Serologic Evidence of Human Herpesvirus 8 Transmission by Homosexual but Not Heterosexual Sex

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Epidemiologic studies link Kaposi's sarcoma with a sexually transmitted agent. Human herpesvirus 8 (HHV-8) is likely to be that agent, but routes of transmission are poorly described. A seroepidemiologic study was conducted to determine whether HHV-8 is transmitted sexually between heterosexuals. Sera from 2718 patients attending a sexually transmitted disease (STD) clinic were tested for antibodies to HHV-8 and herpes simplex virus type 2 (HSV-2). Information on sex partners in the previous 12 months and past STDs were obtained by questionnaire. Relationships between possible risk factors and HHV-8 infection were assessed by logistic regression. Overall, seroprevalence of HHV-8 was 7.3%. Independent risk factors for HHV-8 in the whole group were homo/bisexuality and birth in Africa and, among homo/bisexual men, a history of syphilis and HSV-2 and human immunodeficiency virus seropositivity. Among heterosexuals there was no evidence for sexual transmission; the only independent risk factor for HHV-8 seropositivity was birth in Africa.

Human herpesvirus 8 (HHV-8), also called Kaposi's sarcoma (KS)–associated herpesvirus, is causally linked to KS, primary effusion lymphoma, and certain types of multicentric Castleman disease (reviewed in [1–3]). Recent serologic studies show that HHV-8 infection is frequent among human immunodeficiency virus (HIV)–positive patients with KS or at risk of developing KS (i.e., predominantly homosexual men and African patients) [4–7]. The prevalence of HHV-8 in healthy adults is high in countries with a relatively high incidence of classic or endemic KS [4, 6–10].

Before the discovery of HHV-8, epidemiologic studies suggested that the etiologic agent for KS was sexually transmitted [11]. Later serologic studies showed a higher HHV-8 seroprevalence among persons visiting sexually transmitted disease (STD) clinics than among US and UK blood donors [5, 7]. Sexual transmission among homosexual men was confirmed by recent seroepidemiologic studies [12], but there has been no equivalent study of a large population of heterosexuals. Results of studies in Africa suggest that HHV-8 is not sexually transmitted, as the prevalence of HHV-8 increases steadily with age [10]. There is also serologic evidence for mother-to-child transmission [13].

We determined the seroprevalence of HHV-8 in 2718 predominantly heterosexual patients attending the STD clinic at St. Thomas' Hospital, which is one of the busiest in the United Kingdom. The relationships between HHV-8 seropositivity and 3 measures of sexual activity were investigated: self-reported number of sex partners over the previous 12 months, history of STDs, and herpes simplex virus type 2 (HSV-2) antibody status.

Methods

Study population. Serum was obtained from 2718 patients ⩾14 years old who attended the STD clinic at St. Thomas' Hospital between July 1995 and June 1996, and who agreed to undergo venepuncture as part of an ongoing HIV seroprevalence survey [14]. All patients had a detailed structured interview regarding sexual activity in the past 12 months, past STDs, and demography.

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Table 1. Demographic details and sexual risk behaviors of patients in study.

	Total (n = 2718)[a]	Homo/bisexual (n = 271)	African heterosexual (n = 232)	Non-African, heterosexual (n = 2206)
Demographics				
Sex				
Male	1490 (55)	271 (100)	136 (59)	1074 (49)
Female	1228 (45)	—	96 (41)	1132 (51)
Age in years, median (range)	28 (15–90)	29 (16–66)	29 (18–88)	28 (15–90)
Country of birth				
Europe	2036 (75)	238 (88)	—	1798 (81)
West Africa	123 (5)	—	120 (52)	—
Other Africa	118 (4)	—	112 (48)	—
Caribbean/West Indies	172 (6)	2 (1)	—	170 (8)
Other	269 (10)	31 (11)	—	238 (11)
Country of birth of mother				
Europe	1441 (53)	221 (82)	5 (2)	1213 (55)
West Africa	192 (7)	1 (1)	116 (50)	73 (3)
Other Africa	131 (5)	2 (1)	104 (45)	20 (1)
Caribbean/West Indies	610 (22)	15 (6)	—	595 (27)
Other	344 (13)	32 (12)	7 (3)	305 (14)
Sexual activity				
No. of partners in last 12 mo. in United Kingdom[b]				
0	133 (5)	17 (6)	18 (8)	98 (4)
1	1122 (42)	58 (21)	121 (53)	943 (43)
2–9	1309 (48)	126 (45)	88 (38)	1095 (50)
⩾10	138 (5)	78 (28)	3 (1)	57 (3)
Any partners in prior 12 mo. from				
Africa	230 (8)	11 (4)	116 (50)	103 (5)
United States	103 (4)	23 (8)	1 (—)	79 (4)
Previous sexually transmitted disease				
Gonorrhea	267 (10)	43 (15)	16 (7)	208 (9)
Syphilis	25 (1)	12 (4)	3 (1)	10 (1)
Virology				
Herpes simplex virus type 2 (HSV-2) seropositive[b]	691 (26)	58 (21)	81 (36)	552 (26)
Human immunodeficiency virus seropositive	39 (1)	15 (5)	13 (6)	11 (1)

NOTE. Data are no. (%) unless otherwise indicated.

[a] Includes 9 African homosexual men who were excluded from subsequent analyses.

[b] Based on reduced numbers because of incomplete information: no. of sex partners in the United Kingdom in prior 12 months (n = 2702) and HSV-2 serostatus (n = 2665).

Laboratory methods. Sera were tested for HHV-8 antibodies by indirect immunofluorescence assay using the BCP-1 cell line [15], which is latently infected with HHV-8, as previously described [4, 7]. All positive sera were confirmed on repeat testing and were titrated from 1 : 200 to 1 : 400,000.

Sera from 2665 patients were tested for antibodies to HSV-2 by a competitive ELISA. This test is a modification of the method described by Slomka et al. [16], with similar performance characteristics. The gG2 antigen was prepared by alkaline glycine extraction of Vero cells infected with the well-characterized HSV-2 isolate 186 and centrifuged at 100,000 *g* onto Maxisorp (Nunc, Roskilde, Denmark) ELISA plates that were blocked with nonfat milk. Twofold dilutions of test sera were reacted with antigen-coated wells for 3 h at 37°C, washed, and incubated for 1 h with mouse monoclonal antibody to HSV gG2 (IgG-gG2). Plates were washed before incubation, with anti-mouse IgG horseradish peroxidase, and bound IgG-gG2 was detected with tetramethylbenzidine. Sera were considered positive for HSV-2 antibody if they gave >70% blocking relative to the positive control serum.

Serum was screened for antibodies to HIV-1 and HIV-2 by use of a bead assay, the IMX assay, or the AXSYM system (all Abbott Laboratories, Abbott Park, IL). All positive sera were confirmed with the Serodia HIV-1 gelatin particle agglutination assay (Mast Diagnostics, Bootle, Merseyside, UK) and the Wellcozyme HIV-1 EIA (Wellcome Diagnostics, Beckenham, UK); a second serum sample was taken for confirmation and to exclude labeling errors.

Statistical methods. Logistic regression methods were used to assess the relationships between HHV-8 seropositivity and demographic and sexual risk factors of interest. The following variables were considered: age (both as a continuous variable and categorized into quartiles), sex, homo/bisexual or heterosexual, country of birth of individual and of mother (Europe, Africa, Caribbean/West Indies, or elsewhere), number of sex partners in past 12 months in the United Kingdom (none, 1 only, 2–9, ⩾10, fitted as a continuous variable), whether any sex partners were from Africa or the United States, reported history of gonorrhea and syphilis, and HSV-2 and HIV status. Factors that were significant in univariate analyses were included in a multivariate logistic regression analysis, to identify which factors, if any, were independently associated with HHV-8 seropositivity. Variables were selected by use of the stepwise selection procedure in PROC LOGISTIC in the Statistical Analysis System software package [17].

Because it was clear that the risk factors for HHV-8 seropositivity in homo/bisexual men may differ from those for heterosexuals from

Table 2. Human herpesvirus 8 (HHV-8) seroprevalence (95% confidence interval [CI]) in different subgroups defined by demographic details and sexual behaviors.

	No. of samples	No. (%) HHV-8 seropositive	95% CI
All patients	2718	198 (7.3)	6.3–8.3
Demographics			
Sex			
Male	1490	131 (8.8)	7.4–10.3
Female	1228	67 (5.5)	4.3–6.9
Risk group			
Homo/bisexual	271	50 (18.5)	13.8–23.1
African heterosexual	232	45 (19.4)	14.3–24.5
Non-African heterosexual	2206	102 (4.6)	3.8–5.6
Age group (years)			
⩽23	633	31 (4.9)	2.6–12.9
24–28	735	49 (6.7)	7.7–12.6
29–34	627	50 (8.0)	6.0–10.4
>34	615	61 (9.9)	5.0–8.7
Not known	108	7 (6.5)	3.4–6.9
Country of birth			
Europe	2036	121 (5.9)	5.0–7.1
West Africa	123	24 (19.5)	12.5–26.5
Other Africa	118	22 (18.6)	11.6–25.7
Caribbean/West Indies	172	10 (5.8)	2.8–10.4
Other	269	21 (7.8)	4.9–11.7
Country of birth of mother			
Europe	1441	89 (6.2)	5.0–7.6
West Africa	192	30 (15.6)	10.5– 20.8
Other Africa	131	25 (19.1)	12.4–25.8
Caribbean/West Indies	610	35 (5.7)	4.0–7.9
Other	344	19 (5.5)	3.4–8.5
Sexual activity			
No. of partners in prior 12 mo. in United Kingdom			
0	133	13 (9.8)	5.3–16.1
1	1122	76 (6.8)	5.4–8.4
2–9	1309	86 (6.6)	5.3–8.1
⩾10	138	21 (15.2)	9.2–21.2
Any partners in prior 12 mo. from			
Africa	230	31 (13.5)	9.1–17.9
United States	103	6 (5.8)	2.2–12.2
Previous sexually transmitted disease			
Gonorrhea	267	28 (10.5)	6.8–14.2
Syphilis	25	8 (32.0)	14.9–53.5
Virology			
Herpes simplex virus type 2 seropositive	691	68 (9.8)	7.7–12.3
Human immunodeficiency virus seropositive	39	12 (30.8)	17.0–47.6

Africa and elsewhere, these analyses were repeated separately for homo/bisexual men, African heterosexuals, and non-African heterosexuals. Owing to difficulties in allocation of some persons to a particular group, 9 homo/bisexual subjects from Africa were excluded from all regression analyses. Comparisons of demographic data and sexual history between the 3 groups were done by use of χ^2 tests. HHV-8 antibody titers in the HHV-8–positive subjects in the 3 groups were compared by Wilcoxon test.

Results

The 2718 patients were predominantly young (median age, 28 years), heterosexual (89.7%), and male (55%) (table 1). Seventy-five percent were from Europe, although almost one-third of these individuals were born to mothers from countries outside Europe. Fifty-three percent reported having ⩾2 sex partners in the United Kingdom during the prior 12 months, and 10% reported a previous episode of gonorrhea. Only 1% of the subjects had evidence of HIV infection; 26% were HSV-2 seropositive (table 1).

The overall seroprevalence of HHV-8 was 7.3% (198 samples; 95% confidence interval [CI], 6.3%–8.3%; table 2). In univariate analyses, the seroprevalence of HHV-8 was significantly lower among females (odds ratio [OR], 0.60) and significantly higher among homo/bisexual men (OR, 3.52), older individuals (OR, 1.02 per year older), those born in Africa (OR, 2.84) or those with an African mother (OR, 3.53), in those with sex partners from Africa (OR, 1.77), in those with a history of gonorrhea or syphilis (ORs of 1.58 and 6.60, respectively), and in those HSV-2 (OR, 1.67) or HIV positive (OR, 5.96) (table 2, table 3). In multivariate analyses, being homo/bisexual and born in Africa were independent markers of HHV-8 seropositivity (OR, 9.41 and 8.48, respectively; table 3).

Homo/bisexual men and African heterosexuals had similar seroprevalence rates (18.5% and 19.4%, respectively), whereas

Table 3. Relationships between demographic and sexual risk factors and human herpesvirus 8 infection.

	Odds ratio	95% Confidence interval	*P*
Univariate analysis			
Demographics			
Sex			
Female	0.60	0.44–0.81	.001
Male	1	—	—
Age (per year)	1.02	1.01–1.04	.004
Sexual orientation			
Homo/bisexual	3.52	2.48–4.99	<.001
Heterosexual	1	—	—
Country of birth			
Europe	0.75	0.46–1.21	.23
Africa	2.84	1.64–4.93	<.001
Caribbean/West Indies	0.73	0.34–1.59	.43
Other	1	—	—
Country of birth of mother			
Europe	1.13	0.68–1.88	.64
Africa	3.53	2.04–6.10	<.001
Caribbean/West Indies	1.04	0.59–1.85	.89
Other	1	—	—
Sexual activity			
No. of partners in prior 12 mo. in United Kingdom	1.12	0.90–1.39	.31
Any partners in prior 12 mo. from			
Africa	1.77	1.02–3.07	.04
United States	0.79	0.34–1.83	.58
Previous sexually transmitted disease			
Gonorrhea	1.58	1.03–2.40	.03
Syphilis	6.60	2.79–15.62	<.001
Virology			
Herpes simplex type 2 seropositive	1.67	1.22–2.28	<.001
Human immunodeficiency seropositive	5.96	2.97–11.96	<.001
Multivariate analysis			
Sexual orientation			
Homo/bisexual	9.41	4.14–21.42	<.001
Heterosexual	1	—	—
Country of birth			
Africa	8.48	4.04–17.80	<.001
Other	1	—	—

non-African heterosexuals had a significantly lower seroprevalence rate (4.6%, $P < .001$; table 2, figure 1). As it appeared that the risk factors might be different in these 3 groups, the analyses were repeated separately for each group. All 3 groups were sexually active, but there were differences in sexual risk behaviors (table 1). Homo/bisexual men were more likely to report more sex partners (>10) in the United Kingdom over the previous 12 months than were persons in the other groups ($P < .001$). Homo/bisexual men were also more likely to report a history of gonorrhea ($P < .001$) or syphilis ($P < .001$) but less likely to be HSV-2 seropositive ($P < .001$) than heterosexuals. Homo/bisexual men and African heterosexuals had similar rates of HIV seropositivity, which was greater than that among heterosexuals from countries outside Africa ($P < .001$). In addition, homo/bisexual men tended to be slightly younger than other subjects in this study ($P < .001$, Wilcoxon test).

Among homo/bisexual men, having a history of either gonorrhea or syphilis (ORs of 2.58 and 5.89, respectively) being HSV-2 (OR, 3.19) or HIV positive (OR, 7.87) were each associated with HHV-8 seropositivity in univariate analyses. In

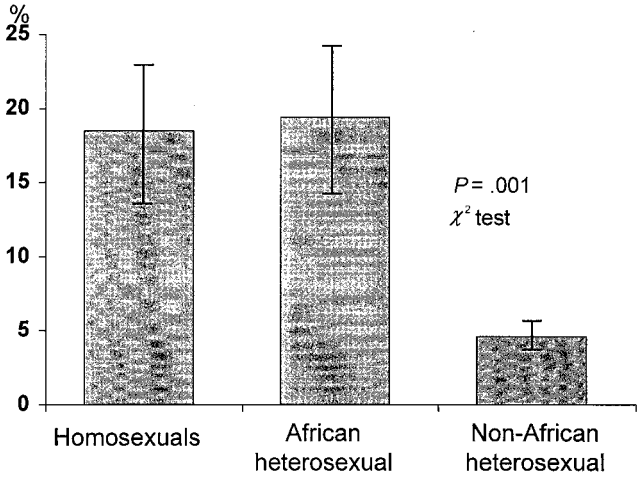

Figure 1. Human herpesvirus 8 seroprevalence (and 95% confidence intervals) for homo/bisexual men, African heterosexuals, and non-African heterosexuals.

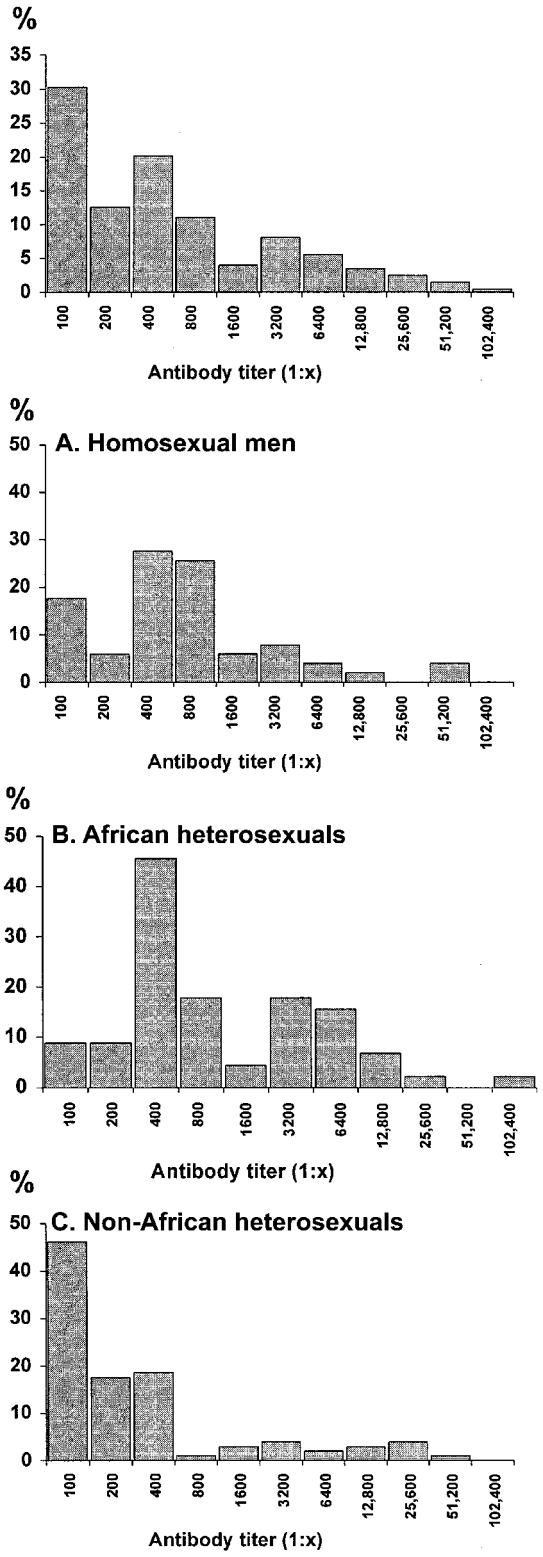

Figure 2. Human herpesvirus 8 (HHV-8) antibody titers for all 198 HHV-8–seropositive subjects (top) and for 3 study groups separately.

a multivariate analysis, a history of syphilis (OR, 3.85), HSV-2 (OR, 2.41), and HIV seropositivity (OR, 5.36) were independently associated with HHV-8 seropositivity (table 4).

In contrast, among heterosexuals from Africa or elsewhere (table 4), there were no sexual risk or demographic factors that predicted HHV-8 seropositivity. The analysis was also performed separately for non-African male and female heterosexuals; although the results were difficult to interpret because of the smaller number of seropositive heterosexuals in each group, there was no evidence for a different epidemiology in males and females.

Antibody titers for the 198 subjects who were HHV-8 seropositive are shown in figure 2. The overall median titer was 400 (range, 100–102,400). Titers were higher in African heterosexuals (median, 800) and homo/bisexual men (median, 400) than in non-African heterosexuals (median, 200; $P = .04$, Wilcoxon test; figure 2).

Discussion

We obtained information on recent and lifetime sexual risks in two ways: subjectively, by asking about numbers of sex partners over the prior 12 months and history of STDs, and objectively, by measuring HSV-2 antibodies [18]. HSV-2 antibodies were higher in those with a history of STDs and with more sex partners, confirming the sexual transmission of HSV-2 in this group.

The HHV-8 seroprevalence in this large study was 7.3%, higher than that reported for UK blood donors (2.7%) in a study using the same assay [7]. The seroprevalence rates were higher among homo/bisexual men (18.5%) and heterosexuals from Africa (19.4%) than among heterosexuals from outside Africa (4.6%; $P = .001$). In the whole group, independent risk factors for HHV-8 seropositivity were being homo/bisexual and born in Africa.

To study these factors in more detail, we separately considered the risk factors for homo/bisexual men and heterosexuals from Africa and elsewhere. Among homo/bisexual men ($n = 380$), we found that HHV-8 seropositivity is associated with sexual activity [12], with higher seroprevalences among those with a history of syphilis and those who are HSV-2 or HIV seropositive. The high seroprevalence of HHV-8 among homo/bisexual men reported here is consistent with that reported in studies using different assays [5, 7, 19, 20]. Our study does not identify the specific sexual activity responsible for transmission. However, among heterosexuals from Africa ($n = 232$) and elsewhere ($n = 2206$), we found little evidence of an association with sexual behavior. The only risk factor for HHV-8 seropositivity was being born in Africa. In both Africa and Italy, the prevalence of HHV-8 increases steadily with age [9, 10, 21], which, together with our data, suggests that nonsexual horizontal transmission occurs.

Sera from KS patients have higher HHV-8 antibody titers

Table 4. Relationships between demographic and sexual risk factors and human herpesvirus 8 infection in homo/bisexual men, African heterosexuals, and non-African heterosexuals.

	Odds ratio	95% Confidence interval	*P*
Homo/bisexual men			
Univariate analysis			
Demographics			
Age (per year)	1.03	(1.00–1.07)	.06
Country of birth			
Outside Europe	1.49	(0.63–3.55)	.36
Europe	1	—	—
Country of birth of mother			
Outside Europe	1.52	(0.73–3.17)	.27
Europe	1	—	—
Sexual activity			
No. of partners in prior 12 mo. in UK	1.10	(0.76–1.58)	.62
Any partners in prior 12 mo. from			
Africa	0.86	(0.16–4.58)	.86
United States	0.68	(0.19–2.39)	.55
Previous sexually transmitted disease			
Gonorrhea	2.58	(1.24–5.35)	.01
Syphilis	5.89	(1.72–20.16)	.005
Virology			
HSV-2 seropositive	3.19	(1.62–6.28)	<.001
HIV seropositive	7.87	(2.66–23.29)	<.001
Multivariate analysis			
Syphilis	3.85	(1.01–14.74)	.05
HSV-2 seropositive	2.41	(1.16–4.98)	.02
HIV seropositive	5.36	(1.66–17.32)	.005
African heterosexuals, univariate analysis			
Demographics			
Sex			
Female	0.83	0.43–1.62	.59
Male	1	—	—
Age (per year)	1.03	0.99–1.06	.12
Country of birth			
West Africa	1.08	0.56–2.08	.81
Other Africa	1	—	—
Country of birth of mother			
West Africa	1.18	0.62–2.27	.62
Other Africa/elsewhere	1	—	—
Sexual activity			
No. of partners in prior 12 mo. in UK	0.93	0.54–1.59	.79
Any partners in last 12 mo. from Africa	1.16	0.12–10.79	.90
Previous sexually transmitted disease			
Gonorrhea	0.26	0.03–2.03	.20
Syphilis	2.10	0.19–23.71	.55
Virology			
HSV-2 seropositive	1.21	0.61–2.39	.59
HIV seropositive	1.26	0.33–4.80	.73
Non-African heterosexuals, univariate analysis			
Demographics			
Sex			
Female	0.91	0.61–1.35	.64
Male	1	—	—
Age (per year)	1.01	0.98–1.03	.55
Country of birth			
Outside Europe	1.30	0.81–2.10	.28
Europe	1	—	—
Country of birth of mother			
Outside Europe	1.23	0.83–1.83	.30
Europe	1	—	—
Sexual activity			
No. of partners in prior 12 mo. in UK	0.95	0.68–1.34	.79
Any partners in last 12 mo. from			
Africa	0.73	0.20–2.71	.64
United States	0.81	0.25–2.61	.72
Previous sexually transmitted disease			
Gonorrhea	1.43	0.78–2.61	.24
Syphilis	2.30	0.29–18.36	.43
Virology			
HSV-2 seropositive	1.44	0.94–2.23	.10

NOTE. UK, United Kingdom; HSV-2, herpes simplex type 2; HIV, human immunodeficiency virus.

than sera from controls [10, 20, 22]. This assocation is similar to those between Epstein-Barr virus and Burkitt's lymphoma in Africa [23] and nasopharyngeal carcinoma in Southeast Asia [24]. Furthermore, high HHV-8 antibody titers in sera from blood donors correlate with HHV-8 prevalence and with KS incidence [8]. We showed that antibody titers are higher in homo/bisexuals and African heterosexuals than in non-African heterosexuals. This is consistent with previous findings that high-titer HHV-8 antibodies are present in groups at higher risk for developing KS [8, 20]. More studies are required to determine whether high antibody titers are due to multiple exposures to the virus, high virus loads, or age at infection.

The subjects in this study represent about one-fifth of all patients seen at our clinic over the same time period. Patients were included in the study if they consented to give a blood sample as part of an ongoing HIV seroprevalence study. Therefore, our sample is likely to underrepresent some demographic groups (e.g., African women) who may have chosen not to give a blood sample. Consequently, our seroprevalence results and the reported relationships between sexual and demographic risk factors and HHV-8 serostatus should be interpreted with this in mind.

Our finding that HHV-8 is associated with sexual activity among homo/bisexuals but not heterosexuals, including those from Africa among whom the prevalence of HHV-8 is high, is both novel and interesting. Other viruses, such as hepatitis B, are transmitted by different routes in different populations. The implication of our findings is that transmission of HHV-8 among heterosexuals is likely to be by nonsexual routes.

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