Article:
Nalwoga, Angela, Nakibuule, Marjorie, Marshall, Vickie et al. (5 more authors) (2020) Risk factors for Kaposi's sarcoma associated herpesvirus (KSHV) DNA in blood and in saliva in rural Uganda. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. ciz916. pp. 1055-1062. ISSN 1058-4838

https://doi.org/10.1093/cid/ciz916

Clinical Infectious Diseases

MAJOR ARTICLE

Risk Factors for Kaposi's Sarcoma–Associated Herpesvirus DNA in Blood and in Saliva in Rural Uganda

Angela Nalwoga,[1,2] Marjorie Nakibuule,[1] Vickie Marshall,[3] Wendell Miley,[3] Nazzarena Labo,[3] Stephen Cose,[1,2] Denise Whitby,[3,a] and Robert Newton[1,4,a]

[1]Medical Research Council/Uganda Virus Research Institute and London School of Hygiene & Tropical Medicine Uganda Research Unit, Entebbe, Uganda; [2]London School of Hygiene and Tropical Medicine, London, United Kingdom; [3]Viral Oncology Section, Acquired Immunodeficiency Syndrome and Cancer Virus Program, Leidos Biomedical Research, Inc., Frederick National Laboratory for Cancer Research, Frederick, Maryland, USA; and [4]University of York, York, United Kingdom

Background: Detectable Kaposi's sarcoma–associated herpesvirus (KSHV) DNA in blood and increased antibody titres may indicate KSHV reactivation, while the transmission of KSHV occurs via viral shedding in saliva.

Methods: We investigated the risk factors for KSHV DNA detection by real-time polymerase chain reaction in blood and by viral shedding in saliva, in 878 people aged 3 to 89 years of both sexes in a rural Ugandan population cohort. Helminths were detected using microscopy and the presence of malaria parasitaemia was identified using rapid diagnostic tests. Regression modelling was used for a statistical analysis.

Results: The KSHV viral load in blood did not correlate with the viral load in saliva, suggesting separate immunological controls within each compartment. The proportions of individuals with a detectable virus in blood were 23% among children aged 3–5 years and 22% among those 6–12 years, thereafter reducing with increasing age. The proportions of individuals with a detectable virus in saliva increased from 30% in children aged 3–5 years to 45% in those aged 6–12 years, and decreased subsequently with increasing age. Overall, 29% of males shed in saliva, compared to 19% of females ($P = .008$).

Conclusions: Together, these data suggest that young males may be responsible for much of the onward transmission of KSHV. Individuals with a current malaria infection had higher levels of viral DNA in their blood ($P = .031$), compared to uninfected individuals. This suggests that malaria may lead to KSHV reactivation, thereby increasing the transmission and pathogenicity of the virus.

Keywords. Kaposi's sarcoma herpesvirus DNA; risk factors; Uganda.

Kaposi's sarcoma-associated herpesvirus (KSHV) causes Kaposi's sarcoma (KS), multicentric Castleman disease, and primary effusion lymphoma [1–6]. The prevalence of KSHV and incidence of KS both vary geographically [7–9], and are endemic in sub-Saharan Africa [10, 11].

Salivary exchange is the main route of transmission of KSHV, normally occurring in early childhood and increasing with age [12–15]. In a rural population cohort in Uganda (the general population cohort [GPC]), we previously reported KSHV infections in children as young as 1 year [11, 16]. In addition to viral shedding in saliva, viral DNA detection in blood and increased antibody titres to lytic antigens are markers of frequent KSHV reactivation [13]. The KSHV-associated oncogenesis and progression of diseases, as well as virus transmission, are all thought to be related to virus reactivation [17].

Viral DNA detection in blood has been associated with KS disease risk and progression [18–21]. Additionally, treatment with combined antiretroviral therapy of KS patients living with acquired immunodeficiency syndrome has been shown to reduce the KSHV load in blood to undetectable levels [18, 22]. Determinants of KSHV DNA detection in blood among KSHV-seropositive people in the general population are not well understood. The presence of a viral load in plasma and peripheral blood mononuclear cells (PBMCs) has been reported mainly in high-risk groups, such as individuals living with human immunodeficiency virus (HIV) and patients with KSHV-related diseases [18, 23–27]. A few studies have reported KSHV viral loads in blood donors (adults) in nonendemic areas [28–30] and another study reported plasma viral loads in a population-based HIV survey in an endemic area [31].

Environmental factors may contribute to high KSHV transmission in endemic areas. We have previously shown that KSHV seroprevalence is associated with malaria parasitaemia [16], higher malaria antibody titres [32], and helminth infections [33, 34]. We and others have reported that KSHV shedding in saliva is more common in males, compared to females [35, 36], but no study has investigated KSHV viral loads in both blood and saliva in the same individuals within a population-based study in a KSHV-endemic area.

This study investigated KSHV viral DNA detection in PBMCs and saliva in KSHV-seropositive individuals aged 3 to 89 years

Received 10 July 2019; editorial decision 9 September 2019; accepted 16 September 2019; published online September 26, 2019.

[a]D. W. and R. N. contributed equally to this work.

Correspondence: A. Nalwoga, MRC/UVRI and LSHTM Uganda Research Unit, P.O. Box 49, Plot 51–59 Nakiwogo Road, Entebbe, Uganda (Angela.nalwoga@mrcuganda.org, angelanalwoga@gmail.com).

Clinical Infectious Diseases® 2019;XX(XX):1–8

DOI: 10.1093/cid/ciz916

from the GPC: a longstanding cohort in rural, southwestern Uganda. We also determined the risk factors associated with viral DNA detection and levels in PBMCs and in saliva, and the relationship between viral DNA detection in PBMCs and saliva and KSHV antibody levels in plasma.

METHODS

Study Population and Ethical Approvals

This work was carried out within the GPC. The GPC is a community-based cohort of 22 000 people in 25 adjacent villages in southwestern Uganda. It was established in 1989 to carry out HIV research; participants from the GPC have been followed ever since. Between July 2017 and November 2017, we nested a cross-sectional study within the GPC, enrolling 975 KSHV-seropositive (tested previously [32]) individuals who were living without HIV and aged 3 to 89 years. Participants were selected randomly after stratification for age, sex, and household. Blood, stool, and saliva samples were collected from these individuals. PBMCs and plasma were obtained from blood for immunological and virological analyses. Stool samples were used for helminth diagnoses, while saliva samples were used for KSHV viral DNA detection and quantification. Sociodemographic data were collected using standard questionnaires. This study was approved by the Uganda Virus Research Institute (UVRI) Research and Ethics Committee (reference number GC/127/16/09/566), the Uganda National Council for Science and Technology (reference number HS2123), and the London School of Hygiene & Tropical Medicine (LSHTM) Ethics Committee (reference number 11881). Written informed consent was obtained from all adults aged 18 years and above. Parents or guardians consented for children below 18 years; additionally, children aged 8–17 years provided written assent.

Laboratory Procedures

KSHV DNA was quantified in PBMCs and saliva from 878 KSHV-seropositive individuals. About 2 million PBMCs and saliva pellets were processed for DNA extraction. Study participants were instructed to rinse with 5 mL of Listerine mouthwash, emptying it, as well as saliva, in a falcon tube. Aliquots (of 1 mL each) of saliva were spun at 13 000 relative centrifugal force for 10 minutes to form saliva pellets. Thereafter, the supernatant was removed, and the saliva pellet was stored at −80°C. Genomic DNA was extracted from PBMCs and saliva pellets using a QIAamp blood kit (Qiagen, Valencia, CA), following the manufacturer's instructions. KSHV DNA was quantified using real-time polymerase chain reaction, following procedures previously reported [13, 37, 38]. KSHV DNA was detected using primers and a probe specific to the K6 gene region [39]. Additionally, the numbers of cellular equivalents in PBMCs were determined using a quantitative assay specific to human endogenous retrovirus 3 [39], which is present in 2 copies per genomic cell. Raw copies were reported for saliva KSHV DNA. Samples were amplified in triplicate; the samples that were positive in 1 or 2 reactions in the KSHV K6 assay were designated as qualitative positives. The sensitivity of the K6 assay is 3 copies.

Using an in-house Luminex assay and enzyme-linked immunosorbent assay, as previously reported [40–42], plasma samples were tested for immunoglobin G (IgG) antibody levels to the KSHV K8.1 (lytic) and open reading frame (ORF) 73 (latent) antigens. The enzyme-linked immunosorbent assay was used to confirm serostatus, while the Luminex assay was used to determine antibody levels, due to its wider dynamic range. The presence of malaria parasitaemia was diagnosed using rapid diagnostic tests (ONE STEP Malaria HRP-II [*Plasmodium falciparum*] and pLDH [*Plasmodium* species] Antigen Rapid Test). A single stool sample was provided by each participant. This was analyzed for helminths (*Schistosoma mansoni*, *Ascaris lumbricoides*, *Tichuris trichiura*, *Trichostrongylus spp*, and hookworm) using the Kato Katz microscopy method, following the manufacturer's instructions. Details of this procedure have been reported elsewhere [43, 44].

Statistical Analysis

A statistical analysis was carried out using STATA version 13 (Statacorp, College Station, TX). Graphs were drawn using STATA and GraphPad Prism version 6. Qualitative, positive samples were given a constant value of 0.04 for saliva and 0.5 for PBMCs, which were below the values of the lowest qualified samples for a quantitative analysis. Viral load levels were $\log_{10}$ transformed. First, the risk factors associated with viral DNA detection (as a categorical outcome variable) in saliva and blood, separately, were obtained using logistic regression modelling. Thereafter, the risk factors associated with increasing levels of viral DNA (as a continuous outcome variable) in saliva and in blood were separately determined using linear regression modelling. Likelihood ratio tests were used to select the best fit models.

RESULTS

Study Participants' Characteristics

We tested 878 individuals for KSHV viral DNA; 49% (410/834) were males and 3% (27/840), 11% (95/840), 13% (110/840), 8% (67/840), 17% (139/840), 14% (121/840), 14% (118/840), 9% (74/840), and 11% (89/840) were aged 3–5, 6–12, 13–18, 19–25, 26–35, 36–45, 46–55, 56–65, and 66–89 years, respectively (Table 1). The proportions of individuals with malaria parasitaemia were 4% (34/834) overall and 11% (13/120) among children aged 3–12 years. Previously, we reported an annual malaria prevalence of 18% in the same population [16]. The lower prevalence of malaria infection in this study might be attributed to sample collection during the dry season. Hookworm was the most prevalent helminth, at 15% (104/686), followed by *Schistosoma mansoni* and *Ascaris lumbricoides* at 1% (8/686) each and *Trichuris trichiura* at 0.1% (1/686). We

Table 1. General Characteristics and Parasite Infection Status

Sex, males	49% (410/834)
Age, mean (range)	36 (3–89)
Age groups	
2–5	3% (27/840)
6–12	11% (95/840)
13–18	13% (110/840)
19–25	8% (67/840)
26–35	17% (139/840)
36–45	14% (121/840)
46–55	14% (118/840)
56–65	9% (74/840)
66–89	11% (89/840)
Malaria parasitaemia	
Overall	4% (34/834)
Children aged 3–12 years	11% (13/120)
Schistosoma mansoni infection status	1% (7/686)
Hookworm infection status	15% (104/686)
Ascaria lumbricoides infection status	1% (8/686)
Trichuris trichiura infection status	0.2% (1/685)

Data are among participants tested for Kaposi's sarcoma–associated herpesvirus viral DNA. The presence of malaria parasitaemia was determined using rapid diagnostic tests. Helminth status was determined from a single stool sample using the Kato Katz method.

may have slightly underestimated the true prevalence of helminths, because a single-sample test was used rather than a triple-sample test.

Blood and Saliva DNA Detection and Levels

We did not observe a correlation between KSHV DNA copy numbers in PBMCs and DNA copy numbers in saliva (Figure 1). The proportion of individuals with detectable viral DNA in saliva was higher than the proportion of people with the detectable viral DNA in PBMCs (Figure 2). Children had the highest proportions of detectable viral DNA in PBMCs (Figure 2A) and in saliva (Figure 2B), decreasing with increasing age in adults. The trends were similar for females and males, with males having higher proportions of detectable viral DNA in saliva.

Associations Between Risk Factors and DNA

The proportion of individuals with detectable viral DNA in PBMCs decreased with increasing age; this trend was significant even after adjusting for sex and parasite infections (Table 2). Individuals infected with malaria parasites had higher levels of KSHV DNA in blood, compared to malaria-uninfected individuals (adjusted regression coefficient 0.79 [confidence interval [CI], .07–1.50]; $P = .031$; Table 3). We observed no statistically significant associations with other measured risk factors, including age, sex, hookworm infection, and *S. mansoni* infection (Table 3).

Associations Between Risk Factors and DNA in Saliva

Overall, males had a higher risk of shedding viral DNA, compared to females (adjusted odds ratio 1.63 [CI, 1.14–2.34]; $P = .008$; Table 4). Similar to PBMCs, the proportion of participants with shedding in saliva diminished with increasing age, even after adjusting for sex and parasite infections ($P = .0001$; Table 4). Additionally, compared to females, males had higher

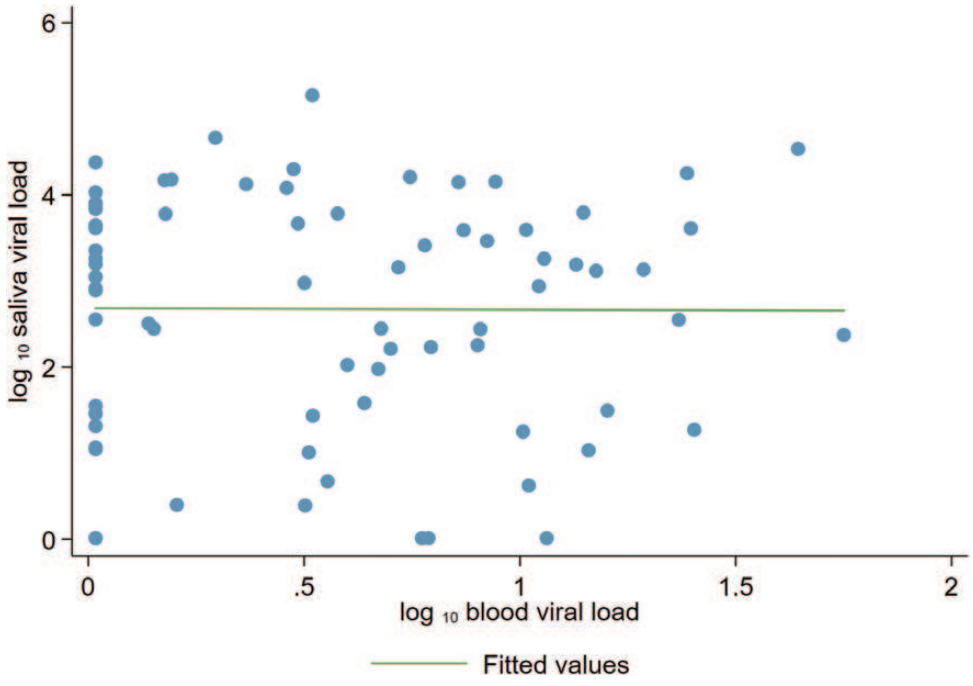

Figure 1. Kaposi's sarcoma–associated herpesvirus (KSHV) viral load in saliva and in peripheral blood mononuclear cells. KSHV viral loads were measured using real-time polymerase chain reaction.

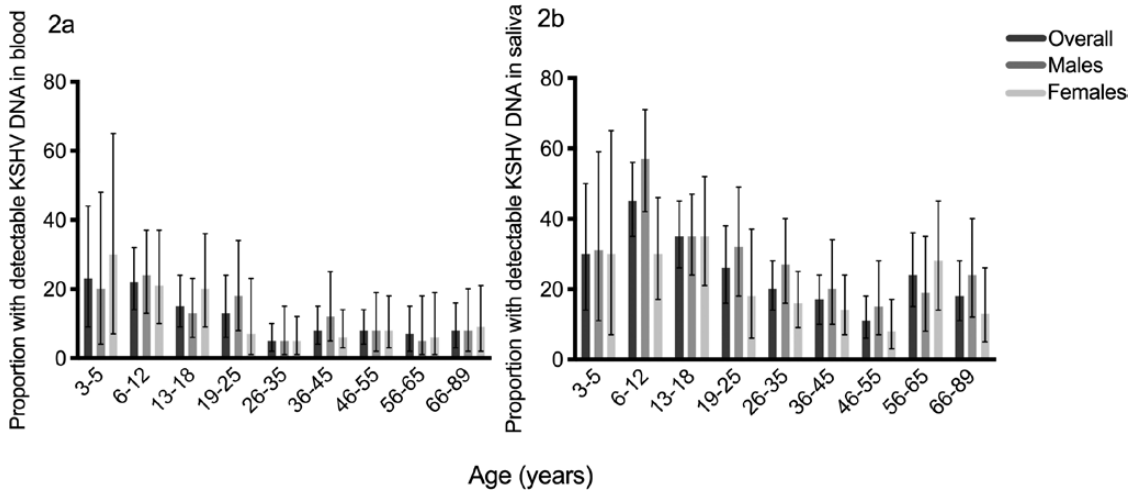

Figure 2. Proportion of individuals with detectable Kaposi's sarcoma–associated herpesvirus (KSHV) in (*A*) peripheral blood mononuclear cells and (*B*) saliva. KSHV viral loads were measured using real time polymerase chain reaction. Abbreviation: KSHV, Kaposi's sarcoma–associated herpesvirus.

levels of KSHV DNA in saliva (adjusted regression coefficient 0.46 [CI, .05–.87]; $P = .027$; Table 5).

DNA Detection and Antibody Levels

Individuals with detectable viral DNA in PBMCs (Supplementary Figure 1*A*) and in saliva (Supplementary Figure 1B) had higher IgG antibodies to the K8.1 antigen ($P < .0001$), as previously reported [13]. There were no differences in IgG antibody levels to the ORF73 antigen between individuals with or without detectable viral DNA in the blood (Supplementary Figure 1*C*) or in saliva (Supplementary Figure 1*D*).

DISCUSSION

This is the first population-based study to report on the presence and levels of KSHV viral DNA in the blood and saliva of apparently healthy people across the life course. The proportion of individuals with detectable viral DNA in saliva was higher than the proportion of individuals with detectable viral DNA in blood, consistent with previous reports [22, 45–47]. We previously reported KSHV and EBV DNA shedding in the saliva of children and their mothers in Uganda, and noted that Epstein-Barr Virus DNA was shed more frequently and at higher levels than KSHV [38].

Table 2. Risk Factors for the Presence of Detectable Kaposi's Sarcoma–Associated Herpesvirus DNA in Blood (Categorically)

	% Detectable Viral DNA in Blood	OR (95% CI)	*P* Value	Adjusted[a] OR (95% CI)	*P* Value
Age group					
3–12	23% (27/120)	1		1	
13–25	15% (26/177)	0.59 (.33–1.08)		0.63 (.33–1.17)	
26–50	7% (20/307)	0.24 (.13–.45)		0.29 (.15–.57)	
50+	8% (18/227)	0.30 (.16–.57)	<.0001	0.34 (.16–.72)	.0014
Sex					
Female	10% (41/419)	1		1	
Male	12% (49/406)	1.27 (.82–1.96)	.294	0.89 (.55–1.45)	.638
Malaria parasitaemia					
Negative	10% (83/791)	1		1	
Positive	21% (7/34)	2.21 (.93–5.24)	.071	1.59 (.64–3.95)	.321
Schistosoma mansoni					
Negative	11% (77/672)	1		1	
Positive	43% (3/7)	5.80 (1.27–26.38)	.023	11.04 (2.16–56.97)	.004
Hookworm					
Negative	13% (74/576)	1		1	
Positive	6% (6/103)	0.42 (.18–.99)	.048	0.41 (.16–1.04)	.061

Logistic regression was used for the statistical analysis. The presence of malaria parasitaemia was determined using rapid diagnostic tests. Helminth status was determined from a single stool sample using the Kato Katz method.

Abbreviations: CI, confidence interval; OR, odds ratio.

[a]Adjusted for age, sex, malaria parasitaemia, *Schistosoma mansoni*, and hookworm infection status.

Table 3. Risk Factors Associated With Increasing Levels of Kaposi's Sarcoma–Associated Herpesvirus DNA in Blood (Continuously)

	Coef (95% CI)	*P* Value	Adjusted[a] Coef (95% CI)	*P* Value
Age group				
3–12	Ref		Ref	
13–25	−0.39 (−.86 to .07)		−0.35 (−.84 to .15)	
26–50	0.19 (−.31 to .69)		0.26 (−.30 to .83)	
50+	−0.32 (−.83 to .19)	.084	−0.06 (−.70 to .57)	.160
Sex				
Female	Ref		Ref	
Male	0.10 (−.27 to .47)	.594	0.21 (−.19 to .61)	.292
Malaria parasitaemia				
Negative	Ref		Ref	
Positive	0.71 (.05–1.38)	.036	0.79 (.07–1.50)	.031
Schistosoma mansoni				
Negative	Ref		Ref	
Positive	0.17 (−.87 to 1.21)	.750	−0.15 (−1.30 to 1.00)	.797
Hookworm				
Negative	Ref		Ref	
Positive	0.30 (−.45 to 1.05)	.79	0.37 (−.46 to 1.21)	.372

Linear regression modelling was performed on $\log_{10}$ transformed KSHV DNA levels for statistical analysis. The presence of malaria parasitaemia was determined using rapid diagnostic tests. Helminth status was determined from a single stool sample using Kato Katz method.

Abbreviations: CI, confidence interval; Coef, linear regression coefficient; KSHV, Kaposi's sarcoma–associated herpesvirus; Ref, reference.

[a]Adjusted for age, sex, malaria parasitaemia, *Schistosoma mansoni*, and hookworm infection status.

In this study, we observed no correlation between levels of KSHV DNA in blood and in saliva. The detection of KSHV DNA in blood and in saliva may reflect a reactivation of the virus or an initial infection that manifests with lytic replication. Viral shedding in saliva leads to transmission of the virus [13], while a viral load in blood has been implicated in disease risk and progression [18, 23]. The lack of a correlation between blood and saliva viral DNA suggests that the mechanisms for reactivation of the virus in blood and in saliva may be different. This may imply that distinct immune control measures are required to prevent viral reactivation in the different compartments. For insistence, immunoglobin A production in saliva may be important for viral control in oral fluids, while T and natural killer cell responses may play a more pivotal role in the control of viral reactivation in peripheral blood. Alternatively, environmental factors, such as plant derivates that have been shown to reactivate KSHV in vitro [48], may play a role in viral reactivation in oral fluids if chewed. Therefore, studies of immune correlates of KSHV DNA detection in saliva and PBMC are warranted.

Table 4. Risk Factors for the Presence of Detectable Kaposi's Sarcoma–Associated Herpesvirus DNA in Saliva (Categorical)

	% Detectable Viral DNA in Saliva	OR (95% CI)	*P* Value	Adjusted[a] OR (95% CI)	*P* Value
Age group					
3–12	42% (50/120)	1			
13–25	31% (55/175)	0.64 (.40–1.40)		0.61 (.37–1.02)	
26–50	18% (56/310)	0.31 (.19–.49)		0.38 (.24–.63)	
50+	17% (39/231)	0.28 (.18–.47)	<.0001	0.30 (.18–.54)	.0001
Sex					
Female	19% (79/423)	1		1	
Male	29% (119/407)	1.80 (1.30–2.49)	<.0001	1.63 (1.14–2.34)	.008
Malaria parasitaemia					
Negative	24% (188/796)	1		1	
Positive	29% (10/34)	1.35 (.63–2.87)	.439	0.98 (.44–2.16)	.952
Schistosoma mansoni					
Negative	26% (174/675)	1		1	
Positive	43% (3/70)	2.16 (.48–9.74)	.317	2.43 (.51–11.52)	.265
Hookworm					
Negative	27% (158/578)	1		1	
Positive	18% (19/104)	0.59 (.35–1.009)	.054	0.66 (.38–1.14)	.136

Logistic regression was used for statistical analysis. The presence of malaria parasitaemia was determined using rapid diagnostic tests. Helminth status was determined from a single stool sample using the Kato Katz method.

Abbreviations: CI, confidence interval; Coef, linear regression coefficient.

[a]Adjusted for age, sex, malaria parasitaemia, *Schistosoma mansoni*, and hookworm infection status.

Table 5. Risk Factors Associated With Levels of KSHV DNA in Saliva (Continuous)

	Coef (95% CI)	*P* Value	Adjusted[a] Coef (95% CI)	*P* Value
Age group				
3–12	Ref		Ref	
13–25	−0.50 (−1.02 to .21)		−0.61 (−.13 to −.09)	
26–50	−0.31 (−.82 to .21)		−0.26 (−.80 to .28)	
50+	−0.78 (−1.35 to −.21)	.049	−0.76 (−1.40 to −.11)	.048
Sex				
Female	Ref		Ref	
Male	0.51 (.12–.89)	.010	0.46 (.05–.87)	.027
Malaria parasitaemia				
Negative	Ref		Ref	
Positive	0.20 (−.67 to 1.07)	.651	0.05 (−.82 to .92)	.909
Schistosoma mansoni				
Negative	Ref		Ref	
Positive	−0.58 (−2.12 to .96)	.460	−0.38 (−1.94 to 1.18)	.635
Hookworm				
Negative	Ref		Ref	
Positive	−0.36 (−.96 to .32)	.326	−0.37 (−1.03 to .29)	.270

Linear regression modelling was performed on $\log_{10}$ transformed KSHV DNA levels for statistical analysis. The presence of malaria parasitaemia was determined using rapid diagnostic tests. Helminth status was determined from a single stool sample using the Kato Katz method. Abbreviations: CI, confidence interval; Coef, linear regression coefficient; KSHV, Kaposi's sarcoma–associated herpesvirus; Ref, reference.

[a]Adjusted for age, sex, malaria parasitaemia, *Schistosoma mansoni*, and hookworm infection status.

The proportion of KSHV DNA detected in blood and saliva was highest in children, compared to adults. Previous studies have reported a high risk of KSHV seropositivity in children born to KSHV-seropositive mothers [13, 35, 49]. However, seropositivity in children whose mothers were seronegative has also been reported [49, 50]. Our current study suggests that siblings or playmates may also be a major source of transmission to uninfected children.

High viral loads among children could be associated with coinfections that are very prevalent in childhood, such as coinfection with malaria; we also observed the highest prevalence of malaria parasitaemia in the same age group. We showed that participants with malaria parasitaemia have higher levels of KSHV DNA in blood, compared to those uninfected with malaria. This is the first study to relate malaria parasitaemia directly with KSHV load in blood. We have previously reported associations between malaria (parasitaemia and antibodies) and KSHV seroprevalence [16, 32–34]. Results from the current study support a potential role of malaria in KSHV pathogenesis. The mechanisms for the association between malaria and KSHV viral load could include immunomodulation and dysfunction associated with repeated malaria infections [51, 52].

In the present study, males (both men and boys) were more likely to shed KSHV DNA in saliva and had higher levels of viral DNA, compared to females (women and girls). This is consistent with previous studies by us and others [31, 36, 38]. Sex differences in the immune controls of KSHV infections might contribute to the higher risk of KS in men [53, 54]. These findings warrant further study.

We previously reported that high KSHV K8.1 antibody titres were associated with and predictive of KS risk [55]. In the current study, we have observed that people with detectable viral DNA in both blood and saliva have higher IgG antibody levels for K8.1, but not for ORF73, when compared to individuals without detectable viral DNA. This association confirms our previous hypothesis [55]: increased lytic antibody levels reflect more frequent KSHV reactivation.

In summary, our data are consistent with high rates of KSHV transmission in rural Uganda. This might be partly attributed to parasite coinfections, such as coinfection with malaria, which interferes with immune control or makes uninfected children susceptible to infections. Studies investigating the mechanism through which malaria affects KSHV are required. Additionally, the characterization of protective immune responses to KSHV is needed to inform vaccine development and to develop strategies to lower KSHV transmission in endemic areas.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Acknowledgements. The authors thank the study participants from Kyamulibwa, Kalungu district and the general population cohort and Clinical Diagnostic Laboratory services teams at the Medical Research Council/Uganda Virus Research Institute (UVRI) who participated in data and sample collection.

Disclaimer. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor

does the mention of trade names, commercial products, or organizations imply endorsement by the US Government.

Financial support. This work was supported by the African Partnership for Chronic Disease Research, University of Cambridge, United Kingdom; federal funds from the National Cancer Institute, National Institutes of Health (contract number HHSN261200800001E); the Intramural Research Program of the National Institutes of Health, National Cancer Institute; and the Makerere University/UVRI Centre of Excellence for Infection and Immunity Research and Training, which is supported through the The Developing Excellence in Leadership, Training And Science Africa Initiative (grant number 107743), an independent funding scheme of the African Academy of Sciences and Alliance for Accelerating Excellence in Science in Africa that is supported by the New Partnership for Africa's Development Planning and Coordinating Agency with funding from the Wellcome Trust (grant number 107743) and the UK government. The Medical Research Council (MRC)/UVRI and London School of Hygiene & Tropical Medicine Uganda Research Unit are jointly funded by the UK MRC and the UK Department for International Development (DFID) under the MRC/DFID Concordat agreement and is also part of the European and Developing Countries Clinical Trials Partnership program, supported by the European Union.

Potential conflicts of interest. The authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

References

1. Schulz TF. Kaposi's sarcoma-associated herpesvirus (human herpesvirus-8). J Gen Virol **1998**; 79(Pt 7):1573–91.
2. Sunil M, Reid E, Lechowicz MJ. Update on HHV-8-associated malignancies. Curr Infect Dis Rep **2010**; 12:147–54.
3. Chang Y, Moore PS. Kaposi's sarcoma (KS)-associated herpesvirus and its role in KS. Infect Agents Dis **1996**; 5:215–22.
4. Cesarman E, Chang Y, Moore PS, Said JW, Knowles DM. Kaposi's sarcoma-associated herpesvirus-like DNA sequences in AIDS-related body-cavity-based lymphomas. N Engl J Med **1995**; 332:1186–91.
5. Soulier J, Grollet L, Oksenhendler E, et al. Kaposi's sarcoma-associated herpesvirus-like DNA sequences in multicentric Castleman's disease. Blood **1995**; 86:1276–80.
6. 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.
7. Rohner E, Wyss N, Trelle S, et al. HHV-8 seroprevalence: a global view. Syst Rev **2014**; 3:11.
8. Weiss RA, Whitby D, Talbot S, Kellam P, Boshoff C. Human herpesvirus type 8 and Kaposi's sarcoma. J Natl Canc Inst Monogr **1998**:51–4.
9. Uldrick TS, Whitby D. Update on KSHV epidemiology, Kaposi Sarcoma pathogenesis, and treatment of Kaposi Sarcoma. Cancer Lett **2011**; 305:150–62.
10. Dedicoat M, Newton R. Review of the distribution of Kaposi's sarcoma-associated herpesvirus (KSHV) in Africa in relation to the incidence of Kaposi's sarcoma. Br J Cancer **2003**; 88:1–3.
11. Newton R, Labo N, Wakeham K, et al. Kaposi sarcoma-associated herpesvirus in a rural Ugandan cohort, 1992-2008. J Infect Dis **2018**; 217:263–9.
12. World Health Organization, International Agency for Research on Cancer. Kaposi's sarcoma herpesvirus/human herpesvirus 8. IARC Monogr **1997**;70:375–492.
13. Dedicoat M, Newton R, Alkharsah KR, et al. Mother-to-child transmission of human herpesvirus-8 in South Africa. J Infect Dis **2004**; 190:1068–75.
14. Minhas V, Crabtree KL, Chao A, et al. Early childhood infection by human herpesvirus 8 in Zambia and the role of human immunodeficiency virus type 1 coinfection in a highly endemic area. Am J Epidemiol **2008**; 168:311–20.
15. 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.
16. Nalwoga A, Cose S, Nash S, et al. Relationship between anemia, malaria coinfection, and Kaposi sarcoma-associated herpesvirus seropositivity in a population-based study in rural Uganda. J Infect Dis **2018**; 218:1061–5.
17. Aneja KK, Yuan Y. Reactivation and lytic replication of Kaposi's sarcoma-associated herpesvirus: an update. Front Microbiol **2017**; 8:613.
18. Broccolo F, Tassan Din C, Viganò MG, et al. HHV-8 DNA replication correlates with the clinical status in AIDS-related Kaposi's sarcoma. J Clin Virol **2016**; 78:47–52.
19. Engels EA, Biggar RJ, Marshall VA, et al. Detection and quantification of Kaposi's sarcoma-associated herpesvirus to predict AIDS-associated Kaposi's sarcoma. AIDS **2003**; 17:1847–51.
20. Jary A, Leducq V, Palich R, et al. Usefulness of Kaposi's sarcoma-associated herpesvirus (KSHV) DNA viral load in whole blood for diagnosis and monitoring of KSHV-associated diseases. J Clin Microbiol **2018**;56.
21. Pellet C, Chevret S, Francès C, et al. Prognostic value of quantitative Kaposi sarcoma–associated herpesvirus load in posttransplantation Kaposi sarcoma. J Infect Dis **2002**; 186:110–3.
22. Tedeschi R, Enbom M, Bidoli E, Linde A, De Paoli P, Dillner J. Viral load of human herpesvirus 8 in peripheral blood of human immunodeficiency virus-infected patients with Kaposi's sarcoma. J Clin Microbiol **2001**; 39:4269–73.
23. Borok M, Fiorillo S, Gudza I, et al. Evaluation of plasma human herpesvirus 8 DNA as a marker of clinical outcomes during antiretroviral therapy for AIDS-related Kaposi sarcoma in Zimbabwe. Clin Infect Dis **2010**; 51:342–9.
24. Campbell TB, Borok M, White IE, et al. Relationship of Kaposi sarcoma (KS)-associated herpesvirus viremia and KS disease in Zimbabwe. Clin Infect Dis **2003**; 36:1144–51.
25. Laney AS, Cannon MJ, Jaffe HW, et al. Human herpesvirus 8 presence and viral load are associated with the progression of AIDS-associated Kaposi's sarcoma. AIDS **2007**; 21:1541–5.
26. Nsubuga MM, Biggar RJ, Combs S, et al. Human herpesvirus 8 load and progression of AIDS-related Kaposi sarcoma lesions. Cancer Lett **2008**; 263:182–8.
27. Polstra AM, Cornelissen M, Goudsmit J, van der Kuyl AC. Retrospective, longitudinal analysis of serum human herpesvirus-8 viral DNA load in AIDS-related Kaposi's sarcoma patients before and after diagnosis. J Med Virol **2004**; 74:390–6.
28. Hulaniuk ML, Torres O, Bartoli S, et al. Increased prevalence of human herpesvirus type 8 (HHV-8) genome among blood donors from North-Western Argentina. J Med Virol **2017**; 89:518–27.
29. 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.
30. Hudnall SD, Chen T, Rady P, Tyring S, Allison P. Human herpesvirus 8 seroprevalence and viral load in healthy adult blood donors. Transfusion **2003**; 43:85–90.
31. Shebl FM, Emmanuel B, Bunts L, et al. Population-based assessment of Kaposi sarcoma-associated herpesvirus DNA in plasma among Ugandans. J Med Virol **2013**; 85:1602–10.
32. Nalwoga A, Cose S, Wakeham K, et al. Association between malaria exposure and Kaposi's sarcoma-associated herpes virus seropositivity in Uganda. Trop Med Int Health **2015**; 20:665–72.
33. Wakeham K, Webb EL, Sebina I, et al. Parasite infection is associated with Kaposi's sarcoma associated herpesvirus (KSHV) in Ugandan women. Infect Agent Cancer **2011**; 6:15.
34. Wakeham K, Webb EL, Sebina I, et al. Risk factors for seropositivity to Kaposi sarcoma-associated herpesvirus among children in Uganda. J Acquir Immune Defic Syndr **2013**; 63:228–33.
35. Newton R, Labo N, Wakeham K, et al. Determinants of gammaherpesvirus shedding in saliva among Ugandan children and their mothers. J Infect Dis **2018**; 218:892–900.
36. Bender Ignacio RA, Goldman JD, Magaret AS, et al. Patterns of human herpesvirus-8 oral shedding among diverse cohorts of human herpesvirus-8 seropositive persons. Infect Agent Cancer **2016**; 11:7.
37. de Sanjosé S, Marshall V, Solà J, et al. Prevalence of Kaposi's sarcoma-associated herpesvirus infection in sex workers and women from the general population in Spain. Int J Cancer **2002**; 98:155–8.
38. Newton R, Labo N, Wakeham K, et al. Determinants of gammaherpesvirus shedding in saliva among Ugandan children and their mothers. J Infect Dis **2018**; 218:892–900.
39. Yuan CC, Miley W, Waters D. A quantification of human cells using an ERV-3 real time PCR assay. J Virol Methods **2001**; 91:109–17.
40. Labo N, Miley W, Marshall V, et al. Heterogeneity and breadth of host antibody response to KSHV infection demonstrated by systematic analysis of the KSHV proteome. PLOS Pathog **2014**; 10:e1004046.
41. Nalwoga A, Miley W, Labo N, et al. Age of infection with Kaposi sarcoma-associated herpesvirus and subsequent antibody values among children in Uganda. Pediatr Infect Dis J **2018**; 37:e225–8.
42. Mbisa GL, Miley W, Gamache CJ, et al. Detection of antibodies to Kaposi's sarcoma-associated herpesvirus: a new approach using K8.1 ELISA and a newly developed recombinant LANA ELISA. J Immunol Methods **2010**; 356:39–46.
43. Bukusuba JW, Hughes P, Kizza M, et al. Screening for intestinal helminth infection in a semi-urban cohort of pregnant women in Uganda. Trop Doct **2004**; 34:27–8.

44. Katz N, Chaves A, Pellegrino J. A simple device for quantitative stool thick-smear technique in *Schistosomiasis mansoni*. Rev Inst Med Trop Sao Paulo **1972**; 14:397–400.
45. Blackbourn DJ, Lennette ET, Ambroziak J, Mourich DV, Levy JA. Human herpesvirus 8 detection in nasal secretions and saliva. J Infect Dis **1998**; 177: 213–6.
46. Pauk J, Huang ML, Brodie SJ, et al. Mucosal shedding of human herpesvirus 8 in men. N Engl J Med **2000**; 343:1369–77.
47. Triantos D, Horefti E, Paximadi E, et al. Presence of human herpes virus-8 in saliva and non-lesional oral mucosa in HIV-infected and oncologic immunocompromised patients. Oral Microbiol Immunol **2004**; 19:201–4.
48. Whitby D, Marshall VA, Bagni RK, et al. Reactivation of Kaposi's sarcoma-associated herpesvirus by natural products from Kaposi's sarcoma endemic regions. Int J Cancer **2007**; 120:321–8.
49. Borges JD, Souza VA, Giambartolomei C, et al. Transmission of human herpesvirus type 8 infection within families in American indigenous populations from the Brazilian Amazon. J Infect Dis **2012**; 205:1869–76.
50. Olp LN, Shea DM, White MK, Gondwe C, Kankasa C, Wood C. Early childhood infection of Kaposi's sarcoma-associated herpesvirus in Zambian households: a molecular analysis. Int J Cancer **2013**; 132:1182–90.
51. Kijogi C, Kimura D, Bao LQ, et al. Modulation of immune responses by *Plasmodium falciparum* infection in asymptomatic children living in the endemic region of Mbita, western Kenya. Parasitol Int **2018**; 67:284–93.
52. Loughland JR, Woodberry T, Boyle MJ, et al. *Plasmodium falciparum* activates CD16+ dendritic cells to produce tumor necrosis factor and interleukin-10 in subpatent malaria. J Infect Dis **2019**; 219:660–71.
53. Biryahwaho B, Dollard SC, Pfeiffer RM, et al. Sex and geographic patterns of human herpesvirus 8 infection in a nationally representative population-based sample in Uganda. J Infect Dis **2010**; 202:1347–53.
54. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin **2011**; 61:69–90.
55. Wakeham K, Johnston WT, Nalwoga A, et al. Trends in Kaposi's sarcoma-associated herpesvirus antibodies prior to the development of HIV-associated Kaposi's sarcoma: a nested case-control study. Int J Cancer **2015**; 136:2822–30.