Brazilian Journal of Medical and Biological Research (2004) 37: 123-127
ISSN 0100-879X Short Communication

Prevalence of antibodies to human herpesvirus-8 in populations with and without risk for infection in São Paulo State

V.A.U.F. Souza[1], L.M. Sumita[1], W. Freire[1], H.K. Sato[2], J.L. Grandi[3], L.C. Pierrotti[4], M.C. Nascimento[5] and C.S. Pannuti[1]

[1]Laboratório de Virologia, Instituto de Medicina Tropical de São Paulo - LIMHC, and Departamento de Moléstias Infecciosas e Parasitárias, Faculdade de Medicina, Universidade de São Paulo, São Paulo, SP, Brasil
[2]Centro de Vigilância Epidemiológica "Alexandre Vranjac", and
[3]Centro de Referência e Treinamento em AIDS, Secretaria dos Negócios da Saúde do Estado de São Paulo, São Paulo, SP, Brasil
[4]Casa da AIDS, Divisão de Moléstias Infecciosas e Parasitárias, Hospital das Clínicas, Faculdade de Medicina, Universidade de São Paulo, São Paulo, SP, Brasil
[5]Instituto de Infectologia Emílio Ribas, São Paulo, SP, Brasil

Abstract

Human herpesvirus 8 (HHV-8) is a newly described herpesvirus that is etiologically associated with all forms of Kaposi's sarcoma (KS). Seroepidemiological studies have shown high prevalence rates of HHV-8 antibodies among men who have sex with men (MSM) and AIDS patients, African children, Brazilian Amerindians, and elderly individuals in certain regions of Europe. The aim of the present study was to determine the prevalence of HHV-8 antibodies in healthy children and young adults from different cities in São Paulo State, and in a population at high risk for HHV-8 infection: HIV-negative MSM, and AIDS patients with and without KS. Antibodies to HHV-8 latency-associated nuclear antigen and lytic-phase antigens were detected by immunofluorescence assays. In 643 healthy children and young adults from the general population attending a vaccination program for yellow fever in ten different cities in São Paulo State, the prevalence of HHV-8 antibodies detected by the presence of latent or lytic antigens ranged from 1.0 to 4.1% in the different age groups (mean = 2.5%). In the MSM group, the prevalence was 31/95 (32.6%). In the group of patients with AIDS, the prevalence was 39.2% (51/130) for non-KS patients and 98.7% (77/78) for AIDS patients with the diagnosis of KS confirmed by histopathological examination. We conclude that HHV-8 has a restricted circulation among healthy children and young adults in the general population of São Paulo State and a high prevalence among MSM and AIDS patients.

Correspondence
C.S. Pannuti
Laboratório de Virologia
Instituto de Medicina Tropical
Universidade de São Paulo
05403-000 São Paulo, SP
Brasil
Fax: +55-11-3063-2659
E-mail: cpannuti@usp.br

Presented at the I Symposium on Advances in Medical Research, Institute of Medical Investigation Laboratories, HC-FMUSP, São Paulo, SP, Brazil, March 21-22, 2003.

Research supported by CNPq (No. 300317/97-2).

Publication supported by FAPESP.

Received June 17, 2003
Accepted September 9, 2003

Key words
- HHV-8
- Seroprevalence
- AIDS
- Kaposi's sarcoma
- Immunofluorescence assay
- Latency-associated nuclear antigen
- Lytic-phase antigens

Human herpesvirus 8 (HHV-8), also known as Kaposi's sarcoma-associated herpesvirus, is a newly described gamma herpesvirus that is etiologically associated with all types of Kaposi's sarcoma (KS).

Despite important advances in our knowledge about the genetic and molecular aspects of the virus, the various modes of transmission of HHV-8 remain somewhat controversial. Seroepidemiological studies have shown high prevalence rates of HHV-8 antibodies among male homosexuals (1-3), African children (4,5), Brazilian Amerindians (6) and elderly individuals in certain regions of Europe (3,7). However, there is no single route of transmission explaining how the virus can infect such different population groups. In the United States, northern Europe, and Japan studies have indicated that the seroprevalence of this virus is low in the general population (8-10). However, in Mediterranean countries there are areas where relatively high prevalence rates of classic KS and HHV-8 infection have been observed (11). In such areas, HHV-8 infection can occur during childhood and adolescence (12), suggesting that transmission of the virus may occur through some form of nonsexual contact. This is particularly evident in African populations, where high prevalence rates have been observed in children. A prevalence rate of 39.8% was found in 5-10-year-old children in northern Cameroon (5). In Uganda approximately 50% of children before the age of puberty were found to be infected by HHV-8 (4).

Men who have sex with men (MSM), regardless of HIV serostatus, have a higher rate of HHV-8 infection than the general male population. In MSM who are HIV negative and asymptomatic, infection rates have ranged from 16.8 to 22.1% (1,3,13). An epidemiological relationship between male homosexual activities and HHV-8 infection has been demonstrated in studies undertaken in different countries (1-3,8,9,13).

In Brazil, 600,000 people are currently living with AIDS and KS is the most prevalent neoplasm affecting these patients. There are few studies regarding the prevalence of HHV-8 infection. Among blood donors, seroprevalence rates of 4.6 and 7.4% were found in Vitória and São Paulo, respectively (14,15). Among HIV patients without KS, the seroprevalence rates observed in these two studies were 16 and 18.5%, respectively.

The aim of the present study was to evaluate the prevalence of HHV-8 antibodies in individuals without risk for HHV-8 infection (healthy children and young adults from the general population from different cities of São Paulo State) and in individuals at risk for sexually transmitted diseases (healthy HIV-negative MSM and AIDS patients with and without KS).

For assessment of the prevalence of HHV-8 antibodies in the population without risk for HHV-8 infection, sera from 643 healthy children and young adults attending a vaccination program for yellow fever in ten different cities of São Paulo State (Campinas, Jardinópolis, Marília, Ribeirão Preto, Jaboticabal, Piracicaba, São José do Rio Preto, Bauru, Dracena, and Presidente Epitácio) were examined for HHV-8-specific antibodies. Sera from MSM were obtained from volunteers of the Bela Vista Project, an HIV seroincidence cohort study undertaken in the city of São Paulo to evaluate the rate of HIV infection in this population. Sera from HIV patients with and without KS were obtained from AIDS patients attending the AIDS Clinic of the Department of Infectious Diseases of the University of São Paulo School of Medicine (Casa da AIDS) and from the Instituto de Infectologia Emílio Ribas, a tertiary-care state hospital for infectious diseases.

Antibodies to HHV-8 latency-associated nuclear antigen (LANA) and lytic-phase antigens were detected by previously described immunofluorescence assays (IFA) (16). IFA were performed employing the BCBL-1 cell

line. A punctate nuclear staining in untreated BCBL-1 cells was considered positive for antibodies to LANA. The viral lytic cycle was induced by incubating BCBL-1 cells with 20 ng/ml of 12-O-tetradecanoylphorbol-13-acetate (TPA; Sigma, St. Louis, MO, USA) for 96 h. Entire cell fluorescence in about 20% of TPA-treated cells was considered positive for antibodies to the lytic-phase antigens.

In individuals from the general population, seropositivity for HHV-8 antibodies against lytic or latent antigens ranged from 1.0 to 4.1% in different age groups. In the group of patients at risk for sexually transmitted diseases, the prevalence rates for HHV-8 antibodies were strikingly higher, ranging from 32.6% in the MSM group to 39.2% in AIDS patients without KS and 98.7% in AIDS patients with KS (Table 1).

Our data suggest that HHV-8 has a restricted circulation in children and young adults from the general population of São Paulo State and a high prevalence in individuals at risk for sexually transmitted diseases. This pattern of seroprevalence parallels the pattern observed in the United States, northern Europe and Japan.

The interpretation of HHV-8 seroepidemiologic studies has been hampered by the lack of a gold standard certifying the absence of infection. On the other hand, the sensitivity of HHV-8 serologic assays is limited by the fact that only KS patients are true positives (17). It is now recognized that antibody titers are higher in KS patients when compared with non-KS HHV-8-infected persons (18), and higher antibody titers are easier to detect. Therefore, the inclusion of KS patients as reference only in calibration groups to establish the sensitivity of a test may yield good performances even though tests may differ in sensitivity.

However, even in KS patients with a diagnosis confirmed by histopathological examination, the anti-LANA IFA has shown a lower sensitivity when compared with the lytic IFA (16). For this reason, using the less sensitive LANA IFA in non-KS HHV-8-infected individuals may underestimate the seroprevalence of HHV-8 infection in these populations (17).

As expected, the lytic IFA showed a higher sensitivity than the LANA IFA for detection of HHV-8 antibodies in the general population. The uncertain accuracy in asymptomatic HHV-8 infection is probably due to the low HHV-8 antibody titers in this group when compared with patients with KS/HIV. Nevertheless, even when subjects who were

Table 1. Prevalence of HHV-8 antibodies in children and adults from the general population of São Paulo State and in high risk groups for HHV-8 infection.

	N	Lytic IFA	95%CI*	LANA IFA	95%CI	Lytic or LANA +	95%CI
Age group							
6-11months	197	8 (4.1%)	1.9-8.1	0	–	8 (4.1%)	1.9-8.1
12-23 months	177	3 (1.7%)	0.4-5.3	0	–	3 (1.7%)	0.4-5.3
2-10 years	98	1 (1.0%)	0.1-6.4	0	–	1 (1.0%)	0.1-6.4
11-17 years	56	1 (1.8%)	0.1-10.8	0	–	1 (1.8%)	0.1-10.8
18-30 years	115	3 (2.6%)	0.7-8.0	0	–	3 (2.6%)	0.7-8.0
Total	643	16 (2.5%)	1.5-4.1	0	–	16 (2.5%)	1.5-4.1
High risk group							
MSM	95	20 (21.1%)	13.6-30.9	19 (20.0%)	12.8-29.7	31 (32.6%)	23.6-43.1
Non-KS HIV+	130	45 (34.6%)	26.6-43.5	32 (24.6%)	17.7-33.1	51 (39.2%)	30.9-48.2
KS AIDS patients	78	76 (97.4%)	90.2-99.6	59 (75.6%)	64.4-84.3	77 (98.7%)	92.1-99.9

*Fleiss quadratic 95% confidence interval (95%CI) calculated using Epi6 software. IFA = immunofluorescence assay; KS = Kaposi's sarcoma; LANA = latency-associated nuclear antigen; Lytic = lytic-phase antigens; MSM = men who have sex with men.

reactive to either of the two IFA were classified as being infected with HHV-8, the prevalence of HHV-8 infection in children and adults in the present series was low.

In a previous study, a blinded comparison of seven immunofluorescence assays carried out at five experienced laboratories has shown that current HHV-8 antibody tests show a better interassay correlation in KS patients and in non-KS HIV-positive patients compared with healthy blood donors (19). In addition, a recent study has shown that titers to both lytic and latent antibodies increased for many years, usually in parallel, in longitudinally followed homosexual men (20). These findings probably explain the better agreement among lytic and LANA IFA in the group of patients at high risk for HHV-8 infection (MSM and AIDS patients with or without KS).

The prevalence of antibodies against this virus varies widely between different countries or within different geographical regions in the same country (11). Brazil is a large country, with a multiple ethnic and cultural composition. Therefore, the data obtained in the present study, relatively limited both in terms of sample number and geographical distribution, cannot be extrapolated to the Brazilian population at large.

References

1. Martin JN, Ganem DE, Osmond DH, Page-Shafer KA, Macrae D & Kedes DH (1998). Sexual transmission and the natural history of human herpesvirus 8 infection. *New England Journal of Medicine*, 338: 948-954.
2. Blackbourn DJ, Osmond D, Levy JA & Lennette ET (1999). Increased human herpesvirus 8 seroprevalence in young homosexual men who have multiple sex contacts with different partners. *Journal of Infectious Diseases*, 179: 237-239.
3. Perna AM, Bonura F, Vitale F et al. (2000). Antibodies to human herpes virus type 8 (HHV8) in general population and in individuals at risk for sexually transmitted diseases in Western Sicily. *International Journal of Epidemiology*, 29: 175-179.
4. Mayama S, Cuevas LE, Sheldon J, Omar OH, Smith DH, Okong P, Silvel B, Hart CA & Schulz TF (1998). Prevalence and transmission of Kaposi's sarcoma-associated herpesvirus (human herpesvirus 8) in Ugandan children and adolescents. *International Journal of Cancer*, 77: 817-820.
5. Rezza G, Tchangmena OB, Andreoni M et al. (2000). Prevalence and risk factors for human herpesvirus 8 infection in northern Cameroon. *Sexually Transmitted Diseases*, 27: 159-164.
6. Biggar RJ, Whitby D, Marshall V, Linhares AC & Black F (2000). Human herpesvirus 8 in Brazilian Amerindians: a hyperendemic population with a new subtype. *Journal of Infectious Diseases*, 181: 1562-1568.
7. 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). *Epidemiology and Infection*, 125: 671-675.
8. Simpson GR, Schulz TF, Whitby D et al. (1996). Prevalence of Kaposi's sarcoma associated herpesvirus infection measured by antibodies to recombinant capsid protein and latent immunofluorescence antigen. *Lancet*, 348: 1133-1138.
9. Fujii T, Taguchi H, Katano H, Mori S, Nakamura T, Nojiri N, Nakajima K, Tadokoro K, Juji T & Iwamoto A (1999). Seroprevalence of human herpesvirus 8 in human immunodeficiency virus 1-positive and human immunodeficiency virus 1-negative populations in Japan. *Journal of Medical Virology*, 57: 159-162.
10. Juhasz A, Remenyik E, Konya J, Veress G, Begany A, Andirko I, Medgyessy I, Hunyadi J & Gergely L (2001). Prevalence and age distribution of human herpesvirus-8 specific antibodies in Hungarian blood donors. *Journal of Medical Virology*, 64: 526-530.
11. Calabro ML, Sheldon J, Favero A, Simpson GR, Fiore JR, Gomes E, Angarano G, Chieco-Bianchi L & Schulz TF (1998). Seroprevalence of Kaposi's sarcoma-associated herpesvirus/human herpesvirus 8 in several regions of Italy. *Journal of Human Virology*, 1: 207-213.
12. Whitby D, Luppi M, Sabin C, Barozzi P, Di Biase AR, Balli F, Cucci F, Weiss RA, Boshoff C & Torelli G (2000). Detection of antibodies to human herpesvirus 8 in Italian children: evidence for horizontal transmission. *British Journal of Cancer*, 82: 702-704.
13. Dukers NH, Renwick N, Prins M, Geskus RB, Schulz TF, Weverling GJ, Coutinho RA & Goudsmit J (2000). Risk factors for human herpesvirus 8 seropositivity and seroconversion in a cohort of homosexual men. *American Journal of Epidemiology*, 151: 213-224.
14. Zago A, Bourboulia D, Viana MC, Collandre H, Dietze R, Boshoff C & Keller R (2000). Seroprevalence of human herpesvirus 8 and its association with Kaposi sarcoma in Brazil. *Sexually Transmitted Diseases*, 27: 468-472.
15. Caterino-de-Araujo A, Calabrò ML, 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 São Paulo, Brazil. *Journal of Infectious Diseases*, 179: 1591-1592.
16. Pierrotti LC, Masami SL, Santos FW, Caiaffa Filho HH & Souza VAUF (2000). Detection of human herpesvirus 8 DNA and antibodies to latent nuclear and lytic-phase antigens in serial samples from aids patients with Kaposi's sarcoma. *Journal of Clinical Virology*, 16: 247-251.
17. Martin JN, Amad Z, Cossen C, Lam PK, Kedes DH, Page-Shafer KA, Osmond DH & Forghani B (2000). Use of epidemiologically well-defined subjects and existing immunofluorescence assays to cali-

brate a new enzyme immunoassay for human herpesvirus 8 antibodies. *Journal of Clinical Microbiology*, 38: 696-701.
18. Chatlynne LG, Lapps W, Handy M et al. (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.
19. Rabkin CS, Schulz TF, Whitby D, Lennette ET, Magpantay LI, Chatlynne L & Biggar RJ (1998). Interassay correlation of human herpesvirus 8 serologic tests. HHV-8 Interlaboratory Collaborative Group. *Journal of Infectious Diseases*, 178: 304-309.
20. Biggar RJ, Engels EA, Whitby D, Kedes DH & Goedert JJ (2003). Antibody reactivity to latent and lytic antigens to human herpesvirus-8 in longitudinally followed homosexual men. *Journal of Infectious Diseases*, 187: 12-18.