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Original Contribution

Early Childhood Infection by Human Herpesvirus 8 in Zambia and the Role of Human Immunodeficiency Virus Type 1 Coinfection in a Highly Endemic Area

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Kaposi's sarcoma occurs at high incidence among Zambian adults and children, but there is a paucity of data on human herpesvirus 8 (HHV-8) incidence and routes of infection, especially in children. Between 1998 and 2004, the authors conducted a prospective study of viral transmission in a cohort of 684 children in Lusaka, Zambia, to estimate the annual incidence of HHV-8 from birth through 48 months of age. Maternal and pediatric human immunodeficiency virus type 1 (HIV-1) infection status was also determined. The results, based on 1,532 child-years of follow-up, showed that HHV-8 seroconversion occurs early in life. The incidence rate of HHV-8 seroconversion was 13.8 infections per 100 child-years by 48 months of age. HIV-1-infected children were at substantially higher risk for HHV-8 seroconversion (adjusted hazard ratio = 4.60, 95% confidence interval: 2.93, 7.22). Maternal HIV-1 and HHV-8 infection status were not independently associated with risk of HHV-8 seroconversion in the child. HHV-8 antibody titers in children followed at all consecutive time points revealed seroreversion of HHV-8 antibodies, with undetectable titers in some children at one or more time points after seroconversion. These results demonstrate that cross-sectional serologic screening probably underestimates true HHV-8 seroprevalence in young Zambian children because of fluctuations in detectable antibody titers.

herpesvirus 8, human; HIV-1; infection; sarcoma, Kaposi; Zambia

Abbreviations: HHV-8, human herpesvirus 8; HIV-1, human immunodeficiency virus type 1; mIFA, monoclonal antibody-enhanced immunofluorescence assay; Sf9, *Spodoptera frugiperda* clone 9.

Human herpesvirus 8 (HHV-8) is the infectious etiologic agent of all forms of Kaposi's sarcoma, primary effusion lymphoma, and multicentric Castleman's disease (1–4). The global distributions of HHV-8 seroprevalence and Kaposi's sarcoma incidence are uneven (5). HHV-8 seroprevalence is generally low in the United States and Northern Europe, but it ranges from 20 percent to 80 percent in adult populations in Africa and the Mediterranean (6–11). In a previous study, He et al. (12) demonstrated that HHV-8 seroprevalence among adolescent and childbearing women in Zambia is approximately 50 percent.

The exact routes of HHV-8 transmission are still unclear and may differ by geographic region and risk group. Horizontal transmission via heterosexual and homosexual contact has been reported in adults (13–15). Vertical transmission to children seems to occur at a very low rate; a likely source of nonsexual transmission is saliva, and the possibility of transmission through breast milk is still controversial

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(16–18). A report from Uganda provided evidence for HHV-8 transmission through blood transfusion (19). In Kaposi's sarcoma-endemic regions, primary HHV-8 infection has been reported to occur during early childhood, suggesting that transmission occurs early in life (20, 21). Among children, HHV-8 seroprevalence generally increases with age, which suggests that horizontal transmission may play an important role (9, 21, 22). Epidemiologic studies from sub-Saharan Africa report a seroprevalence of 20–68 percent in adolescents (22–24). Socioeconomic factors such as low parental education, low household income, and use of a communal water source are associated with HHV-8 infection in African children; in addition, maternal coinfection with HHV-8 and human immunodeficiency virus type 1 (HIV-1) may be an important risk factor (20, 22, 25–27).

In Zambia, coincident with the emergence of the HIV-1 epidemic, there was a significant increase in the incidence of Kaposi's sarcoma in adults and children (28–30). By 1992, Kaposi's sarcoma accounted for approximately 25 percent of all childhood cancers diagnosed in Lusaka, the capital of Zambia (31). Previously, Mantina et al. (18) reported infrequent detection of HHV-8 DNA in Zambian infants born to HHV-8-infected mothers, suggesting a low level of in utero transmission. However, this fails to account for the high seroprevalence levels observed in early childhood. Zambian children appear to contract HHV-8 infection early in life, but the extent of HHV-8 infection, how children acquire the virus, and whether HIV-1 infection in the child is a risk factor remain unclear.

We evaluated early childhood incidence of HHV-8 seroconversion prospectively in a longitudinal cohort study of infants followed from birth through age 48 months. Furthermore, we assessed whether maternal and pediatric HIV-1 infections were associated with higher risk of HHV-8 acquisition during early childhood in Zambia.

MATERIALS AND METHODS

Setting

Between October 1998 and April 2004, pregnant women visiting the labor ward at University Teaching Hospital in Lusaka, Zambia, were screened for HHV-8 and HIV-1 (32). Women in early stages of labor were enrolled in a prospective cohort study after being counseled and educated about the study and giving written informed consent. The study was approved by the institutional review boards of the University of Zambia, the University of Nebraska, and the University of Miami.

Study population

At delivery, mothers were divided into four groups based on single or dual seropositivity for HIV-1 and/or HHV-8. After delivery, mothers were encouraged to return with their children for follow-up visits. A total of 1,424 mother-infant pairs who returned for at least one postpartum visit constituted our longitudinal cohort (figure 1). This analysis included 684 children who survived and were followed for at least 24 months. Children who did not return at age 24 months ($n = 740$) were excluded from this analysis; reasons for exclusion included early mortality, early withdrawal, and loss to follow-up before HIV-1 serostatus could be reliably established. Children born to HIV-1-positive mothers were tested at 24 months or later to determine HIV-1 status. Here we report data collected from the 684 children who survived beyond 24 months of age and were followed prospectively for evaluation of both HHV-8 and HIV-1 seropositivity between 12 and 48 months of age. Of these 684 children, 54 percent (370/684) of the infants were born to HHV-8-seropositive mothers and 22 percent (151/684) were born to HIV-1-seropositive mothers. By 24 months of age, 6 percent (41/684) of the children tested positive for HIV-1.

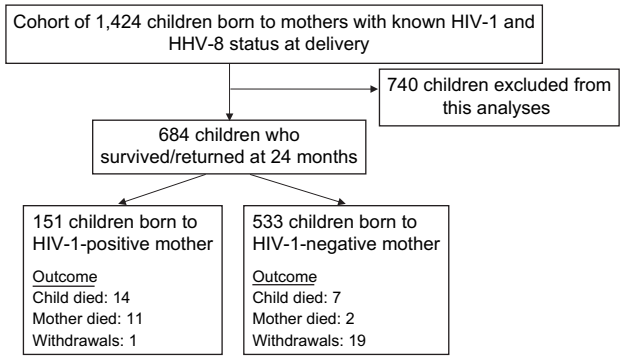

FIGURE 1. Outline of a longitudinal study of human herpesvirus 8 (HHV-8) among children in Lusaka, Zambia, 1998–2004. Of the total cohort, 740 children were excluded from the analysis because of early mortality, early withdrawal, or loss to follow-up before human immunodeficiency virus type 1 (HIV-1) serostatus could be reliably established. "Outcome" indicates the reasons for attrition between 24 and 48 months of age.

Serologic testing for HHV-8 and HIV-1

HHV-8 serology. Blood specimens were collected annually from children at birth and 12, 24, 36, and 48 months after birth. Specimens were coded by means of a unique identification number assigned to each mother-infant pair and were analyzed without knowledge of the personal identity of the study participants. Plasma was screened for evidence of HHV-8 seroconversion. Age at HHV-8 seroconversion was defined as the age at which the first HHV-8-positive test result was obtained using the assays described. To rule out detection of transplacental maternal HHV-8 antibodies, plasma from children younger than 12 months of age was not tested. In addition, the plasma of all HHV-8-seropositive children at 12 months who were born to HHV-8-seropositive mothers was titered at birth, at 6 months, and at 12 months to rule out detection of maternal antibodies.

BC-3 monoclonal antibody-enhanced immunofluorescence assay. Antibodies against HHV-8 were detected by monoclonal antibody-enhanced immunofluorescence assay (mIFA) as described previously (33). BC-3 cells (American Type Culture Collection, Manassas, Virginia) stimulated by tetradecanoyl phorbol acetate were fixed and permeabilized, and mIFA was carried out as described (32). To reduce

subjectivity in observing specific fluorescence, slides were read independently by two laboratory workers. All plasma determined to be positive by BC-3 mIFA was confirmed using *Spodoptera frugiperda* clone 9 (Sf9) mIFA as described below. For determination of HHV-8 antibody titers, serial two-fold dilutions of plasma were performed, and each dilution was assayed using the BC-3 mIFA. The inverse of the last dilution that tested positive was taken as the endpoint titer.

Sf9 monoclonal antibody-enhanced immunofluorescence assay. Recombinant baculoviruses expressing the glutathione *S*-transferase-tagged lytic proteins ORF65 and K8.1A and the latent protein ORF73 (provided by Dr. Bala Chandran, Rosalind Franklin University of Medicine and Science, Chicago, Illinois), were used to develop an Sf9 mIFA. Baculovirus-infected Sf9 cells expressing glutathione *S*-transferase alone were used as a negative control to detect background and nonspecific fluorescence. All infections were initiated separately, harvested at 72 hours post-infection, and fixed using the BC-3 cell method. The Sf9 mIFA procedure was similar to the BC-3 mIFA. A sample was considered HHV-8-seropositive only if it was positive at a standard serum dilution of 1:40 for both the BC-3 mIFA and the Sf9 mIFA (with at least one antigen). The quality of the slides was monitored for every batch, and appropriate positive and negative controls were used every time mIFAs were conducted.

HIV-1 serology. Plasma from mothers (at delivery) and from children born to HIV-1-positive mothers (at 24 months or older) was screened for HIV-1 antibodies. Human immunodeficiency virus type 2 infection has not been reported in Zambia (34, 35). Children born to HIV-1-negative mothers were assumed to be HIV-1-negative. Children younger than 24 months were not screened for HIV-1 antibodies because of the risk of detecting persisting transplacental maternal antibodies. Plasma was screened by means of a standard rapid HIV-1 kit (Capillus HIV-1/2 agglutination test kit; Trinity Biotech PLC, Bray, Ireland) and confirmed by the Abbott Determine HIV-1/2 enzyme immunoassay test kit (Abbott Laboratories, Chicago, Illinois).

Statistical and analytic methods

The crude incidence rate per 100 child-years was calculated by dividing the number of new HHV-8 seroconverters by the total number of child-years at risk and multiplying by 100. Children contributed HHV-8-free child-years at risk until they tested positive for HHV-8. Because the actual date of seroconversion within the 1-year interval is unknown, a child was considered at risk for only half of the year in which he/she tested positive. All data were right-censored at 48 months of age. We present the crude incidence rate per 100 child-years according to covariates. We also compared stratum-specific incidence rates using the crude incidence rate ratio and its 95 percent confidence interval. To evaluate the risk of HHV-8 seroconversion over time, we estimated hazard rate ratios and 95 percent confidence intervals using Cox proportional hazards modeling in which we examined various characteristics individually and simultaneously to obtain adjusted hazard rate ratios and to generate hazard curves that represented the child's risk of seroconversion for HHV-8 over time (36). All comparisons were considered statistically significant at $p \leq 0.05$. Data were analyzed using the statistical software packages SAS, version 9.1 (SAS Institute, Inc., Cary, North Carolina), and SPSS, version 15 (SPSS, Inc., Chicago, Illinois).

RESULTS

HHV-8 incidence and associated risk factors

Based on 1,532 total child-years of follow-up, the incidence rate of HHV-8 seroconversion in Zambian children was 13.8 infections per 100 child-years over 48 months (table 1). We observed a statistically significant increased risk of seroconversion among HIV-1-positive children after adjusting for multiple covariates (adjusted hazard rate ratio = 4.60, 95 percent confidence interval: 2.93, 7.22). No statistically significant difference in hazard rates was observed by sex of the child or mother's HHV-8 infection status at delivery. The association between HHV-8 seroconversion in children and maternal HIV-1 seropositivity was no longer statistically significant when results were adjusted for HIV-1 seropositivity of the child. Similar results were observed in children born to HIV-1-positive, HHV-8-negative mothers in comparison with children born to HHV-8- and HIV-1-negative mothers.

Table 2 shows a constant rate of annual HHV-8 infection occurring in children, reported as the crude incidence rate per 100 child-years. We also examined these crude incidence rates by maternal and child HIV-1 coinfection status and maternal HHV-8 serostatus and observed results similar to those presented in table 1. Note that none of the HIV-1-infected children in the cohort returned for follow-up after 36 months.

Figure 2 presents the probabilities of HHV-8 seroconversion as hazard curves, stratified by covariates and adjusted for confounders. These graphs show that there is little difference in the probability of HHV-8 seroconversion according to maternal HHV-8 status ($p = 0.2$), maternal HIV-1 infection ($p = 0.41$), or maternal HIV-1 and HHV-8 coinfection status ($p = 0.38$) (panels *A*, *B*, and *C*). The most important independent risk factor was HIV-1 infection in children, where the probability of HHV-8 seroconversion was significantly lower in the HIV-1-uninfected children than in the infected children ($p < 0.001$) (panel *D*).

Figure 3 (panel *A*) shows the annual (12-month) percentage of children newly acquiring HHV-8 infection within each period. Because HIV-1 infection of the child was the most prominent risk factor observed for HHV-8 seroconversion, we also present annual percentages of seroconversion according to the child's HIV-1 status at 24 months (panel *B*). This figure shows that HHV-8 seroconversion in HIV-1-uninfected children was essentially constant during each annual period, while HHV-8 seroconversion in HIV-1-infected children was significantly higher and increased during each annual period.

Variations in antibody titers in HHV-8-positive children over time

The different HHV-8 seroreactivity patterns are summarized in table 3. We monitored the HHV-8 antibody

TABLE 1. Incidence of human herpesvirus 8 infection per 100 child-years and associated hazard rate ratios in a longitudinal cohort study of 684 children, by maternal and child characteristics, Lusaka, Zambia, 1998–2004

Characteristic	No. of children	%	No. of HHV-8*-positive children	No. of HHV-8-free child-years	Incidence rate per 100 child-years	Unadjusted HRR*	95% CI*	Adjusted HRR	95% CI
Total cohort	684		212	1,532.1	13.8				
Sex of child									
Male	349	51	101	799.3	12.6	0.84	0.64, 1.10	0.84†	0.64, 1.10
Female	335	49	111	732.8	15.2	1.00‡		1.00‡	
Mother's HIV-1* status at delivery									
Uninfected	533	78	140	1,194.7	11.7	1.00‡		1.00‡	
Infected	151	22	72	337.5	21.3	1.79	1.35, 2.38	1.16†	0.82, 1.63
Mother's HHV-8 status at delivery									
Negative	314	46	104	672.5	15.5	1.00‡		1.00‡	
Positive	370	54	108	859.7	12.6	0.82	0.62, 1.07	0.84†	0.64, 1.10
Mother's HIV-1 and HHV-8 status§									
HIV-1− and HHV-8−	240	35	63	511.8	12.3	1.00‡		1.00‡	
HIV-1− and HHV-8+	293	43	77	682.9	11.3	0.92	0.66, 1.29	0.91¶	0.65, 1.26
HIV-1+ and HHV-8−	74	11	41	160.7	25.5	2.05	1.38, 3.03	1.29¶	0.83, 2.02
HIV-1+ and HHV-8+	77	11	31	176.8	17.5	1.40	0.91, 2.15	0.94¶	0.59, 1.49
Child's HIV-1 status at age 24 months									
Uninfected	643	94	178	1,478.1	12.0	1.00‡		1.00‡	
Infected	41	6	34	54.0	63.0	5.17	3.55, 7.52	4.60†	2.93, 7.22

* HHV-8, human herpesvirus 8; HRR, hazard rate ratio; CI, confidence interval; HIV-1, human immunodeficiency virus type 1.
† Adjusted for sex of the child, mother's HHV-8 status, mother's HIV-1 status, and child's HIV-1 status at age 24 months.
‡ Reference category.
§ A positive sign (+) indicates seropositivity and a negative sign (−) indicates seronegativity.
¶ Adjusted for sex of the child and child's HIV-1 status at age 24 months.

responses of 171 children who returned for all four follow-up visits to study the temporal persistence of HHV-8 antibodies. Sixty percent (103/171) of these children were persistently HHV-8-seronegative and 68 of the 171 children (40 percent) seroconverted by 48 months, but only three of the 68 children remained persistently HHV-8-seropositive at all time points. We frequently observed fluctuations in antibody titers, which dropped below the detection limit at one or more time points, leading to seroreversion.

The titers of four representative patients are shown in figure 4 to demonstrate the fluctuations in anti-HHV-8 antibody titers over time. Patients A and B were born to HHV-8-seropositive mothers, and patients C and D were born to HHV-8-seronegative mothers.

DISCUSSION

The major strengths of the present study were its size, its prospective nature, and the availability of HHV-8 and HIV-1 coinfection data from mother-infant pairs collected at multiple time points from birth to 48 months of follow-up. These strengths enabled us to provide the first documentation of annual HHV-8 incidence rates in early childhood in an African endemic area. Our results indicate that the HIV-1 status of the child is a strong predictor of HHV-8 seroconversion. Incidence rates were generally high among these children between birth and 48 months of age. In addition, fluctuations in detectable HHV-8 titers leading to seroreversions among HHV-8-seroconverted children may produce frequent underestimation of childhood HHV-8 seroprevalence in cross-sectional studies.

The observed HHV-8 seroprevalence in Zambian children is generally comparable to prevalences reported in cross-sectional studies from other parts of sub-Saharan Africa (17, 23, 26). While the prospective, longitudinal design of this study makes it difficult to compare its results directly with those of published cross-sectional studies conducted in the region, HHV-8 seroprevalence of 20–60 percent has been reported among young children (22–24). Kaplan-Meier analysis (data not shown) estimating the probability of HHV-8 seroconversion in this longitudinally followed cohort revealed that more than 40 percent of the children seroconverted for HHV-8 by age 48 months, with a clear increase in HHV-8 seroprevalence with age. These results show that children become infected at a young age in Zambia and that adult seroprevalence levels may be reached relatively early in life. These results indicate that HIV-1-infected children are more likely to become HHV-8-infected, but it remains unclear whether increased risk is due to HIV-1-infected

TABLE 2. Crude incidence of human herpesvirus 8 per 100 child-years and associated incidence rate ratios in a longitudinal cohort study of 684 children, by maternal and pediatric human immunodeficiency virus type 1 status and maternal human herpesvirus 8 status, Lusaka, Zambia, 1998–2004

Characteristic and study period	No. of HHV-8*-positive children	No. of HHV-8-free child-years	Crude incidence rate per 100 child-years	No. of HHV-8-positive children	No. of HHV-8-free child-years	Crude incidence rate per 100 child-years	IRR*	95% confidence interval
Total cohort		*All children*						
Birth to 12 months	92	638.4	14.4					
Birth to 24 months	152	1,086.6	14.0					
Birth to 36 months	195	1,393.2	14.0					
Birth to 48 months	212	1,532.1	13.8					
Sex of child		*Female*			*Male*		$IRR_{female/male}$	
Birth to 12 months	51	309.5	16.5	41	329.0	12.5	1.32	0.88, 1.99
Birth to 24 months	78	521.2	15.0	74	565.4	13.1	1.14	0.83, 1.57
Birth to 36 months	101	668.3	15.1	94	724.9	13.0	1.17	0.88, 1.54
Birth to 48 months	111	732.8	15.2	101	799.3	12.6	1.20	0.92, 1.57
Mother's HIV-1* status at delivery		*Negative*			*Positive*		$IRR_{positive/negative}$	
Birth to 12 months	62	501.5	12.4	30	136.9	21.9	1.77	1.15, 2.74
Birth to 24 months	98	844.7	11.6	54	241.9	22.3	1.92	1.38, 2.68
Birth to 36 months	131	1,081.3	12.1	64	311.9	20.5	1.69	1.26, 2.28
Birth to 48 months	140	1,194.7	11.7	72	337.5	21.3	1.82	1.37, 2.42
Mother's HHV-8 serostatus at delivery		*Negative*			*Positive*		$IRR_{positive/negative}$	
Birth to 12 months	48	290.6	16.5	44	347.9	12.6	0.77	0.51, 1.15
Birth to 24 months	79	485.3	16.3	73	601.3	12.1	0.75	0.54, 1.02
Birth to 36 months	94	614.9	15.3	101	778.4	13.0	0.85	0.64, 1.12
Birth to 48 months	104	672.5	15.5	108	859.7	12.6	0.81	0.62, 1.06
Child's HIV-1 status at age 24 months		*Negative*			*Positive*		$IRR_{positive/negative}$	
Birth to 12 months	76	604.9	12.6	16	33.6	47.7	3.79	2.21, 6.50
Birth to 24 months	124	1,909.9	6.5	28	50.0	56.0	8.62	5.72, 13.00
Birth to 36 months	161	2,212.5	7.3	34	54.0	63.0	8.65	5.98, 12.53
Birth to 48 months	178	2,351.4	7.6	34	54.0	63.0	8.32	5.76, 12.00

* HHV-8, human herpesvirus 8; IRR, incidence rate ratio; HIV-1, human immunodeficiency virus type 1.

children's having 1) a higher likelihood of being exposed to HHV-8, 2) a higher likelihood of becoming infected when exposed, or 3) a higher likelihood of antibody detection when infected. In addition, the specific risk factors associated with each of these possibilities have yet to be determined.

Potential routes of horizontal HHV-8 transmission are poorly understood, but salivary contact may be the major route of transmission in early childhood. Our laboratory and others have previously found that HHV-8 DNA can be readily detected in the saliva of infected persons and is detected more frequently in persons with higher antibody titers (16, 37–39). While Zambian children are usually breast-fed up to the age of 18–24 months, HHV-8 cannot be easily detected in breast milk, suggesting that it is an unlikely source of infection (16). We frequently observed HHV-8 seroconversion in children born to mothers who were HHV-8-seronegative at delivery, indicating that horizontal transmission is possible in early childhood. These results are consistent with Mantina et al.'s finding that in utero infection of infants is infrequent (18). However, we cannot rule out the possibility that some children who are HHV-8-seropositive at 12 months have HHV-8 that is due to perinatal transmission. Incidence in this cohort could still be underestimated if the perinatally infected children seroreverted before age 12 months. Determining serostatus in children below age 12 months is difficult because of the presence of maternal antibodies.

In this cohort, maternal HHV-8 infection was not an independent risk factor associated with transmission of HHV-8 to children—a finding which was somewhat unexpected, because in the early phase of life the mother is usually the primary caregiver and has close contact with the child. This suggests that mothers may not be the only source of transmission to children and that other members of the household or nonfamilial contacts could be responsible for horizontal

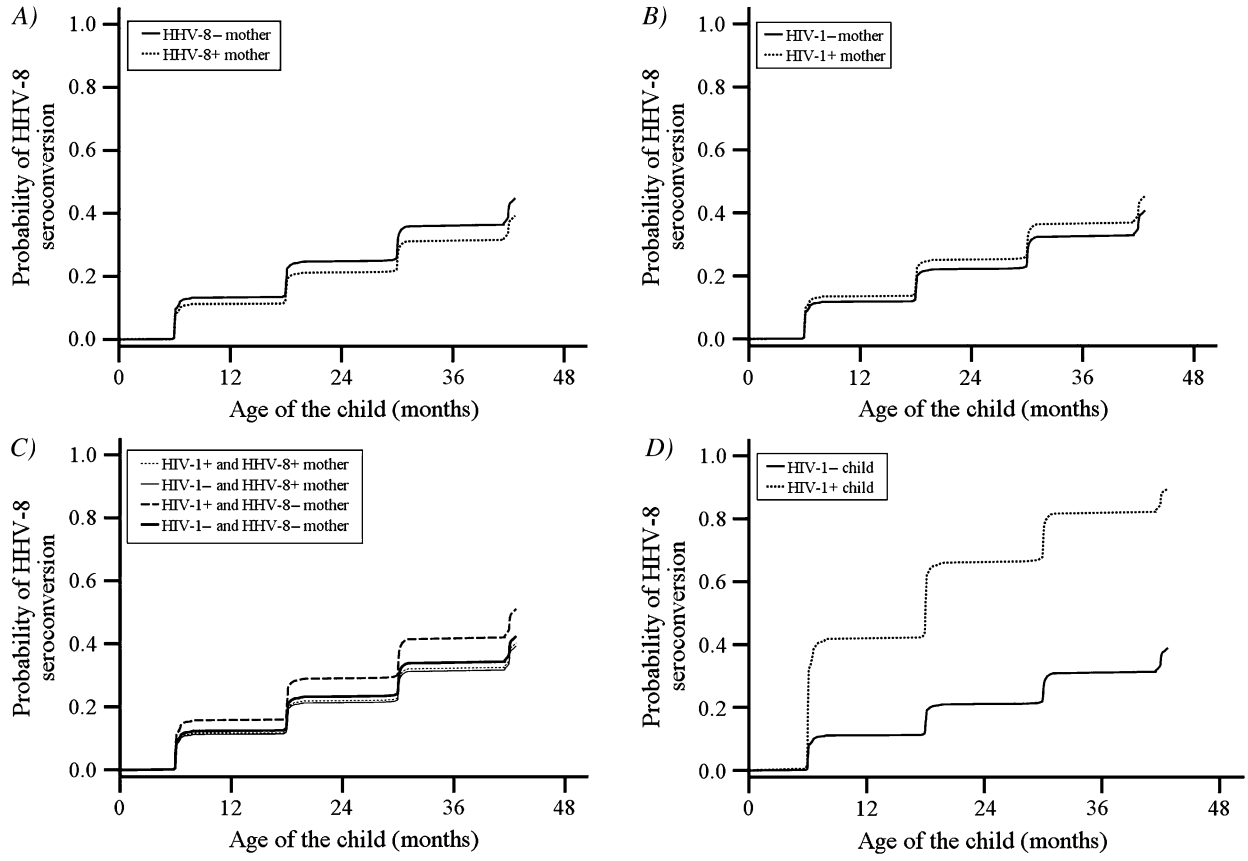

FIGURE 2. Results from survival analysis estimating the probability of becoming seropositive for human herpesvirus 8 (HHV-8) in a longitudinal cohort of 684 children followed from birth to 48 months of age, Lusaka, Zambia, 1998–2004. Results are presented by the HHV-8 serostatus of the mother at delivery (panel *A*); the human immunodeficiency virus type 1 (HIV-1) infection status of the mother at delivery (panel *B*); maternal coinfection with HIV-1 and HHV-8 at delivery (panel *C*); and the HIV-1 infection status of the child at age 24 months (panel *D*).

transmission to young children. Some reports from sub-Saharan Africa have shown a positive correlation between the HHV-8 status of the mother and that of the child (24, 26). However, other studies reported only a marginal-to-weak correlation (25, 40), and a recent study demonstrated that young infants' risk of acquiring HHV-8 infection in South Africa was not dependent on maternal serostatus (41). Molecular evidence for this has come from Uganda, where a mother and child were demonstrated to have different HHV-8 subtypes (42). The strength of this association could depend on locally common child-care practices, such as kissing, premastication of food, or sharing of food and utensils (43). The impact of such practices has not been explored fully and may be different in Zambia than in other HHV-8-endemic countries.

Brayfield et al. (16) previously reported risk factors associated with HHV-8 infection in a much smaller group of infants at 12 months while active follow-up of mother-infant pairs was still ongoing. A commercially available enzyme-linked immunosorbent assay was used to validate BC-3 mIFA results, and we observed that it underestimated HHV-8 seroprevalence by missing patients with distinct punctate nuclear staining, which led us to develop the Sf9 mIFA. We believe that patients with specific punctate staining could have low-titer antibodies that were missed by enzyme-linked immunosorbent assay because of the higher optical density cutoff values. Employing two assays as part of an algorithm provided us with a reliable, highly specific and conservative method. Sf9 mIFAs have matched negative controls that are lacking for BC-3 mIFAs, thus contributing to a low number of false-positive results. Analysis of a panel of positive and negative control serum samples revealed a high concordance between the two mIFAs ($\kappa = 0.75$; unpublished data). Zhu et al. (44) have suggested that confirmation of HHV-8 serostatus should not be based on a single antigen, since infected persons may demonstrate variable reactivity against different antigens. This variability may explain the fluctuations in the anti-HHV-8 antibody titers seen in this study and may explain why certain children have undetectable antibody titers during follow-up. Such variation has been observed in adults, and seroreversion has been reported for other herpesviruses and for hepatitis C virus (45–49). HHV-8 DNA has been detected in certain HHV-8-seronegative patients, and biopsy-proven Kaposi's sarcoma patients in full remission have been reported to undergo HHV-8 seroreversion (50, 51).

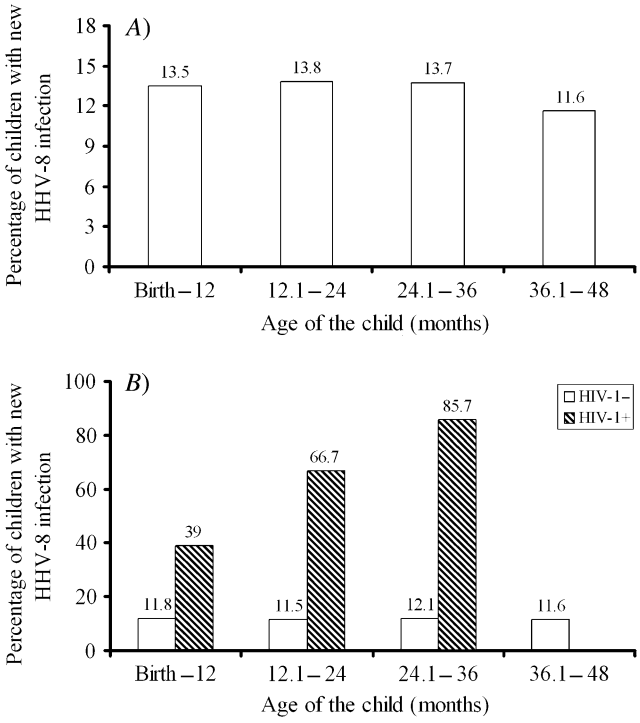

FIGURE 3. Proportions (%) of 684 children seroconverting for human herpesvirus 8 (HHV-8) during each annual follow-up period, Lusaka, Zambia, 1998–2004. Results are presented for all children followed to 48 months of age (panel *A*) and stratified by the human immunodeficiency virus type 1 (HIV-1) infection status of the child at age 24 months (panel *B*). None of the HIV-1-infected children survived or returned beyond 36 months of age.

TABLE 3. Human herpesvirus 8 seroreactivity patterns among 171 children with completion of all planned study visits at 12, 24, 36, and 48 months of age, Lusaka, Zambia, 1998–2004

HHV-8* seroreactivity pattern†				No. of children	%
12 months	24 months	36 months	48 months		
−	−	−	−	103	60.2
−	−	−	+	11	6.4
−	−	+	+	7	4.1
−	+	+	+	4	2.3
+	+	+	+	3	1.8
+	+	+	−	1	0.6
+	+	−	−	2	1.2
+	−	−	−	10	5.8
+	−	−	+	4	2.3
−	+	−	−	9	5.3
−	+	+	−	4	2.3
−	+	−	+	7	4.1
−	−	+	−	6	3.5
Total for HHV-8-negative children				103	60.2
Total for HHV-8-positive children				68	39.8
Total for all children				171	100

* HHV-8, human herpesvirus 8.

† A positive sign (+) indicates HHV-8 seropositivity at that age, and a negative sign (−) indicates HHV-8 seronegativity.

Seroreversion may be partial or complete, resulting from a loss of detectable titer to one or more antigens or a total loss of all specific antibodies. We believe that the observed seroreversions in our cohort could be both partial and complete seroreversions. It is unlikely that the HHV-8 seropositivity of children who tested positive at 12 months and subsequently tested negative was due to residual maternal HHV-8 antibodies, because all children born to HHV-8-seropositive mothers were titered at birth and at age 6 months. Eight of 10 children who were seropositive at 12 months and were seronegative at all later time points were born to HHV-8-seronegative mothers and were themselves seronegative at birth. It is possible that the observed seroreversions in our cohort were due to antibody titers that were below the limit of detection of our assays or due to the stringent detection criteria used. However, seroreversion has also been reported in studies using enzyme-linked immunosorbent assay (45). The lack of a gold-standard assay with established 100 percent accuracy makes it difficult to confirm these results. Performance studies of type-specific commercial assays designed to distinguish between herpes simplex virus types 1 and 2 have also reported that seroreversions were introduced because of poor assay sensitivity (52). An absence of antigenic stimulation after establishment of latency or viral clearance could lead to seroreversion. In addition, immunocompetent children may be able to efficiently control further reactivation, and thus the antibody titers drop below detection levels. It has been proposed that HHV-8-specific antibodies might be more readily detectable due to broadening of epitope recognition over time or due to subsequent reactivation after primary HHV-8 infection (45). HIV-1-related immunosuppression has also been proposed to be a factor responsible for HHV-8 seroreversion, especially in the Zambian population, which is experiencing a generalized HIV-1 epidemic. We observed that only four out of 41 HIV-1-infected children underwent seroreversion at one or more time points, but this number was too small for us to draw any conclusion, and a much larger cohort of HIV-1-infected children will be needed in order to understand this phenomenon.

Our study did have some important limitations. Although the number of HIV-1-infected children followed was limited, we found significantly higher HHV-8 incidence among HIV-1-infected children than among uninfected children. The high rate of attrition in HIV-1-infected children probably led to an underestimate of the true HHV-8 incidence and of the impact of child HIV-1 infection status on the risk of HHV-8 acquisition during early childhood. The number of children born to HIV-1-positive mothers was higher in the group that was not included in the analysis. To be eligible for inclusion in the analysis, a child had to survive and return at 24 months of age for reliable detection of HIV-1 antibodies. In addition, neither degree of clinical

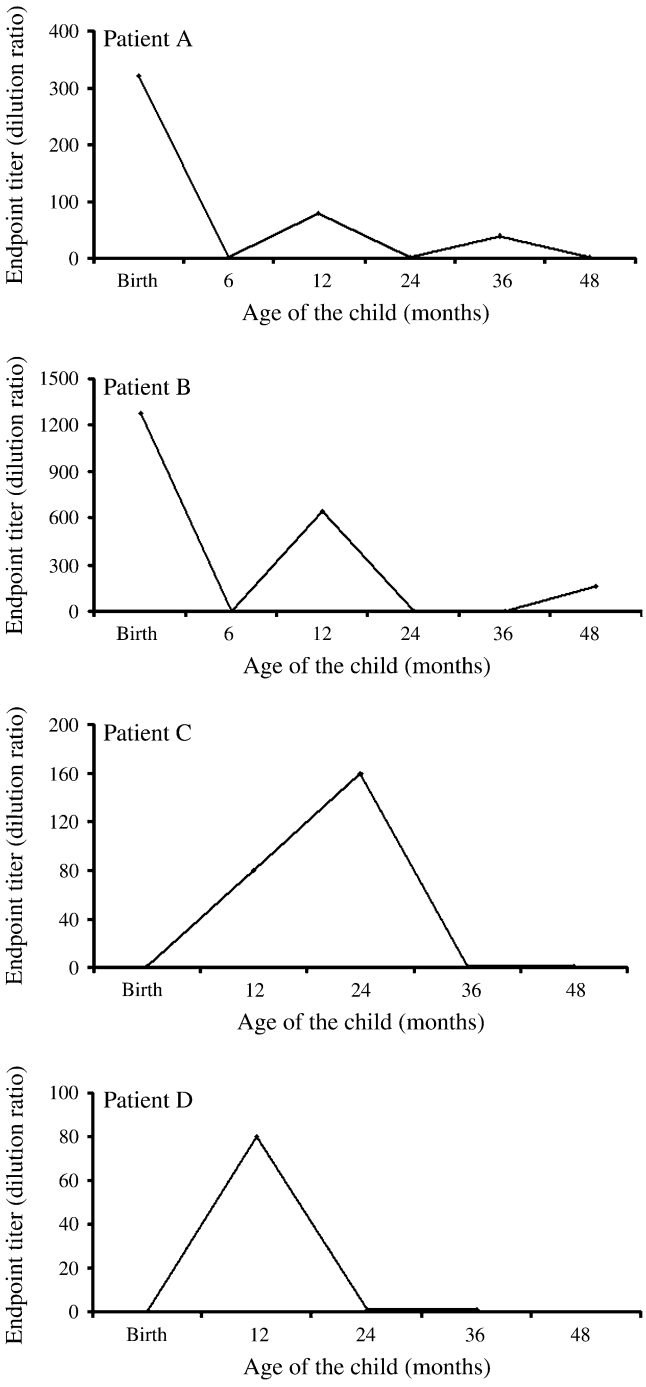

FIGURE 4. Observed fluctuations in titers of antibodies to human herpesvirus 8 (HHV-8) during early childhood, Lusaka, Zambia, 1998–2004. Children who had detectable antibody responses were titered by serial twofold dilutions of each test serum sample, beginning with 1:40, as described in Materials and Methods. The inverse of the last positive dilution was considered to be the endpoint titer. Patients A and B were born to HHV-8-seropositive mothers, and patients C and D were born to HHV-8-seronegative mothers.

immunosuppression nor HIV-1 viral load could be assessed, because methods for determining CD4 cell count and viral load were not yet available in Zambia. Transmission from siblings could not be examined because siblings were not recruited. Attrition due to mortality was high in children, especially those who were HIV-1-infected, because of lack of availability of antiretroviral therapy at the time of the study (53). None of the HIV-1-infected children returned after 36 months, probably because of high mortality in this group, which was without access to antiretroviral agents during the time period of this study. Pediatric antiretroviral therapy has since been implemented at primary health-care clinics throughout Lusaka (54). Common reasons for withdrawal from the study were religious beliefs, disapproval of a spouse or family, or lack of interest. Some families were untraceable because both the mother and the child died, relocated, or provided a wrong address. Most members of the study population were of lower socioeconomic status, which contributed to their being highly mobile.

In conclusion, horizontal transmission appears to be the major route of HHV-8 transmission in early childhood, and HIV-1 infection of the child is an important risk factor for HHV-8 acquisition in an area that is highly endemic for both viruses. The frequent seroreversions observed demonstrate that cross-sectional serologic screening for HHV-8 underestimates true rates of HHV-8 infection and may not provide a true representation of HHV-8 prevalence and incidence in a population.

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