

Human Herpesvirus 8: Seroprevalence and Correlates in Prostitutes in Mombasa, Kenya

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Human herpesvirus 8 (HHV-8) infection is very prevalent in sub-Saharan Africa, but the role of sexual transmission has not been well characterized. HHV-8 seroprevalence and correlates were evaluated in a cohort of female prostitutes in Mombasa, Kenya. Between February 1993 and January 2000, stored plasma samples taken from 736 women were tested, by whole-virus ELISA assay, for the presence of HHV-8 antibodies; of these 736 women, 633 were included in the analysis of correlates of HHV-8 infection; and, of these 633, 44.1% were seropositive for HHV-8 antibodies. In univariate analysis, age, years of education, years of prostitution, workplace, hormonal contraception, intrauterine-device use, alcohol consumption, syphilis, and gonorrhea were all significantly associated with the presence of HHV-8 antibodies. In a multivariate model, older age, fewer years of education, and 2 markers of high-risk sexual behavior—namely, alcohol consumption and gonorrhea—were each independently associated with HHV-8 seropositivity. These results suggest that heterosexual transmission may contribute to acquisition of HHV-8 infections in this African population of prostitutes.

Human herpesvirus 8 (HHV-8), or Kaposi sarcoma (KS)-associated herpes virus, was first identified in 1994, in lesions in patients with KS [1]. Since then, much has been learned about the virus, but the epidemiology of HHV-8 transmission remains incompletely understood. Transmission appears to occur by both sexual and nonsexual routes, and epidemiologic patterns differ by population and geography [2].

In the United States and Europe, the seroprevalence of HHV-8 in blood donors and other parts of the general population has been 0%–2% [3]. This is in contrast to high seroprevalence in homosexual men, as high as 26% among young gay men in San Francisco [4]. Women at risk for sexually transmitted disease (STD) or human immunodeficiency type 1 (HIV-1) appear to have intermediate rates, with HHV-8 seroprevalence being 11.5% for non-African women attending HIV/STD clinics in London and 18.2% for HIV-1 seropositive women in a large multicenter study in the United States [5, 6]. Few studies in industrialized countries have included children, and HHV-8 infections have been rare [7–9].

A number of studies have provided evidence for sexual transmission of HHV-8 among homosexual men [10–12], although the precise mechanism remains unclear [13]. Epidemiological evidence for heterosexual transmission has been provided by some studies but, overall, has been less strong than that for homosexual transmission [14–16].

Received 18 June 2002; revised 7 October 2002; electronically published 24 January 2003.

Presented in part: 12th International Conference on STD/AIDS in Africa, Ouagadougou, Burkina Faso, 9–13 December 2001 (abstract 10BT4-4).

Informed consent was obtained from all the participating subjects before they were enrolled in the study, and the study was approved by the Ethical Review Committees of the University of Nairobi and the University of Washington.

Financial support: National Institutes of Health (grants CA-86795, DE-14149, D43-TW00007, and CA-15704).

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The Journal of Infectious Diseases 2003;187:359–63

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0022-1899/2003/18703-0003\$15.00

In sub-Saharan Africa, HHV-8 infection is much more prevalent than it is in the industrialized world, both in children and in adults. An age-specific prevalence study in Uganda found high infection rates in young children, which subsequently increased during the prepubertal years, showing that nonsexual horizontal transmission occurred with some frequency [17]. In Cameroon, HHV-8 seroprevalence was 40% among children, 52% in adolescents, and 62% in adults, showing that acquisition of HHV-8 infections continues during adulthood [18]. Whether most adult infections are attributable to sexual or nonsexual routes is unknown.

In 1993, we began a cohort study of female prostitutes in Mombasa, Kenya, to study the epidemiology of HIV-1 infection [19]. This study provided an opportunity to determine HHV-8 seroprevalence and to identify possible demographic and behavioral correlates of HHV-8 infection in a cohort of female prostitutes in sub-Saharan Africa.

SUBJECTS AND METHODS

Study population and procedures. In 1993, a cohort study of female prostitutes was initiated in Mombasa, Kenya, as part of the Preparation of AIDS Vaccine Evaluation/HIV Network for Prevention Trials initiative of the US National Institutes of Health, as described elsewhere [19]. After providing informed consent, women attending a municipal clinic were screened for HIV-1 antibodies. Those who were HIV-1 seronegative were invited to enroll in the prospective cohort study. At enrollment, patients were interviewed with regard to demographic and behavioral variables, and a physical examination was conducted, including screening for STD. The women were asked questions regarding the frequency, in terms of average figures per week during the weeks preceding the study enrollment, of sexual activities and use of alcohol and drugs. A short questionnaire regarding kissing practices was introduced in September 2000, asking whether and at what frequency the women practiced deep kissing with clients and/or steady partners/boyfriends.

Frozen serum samples from the screening visit of HIV-1-seronegative women with ≥ 3 months of follow-up in the cohort study were used to determine HHV-8 seroprevalence. The criterion of ≥ 3 months follow-up was established because the current cross-sectional study was a prelude to a larger study of HHV-8 seroincidence, which would require two time points. Behavioral, demographic, physical-examination, and STD data from the enrollment visit were used to analyze correlates of HHV-8 seropositivity. Data regarding kissing practices were from a follow-up visit. The study was reviewed and approved by the Ethical Review Committees of the University of Washington and the University of Nairobi.

Laboratory procedures. Serum samples were stored at -70°C in Mombasa and subsequently were shipped to Seattle.

Antibodies to HHV-8 were detected by an ELISA based on whole-virus lysate (Advanced Biotechnologies), as described elsewhere [20]. The serologic result was defined as negative if the optical-density value (OD) was <0.2 , positive if >0.35 , and equivocal if $0.2-0.35$ (inclusive). In a previous study of clinically characterized patients [20], these cutoff values had been established as being optimal for indication, in sera, of both lack of infection (OD <0.2) and infection with HHV-8 (OD >0.35); equivocal values were found to be poorly predictive of infection status, and, therefore, patients with such values were not evaluated further in the present study.

Screening for HIV-1 infection was conducted by an ELISA assay (Detect-HIV; Biochem ImmunoSystem). A second confirmatory ELISA (Recombigen; Cambridge Biotech) was performed if a sample tested positive by the screening ELISA. Syphilis, including both past and current cases, was defined as a positive result by a *Treponema pallidum* hemagglutination assay (TPHA; Biotech Laboratories). Diagnosis of *Neisseria gonorrhoeae* was established by culture on modified Thayer-Martin media, *Chlamydia trachomatis* by antigen detection (Microtrak; Syva). Wet mount was used to diagnose *Trichomonas vaginalis* and vaginal candidiasis, and bacterial vaginosis was defined as a Nugent score ≥ 7 by Gram stain [21].

Statistical analyses. Data were entered into SPSS PC software and were analyzed by SPSS for Windows (SPSS). Univariate analysis using either Pearson's χ^2 test, for binary variables, or logistic regression, for multiple categorical variables, was performed to examine possible associations between variables determined at enrollment and the presence of HHV-8 antibodies. Variables with a statistically significant association ($P \leq .05$) in univariate analysis were then entered into a multivariate logistic-regression model. Backward stepwise elimination was used to determine those factors that were independently associated with HHV-8 (the P value for elimination was >0.1). Statcalc (EpiInfo version 6) was used to calculate the χ^2 for trend for HHV-8 seroprevalence, by age group and by years of prostitution.

RESULTS

Enrollment characteristics of the study population. Between February 1993 and January 2000, 4659 prostitutes were screened for HIV-1 antibodies, at Ganjoni Municipal Clinic. Of these women, 53% were HIV-1 seropositive; 1225 HIV-1-seronegative women were enrolled in the open cohort, and, of these, 736 had ≥ 3 months of follow-up.

Stored serum samples from the screening visit of these 736 women were analyzed for HHV-8 antibodies. Of these, 633 (86.0%) had a definitive (i.e., negative or positive) result for HHV-8 antibody and therefore were included in the analysis data set. Enrollment characteristics of these 633 women, in-

cluding demographic and sexual-behavioral information, are summarized in table 1. Sex clients of women who worked in bars were mostly local men, whereas the clients of women in nightclubs included foreigners (e.g., tourists and sailors). Deep kissing was reported by 24% of women who worked in nightclubs and by 5% of those who worked in bars.

HHV-8 seroprevalence and correlates. Of the subgroup of 633 defined above, 279 (44.1%) had HHV-8 antibodies. HHV-8 seroprevalence increased with age, from 37.1% for women 15–24 years old to 47.2% for women 25–34 years old to 57.3% for women ≥ 35 years old (P for linear trend, $\leq .001$). There was a decrease in HHV-8 seroprevalence with increasing level of education. Women who had received only primary-school education (≤ 8 years) had a seroprevalence of 52.4%, compared with 28.2% for women with > 8 years of school education ($P < .001$). The number of years in prostitution was positively associated with seroprevalence of HHV-8, increasing from 34.8% for women with < 1 year in prostitution to 47.2% for those with 1–4 years in prostitution to 55.1% for those with ≥ 5 years in prostitution (P for linear trend, $\leq .001$). There was no association between seroprevalence of HHV-8 and number of sex partners, number of sex acts per week, or condom use. HHV-8 seroprevalence was higher in women who worked in a bar (47.7%) than

in women who worked in nightclubs (34.4%) ($P = .002$). HHV-8 seroprevalence was lower among women using oral contraceptives, depot medroxyprogesterone acetate (DMPA), or an intrauterine device, compared with those who did not use one of these contraceptive methods. The use of alcohol was associated with HHV-8 seropositivity, but this was not the case for miraa, marijuana, or cigarette smoking. Both syphilis at enrollment and a positive culture for *N. gonorrhoeae* at enrollment were significantly associated with HHV-8 seropositivity. No significant association was found between HHV-8 seroprevalence and *T. vaginalis* at enrollment, chlamydia infection at enrollment, bacterial vaginosis at enrollment, or vaginal candidiasis at enrollment. In the subgroup of 136 women who provided information on deep-kissing practices with male clients, deep kissing was inversely related to HHV-8 seroprevalence. This relationship persisted after adjustment for workplace (odds ratio [OR], 0.26; 95% confidence interval [95% CI], 0.07–1.02; $P = .05$).

Variables that were significantly associated with HHV-8 infection in the univariate analysis—that is, age, years of education, workplace, use of oral contraception or DMPA, alcohol consumption, syphilis, and infection with *N. gonorrhoeae*—were entered into a backward stepwise logistic-regression model. The number of years of prostitution was not included in the model

Table 1. Univariate analysis of correlates of human herpesvirus 8 (HHV-8) seropositivity in prostitutes in Mombasa, Kenya.

Characteristic	All patients (<i>n</i> = 633)	HHV-8 seropositive (<i>n</i> = 279)	HHV-8 seronegative (<i>n</i> = 354)	<i>P</i>
Age, median (interquartile range), years	26 (22–31)	27.5 (23–32)	25 (21–30)	.001
Education, median (interquartile range), years	8 (6–10)	7 (5–8)	8 (7–10)	$< .001$
Prostitution, median (interquartile range), years	1 (0.3–4)	2 (0.4–5)	1 (0.25–3)	$< .001$
Sex partners per week, median (interquartile range), no.	1 (1–2)	1 (1–2)	1 (1–2)	$< .7$
Sex acts per week, median (interquartile range), no.	2 (1–3)	2 (1–3)	2 (1–3)	1.0
Condom use at all sex acts, proportion (%)	393/633 (62.1)	174/279 (62.4)	219/354 (61.9)	.9
Workplace bar (vs. nightclub), proportion (%)	413/599 (68.9)	197/261 (75.5)	216/338 (63.9)	.002
Ever practiced deep kissing, proportion (%)	15/136 (11.0)	3/59 (5.1)	12/77 (15.6)	.06
Contraceptive use, proportion (%) ^a				
Oral	108/516 (20.9)	42/238 (17.6)	66/278 (23.7)	.09
DMPA	98/506 (19.4)	35/231 (15.2)	63/275 (22.9)	.03
IUD	19/427 (4.4)	6/202 (3.0)	13/225 (5.8)	.2
Oral or DMPA	206/614 (33.6)	77/273 (28.2)	129/341 (37.8)	.01
Use of alcohol, proportion (%)	496/633 (78)	237/279 (84.9)	259/354 (73.2)	$< .001$
STDs, proportion (%)				
Syphilis	113/601 (19)	63/261 (24.1)	50/340 (14.7)	.004
<i>Neisseria gonorrhoeae</i>	45/629 (7.1)	29/276 (10.5)	16/353 (4.5)	.005
<i>Chlamydia trachomatis</i>	15/599 (3.2)	8/261 (3.1)	11/338 (3.3)	1.0
<i>Trichomonas vaginalis</i>	38/632 (6.0)	15/278 (5.4)	23/354 (6.5)	.6
Vaginal candidiasis	86/632 (13.6)	33/278 (11.9)	53/354 (15.0)	.3
Bacterial vaginosis	237/609 (38.9)	111/268 (41.4)	126/341 (37.0)	.3

NOTE. DMPA, depot medroxyprogesterone acetate; IUD, intrauterine device.

^a Compared HHV-8 seroprevalence in women who used contraceptive pills, injectable progesterone, or an intrauterine device, or one of the two hormonal methods with the seroprevalence in women who did not use them.

because of its colinearity with age. Deep kissing was not included because data were available for only 22% of the patients. In this model, older age (OR, 1.04; 95% CI, 1.01–1.07), ≤ 8 years of education (OR, 2.61; 95% CI, 1.76–3.87), alcohol consumption (OR, 1.74; 95% CI, 1.11–2.71), and infection with *N. gonorrhoeae* (OR, 2.42; 95% CI, 1.19–4.90) were each independently associated with higher HHV-8 seroprevalence (table 2).

DISCUSSION

In this cross-sectional study in Mombasa, we found high (44%) HHV-8 seroprevalence among HIV-1-seronegative prostitutes. In 2 other surveys of prostitutes in Africa, HHV-8 seroprevalence was 52% (in Cameroon) and 8% (in Eritrea) [14, 22]. In a number of countries, HHV-8 seroprevalence in prostitutes has been higher than that in nonprostitute women, suggesting that the former are a high-risk population [14, 23]. In Kenya, HHV-8 seroprevalence in the general population is not available for comparison. However, we found that trucking-company employees in Mombasa had a seroprevalence of 43%, a rate almost identical to that observed among these Mombasa prostitutes [24]. Our study included only HIV-1-seronegative women, and it is possible that HHV-8 seroprevalence might be higher among HIV-1-seropositive women.

The increase in HHV-8 seroprevalence with increasing age suggests that, in our study population, new infections occur during adulthood. This is consistent with what has been found in other age-specific prevalence studies, both in Africa and in the United States [14, 17, 25].

Our risk-factor analysis identified a number of variables that suggest that heterosexual transmission of HHV-8 may occur. In univariate analysis, both syphilis and gonorrhea infection were strongly associated with increased HHV-8 seroprevalence; in multivariate analysis, only gonorrhea infection was. HHV-8 seropositivity also has been associated both with HIV-1 seropositivity [6, 26] and, in studies in both developing and industrialized countries, with STDs such as gonorrhea, syphilis, chlamydia infection, and condyloma [6, 11, 15, 18]. Whether these STDs facilitate HHV-8 transmission or are markers of shared high-risk behavior cannot be determined by a cross-sectional study design. Length of time in the practice of prostitution was highly associated with HHV-8 seropositivity in univariate analysis, but, because of colinearity with age, it was not used in the multivariate model. Alcohol use, which has been associated with increased high-risk sexual behavior [27], was independently associated with HHV-8 seroprevalence in our study population. On the other hand, we found no association between HHV-8 seroprevalence and frequency of sex acts or sex partners and condom use. Prior studies have been inconsistent in their analyses of sexual-behavior variables and HHV-8 seroprevalence in women [6, 15, 28].

In contrast to HHV-8 transmission among male homosex-

Table 2. Multivariate analysis of covariates associated with seroprevalence of human herpesvirus-8 in prostitutes in Mombasa, Kenya.

Characteristic	OR (95% CI)	P
Age, year	1.04 (1.01–1.07) ^a	.02
Education ≤ 8 years	2.61 (1.76–3.87)	<.001
Consumption of alcohol	1.74 (1.11–2.71)	.02
<i>Neisseria gonorrhoeae</i> infection	2.42 (1.19–4.90)	.01

NOTE. CI, confidence interval; OR, odds ratio.

^a Reflects the increased odds of HHV-8 for each year of age.

uals, evidence for heterosexual transmission has been more difficult to establish. The low prevalence of HHV-8 in heterosexual-female study populations in industrialized countries makes it difficult to obtain adequate statistical power to conduct risk-factor analyses. In African countries, the prevalence is very high, as was observed in our population of prostitutes. However, our study cohort included many women who had likely acquired HHV-8 during childhood or presexual adolescence. The inclusion of these women in our analysis of sexual risk factors would have the effect of diminishing the strength of true associations and reducing the power to identify significant correlates. Large prospective cohort studies would be needed to characterize those risk factors that are present at the time of HHV-8 acquisition.

Our study had several limitations. First, the sensitivity and specificity for the presence of HHV-8 antibodies in our ELISA were 91% and 98%, respectively, for undiluted sera, compared with an indirect immunofluorescence assay for lytic antibody (authors' unpublished data). The same test, when exhaustively validated in patients with PCR-documented infections and in a large group of low-risk adults, had a sensitivity of 72% and a specificity of 98% for detection of HHV-8 infection [20]. An additional 14% could not be included in the analysis because the ELISA results fell into the equivocal range, in which definitive antibody status is not obtained. Second, our questions regarding sexual activity and condom use pertained to recent sexual behavior, rather than to activities that might have been operative at the time of HHV-8 acquisition. Third, data on kissing practices were available only for a subset of the women, since these questions were added at a later date. Among gay men, deep kissing has been found to be significantly associated with HHV-8 infection [29]. In our study cohort, we found an inverse association. Deep kissing was more commonly reported by nightclub workers, whose HHV-8 exposure risk may have been low because more of their sex clients are foreign born; however, in a model that adjusted for workplace, deep kissing was still inversely associated with HHV-8 seropositivity. We hope that results from our prospective study will shed light on this relationship.

In this cohort of female sex workers in Mombasa, Kenya, we found (1) a 44.1% seroprevalence of HHV-8, (2) evidence that

ongoing infections occur during the adult years, and (3) some risk factors consistent with heterosexual transmission of HHV-8. However, other factors that would be suggestive of heterosexual transmission were not associated with HHV-8. The contribution of heterosexual transmission to HHV-8 infection in this population cannot be definitively addressed in a cross-sectional study. Future data from our prospective cohort study will allow us to establish HHV-8 seroincidence and risk factors, including sexual behavior, for incident infection. In light of both the high (53%) HIV-1 seroprevalence in Mombasa prostitutes and the attendant risk of Kaposi sarcoma in dually infected women, it is important to determine risk factors for HHV-8, so that appropriate preventive interventions can be introduced.

Acknowledgments

The authors wish to thank the research physicians (Drs. W. Hassan, M. Taylor, H. L. Martin, and G. Ogwen) and staff (A. Abdalla, Z. Basheikh, J. Gow, F. Kashonga, G. Maina, C. Mpoya, E. Mutunga, K. Mwinyikai, J. Saha, and H. Saleh) in Mombasa, for their hard work; the Public Health Department and the Ganjoni Municipal Clinic administration (Dr. M. Lugogo, Dr. P. Musya, P. Njogu, and A. Mungalu), for their cooperation; the administration of Coast Provincial General Hospital, for provision of laboratory space; and Heather Taylor at Children's Hospital Medical Center, in Seattle, Washington, for her help with HHV-8 ELISA testing. Special thanks go to the women who have participated in this study.

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