Epidemiology of HIV and selected blood-borne infections in East-Africa
Hladik, W.

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Kaposi’s sarcoma in Uganda: risk factors for human herpesvirus 8 infection among blood donors

W. HLADIK1,2, S.C. DOLLARD3, R. DOWNING2,4, P. KATAAHA5, P.E. PELLETT6, J.M. KARON2, J. MERMIN2,4, E.M. LACKRITZ7

1 Epidemic Intelligence Service, Division of Applied Public Health Training, Epidemiology Program Office, Centers for Disease Control and Prevention, Atlanta, GA, USA
2 Global AIDS Program, National Center for HIV, Viral Hepatitis, STD and TB Prevention, Centers for Disease Control and Prevention, Atlanta, GA, USA
3 Division of Viral and Rickettsial Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA, USA
4 Uganda Virus Research Institute, Entebbe, Uganda
5 Nakasero Blood Bank, Kampala, Uganda
6 Department of Virology, Lerner Research Institute, Cleveland Clinic Foundation, Cleveland, Ohio, USA
7 Division of Reproductive Health, National Center for Chronic Diseases Prevention and Health Promotion, Centers for Disease Control and Prevention, Atlanta, GA, USA

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Abstract

Human herpesvirus 8 (HHV-8) is etiologically linked to Kaposi’s sarcoma, a common cancer in Uganda. The authors assessed HHV-8 seroprevalence, risk factors for infection, and HHV-8 assays in a cross-sectional study of Ugandan blood donors. Of 3,736 specimens, the authors selected 203 reactive for HIV, hepatitis B surface antigen (HBsAg), or syphilis, and, randomly, 203 nonreactive specimens. For HHV-8 testing, the authors used two peptide-based enzyme-linked immunosorbent assays (EIAs), ORFK8.1 and ORF65, and an immunofluorescence assay (IFA). Specimens reactive in at least two assays or on IFA alone were considered HHV-8 seropositive. Prevalence estimates were weighted to account for the sampling scheme. Overall HHV-8 seroprevalence was 40%. HHV-8 seroprevalence was higher among HBsAg-positive donors (53%) than HBsAg-negative donors (39%; \( p = .02 \)) and higher among HIV-positive donors (63%) than HIV-negative donors (39%; \( p < .001 \)). HHV-8 sero-reactivity showed no trend with age. Kappa values for assay concordances were 0.68 (ORFK8.1 EIA and IFA), 0.37 (ORF65 EIA and K8.1 EIA), and 0.29 (ORF65 EIA and IFA). The association between HHV-8 and HBsAg positivity and the lack of association between HHV-8 and age point to primarily nonsexual HHV-8 transmission during childhood. The association with HIV indicates sexual transmission may also occur. The role of ORF65 EIA in testing specimens from Africa warrants further evaluation.
In Uganda, half of all reported cancers are Kaposi’s sarcoma (KS) (1), a malignancy caused by human herpesvirus 8 (HHV-8, also known as Kaposi’s sarcoma–associated herpesvirus) (2–4). KS incidence in sub-Saharan Africa increased rapidly during the last two decades owing to the high incidence of AIDS in the region (5,6). Seroprevalence estimates for HHV-8 vary greatly worldwide (7,8), from less than 5% among blood donors in North America and northern Europe (4,9,10) to more than 50% in some study populations in sub-Saharan Africa (9,11–14).

Strategies to prevent HHV-8 infection have not been identified, because the precise transmission modes are uncertain. In the United States and northern Europe, risks for infection among the general population are unknown, but male-male sexual contact is associated with HHV-8 seropositivity (15,16). A recent US study (17) identified possible blood-borne transmission by injection drug use. Among Africans, seroprevalence increases during childhood (18–22) but apparently is infrequently transmitted vertically (20,23,24). To evaluate risk factors for HHV-8 infection, we investigated specimens from volunteer blood donors in Uganda.

The development of HHV-8 serologic assays is ongoing. Assay performance has been more thoroughly evaluated using specimens obtained from industrialized countries than from highly endemic areas such as sub-Saharan Africa (12,25–27). We investigated performance of established diagnostic assays in this Ugandan population.

**Materials and Methods**

**Blood Samples**

We evaluated stored plasma specimens from 3736 voluntary blood donors. Specimens unlinked to patient identifiers had been collected by Nakasero Blood Bank, Kampala, Uganda, from November 1998 through May 1999 within Kampala and central Uganda. Specimens were processed and stored at −70°C within
8 hours of collection. Information routinely collected included age, sex, and number of previous donations. We selected 203 specimens that were identified by routine screening as reactive for HIV, hepatitis B, or syphilis and selected randomly another 203 specimens that were nonreactive in routine screening.

Screening Tests

As part of its routine screening program, Nakasero Blood Bank in Uganda had tested all 3736 specimens for HIV, hepatitis B surface antigen (HBsAg), and syphilis. Specimens were tested for HIV anti-bodies using an enzyme immunosorbent assay (EIA) (MUREX HIV-1.2.O; Murex Biotech, Kent, United Kingdom). Nonreactive specimens were reported as HIV-negative; repeatedly reactive specimens were reported as HIV-positive. Specimens with discordant EIA results were further evaluated using Uni-Form II plus O (Vironostika, Boxtel, The Netherlands) and Western blot analysis. For HBsAg testing, the MUREX HBsAg assay was used; specimens reactive in duplicate were reported as positive, and all others were reported as negative. Screening for syphilis was done by the Venereal Disease Research Laboratory test; reactive samples were confirmed by the Treponema pallidum hemagglutination (TPHA) test.

Human Herpesvirus 8 Testing

Human herpesvirus 8 serologic testing was performed at the Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia, using three assays. Two of these assays were EIAs using peptides based on open reading frames (ORFs) 65 and K8.1 and were performed as described (26,28). The cutoff chosen was the mean corrected optical density at 450 nm of 20 negative control specimens plus 5 standard deviations (SDs). The third test was a lytic monoclonal antibody-enhanced immunofluorescence assay (IFA) using a body cavity–based lymphoma cell line harboring HHV-8. IFA testing was blinded from EIA results and performed as described (14), except that specimens were tested at a higher
dilution, 1:40. Specimens clearly reactive at 1:40 were scored as positive. Specimens marginally reactive were scored as equivocal and counted as negative in the analyses. Approximately 3% to 4% of the specimens showed interference or background with the IFA testing and were retested at 1:80 dilution and interpreted as described for the 1:40 dilution.

Analysis

Data were entered using EPI INFO 6.04c (CDC, Atlanta, GA) and analyzed with SAS 8.2 (SAS Institute, Cary, NC). To adjust for the preferential selection of specimens reactive for HIV, HBsAg, or syphilis, we used SAS PROC SURVEYMEANS to estimate weighted HHV-8 seroprevalences and their standard errors (SEs). We used the z test to evaluate differences between HHV-8 seroprevalences and the kappa statistic (29) to evaluate interassay agreement.

Results

Study Population

Routine screening of 3736 blood donors (median age: 22 years, interquartile range [IQR]: 19–28 years, 80% male) had identified 72 (1.9%) HIV-positive, 130 (3.5%) HBsAg-positive, 4 (0.1%) positive for both HIV and HBsAg, 6 (0.2%) reactive for syphilis, and 1 (0.03%) positive for both HBsAg and syphilis. Of these 213 reactive specimens, 203 were available for HHV-8 testing. Including the randomly selected 203 specimens that were nonreactive on all screening tests, the weighted median age of the 406 donors selected for HHV-8 testing was 24 years (IQR: 19–30 years); 84% were male.
Patterns of reactivity in serologic human herpesvirus 8 (HHV-8) assays from blood donors in central Uganda, November 1998–May 1999

Of the 406 specimens tested, 143 (35%) were ORF65 EIA-reactive, 156 (38%) were ORFK8.1 EIA-reactive, and 182 (45%) were IFA-reactive (Table 8.1). Fifty-nine percent of specimens were reactive in at least one of the three assays. Concordances for test results were 85% (κ=0.68) between the ORFK8.1 EIA and IFA, 71% (κ=0.37) between the ORFK8.1 EIA and the ORF65 EIA, and 67% (κ=0.29) between the ORF65 EIA and the IFA.

**Human Herpesvirus 8 Seroprevalence**

Because these HHV-8 assays had not previously been used in our laboratory to test African populations, we used a testing algorithm that emphasized specificity more than sensitivity. Specimens reactive in at least two of the three assays or reactive in the IFA alone were classified as HHV-8-seropositive, and all others were nonreactive.
HHV-8 prevalence in blood donors

Figure 8.1 Human herpesvirus 8 seroprevalence, blood donors in central Uganda, November 1998 through May 1999. *Prevalence and 95% confidence intervals are adjusted for the sampling scheme. Age information was available for 393 donors.

classified as HHV-8-seronegative. Based on this algorithm, 192 (47%) specimens were HHV-8-seropositive. All seroprevalence estimates described hereafter are weighted to adjust for the specimen-sampling scheme. Extrapolating these results to the overall sample of 3736 blood donors, the estimated weighted HHV-8 seroprevalence was 40% (95% CI: 34%–46%). Seroprevalence was 41% for the youngest age group (15–17 years), with no age trend (Fig. 8.1). HHV-8 seroprevalence was higher among HBsAg-positive donors (53%) than among HBsAg-negative donors (39%; \( p = 02 \)) and higher among HIV-positive donors (63%) than among HIV-negative donors (39%; \( p < .001 \)). Seroprevalence was similar among first-time (42%) and repeat donors (38%; \( p = 29 \)) and among male (40%) and female donors (36%; \( p = .67 \)). Overall HHV-8 seroprevalence was higher than the weighted single assay reactivities (ORF65 EIA: 30%, K8 EIA: 33%, and IFA: 37%). HHV-positive or HBsAg-positive specimens showed higher reactivity in all three assays than HIV-negative or HBsAg-negative specimens, but these associations were significant only for the IFA comparing HIV-positive with HIV-negative specimens. There was no association between age and single assay reactivity, except for a decreasing trend in ORF65 EIA reactivity with increasing age (data not shown).
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Discussion

This work represents the largest serologic study to date of HHV-8 infection in African blood donors. In a Ugandan blood donor population, we estimated HHV-8 seroprevalence, investigated potential risk factors for HHV-8 seropositivity, and compared test results obtained with three serologic assays. Results in the current study are relevant to blood safety regarding HHV-8 in African populations.

HHV-8 seroprevalence was high, even though our testing algorithm emphasized specificity and therefore may have underestimated the true HHV-8 seroprevalence in Ugandan blood donors. Because blood donors in Uganda tend to be healthy young adult men who are prescreened for HIV-related risk behavior, our findings cannot be extrapolated to the general population. Nonetheless, the estimated HHV-8 seroprevalence of 40% is comparable to that in other study populations of KS-negative adults in sub-Saharan Africa (12,13,18,21,27, 30,31). Interestingly, another recent study of blood samples from the same blood bank in Uganda reported a nearly contemporaneous HHV-8 seroprevalence of 74% (31), substantially higher than our estimate. This difference is likely due to differences in the assays, cutoffs, and algorithms used for interpreting assay results.

Although the high HHV-8 seroprevalence in many African countries is well documented, HHV-8 transmission modes are still unclear. In Uganda, prevalence of sexually transmitted infections increases from adolescence to early adulthood. We did not see such a trend for HHV-8. HHV-8 seroprevalence was already high in the youngest age group (15–17 years), suggesting that most HHV-8 infections occurred prior to that age. Our findings corroborate studies from Uganda, Cameroon, and other African countries reporting steep rises in HHV-8 seroprevalence during childhood, often reaching adult levels at or before the age of puberty, indicating primarily nonsexual transmission (18,20–22). Likewise, the association of HHV-8 infection with HBsAg in our study and
others (18) points toward nonsexual HHV-8 transmission, because hepatitis B virus in Africa is thought to be primarily transmitted horizontally during childhood (32,33). Also, Kakoola et al. (31) reported similar HHV-8 seropositivity among Ugandan blood donors across adult age groups, whereas Wawer et al. (34) found that HHV-8 seropositivity decreased with age among 15- to 29-year-old Ugandans and was not associated with sexually transmitted diseases. Our finding of an association between HHV-8 and HIV seropositivity suggests that sexual HHV-8 transmission may occur, however. Thus, our observation supports similar conclusions in some (30) but not other (11,34) studies in Africa.

Our findings add to the body of evidence that HHV-8 epidemiology differs between Africa and industrialized countries. These distinctions are sharpest relative to age distribution (early childhood infection in Africa) and some risk factors (homosexual contact in industrialized countries).

Little has been published about performance of HHV-8 assays using specimens from Africa. Engels et al. (12) used latent class methods to estimate sensitivity and specificity of an IFA and two EIAs based on ORFK8.1 and ORF65; sensitivity and specificity were the lowest for the ORF65 EIA. Schatz et al. (35) evaluated nine HHV-8 serologic assays and found much lower concordance among specimens from Uganda compared with those from Europe. Studies have consistently found the HHV-8 lytic IFA to be among the most sensitive serologic assays (26,27). Our laboratory has found that diluting the serum specimen further renders the lytic IFA more specific while retaining much of the higher sensitivity (unpublished). In our study, unweighted HHV-8 seroreactivities were similar for the three assays, varying from 35% to 45%. Assay concordance was modest and lowest with the ORF65 EIA. Spira et al. (26) reported higher concordances for the same three assays when testing US KS patients and healthy volunteers. The lower concordance between the ORF65 EIA and the other assays may be due to healthy HHV-8-infected persons developing antibody to fewer antigens, or ORF65 assays may be more susceptible to false-
positive reactivity in some populations. The role of the ORF65 EIA in testing algorithms for specimens from Africans needs further evaluation.

The high HHV-8 seroprevalence among blood donors raises questions about blood safety in Africa. Although there have not been any documented cases of HHV-8 transmission by blood transfusion, HHV-8 DNA has been detected in circulating lymphocytes obtained from blood donors in the Central African Republic (13), and injecting drug use has been associated with HHV-8 infection in a US study (17). This warrants further evaluation of potential HHV-8 transmission by blood transfusion in African settings. Nevertheless, results from this and other studies provide evidence that HHV-8 transmission in Africa predominantly occurs during childhood and that future research should focus on risk factors for infection during childhood. This may lay the groundwork for identifying ways to prevent HHV-8 infection and reduce the great burden of KS disease in African populations.

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References


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