

MUCOSAL SHEDDING OF HUMAN HERPESVIRUS 8 IN MEN

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ABSTRACT

Background Epidemiologic studies suggest that human herpesvirus 8 (HHV-8) is sexually transmitted among men who have sex with men; however, the mode of transmission is unclear.

Methods To evaluate the patterns of shedding of HHV-8, we obtained mucosal-secretion samples from a cohort of HHV-8-seropositive men who had sex with men and had no clinical evidence of Kaposi's sarcoma. Quantitative polymerase-chain-reaction (PCR) assays, in situ PCR assays, and in situ RNA hybridization were used to identify potential sources of infectious HHV-8.

Results We detected HHV-8 in at least one mucosal sample from 30 of 50 men who were seropositive for HHV-8 (60 percent). Overall, HHV-8 was detected in 30 percent of oropharyngeal samples, as compared with 1 percent of anal and genital samples ($P < 0.001$). In 39 percent of the HHV-8-seropositive men, HHV-8 was detected in saliva on more than 35 percent of the consecutive days on which samples were obtained. The median log titer of HHV-8 from the oral cavity was approximately 2.5 times as high as the titer at all other sites. In situ hybridization studies indicated that HHV-8 DNA and messenger RNA were present in oral epithelial cells. Among 92 men who had sex with men and who were seronegative for the human immunodeficiency virus (HIV), a history of sex with a partner who had Kaposi's sarcoma, deep kissing with an HIV-positive partner, and the use of amyl nitrite capsules ("poppers") or inhaled nitrites were independent risk factors for infection with HHV-8.

Conclusions Oral exposure to infectious saliva is a potential risk factor for the acquisition of HHV-8 among men who have sex with men. Hence, currently recommended safer sex practices may not protect against HHV-8 infection. (N Engl J Med 2000;343:1369-77.)

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AMONG men who have sex with men, Kaposi's sarcoma is the most common cancer associated with infection with the human immunodeficiency virus (HIV).^{1,2} Epidemiologic evidence and molecular evidence increasingly suggest that human herpesvirus 8 (HHV-8) is the cause of Kaposi's sarcoma.³⁻⁵ Although HHV-8 is uncommon in the general population in North America, among men who have sex with men, 11 to 20 percent of those who are seronegative for HIV and 30 to 54 percent of those who are seropositive for HIV have detectable antibodies against HHV-8.⁶⁻⁹

The seroprevalence of HHV-8 is highest among men in this group who report a large number of sexual partners or a history of sexually transmitted diseases, findings that support the idea that HHV-8 is sexually transmitted in this population.^{5,10} In contrast, the seroprevalence of HHV-8 in certain regions of Africa and Italy ranges from 15 to 58 percent. In these regions, epidemiologic data support the existence of both sexual and nonsexual modes of transmission, since HHV-8 infection is found in both men and women and appears often to be acquired during childhood.¹¹⁻¹⁶

Since HHV-8 cannot yet be isolated from mucosal cultures, the detection of HHV-8 DNA by the polymerase-chain-reaction (PCR) assay has been the primary method used to identify the virus in mucosa and other locations.^{3,4} PCR-based studies have shown that HHV-8 is uncommon in semen, especially when stringent methods are used to eliminate contamination of samples in the laboratory.¹⁷⁻¹⁹ In previous studies of persons with Kaposi's sarcoma, we found that HHV-8 was more common in saliva than in genital secretions and that the titers in saliva were high.^{17,20,21} Therefore, we conducted a study to determine the source of HHV-8 in the body and the patterns of viral shedding among men who had sex with men but who did not have Kaposi's sarcoma.

METHODS**Study Population**

Between 1994 and 1998, two cohorts of men who have sex with men were recruited for studies of the patterns of shedding of herpesviruses at the University of Washington Virology Research Clinic. All protocols were approved by the institutional review board of the University of Washington. After providing written informed consent, the men underwent a physical examination and answered a questionnaire that included detailed questions concerning the frequency and type of sexual practices with partners, illicit drug use, and medical history, including sexually transmitted diseases. Serum was tested for antibodies against HIV, HHV-8, and other human herpesviruses, and the men were enrolled in protocols that evaluated patterns of viral shedding from mucosal sites.

One cohort collected daily samples at home by rubbing sepa-

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rate swabs over the buccal and lingual surfaces, anal mucosa, and urethra.²² The second cohort, enrolled in 1998 specifically for the study of HHV-8, provided samples in the clinic at four visits at intervals of one week. At each clinic visit, whole saliva was collected in a sterile cup. In addition, we collected pharyngeal, nasal, urethral, and anal swabs; semen; urine; peripheral-blood mononuclear cells; plasma; and prostatic secretions. Prostatic secretions were obtained in the morning by prostatic massage after the collection of the first urine voided that day. All pharyngeal, nasal, urethral, and anal specimens were obtained with Dacron swabs and then placed in PCR buffer. Mononuclear cells were separated by density-gradient centrifugation and assayed as described previously.²⁰

To determine the site of HHV-8 replication in oral mucosal cells, we collected additional samples from men in whom HHV-8 DNA was detected in saliva. We obtained superficial buccal and lingual epithelial cells by rubbing the mucosal surface with a plastic spatula and suspending the cells in buffered saline. We collected free cells in the oral cavity by having the men swish, gargle, and spit out a small amount of saline into a sterile cup. These samples then were split and sent to separate laboratories. The samples were centrifuged, and the resulting cell pellets (which contained essentially only epithelial cells) were prepared for solution-phase PCR assays and for *in situ* studies.

Serologic Analysis

In serologic tests for HHV-8, we used an immunofluorescence assay that is based on the HHV-8–positive BCBL-1 lymphoma-cell line. Seropositivity was defined as the presence of both latent and lytic antibodies or of lytic antibodies alone at a serum dilution of at least 1:40.²³ All serum was reacted against a BJAB cell line to rule out nonspecific reactivity.

Measurement of HHV-8 DNA

The method of detecting HHV-8 DNA by PCR assay has been described previously, and independent proficiency tests showed that the method was specific, reproducible, and sensitive.^{17,18,20,24} We used two solution-based PCR methods, in which the PCR primers were derived from the KS330 Bam₁233 fragment of an open-reading-frame protein (ORF 26), to detect HHV-8 DNA.³ The semi-quantitative liquid hybridization method was capable of detecting as few as 1 to 3 copies of HHV-8 DNA in as much as 5 μ g of cellular DNA.^{17,18,20} We also used a fluorescent-probe–based PCR assay (TaqMan assay, Applied Biosystems, Foster City, Calif.) to quantitate HHV-8 DNA.²⁵⁻²⁷

The methods of specimen collection and DNA extraction were similar for both assays. Swab samples were collected in 1 ml of digestion buffer. DNA was extracted from 400 μ l of the swab buffer, a sample containing 3 million peripheral-blood mononuclear cells, or 400 μ l of saliva, semen, prostatic secretions, or plasma. The total DNA in each sample was extracted with Qiagen columns (Qiagen, Santa Clarita, Calif.), and one fifth of the DNA extracted was used for each PCR assay. β -Globulin primers were used for DNA standardization to quantify the amount of human genomic material in each sample.²⁸ To identify false positive reactions, specimens were processed in parallel with control samples.

The primers (KS-1 and KS-2), probes, and conditions used in the quantitative fluorescent-probe PCR assay were similar to those described previously.^{3,25,26*} The KS-NP probe was labeled at the 5' end with 6-carboxyfluorescein and at the 3' end with 6-carboxytetramethylrhodamine (Synthegen, Houston). To ensure that negative results were not due to nonspecific inhibition of the assay, each PCR was spiked with 50 copies of jellyfish gene DNA (EXO) as an internal control, 30 nM of primers (EXO186F and EXO314R), and 50 nM of probe (PiMP-242T). All of the negative PCR results for the KS-1 and KS-2 primers required the detection of EXO DNA to be considered valid.

*See NAPS document no. 05571 for 1 page of supplementary material. To order, contact NAPS, c/o Microfiche Publications, 248 Hempstead Tpke., West Hempstead, NY 11552.

PCR *In Situ* Hybridization

Cell scrapings from the oral cavity were centrifuged onto glass microscope slides, fixed, treated with Permeafix (Ortho Diagnostics, Raritan, N.J.), and subjected to PCR *in situ* hybridization as previously described.^{24,29} The PCR product was detected by *in situ* hybridization with a cocktail of three digoxigenin (DIG)–labeled oligonucleotides, all of which were in the sense orientation and complementary to DNA encoding the minor capsid protein of HHV-8. The cells were also stained with monoclonal antibodies against high-molecular-weight cytokeratin (clone 35 β H11, Dako, Carpinteria, Calif.) and low-molecular-weight cytokeratin (clone 34 β E12, Dako) to determine their epithelial origin. The presence of viral nucleic acid was indicated by a cell-associated purple precipitate on light microscopy.

In Situ Hybridization

For the detection of HHV-8 RNA, we used DIG-labeled riboprobes encoding the HHV-8 T0.7 messenger RNA (155 bp) and T1.1 messenger RNA (211 bp).^{24,29-32} The riboprobes (sense and antisense) were applied to cytospin preparations of oral epithelium. RNA hybrids were detected with the use of alkaline phosphatase–conjugated antibodies against DIG and nitroblue tetrazolium substrate, as described previously.^{29,33-37} The presence of viral nucleic acid was indicated by a cell-associated purple precipitate. The T0.7 and T1.1 riboprobes detect as few as 20 copies of virus per cell.^{24,29}

Statistical Analysis

We used the Wilcoxon rank-sum test to compare differences in continuous variables and a chi-square test to compare differences in categorical variables. Odds ratios were calculated with 95 percent confidence intervals. We performed a multivariate stepwise logistic-regression analysis to identify independent predictors of HHV-8 seropositivity in a model that included variables that were significant in a univariate analysis ($P < 0.05$) or of biologic interest.

RESULTS

Sites of HHV-8 Shedding

To identify the sites of HHV-8 infection among seropositive men, we used the samples obtained from a cohort of 112 men who had sex with men and who were enrolled from 1998 to 1999. Among these 112 men, 20 (18 percent) were seropositive for HIV and 39 (35 percent) were seropositive for HHV-8. Of the 39 HHV-8–seropositive men, 27 (69 percent) agreed to participate in the HHV-8 study, 16 of whom were seronegative for HIV and 11 of whom were seropositive. We collected 880 samples from these 27 men, of which 49 samples (6 percent) had detectable HHV-8 DNA (Table 1). HHV-8 DNA was detected in at least one sample from 15 of the 27 men (56 percent). HHV-8 DNA was detected most frequently in samples from the oral cavity (i.e., saliva, pharyngeal swabs, or both), followed by peripheral-blood mononuclear cells, semen samples, prostatic secretions, and anal swabs (Table 1). Overall, HHV-8 was detected in oral-cavity secretions in seven of the HHV-8–seropositive men and in semen, prostatic secretions, or urethral swabs in eight of these men. Two of these men had HHV-8 in both oral and genital sites.

Oral-cavity secretions had both the highest overall frequency of HHV-8 DNA and the highest number of copies of HHV-8 DNA; 12 of 103 saliva samples

TABLE 1. RATE OF DETECTION OF HUMAN HERPESVIRUS 8 (HHV-8) DNA BY A QUANTITATIVE PCR ASSAY AMONG 27 HHV-8-SEROPOSITIVE MEN WHO HAD SEX WITH MEN, ACCORDING TO THEIR HIV STATUS.*

TYPE OF SAMPLE	HIV-SERONEGATIVE MEN (N=16)		HIV-SEROPOSITIVE MEN (N=11)	
	STRONGLY POSITIVE	TRACE POSITIVE	STRONGLY POSITIVE	TRACE POSITIVE
	no. positive/total no. (%)			
Saliva	5/61 (8)	1/61 (2)	5/42 (12)	1/42 (2)
Pharyngeal swab	4/60 (7)	0/60	4/41 (10)	3/41 (7)
Nasal swab	0/62	1/62 (2)	0/41	1/41 (2)
Peripheral-blood mononuclear cell	2/58 (3)	1/58 (2)	3/40 (8)	4/40 (10)
Plasma	0/55	0/55	1/41 (2)	1/41 (2)
Semen	1/53 (2)	2/53 (4)	1/38 (3)	1/38 (3)
Prostatic secretions	0/58	2/58 (3)	0/33	1/33 (3)
Urethral swab	1/61 (2)	0/61	0/40	1/40 (2)
Anal swab	0/61	2/61 (3)	0/35	0/35

*Samples were collected during four consecutive weekly visits. Trace positive was defined as a finding of 1 to 10 copies of HHV-8 DNA per milliliter, and strongly positive as a finding of more than 10 copies per milliliter.

(12 percent) and 11 of 101 pharyngeal swabs (11 percent) were positive for HHV-8 DNA, as compared with 10 of 283 genital tract samples (4 percent) and 2 of 96 anal swabs (2 percent) ($P < 0.01$ for each comparison). Five of 91 semen samples (5 percent) were positive. In addition, the geometric mean titer of virus detected in saliva samples (4.3 log copies of HHV-8 DNA per milliliter) and pharyngeal swabs (3.1 log copies per milliliter) was higher than the titer at all other sites (overall, 1.6 log copies per milliliter) (Fig. 1). These relative differences remained the same when HHV-8 DNA was quantified according to the log number of copies of viral DNA per copy of hemoglobin gene. The amount of HHV-8 DNA in specimens was not correlated with the number of copies of the hemoglobin gene, indicating that the method of sample collection did not influence the differences in titer.

Pattern of HHV-8 Shedding

To evaluate the pattern of reactivation of HHV-8 in the oral cavity, we assayed the samples from the cohort of 67 men who had sex with men and were enrolled in earlier studies, from whom samples were collected daily from the buccal and lingual surfaces, urethra, and anal mucosa. The seroprevalence of HHV-8 in this cohort (34 percent [23 of 67 men]) was similar to the seroprevalence (35 percent) in the cohort enrolled four years later. We assayed 1134 oropharyngeal swabs that had been obtained daily from

these 23 HHV-8-seropositive men for a mean of 49.3 days (median, 47; range, 15 to 75); 14 of these men were seropositive for HIV and 9 were seronegative for HIV.

As was the case for the samples from the other cohort, HHV-8 DNA was found most frequently in the oropharynx among the 23 HHV-8-seropositive men who collected daily samples. HHV-8 DNA was detected in at least one oropharyngeal sample from 13 of the 23 men (57 percent), whereas 2 men had viral shedding only in the anal region. Overall, HHV-8 DNA was detected in 34 percent of oropharyngeal samples (382 of 1134), 0.4 percent of urethral samples (3 of 848), and 1 percent of anal samples (14 of 1087) ($P < 0.001$ for the comparison of oral samples with urethral samples and with anal samples). HHV-8 DNA was detected in oropharyngeal samples on more than 5 percent of days in the case of 6 of 14 HIV-seropositive men and in the case of 3 of 9 HIV-seronegative subjects (Fig. 2); the geometric mean titer was 4.0 log copies per positive swab.

The pattern of detection of HHV-8 in the oral cavity among the HHV-8-seropositive men was dichotomous; the virus either was undetectable or was detectable on a high frequency of days (Fig. 2). Of the 13 men with any positive samples, 9 had detectable HHV-8 in saliva on more than 35 percent of days on which samples were obtained. Figure 3 shows representative patterns of shedding in two HIV-seronegative men and one HIV-seropositive man.

To confirm the specificity of the serologic assay and PCR assay, we also tested oropharyngeal samples obtained on 10 consecutive days from 10 HHV-8-seronegative men in this cohort (titer on immunofluorescence assay, $< 1:20$). In none of the 100 samples was HHV-8 DNA detected by PCR assay.

Location of HHV-8 DNA in Oral Epithelial Cells

We performed in situ hybridization studies among HIV-positive and HIV-negative men who had shedding of HHV-8 DNA in the oral cavity according to both quantitative and qualitative PCR assays. An in situ PCR assay and in situ hybridization for HHV-8 were performed on cytospin preparations of oropharyngeal-lavage fluid and scrapings of the buccal and lingual epithelial surfaces (Fig. 4). PCR in situ hybridization demonstrated HHV-8 DNA in epithelial cells from all three types of samples on the basis of both cytokeratin staining and morphologic analysis. The highest concentration of HHV-8-positive cells was in samples of buccal mucosa. In situ hybridization identified transcripts associated with viral latency (T0.7 RNA) in epithelial cells from all sites and transcripts associated with lytic replication, as evidenced by the expression of a nuclear transcript (T1.1 RNA) in buccal epithelial cells. Similar patterns were seen in all samples with a high titer of HHV-8 DNA that were evaluated.

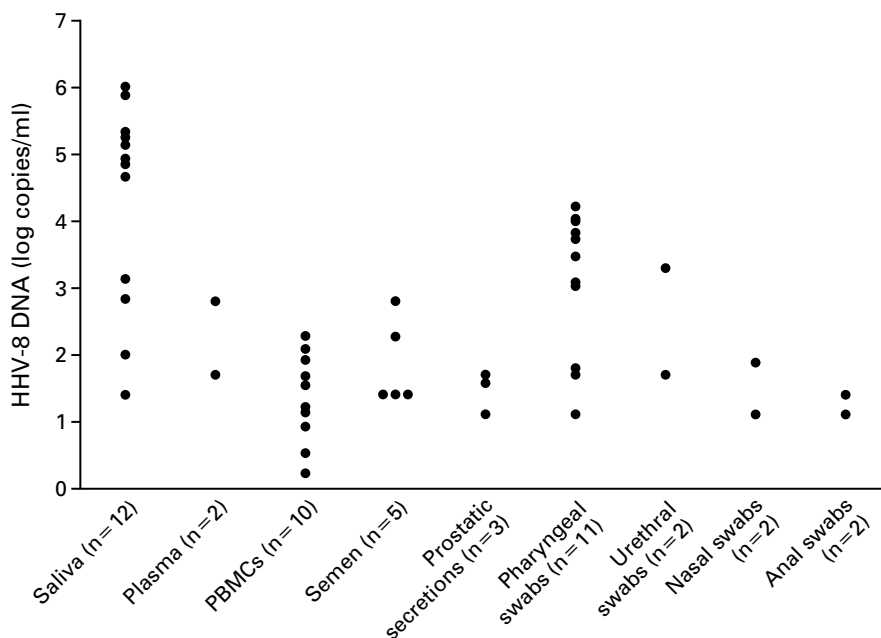


Figure 1. Quantity of Human Herpesvirus 8 (HHV-8) DNA in the Various Samples. Values are expressed as the log number of copies of HHV-8 DNA per milliliter of sample or per milliliter of PCR digestion buffer into which each specimen swab was placed. PBMCs denotes peripheral-blood mononuclear cells. The numbers of positive samples are given in parentheses.

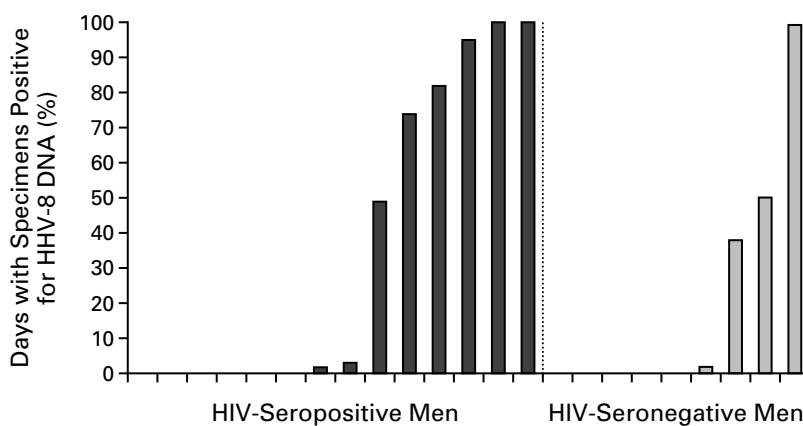


Figure 2. The Frequency of Detection of Human Herpesvirus 8 (HHV-8) DNA in Swabs of Buccal and Lingual Mucosa Obtained Daily from 23 HHV-8-Seropositive Men Who Had Sex with Men. Fourteen of the men were seropositive for HIV, and nine were seronegative for HIV. The median duration of collection was 47 days (range, 15 to 75).

Risk Factors for HHV-8 Infection

In an analysis of risk factors for HHV-8 infection, we included only the HIV-seronegative men, since HIV-related immunosuppression may confound risk factors for the acquisition of HHV-8. Thus, our study group consisted of 92 HIV-seronegative men in the

cohort enrolled in 1998 and 1999, 66 of whom were seronegative for HHV-8 and 26 of whom were seropositive. On univariate analysis, seropositivity for HHV-8 was significantly associated ($P < 0.05$) with older age, a greater number of years of sexual activity with men, a higher number of male sexual partners,

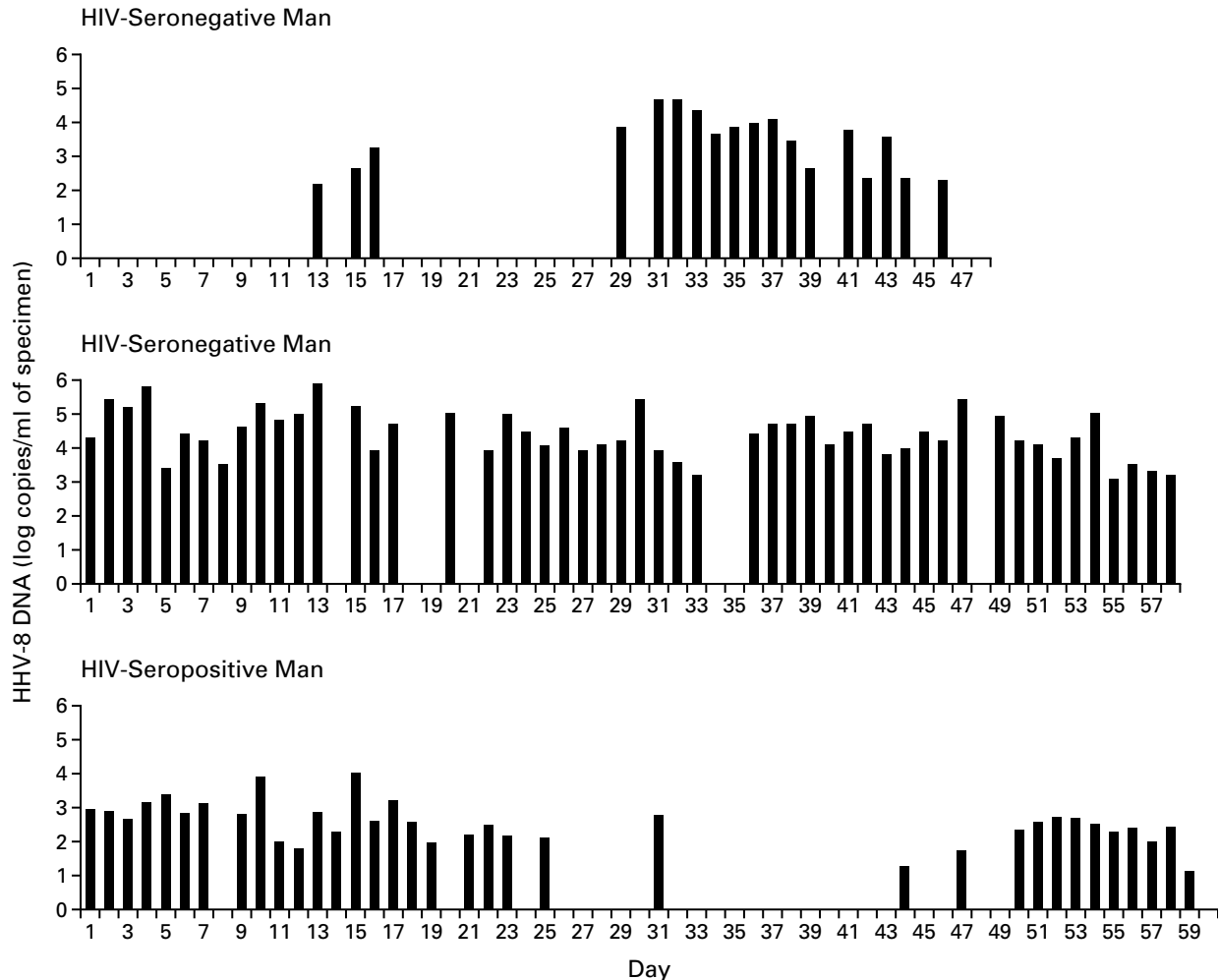


Figure 3. Patterns of Shedding of Human Herpesvirus 8 (HHV-8) DNA in Oropharyngeal Swabs from Three HHV-8–Seropositive Men, According to Their HIV Status.

The man who was seropositive for HIV had a CD4 cell count of 207 per cubic millimeter and 77,000 copies of HIV RNA per milliliter of plasma at study entry.

a history of sexually transmitted diseases (hepatitis B, gonorrhea, genital or anal warts, genital or anal herpes), and the presence of herpes simplex virus type 2 antibody. Having a larger number of HIV-seropositive partners and having had a partner with Kaposi's sarcoma were also strongly correlated with seropositivity for HHV-8 ($P < 0.001$). Among specific sexual behaviors with HIV-positive partners, only deep kissing was significantly associated with seropositivity for HHV-8 (odds ratio, 7.0; 95 percent confidence interval, 2.2 to 23.0). The reported frequency of illicit-drug use in association with sex was correlated with HHV-8 serostatus for a number of drugs, but the association was especially marked in the case of amyl nitrite capsules ("poppers") and inhaled nitrites: 77 percent of HHV-8–seropositive men had used these drugs, as

compared with 32 percent of HHV-8–seronegative men (odds ratio, 3.7; 95 percent confidence interval, 1.5 to 17.7).

On multivariate analysis, three factors were independently predictive of seropositivity for HHV-8: a history of deep kissing with an HIV-positive partner (odds ratio, 5.4; 95 percent confidence interval, 1.3 to 22.7), a history of having a partner with Kaposi's sarcoma (odds ratio, 4.8; 95 percent confidence interval, 1.5 to 17.7), and the use of poppers or inhaled nitrites in association with sex (odds ratio, 5.1; 95 percent confidence interval, 1.5 to 17.7).

DISCUSSION

We studied a cohort of men who had sex with men, who were seropositive for HHV-8, but who had no

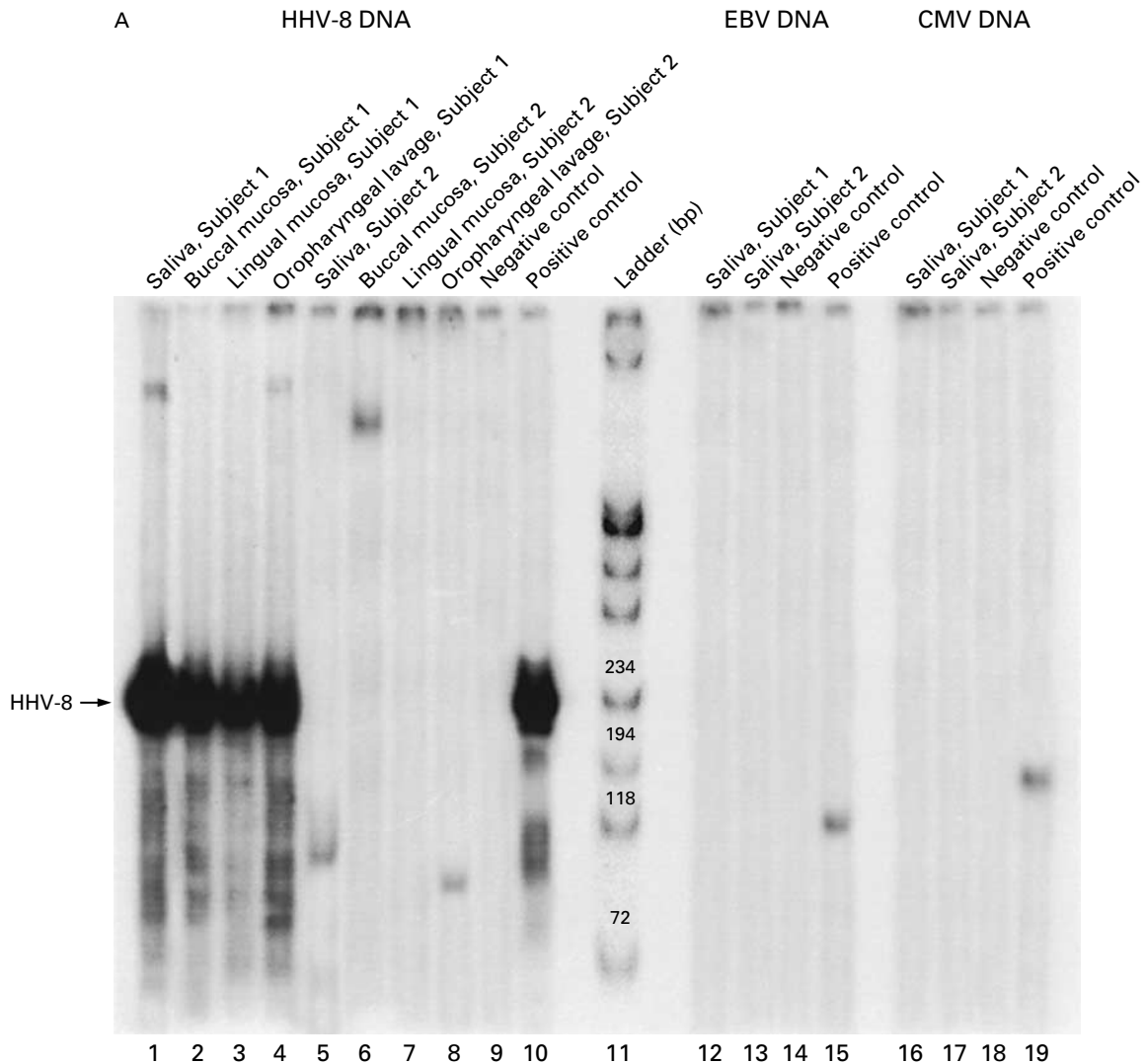
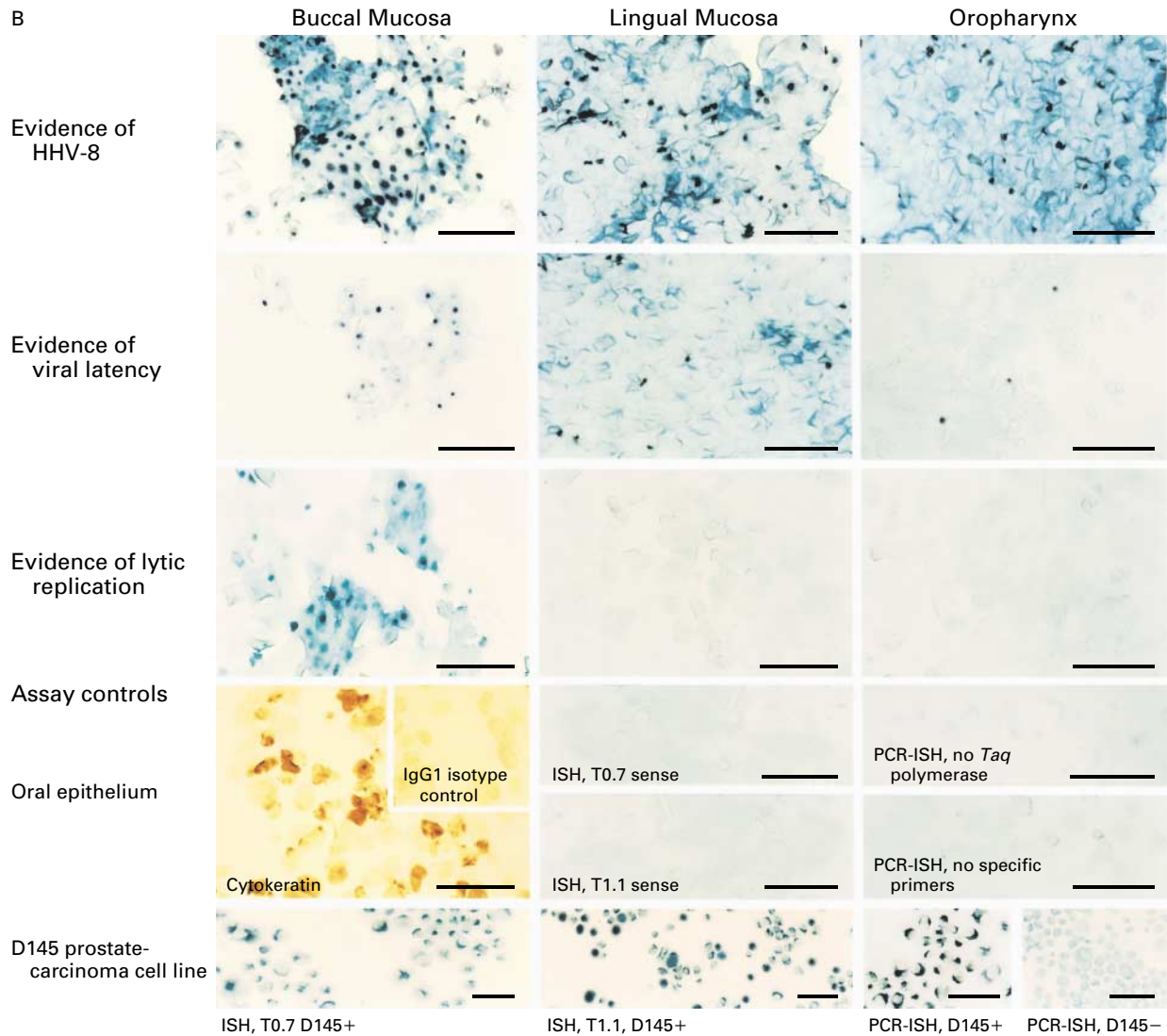


Figure 4. Detection and Location of Human Herpesvirus 8 (HHV-8) DNA in Oropharyngeal-Lavage Fluid and Scrapings of Buccal and Lingual Epithelial Surfaces.

In Panel A solution-based PCR with liquid hybridization demonstrated HHV-8 DNA (ORF 26) in saliva, buccal-mucosa scrapings, lingual-mucosa scrapings, and cells obtained from oropharyngeal-lavage fluid from an HHV-8-seropositive man who was seronegative for HIV (Subject 1) but not from an HHV-8-seronegative man who was also seronegative for HIV (Subject 2). Neither man had evidence of Epstein-Barr virus (EBV) DNA or cytomegalovirus (CMV) DNA in saliva. Subject 1 had 10,000 copies of HHV-8 DNA per milliliter of saliva sample on a fluorescent-probe PCR assay. The positive control was a D145 prostate-carcinoma epithelial cell line infected in vitro with HHV-8.³⁸ The negative control was an uninoculated D145 cell line.

Panel B (facing page) shows the epithelial cells from Subject 1 in which HHV-8 DNA (ORF 26) and RNA were identified. PCR in situ hybridization (PCR-ISH) showed that HHV-8 DNA was present in buccal and lingual epithelial cells as well as epithelial cells from oropharyngeal-lavage fluid. Levels of expression of HHV-8 RNA were evaluated with in situ hybridization (ISH). Transcripts associated with viral latency (T0.7 RNA) were observed within epithelial cells from all sites. Cells harboring HHV-8 RNA were observed less frequently than those containing HHV-8 DNA. Evidence of HHV-8 lytic replication, as indicated by the expression of nuclear transcript (T1.1 RNA), was rare and was found only in buccal mucosa. All cells that were positive for HHV-8 DNA, RNA, or both were also positive for low-molecular-weight cytokeratin, a marker specific for epithelium. Assay controls for the PCR component of the PCR-in situ hybridization procedure consisted of specimens in which *Taq* polymerase or sequence-specific primers were absent. Controls for in situ hybridization included digoxigenin (DIG)-labeled oligoprobes specific for the visna-maedi virus *gag* gene (nonsense probe), DIG-labeled complementary (sense strand) RNA T0.7 and T1.1, and irrelevant isotype-specific alkaline phosphatase-conjugated monoclonal antibody (irrelevant IgG₁) against the visna-maedi virus *gag* p27 antigen.³⁹ Additional controls consisted of cytospin preparations of HHV-8-infected D145 prostate-carcinoma cell line (D145+),³⁸ an uninfected D145 prostate-carcinoma cell line (D145-),³⁸ cytospin preparations of an HHV-8-infected BCBL-1 cell line (not shown),^{24,29} and oral epithelium from HHV-8-negative men (not shown). These controls have been described in detail in previous studies.^{29,33-37} In each sample, the bar indicates 100 μ m.



clinical evidence of Kaposi's sarcoma in order to determine the source of mucosal HHV-8 infection — the genital tract or the oropharynx. We could detect shedding of HHV-8 in mucosal sites in 60 percent of the 50 men who had sex with men whom we studied extensively. HHV-8 was detected significantly more often in oropharyngeal samples than in genital tract samples. Moreover, we found that HHV-8 DNA was present in higher titers in samples of saliva than in other samples. Some men consistently shed HHV-8 at high titers (>10,000 copies per milliliter of sample) from the oral cavity for extended periods but had no evidence of HHV-8 at other sites. This group of men included both HIV-seropositive men and healthy, HIV-seronegative men. In situ PCR and hybridization techniques showed that HHV-8 was present in epithelial cells in the oral cavity.

There are a number of puzzling epidemiologic aspects of HHV-8 infection. HHV-8 infection has a very low prevalence in the general population in North America and northern Europe but is highly prevalent among men who have sex with men.⁵⁻⁹ There is also a marked geographic variation, since many regions of Africa and Italy have a high rate of seropositivity for the virus and a relatively wide distribution of infection among the population. In Africa and southern Italy, seropositivity for HHV-8 is often initially detected in early childhood, and the rates of seropositivity increase with age, suggesting that nonsexual modes of transmission may also be important in these regions.^{11,13-16} Among men who have sex with men, sexual activity has a predominant role in the acquisition of HHV-8.⁵⁻⁷ However, HHV-8 has been found only infrequently in semen, the anal canal,

and prostatic secretions.^{17-19,21,24,31,40,41} We have focused on the genital tract as the most likely source of shedding of HHV-8.^{17,20,21} However, our latest results indicate that the oral cavity is an important, if not the preeminent, source of infectious virus.^{20,21,42,43}

We obtained samples from multiple mucosal sites to identify the predominant pattern and sites of shedding of HHV-8 in seropositive persons. Overall, we detected HHV-8 DNA in 34 percent of oropharyngeal samples (382 of 1134), 0.3 percent of urethral samples (3 of 848), 1 percent of anal samples (11 of 1087), and 5 percent of semen samples ($P < 0.001$ for the comparison of oral samples with genital samples and with anal samples). Perhaps the most surprising finding was that nearly 50 percent of the HHV-8-seropositive men had detectable HHV-8 in oropharyngeal samples on more than 35 percent of the days on which samples were obtained. Our finding of HHV-8 in oral epithelial cells suggests that these cells serve as a replication-competent source of the virus. Whether these are the only cells that contribute to the HHV-8 in saliva is unclear. We did not find HHV-8 in biopsy specimens of submandibular salivary glands or in samples collected directly from salivary ducts from subjects who had Kaposi's sarcoma or were HIV-positive (unpublished data). The finding of epithelial reservoirs of HHV-8 supports our previous observations of high titers of HHV-8 virions in saliva.²¹ Thus, it appears that HHV-8 is similar to the related gamma herpesvirus Epstein-Barr virus, which replicates in both epithelial cells and B cells.⁴⁴

Our laboratory findings suggest that HHV-8 may be acquired by oral-oral contact, especially if the seropositive sexual partner has a high titer of HHV-8 and a high frequency of viral shedding. These observations were supported by behavioral data showing that deep kissing with an HIV-seropositive partner was an independent risk factor for the acquisition of HHV-8 (odds ratio, 5.4). That oral contact may not be the sole mode of transmission is suggested by our intermittent detection of HHV-8 in genital secretions and anal swabs. Thus, our findings are consistent with previous studies associating increased sexual activity, including orogenital contact, with the acquisition of HHV-8.^{5-8,45} Interestingly, a primate gamma herpesvirus that is closely related to HHV-8 has been detected at high levels in the tongue and cheek tissues.⁴⁶ Since many sexual activities are practiced together, identification of the relative contributions of oral or genital contact in the acquisition of HHV-8 will require prospective analysis of sexual activities.

If HHV-8 is transmitted through a common behavior such as kissing, then why is the infection in North America mainly restricted to men who have sex with men? The observation that HHV-8 is not ubiquitous among all populations suggests that it may not be easily transmitted and that acquisition may depend on the degree of exposure to infected persons, especially

to those who are immunocompromised. Alternatively, there may be unidentified cofactors that greatly increase either the shedding of infectious virus in HHV-8-positive persons or the risk of infection in uninfected persons. Our cohort included both men with persistent oral shedding of HHV-8 and men without detectable shedding of the virus. Whether such patterns occur in other populations, such as women and children, is unclear.¹⁹

Defining the mechanism of infection with HHV-8 is an important clinical issue. HHV-8 is a severe infection in HIV-seropositive persons, and Kaposi's sarcoma ultimately develops in over 39 percent of those infected with both viruses.^{7,47,48} Few men who have sex with men practice protected oral sex, and oral-oral contact is not generally considered a high-risk behavior for the transmission of sexually transmitted diseases. Until we have a better understanding of the mechanisms of transmission, it will be difficult to define the most effective approach to prevention. Our findings suggest that safer sex practices, such as consistent use of condoms, although important in preventing other sexually transmitted infections, may not protect against HHV-8 infection.

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