CONCISE COMMUNICATION

Detection of Kaposi's Sarcoma–Associated Herpesvirus in Oral and Genital Secretions of Zimbabwean Women

Thomas M. Lampinen,[1,3] Shalini Kulasingam,[1] Juno Min,[4] Margaret Borok,[6] Lovemore Gwanzura,[7] Julie Lamb,[4] Kassam Mahomed,[8] Godfrey B. Woelk,[9] Kurt B. Strand,[2] Marnix L. Bosch,[2] Daniel C. Edelman,[10] Niel T. Constantine,[10,11] David Katzenstein,[4,5] and Michelle A. Williams[1]

[1]Departments of Epidemiology and [2]Pathobiology and [3]Center for AIDS and STD, University of Washington, Seattle; [4]Department of Medicine and [5]Center for AIDS Research, Stanford University, Stanford, California; Departments of [6]Medicine, [7]Medical Laboratory Sciences, [8]Obstetrics and Gynecology, and [9]Community Medicine, University of Zimbabwe, Harare; [10]Department of Pathology, University of Maryland and [11]Institute of Human Virology, Baltimore, Maryland

Kaposi's sarcoma–associated herpesvirus (KSHV) in oral and genital secretions of women may be involved in horizontal and vertical transmission in endemic regions. Nested polymerase chain reaction assays were used to detect KSHV DNA sequences in one-third of oral, vaginal, and cervical specimens and in 42% of peripheral blood mononuclear cell (PBMC) specimens collected from 41 women infected with human immunodeficiency virus type 1 who had Kaposi's sarcoma (KS). KSHV DNA was not detected in specimens from 100 women without KS, 9 of whom were seropositive for KSHV. A positive association was observed between KSHV DNA detection in oral and genital mucosa, neither of which was associated with KSHV DNA detection in PBMC. These data suggest that KSHV replicates in preferred anatomic sites at levels independent of PBMC viremia. Detection of genital-tract KSHV only among relatively immunosuppressed women may provide an explanation for infrequent perinatal transmission of KSHV.

Kaposi's sarcoma–associated herpesvirus (KSHV), also known as human herpesvirus 8 (HHV-8), appears to be causally associated with Kaposi's sarcoma (KS) [1, 2]. The modes of KSHV transmission are incompletely understood. However, like other pathogenic herpesviruses, KSHV might be transmitted via mucosal secretions. In northern Europe and the United States, where KS is not endemic and childhood KSHV infections seem rare, epidemiological studies have found evidence for transmission of KSHV between male homosexual partners. In contrast, in many regions where KS is endemic, KSHV infection is commonly acquired during childhood [1, 2]. In Harare, Zimbabwe, the incidence of KS has increased 20-fold during the past 10–15 years, making it the most common malignancy in children [3], men [4, 5], and women [5]. The high incidence of KS and the possible role of women in KSHV transmission led us to screen for KSHV DNA present in genital and oral specimens collected from Zimbabwean women with and without clinically evident KS.

Methods

Recruitment, interview, and evaluation of study participants. All patients with clinical evidence of KS referred to central hospitals (Parirenyatwa and Harare Hospitals) in Harare, Zimbabwe were assessed at a designated clinic by 1 physician (M.B.). Nonmenstruating patients with intact uteri who were ⩾18 years of age and able to provide informed consent were eligible to participate in this study, regardless of their KS treatment history. Between July and October 1996, we enrolled 41 women with clinical diagnoses of KS; 33 (80%) had histologic confirmation of the diagnosis recorded in their medical record. Clinically defined stages of KS and human immunodeficiency virus type 1 (HIV-1) serostatus were abstracted from the medical record. All but 2 eligible women participated.

For comparison, 100 women without clinical KS were recruited between September 1996 and January 1997 from the outpatient gynecology department of Harare Maternity Hospital. Over 90%

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Informed consent was obtained from all participants, and human experimentation guidelines of the US Department of Health and Human Services and the Medical Research Council of Zimbabwe were followed in the conduct of this investigation.

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Reprints or correspondence: Thomas M. Lampinen, University of Washington, Dept. of Epidemiology, Box 357236, Seattle, WA 98195 (tlamp@u.washington.edu).

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of invited women agreed to participate. The high prevalence of HIV-1 among women seeking routine gynecologic care in Harare allowed comparison of the frequency of KSHV among women with ($n = 47$) and without ($n = 53$) HIV-1 infection. Women participating in the study were generally unaware of their HIV-1 or KSHV serologic status at the time of enrollment and interview. Posttest counseling was provided with HIV-1 serologic results.

Demographic, medical, reproductive, and sexual histories were collected during in-person interviews using a structured questionnaire. Two experienced nurse-midwives performed physical and pelvic examinations with sample collection.

Blood samples. Lymphocyte subset analyses were performed on 1-mL aliquots of EDTA-anticoagulated blood specimens using a fluorescence-activated cell sorter counter (Becton Dickinson, Cockeysville, MD). Anticoagulant acid-citrate-dextrose–blood specimens were collected for peripheral blood mononuclear cell (PBMC) DNA extraction after Ficoll-Hypaque cell separation. Sera were collected for syphilis (*Treponema pallidum* hemagglutination assay), herpes simplex virus (HSV)–1, HSV-2, and HIV-1 testing (the latter by EIA with Western blot confirmation). Antibodies to KSHV were identified in 1 : 50 serum dilutions using an ELISA based on a recombinant antigen derived from the open-reading frame (ORF) 65 capsid region [6].

Vaginal, cervical, and oral samples. After insertion of a speculum, Dacron swabs were used to collect specimens for microbiological and polymerase chain reaction (PCR) assays. Three swabs were separately collected from the anterolateral vaginal wall (with avoidance of pooled cervical secretions), 2 were collected from the ectocervix, and 2 were collected from the cervical os. Genital swabs were placed in 2 mL of RPMI media (Life Technologies, Gaithersburg, MD) and vortexed to remove cellular material. Vaginal secretions adhering to the speculum were used for pH measurements and for potassium hydroxide (amine "whiff") tests. Oral-rinse samples were collected using 10 mL of sterile saline. Genital and oral samples were frozen at −70°C for later DNA extraction.

DNA testing. Vaginal, cervical, oral rinse, and PBMC samples were centrifuged at 2000 *g* for 1 min. Pellets were resuspended in 200 μL of PBS, and DNA was extracted using QIAGEN Blood Kits (QIAGEN, Chatsworth, CA), then quantitated by spectrophotometry. PCR amplification assays were performed as described elsewhere, using 0.5 μg of DNA [7]. Briefly, 2 distinct, nested PCR primer pairs that amplify nonoverlapping regions of the KSHV major capsid gene were used to detect KSHV DNA. Only samples that consistently amplified with both sets of primers were considered to be positive for KSHV DNA. DNA integrity and the absence of inhibitors were confirmed in each sample by PCR amplification of human betaglobin DNA fragments. Positive (BC-1 cell line) and negative (human placental DNA) controls were included in every PCR reaction.

DNA gene sequences. Among PCR-positive DNA extract samples, 30 with sufficient DNA were duplicated, then coded with negative controls for blind analysis. We used PCR to amplify a 258–bp fragment of the KSHV ORF K1 gene using a nested set of primers: for the outer pair, YVVCA (5′-TGTATGTTGTCTGCAGTCTGGC-3′) and RCANB (5′-GGAGTTATATTGGCACAACGC-3′), and for the inner pair, CPGVA (5′-CCCTTGAGTGATTTCAACG-3′) and VTCGB (5′-AACATGCTGACCGGATCC-3′). Each 20-μL PCR reaction contained 20–400 ng of DNA, 5.0 pmol of each primer, 1.0 U of Taq polymerase, 50 μ*M* each dNTP, 67 m*M* Tris (pH 8.8), 2 m*M* $MgCl_2$, 11 m*M* $(NH_4)_2SO_4$, 10 m*M* 2-mercaptoethanol, and bovine serum albumin (100 μg/mL). PCR conditions were as follows: 94°C for 1 min, 60°C for 1 min, 72°C for 1 min (40 cycles); and 72°C for 30 min (1 cycle). PCR products were purified over gel extraction columns (QIAquick Gel Extraction Kit; QIAGEN), were then either sequenced directly or cloned using a T-A cloning system (pCR2.1/INV∝F'; Invitrogen, Carlsbad, CA), and were then sequenced using M-13 universal primers.

Data analysis. $CD4^+$ T cell counts <50 per cubic mL (the lower limit of detection, $n = 3$), were assigned a midpoint value of 25. KSHV-seropositive women with unmeasurable antibody levels ($n = 2$) were assigned a titer of 2. KSHV antibody titers were $\log_2$ transformed, and geometric mean titers (GMTs) were calculated after exclusion of nonreactive specimens. Kruskal-Wallis tests were used to compare continuous variables across multiple groups; pairwise (Mann-Whitney) comparisons were performed only when overall tests were significant. Pearson's χ^2, Fisher's exact test, and odds ratios (ORs) with 95% confidence intervals (CIs) were calculated in analyses involving dichotomous and categorical variables.

Results

Description of the study population. The 41 women with KS were HIV-1 seropositive. Most (39 [95%] of 41) had generalized KS lesions, with visceral KS noted in 10 (24%). The average self-reported duration of KS lesions was 18 months (range, 1–62). Among those with available information, half (17/34) had received some form of treatment for their KS before study enrollment (9, chemotherapy; 7, radiation therapy; 1, chemotherapy and radiation therapy).

Women with KS had significantly fewer peripheral $CD4^+$ lymphocytes per milliliter (median, 140; range, 25–391; $n = 32$) than either HIV-1–seropositive women without KS (median, 274; range, 63–949; $n = 37$; $P < .001$) or HIV-1–seronegative women without KS (median, 719; range, 33–1888; $n = 45$; $P < .001$). Women with KS were more likely than women without KS to have antibodies to KSHV ORF 65 (table 1), and the mean antibody titer was 5- to 6-fold higher in women with KS (GMT, 761; range, 2–32,768), than in women without KS (GMT, 138, range, 2–1024; $P = .05$). HIV-1–seropositive women with and without KS differed from HIV-1–seronegative women; the latter were more likely to be married, reported fewer lifetime sexual partners, and had a lower prevalence of HSV-2 antibodies (table 1). None of the women enrolled in the study were receiving antiviral medication.

Detection of KSHV DNA. Variable success in specimen collection and nucleic-acid extraction resulted in different numbers of PCR results for each type of specimen. Overall, KSHV DNA sequences were detected in ⩾1 genital, oral, or PBMC specimen obtained from 23 (56%) of the 41 women with KS:

Table 1. Selected characteristics of Zimbabwean women, by clinical Kaposi's sarcoma and human immunodeficiency virus type 1 serostatus.

Characteristic	With KS/HIV$^+$ (n = 41)	Without KS/HIV$^+$ (n = 47)	Without KS/HIV$^-$ (n = 53)	P
Age, in years (mean ± SD)	36.2 ± 10.6	33.7 ± 9.3	35.4 ± 10.5	.26
Education, no. (%)				.27
None	5 (12)	6 (13)	2 (4)	
Primary–O-level	26 (63)	23 (49)	31 (58)	
>O-level	10 (24)	18 (38)	20 (38)	
Marital status, no. (%)[a]				.01
Married	21 (54)	30 (67)	42 (79)	
Widowed	7 (18)	5 (11)	2 (4)	
Divorced/separated	11 (28)	3 (16)	5 (9)	
Single	0 (0)	4 (7)	4 (8)	
Lifetime sex partners, median (range)	2 (1–8)	2 (1–9)	1 (1–5)	.001
Age at first intercourse, in years, median (range)	18 (13–25)	18 (12–30)	18.5 (13–28)	.33
Pregnancies, median (range)	4 (0–12)	2 (0–12)	2 (0–11)	.08
Nulliparous, no. (%)	2 (5)	6 (13)	9 (17)	.20
Clinical/lab results, no. (% positive)[a]				
Bacterial vaginosis[b]	7 (21)	7 (16)	9 (18)	.88
Chlamydia trachomatis	0 (0)	0 (0)	0 (0)	—
Lactobacillus species	12 (30)	11 (24)	14 (27)	.85
Yeast	12 (29)	11 (24)	7 (14)	.16
Trichomonas vaginalis	1 (3)	2 (4)	0 (0)	.32
Neisseria gonorrhea	0 (0)	1 (2)	1 (2)	.65
Serologic results, no. (% positive)[a]				
KSHV ORF 65	35 (85)	4 (9)	5 (9)	<.001
HSV-1	35 (85)	41 (93)	51 (98)	.06
HSV-2	39 (95)	41 (93)	29 (56)	.001
TPHA	11 (27)	11 (23)	12 (23)	.89

NOTE. KS, Kaposi's sarcoma; HIV, human immunodeficiency virus; KSHV, Kaposi's sarcoma–associated herpesvirus; ORF, open-reading frame; HSV, herpes simplex virus; TPHA, *Treponema pallidum* hemagglutination assay.

[a] Percentages of those with available data.

[b] Bacterial vaginosis defined as presence of ⩾2 of the following: positive "whiff" test, presence of clue cells by Gram stain, or vaginal pH <4.5.

KSHV DNA sequences were detected in 13 (34%) of 38 vaginal specimens, in 9 (28%) of 32 ectocervical specimens, in 10 (37%) of 27 endocervical specimens, in 14 (37%) of 38 oral-rinse specimens, and in 11 (42%) of 26 PBMC specimens (table 2). Among the 27 women with KS for whom all genital (vaginal, ectocervical, and endocervical) PCR results were available, 7 (26%) were positive at all 3 sites, and 16 (59%) were negative at all 3 sites (table 2). Two of 4 women with vaginal lesions suggestive of KS had KSHV DNA detected in their vaginal-swab specimens.

KSHV DNA was not detected in oral or ectocervical specimens obtained from each of 100 women without clinical KS, including 9 who were who KSHV seropositive (4 were HIV-1 seropositive, and 5 were HIV-1 seronegative). PBMC, vaginal, and endocervical specimens collected from each of these 9 KSHV-seropositive women were also negative for KSHV DNA. The higher frequency of KSHV DNA detection in oral, vaginal, endocervical, and PBMC specimens collected from KSHV-seropositive women with, compared with those without, KS was statistically significant ($P < .05$).

Relation of KSHV DNA detection among specimens obtained from women with KS. Women with oral KSHV DNA were significantly more likely to have KSHV DNA detected in their vaginal (8 [57%] of 14 vs. 5 [23%] of 22; odds ratio [OR], 4.5; 95% confidence interval [95% CI], 1.1–19.4; $P = .04$), ectocervical (7 [50%] of 14 vs. 2 [12%] of 17; OR, 7.5; 95% CI, 1.2–45.8; $P = .04$), and endocervical (7 [58%] of 12 vs. 3 [20%] of 15; OR, 5.6; 95% CI, 1.0–30.9; $P = .04$) specimens, respectively, compared with women without detectable oral KSHV. Among 32 women with KS for whom both a vaginal and cervical result were available, detection of KSHV DNA in these sites was completely concordant (11 were KSHV DNA positive; table 2). In contrast, KSHV DNA detection in PBMC was not significantly associated with its detection in vaginal, ectocervical, endocervical, or oral specimens.

Relation of KSHV DNA detection to KSHV antibody titers, CD4$^+$ lymphocyte count, and other potential correlates in women with KS. Titers of antibody to ORF 65 were significantly lower among women in whom KSHV DNA was detected in ectocervical ($P = .02$) and endocervical ($P = .01$) specimens, and GMTs tended to be lower among women with vaginal ($P = .09$) and oral-rinse ($P = .15$) KSHV DNA

Table 2. Selected characteristics and Kaposi's sarcoma–associated herpesvirus DNA polymerase chain reaction results among 41 human immunodeficiency virus type 1–positive Zimbabwean women with clinical Kaposi's sarcoma, by stage of Kaposi's sarcoma and $CD4^+$ count.

Patient	Age, years	KS stage[a]	$CD4^+$ count, cells/mm^3	Anti-ORF65 titer	Oral KSHV	Vaginal KSHV	Ectocervical KSHV	Endocervical KSHV	PBMC KSHV
38	39	1	63	4096	−	−	−	−	+
36	43	2	ND	<2	−	−	−	−	−
10	36	3	145	8192	ND	ND	ND	ND	−
35	32	3	142	2048	+	−	−	−	+
12	31	3	138	32	−	−	ND	ND	+
14	35	3	122	8192	ND	−	ND	ND	ND
30	48	3	84	1024	−	−	−	−	−
29	29	3	ND	2048	−	−	−	−	ND
40	32	3	ND	2048	−	−	−	−	−
20	39	3	ND	1024	−	−	−	−	ND
42	32	3	ND	256	+	+	+	+	−
26	35	3B	348	512	−	−	ND	ND	ND
15	39	3B	211	2048	−	−	−	−	−
43	43	4	391	4096	−	−	−	−	−
41	23	4	355	<2	+	−	−	−	−
13	40	4	332	8192	−	+	ND	ND	+
24	27	4	274	4096	−	−	ND	ND	ND
33	77	4	205	2048	+	+	+	−	+
16	NA	4	159	<2	−	−	−	−	−
9	29	4	154	16384	−	ND	ND	ND	+
28	33	4	142	1024	−	+	−	+	ND
2	26	4	141	32	−	+	+	ND	ND
25	36	4	136	8192	+	−	−	−	ND
11	26	4	131	128	−	ND	ND	ND	−
27	32	4	102	4096	+	−	−	ND	ND
34	45	4	96	64	−	+	+	+	+
21	40	4	86	2	+	+	−	+	ND
32	38	4	69	32768	−	−	−	−	−
1	44	4	50	128	ND	−	−	ND	ND
22	41	4	<50	2048	−	−	−	−	ND
3	24	4	ND	256	+	−	−	ND	+
23	32	4	ND	32	+	+	+	+	ND
39	54	4	ND	<2	−	−	−	−	−
17	29	4B	237	16384	−	+	−	+	−
44	23	4B	217	32	+	+	+	+	−
37	23	4B	165	128	+	+	+	+	+
31	25	4B	74	<2	+	+	+	+	−
4	38	4B	71	<2	−	−	−	ND	+
18	30	4B	<50	64	−	−	ND	ND	ND
19	59	4B	<50	64	+	−	−	−	ND
7	35	4B	ND	2048	+	+	+	+	+
KSHV DNA[b]					14/38 (37)	13/38 (34)	9/32 (28)	10/27 (37)	11/26 (42)

NOTE. KS, Kaposi's sarcoma; ORF, open-reading frame; KSVH, Kaposi's sarcoma–associated herpesvirus; PBMC, peripheral blood mononuclear cells; ND, not done; NA, not available.

[a] KS disease stage: 1, indolent localized; 2, locally aggressive; 3, generalized cutaneous or lymphadenopathic; 4, generalized cutaneous or lymphadenopathic with evidence of visceral involvement; A, without systemic symptoms; B, with systemic symptoms including fever, weight loss, or night sweats.

[b] Data are no. positive for KSHV DNA/total no. tested (%).

detection, compared with titers in women in whom KSHV DNA was not detected in each specimen type, respectively. Antibody GMTs were not significantly different between women with and without detectable KSHV DNA in PBMC ($P = .59$; table 2).

KSHV DNA detection in vaginal, cervical, oral, and PBMC specimens was not significantly associated with peripheral $CD4^+$ lymphocyte count, self-reported duration of KS lesions, stage of KS, or receipt of chemotherapy or radiation therapy. Neither vaginal nor cervical KSHV DNA detection was associated with vaginal pH; clinically evident genital bleeding, inflammation, discharge, or ulceration; sexual intercourse the night before the clinic visit; or laboratory evidence of past or current sexually transmitted infection (data not shown). However, our statistical power to detect correlates of KSHV DNA detection was considerably limited, because of small sample sizes and a relatively narrow range of $CD4^+$ cell counts.

KSHV K1 gene sequences. Of 30 cell extracts screened using the KSHV K1V1–specific primers, 6 yielded PCR product bands of the expected size. Sequence analysis of the 6 PCR

fragments indicated the presence of 2 unique K1V1 sequences, which we called Zw1 (GenBank accession number AF152366) and Zw2 (GenBank accession number AF152367), that have not been reported elsewhere. The predicted amino acid sequences of the 123-bp V1 region show 82% identity between Zw1 and Zw2, whereas Zw1/2 identities range from 46% to 98% with other published K1V1 sequences [8]. Phylogenetically, Zw1 and Zw2 most closely resemble KSHV subgroup A5 [9].

The 6 positive samples were derived from only 3 patients, with 2 patients (34 and 44) having multiple sites that tested positive. Sequence Zw1 was found in patient 23 (vaginal sample), and Zw2 was identified in patient 34 (vaginal, ectocervical, and PBMC samples) and in patient 44 (vaginal and endocervical samples). In each of the latter patients, the KSHV K1 sequences obtained from different types of specimens were identical.

Discussion

In this study, we detected KSHV DNA in one-third of oral and genital specimens obtained from Zimbabwean women with KS but in none of the specimens obtained from 100 women without KS, including 9 who were KSHV seropositive. Detection of KSHV DNA among immunocompromised women with KS lesions, but not in healthier KSHV-seropositive women, was unexpected. Previous studies did not detect KSHV DNA in genital-tract specimens collected from 3 women with KS [10, 11]. In a small number of KSHV-seropositive women without KS, KSHV DNA was recently detected in the genital tract of African [12], but generally not European [10, 12], women. The immune status of women included in those studies was not described. Our results suggest a plausible explanation for the apparently infrequent perinatal transmission of this herpesvirus [13, 14]: genital-tract shedding may be limited to a small minority of childbearing women who lack immunologic control over KSHV replication. The impact of pregnancy on KSHV viremia and shedding requires further study.

Among men with HIV-1 infection, detection of KSHV DNA in saliva is more frequent in those with, compared with those without, clinically evident KS [15]. In the present study, detection of KSHV DNA in oral-rinse specimens was limited to women with KS. The infectivity of oral specimens containing KSHV DNA is not known, but these results suggest that nonmaternal sources, perhaps other children, may be important causes of primary infections that occur during childhood in endemic areas [2, 13, 14].

In an analysis that excluded women with KS lesions (a risk factor for lesions being HIV-1 infection), we found that HSV-2 serostatus was associated with HIV-1 infection (OR, 10.8; 95% CI, 3.0–40.0; $P < .001$) and prior syphilis (OR, 3.1; 95% CI, 0.8–11.4; $P = .08$). In contrast, neither HSV-2, nor HIV-1, nor syphilis was positively associated with KSHV infection (data not shown). Cross-sectional surveys conducted in endemic areas may not identify risk factors for sexual acquisition of KSHV, underscoring the importance of longitudinal studies to determine whether KSHV is sexually acquired among African adults.

An important finding in the present study was compartmentalized detection of KSHV in oral and genital mucosa, distinct from PBMC. This observation suggests there may be preferred anatomic sites for viral replication not reflected by KSHV viremia. Transmission studies should characterize KSHV shedding from mucosal sources among KSHV-seropositive individuals. Additional studies will be needed to define the temporal patterns and immunologic correlates of genital and oral KSHV shedding in larger, representative samples of KSHV-seropositive African women and children.

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References

1. International Agency for Research on Cancer (IARC). Kaposi's sarcoma herpesvirus/human herpesvirus 8. IARC Monogr Eval Carcinog Risks Hum **1997**; 70:375–492.
2. Schulz TF. Kaposi's sarcoma-associated herpesvirus (human herpesvirus 8). J Gen Virol **1998**; 79:1573–91.
3. Chitsike I, Chokunonga E. Kaposi's sarcoma: commonest childhood malignancy in Zimbabwe [abstract 3]. In: Program and abstracts of the 3rd National AIDS Malignancy Conference. J Acquir Immune Defic Syndr Hum Retrovirol **1999**; 21:A10.
4. Chokunonga E, Levy LM, Bassett MT, Mauchaza BG, Thomas DB, Parkin DM. Cancer incidence in the African population of Harare, Zimbabwe: second results from the cancer registry 1993–1995. Int J Cancer **2000**; 85: 54–9.
5. Chokunga E, Levy LM, Bassett MT, eds. Pattern of cancer in Zimbabwe. In: Annual Report of the Zimbabwe National Cancer Registry **1996**.
6. Edelman DC, Ketema F, Saville RD, et al. Specifics on the refinement and application of two serological assays for the detection of antibodies to HHV-8. J Clin Virol **2000**; 16:225–37.
7. Min J, Katzenstein DA. Detection of Kaposi's sarcoma-associated herpesvirus in peripheral blood cells in human immunodeficiency virus infection: association with Kaposi's sarcoma, CD4 cell count, and HIV RNA levels. AIDS Res Hum Retroviruses **1999**; 15:51–5.
8. Nicholas J, Zong JC, Alcendor DJ, et al. Novel organizational features, captured cellular genes, and strain variability within the genome of KSHV/HHV-8. J Natl Cancer Inst Monogr **1998**; 23:79–88.
9. Cook PM, Whitby D, Calabro M, et al. Variability and evolution of Kaposi's

sarcoma–associated herpesvirus in Europe and Africa. AIDS **1999**;13: 1165–76.

10. Calabro ML, Fiore JR, Favero A, et al. Detection of human herpesvirus 8 in cervicovaginal secretions and seroprevalence in human immunodeficiency virus type 1-seropositive and -seronegative women. J Infect Dis **1999**;179:1534–7.
11. Pulik M, Dupin N, Gorin I. Herpesvirus-like DNA sequences in Kaposi's sarcoma lesions from two women with AIDS. Clin Infect Dis **1997**;25: 740–1.
12. Whitby D, Smith NA, Matthews S, et al. Human herpesvirus 8: seroepidemiology among women and detection in the genital tract of seropositive women. J Infect Dis **1999**;179:234–6.
13. Lyall EGH, Patton GS, Sheldon J, et al. Evidence for horizontal and not vertical transmission of human herpesvirus 8 in children born to human immunodeficiency virus-infected mothers. Pediatr Infect Dis J 1999;18: 795–9.
14. Gessain A, Mauclere P, van Beveren M, et al. Human herpesvirus 8 primary infection occurs during childhood in Cameroon, Central Africa. Int J Cancer **1999**;81:189–92.
15. Koelle DM, Huang M, Chandran B, Vieira J, Piepkorn M, Corey L. Frequent detection of Kaposi's sarcoma–associated herpesvirus (human herpesvirus 8) DNA in saliva of human immunodeficiency virus-infected men: clinical and immunologic correlates. J Infect Dis **1997**;176:94–102.