# Human Herpesvirus 8 Infection and Transfusion History in Children With Sickle-Cell Disease in Uganda

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***Background:*** **Although human herpesvirus 8 (HHV-8), the etiologic agent for Kaposi's sarcoma, can be detected in peripheral blood, blood-borne transmission of this virus has not been demonstrated. We studied the association between HHV-8 seropositivity and transfusion history among children with sickle-cell disease in Uganda, where HHV-8 infection is common in blood donors.** ***Methods:*** **We studied 600 children (aged 0–16 years) with sickle-cell disease at Mulago Hospital, Kampala, from November 2001 through April 2002. By design, about half had previously been transfused. HHV-8 serostatus was determined using enzyme-linked immunosorbent assays for antibodies against HHV-8 proteins K8.1 and orf73. We used logistic regression to test for an association between HHV-8 serostatus and transfusion history and a Markov model to estimate the transmission risk per transfusion and the cumulative risk from community (i.e., nontransfusion) sources. Statistical tests were two-sided.** ***Results:*** **HHV-8 antibodies were detected in 117 of 561 (21%) children with unambiguous K8.1 results. HHV-8 seroprevalence among the never-transfused children increased with age from 7% in children aged 0–2 years to 32% in those aged 13–16 years ($P_{trend}$<.001). HHV-8 seropositivity was more frequent in transfused than never-transfused children (24% versus 17%, odds ratio = 1.48, 95% confidence interval [CI] = 0.97 to 2.26; $P$ = .07). Seropositivity increased with number of reported transfusions, with age-adjusted odds ratios of 0.97 (95% CI = 0.54 to 1.75), 1.13 (95% CI = 0.59 to 2.17), 1.76 (95% CI = 0.81 to 3.83), and 2.17 (95% CI = 1.18 to 3.99) for children with one, two, three, or four or more transfusions, respectively ($P_{trend}$ = .007). Overall, the estimated HHV-8 transmission risk was 2.6% per transfusion (95% CI = 1.9% to 3.3%), whereas the annual risk of infection unrelated to transfusion was 2.7% (95% CI = 1.7% to 3.7%).** ***Conclusion:*** **Our study suggests that blood transfusion is associated with a small risk of HHV-8 transmission. In Uganda, this risk is approximately equivalent to the 1-year cumulative risk of infection from community sources.** **[J Natl Cancer Inst 2003;95:1330–5]**

Human herpesvirus 8 (HHV-8, also called Kaposi's sarcoma–associated herpesvirus) is the infectious etiologic agent of Kaposi's sarcoma *(1).* HHV-8 may be transmitted through saliva *(2)* or sexual contact *(3–5).* It is uncertain whether blood-borne transmission occurs, although infectious HHV-8 has been detected in the peripheral blood of a blood donor *(6).* HHV-8 transmission was not observed in two studies of transfusion recipients conducted in Jamaica and in the United States *(7,8),* although both studies were small and could not exclude a transmission risk as high as 11% per transfusion of HHV-8–seropositive blood. Recent studies *(9–11)* have reported an association between HHV-8 seroprevalence and intravenous drug use, suggesting that blood-borne transmission might occur.

The possibility of blood-borne HHV-8 transmission has not been directly studied in Africa, where HHV-8 prevalence is estimated to range from 20% to 80% in adults *(12–15).* HHV-8

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DNA is detected more readily and at higher levels in the blood of Africans with asymptomatic HHV-8 infection than in people with HHV-8 infection from elsewhere *(16,17),* which may suggest a higher transmission risk associated with blood transfusions in Africa. In the present study, we examined the association between HHV-8 seropositivity, i.e., the presence of detectable antibodies to HHV-8, and reported history of blood transfusion among children with sickle-cell disease in Uganda.

## Subjects and Methods

### Study Subjects

From November 2001 through April 2002, we studied 600 children with sickle-cell disease aged 0–16 years who attended the Sickle Cell Day Care Center at Mulago Hospital, Kampala, Uganda. By design, approximately half of the children had a history of blood transfusion and half had never been transfused. Eligible children in each transfusion group were enrolled consecutively. Study staff elicited demographic information and transfusion history (ever versus never transfused, number of transfusions, date of transfusion, and hospital where the transfusion was given) from the mothers of participating children by using a structured questionnaire. To validate the mothers' recall, we performed a structured chart abstraction to verify that the transfusions reported by the mothers had occurred at Mulago Hospital, the largest hospital in Kampala. Data forms were checked for completeness before double-keying into an Access 2000 database (Microsoft, Redmond, WA).

### Ethical Issues

The study staff explained the study procedures to mothers of potentially eligible children and obtained written informed consent for their children to participate. Children who were 7 years old or older were also asked to provide documented assent. Permission to test for human immunodeficiency virus (HIV) infection was not requested because the prevalence of infection was thought to be low (<2%) in children *(18).* The Uganda National Council for Science and Technology and the Institutional Review Board of the U.S. National Cancer Institute approved this study.

### Laboratory Methods

Plasma was separated from blood samples that were obtained by venipuncture and stored at –80 °C until testing with two enzyme-linked immunosorbent assays for HHV-8 antibodies. The first enzyme-linked immunosorbent assay measured antibodies against K8.1, an HHV-8 structural glycoprotein, using plasma diluted 1 : 20 *(19).* Each sample was tested once on a 96-well plate; each plate incorporated three positive and three negative control sera. A titration of positive control sera was included with each batch of six plates. Batches or plates for which control samples gave anomalous results were repeated. This assay has an estimated sensitivity of 91%–100% and a specificity of 92%–100% in African populations *(19).* We used the distribution of assay optical density readings, which was bimodal, to choose cutoff points for this population, as previously described *(14).* Subjects were classified as seronegative (optical density ≤0.90), indeterminate (0.91–1.20), or seropositive (>1.20).

For the second assay, we used our recently developed enzyme-linked immunosorbent assay that detects antibodies to LANA, the orf73-encoded HHV-8 latent nuclear antigen, using a full-length baculovirus-expressed recombinant orf73 protein (provided by Dr. Thomas Schultz, University of Hanover, Hanover, Germany). Microtiter plates were coated with the recombinant orf73 protein diluted 1 : 1500 in 0.5 *M* bicarbonate buffer (pH 10.0) and incubated overnight at 4 °C. The plates were then washed, blocked with assay buffer (2.5% bovine serum albumin, 2.5% normal goat serum, 0.005% Tween 20 and 0.005% Triton X-100 in phosphate-buffered saline), and stored at –80 °C until needed. Plates were warmed to 37 °C before use. Plasma was applied to plates at a 1 : 100 dilution in assay buffer and incubated for 1.5 hours at 37 °C. The plates were then washed, and antibodies were detected with a goat anti-human immunoglobulin G alkaline phosphatase–conjugated antibody (Roche, Indianapolis, IN), diluted 1 : 3000 in the assay buffer, and then incubated for 30 minutes at 37 °C. After further washing, substrate (para-nitrophenylphosphate at 1 mg/mL in 10% diethanolamine buffer with 0.0002% $NaN_3$ and 0.0001% $MgCl\text{-}6H_2O$, pH 9.8) was added, and the plates were incubated for 30 minutes at 37 °C. The reaction was stopped with a solution of 3N sodium hydroxide, and the reactions were read at 405 nm. The optical density readings were bimodally distributed, which allowed the subjects to be classified as seronegative (if the optical density was ≤0.50), indeterminate (if the optical density was 0.51–0.70), or seropositive (if the optical density was >0.70). As with the K8.1 assay, each sample was tested once on a 96-well plate, and each plate included three positive and three negative control sera. A titration of positive control sera was included with each batch of six plates. Tests were repeated for batches or plates for which control samples gave anomalous results. This assay has a specificity of 99% in U.S. blood donors and sensitivity of 84% for detecting antibodies in individuals with AIDS-related Kaposi's sarcoma (Whitby D: unpublished data).

### Statistical Methods

For our primary analyses of HHV-8 seroprevalence, we used results from the established K8.1 enzyme-linked immunosorbent assay. In these analyses, we excluded 39 (6.5%) children whose K8.1 results were indeterminate. In secondary analyses using results from the orf73 assay, we excluded 50 (8.3%) children whose results were indeterminate. Three subjects had results that were indeterminate on both assays. For each assay, we estimated the association (i.e., odds ratios [ORs]) between the children's HHV-8 serostatus and blood transfusion history (ever versus never), and we tested for trend between HHV-8 seropositivity and the reported number of transfusions (zero, one, two, three, or four or more) using logistic regression, adjusting for current age and the mother's HHV-8 status. We also examined the associations between HHV-8 seropositivity and several other social and demographic characteristics. All *P* values and confidence intervals (CIs) are two-sided but are not adjusted for multiple comparisons. The Spearman correlation coefficient was used to compare the values of continuous variables.

To estimate the absolute risk of HHV-8 transmission associated with each transfusion, we used a two-state Markov model, in which the states correspond to "infected" and "uninfected." A detailed description of the model and a general overview of Markov and multistate models in epidemiology are provided elsewhere *(20).* In our model, each transfusion was considered to confer a fixed probability ($P_T$) of transmitting HHV-8 infection to the child. In the time intervals between transfusions, the risk

**Table 1.** Social and demographic characteristics of study participants by transfusion history

| Characteristic | All subjects (N = 600) | Never transfused (N = 256) | Transfused (N = 344) | *P* value* |
|---|---|---|---|---|
| Sex, n (%) | | | | .002 |
| Female | 306 (51) | 149 (58) | 157 (46) | |
| Male | 294 (49) | 107 (42) | 187 (54) | |
| Age group, n (%)† | | | | .02 |
| 0–2 y | 97 (16) | 50 (20) | 47 (14) | |
| 3–4 y | 95 (16) | 44 (17) | 51 (15) | |
| 5–6 y | 116 (19) | 49 (19) | 67 (20) | |
| 7–9 y | 108 (18) | 46 (18) | 62 (18) | |
| 10–12 y | 108 (18) | 37 (14) | 71 (21) | |
| 13–16 y | 75 (13) | 30 (12) | 45 (13) | |
| Mean age, y (SD) | 7.5 (4.0) | 7.1 (4.0) | 7.9 (4.0) | |
| Religion, n (%) | | | | .31 |
| Catholic | 196 (33) | 83 (33) | 113 (33) | |
| Protestant | 228 (38) | 107 (42) | 121 (35) | |
| Muslim | 132 (22) | 49 (19) | 83 (24) | |
| Other | 41 (7) | 16 (6) | 25 (7) | |
| Tribe, n (%) | | | | .33 |
| Ganda | 408 (68) | 166 (65) | 242 (70) | |
| Luo | 32 (5) | 18 (7) | 14 (4) | |
| Soga | 50 (8) | 23 (9) | 27 (8) | |
| Other | 110 (18) | 49 (19) | 61 (18) | |

*$P$ values are for tests of heterogeneity in human herpesvirus 8 seroprevalence between never-transfused and transfused children, except for age, where $P$ value was derived from a two-sample $t$ test. SD = standard deviation.

†Numbers do not always add to column totals because of missing data.

of infection was described by a baseline hazard function that characterized the infection risk from community sources. We assumed that this baseline hazard function was constant and was equivalent to a simple exponential model for the time to infection among never-transfused children. The two parameters specified by the model, namely the per transfusion probability of infection $P_T$ and the background hazard rate, were estimated by maximum likelihood methods. We obtained two-sided model–based 95% confidence intervals for the parameter estimates using the model information matrix. Bootstrapped confidence intervals *(21),* although wider, produced qualitatively similar results (data not shown).

## RESULTS

We studied 600 children, of whom 256 (43%) had no prior transfusions reported by their mothers. Compared with these never-transfused children, the 344 children (57%) who had previously been transfused were more likely to be male and were slightly older, but these groups did not differ by religion ($P$ = .31) or tribe ($P$ = .33) (Table 1). None of the children was known to have Kaposi's sarcoma or to be infected with HIV.

There were a total of 921 transfusions reported for 344 transfused children (mean = 2.7 per child, maximum 10). The median age at first transfusion was 2.9 years (interquartile range = 1.2–5.8 years). Of the 921 transfusions, 689 (75%) in 270 children had been given at Mulago Hospital. We reviewed medical records available at that hospital but were unable to find any records for 19 of the children and found that the records were incomplete regarding whether transfusions were given for 85 of the children. We identified 334 transfusions that were given to the remaining 166 children. For these children, the number of transfusions reported by mothers correlated well with the number of transfusions documented in the medical records (Spearman correlation coefficient = 0.51, $P$<.001).

Of 561 children with unambiguous K8.1 assay results, HHV-8 antibodies were detected in 117 (21%) children. The frequency of HHV-8 seropositivity was similar among males and females (23% versus 19%, $P$ = .27) (Table 2), but it underwent a statistically significant increase with age, from 7% in children aged 0–2 years to 32% in children aged 13–16 years ($P_{trend}$<.001). Among mothers, 166 (34%) of 485 with evaluable results were seropositive. Children whose mothers were seropositive were themselves more often seropositive than children whose mothers were seronegative (26% versus 18%; OR = 1.57, 95% CI = 0.98 to 2.49).

Transfused children were more likely to be HHV-8–seropositive than never-transfused children (24% versus 17%; OR = 1.48, 95% CI = 0.97 to 2.26; $P$ = .07). HHV-8 seropositivity increased steadily with the number of reported transfusions, from 17% among children who had never been transfused to 33% among children reporting four or more transfusions (for four or more transfusions, OR = 2.36, 95% CI = 1.31 to 4.23, compared with never-transfused children; Table 2). Although the number of reported transfusions also increased with age (Spearman correlation coefficient = 0.10, $P$ = .01), the association between HHV-8 serostatus and number of re-

**Table 2.** Association between human herpesvirus 8 (HHV-8) serostatus and selected characteristics

| Characteristic | HHV-8 seropositive, n (%) | Odds ratio (95% CI)* |
|---|---|---|
| Sex | | |
| Female | 55 (19) | 1.00 (referent) |
| Male | 62 (23) | 1.26 (0.84 to 1.89) |
| Age group | | |
| 0–2 y | 6 (7) | 1.00 (referent) |
| 3–4 y | 13 (14) | 2.36 (0.85 to 6.50) |
| 5–6 y | 15 (15) | 2.50 (0.93 to 6.75) |
| 7–9 y | 30 (29) | 5.97 (2.35 to 15.2) |
| 10–12 y | 31 (30) | 6.00 (2.37 to 18.9) |
| 13–16 y | 22 (32) | 6.86 (2.60 to 18.1) |
| Religion | | |
| Catholic | 35 (19) | 1.00 (referent) |
| Protestant | 52 (24) | 1.32 (0.82 to 2.14) |
| Muslim | 23 (19) | 0.96 (0.53 to 1.72) |
| Other | 6 (16) | 0.78 (0.30 to 2.01) |
| Tribe | | |
| Ganda | 89 (23) | 1.00 (referent) |
| Luo | 7 (22) | 0.93 (0.39 to 2.12) |
| Soga | 9 (18) | 0.75 (0.34 to 1.60) |
| Other | 24 (22) | 0.95 (0.57 to 1.58) |
| Mother's status | | |
| HHV-8 seronegative | 54 (18) | 1.00 (referent) |
| HHV-8 seropositive | 40 (26) | 1.57 (0.98 to 2.49) |
| Indeterminate | 8 (26) | 1.57 (0.66 to 3.69) |
| Unknown | 11 (18) | 0.99 (0.48 to 2.03) |
| Transfusion status | | |
| Never-transfused | 41 (17) | 1.00 (referent) |
| Transfused | 76 (24) | 1.48 (0.97 to 2.26) |
| No. of transfusions | | |
| 0 | 41 (17) | 1.00 (referent) |
| 1 | 22 (18) | 1.03 (0.58 to 1.81) |
| 2 | 17 (22) | 1.32 (0.70 to 2.48) |
| 3 | 12 (28) | 1.86 (0.88 to 3.92) |
| 4–10 | 25 (33) | 2.36 (1.31 to 4.23) |

*An enzyme-linked immunosorbent assay to detect antibodies against K8.1, an HHV-8 structural glycoprotein, was used to determine HHV-8 serostatus. Subjects were classified as seronegative if the optical density readings were less than or equal to 0.90, indeterminate if the optical density readings were between 0.90 and 1.20, and seropositive if the optical density readings were greater than 1.20. We excluded from the analysis 39 children with indeterminate K8.1 HHV-8 serostatus and one child with missing age. CI = confidence interval.

ported transfusions remained statistically significant when we adjusted for age. Specifically, age-adjusted odds ratios for HHV-8 seropositivity were 0.97 (95% CI = 0.54 to 1.75), 1.13 (95% CI = 0.59 to 2.17), 1.76 (95% CI = 0.81 to 3.83), and 2.17 (95% CI = 1.18 to 3.99) for children with one, two, three, or four or more transfusions, respectively, compared with never-transfused children ($P_{trend}$ = .007). The relationship between HHV-8 and number of transfusions remained statistically significant when the mother's HHV-8 status was also included in the model ($P_{trend}$ = .01).

Using the Markov model, we estimated the risk of HHV-8 infection among children for community-acquired infection, unrelated to transfusion, to be 2.7% per year (95% CI = 1.7% to 3.7%). The additional infection risk associated with a single blood transfusion ($P_T$) was estimated to be 2.6% (95% CI = 1.9% to 3.3%). The HHV-8 seroprevalence predicted by the Markov model for transfused and never-transfused children closely resembled the observed seroprevalence (Fig. 1; $\chi^2$ goodness of fit = 0.41, $P$>.9, 9 degrees of freedom).

We assessed whether the results obtained by K8.1 and orf73 assays were correlated and found that they were (Spearman's correlation coefficient = 0.68). In a second analysis of 550 children with unambiguous orf73 assay results, HHV-8 antibodies were detected in 130 (24%) children. HHV-8 seropositivity determined by the orf73 assay was more frequent in transfused than in never-transfused children (26% versus 19%; OR = 1.51, 95% CI = 1.00 to 2.28). The age-adjusted odds ratios for HHV-8 seropositivity using the orf73 assay were 1.23 (95% CI = 0.71 to 2.14), 0.98 (95% CI = 0.51 to 1.87), 2.01 (95% CI = 0.94 to 4.32), and 1.58 (95% CI = 0.84 to 2.96) for children with one, two, three, and four or more transfusions, respectively, compared with never-transfused children ($P_{trend}$ = .09). The infection risk associated with a single blood transfusion ($P_T$) was estimated to be 2.4% (95% CI = 0.4% to 4.5%) using the orf73 assay.

## Discussion

We observed a statistically significant association between HHV-8 seropositivity and transfusion history in children with sickle-cell disease in Uganda. The frequency of HHV-8 seropositivity increased with the number of reported transfusions, although this trend was statistically significant only when using the assay that detected antibodies to the HHV-8 K8.1 protein and not when using the orf73 assay. HHV-8 seropositivity also increased with age and was more frequent in the children of HHV-8–seropositive mothers than in those of HHV-8–seronegative mothers. Nonetheless, in analyses adjusting for these factors, the association between HHV-8 seropositivity as detected by the K8.1 assay and number of transfusions remained statistically significant. On the basis of the Markov model, we estimated the risk of HHV-8 transmission with each transfusion to be 2.6%.

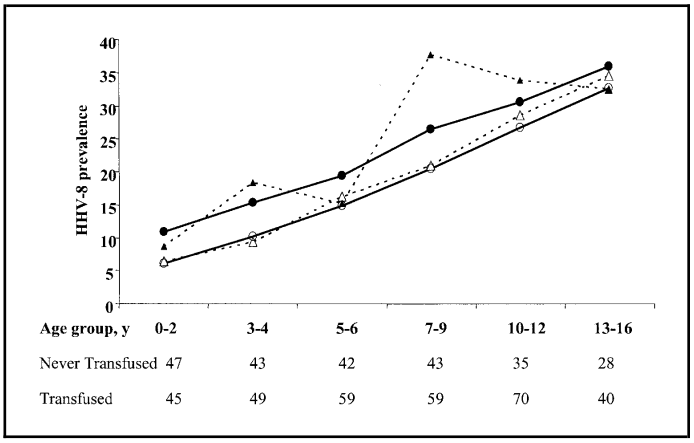


**Fig. 1.** Observed and modeled human herpesvirus 8 (HHV-8) seroprevalence, using K8.1 antibody results, as a function of children's age and transfusion status. Modeled results were derived from a two-state Markov model *(19,20)* as described in the "Subjects and Methods" section. The numbers of never-transfused and ever transfused children are shown below the graph. **Open triangles** = never transfused (observed); **filled triangles** = transfused (observed); **open circles** = never transfused (modeled); **filled circles** = transfused (modeled).

HHV-8 prevalence among Ugandan blood donors is not known with certainty, but three studies *(22–24)* have reported a seroprevalence ranging from 65% to 74% based on serologic assays and algorithms that were different from ours. We observed a seroprevalence of 34% in the mothers of our subjects, using the same tests and algorithms as in the children, but we do not know if the prevalence in Ugandan donors, who are mostly young adult males, would be similar to that in these mothers. If we assume, somewhat arbitrarily, that HHV-8 seroprevalence in Ugandan donors is approximately 50%, then the risk associated with transfusion from an HHV-8–seropositive donor would be twice our estimate, i.e., approximately 5%.

Our study results are consistent with a small risk of HHV-8 infection associated with blood transfusion. Two prospective studies *(7,8)* that investigated the possibility of blood-borne HHV-8 transmission in the United States and in Jamaica did not observe seroconversions in transfused subjects. However, these studies were small (a total of 35 recipients of HHV-8–seropositive blood) and used an earlier generation of HHV-8 assays. Additionally, these studies were conducted among populations with a low prevalence of HHV-8, so that misclassification of HHV-8 exposure could partly explain their null results. Moreover, some transfusion recipients in these studies received fractionated blood (which could have been stored for relatively long periods) or only plasma products, both of which might have reduced the probability of HHV-8 transmission. By comparison, we studied a population with a relatively high HHV-8 prevalence and used state-of-the-art serologic assays *(19)*. Furthermore, because transfusion recipients in Uganda receive unprocessed whole blood, often on the day of collection, our study was well-suited to detect an association between transfusion and HHV-8 seropositivity.

Transmission of HHV-8 through transfusions appears to be less efficient than that of other blood-borne viruses. For example, our estimate for HHV-8 blood-borne transmission risk of 2.6% is lower than the reported 12% per transfusion risk for acquiring cytomegalovirus (another herpesvirus) from unscreened blood *(25)*. The transfusion transmission risk is also much lower than that associated with HIV or hepatitis C virus *(26)*. These differences in risk may partly reflect a comparatively low HHV-8 virus load in blood. We did not take into account the volume of blood transfused, which was not known to us and would have varied by the amount needed for each child. Additional studies are needed to better quantify the absolute risk associated with blood transfusion, particularly in donors with detectable HHV-8 viremia, who might be more likely to transmit HHV-8.

Some studies of intravenous drug users *(10,27)* have reported a positive association between HHV-8 serostatus and duration or

frequency of drug use. Among HIV-infected women in the United States, HHV-8 seroprevalence is higher in those who use intravenous drugs or are infected with hepatitis C virus (predominantly transmitted by blood inoculation or transfusion) *(11).* Nonetheless, other studies *(4,28,29)* have not identified an association between intravenous drug use and HHV-8 seropositivity. Overall, these varying results are consistent with our finding that HHV-8 transmission via transfusion is inefficient.

We estimated that the risk of HHV-8 infection unrelated to transfusion was 2.7% per year among children in our study. This risk represents the aggregate risk of acquiring HHV-8 infection from family and community sources in 1 year, although it could also include a small contribution from unsterile medical injections or traditional treatment practices, such as scarification, which might be more common in children with sickle-cell disease than in healthy Ugandan children. Other studies *(30,31)* have clearly demonstrated the importance of the HHV-8 infection status of a child's family members, especially the infection status of the mother and siblings. In the current study, mothers' HHV-8 status was marginally associated with infection status in their children. Because HHV-8 is present in saliva *(32),* children could acquire HHV-8 from family members if they eat prechewed food, are cleaned with saliva, or share utensils or toys with their siblings.

We note several limitations to our study. First, our measurement of exposure to blood transfusions relied on mothers' recall, which may have been inaccurate. Ideally, we would have used medical record chart abstraction to ascertain exposure to blood, but this was possible only for children who were transfused at Mulago Hospital. Even for these children, hospital records were not always available or complete. However, for children with transfusions documented in their medical records, the number of recorded transfusions correlated well with the number reported by mothers, and the correlation was similar in HHV-8–seropositive and –seronegative children (data not shown).

Second, we used a cross-sectional design that precluded us from observing HHV-8 seroconversion in transfused children. A prospective study of transfusion recipients would provide the most convincing data. Such a study, by another group, is now underway in Uganda, but preliminary analyses based on about one-fourth of the data have not been conclusive *(23,24).* Given the low absolute risk for transmission that we estimated and the difficulties associated with accurately assessing serostatus in both donors and recipients, conclusions from that study must await an analysis of all data.

Third, we used assays for HHV-8 seropositivity that do not have 100% sensitivity and specificity *(33).* Nonetheless, our K8.1 enzyme-linked immunosorbent assay has been shown to be highly accurate in Africa *(19,34)* and our orf 73 assay produced qualitatively similar results.

Finally, we considered the possibility that confounding might partly explain our results. Children with sickle-cell disease receive blood transfusions for a variety of reasons, such as severe malaria or sickle-cell crisis, but we are aware of no data suggesting that these conditions predispose children to HHV-8 infection. Still, heterologous blood transfusions have been reported to cause transient immunosuppression *(35),* and the higher prevalence of HHV-8 infection could conceivably be the result of heightened susceptibility in transfused children to infection from other sources. Nonetheless, in additional analyses stratified on number of blood transfusions, we did not obtain differing estimates of the risk for community-acquired infection (data not shown), suggesting that it is unlikely that transfusions serve merely as a marker for poor overall health and increased risk for community-acquired HHV-8 infection. Alternatively, it is possible that primary or chronic HHV-8 infection in these children could lead to anemia, which would in turn lead to the need for a blood transfusion. Although acute HHV-8 infection was associated with fever and cytopenia in transplant recipients *(36),* this syndrome has not been described in children *(37),* and this type of confounding could not explain the especially high seroprevalence noted in children who received multiple transfusions over an extended period.

In summary, our study suggests that HHV-8 can be transmitted through transfusion in sub-Saharan Africa. However, additional studies are needed to address the need for screening of blood for HHV-8. In Uganda, the risk of acquiring HHV-8 via seropositive transfusion appears small relative to community sources of infection. Moreover, the risk of Kaposi's sarcoma, even given HHV-8 infection, is low in immunocompetent persons *(38).* The high prevalences of HIV and hepatitis B virus already impose a heavy burden on blood transfusion services in Uganda and elsewhere in Africa *(39–41).* Therefore, based on current evidence, HHV-8 screening may not be warranted in Uganda. Our study does not provide data on the risk of transmission of HHV-8 in developed countries, such as the United States, where HHV-8 prevalence is low.

## REFERENCES


*(1)* Cesarman E, Knowles DM. The role of Kaposi's sarcoma-associated herpesvirus (KSHV/HHV-8) in lymphoproliferative diseases. Semin Cancer Biol 1999;9:165–74.

*(2)* Pauk J, Huang ML, Brodie SJ, Wald A, Koelle DM, Schacker T, et al. Mucosal shedding of human herpesvirus 8 in men. N Engl J Med 2000; 343:1369–77.

*(3)* 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.

*(4)* 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.

*(5)* O'Brien TR, Kedes D, Ganem D, Macrae DR, Rosenberg PS, Molden J, et al. Evidence for concurrent epidemics of human herpesvirus 8 and human immunodeficiency virus type 1 in US homosexual men: rates, risk factors, and relationship to Kaposi's sarcoma. J Infect Dis 1999;180: 1010–7.

*(6)* Blackbourn DJ, Ambroziak J, Lennette E, Adams M, Ramachandran B, Levy JA. Infectious human herpesvirus 8 in a healthy North American blood donor. Lancet 1997;349:609–11.

*(7)* Engels EA, Eastman H, Ablashi DV, Wilks RJ, Braham J, Manns A. Risk of transfusion-associated transmission of human herpesvirus 8. J Natl Cancer Inst 1999;91:1773–5.

*(8)* Operskalski EA, Busch MP, Mosley JW, Kedes DH. Blood donations and viruses. Lancet 1997;349:1327.

*(9)* Cannon MJ, Dollard SC, Smith DK, Klein RS, Schuman P, Rich JD, et al. Blood-borne and sexual transmission of human herpesvirus 8 in women with or at risk for human immunodeficiency virus infection. N Engl J Med 2001;344:637–43.

*(10)* Atkinson J, Edlin BR, Engels EA, Kral AH, Seal K, Gamache CJ, et al. Seroprevalence of human herpesvirus 8 among injection drug users in San Francisco. J Infect Dis 2003;187:974–81.

*(11)* Goedert JJ, Charurat M, Blattner WA, Hershow RC, Pitt J, Diaz C, et al. Risk factors for Kaposi's sarcoma-associated herpesvirus infection among HIV-1-infected pregnant women in the USA. AIDS 2003;17:425–33.

*(12)* Chang Y, Ziegler J, Wabinga H, Katangole-Mbidde E, Boshoff C, Schulz T, et al. Kaposi's sarcoma-associated herpesvirus and Kaposi's sarcoma in

Africa. Uganda Kaposi's Sarcoma Study Group. Arch Intern Med 1996; 156:202–4.
(13) 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.
(14) Eltom MA, Mbulaiteye SM, Dada AJ, Whitby D, Biggar RJ. Transmission of human herpesvirus 8 by sexual activity among adults in Lagos, Nigeria. AIDS 2002;16:2473–8.
(15) Mayama S, Cuevas LE, Sheldon J, Omar OH, Smith DH, Okong P, 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.
(16) Belec L, Cancre N, Hallouin MC, Morvan J, Si Mohamed A, Gresenguet G. High prevalence in Central Africa of blood donors who are potentially infectious for human herpesvirus 8. Transfusion 1998;38:771–5.
(17) Enbom M, Urassa W, Massambu C, Thorstensson R, Mhalu F, Linde A. Detection of human herpesvirus 8 DNA in serum from blood donors with HHV-8 antibodies indicates possible bloodborne virus transmission. J Med Virol 2002;68:264–7.
(18) Kengeya-Kayondo JF, Malamba SS, Nunn AJ, Seeley JA, Ssali A, Mulder DW. Human immunodeficiency virus (HIV-1) seropositivity among children in a rural population of south-west Uganda: probable routes of exposure. Ann Trop Paediatr 1995;15:115–20.
(19) Engels EA, Sinclair MD, Biggar RJ, Whitby D, Ebbesen P, Goedert JJ, et al. Latent class analysis of human herpesvirus 8 assay performance and infection prevalence in sub-Saharan Africa and Malta. Int J Cancer 2000; 88:1003–8.
(20) Commenges D. Multi-state models in epidemiology. Lifetime Data Anal 1999;5:315–27.
(21) Foster DH, Bischof WF. Bootstrap variance estimators for the parameters of small-sample sensory-performance functions. Biol Cybern 1987;57: 341–7.
(22) Kakoola DN, Sheldon J, Byabazaire N, Bowden RJ, Katongole-Mbidde E, Schulz TF, et al. Recombination in human herpesvirus-8 strains from Uganda and evolution of the K15 gene. J Gen Virol 2001;82:2393–404.
(23) Hladik W, Dollard S, Downing R, Nzaro E, Kataaha P, Banage F, et al. Estimated risk of human herpesvirus 8 transmission by blood transfusion, Uganda. XIV International AIDS Conference; 2002 July 7–12; Barcelona (Spain). Marathon Multimedia. p. WeOrC1374.
(24) Hladik W, Dollard SC, Downing RG, Kataaha P, Pellett PE, Karon JM, et al. Kaposi's sarcoma in Uganda: risk factors for human herpesvirus 8 infection among blood donors. J Acquir Immune Defic Syndr 2003;33: 206–10.
(25) Ho M. Epidemiology of cytomegalovirus infections. Rev Infect Dis 1990;12 Suppl 7:S701–10.
(26) Goodnough LT, Shander A, Brecher ME. Transfusion medicine: looking to the future. Lancet 2003;361:161–9.
(27) Cannon MJ, Dollard SC, Smith DK, Klein RS, Schuman P, Rich JD, et al. Blood-borne and sexual transmission of human herpesvirus 8 in women with or at risk for human immunodeficiency virus infection. N Engl J Med 2001;344:637–43.
(28) Greenblatt RM, Jacobson LP, Levine AM, Melnick S, Anastos K, Cohen M, et al. Human herpesvirus 8 infection and Kaposi's sarcoma among human immunodeficiency virus-infected and -uninfected women. J Infect Dis 2001;183:1130–4.
(29) Renwick N, Dukers NH, Weverling GJ, Sheldon JA, Schulz TF, Prins M, et al. Risk factors for human herpesvirus 8 infection in a cohort of drug users in the Netherlands, 1985–1996. J Infect Dis 2002;185:1808–12.
(30) Plancoulaine S, Abel L, van Beveren M, Tregouet DA, Joubert M, Tortevoye P, et al. Human herpesvirus 8 transmission from mother to child and between siblings in an endemic population. Lancet 2000;356:1062–5.
(31) Mbulaiteye SM, Pfeiffer RM, Whitby D, Brubaker GR, Shao J, Biggar RJ. Human herpesvirus 8 infection within families in rural Tanzania. J Infect Dis 2003;187:1780–5.
(32) Pauk J, Huang ML, Brodie SJ, Wald A, Koelle DM, Schacker T, et al. Mucosal shedding of human herpesvirus 8 in men. N Engl J Med 2000; 343:1369–77.
(33) Rabkin CS, Schulz TF, Whitby D, Lennette ET, Magpantay LI, Chatlynne L, et al. Interassay correlation of human herpesvirus 8 serologic tests. HHV-8 Interlaboratory Collaborative Group. J Infect Dis 1998;178:304–9.
(34) Engels EA, Whitby D, Goebel PB, Stossel A, Waters D, Pintus A, et al. Identifying human herpesvirus 8 infection: performance characteristics of serologic assays. J Acquir Immune Defic Syndr 2000;23:346–54.
(35) Takemura M, Osugi H, Takada N, Kinoshita H, Higashino M. Immunologic effects of allogeneic versus autologous blood transfusion in patients undergoing radical oesophagectomy. Eur Surg Res 2003;35:115–22.
(36) Luppi M, Barozzi P, Schulz TF, Setti G, Staskus K, Trovato R, et al. Bone marrow failure associated with human herpesvirus 8 infection after transplantation. N Engl J Med 2000;343:1378–85.
(37) Andreoni M, Sarmati L, Nicastri E, El Sawaf G, El Zalabani M, Uccella I, et al. Primary human herpesvirus 8 infection in immunocompetent children. JAMA 2002;287:1295–300.
(38) Vitale F, Briffa DV, Whitby D, Maida I, Grochowska A, Levin A, et al. Kaposi's sarcoma herpes virus and Kaposi's sarcoma in the elderly populations of 3 Mediterranean islands. Int J Cancer 2001;91:588–91.
(39) Herve P. [Transfusion safety: emergent or hypothetical risks] Transfus Clin Biol 2000;7:30–8.
(40) Watson-Williams EJ, Kataaha PK. Revival of the Ugandan Blood Transfusion System 1989: an example of international cooperation. Transfus Sci 1990;11:179–84.
(41) Okware S, Opio A, Musinguzi J, Waibale P. Fighting HIV/AIDS: is success possible? Bull World Health Organ 2001;79:1113–20.

## NOTES

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