MAJOR ARTICLE

Postnatal Human Herpesvirus 8 and Human Immunodeficiency Virus Type 1 Infection in Mothers and Infants from Zambia

Brad P. Brayfield,[1] Saul Phiri,[1,4] Chipepo Kankasa,[4] Jubra Muyanga,[4] Hamakwa Mantina,[4] Geoffrey Kwenda,[1,4] John T. West,[1] Ganapati Bhat,[4] Dave B. Marx,[2] Winslow Klaskala,[1] Charles D. Mitchell,[3] and Charles Wood[1]

[1]Nebraska Center for Virology, School of Biological Sciences, and [2]Department of Biometry, University of Nebraska, Lincoln; [3]University of Miami School of Medicine, Miami, Florida; [4]University Teaching Hospital, Lusaka, Zambia

The specific route and timing of human herpesvirus (HHV) 8 infection in regions where Kaposi sarcoma is endemic are not known. HHV-8 infection and any risk factors that may be associated with HHV-8, including human immunodeficiency virus (HIV) type 1 infection, were monitored during the 12-month postdelivery period for 416 mothers and 485 infants from Lusaka, Zambia. HHV-8 incident infection rates during this period were 3.2 and 5.3 infections/100 person-years for infants and mothers, respectively. HHV-8 infection among infants was not associated with HHV-8 or HIV-1 infection in the mother. Among the HHV-8–positive infants, 2 of 12 tested were found to have HHV-8 DNA in their peripheral blood mononuclear cells at birth, which suggests that in utero infection is possible. However, most HHV-8–positive infants appeared to have acquired infection either intrapartum or postpartum. The present study indicates that transmission of HHV-8 to infants can occur early and is likely via multiple routes.

Human herpesvirus (HHV) 8, or Kaposi sarcoma (KS)–associated herpesvirus, a gamma herpesvirus first identified in 1994 in biopsy tissue from a patient with AIDS and KS [1], is now thought to play a critical role in the development of KS, as well as other lymphoproliferative diseases, such as primary effusion lymphoma and multicentric Castleman disease [2]. Four epidemiological forms of KS have been recognized: classic, endemic, iatrogenic, and AIDS associated [3]. The number of reported cases of KS varies according to geographic regions and the specific type of KS. Before the early 1980s, KS was rare in Europe and the United States but was prominent in regions of equatorial Africa. This form of African KS, known as "endemic KS," is an aggressive disease that usually involves multiple regions of the body, such as the lymph nodes and the extremities. In addition, endemic KS presents between the ages of 25–40 years, in contrast to classic KS, which is largely observed in elderly men of Mediterranean or Jewish descent. Since the early 1980s, as human immunodeficiency virus (HIV) type 1 infection has increased, AIDS-associated KS has become prominent in several regions of the world, including Africa. This form of KS is more aggressive than endemic KS and is often multifocal, involving the lymph nodes, visceral organs, and other mucosal surfaces. AIDS-associated KS is predominantly found in heterosexual adults and children in parts of Africa but is most prevalent among HIV-1–positive homosexual men in the Western world [3].

Received 22 August 2002; revised 7 November 2002; electronically published 7 February 2003.

Informed written consent was obtained from all study participants. Approval for the study was obtained from the Ministry of Health of Zambia, the Research and Ethics Committee of the University of Zambia School of Medicine in Lusaka, Zambia, and the institutional review boards of the University of Nebraska, Lincoln, and the University of Miami, Miami, Florida.

Financial support: Public Health Service (grants CA75903 and CA76958); Fogarty International Training and the Fogarty International Fellowship (grant TW01492 to S.P., J.M. and G.K.); National Center for Research Resources, Center of Biomedical Research Excellence (grant RR 15635 to C.W.).

Reprints or correspondence: Dr. Charles Wood, Nebraska Center for Virology, School of Biological Sciences, University of Nebraska, Lincoln, E318 Beadle Center, 1901 Vine St., Lincoln, NE 68588 (cwood1@unl.edu).

The Journal of Infectious Diseases 2003; 187:559–68

0022-1899/2003/18704-0005$15.00

The number of reported cases of childhood KS has increased dramatically in several countries in sub-Saharan Africa since the onset of the AIDS epidemic [4, 5]. This contrasts with reports in the United States and northern Europe, where KS is rarely seen in children [3]. In Zambia, the number of reported cases of childhood KS has increased significantly since the onset of the AIDS epidemic, accounting for 0%–2% of childhood malignancies in the early 1980s and 20%–25% of childhood malignancies by 1992 [5–8]. HHV-8 seroprevalence follows a similar pattern, with high seroprevalence in the general population observed in regions of high KS incidence, such as Italy, sub-Saharan Africa, and regions of South America. Several cross-sectionally designed studies of HHV-8 seroprevalence in several age groups from regions of Africa where KS is endemic suggest that primary infection with HHV-8 occurs during childhood, with increasing seroprevalence observed through puberty [9–16]. In Zambia, an HHV-8 seroprevalence rate near 50% has been reported among adolescents and childbearing women, and the seroprevalence rate increases with age [17, 18]. Viral DNA has also been detected in biopsy tissue and peripheral blood mononuclear cells (PBMC) of infants and children with febrile illnesses in Zambia, further supporting the concept that HHV-8 infection occurs early in life in Africa [9, 14]. Similar studies conducted in the United States have shown that primary infection likely occurs in adults, with low seroprevalence observed in children [19].

In contrast to what is known about HHV-8 seroprevalence, information concerning the route of transmission and any risk factors that may influence transmission is still limited. Transmission of HHV-8 has been strongly linked to homosexual contact in North America and western Europe. HHV-8 seropositivity has been reported to be correlated with receptive oral sex, the number of sex partners, and the use of amyl nitrate [20–24]. In addition, a study of patients from public health clinics reported a higher seroprevalence among commercial sex workers than non–commercial sex workers [25]. The absence of antibodies and viral DNA in children and a high seroprevalence among children in an isolated region of southern Texas indicate that nonsexual transmission is not a significant route of infection in the United States [19, 26]. In some parts of South America and Africa and in regions where low socioeconomic conditions exist, a higher proportion of the general population is infected with HHV-8 than in the United States [10, 12, 14, 16, 26, 27]. In these studies, children were shown to have a high seroprevalence of HHV-8, ranging from 13% to 48% among children aged 2–15 years [10, 14]. One study also indicated significant familial correlations in HHV-8 seroprevalence between mother and child and among siblings [16]. Furthermore, a recent study identified matching sequences of the variable K1 gene in saliva collected from various family members and an index case patient with KS [28]. These studies strongly suggest that nonsexual means of infection, possibly intrafamilial transmission via saliva or other close contact, may be a significant source of infection in some regions.

Although cross-sectional studies have established the seroprevalence of HHV-8 in children from regions where KS is endemic, several questions about HHV-8 infection still need to be addressed. The incidence of HHV-8 infection among children, the specific routes of transmission in regions where KS is endemic, the role of HIV-1 and socioeconomic and behavioral factors in the transmission of HHV-8 to children, and the impact other diseases have on HHV-8 transmission have not been fully examined. To address some of these questions, a large study was initiated with the University Teaching Hospital (UTH) in Lusaka, Zambia, to study both HHV-8 and HIV-1 infections in a population of childbearing women and their infants. The present study examined HHV-8 and HIV-1 infections in infants and mothers in an effort to increase understanding of the increasing incidence of KS among young children in Zambia. More important, this study aimed to increase understanding of the pathogenesis of KS, which will be useful in identifying potential treatments and/or preventative measures to decrease the incidence of KS. Here, we report a prospective study of the incidence of HHV-8 and HIV-1 infection among infants during the first 12 months after delivery and the risk factors associated with HHV-8 infection in a group of mothers and infants from a region where HHV-8 is endemic.

SUBJECTS AND METHODS

Patient population. Study participants were part of an ongoing cohort study of HHV-8 transmission at the UTH in Lusaka, Zambia. In this cohort, healthy childbearing women were screened for HHV-8 and HIV-1 after admission to the labor ward of UTH. Before giving birth, the women were counseled and informed about HIV-1, HHV-8, and the ongoing study; then written consent to participate was obtained. Women who were in active labor were excluded, and only the first infant from multiple births was included. In the present study, a group of 416 mothers and 485 infants who returned for follow-up analysis after delivery and with an available blood sample taken 12 months after delivery were tested for infection with HHV-8 and HIV-1 at 12 months after delivery, with primary focus on HHV-8 incident infection and attention to HIV-1 only as a correlate to HHV-8 infection.

Sample collection and serological testing for HHV-8 and HIV-1. Blood was collected from mothers before delivery and from infants within 24 h of delivery, by venipuncture into acid citrate dextrose tubes, and was processed as described elsewhere [29]. In brief, whole blood was separated into plasma and cell fractions by centrifugation, and PBMC were isolated from the cell fraction using lymphocyte separation media (In-

vitrogen), according to the manufacturer's instructions. An aliquot of the plasma and the PBMC was then stored at −80°C for future use. Lytic and latent antibodies to HHV-8 were detected using a monoclonal antibody–enhanced immunofluorescence assay (mIFA) with minor modifications, as described elsewhere [29, 30]. For the lytic mIFA, BC-3 cells [31] (provided by Dr. Ethel Cesarman, Weill Medical College, Cornell University, New York, NY), a primary effusion lymphoma-derived cell line that harbors HHV-8 and is free of Epstein-Barr virus (EBV), were induced into lytic replication by addition of TPA to the culture medium at a final concentration of 20 ng/mL for 72 h. The cells were washed with PBS and spotted onto slides, air dried, and fixed in cold acetone at −20°C. Prior to serological testing, an aliquot of each test sample was heat inactivated at 56°C for 45 min and used for subsequent serological analysis. Each test sample was assayed at a dilution of 1:40 and was examined by fluorescence microscopy. For the latent mIFA, BC-3 cells not induced with TPA were spotted onto slides and assayed as above. Test samples that exhibited punctate nuclear staining were considered to be positive for HHV-8. To exclude background fluorescence, all positive serum samples were tested as above, using BJAB cells, an HHV-8– and EBV-negative B cell lymphoma cell line [32]. For plasma to be considered positive for HHV-8, concordant positive results from 2 independent mIFA tests and a negative result from the BJAB mIFA had to be achieved. For lytic antibody titer determination, serial 2-fold dilutions of the test serum were performed, and each dilution was assayed using mIFA. The inverse of the last dilution that tested positive was considered to be the end-point titer. Test plasma that was positive by mIFA was further tested using an ELISA made from a solubilized HHV-8 whole-virus extract (Advanced Biotechnologies). The ELISA was used according to the manufacturer's instructions with a test serum dilution of 1:40.

Serological testing for HIV-1 was performed as described elsewhere [29]. Plasma was screened for HIV-1 antibodies using Capillus (Trinity Biotech) and Determine (Abbott Laboratories) rapid assays, according to the manufacturers' instructions, and by IFA. Positive test samples were then analyzed by Western blot, using recombinant HIV-1 clade C p24 and gp160 (provided by Drs. Daniel Perez-Filgueira [Department of Biotechnology, INIA, Madrid] and Shiu-Lok Hu [University of Washington, Seattle], respectively). The reactive bands were detected using nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyphosphate colorimetric substrates, according to the manufacturer's instructions (Roche Laboratories).

DNA extraction, polymerase chain reaction (PCR), and Southern blot analysis. DNA was extracted using phenol-chloroform-isoamyl alcohol (PCA) and proteinase K, according to standard methods, or by Puregene DNA Purification System (Gentra Systems), according to the manufacturer's instructions. In brief, PBMC pellets were resuspended in standard cell lysis solution or Gentra Systems cell lysis solution and incubated overnight. For PCA extraction, 2 rounds of PCA and 1 chloroform-isoamyl alcohol extraction were performed on all samples. This was followed by ethanol precipitation at −20°C. For Gentra Systems lysis, cell pellets were extracted according to manufacturer's procedure and resuspended in Tris-EDTA. PCR for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was performed on all extracted samples, and PCR-positive samples were then used for HHV-8 PCR. HHV-8 PCR was performed using primers specific for 3 genes—*ORF26, gB,* and *K1*—as described elsewhere [29]. Screening of DNA samples was performed initially with *ORF26* primers and 400 ng of genomic DNA. For confirmation of *ORF26*-positive DNA samples, primers specific for *gB* and *K1* were used on those samples initially found to be *ORF26* positive. Southern blot of all PCR products was performed for confirmation using digoxygenin-labeled probes specific for these genes, according to the manufacturer's protocols (Roche Laboratories).

Risk factor analysis for HHV-8 and HIV-1 infection. All statistical analysis was carried out using SAS software (version 8; SAS Institute). Odds ratios (ORs), 95% confidence intervals (CIs), and *P* values were generated for each risk factor, and factors with $P < .05$ and CIs not containing 1 were considered to be significant. Information regarding sociodemographic characteristics, behavior, and prior exposure status to sexually transmitted diseases (STDs) were obtained by an attending physician using standardized questionnaires at the time of recruitment into the study and at each subsequent visit to the follow-up clinic, as described elsewhere [29].

RESULTS

Follow-up cohort for HHV-8 and HIV-1 incident infection. To analyze our population for infection during the 12-month postdelivery period, we first analyzed the seroprevalence of HHV-8 and HIV-1 at the time of delivery. From an ongoing cohort at UTH, 3150 healthy women had been screened previously for HHV-8 and HIV-1 at the time of delivery at UTH. At the time of sample testing, mIFA was performed because it was determined to be the most specific and sensitive assay for analyzing HHV-8 seroprevalence. Using the mIFA, a high seroprevalence of both HHV-8 and HIV-1 was observed in this population (table 1). Women from this cohort showed a seroprevalence of 40% (1256/3150) and 30% (957/3150) for HHV-8 and HIV-1, respectively. Women who were HIV-1 positive were more likely to be HHV-8 positive than those who were HIV-1 negative (OR, 1.42; 95% CI, 1.22–1.66), thus indicating a possible association between HIV-1 and HHV-8 infection. The mothers and infants were divided into 4 groups on the basis of maternal serostatus for HHV-8 and HIV-1 at

Table 1. Human herpesvirus (HHV) 8 and human immunodeficiency virus (HIV) type 1 seroprevalence in a population of women who gave birth at the University Teaching Hospital, Lusaka, Zambia.

HHV-8 serostatus	HIV-1 serostatus		Total
	Positive	Negative	
Positive	438[a]	818	1256 (40)
Negative	519	1375	1894
Total	957 (30)	2193	3150

NOTE. Data are no. (%) of women.

[a] HIV-1–seropositive mothers were significantly more likely to be HHV-8 seropositive than were HIV-1–seronegative mothers (odds ratio, 1.42; 95% confidence interval, 1.22–1.66).

the time of delivery: group 1 included mothers who were seropositive for both viruses, group 2 included mothers who were HHV-8 positive and HIV-1 negative, group 3 included mothers who were HIV-1 positive and HHV-8 negative, and group 4 included mothers who were negative for both viruses. Of the screened population of 3150 mother-infant pairs (MIPs), 1431 returned to UTH for at least one follow-up visit and were recruited for longitudinal follow-up; 740 of these were still available for follow-up ("active") at the time of analysis. The rest of the screened MIPs were considered to be inactive because they did not return for follow-up analysis; they were untraceable, had relocated, or had died before follow-up analysis. Of the 740 MIPs who were active for follow-up, 485 infants and 416 mothers with available specimens at 12 months after delivery were monitored for HHV-8 and HIV-1 infection within this time period. The sociodemographic characteristics of the study population are shown in table 2, according to the HHV-8 serostatus of the mothers of the 485 infants tested. HHV-8–positive mothers were more likely to be unemployed than were HHV-8–negative mothers (OR, 0.51; 95% CI, 0.32–0.84). No other characteristics examined were significantly different between HHV-8–positive and –negative mothers (table 2). The number of infants and mothers in the follow-up cohort, the number of deaths, and the number of patients lost to follow-up by withdrawal from the study or relocating during the study period are shown in table 3. A total of 54 infants and 3 mothers had died by 12 months after delivery, with significantly more deaths among infants born to HIV-1–positive mothers (groups 1 and 3) than HIV-1–negative mothers (groups 2 and 4) (OR, 10.1; 95% CI, 5.08–20.2). No significant difference in the number of infant deaths was observed between those born to HHV-8–positive and –negative mothers (table 3; OR, 1.14; 95% CI, 0.65–1.99). Mothers in group 4 were the least likely to be lost to follow-up, which reflects the fact that they were dual negative at the time of delivery and were the most healthy mothers with the most healthy infants in the study.

HHV-8 infection by 12 months after delivery. For initial determination of HHV-8 infection, plasma samples taken at 12 months after delivery from both mothers and infants were analyzed by mIFA, as described elsewhere [29, 30], followed by an IFA for latent antibodies to HHV-8. The mIFA has been found to be sensitive in detecting antibodies to HHV-8 and most practical for testing large numbers of samples, even though the interpretations of the results could be subjective. Recently, an HHV-8–specific ELISA was developed and it is now commercially available [33]; therefore, all IFA-positive samples were further confirmed by ELISA, and only those patients who tested positive by both IFA and ELISA were considered to have seroconverted. Only mothers in groups 3 and 4 who had negative serological test results at delivery were tested for HHV-8 infection. Mothers who tested negative at the time of delivery but then tested positive 12 months later were con-

Table 2. Sociodemographic characteristics of 485 mother-infant pairs, by results of human herpesvirus (HHV) 8 serological analysis.

Characteristic	HHV-8$^+$ mothers (n = 236)	HHV-8$^-$ mothers (n = 249)
Age, mean years (range)	24.9 (14–41)	24.9 (14–43)
Household size, mean no. of members (range)	4.8 (1–14)	5.0 (1–17)
Household income		
>$75.00/month	15	18
$75.00/month	199	204
NA	22	27
Marital status		
Single	15	20
Married	214	221
Other[a]	7	8
Duration of education, years		
7	140	128
>7	96	121
Tribe		
Bemba	62	78
Nyanja	122	112
Other	52	59
Employment status		
Employed	30	55
Unemployed	206	194
Sex of infant		
Female	119	116
Male	117	133
No. of infants breast-fed/ total no. of infants (%)	232/236 (98)	248/249 (99)

NOTE. Data are no. of mothers, except where noted. NA, not available.

[a] Includes divorced, widowed, and cohabiting mothers.

Table 3. Twelve-month follow-up cohort analyzed for human herpesvirus (HHV) 8 and human immunodeficiency virus (HIV) type 1 infection, by maternal serostatus.

Group (serostatus)	Infants (n = 485)	Mothers (n = 416)	Deaths[a]		Lost to follow-up[b]
			Infants	Mothers	
1 (HHV-8$^+$/HIV-1$^+$)	52	52[c]	21[d]	1	8
2 (HHV-8$^+$/HIV-1$^-$)	184	156	7	0	26
3 (HHV-8$^-$/HIV-1$^+$)	83	52	22[e]	2	23
4 (HHV-8$^-$/HIV-1$^-$)	166	156	4	0	2

NOTE. Data are no. of subjects.

[a] No. of deaths within 12-month postdelivery period from the active follow-up cohort.

[b] No. of patients from active cohort who either withdrew from the study or relocated to an untraceable location by 12 months after delivery; they were not tested for HHV-8 infection.

[c] Mothers not tested for infection; they were positive at delivery but still were included in cohort.

[d] A significant increase in the no. of deaths by 12 months after delivery was observed among infants born to HIV-1–positive mothers (groups 1 and 3) vs. HIV-1–negative mothers (groups 2 and 4) (odds ratio [OR], 10.1; 95% confidence interval [CI], 5.08–20.2).

[e] No significant difference in deaths was observed among infants born to HHV-8–positive mothers (groups 1 and 2) vs. HHV-8–negative mothers (groups 3 and 4) (OR, 1.14; 95% CI, 0.65–1.99).

sidered to have seroconverted. Infants from all groups were tested for infection with HHV-8. To exclude the possibility that maternal antibodies acquired transplacentally and/or through breast-feeding were producing false positive results in our infant population at 12 months, the end-point titer for all positive infant samples was compared with the corresponding sample taken at delivery or, if available, with a sample taken at a time point in between. An infant with an end-point antibody titer at 6 or 12 months after delivery higher than that at delivery was considered to have decreasing maternal antibodies being replaced by increasing levels of infant antibodies as a result of HHV-8 infection. These infants were then considered to be infected with HHV-8. The end-point titration for all infected infants is shown in figure 1. Similarly, titers were measured for mothers who became infected (data not shown).

The incident infection rates of HHV-8 for all mothers and infants in our study are shown in table 4. The incident infection rate was calculated for the total infant and mother population and within each group by dividing the number of IFA- and ELISA-positive samples by the number of person-years. Overall, the HHV-8 infection rate for the infant population during the first 12 months after delivery was 3.2 infections/100 person-years, with 18 infections/558 person-years . In our study groups, HHV-8 incident infection rates were 2.9 (2 infections/69.2 person-years) and 4.8 (10 infections/206.6 person-years) infections/100 person-years for infants in groups 1 and 2, respectively, and 2.7 (3 infections/112 person-years) and 1.8 (3 infections/170.2 person-years) infections/100 person-years for infants in groups 3 and 4, respectively. No association was observed between the infection rates among infants born to HHV-8–positive mothers (groups 1 and 2) and those born to HHV-8–negative mothers (groups 3 and 4) (OR, 2.09; 95% CI, 0.77–5.66; table 4). Of the 6 infants from groups 3 and 4 who were infected, only 1 had a mother who seroconverted in the same time period, although samples for 2 of the mothers were not available for testing at 12 months after delivery. The infection rate for the mothers was 5.3 infections/100 person-years, with 4.4 (3 infections/67.5 person-years) and 5.7 (9 infections/157.8 person-years) infections/100 person-years in groups 3 and 4, respectively. To analyze the role of maternal HIV-1 infection at delivery in HHV-8 infection by 12 months after delivery, the number of infections was compared between our study groups for both infants and mothers. For the infants, the number born to HHV-8– and HIV-1–positive mothers (group 1) was compared with the number born to HHV-8–positive mothers only (group 2). No significant difference between group 1 (2 infections/69.2 person-years) and group 2 (10 infections/206.6 person-years) was observed (OR, 0.59; 95% CI, 0.13–2.74; table 4). In addition, the incident HHV-8 infection rates among infants born to HIV-1–positive mothers in groups 1 and 3 (2.9 and 2.7, respectively) were similar to those among infants born to HIV-1–seronegative mothers in groups 2 and 4 (4.8 and 1.8, respectively). This was also true for the mothers examined. The incident HHV-8 infection rate by 12 months after delivery was not significantly different for group 3 HIV-1–positive mothers (4.4) and group 4 HIV-1–negative mothers (5.7) (OR, 0.77; 95% CI, 0.20–2.93).

Antibody profile of infants who seroconverted to HHV-8 positive. After examining the infant population for HHV-8 infection, we next wanted to determine the patterns of antibodies to HHV-8 over the 12-month postdelivery period and to examine whether these patterns indicated a specific route of transmission. The end-point titer at 0 (delivery), 6, and 12 months after delivery was determined using the mIFA for 14 of the 18 seropositive infants. All infants had at least 2 samples available for the 12-month postdelivery period, but 4 infants did not have a baseline sample available for titration, and 6 infants did not have a 6-month sample available. The antibody titers for the HHV-8–positive infants are shown in figure 1. In contrast to infants born to HIV-1–positive mothers, infants born to HHV-8–positive mothers generally had low titers of passively acquired HHV-8 antibodies at delivery (40–160 or 5–7 $\log_2$ U). Three main antibody response patterns emerged. First, a rapid increase in antibody titer by 6 months after delivery, followed by a decrease through month 12, was seen in infants 1, 7, 9, and 14. Second, a slow increase in antibody titer throughout the 12 months was seen in infant 10. Third, an increase in antibody titer from 6 to 12 months after delivery was seen in infants 2, 4, 8, and 12. Previously, we did not observe a relationship between the lytic antibody titer of the

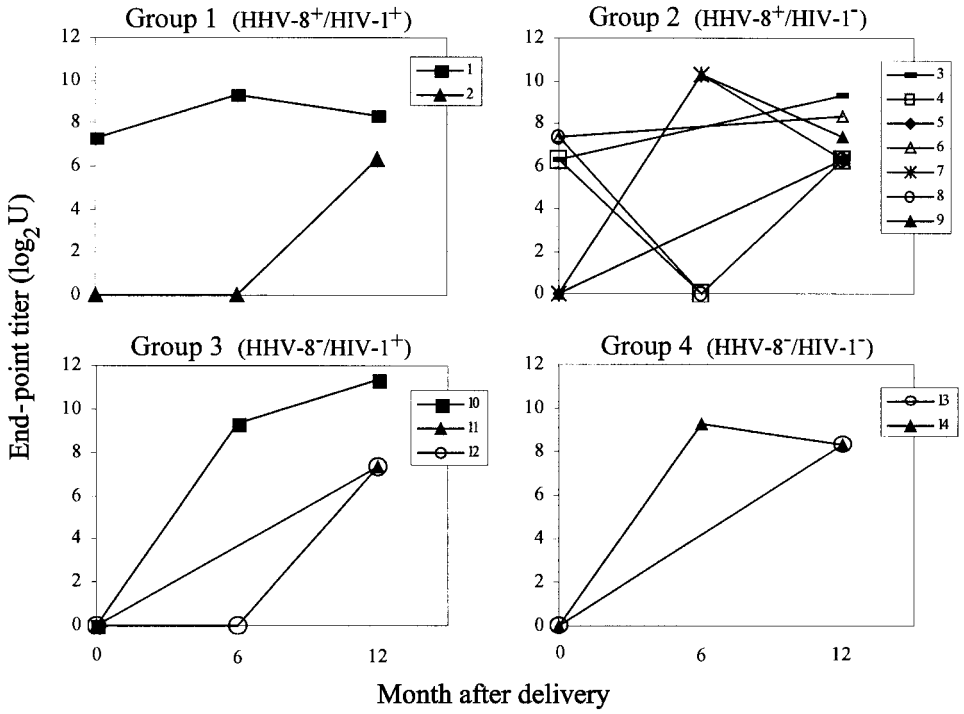

Figure 1. Human herpesvirus (HHV) 8 IgG antibody titer at delivery and at 6 and 12 months after delivery for infants who became HHV-8–positive by age 12 months. Serial 2-fold dilutions of each test serum sample were tested by monoclonal antibody–enhanced immunofluorescence assay, beginning with 1:40, as in described in Materials and Methods. The inverse of the last positive dilution was considered to be the end-point titer and was expressed as a $\log_2$ value. Infants with no 6-month sample available for analysis have a single line from delivery to 12 months. Each point represents an individual patient (patient nos. are given in the key on the right side of each panel). HIV-1, human immunodeficiency virus type 1.

mother and transmission to the infant; therefore, we did not measure antibody titers of the mothers.

HHV-8 PCR analysis of seroconverting infants from groups 1 and 2. To determine whether mother-to-child transmission had occurred in utero for the infants who became infected, HHV-8 PCR was performed on PBMC DNA from all the infected infants of HHV-8–seropositive mothers (groups 1 and 2). Available PBMC from infected infants from both groups were obtained at the time of delivery, and DNA was extracted. Of those infants who had control *GAPDH*-amplifiable DNA, 2 (16%) of 12 were found to be positive for HHV-8 DNA at the time of delivery and were born to mothers in group 2. In addition to *ORF26*, the 2 DNA-positive samples were confirmed with primers specific for *gB* and *K1* genes.

Risk factors for HHV-8 infection during the 12-month post-delivery period. Statistical analysis was performed to examine any potential risk factors that may be associated with HHV-8 infection in both infants and mothers and to further investigate possible transmission routes (table 5). For HHV-8–positive infants, we observed a significant relationship with the mother's being employed outside the home (OR, 5.14; 95% CI, 1.98–13.4) and an inverse association with the mother's being a homemaker (OR, 0.17; 95% CI, 0.07–0.46). On the other hand, all other potential demographic or health-related factors examined—including infant sex; mother's history of genital warts, genital herpes, and syphilis; the presence of skin lesions on the mother; blood transfusion in the infant; bleeding during labor; number of mother's sex partners; positive HIV-1 and/or HHV-8 serostatus in the mother; household size; and number of pregnancies—were not significantly related to HHV-8 infection in the infants. In addition, HIV-1 infection in the infants during the study period was found to be significantly related to 3 examined risk factors: the mother bleeding during labor, the presence of new skin rashes or lesions, and swollen lymph nodes by 12 months after delivery (data not shown). Infection with HHV-8 among the mothers was not significantly associated with any of the factors examined, including monthly income, education, occupation, household size, history of STDs, >1 sex partner, history of blood transfusion, and the presence of new skin rashes or lesions by 12 months after delivery (table 5), as well as age, number of pregnancies, and marital status (data not shown).

DISCUSSION

The incidence of KS has risen dramatically since the onset of the AIDS epidemic in Africa, especially among children. The prevalence of HHV-8, which is now implicated in the patho-

Table 4. Human herpesvirus (HHV) 8 and human immunodeficiency virus (HIV) type 1 incident infection among infants and mothers within the 12-month postdelivery period, by maternal serostatus.

Group (serostatus)	Infants		Mothers	
	HHV-8	HIV-1	HHV-8	HIV-1
1 (HHV-8$^+$/HIV-1$^+$)	2.9 (2/69.2)[a]	18.8 (13/69.2)	NA	NA
2 (HHV-8$^+$/HIV-1$^-$)	4.8 (10/206.6)	1.9 (4/206.6)	NA	2.3 (4/174)
3 (HHV-8$^-$/HIV-1$^+$)	2.7 (3/112)[b]	17.6 (20/112)	4.4 (3/67.5)[c]	NA
4 (HHV-8$^-$/HIV-1$^-$)	1.8 (3/170.2)	0 (0/170.2)	5.7 (9/157.8)	1.9 (3/157.8)
Total	3.2 (18/558)	6.6 (37/558)	5.3 (12/225.3)	2.1 (7/331.8)

NOTE. Data are incident infections/100 person-years (actual infections/person-years). Deaths and loss to follow-up, including both mothers and infants, were used for person-year data. NA, not applicable (i.e., participants already were positive at delivery).

[a] No significant difference in HHV-8 infection between infants born to HHV-8/HIV-1 dually infected mothers and mothers who were HHV-8 positive only (odds ratio [OR], 0.59; 95% confidence interval [CI], 0.13–2.74).

[b] No significant difference in HHV-8 infection between infants born to HHV-8–positive (groups 1 and 2) and HHV-8–negative (groups 3 and 4) mothers (OR, 2.09; 95% CI, 0.77–5.66).

[c] No significant difference in HHV-8 infection between HIV-1–positive and –negative mothers (OR, 0.77; 95% CI, 0.20–2.93).

genesis of KS, has been shown to be high in adult women and children in several regions of Africa, including Zambia. Although several studies have indicated that infection with HHV-8 is likely to occur in childhood, how and when children acquire HHV-8 is still unclear. Unlike previous reports [9, 10, 12–14, 16], the present study is a longitudinal analysis of HHV-8 and HIV-1 infection among infants born to infected and uninfected mothers in a region where HHV-8 is endemic.

Very few studies have examined the incident infection rate of HHV-8, and the study presented here, to our knowledge, is the first to document this in an area where HHV-8 is endemic. Here, we report the incident infection rate of HHV-8 during the first 12 months of life in a population of MIPs examined at 12 months after delivery and for MIPs whom a documented death or loss to follow-up was observed during the study period. In our study population, a large number of patients enrolled at delivery from an ongoing study in Zambia were lost to follow-up and, thus, were not examined at 12 months after delivery. This loss was primarily due to the death of a mother or infant or because the patient was untraceable after having relocated. Therefore, interpretation of the observed incident infection rate is limited to those patients who were still active at the end of the study, those with documented deaths, or those who were lost to follow-up and may not be representative of the entire cohort. In addition, because this is the only study to examine incident infection in a region where HHV-8 is endemic, it cannot be compared directly with cross-sectional studies of seroprevalence. Nevertheless, an HHV-8 infection rate of 18 of 485 infants is in agreement with several cross-sectional studies of HHV-8 infection in hospitalized Ugandan and Italian children <12 months old [12, 13]. However, other studies using cross-sectional sampling have reported seroprevalence rates near 17% in the same age group as our study population [14]. The high seroprevalence reported could have been influenced by detection of passively acquired antibodies from the mother in these infants and/or cross-sectional sampling. The infants in the present study could have acquired HHV-8 infection either vertically via in utero or intrapartum routes or horizontally by way of postnatal infection. Postnatal transmission of HHV-8 could occur via exchange of saliva with the mother or father or via household contact with family members, such as siblings. Indeed, several studies have indicated that HHV-8 can be detected in the saliva of HHV-8–positive individuals [34]. Another possible route of postnatal transmission of HHV-8 from mother to child is via breast milk or premastication of food; to date, however, there is no direct evidence demonstrating infectious HHV-8 in breast milk or a strong association between infection and premastication.

Previous studies have suggested that mother-to-child transmission can occur either vertically or horizontally but that it is an infrequent event [16, 28, 29, 35]. In addition to serological analyses from several groups [16, 35, 36], recent detection of identical sequences of a highly variable region in the K1 gene of HHV-8 in mother-child pairs from Malawian families that exhibited a case of KS further supports the notion that mother-to-child transmission of HHV-8 exists [28]. Furthermore, our laboratory has recently reported elsewhere [29] the detection of viral DNA in PBMC at the time of delivery for 2 of 89 infants born to HHV-8–positive mothers, which suggests that in utero infection occurs but that the incidence may be low. In contrast, several studies have indicated that vertical transmission of HHV-8 is unlikely to occur. Analysis of 32 HIV-1–positive mothers and their infants did not find any evidence of HHV-8 viral DNA or antibodies at 24 months after delivery

Table 5. Sociodemographic, behavioral, and health-related risk factors by 12 months after delivery associated with human herpesvirus 8 infection among infants and mothers.

Risk factor	Infants (n = 485)			Mothers (n = 208)		
	OR	95% CI	P	OR	95% CI	P
Mother employed outside home	5.14	1.98–13.4	.0002	2.62	0.79–8.67	.10
Mother is homemaker	0.17	0.07–0.46	<.0001	0.67	0.19–2.38	.53
Education ⩽7 years	0.80	0.31–2.06	.65	0.70	0.21–2.28	.55
Household size ⩽3 members	1.21	0.47–3.12	.69	1.61	0.5–5.19	.42
Mother HIV-1$^+$	0.65	0.15–3.28	.58	1.00	0.26–3.84	1.00
Abnormal delivery[a]	0.61	0.17–2.19	.45	0.63	0.13–3.07	.57
Bleeding during labor[b]	2.05	0.25–16.6	.49	3.47	0.37–32.3	.25
Blood transfusion during delivery[c]	2.23	0.27–18.1	.44	1.03	1.01–1.06	.55
Maternal lesions or sores 12 months after delivery	1.01	0.99–1.04	.66	1.01	0.99–1.04	.68
Maternal syphilis	0.47	0.06–3.60	.46	0.65	0.08–5.27	.69
Antibiotics[d]	0.72	0.26–1.96	.51	1.14	0.23–5.63	.87
>1 Sex partner in last 3 years	1.12	0.25–5.01	.92	1.15	1.09–1.22	.17
New lesion or rash on infant 12 months after delivery	0.33	0.04–2.53	.26	NA	NA	NA
Swollen nodes in infant 12 months after delivery	1.02	1.00–1.03	.60	NA	NA	NA

NOTE. CI, confidence interval; HIV-1, human immunodeficiency virus type 1; NA, not applicable (variable is only associated with infant); OR, odds ratio.

[a] Assisted or breach delivery.
[b] History of bleeding during labor.
[c] Mother received blood transfusion during delivery.
[d] Taken by the mother during study period.

[37], whereas serological analysis of 11 infants from HHV-8–positive mothers revealed no seroconversion by 7 months after delivery [11]. Given the fact that mother-to-child transmission is likely to occur infrequently, the lack of evidence supporting mother-to-child transmission in these studies could be due to the small population size analyzed. In the present study, several HHV-8–positive infants lacked any antibody titer at delivery but had high antibody titers at 6 months after delivery. This result, combined with the detection of HHV-8 viral DNA at delivery for 2 seroconverting infants, suggests that HHV-8 infection occurred perinatally or soon after delivery and further supports the notion that perinatal and in utero HHV-8 infection can occur. Furthermore, 1 of the mothers of the 2 PCR-positive infants was also found to be HHV-8 DNA positive, whereas all other mothers were PCR negative (data not shown). Whether perinatal infection is the major route of HHV-8 transmission in the Zambian population is a current focus of investigation.

HHV-8 infection in infants during the 12-month post-delivery study period was associated with the mother's employment status and inversely associated with the mother's being a homemaker. The significance of this association is not currently known, but a similar observation was reported in relation to KS incidence [38]. HHV-8 infection may follow a pattern similar to that of other infectious agents, in which transmission is more likely in urban settings where large numbers of people are continually interacting with others, compared with rural settings with limited personal contacts, as suggested by other researchers [14, 26, 39]. Of interest, we observed no significant association between HHV-8 and HIV-1 infection in the infant population studied, nor was there any significant association between the HIV-1 and HHV-8 status of the mothers. Infants born to mothers who were HIV-1 positive, HHV-8 positive, or both did not have significantly different HHV-8 infection rates. In fact, the infection rates observed in infants born to mothers in group 4 (HHV-8– and HIV-1–negative) were similar to those seen in the other groups, which further supports the concept that HIV-1 infection in the mothers may not influence HHV-8 infection in the infants and that mother-to-child transmission of HHV-8 may be infrequent. In addition, only 1 HHV-8–positive infant from group 4 had a mother who also was infected with HHV-8. Our findings indicate that household contacts could be a source of HHV-8 infection, but other sources outside of the family may also be involved in transmission. The relationship between HHV-8 transmission and household contact is currently being assessed.

Although primary infection with HHV-8 is considered to occur early in life in regions where HHV-8 is endemic, such as Zambia, our analysis of HHV-8 infection in previously seronegative mothers revealed an HHV-8 infection rate of 5.3 infections/100 person-years, which indicates that HHV-8 transmission to adults does occur. These results are congruent with reports from other regions of Africa, where the cross-sectional seroprevalence of HHV-8 increases with age, suggesting that

there is continued infection in the adult population [17, 39–41]. Again, because the present study is prospective in design, direct comparison with other cross-sectional studies is difficult. Nevertheless, the cross-sectional seroprevalence among our mothers at delivery was 40%, which is in agreement with other age-matched populations in Africa. Sexual transmission of HHV-8 may be the route of infection in this maternal population, because these mothers were in a sexually active age group. Alternatively, nonsexual routes of infection via familial contact with household members, including partners, children, or other family members, could also be possible. Contact outside the household with infected persons other than family members may also be sources of HHV-8 transmission to the women studied here.

In the present study, no significant association was observed between HHV-8 infection in the mothers and any of the risk factors examined, including HIV-1, other STDs, and >1 sex partner. In addition, similar HHV-8 seroconversion rates were observed for HIV-1–positive and –negative mothers (groups 3 and 4), which further indicates that HIV-1 infection is not associated with HHV-8 infection in the mothers. This is consistent with reports from Uganda and French Guiana, which found no association with several STDs or number of sex partners [16, 41]. The lack of an observed association between HIV-1 infection and HHV-8 seroconversion in the mothers during the 12-month study period is in contrast to our baseline cross-sectional screening of mothers delivery at UTH, where HIV-1 was found to be a risk factor for HHV-8 infection. This discrepancy could be due to the short follow-up period of 12 months in which the mothers were examined.

Some limitations in our study exist and should be considered. Although the socioeconomic characteristics of our study population are not significantly different between the HHV-8–positive and –negative mothers, our study participants were recruited from the main referral hospital in Zambia, which accepts mothers who have complications during pregnancy from the local clinics throughout Lusaka province. Nevertheless, most of the births in our study were normal, with few assisted or breach deliveries. In addition, the 12-month time period used to monitor seroconversion is likely to be a small window for analysis of adult infection. This fact, combined with the limited population size available for study, could have influenced our observed infection rates and subsequent risk analysis for HHV-8 and HIV-1 in adults in this population. Although our initial determination of HHV-8 infection was based on the mIFA analysis and only those confirmed by commercial ELISA were considered to be positive in the present study, the mIFA showed a higher infection rate in the infants, but many results could not be confirmed by the ELISA. Whether this result could be due to the differences in sensitivity and specificity between the assays and, more important, whether the incidence rates reported here are underestimated on the basis of our criteria is currently being investigated.

The present study was the first to document the number of incident infections of HHV-8 in an area where it is endemic and examined several risk factors that may be involved in transmission. Evidence was provided indicating that infection with HHV-8 can occur within the first 12 months of life in an area where HHV-8 is endemic and that transmission likely occurs through multiple routes, both vertical and horizontal. Data from risk factor analysis, PCR, and serum antibody titers also suggest that in utero transmission occurs but is infrequent and that postnatal transmission is a more likely route of HHV-8 infection for infants in Zambia.

Acknowledgments

We thank James Goedert and Robert Biggar for help with sample testing and experimentation; Jordan Kautz and Evan Burkala for technical assistance and manuscript preparation; Jim Campbell and Chafye Siuluta for help with statistical analyses; Daniel Perez-Filgueira and Shiu-Lok Hu for providing recombinant human immunodeficiency virus type 1 proteins; the study women and their infants; and the Zambian study team for their tireless effort in keeping the study running.

References

1. Chang Y, Cesarman E, Pessin MS, et al. Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma. Science **1994**; 266:1865–9.
2. Boshoff C, Weiss RA. Epidemiology and pathogenesis of Kaposi's sarcoma–associated herpesvirus. Philos Trans R Soc Lond B Biol Sci **2001**; 356:517–34.
3. Friedman-Kien AE, Saltzman BR. Clinical manifestations of classical, endemic African, and epidemic AIDS-associated Kaposi's sarcoma. J Am Acad Dermatol **1990**; 22:1237–50.
4. Wabinga HR, Parkin DM, Wabwire-Mangen F, Mugerwa JW. Cancer in Kampala, Uganda, in 1989–91: changes in incidence in the era of AIDS. Int J Cancer **1993**; 54:26–36.
5. Bayley AC. Occurrence, clinical behaviour and management of Kaposi's sarcoma in Zambia. Cancer Surv **1991**; 10:53–71.
6. Patil P, Elem B, Zumla A. Pattern of adult malignancies in Zambia (1980–1989) in light of the human immunodeficiency virus type 1 epidemic. J Trop Med Hyg **1995**; 98:281–4.
7. Chintu C, Athale UH, Patil PS. Childhood cancers in Zambia before and after the HIV epidemic. Arch Dis Child **1995**; 73:100–5.
8. Patil PS, Elem B, Gwavava NJ, Urban MI. The pattern of paediatric malignancy in Zambia (1980–1989): a hospital-based histopathological study. J Trop Med Hyg **1992**; 95:124–7.
9. Kasolo FC, Mpabalwani E, Gompels UA. Infection with AIDS-related herpesviruses in human immunodeficiency virus–negative infants and endemic childhood Kaposi's sarcoma in Africa. J Gen Virol **1997**; 78: 847–55.
10. 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.
11. Lyall EG, Patton GS, Sheldon J, et al. Evidence for horizontal and not vertical transmission of human herpesvirus 8 in children born to hu-

man immunodeficiency virus–infected mothers. Pediatr Infect Dis J **1999**; 18:795–9.
12. Whitby D, Luppi M, Sabin C, et al. Detection of antibodies to human herpesvirus 8 in Italian children: evidence for horizontal transmission. Br J Cancer **2000**; 82:702–4.
13. Mayama S, Cuevas LE, Sheldon J, et al. Prevalence and transmission of Kaposi's sarcoma–associated herpesvirus (human herpesvirus 8) in Ugandan children and adolescents. Int J Cancer **1998**; 77:817–20.
14. Andreoni M, El-Sawaf G, Rezza G, et al. High seroprevalence of antibodies to human herpesvirus–8 in Egyptian children: evidence of nonsexual transmission. J Natl Cancer Inst **1999**; 91:465–9.
15. Serraino D, Locatelli M, Songini M, et al. Human herpes virus–8 infection among pregnant women and their children: results from the Sardinia-IDDM Study 2. Int J Cancer **2001**; 91:740–1.
16. Plancoulaine S, Abel L, van Beveren M, et al. Human herpesvirus 8 transmission from mother to child and between siblings in an endemic population. Lancet **2000**; 356:1062–5.
17. Olsen SJ, Chang Y, Moore PS, Biggar RJ, Melbye M. Increasing Kaposi's sarcoma–associated herpesvirus seroprevalence with age in a highly Kaposi's sarcoma endemic region, Zambia in 1985. AIDS **1998**; 12:1921–5.
18. He J, Bhat G, Kankasa C, et al. Seroprevalence of human herpesvirus 8 among Zambian women of childbearing age without Kaposi's sarcoma (KS) and mother-child pairs with KS. J Infect Dis **1998**; 178:1787–90.
19. Blauvelt A, Sei S, Cook PM, Schulz TF, Jeang KT. Human herpesvirus 8 infection occurs following adolescence in the United States. J Infect Dis **1997**; 176:771–4.
20. Dukers NH, Renwick N, Prins M, et al. Risk factors for human herpesvirus 8 seropositivity and seroconversion in a cohort of homosexual men. Am J Epidemiol **2000**; 151:213–24.
21. Martin JN, Ganem DE, Osmond DH, Page-Shafer KA, Macrae D, Kedes DH. Sexual transmission and the natural history of human herpesvirus 8 infection. N Engl J Med **1998**; 338:948–54.
22. Melbye M, Cook PM, Hjalgrim H, et al. Risk factors for Kaposi's-sarcoma–associated herpesvirus (KSHV/HHV-8) seropositivity in a cohort of homosexual men, 1981–1996. Int J Cancer **1998**; 77:543–8.
23. Casper C, Wald A, Pauk J, Tabet SR, Corey L, Celum CL. Correlates of prevalent and incident Kaposi's sarcoma–associated herpesvirus infection in men who have sex with men. J Infect Dis **2002**; 185:990–3.
24. Challine D, Roudot-Thoraval F, Sarah T, et al. Seroprevalence of human herpes virus 8 antibody in populations at high or low risk of transfusion, graft, or sexual transmission of viruses. Transfusion **2001**; 41: 1120–5.
25. Sosa C, Klaskala W, Chandran B, et al. Human herpesvirus 8 as a potential sexually transmitted agent in Honduras. J Infect Dis **1998**; 178:547–51.
26. Baillargeon J, Leach CT, Deng JH, Gao SJ, Jenson HB. High prevalence of human herpesvirus 8 (HHV-8) infection in south Texas children. J Med Virol **2002**; 67:542–8.
27. Calabro ML, Sheldon J, Favero A, et al. Seroprevalence of Kaposi's sarcoma–associated herpesvirus/human herpesvirus 8 in several regions of Italy. J Hum Virol **1998**; 1:207–13.
28. Cook RD, Hodgson TA, Waugh AC, et al. Mixed patterns of transmission of human herpesvirus–8 (Kaposi's sarcoma–associated herpesvirus) in Malawian families. J Gen Virol **2002**; 83:1613–9.
29. Mantina H, Kankasa C, Klaskala W, et al. Vertical transmission of Kaposi's sarcoma–associated herpesvirus. Int J Cancer **2001**; 94:749–52.
30. Lennette ET, Blackbourn DJ, Levy JA. Antibodies to human herpesvirus type 8 in the general population and in Kaposi's sarcoma patients. Lancet **1996**; 348:858–61.
31. Arvanitakis L, Mesri EA, Nador RG, et al. Establishment and characterization of a primary effusion (body cavity–based) lymphoma cell line (BC-3) harboring Kaposi's sarcoma–associated herpesvirus (KSHV/HHV-8) in the absence of Epstein-Barr virus. Blood **1996**; 88:2648–54.
32. Clements GB, Klein G, Povey S. Production by EBV infection of an EBNA-positive subline from an EBNA-negative human lymphoma cell line without detectable EBV DNA. Int J Cancer **1975**; 16:125–33.
33. Chatlynne LG, Lapps W, Handy M, et al. Detection and titration of human herpesvirus–8–specific antibodies in sera from blood donors, acquired immunodeficiency syndrome patients, and Kaposi's sarcoma patients using a whole virus enzyme-linked immunosorbent assay. Blood **1998**; 92:53–8.
34. Corey L, Brodie S, Huang ML, Koelle DM, Wald A. HHV-8 infection: a model for reactivation and transmission. Rev Med Virol **2002**; 12:47–63.
35. Bourboulia D, Whitby D, Boshoff C, et al. Serologic evidence for mother-to-child transmission of Kaposi sarcoma–associated herpesvirus infection. JAMA **1998**; 280:31–2.
36. Sitas F, Newton R, Boshoff C. Increasing probability of mother-to-child transmission of HHV-8 with increasing maternal antibody titer for HHV-8. N Engl J Med **1999**; 340:1923.
37. Calabro ML, Gasperini P, Barbierato M, et al. A search for human herpesvirus 8 (HHV-8) in HIV-1 infected mothers and their infants does not suggest vertical transmission of HHV-8. Int J Cancer **2000**; 85:296–7.
38. Ziegler JL, Newton R, Katongole-Mbidde E, et al. Risk factors for Kaposi's sarcoma in HIV-positive subjects in Uganda. AIDS **1997**; 11: 1619–26.
39. Sitas F, Carrara H, Beral V, et al. Antibodies against human herpesvirus 8 in black South African patients with cancer. N Engl J Med **1999**; 340:1863–71.
40. Rezza G, Tchangmena OB, Andreoni M, et al. Prevalence and risk factors for human herpesvirus 8 infection in northern Cameroon. Sex Transm Dis **2000**; 27:159–64.
41. Wawer MJ, Eng SM, Serwadda D, et al. Prevalence of Kaposi sarcoma–associated herpesvirus compared with selected sexually transmitted diseases in adolescents and young adults in rural Rakai District, Uganda. Sex Transm Dis **2001**; 28:77–81.