 (3.9)	37.5 (18–61)	36.0 (17–76)

TABLE III. Blood Bank Results of HHV-8 Seropositive Samples

Blood bank	None	Brucellosis	Chagas	HBV	HCV	Total
Hospital Británico, Buenos Aires	5	0	1	0	0	1/6
Hospital F. Muñiz, Buenos Aires	31	1	4	1	0	6/37
Bahía Blanca	6	0	0	0	0	0/6
Córdoba	23	0	1	0	1	2/25
Hemocentro da Campinas	9	0	0	0	0	0/9
Santiago de Chile	9	ND[a]	0	0	0	0/9
Total	83	1	6	1	1	9/92

[a]Brucellosis was not tested in Chilean donors.

et al., 2002; Hudnall et al., 2003]. We analyzed a significant number of samples from different regions and all the assays were carried out at the same center, which avoided interlaboratory differences. Two tests were added to prevent cross-reactivity; an IFA performed on Ramos cells and the adsorption of EBV antibodies [Moore et al., 1996]. According with previous reports [Chatlynne et al., 1998; Cattani et al., 1999; Edelman et al., 2000; Sosa et al., 2001], we found no evidence of cross-reaction with EBV antibodies. In contrast to these studies, some of our samples showed fluorescence in Ramos cells and were excluded from analysis.

To our knowledge, the present study is the first approach in South America aimed to assessing the immune status of blood donors to HHV-8 in several regions. The results herein show similar prevalence rates in the five different regions studied. However, a more detailed analysis of each blood bank results reveals that anti-HHV-8 rate of Hospital F. Muñiz (6.7%) was higher than the others ($P < 0.05$) and this gap is more remarkable ($P < 0.01$) with the Hospital Británico (1.9%), although both are located at the same neighborhood in Buenos Aires city. Both populations differ widely, Hospital Británico is a private care facility that gives service to patients with higher socio-economic level than Hospital F. Muñiz which is a public hospital focused mainly in infectious diseases. The latter has a high rate of HIV-infected patients (61%) (Hospital F. Muñiz, Control Infections Committee Note No. 2965/01, September 26, 2001), that constitute a high-risk group for HHV-8 infection. Remarkable data are that most of the donors studied at this hospital had some relationship with HIV patient. Thus, the increased prevalence found in this blood bank might be linked to non-sexual routes of transmission like saliva or nasal secretions, as has been previously proposed [Cattani et al., 1999; Pauk et al., 2000; Plancoulaine et al., 2000; Vitale et al., 2000]. This blood donor population also showed a great migratory movement and none of the HHV-8 positive samples belonged to a Buenos Aires city resident. Therefore, the rate found at the Hospital F. Muñiz may not be representative of the HHV-8 prevalence in Buenos Aires city.

In Brazil, recent reports have found that seroprevalences among Amerindians (53%) [Biggar et al., 2000] and urban communities (16%) [Freitas et al., 2002] from the northern states are higher than those from southern states. In blood donors from Brazilian southern states a 4.6% prevalence has been reported [Zago et al., 2000]. The latter is similar to our finding of 2.8% (95% CI 1.0–4.6) in Campinas, São Paulo.

Most studies showed that HHV-8 seroprevalence was almost equally distributed between men and women but was increased in the older age groups [Calabro et al., 1998; Huang et al., 2000; Juhasz et al., 2001; Hudnall et al., 2003]. In the other hand, Pellet et al. [2003] found no significant relationships between HHV-8 seropositivity and demographic variables differences. The results herein show no gender or age differences, except in Brazil, where positive results were found only among males with age ranged between 30 and 50 years old. The similar prevalence observed among the different age groups may be associated to the absence of enough power of our study to demonstrate these differences if they exist, which will probably require an increased sample size to analyze age related differences. Another possibility is that these results reflect an epidemiological situation related to different environmental and cultural factors. Our results from Brazilian donors agree with the observation of Caterino-de-Araujo et al. [1999] who found that seropositive blood donors in São Paulo, Brazil, were all men with a mean age of 37.5 years.

An unresolved question with public health implications is whether blood transfusion can transmit HHV-8. Cytomegalovirus (CMV) and another cell-associated herpesvirus are transmitted via blood transfusion and both, HHV-8 and CMV are transmitted by solid organ transplantation [Parravicini et al., 1997]. Even though infectious HHV-8 was found in CD19+ lymphocytes of a healthy US blood donor [Blackbourn et al., 1997], no study to date has documented HHV-8 through transfusion [Operskalski et al., 1997; Engels et al., 1999; Hudnall et al., 2003]. The likelihood of finding such infected cells varies with the seroprevalence for a particular region [Ablashi et al., 2002]. Recently some studies were carried out in order to evaluate possible bloodborne HHV-8 transmission in blood donors. Two of them were done in samples from US, one included patients solely from Texas [Hudnall et al., 2003] and the other included specimens from diverse geographic areas

[Pellet et al., 2003]. In both studies, none of the blood donors was positive for HHV-8 DNA but a higher seroprevalence rate was found in Texas. It remains to be seen whether the differences are due to the assays used or the populations studied [Pellet et al., 2003]. Nonetheless, HHV-8 DNA was found in studies carried out in endemic zones and the viral load correlated with antibody titre to lytic antigen, suggesting that transmission of HHV-8 by blood contact could be of importance [Enbom et al., 2002]. In Uganda, where HHV-8 is common in blood donors, the risk of HHV-8 transmission by transfusion is equivalent to the 1-year cumulative risk of infection from community sources [Mbulaiteye et al., 2003]. The association between injection drug use and HHV-8 also supported the notion that blood exposure is an alternative route of transmission [Sosa et al., 2001; Atkinson et al., 2003; Goedert et al., 2003]. In the present study, 90% (83/92) of HHV-8 positive samples were non-reactive to all other infectious agents tested in blood banks and consequently these blood units were available for transfusion. A follow-up study would be necessary to determine their infectious capability.

In conclusion, this study shows that the five regions studied correspond to low-HHV-8 seroprevalence areas. The high prevalence found at Hospital F. Muñiz blood bank could be explained by the close relationship between HIV patients and blood donors, which might put them at an increased risk for HHV-8 infection in comparison with donors from the other centers. The latter points out that the safety of the blood supply in blood banks of infectious disease hospitals could be questioned and warrants further investigations. Special caution should be taken with immuno-suppressed recipient patients who are prone to acquire infections and to develop the associated diseases. South America is a large sub-continent with a wide spectrum of population characteristics including cultural and geographical differences, native and immigratory populations, isolated communities, and different socio-economic conditions. More HHV-8 prevalence studies should be carried out to establish possible regional differences.

ACKNOWLEDGMENTS

The authors thank the AIDS Research and Reference Reagent Program (Division of AIDS, NIAID, NIH) for providing the BCBL-1 cell line, Benjamin Koziner (Oncolab, Buenos Aires) for providing Ramos cell line, Marta Maza for statistical analysis, and Shou-Jiang Gao for critical reading of the manuscript.

REFERENCES

Ablashi D, Chatlynne L, Whitman J, Cesarman E. 2002. Spectrum of Kaposi's sarcoma-associated Herpesvirus, or human Herpesvirus 8. Clin Microbiol Rev 15:439–464.

Atkinson J, Edlin BR, Engels EA, Kral AH, Seal K, Gamache CJ, Whitby D, O'Brien TR. 2003. Seroprevalence of human herpesvirus 8 among injection drug users in San Francisco. J Infect Dis 187:974–981.

Baillargeon J, Deng JH, Hettler E, Harrison C, Grady JJ, Korte LG, Alexander J, Montalvo E, Jenson HB, Gao SJ. 2001. Seroprevalence of Kaposi's sarcoma-associated herpesvirus infection among blood donors from Texas. Ann Epidemiol 11:512–518.

Baillargeon J, Leach CT, Deng JH, Gao SJ, Jenson HB. 2002. High prevalence of human Herpesvirus 8 (HHV-8) infection in South Texas children. J Med Virol 67:542–548.

Barozzi P, Luppi M, Facchetti F, Mecucci C, Alù M, Sarid R, Rasini V, Ravazzini L, Rossi E, Festa S, Crescenzi B, Wolf DG, Schulz TF, Torelli G. 2003. Post-transplant Kaposi sarcoma originates from the seeding of donor-derived progenitors. Nat Med 5:554–561.

Biggar RJ, Whitby D, Marshall V, Linhares AC, Black F. 2000. Human herpesvirus 8 in Brazilian Amerindians: A hyperendemic population with a new subtype. J Infect Dis 173:542–549.

Blackbourn DJ, Ambroziak J, Lennette E, Adams M, Ramachandran B, Levy JA. 1997. Infectious human herpesvirus 8 in a healthy North American blood donor. Lancet 349:609–611.

Boshoff C, Whitby D, Hatziioannou T, Fisher C, Walt J, Hatzakis A, Weis R, Schultz T. 1995. Kaposi's sarcoma associated herpesvirus in HIV-negative Kaposi's sarcoma. Lancet 345:1030–1044.

Calabro ML, Sheldon J, Favero A, Simpson GR, Fiore JR. 1998. Seroprevalence of Kaposi's sarcoma-associated herpesvirus/human herpesvirus 8 in several regions of Italy. J Hum Virol 1:207–213.

Cannon MJ, Dollard SC, Smith DK, Klein RS, Schuman P, Rich JD, Vlahov D, Pellet PE. 2001. Bloodborne and sexual transmission of human Herpesvirus 8 in women with or at risk for human immunodeficiency virus infection. N Engl J Med 344:637–643.

Carbone A, Gloghini A, Vacher E. 1996. Kaposi's sarcoma-associated herpesvirus DNA sequences in AIDS-related and in AIDS-unrelated lymphomatosus effusions. Br J Haematol 94:533–543.

Casper C, Krants E, Taylor H, Dalessio J, Carrel D, Wald A, Corey L, Ashley R. 2002. Assessment of a combined testing stratgy for detection of antibodies to human Herpesvirus 8 (HHV-8) in persons with asymptomatic HHV-8 infection, and in persons at low risk for HHV-8 infection. J Clin Microbiol 40:3822–3825.

Caterino-de-Araujo A, Calabro ML, de los Santos-Fortuna E, Suleiman J, Chieco-Bianchi L. 1999. Searching for human Herpesvirus 8 antibodies in serum samples from patients infected with human immunodeficiency virus type 1 and blood donors from Sao Paulo, Brazil. J Infect Dis 179:1591–1592.

Cattani P, Capuano M, Cerimele F, La Parola IL, Santangelo R, Masini C, Cerimele D, Fadda G. 1999. Human herpesvirus 8 seroprevalence and evaluation of nonsexual transmission routes by detection of DNA in clinical specimens from human immunodeficiency virus-seronegative patients from central and southern Italy, with and without Kaposi's sarcoma. J Clin Microbiol 37: 1150–1153.

Challine D, Roudot-Thoraval F, Sarah T, Laperche L, Boisson B, Mauberquez S, Dubernet F, Rigot P, Lefrere F, Mercier B, Brossard Y, Rouet F, Girot R, Loiseau P, Girard D, Claquin J, Loty B, Lerable J, Mariotti M, Pawlotsky JM, Lefrere JJ. 2001. Seroprevalence of human herpes virus 8 antibody in populations at high or low risk of transfusion, graft, or sexual transmission of viruses. Transfusion 41:1120–1125.

Chang Y, Cesarman E, Pessin MS, Lee F, Culpepper J, Knowles DM, Moore PS. 1994. Identification of an herpesvirus-like DNA sequences in AIDS associated Kaposi's sarcoma. Science 266: 1865–1869.

Chatlynne LG, Lapps W, Handy M, Huang YQ, Massod R, Hamilton AS, Said JW, Koeffer HP, Kaplan MH, Friedman-Kien A, Gill PS, Whitman JE. 1998. Detection and titration of human herpesvirus-8-specific antibodies in sera from blood donors, acquired immunodeficiency syndrome patients, and Kaposi's sarcoma patients using a whole virus enzyme-linked immunosorbent assay. Blood 92:53–58.

Chen TR. 1977. In situ detection of mycoplasma contamination in cell cultures by Fluorescent Hoechst 33258. Exp Cell Res 104:255–262.

Cottoni F, De Marco R, Montesu MA. 1996. Classical Kaposi's sarcoma in north-east Sardinia: An overview from 1977 to 1991. Br J Cancer 73:1132–1133.

Dilnur P, Katano H, Wang ZH, Osakabe Y, Kudo M, Sata T, Ebihara Y. 2001. Classic type of Kaposi's sarcoma and human herpesvirus 8 infection in Xinjiang, China. Pathol Int 51:845–852.

Dupin N, Grandadam M, Calvez V, Gorin I, Aubin JT, Havard S, Lamy F, Leibowicht M, Huraux JM, Escande JP. 1995. Herpesvirus-like DNA sequences in patients with Mediterranean Kaposi's sarcoma. Lancet 345:761–763.

Edelman DC, Ketema F, Saville RD, Herman J, Still AM, Gill PS, Blattner W, Constnantine NT. 2000. Specifics on the refinement and application of two serological assays for the detection of antibodies to HHV-8. J Clin Virol 16:225–237.

Enbom M, Sheldon J, Lennette E, Schulz T, Ablashi DV, Neipel F, Biberfeld P, Carlberg H, Ljungman P, Nilsson A, Soderstrom T, Wadstrom J, Linde A. 2000. Antibodies to human herpesvirus 8 latent and lytic antigens in blood donors and potential high-risk groups in Sweden: Variable frequencies found in a multicenter serological study. J Med Virol 62:498–504.

Enbom M, Urassa W, Massambu C, Thorstensson R, Mhalu F, Linde A. 2002. Detection of human herpesvirus 8 DNA in serum from blood donors with HHV-8 antibodies indicates possible bloodborne virus transmission. J Med Virol 68:264–267.

Engels EA, Eastman H, Ablashi DV, Wilks RJ, Braham J, Manns A. 1999. Risk of transfusion-associated transmission of human herpesvirus 8. J Natl Cancer Inst 91:1773–1175.

Freitas RB, Freitas MR, Linhares AC. 2002. Prevalence of herpesvirus 8 antibodies in the population of Belém, Pará, Brazil. Rev Inst Med Trop 44:309–313.

Gambus G, Bourboulia D, Esteve A, Lahoz R, Rodriguez C, Bolao F, Sirera G, Muga R, del Romero J, Boshoff C, Whitby D, Casabona J. 2001. Prevalence and distribution of HHV-8 in different subpopulations, with and without HIV infection, in Spain. AIDS 15:1167–1174.

Gao SJ, Kingsley L, Li M, Zheng W, Parravicini C, Ziegler J, Newton R, Rinaldo C, Saah A, Phair J, Detels R, Chang Y, Moore PS. 1996. KSHV antibodies among Americans, Italians, and Ugandans with and without Kaposisi's sarcoma. Nat Med 2:925–928.

Goedert JJ, Charurat M, Blattner WA, Hershow RC, Pitt J, Diaz C, Mofenson LM, Green K, Minkoff H, Paul ME, Thomas DL, Whitby D, The Women and Infants Transmission Study. 2003. Risk factors for Kaposi's sarcoma-associated herpesvirus infection among HIV-1-infected pregnant women in the USA. AIDS 17:425–433.

Harasawa R, Mizusawa H, Nozawa K. 1993. Detection and tentative identification of dominant mycoplasma species in cell cultures by restriction analysis of the 16S-23S rRNA intergenic spacer regions. Res Microbiol 144:489–493.

Hladik W, Dollard SC, Downing RG, Kataaha P, Pellett PE, Karon JM, Mermin J, Lackritz EM. 2003. Kaposi's sarcoma in Uganda: Risk factors for human herpesvirus 8 infection among blood donors. J AIDS 33:206–210.

Huang LM, Huang SY, Chen MY, Chao MF, Lu CY, Tien HF, Lee CY, Jeang KT. 2000. Geographical differences in human herpesvirus 8 seroepidemiology: A survey of 1201 individuals in Asia. Med Virol 60:290–293.

Hudnall SD, Chen T, Rady P, Tyring S, Allison P. 2003. Human Herpesvirus-8 seroprevalence and viral load in healthy adult blood donors. Transfusion 43:85–90.

Juhasz A, Remenyik E, Konya J, Veres G, Begany A, Andirko I, Medgyessy I, Hunyadi J, Gergerly L. 2001. Prevalence and age distribution of human Herpesvirus-8 specific antibodies in Hungarian blood donors. J Med Virol 64:526–530.

Kedes D, Operkalski E, Busch M, Kohn R, Flood J, Gandem D. 1996. The seroepidemiology of human herpesvirus 8 (Kaposi's sarcoma-associated herpesvirus): Distribution of infection in KS risk groups and evidence for sexual transmission. Nat Med 2:918–924.

Lennete ET, Blackbourn DJ, Levy JA. 1996. Antibodies to human herpesvirus type 8 in the general population an in Kaposi's sarcoma patients. Lancet 348:858–861.

Luppi M, Barozzi P, Schultz TF, Bone A. 2000. Marrow failure associated with human herpesvirus 8 infection after transplantation. N Engl J Med 343:1378–1385.

Mbulaiteye SM, Biggar RJ, Bakaki PM, Pfeiffer RM, Whitby D, Owor AM, Katongole-Mbidde E, Goedert JJ, Ndugwa CM, Engels EA. 2003. Human herpesvirus 8 infection and transfusion history in children with sickle-cell disease in Uganda. J Natl Cancer Inst 95:1330–1335.

Moore P. 2000. The emergence of Kaposis's sarcoma-associated herpesvirus (human herpesvirus 8). New Engl J Med 343:1411–1413.

Moore P, Chang Y. 1995. Detection of herpesvirus-like DNA sequences in Kaposi's sarcoma in patients with and those without HIV infections. New Engl J Med 332:1181–1185.

Moore P, Gao SJ, Dominguez G, Cesarman E, Lungu O, Knowles DM, Garber R, Pellet PE, McGeoch D, Chang Y. 1996. Primary characterization of a herpesvirus agent associated with Kaposi's sarcoma. J Virol 70:549–558.

Nador RG, Cesarman E, Chadburn A, Dawson DB, Ansari MQ, Sald J, Knowles DM. 1996. Primary effusion lymphoma: A distinct clinicopathologic entity associated with the Kaposi's sarcoma-associated herpes virus. Blood 88:645–656.

Operskalski EA, Busch MP, Mosley JW, Kedes DH. 1997. Blood donations and viruses. Lancet 349:1327.

Parravicini C, Olsen SJ, Capra M, Poli F, Sirchia G, Gao SJ. 1997. Risk of Kaposi's sarcoma-associated herpesvirus transmission from dono allografts among Italian post-transplant Kaposis's sarcoma patients. Blood 90:2826–2829.

Pau CP, Lam LL, Spira TJ, Black JB, Stewart JA, Pellet PE, Respes RA. 1998. Mapping and serodiagnosis application of a dominant epitope within the human herpesvirus 8 ORF 65-encodede protein. J Clin Microbiol 36:1574–1577.

Pauk J, Huang ML, Brodie SJ, Wald A, Koelle DM, Schacker T, Celum C, Selke S, Corey L. 2000. Mucosal shedding of human herpesvirus 8 in men. N Engl J Med 343:1369–1377.

Pellet PE, Wright DJ, Engels EA, Ablashi DV, Dollard SC, Forghani B, Glynn SA, Goedert JJ, Jenkins FJ, Lee TH, Neipel F, Todd DS, Whitby D, Nemo GJ, Busch MP, Retrovirus Epidemiology Donor Study. 2003. Multicenter comparison of serologic assays and estimation of human herpesvirus 8 seroprevalence among US blood donors. Transfusion 43:1260–1268.

Plancoulaine S, Abel L, van Beveren M, Tregouet DA, Joubert M, Tortevoye P, de The G, Gessain A. 2000. Human herpesvirus 8 transmission from mother to child and between siblings in an endemic population. Lancet 356:1062–1065.

Preiser W, Szep NI, Lang D, Doerr HW, Rabenau HF. 2001. Kaposi's sarcoma-associated herpesvirus seroprevalence in selected German patients: Evaluation by different test systems. Med Microbiol Immunol 190:121–127.

Pérez CL, Rudoy S. 2001. Anti-CD20 monoclonal antibody treatment of human Herpesvirus 8—associated, body cavity-based lymphoma with an unusual phenotype in a human immunodeficiency virus-negative patient. Clin Diagn Lab Immunol 5:993–996.

Rabkin CS, Schulz TF, Whitby D, Lennette ET, Magpantay LL, Chatlynne L, Biggar RJ. 1998. Interassay correlation of human herpesvirus 8 serological tests. HHV-8. Interlaboratory Collaborative Group. J Infect Dis 178:304–309.

Renne R, Zhong W, Herndier B, McGrath M, Abbey N, Kedes D, Ganem D. 1996. Lytic growth of Kaposi's sarcoma-associated herpesvirus (human herpesvirus 8) in culture. Nat Med 2:342–346.

Rezza G, Tchangmena OB, Andreoni M, Bugarini R, Toma L, Bakary DK, Glikoutou M, Sarmati L, Monini P, Pezzotti P, Ensoli B. 2000. Prevalence and risk factors for human herpesvirus 8 infection in northern Cameroon. Sex Transm Dis 27:159–164.

Santarelli R, De Marco R, Masala MV, Angeloni A, Uccini S, Pacchiarotti R, Montesu MA, Satta R, Cerimele D, Faggioni A, Cottoni F. 2001. Direct correlation between human herpesvirus-8 seroprevalence and classic Kaposi's sarcoma incidence in Northern Sardinia. J Med Virol 65:368–372.

Satoh M, Toma H, Sato Y, Futenma C, Kiyuna KS, Shiroma Y, Kokaze A, Sakurada S, Sata T, Katano H. 2001. Seroprevalence of human Herpesvirus 8 in Okinawa, Japan. Jpn J Infect Dis 54:125–126.

Simpson GR, Schulz TF, Whitby D, Cook PM, Boshoff C, Rainbow L, Howard MR, Gao SJ, Bohenzky RA, Simmonds P, Lee C, de Ruiter A, Hatzakis A, Tedder RS, Weller IV, Weiss RA, Moore PS. 1996. Prevalence of Kaposi's sarcoma associated herpesvirus infection measured by antibodies to recombinant capsid protein and latent immunofluorescence antigen. Lancet 348:1133–1138.

Sosa C, Klascala W, Chandran B. 1998. Human herpesvirus 8 as an potential sexually transmitted agent in Honduras. J Infect Dis 178:547–551.

Sosa C, Benetucci J, Colleen H, Sierczkowski L, Deluchi G, Canizal AM, Hamaka M, Winslow K, Baum M, Wood C. 2001. Human herpesvirus 8 can be transmitted through blood in drug addicts. Medicina 61:291–294.

Vitale F, Viviano E, Perna AM, Bonura F, Mazzola G, Ajello F, Romano N. 2000. Serological and virological evidence of non-sexual transmission of human herpesvirus type 8 (HHV8). Epidemiol Infect 125:671–675.

Zago A, Bourboulia K, Viana MC, Collandre H, Dietze R, Boshoff C, Keller R. 2000. Seroprevalence of human herpesvirus 8 and its association with Kaposi sarcoma in Brazil. Sex Transm Dis 27:468–472.

Zhang X, Fitzpatrik L, Campbell TB, Campbell TB, Badaro R, Schechter M, Melo MF, Brites C, Sampaio D, Schooley RT. 1998. Comparison to the prevalence of antibodies to human herpesvirus 8 (Kaposi's sarcoma-associated herpesvirus) in Brazil and Colorado. J Infect Dis 178:1488–1491.