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MAJOR ARTICLE

Clinical and Demographic Factors Associated With Kaposi Sarcoma–Associated Herpesvirus Shedding in Saliva or Cervical Secretions in a Cohort of Tanzanian Women

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Background. Reasons for the high prevalence of Kaposi sarcoma–associated herpesvirus (KSHV) in sub-Saharan Africa, and risk factors leading to viral reactivation and shedding, remain largely undefined. Preliminary studies have suggested that schistosome infection, which has been associated with impaired viral control, is associated with KSHV. In this study we sought to determine the relationship between active *Schistosoma mansoni* or *Schistosoma haematobium* infection and KSHV shedding.

Methods. We quantified KSHV DNA in saliva and cervical swabs from 2 cohorts of women living in northwestern Tanzanian communities endemic for *S mansoni* or *S haematobium* by real-time polymerase chain reaction. χ^2 and Fisher exact tests were used to determine differences in clinical and demographic factors between those who were and were not shedding KSHV.

Results. Among 139 total women, 44.6% were KSHV seropositive. Six percent of those with *S mansoni* and 17.1% of those with *S haematobium* were actively shedding KSHV in saliva and none in cervical samples. Women from the *S mansoni* cohort who were shedding virus reported infertility more frequently (80% vs 19.5%, $P = .009$). There was no difference in frequency of KSHV salivary shedding between schistosome-infected and -uninfected women.

Conclusions. In an area with high KSHV seroprevalence and endemic schistosome infections, we provide the first report with data demonstrating no association between schistosome infection and salivary or cervical herpesvirus shedding. KSHV salivary shedding was associated with infertility, a known effect of another herpesvirus, human herpesvirus 6.

Keywords. Kaposi sarcoma–associated herpesvirus; *S haematobium*; *S mansoni*; schistosomiasis; viral reactivation.

For reasons poorly understood, the distribution of Kaposi sarcoma–associated herpesvirus (KSHV) varies geographically [1, 2] with reports documenting seroprevalences as high as 90% in some areas of sub-Saharan Africa and <10% in the United States and most of Europe [3, 4]. Some evidence suggests that coinfections, including schistosome and sexually transmitted infections (STIs), may contribute to reactivation of KSHV from its latent state, leading to shedding of virus in saliva and transmission of KSHV [5–12]. For example, Ugandan adults with *Schistosoma mansoni* infection were more frequently KSHV seropositive and specifically had high rates of K8.1 antigen seropositivity, which has been associated with viral reactivation [13–15]. In a small number of individuals with *S mansoni* infection, the number with detectable KSHV in blood and saliva was higher than in those without *S mansoni*, but this did not reach significance [8].

Particularly in areas endemic for KSHV, transmission via salivary shedding appears to play an important role [9, 16, 17], with approximately one-third of children being KSHV seropositive by 8 years of age [18]. Behavioral habits that could contribute to transmission in saliva include sharing of candy, food premastication by adults for their infants, toothbrush sharing, and application of others' saliva to insect bites [19, 20]. Limited data exist on factors that influence KSHV reactivation and salivary shedding, which occurs intermittently and can be detected in 20%–23% of KSHV-seropositive individuals at a given time [21, 22]. There is evidence suggesting sexual intercourse could be a mode of KSHV transmission. Men who have sex with men with multiple sexual partners in the United States [12, 23] and commercial sex workers in sub-Saharan Africa [5, 24]

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have been shown to have higher seroprevalence, suggesting KSHV transmission may occur through bodily fluids other than saliva. However, there have not been any studies specifically examining KSHV shedding in the genital tract.

We hypothesized that *S mansoni* infection, and to a lesser extent *Schistosoma haematobium* infection, would be associated with KSHV shedding given that multiple human and animal studies have demonstrated impaired mucosal control of viruses in schistosome infection [25]. No study has previously investigated KSHV viral loads in both saliva and cervical samples in women within a population in a KSHV-endemic area. In this study, we compared KSHV salivary and cervical shedding in stored saliva and cervical swab samples from 2 different cohorts of Tanzanian women who did or did not have *S mansoni* or *S haematobium* infection. Our goal was to quantify associations between schistosome infection, other clinical and demographic factors, and KSHV salivary and genital tract shedding.

METHODS

Patient Consent Statement

Prior to study enrollment, nurses obtained written informed consent for study participation in a private setting.

Ethics

All women enrolled in this study were adults. All treatment and studies were provided free of charge. Permission to conduct this study was obtained from the joint research ethics committee of Bugando Medical Centre/Catholic University for Health and Allied Sciences (CREC/171/2017) and the National Institute for Medical Research (NIMR/HQ/R.8a/Vol.IX/2446) (both in Tanzania), and from Weill Cornell Medicine in New York (1612017800).

Study Population

As part of a longitudinal study of interactions of schistosomes with viral infections, saliva was collected in 2017 and 2018 at a baseline visit from women living in rural villages of northwest Tanzania in which either *S mansoni* or *S haematobium* was endemic. Women confirmed to be schistosome infected and uninfected were enrolled from the villages. We prespecified that we would assess the 2 cohorts of women separately prior to enrollment given differences in demographics and environmental exposures, as well as species-specific differences reported in host immune responses, gene expression, and microbiota with *S mansoni* versus *S haematobium* [26, 27].

Screening Procedures and Cohort Formation

Working in communities in which prior studies had indicated a prevalence of *S mansoni* or *S haematobium* of approximately 30%–50%, we invited women to be screened for study eligibility with urine, stool, and serum testing, as previously described in detail [28]. Microscopic examinations of stool processed using the Kato-Katz method and of filtered urine were performed. Schistosome circulating anodic antigen (CAA) was quantified in serum, with CAA ≥30 pg/mL considered positive [27, 28]. In *S mansoni*–endemic villages, women who were confirmed to be positive for *S mansoni* infection by both stool ova and serum CAA and women confirmed to be negative for schistosomes by stool and urine microscopy and serum CAA were enrolled in the *S mansoni* cohort. In *S haematobium*–endemic villages, women who were confirmed to be positive for *S haematobium* infection by both urine microscopy and serum CAA and women confirmed to be negative for schistosomes by stool and urine microscopy and serum CAA were enrolled into the *S haematobium* cohort. Due to difficulty finding women excreting *S haematobium* eggs [29], women living in *S haematobium*–endemic villages who had no ova seen in urine or stool but had CAA concentrations >300 pg/mL (at least 10 times greater than the cut-off) were considered to be *S haematobium* infected. Participants who were eligible for cohort participation underwent structured sociodemographic interviews in a private setting with a female nurse. Answers were recorded on data collection forms in the field. Women also underwent human immunodeficiency virus (HIV) voluntary counseling, testing, and referrals for free HIV care and treatment, if needed, in accordance with Tanzanian national guidelines as previously described [28] and had rapid *Treponema pallidum* hemagglutination tests performed, both on venous blood. Subsequent gynecologic examinations included point-of-care testing for *Trichomonas vaginalis* (OSOM, Sekisui Diagnostics) and endocervical swab collection for polymerase chain reaction (PCR) testing for *Neisseria gonorrhoeae* and *Chlamydia trachomatis*. Women also received visualization with acetic acid screening for cervical cancer in accordance with Tanzanian national guidelines. All infections diagnosed were treated, and sexual partners also received simultaneous free treatment for STIs. Infertility was defined as a woman's report of no successful pregnancy despite at least 1 year of regular unprotected intercourse as per the World Health Organization definition [30].

Saliva Collection and KSHV Testing

During the baseline visit, women provided whole-mouth stimulated saliva. After not eating for at least 90 minutes, women rinsed their mouths with water and were given 2–3 neutral gum base pellets (Glee Gum, Providence, Rhode Island) to chew. They were asked to provide 5 mL saliva into a 15 mL conical tube. Tubes were kept on ice in the field and had protease inhibitor (Sigma-Aldrich) added in a 1:100 dilution upon arrival to the laboratory. Samples were then centrifuged at 2500 RPM for 20 minutes and the supernatant was aliquoted and frozen at −80°C. DNA was extracted from saliva and endocervical swabs using a QIAamp DNA Mini Kit (Qiagen, Germany). Real-time quantitative PCR to quantify KSHV in

salivary and endocervical DNA was done on a Rotor-Gene Q machine in Mwanza, Tanzania, using the VIASURE kit (CerTest Biotech, Zaragoza, Spain) according to the manufacturer's instructions and each run was performed once per sample. A total of 250 µL of supernatant was used for each sample. Any sample with a cycle threshold (Ct) value <50 was considered positive. As quality control, each assay was run with a positive and negative control.

Serum Collection and KSHV Antibody Testing

Blood samples were collected at baseline visits and centrifuged to separate serum, and the serum was stored at −80°C. Serum was tested for the presence of KSHV K8.1 and Orf73 antibodies. A commercially available enzyme-linked immunosorbent assay (ELISA) kit (Abbexa, Cambridge, United Kingdom) was used to detect antibody against Orf73 antigen, according to the manufacturer's instructions. The Whitby laboratory at the National Institutes of Health kindly provided our laboratory with their developed K8.1 ELISA kit to detect immunoglobulin G (IgG) antibody against K8.1 recombinant proteins, as previously described [31]. This ELISA for K8.1 antigen has been shown to be specific and sensitive for KSHV infection [31]. Each plate contained 3 negative and positive controls. De-identified positive KSHV control plasma samples from African patients were graciously provided by the AIDS and Cancer Specimen Resource, funded by the US National Cancer Institute. Serum samples from patients confirmed negative for KSHV were used as negative controls to determine the cut-off value. Seropositivity was defined as reactivity to either K8.1 or Orf73 antigen or both, unless otherwise specified.

Statistics and Software

Stata software version 17 (StataCorp, College Station, Texas) was used for data analysis. Demographic and clinical characteristics were quantified as median (interquartile range [IQR]) or number (percentage). We performed univariable logistic regressions for continuous variables, and Fisher exact test for binary variables given the small sample size. Factors associated with KSHV viral shedding on univariable analysis ($P < .10$) were included in a multivariable logistic regression analysis. Age was included in multivariable analysis given that KSHV seroprevalence increases with age [13]. The displayed graph was generated using GraphPad Prism.

Water Insecurity Quantification

To estimate and quantify experiences of household water insecurity, we used a prior version of the Household Water Insecurity Experiences Scale (HWISE) [32]. We matched our questionnaire to estimate water insecurity in alignment with indicators used by the HWISE scale. If an HWISE indicator matched multiple answers on our questionnaire, we averaged the score of all the matching answers. Averaging was performed for the HWISE indicators *Plans* and *Angry*. The HWISE indicators that we could not pair to any of our questions were *Interrupt* and *Shame*. We were able to match 10 of the 12 indicators of the HWISE scale to our questionnaire for a total of 30 possible points and analyzed the score as a continuous variable. The HWISE scale considers water insecurity score to be a score ≥12 of the total 36 points. Hence, we generated a binary water insecurity variable for a score ≥10 of the total 30 points of our scale, which also equals one-third of the total possible points.

RESULTS

Participant Characteristics

In total, 98 women from the *S mansoni* cohort and 41 women from the *S haematobium* cohort, representing 95% of total enrolled participants, had saliva and cervical samples available for KSHV viral PCR and serum for KSHV antibody testing. The baseline characteristics of both cohorts are shown in Table 1.

A total of 39 (39.8%) women in the *S mansoni* cohort were found to be infected with *S mansoni* with a median egg count of 24 eggs/g of stool and a median CAA value of 2091 pg/mL. In the *S haematobium* cohort there were 17 (41.5%) diagnosed with *S haematobium* infection. Four of these had eggs seen in urine and had a median CAA of 919 (IQR, 482–5480) pg/mL. Among the 13 who had no eggs seen in urine, the median CAA was 581 (IQR, 399–10 000) pg/mL.

Women in the *S haematobium* cohort more frequently reported water insecurity, while women in the *S mansoni* cohort more frequently reported food insecurity.

KSHV Seropositivity and Viral Shedding

A total of 44 (45%) women of the 98 women in the *S mansoni* cohort were found to be KSHV seropositive (Supplementary Table 1). Of these seropositive women, 5 solely had antibodies against antigen Orf73, 32 only against K8.1, and 7 had antibodies against both K8.1 and Orf73 antigens (Figure 1). In the *S haematobium* cohort, 18 women (44%) were KSHV seropositive, of which a total of 16 (39%) had antibodies against K8.1 alone, 1 had antibodies solely against Orf73, and 1 had antibodies against both K8.1 and Orf73.

Out of the 62 seropositive women, 11 (17.7%) had KSHV DNA in saliva, while 2 (2.6%) seronegative women from a total of 77 seronegative were shedding KSHV in saliva. The median Ct value for all actively shedding women was 31.0 (IQR, 29.2–33.8).

Notably, the 2 seronegative women did not have unique clinical or demographic characteristics that might explain their KSHV shedding while KSHV seronegative. Their KSHV PCR Ct values were 34.9 and 31, neither had HIV infection, and neither reported involvement in transactional sex. One of the

Table 1. Study Participant Characteristics

Characteristic	*Schistosoma mansoni* Cohort (n = 98) No. (%) or Median (IQR)	*Schistosoma haematobium* Cohort (n = 41) No. (%) or Median (IQR)
Demographic and behavioral characteristics		
Age, y	30 (23–40)	30 (23–40)
Years attended school	7 (4–7)	7 (5–7)
Water and food insecurity		
Water insecurity score (out of 30 possible points)	0 (0–10)	9 (0–16)
Water insecurity (≥10 of 30 points on scale)	24 (24.5)	19 (46.3)
Reports missing lunch or dinner due to lack of food in past month	51 (56)	11 (26.8)
Reproductive health		
Currently breastfeeding	17 (17.4)	12 (29.3)
Reported infertility	21 (22.8)	7 (17.5)
No. of babies born	3 (2–5)	4 (2–6)
Sexual behavior		
Age at first sexual intercourse, y	17 (16–18)	17 (15–18)
Condom use in the last 3 mo	30 (33.7)	9 (22.5)
Has been paid for sex in the past year	13 (14.1)	1 (2.4)
Accepted money or gifts for sex in the past year	17 (18.5)	7 (17.1)
Helminth infections		
S mansoni	39 (39.8)	0
S haematobium	0	17 (41.5)
Hookworm	18 (18.4)	1 (2.7)
Coinfections		
HIV	9 (9.9)	0 (0)
Treponema pallidum	12 (13.2)	Not tested
Trichomonas vaginalis	17 (18.3)	8 (19.5)
Chlamydia trachomatis	8 (8.4)	1 (2.4)
Neisseria gonorrhoeae	5 (5.3)	2 (4.9)
Any coinfection[a]	38 (38.8)	11 (26.8)
Past treatment for an STI, genital discharge/itching, or other genital infection	31 (34.1)	6 (14.6)

Data are presented as No. (%) unless otherwise indicated.

Abbreviations: HIV, human immunodeficiency virus; IQR, interquartile range; STI, sexually transmitted infection.

[a]Some participants were coinfected with HIV, *T pallidum*, *T vaginalis*, *C trachomatis*, and/or *N gonorrhoeae*.

2 women was from the *S mansoni* cohort, was 33 years old, reported food insecurity, had 1 child, reported infertility, and was infected with *S mansoni*. The other, from the *S haematobium* cohort, aged 21, had no acute *S haematobium* infection, had 3 children, and was breastfeeding.

Among the 39 KSHV-seropositive women in the *S mansoni* cohort, only 5 (11.4%) were actively shedding virus in saliva, whereas in the *S haematobium* cohort, 6 of the 18 (33.3%) KSHV-seropositive women had active salivary shedding ($P = .031$).

We further examined whether K8.1 positivity correlated with reactivation of KSHV in saliva as previously described [13–15]. Overall, 56 women were K8.1 positive, of whom 39 were in the *S mansoni* cohort and 17 were in the *S haematobium* cohort. Of these 56 women, 11 (19.6%) were shedding KSHV in saliva, versus 2 (2.4%) shedding KSHV in saliva among the 83 women who were K8.1 seronegative ($P = .001$). No woman in either cohort had KSHV detected in her cervical sample.

Associations Between KSHV Seropositivity and Sociodemographic Factors

We did not find any association between KSHV seropositivity or KSHV shedding and schistosome infection in either cohort (Tables 2 and 3).

Interestingly, 80% of women in the *S mansoni* cohort who were shedding KSHV reported infertility, versus 19.5% who were not shedding KSHV (4 vs 17 women, $P = .009$). In a sensitivity analysis, this remained significant even if the 2 KSHV shedding participants whose infertility data were missing were assumed to be fertile ($P = .035$). In support of this, women in the *S mansoni* cohort with active viral shedding had a trend toward having a lower median number of children (2 vs 3, $P = .076$), which persisted when controlling for age ($P = .096$). We observed a trend toward KSHV shedding being more frequent in those without STIs (0 STIs in those with KSHV shedding vs 41% in those without KSHV shedding, $P = .079$). On multivariable regression analysis in the *S mansoni* cohort that included age, any STI, and infertility, only infertility remained

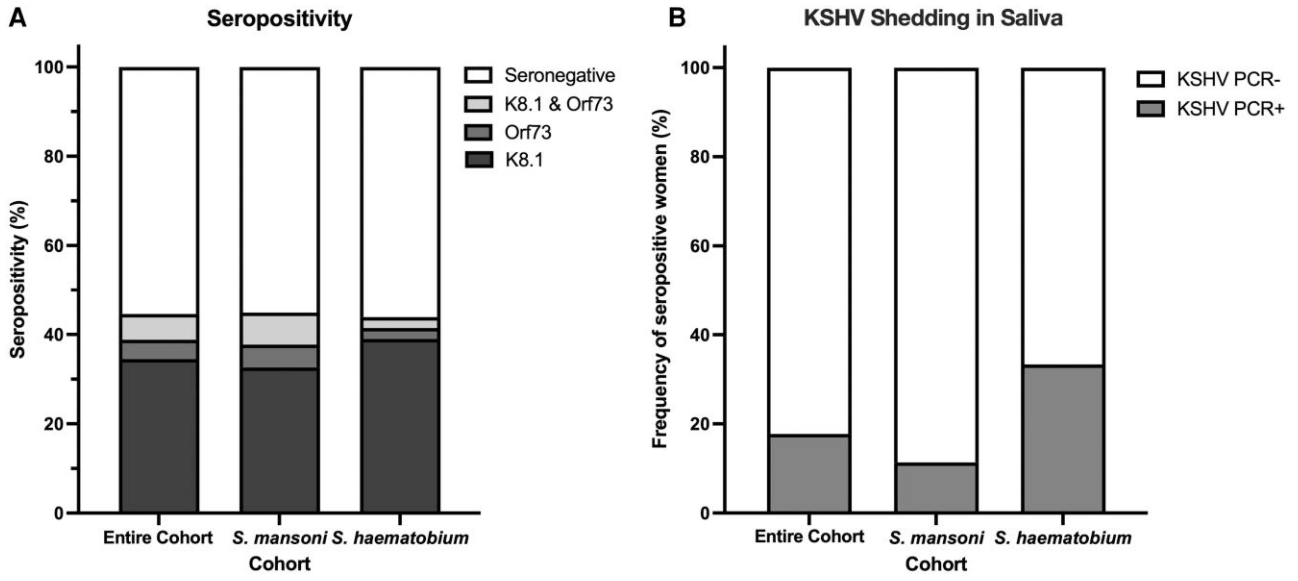

Figure 1. Kaposi sarcoma–associated herpesvirus (KSHV) test results of participants. *A*, Serology test results of KSHV-seropositive participants. Of the 139 participants, a total of 62 (44.6%) women were seropositive with 56 (40.3%) women having antibodies against K8.1, 6 (4.3%) against Orf73, and 8 (5.8%) against both KSHV antigens. A total of 44 of 98 women in the *Schistosoma mansoni* cohort were seropositive, of whom 32 (32.7%) were K8.1 positive, 5 (5.1%) ORf73 positive, and 7 (7.1%) both K8.1 and Orf73 positive. Eighteen of 41 (43.9%) women in the *Schistosoma haematobium* cohort were seropositive, of whom 16 (39%) were K8.1 positive, 1 (2.4%) ORf73 positive, and 1 (2.4%) both K8.1 and Orf73 positive. *B*, KSHV polymerase chain reaction (PCR) test results in saliva of all KSHV-seropositive participants. Of the 62 seropositive women, 11 (17.7%) were actively shedding KSHV in saliva. In the *S mansoni* group, 5 of the 44 (11.4%) seropositive women had KSHV DNA detected in their saliva, and 6 (33.3%) of the 18 seropositive women in the *S haematobium* cohort had KSHV DNA detected in their saliva.

significantly associated with KSHV shedding (odds ratio, 33.8, 95% CI [2.2–520.9], $P = .012$). Of the 9 HIV infected women, 5 were seropositive (55.6%, $P = .73$), but none had KSHV DNA detected in saliva.

There were no other significant findings in the *S mansoni* cohort between the 2 groups (Table 2).

In the *S haematobium* cohort, women with KSHV detected in saliva were significantly less likely to report water insecurity (0/34 vs 19/34 [55.9%] who did not have KSHV detected in saliva, $P = .010$), with a similar trend observed in KSHV-seropositive women (5/18 [29.8%] vs 14/23 [60.9%], $P = .058$). Seropositive women also reported less frequent food insecurity compared to negative women in the *S haematobium* cohort (1/18 [5.6%] vs 10/23 [43.5%] who were KSHV seronegative, $P = .011$).

No other significant differences were found in the *S haematobium* cohort between the 2 groups (Table 3).

DISCUSSION

In a population of adult women living in rural Tanzania, we found that nearly 10% were shedding KSHV in saliva at a given time-point when they enrolled in a longitudinal study. Little is known about KSHV viral shedding in people living with schistosome infections, and our findings provide new data on the prevalence of shedding. Furthermore, to our knowledge this is the first study assessing possible association of schistosome infections and cervical shedding, as well as the first to examine KSHV shedding in *S haematobium* infection. Our focus on salivary shedding, which plays a major role in viral transmission, is novel given that current knowledge of the association between helminth and KSHV infections is derived almost entirely from studies that measured KSHV seroprevalence [9, 16, 17].

Our findings contribute to an existing body of literature in which some studies have indicated a possible association between KSHV seropositivity and helminth infections including hookworms, *S mansoni*, and *Schistosoma japonicum* [10, 13, 33], while others have not [8, 34]. Children with helminthiases appear to have higher odds of KSHV salivary shedding than children without helminthiases [6]. In contrast, in the current study our cohorts of adult women did not reveal any association of KSHV salivary shedding with *S mansoni* or *S haematobium* infections. The difference between children and adult women in this association could be due to children being less able to control viral replication, as shown with other viral infections [35], or to distinct behavioral or environmental factors that cause children to shed KSHV more frequently than adults.

Analyzing our findings by cohort separated by *S mansoni* and *S haematobium* endemicity was crucial since each can lead to distinct pathophysiology in the infected host. Studies have shown unique alterations of the immune system and microbiome compositions differing between *S mansoni* and *S haematobium* [36, 37], highlighting the importance of assessing viral control separately in the 2 cohorts of women in this present study.

Table 2. Factors Associated With Kaposi Sarcoma–Associated Herpesvirus Viral Salivary Shedding in Women With and Without *Schistosoma mansoni* Infection

Factor	KSHV Salivary Shedding (n = 6) No. (%) or Median (IQR)	No KSHV Salivary Shedding (n = 92) No. (%) or Median (IQR)	Odds Ratio (95% CI)	*P* Value or Fisher Exact *P* Value
Helminth infections				
Schistosoma mansoni infection	3 (50)	36 (39.1)	…	.68
Hookworm infection	0	18 (19.6)	…	.59
Coinfections				
HIV infection	0	9 (10.3)	…	1.0
Treponema pallidum infection	0	12 (13.8)	…	1.0
Trichomonas vaginalis infection	0	17 (19.3)	…	.58
Chlamydia trachomatis infection	0	8 (9)	…	1.0
Neisseria gonorrhoeae infection	0	5 (5.6)	…	1.0
Any coinfection[a]	0	38 (41.3)	…	.079
Past treatment for an STI, genital discharge/itching, or other genital infection	1 (20)	30 (34.9)	…	.66
Demographic and behavioral characteristics				
Age, y	27 (23–30)	30 (23–40)	0.76 (.56–1.03)	.067
Years in school	4 (0–4)	7 (4–7)	0.93 (.82–1.0)	.2
Water and food insecurity				
Water insecurity score (out of 30 possible points)	4.8 (1–5.3)	0 (0–10)	0.6 (.07–5.4)	.63
Water insecurity (≥10 of 30 points on scale)	1 (16.7)	23 (25)	…	1.0
Reports missing lunch or dinner due to lack of food in past month	2 (40)	49 (57.9)	…	.65
Reproductive health				
Breastfeeding	0 (0)	17 (18.5)	…	.59
Infertility	4 (80.0)	17 (19.5)	…	**.009**
No. of babies born	2 (1–2)	3 (2–5)	0.55 (.29–1.06)	.12
Sexual behavior				
Age at first sexual intercourse, y	17 (17–18)	17 (16–18)	1.03 (.72–1.47)	.89
Condom use in the last 3 mo	1 (20)	29 (34.5)	…	.66
Has been paid for sex in the past year	0	13 (14.9)	…	1.0
Accepted money or gifts for sex in the past year	0	17 (19.5)	…	.58

Data are presented as No. (%) unless otherwise indicated. Nonmissing data were included in each calculation (no variable was missing >7 values). *Treponema pallidum* and HIV test results were missing from 7 participants total.

Data in bold indicate a *p* value of less than or equal to 0.05.

Abbreviations: CI, confidence interval; HIV, human immunodeficiency virus; IQR, interquartile range; KSHV, Kaposi sarcoma–associated herpesvirus; STI, sexually transmitted infection.

[a]Participants were coinfected with HIV, *T pallidum*, *T vaginalis*, *C trachomatis*, and/or *N gonorrhoeae*.

Intriguingly, we found that reported infertility was strongly associated with higher odds of KSHV shedding (80% vs 19.5%, $P = .009$) among women in our *S mansoni* cohort. Human herpesvirus 6 (HHV6), another herpesvirus that infects endometrial cells, has been previously associated with infertility and is believed to cause a dysfunctional uterine environment marked by an influx of natural killer cells and altered cytokine levels [38]. Similarly to HHV6, KSHV can be found in the genital tract [39]. We postulate that salivary KSHV viral shedding could signify replication of the virus not only in the oropharynx but also in the endometrium, where it could trigger a local immune response that, like HHV6, causes an inhabitable uterine environment for a fetus. Our data indicate that this shedding does not appear to be transmitted to cervical secretions.

In the *S haematobium* cohort, lack of food and water insecurity was significantly associated with KSHV salivary shedding. These findings contrast with a prior study showing a correlation between KSHV seroprevalence and the use of surface water [40], which was thought to be a marker of poor water security and/or poor hygiene, and possibly to higher KSHV transmission rates. Other studies have not found any correlation between water source and KSHV seropositivity [9, 41]. Of note, these particular studies investigated seropositivity and not KSHV viral shedding. It is possible that participants who lacked food and water security had access to ample surface water to use for crops and drinking, which may have contained microorganisms that triggered inadequate immune control of KSHV itself and permitted viral reactivation.

It has been reported that KSHV seroprevalence is higher in men who have sex with men with multiple sexual partners in the United States [12, 23] and in commercial sex workers in sub-Saharan Africa [5, 24] as compared to other groups. Since kissing leading to saliva exchange is common during sexual contact, it is difficult to delineate if transmission occurred

Table 3. Factors Associated With Kaposi Sarcoma–Associated Herpesvirus Viral Salivary Shedding in Women With and Without *Schistosoma haematobium* Infection

Factors	KSHV Salivary Shedding (n = 7) No. (%) or Median (IQR)	No KSHV Salivary Shedding (n = 34) No. (%) or Median (IQR)	Odds Ratio (95% CI)	*P* Value or Fisher Exact *P* Value
Helminth infection				
Schistosoma haematobium infection	4 (57.1)	13 (38.2)	…	.42
Hookworm infection	0	1 (3.2)	…	1.0
Coinfections				
HIV infection	0	0	…	
Trichomonas vaginalis infection	1 (14.3)	7 (20.6)	…	1.0
Chlamydia trachomatis infection	0	1 (2.9)	…	1.0
Neisseria gonorrhoeae infection	1 (14.3)	1 (2.8)	…	.54
Any coinfection[a]	2 (28.6)	9 (26.5)	…	1.0
Past treatment for an STI, genital discharge/itching, or other genital infection	1 (14.3)	5 (14.7)	…	1.0
Demographic and behavioral characteristics				
Age, y	31 (24–38)	29.5 (22–40)	1.00 (.92–1.11)	.91
Years in school	7 (5–7)	7 (5–7)	1.15 (.86–1.53)	.31
Water and food insecurity				
Water insecurity score (out of 30 possible points)	0 (0–4.3)	10 (0–18.67)	0.81 (.64–1.00)	.0032
Water insecurity (≥10 of 30 points on scale)	0	19 (55.9)	…	.01
Reports missing lunch or dinner due to lack of food in past month	0 (0)	11 (23.4)	…	.16
Reproductive health				
Breastfeeding	3 (42.9)	9 (26.5)	…	.4
Infertility	0	7 (21.2)	…	.32
No. of babies born	5 (3–8)	4 (2–5)	1.18 (.86–1.61)	.3
Sexual behavior				
Age at first sexual intercourse, y	16.5 (16–18)	17 (15–18)	1.18 (.74–1.88)	.5
Condom use in the last 3 mo	3 (42.9)	6 (18.2)	…	.32
Has been paid for sex in the past year	0	1 (2.9)	…	1.0
Accepted money or gifts for sex in the past year	1 (14.3)	6 (17.7)	…	1.0

Data are presented as No. (%) unless otherwise indicated.

Abbreviations: CI, confidence interval; HIV, human immunodeficiency virus; IQR, interquartile range; KSHV, Kaposi sarcoma–associated herpesvirus; STI, sexually transmitted infection.

[a]Participants were coinfected with HIV, *T vaginalis*, *C trachomatis*, and/or *N gonorrhoeae*. *Treponema pallidum* testing was not performed in the *S haematobium* cohort.

through saliva or sexual intercourse. Consistent with prior studies [6, 9, 16, 17, 22, 41, 42], we did not elucidate an association between KSHV shedding nor seropositivity and risky sexual behavior. Furthermore, we did not detect any KSHV DNA in cervical specimens, arguing that transmission likely occurs more through saliva exchange rather than actual sexual intercourse, at least in women. Though prior work has shown a positive correlation in KSHV seroprevalence and STIs [5, 12], in our study, women in the *S mansoni* cohort in our study who had HIV and/or STIs had a trend toward less, rather than more, KSHV salivary shedding. Although HIV infection has an impact on KSHV reactivation [11], we did not find an association in our study, which may be due to the low number of HIV-infected women in our cohort.

Our findings highlight the complexity of KSHV seropositivity assessment, particularly since KSHV expresses multiple different antigens and antibody responses are highly variable among individuals [31]. We found a KSHV seropositivity of approximately 45% in both cohorts. This is in line with similar KSHV seroprevalences described in other studies in the region, including among Tanzanian blood donors (56.9%) and on the other side of Lake Victoria in Uganda (84%) [13, 43]. Prior studies [13–15] have also associated K8.1 seropositivity with KSHV reactivation with salivary shedding. We also documented importance of measuring multiple antibodies to KSHV when determining seropositivity. We found that seropositivity was more common against antigen K8.1 than Orf73. Most women had antibodies against both Orf73 and K8.1, but 6 women had antibodies against Orf73 alone. Furthermore, the fact that 2 women were actively shedding KSHV in saliva but were found to be KSHV seronegative suggests limitations of these antibody measurements, particularly as neither woman had obvious distinctive clinical or demographic features that could predispose her to KSHV shedding. A possibility is that these 2 women were recently infected with KSHV and had not yet mounted a humoral immune response at the time of sample collection. Our findings and conclusions may be caused by diverse human individual antibody responses, natural variation in antibody response to

primary infection as well as reactivation, known fluctuations of KSHV seropositivity, and seroconversion over time [44].

Our study has strengths and limitations. We provide a novel analysis of KSHV shedding among 141 adult women in East Africa. Given the small numbers who were actively shedding KSHV (only 6–7 women in each cohort), we had limited power to establish associations between viral shedding and other factors. Additionally, our cohorts consisted solely of adult women. Studies have indicated that both adult men and male children have higher odds of shedding KSHV compared to women and girls [6, 13], which is an additional limitation of this study. We did not collect whole blood samples and therefore could not include malaria testing in our longitudinal cohort study, though past research has linked KSHV seropositivity to malaria infection [7] and a recent study showed an association between KSHV viral load in blood and active malaria infection [8]. Despite the lack of malaria testing in our study, all women in our cohort were symptom-free and had no clinical evidence of an acute illness when samples were obtained; therefore, active malaria infection was unlikely. Finally, since it is known that KSHV is only intermittently shed [21, 22], it would be informative to monitor KSHV viral shedding over time.

In conclusion, we present novel data investigating factors associated with KSHV salivary viral shedding in an East African population. Our work highlights the importance of identifying and enhancing our current knowledge on modes of KSHV transmission, rather than solely KSHV seroprevalence, which can have important public health implications for antiviral control strategies globally. Associations of KSHV shedding with infertility, and lack of association with schistosome infections, contribute to the knowledge about the transmission and epidemiology of KSHV infection in a region of the world in which KSHV and schistosomiasis are endemic.

Supplementary Data

Supplementary materials are available at *Open Forum 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

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Potential conflicts of interest. All authors: No reported conflicts.

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