Clinical Infectious Diseases Advance Access published November 27, 2014

MAJOR ARTICLE

Prevalence and Risk Factors for Betaherpesvirus DNAemia in Children >3 Weeks and <2 Years of Age Admitted to a Large Referral Hospital in Sub-Saharan Africa

John Tembo,[1,2,3,a] Mwila Kabwe,[1,2] Lophina Chilukutu,[1,2] Moses Chilufya,[1,2] Nyaxewo Mwaanza,[4] Chishala Chabala,[4] Alimuddin Zumla,[1,5,6] and Matthew Bates[1,2,5,a]

[1]University of Zambia - University College London Medical School Research and Training Programme, and [2]HerpeZ, University Teaching Hospital, Lusaka, Zambia; [3]Institute for Infectious Diseases, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China; [4]Department of Paediatrics and Child Health, University Teaching Hospital, Lusaka, Zambia; [5]Division of Infection and Immunity, University College London, and [6]National Institute for Health Research Biomedical Research Centre, University College London Hospitals, United Kingdom

Background. Betaherpesviruses are established causes of morbidity and mortality in immunosuppressed patient groups but have been little studied in sub-Saharan Africa, the epicenter of the human immunodeficiency virus (HIV) pandemic. In this region, primary infections with human cytomegalovirus (HCMV) and human herpesvirus type 6 (HHV-6) type 6 are endemic in infancy, but the clinical impact of these infections among pediatric inpatient groups is poorly characterized and assumptive, based largely on data from Western populations.

Methods. We used TaqMan polymerase chain reaction to screen sera from a group of 303 pediatric inpatients aged between 3 weeks and 2 years, at the University Teaching Hospital in Lusaka, Zambia. We report the prevalence of DNAemia and viral loads within this patient group, and evaluate possible clinical associations/risk factors for betaherpesvirus infections in these hospitalized children.

Results. We detected betaherpesvirus DNAemia in 59.1% (179/303) of children. HCMV was the most prevalent (41.3%), followed by HHV-6B (20.5%), HHV-7 (20.1%), and HHV-6A (0.3%). HIV infection (odds ratio [OR], 2.31; 95% confidence interval [CI], 1.37–3.90; $P = .002$), being underweight (OR, 1.82; 95% CI, 1.06–3.12; $P = .03$), and an admission diagnosis of suspected meningitis (OR, 5.72; 95% CI, 1.07–30.5; $P = .041$) were independently associated with an increased odds of HCMV DNAemia. Conversely, HHV-6B and HHV-7 DNAemia were not associated with HIV, underweight, or admission diagnosis. Median HCMV viral load was moderately but significantly higher in HIV-infected children.

Conclusions. Highly prevalent HCMV DNAemia was independently associated with HIV infection and being underweight across all age groups, and was also associated with meningitis, with previously underappreciated implications for the health and development of African children.

Keywords. CMV; HHV-6; HIV; malnutrition; meningitis.

The human betaherpesviruses (human cytomegalovirus [HCMV] and human herpesvirus [HHV] types 6A, 6B, and 7) are important causes of morbidity in children, and HCMV and HHV-6 are well-established causes of morbidity and mortality in immunocompromised patients [1, 2], but they have been little studied in sub-Saharan Africa, the World Health Organization region with the highest pediatric disease burden and also the epicenter of the human immunodeficiency virus (HIV) pandemic. Primary HCMV and HHV-6 infections are endemic in African infants and children [3–5], establishing lifelong latency with periodic reactivations or reinfections [1]. The great challenge in herpes virology

Received 30 August 2014; accepted 19 October 2014.

[a]J. T. and M. B. contributed equally to this work.

Correspondence: Matthew Bates, MSc, PhD, Programme Director, UNZA-UCLMS Research and Training Programme, D-Block Research Office, University Teaching Hospital, Lusaka, Zambia (matthew.bates@ucl.ac.uk).

Clinical Infectious Diseases®

DOI: 10.1093/cid/ciu853

is to differentiate active infections causing pathology from background subclinical viremia. To this end, the latest quantitative molecular diagnostics are being used to establish clinically informative viral load cutoffs in different patient groups and within different compartments, which can inform treatment [6–8]. The betaherpesviruses are highly cell-associated and within vulnerable patient groups, detection of high HCMV and HHV-6 DNA loads in serum is indicative of active infections linked with poor outcomes [8, 9], but absence or low viral loads in serum does not rule out localized infection restricted to specific compartments [10].

Recent prospective studies and careful review of some older but overlooked studies has led to a growing awareness of the importance of HCMV infections in high-seroprevalence settings [11–13], as a major cause of mortality in HIV-infected children [14, 15] and as a cause of developmental delay [5] and hearing loss [16–18] in children through either congenital or early infant infections, with maternal HIV infection and lower CD4 count associated with higher prevalence of congenital infection [15, 19]. HHV-6A, HHV-6B, and HHV-7 have been less well studied in sub-Saharan Africa. HHV-6B primary infection (and to a lesser extent, HHV-7) is the etiological agent of exanthema subitum, an endemic childhood illness characterized by fever, diarrhea, and skin rash [20, 21], and is strongly associated with febrile seizures [22] including febrile status epilepticus [23]. HHV-6A is rarely found in children in Europe, the United States, and Japan, but a recent study found HHV-6A to be endemic in healthy Zambian infants [3]. Elsewhere, HHV-6A is more common in immunocompromised adults, and recent animal model studies have suggested possible roles for HHV-6A in immunodeficiency [24] and AIDS progression [25]. A study in US children also demonstrated an association between HHV-6B and AIDS progression [26].

In sub-Saharan Africa, tertiary referral hospitals concentrate large numbers of seriously ill children from impoverished urban communities. At such centers, drugs to control HIV, tuberculosis, malaria, and other common bacterial, fungal, and parasite infections are broadly available, yet mortality rates remain high [27]. Rapid diagnostics and treatment for viral infections are broadly unavailable, but HCMV is an important cause of respiratory disease in HIV-infected African children [8, 14, 28, 29], and HHV-6 and HHV-7 are possible causes of nonmalarial central nervous system (CNS) infections [30]. HCMV pneumonia can be treated successfully with intravenous ganciclovir in both immunocompromised (HIV-infected South African infants with confirmed HCMV infection) [28, 29] and immunocompetent children [31]. There are generic anticytomegalovirus drugs in the pipeline, and antibetaherpesvirus activity has been reported for several low-cost alternatives such as artesunate [32, 33] and the immunostimulant active hexose correlated compound [34]. Here we present data from a broad group of admitted children at the University Teaching Hospital in Lusaka, Zambia, with the aim of determining prevalence of betaherpesvirus DNAemia and viral loads, and identifying possible clinical associations and risk factors.

METHODS

Ethics Approval

The study was approved by the Biomedical Research Ethics Committee of the University of Zambia School of Medicine, Lusaka. The mothers/guardians of all participants gave written informed consent.

Study Design and Setting

We conducted a retrospective observational study to determine the prevalence, viral loads, and risk factors associated with betaherpesvirus DNAemia in admitted children at the University Teaching Hospital, Lusaka, Zambia. We selected biobanked sera specimens from children aged between 3 weeks and 2 years of age, admitted to the general pediatric inpatient wards, who had taken part in a tuberculosis diagnostic trial [35], and screened them for all 4 betaherpesviruses and measured viral loads. The degree to which the recruited children were representative of the broader inpatient population was evaluated by comparing key demographic data with data from general admission records.

DNA Extraction and Polymerase Chain Reaction Analysis

Laboratory analysis was conducted in our dedicated 3-room molecular diagnostics laboratory. DNA from serum was extracted using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) as previously described [4, 15]. Extraction controls were included with every 11 samples. DNA extraction quality was monitored on every 11th sample using a Nanodrop (Thermo Fisher Scientific, Waltham, Massachusetts). Samples were tested for presence of HCMV, HHV-6A, HHV-6B, and HHV-7 using 3 different quantitative real-time TaqMan polymerase chain reaction (PCR) assays as described [7, 36, 37]. Oligonucleotide sequences and thermocycling conditions were as indicated (Table 1). Real-time PCR was undertaken on a Rotor-Gene 6000 (Qiagen, Hilden, Germany). The fidelity of the PCR enzyme and purity of the DNA extraction was controlled through amplification of the housekeeping gene, β-actin, from every 11th sample. Positive (commercially produced standards), negative (molecular grade water), reagent (no template), and extraction controls were included with each run. HCMV genomic DNA was purchased from the National Institute for Biological Standards and Control (Potters Bar, United Kingdom). Genomic DNA for HHV-6A, HHV-6B, and HHV-7 were donated from the reagent repository of the HHV-6 Foundation (Santa Barbara, California). Standard curves for quantitation of betaherpesvirus

Table 1. Oligonucleotide Sequences and Thermocycling Conditions

Target	Primers and Probes 5′-3′	Cycling Conditions
HCMV UL83 [37]	Forward Primer CAGTCCCGAGACMGTGAGAC	Hold @ 95°C, 10 min
	Reverse Primer TGAACATCCCCAGCATCAACG	Cycling (45 repeats)
	Probe: FAMb-TGCCACATCTGCTTGCCCGACGC-BBQb	Step 1 @ 95°C, hold 10 s
		Step 2 @ 58°C, hold 20 s
		Step 3 @ 72°C, hold 1 s
HHV-6A and HHV-6B [7]	Forward primer GGAGTGCCTGTGGGTATTC	Hold @ 94°C, 5 min
	Reverse primer CTAAGGTGAGCCAGATTCG	Cycling (55 repeats)
	HHV-6A probe: HEX-TGCAGCCATTTCTTTGGAAAGC-TAMRA	Step 1 @ 94°C, hold 15 s
	HHV-6B probe: FAM-TGCAGCCACCTCCTTGGAAAG-TAMRA	Step 2 @ 60°C, hold 60 s
HHV-7 [36]	Forward primer TTTCCTGTGACAAAAGAAGCAGTTA	Hold @ 50°C, 2 min
	Reverse primer ATCCCACACGCTTTACGGG	Cycling (60 repeats)
	Probe: FAM-TTCCTGCGCAATAAAGTGAAAACTGTTAGCATT-TAMRA	Step 1 @ 95°C, hold 20 s
		Step 2 @ 60°C, hold 60 s
β-actin	Forward primer: CACACTGTGCCCATCTACGA	Hold 94°C 3 min
	Reverse primer: CTCAGTGAGGATCTTCATGAGGTAGT	Cycling (45 cycles)
	Probe: FAM-ATGCCCTCCCCCATGCCATCCTGCGT-TAMRA	Step 1 @ 94°C
		Step 2 @ 65°C hold 50 s

Abbreviations: HCMV, human cytomegalovirus; HHV, human herpesvirus.

viral loads were prepared as follows: for HCMV, lyophilized standard was resuspended in elution buffer according to the manufacturer's instructions to create a stock solution of 5×10^6 copies/mL. HHV-6A, HHV-6B, and HHV-7 standards were human sera containing 5×10^6 copies/mL. Two hundred microliters was extracted and reconstituted in 200 μL of elution buffer. For all 4 viruses, 10-fold serial dilutions were made down to 5×10^{-1} copies/mL. Five microliters of each dilution (25 000, 2500, 250, 25, and 2.5 absolute copies) was then amplified in triplicate. The manufacturer's software was then used to generate standard curves against which cycle threshold values of clinical specimens were converted to copies per microliter of extracted DNA. Extracted DNA from clinical specimens was quantified (ng/μL) using a Nanodrop (Thermo Scientific), and viral genome equivalents per microgram of extracted DNA were calculated.

Statistical Analysis

Data analysis was undertaken using SPSS software, version 21 (IBM, Armonk, New York). Binary and continuous variables were compared by Pearson χ^2 test and Mann–Whitney *U* test respectively, between study groups and the general admitted population (Table 2). For under- or overrepresented covariates, weighting variables were calculated using the formula [prevalence in hospital population / prevalence in sample] and then multiplied together and divided by the mean weight to make a composite weighting variable. This composite weight was then used to estimate hospital population-weighted prevalences for each of the betaherpesviruses. Multivariate binary logistic regression analysis (on nonweighted data) was used to evaluate possible associations between various clinical and demographic factors and betaherpesvirus infection and virus loads. The Wald–Wolfowitz runs test was used to evaluate the randomness of the distribution of positive samples. Median viral loads were compared by HIV status and underweight status using the Mann–Whitney *U* test.

Table 2. Comparison of Key Descriptive Variables Between the Study Group and Admitted Population

Variable	Study Group	Admitted Population
No.	303	1176
Dates	June 2011–April 2012	April–May 2011
Female sex	128/301 (42.5%)	532/1167 (44.8%)
Median age (IQR), mo	12 (7–17)[a]	8 (3–13)[a]
Infant HIV infection	101/290 (34.8%)[b]	147/1142 (12.9%)[b]
Mortality	ND	128/1171 (10.9%)
Malnutrition noted on admission	110/303 (36.3%)[c]	117/1176 (9.9%)[c]

Where denominators differ from total sample size, this represents missing data for that variable.

Significance was by Pearson χ^2 test for binomial variables, Mann–Whitney *U* test for age, and Student *t* test for birth weight.

Abbreviations: HIV, human immunodeficiency virus; IQR, interquartile range; ND, no data.

[a] $P < .001$.

[b] $P < .001$.

[c] $P < .001$.

RESULTS

Patient Recruitment

We screened DNA-extracted sera from 303 children aged between 3 weeks and 2 years of age. Median age was 12 months (interquartile range [IQR], 7–17 months), 42.5% of the children were female, 34.8% were HIV infected, and 36.3% were considered to be malnourished on admission (ie, admitted to the malnutrition ward due to weight-for-height z score < −2 or on clinical grounds at the discretion of the attending physician). Comparison with these routine admissions data showed that although sex was representative, the study children were significantly older, more likely to be HIV infected, and more likely to be malnourished (Table 2), consistent with suspicion of tuberculosis or tuberculosis risk factors (HIV infection, household contact, etc), which were key inclusion criteria on the parent trial [35].

Prevalence of Betaherpesvirus Infections

Betaherpesvirus DNAemia was detected by PCR in sera from 59.1% (179/303) of children (Table 3). All negative controls were negative, and positive specimens were randomly distributed (analysis not shown). HCMV was the most prevalent virus (41.3% [125/303]), significantly more prevalent than HHV-6B (20.5% [62/303]) ($P < .001$) and HHV-7 (20.1% [61/303]) ($P < .001$). Weighting for the overrepresentation of HIV-infected children, older median age, and increased prevalence of malnutrition did not significantly affect betaherpesvirus prevalence (Table 3). HHV-6A was rare, with just 1 case—a male infant 9 months of age, admitted with suspected pneumonia, tuberculosis, or pertussis. A gastric aspirate was culture negative for tuberculosis; the baby was HIV negative and had a weight-for-age z score (WAZ) of −1.22. The mother reported recent loss of appetite and weight loss, fevers, productive cough, difficulties breathing, and lethargy. This baby was also positive for HCMV and HHV-7, 1 of just 9 babies positive for 3 betaherpesviruses. Fifty-two cases were coinfected with 2 viruses (Table 3).

Risk Factors for Betaherpesvirus Infections

HCMV DNAemia was significantly more prevalent among HIV-infected children than among HIV-uninfected children (54.5% [55/101] vs 34.0% [65/191]; $P = .001$), a univariate effect not seen for HHV-6B or HHV-7 (Table 3). Multivariate binary logistic regression analysis demonstrated that HCMV DNAemia in children was independently associated with HIV infection (odds ratio [OR], 2.31; 95% confidence interval [CI], 1.37–3.90; $P = .002$) and being underweight (OR, 1.82; 95% CI, 1.06–3.12; $P = .03$) (Table 4). Among 8 children admitted with suspected meningitis, 6 were positive for HCMV DNAemia (OR, 5.72; 95% CI, 1.07–30.5; $P = .041$). In our study group, betaherpesvirus DNAemia was not associated with fever (≥38°C), breastfeeding (an established route of transmission for HCMV, although only 13 children were not being, or had not been, breast fed), or reported recent rash (none of the children presented with a rash) (Table 4).

The odds of HCMV DNAemia decreased significantly with age (OR, 0.95; 95% CI, .91–.99; $P = .008$), with a similar trend for HHV-6B. Conversely, there was a trend for increased odds of HHV-7 DNAemia with age. To investigate these age trends in

Table 3. Betaherpesvirus Prevalence Stratified by HIV Status

Virus	Unweighted, No. (%, SE)	Weighted[a], No. (%, SE)	HIV Uninfected[b], No. (%, SE)	HIV Infected[b], No. (%, SE)	*P* Value
Any betaherpesvirus	179/303 (59.1, 2.8)	170/303 (56.0, 2.9)	105/191 (55, 3.6)	68/101 (67.3, 4.7)	.041
Totals					
HCMV	125/303 (41.3, 2.8)[c,d]	111/303 (36.5, 2.8)	65/191 (34, 3.4)	55/101 (54.5, 5.0)	.001
HHV-6A	1/303 (0.3, 0.3)	2/303 (0.6, 0.4)	1/191 (0.5, 0.5)	0/101 (0%)	.466
HHV-6B	62/303 (20.5, 2.3)[c]	73/303 (24.0, 2.5)	44/191 (23, 3.1)	17/101 (16.8, 3.7)	.215
HHV-7	61/303 (20.1, 2.3)[d]	53/303 (17.4, 2.2)	42/191 (22, 3.0)	17/101 (16.8, 3.7)	.296
Coinfections					
HCMV + HHV-6B	27/303 (8.9, 1.6)	33/303 (10.8, 1.8)	18/191 (9.4, 2.1)	8/101 (7.9, 2.7)	.668
HCMV + HHV-7	16/303 (5.3, 1.3)	9/303 (2.9, 1.0)	11/191 (5.8, 1.7)	5/101 (5.0, 2.2)	.773
HHV-6B + HHV-7	9/303 (3, 0.9)	7/303 (2.4, 0.9)	7/191 (3.7, 1.4)	2/101 (2.0, 1.4)	.428
HCMV + HHV-6B + HHV-7	8/303 (2.6, 0.9)	8/303 (2.4, 0.9)	5/191 (2.6, 1.2)	3/101 (3.0, 1.7)	.861
HCMV + HHV-6A + HHV-7	1/303 (0.3, 0.3)	2/303 (0.6, 0.5)	1/191 (0.5, 0.5)	0/101 (0, 0)	.466

Significance is by Pearson χ^2 test.

Abbreviations: HCMV, human cytomegalovirus; HHV, human herpesvirus; HIV, human immunodeficiency virus; SE, standard error.

[a] Weighted for recruitment biases in age, infant HIV status, and malnutrition status on admission.

[b] Unweighted data stratified by HIV status.

[c] $P < .001$.

[d] $P < .001$.

Table 4. Multivariate Binary Logistic Regression Analysis of Risk Factors Associated With Betaherpesvirus DNAemia in Pediatric Admissions <2 Years of Age

	HCMV (>200 Copies/mL Sera)		HHV-6B (>200 Copies/mL Sera)		HHV-7	
Risk Factor	aOR (95% CI)	*P* Value	aOR (95% CI)	*P* Value	aOR (95% CI)	*P* Value
Male sex	1.12 (.68–1.85)	.654	0.59 (.34–1.06)	.076	0.69 (.38–1.27)	.689
HIV infected	2.31 (1.37–3.90)	.002	0.74 (.39–1.41)	.361	0.60 (.31–1.15)	.125
Fever (≥38°C)	1.01 (.47–2.19)	.976	0.54 (.18–1.63)	.277	1.02 (.41–2.53)	.963
Underweight (WAZ < −2)	1.82 (1.06–3.12)	.030	0.92 (.50–1.71)	.791	1.01 (.55–1.88)	.967
Age, mo	0.95 (.91–.99)	.012	0.96 (.92–1.01)	.080	1.04 (1.0–1.09)	.064
Breast-fed	2.53 (.72–8.87)	.147	3.59 (.45–28.9)	.230	2.39 (.30–19.2)	.414
Rash in the past month	2.27 (.77–6.68)	.136	1.56 (.44–5.57)	.495	0.61 (.22–1.70)	.345
Diagnosis at admission						
Pneumonia	0.74 (.45–1.24)	.251	0.97 (.53–1.76)	.913	1.39 (.76–2.53)	.281
Acute diarrhea + dehydration	1.17 (.53–2.60)	.701	0.73 (.26–2.03)	.549	1.73 (.71–4.19)	.846
Malaria	0.66 (.17–2.58)	.546	1.34 (.34–5.32)	.675	0.35 (.40–2.81)	.323
Meningitis[a]	5.72 (1.07–30.5)	.041	0.40 (.50–3.36)	.396	NC	NC

Odds of betaherpesvirus infections were unaffected by infant tuberculosis status. Analysis of all 3 viruses was adjusted for the effects of age, infant HIV status, and being underweight, as defined by having a WAZ < −2.

Abbreviations: aOR, adjusted odds ratio; CI, confidence interval; HCMV, human cytomegalovirus; HHV, human herpesvirus; HIV, human immunodeficiency virus; NC, not calculable; WAZ, weight-for-age *z* score.

[a] Of 8 children admitted with suspected meningitis, 6 were positive for HCMV, 1 was positive for HHV-6B, and none were positive for HHV-7.

more detail, we plotted betaherpesvirus DNAemia prevalence by age in months (Figure 1*A*). We observed peaks of HCMV prevalence at 3–5 months and 18–20 months, indicative of early primary infection (with some residual DNAemia from congenital infections) followed by reactivation/reinfection in older children. A higher prevalence of HCMV DNAemia was associated with HIV infection (Figure 1*B*) and being underweight (Figure 1*C*) across all age groups.

HHV-6B displayed a similar temporal profile to HCMV, with peaks suggestive of primary and secondary infections (Figure 1*A*). The temporal signature of HHV-7 DNAemia was clearly distinct from HCMV and HHV-6B, dropping from 20% to 10% over the first 6 months of life, but then steadily increasing back to 20% by 12 months, and remaining stable (Figure 1*A*). Coinfections with >1 betaherpesvirus were common, detected in 33.5% (60/179) of all cases (51 cases with 2 viruses, and 9 cases with 3 viruses), but HIV-infected children were no more likely to have a double or triple infection than HIV-uninfected children (data not shown). There was a significant correlation between HCMV and HHV-6B (Pearson correlation 0.157; $P = .006$), with HHV-6B DNAemia being associated with increased odds of HCMV DNAemia, controlled for HIV status, age, and WAZ score (OR, 2.20; 95% CI, 1.36–4.62; $P = .003$) (data not shown).

Viral Loads

Comparing viral loads, as defined by betaherpesvirus genome equivalents per microgram of extracted DNA, we found the highest viral loads for HHV-7 (median, 13 218 copies/μg DNA; IQR, 5185–25 195) followed by HCMV (median, 2993 copies/μg DNA; IQR, 991–10 630). HHV-6B median viral load was more than a log lower than that of HCMV (median, 91 copies/μg DNA; IQR, 42–192) (Table 5 and Figure 2*A*). The single HHV-6A positive sample had a relatively high viral load of 15 400 genome equivalents per microgram extracted DNA. Stratifying by HIV status, median viral load for HCMV was significantly higher among HIV-infected children (Figure 2*B*) (HIV uninfected: median, 1985 [95% CI, 884–6510] vs HIV infected: median, 3639 [95% CI, 1567–18 980]; $P = .024$). Conversely, median HCMV viral load did not differ significantly by WAZ ($P = .120$; data not shown).

DISCUSSION

Betaherpesvirus DNAemia was detected in two-thirds of admitted Zambian infants with HCMV DNAemia (41.3% [125/303]), being roughly twice as prevalent as both HHV-6B (20.5% [62/303]) and HHV-7 (20.1% [61/303]). HCMV DNAemia was twice as prevalent among HIV-infected children, consistent with previous studies [4], which have also shown links with AIDS progression [38] and death [14]. The persistent association of HCMV infection with HIV infection and being underweight is consistent with a previous population-based study, which showed that early infant HCMV infection is independently linked with impaired physical development of Zambian children [5].

The median HCMV viral load (3.9 $\log_{10}$ copies/mL whole blood) was similar to a previous study of hospitalized Zambian

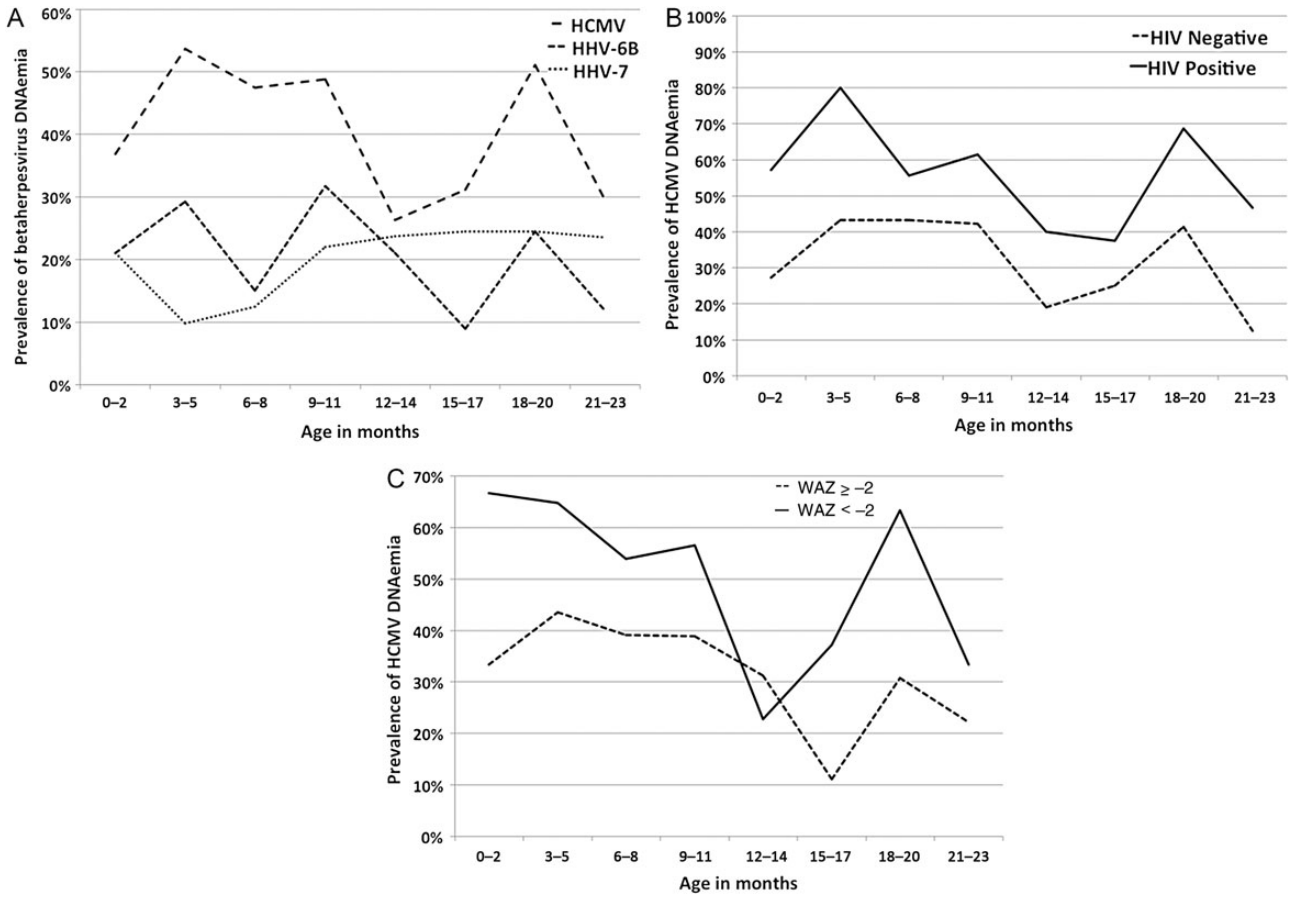

Figure 1. *A*, Betaherpesvirus prevalence stratified by age among admitted children <2 years of age. *B*, Human cytomegalovirus (HCMV) prevalence stratified by age and human immunodeficiency virus (HIV) status among admitted children. *C*, HCMV prevalence stratified by age and underweight among admitted children. Abbreviations: HHV, human herpesvirus; WAZ, weight-for-age *z* score.

children [4]. A recent South African study of HCMV pneumonia in infants used receiver operating characteristic analysis to determine that a viral load of 4.1 $\log_{10}$ copies/mL whole blood could isolate 70% of cases with a positive urine culture [8]. Here we also analyzed copies per microgram of extracted DNA (normalizing for the amount of cell DNA present in the specimen) and found that although the range of viral loads was extremely broad, median HCMV viral load (copies/μg extracted DNA) was significantly higher (by 1 log) among HIV-infected children, an effect not evident for HHV-6 or HHV-7.

Table 5. Betaherpesvirus Loads

	Genome Equivalents/μg Extracted DNA				Genome Equivalents/mL Whole Blood			
	Median	IQR	Min	Max	Median	IQR	Min	Max
HCMV	2993[a,b]	991–10 630	31	207 262 671	9404	3739–38 640	207	775 162 391
HHV-6A	15 400	15 400–15 400	15 400	15 400	172 812	172 812–172 812	172 812	172 812
HHV-6B	91[a,c]	42–192	2	27 389	498	296–1062	201	27 115
HHV-7	13 218[b,c]	5185–25 195	247	5 267 277	56 868	23 772–140 704	4005	9 547 567

Abbreviations: HCMV, human cytomegalovirus; HHV, human herpesvirus; IQR, interquartile range.

[a] $P < .001$ Mann–Whitney *U* test.

[b] $P < .001$ Mann–Whitney *U* test.

[c] $P < .001$ Mann–Whitney *U* test.

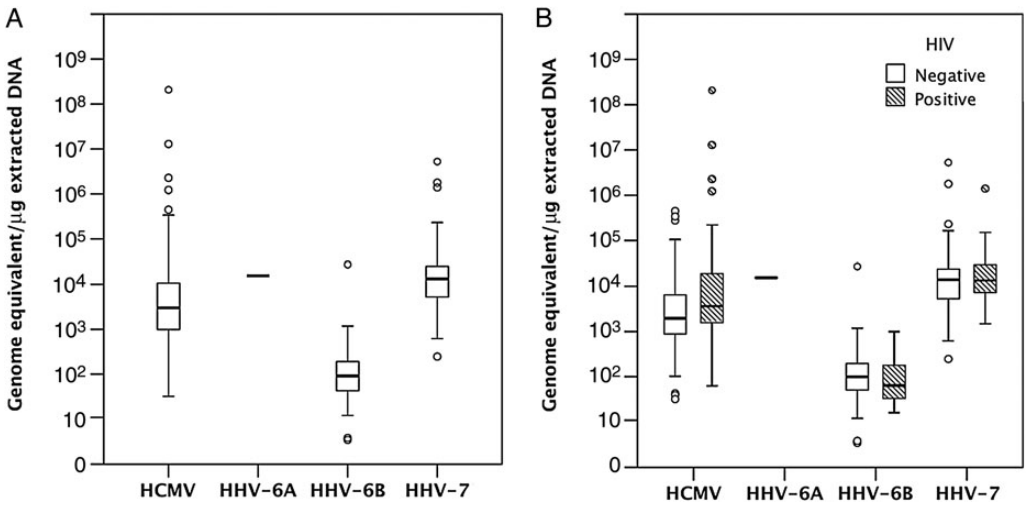

Figure 2. *A*, Betaherpesvirus genomes copies per microgram of extracted DNA. *B*, Betaherpesvirus genome copies stratified by human immunodeficiency virus (HIV) status. In both panels, boxes indicate interquartile range, and whiskers indicate 5th and 95th percentiles; circles represent outliers. Abbreviations: HCMV, human cytomegalovirus; HHV, human herpesvirus.

HCMV is an established cofactor linked with AIDS progression and neurological sequelae [38], and so the association of HCMV infection with an admission diagnosis of meningitis is interesting and raises the question of to what degree HCMV is causing meningitis in African children, compared with bacterial pathogens, and whether it should be diagnosed and treated. A previous study also found that among HIV-exposed children (those who remain HIV negative but who have HIV-infected mothers), HCMV was linked with impaired psychomotor development [5]. Other reports from sub-Saharan Africa have identified both HCMV and HHV-6 among other viral infections as important causes of hospitalization and mortality among children with nonmalarial CNS infections [30, 39]. Studies have shown that detection of HCMV DNA in the CSF of patients with suspected meningitis is associated with mortality in both adults [40, 41] and children [42].

With respect to HHV-6B, our findings are consistent with a large study from the United States that demonstrated active HHV-6 infection in 20% of infants admitted with febrile illness [22]. Previous studies from Zambia with smaller sample sizes have detected HHV-6 DNA in the whole blood of 5%–30% of children admitted with febrile illness [3, 43]. Classical sequencing over a hypervariable region within the U47 locus identified HHV-6A in 37.5%–57% of those that were successfully sequenced [3, 43]. This indication that HHV-6A might be a prevalent infant infection in Africa was followed up by a population-based study of healthy children, which identified HHV-6A in 99% (55/56) (of which 7 cases were coinfected with HHV-6B) and HHV-6B in 15% (8/56) of infants in which HHV-6 was detected and successfully typed [3]. In light of these previous findings, we were surprised to find such a low prevalence of HHV-6A. We detected HHV-6A and HHV-6B using a highly sensitive and specific multiplex TaqMan-based real-time PCR assay with conserved primers but species-specific probes targeting the HHV-6 DNA polymerase gene (U38) [7]. In coinfected patients, the use of conserved primers may result in the masking of the species with lower copy number, but this alone is unlikely to explain the stark contrast between the 2 studies. There is some limited evidence within the U47 gene for isolates containing both HHV-6A and HHV-6B elements [3, 44], although globally, complete genomic sequences for HHV-6 have only been published for 4 strains: [45–52]. HHV-6 is integrated into the telomeric repeats of roughly 1% of European and 0.2% of Japanese populations, typically characterized by high loads of HHV-6 DNA in clinical samples [53]. None of the HHV-6-positive samples on this study displayed viral loads (5 log/μg DNA) that might be consistent with chromosomal integration [54].

The distribution of betaherpesvirus prevalence by age showed profiles indicative of early primary infection with HCMV and HHV-6B peaking between 3 and 6 months of age, followed by secondary infection or reactivation in children >12 months of age. For HCMV this is consistent with previous African studies [5] but different from high-income populations where HCMV infection occurs later [55]. For HHV-6B, our data are consistent with a US study [22]. The distribution of HHV-7 infections was distinct from HCMV and HHV-6B, with most primary infections appearing to occur in young neonates, followed by a steady increase in probable reactivated infections during the first year of life, and a very stable prevalence of around 20% among admitted children >12 months of age. We present the first data on HHV-7 infections in African children, and the age distribution is consistent with serological data from Brazil

[56], yet contrary to US data, which suggest that primary infection with HHV-7 peaks later than HHV-6B [57].

There were several limitations to this study: Analysis of a single serum sample prevents detailed determination of the clinical and virological course of individual betaherpesvirus infections. The diagnostic tests used cannot accurately differentiate between active infections causal of pathology, and subclinical DNAemia arising from reactivated infections secondary to other infections and diseases. Similarly, in the absence of serological data we cannot confirm whether individual infections were primary or secondary, although we could infer some indication of the presence of primary and secondary infections from the age distribution. The use of commercial assays would have greatly strengthened comparisons of prevalence and viral loads with other studies. We did not have access to maternal HIV status preventing comparison of the relevance of HCMV infections in HIV-exposed children, seen in previous studies [5], and could not determine how many of the infections were acquired in utero. We did not have infant height data and so could not calculate z scores for stunting and wasting, which are more accurate markers of growth delay than underweight (WAZ).

Despite these limitations, our findings add weight to previous studies which demonstrate that HCMV infections (both congenital [15, 16, 19] and infant infections [5, 8, 14, 28, 29]) are emerging as important determinants of health and development in African children.

Notes

Acknowledgments. The authors acknowledge the support of Kristin Loomis, executive director of the Human Herpesvirus 6 Foundation, California, and Dr Sylvester Sinyangwe, head of the department of pediatrics at the University Teaching Hospital.

Author contributions. J. T. and M. B. wrote the first draft. M. K., M. C., L. C., and C. C. recruited the patients and undertook laboratory analysis. N. M. and A. Z. made substantial contributions to study design, data acquisition, analysis, and interpretation, and all authors made significant revisions to the final draft.

Financial support. The parent study was funded by EuropeAid through the Active Detection of Active Tuberculosis project (SANTE/2006/129–131). J. T., M. K., M. C., L. C., M. B., and A. Z. also received support from the European and Developing Countries Clinical Trials Partnership through the Tuberculosis NEAT grant and the UBS Optimus Foundation, Switzerland. A. Z. also acknowledges support from the UK Medical Research Council; University College London Hospitals (UCLH) Comprehensive Biomedical Research Centre; EU-FP7; and the UCLH National Health Service Foundation Trust. Reagents for the screening of betaherpesviruses were funded by philanthropic donations from Kids Here & There (Columbus, Ohio).

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

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Mocarski ES Jr, Shenk T, Pass RF. Cytomegaloviruses. In: Knipe DM, Howley PM, eds. Fields virology. Vol 2. 5th ed. Philadelphia: Lippincott, Williams and Wilkins, **2007**:2701–72.
2. Krueger GRF, Ablashi DV. Human herpesvirus-6 : general virology, epidemiology, and clinical pathology. 2nd ed. Amsterdam, Boston: Elsevier; **2006**.
3. Bates M, Monze M, Bima H, et al. Predominant human herpesvirus 6 variant A infant infections in an HIV-1 endemic region of sub-Saharan Africa. J Med Virol **2009**; 81:779–89.
4. Bates M, Monze M, Bima H, Kapambwe M, Kasolo FC, Gompels UA. High human cytomegalovirus loads and diverse linked variable genotypes in both HIV-1 infected and exposed, but uninfected, children in Africa. Virology **2008**; 382:28–36.
5. Gompels UA, Larke N, Sanz-Ramos M, et al.; CIGNIS Study Group. Human cytomegalovirus infant infection adversely affects growth and development in maternally HIV-exposed and unexposed infants in Zambia. Clin Infect Dis **2012**; 54:434–42.
6. Cassina G, Russo D, De Battista D, Broccolo F, Lusso P, Malnati MS. Calibrated real-time polymerase chain reaction for specific quantitation of HHV-6A and HHV-6B in clinical samples. J Virol Methods **2013**; 189:172–9.
7. Lou J, Wu Y, Cai M, Wu X, Shang S. Subtype-specific, probe-based, real-time PCR for detection and typing of human herpesvirus-6 encephalitis from pediatric patients under the age of 2 years. Diagn Microbiol Infect Dis **2011**; 70:223–9.
8. Hsiao NY, Zampoli M, Morrow B, Zar HJ, Hardie D. Cytomegalovirus viraemia in HIV exposed and infected infants: prevalence and clinical utility for diagnosing CMV pneumonia. J Clin Virol **2013**; 58:74–8.
9. de Pagter PJ, Schuurman R, Visscher H, et al. Human herpes virus 6 plasma DNA positivity after hematopoietic stem cell transplantation in children: an important risk factor for clinical outcome. Biol Blood Marrow Transplant **2008**; 14:831–9.
10. Achour A, Boutolleau D, Slim A, Agut H, Gautheret-Dejean A. Human herpesvirus-6 (HHV-6) DNA in plasma reflects the presence of infected blood cells rather than circulating viral particles. J Clin Virol **2007**; 38:280–5.
11. Bates M, Kunda M, Zumla A. Human cytomegalovirus (HCMV) infection in sub-Saharan Africa. In: Price PP, ed. Manifestations of cytomegalovirus infection. Rijeka, Croatia: InTech, **2013**.
12. Bates M, Tembo J, Zumla A. Congenital cytomegalovirus infections in sub-Saharan Africa - a neglected and growing problem. Trop Med Int Health **2014**; 19:996–8.
13. Manicklal S, Emery VC, Lazzarotto T, Boppana SB, Gupta RK. The "silent" global burden of congenital cytomegalovirus. Clin Microbiol Rev **2013**; 26:86–102.
14. Chintu C, Mudenda V, Lucas S, et al. Lung diseases at necropsy in African children dying from respiratory illnesses: a descriptive necropsy study. Lancet **2002**; 360:985–90.
15. Mwaanza N, Chilukutu L, Tembo J, et al. High rates of congenital cytomegalovirus infection linked with maternal HIV infection among neonatal admissions at a large referral centre in sub-Saharan Africa. Clin Infect Dis **2014**; 58:728–35.
16. Bello C, Whittle H. Cytomegalovirus infection in Gambian mothers and their babies. J Clin Pathol **1991**; 44:366–9.
17. Mussi-Pinhata MM, Yamamoto AY, Moura Brito RM, et al. Birth prevalence and natural history of congenital cytomegalovirus infection in a highly seroimmune population. Clin Infect Dis **2009**; 49:522–8.
18. Yamamoto AY, Mussi-Pinhata MM, Isaac Mde L, et al. Congenital cytomegalovirus infection as a cause of sensorineural hearing loss in a highly immune population. Pediatr Infect Dis J **2011**; 30:1043–6.
19. Manicklal S, van Niekerk AM, Kroon SM, et al. Birth prevalence of congenital cytomegalovirus among infants of HIV-infected women on prenatal antiretroviral prophylaxis in South Africa. Clin Infect Dis **2014**; 58:1467–72.
20. Asano Y, Yoshikawa T, Suga S, et al. Clinical features of infants with primary human herpesvirus 6 infection (exanthem subitum, roseola infantum). Pediatrics **1994**; 93:104–8.

21. Yamanishi K, Okuno T, Shiraki K, et al. Identification of human herpesvirus-6 as a causal agent for exanthem subitum. Lancet **1988**; 1:1065–7.
22. Hall CB, Long CE, Schnabel KC, et al. Human herpesvirus-6 infection in children. A prospective study of complications and reactivation. N Engl J Med **1994**; 331:432–8.
23. Epstein LG, Shinnar S, Hesdorffer DC, et al. Human herpesvirus 6 and 7 in febrile status epilepticus: the FEBSTAT study. Epilepsia **2012**; 53:1481–8.
24. Tanner A, Carlson SA, Nukui M, Murphy EA, Berges BK. Human herpesvirus 6A infection and immunopathogenesis in humanized Rag2-/-gammac-/- mice. J Virol **2013**; 87:12020–8.
25. Lusso P, Crowley RW, Malnati MS, et al. Human herpesvirus 6A accelerates AIDS progression in macaques. Proc Natl Acad Sci U S A **2007**; 104:5067–72.
26. Kositanont U, Wasi C, Wanprapar N, et al. Primary infection of human herpesvirus 6 in children with vertical infection of human immunodeficiency virus type 1. J Infect Dis **1999**; 180:50–5.
27. Liu L, Johnson HL, Cousens S, et al. Global, regional, and national causes of child mortality: an updated systematic analysis for 2010 with time trends since 2000. Lancet **2012**; 379:2151–61.
28. Goussard P, Kling S, Gie RP, et al. CMV pneumonia in HIV-infected ventilated infants. Pediatr Pulmonol **2010**; 45:650–5.
29. Zampoli M, Morrow B, Hsiao NY, Whitelaw A, Zar HJ. Prevalence and outcome of cytomegalovirus-associated pneumonia in relation to human immunodeficiency virus infection. Pediatr Infect Dis J **2010**; 30:413–7.
30. Mallewa M, Vallely P, Faragher B, et al. Viral CNS infections in children from a malaria-endemic area of Malawi: a prospective cohort study. Lancet Glob Health **2013**; 1:e153–60.
31. Doan TT, Phung TT, Pham HV, Pham SH, Nguyen LT. Effect of ganciclovir for the treatment of severe cytomegalovirus-associated pneumonia in children without a specific immunocompromised state. BMC Infect Dis **2013**; 13:424.
32. Efferth T, Romero MR, Wolf DG, Stamminger T, Marin JJ, Marschall M. The antiviral activities of artemisinin and artesunate. Clin Infect Dis **2008**; 47:804–11.
33. Milbradt J, Auerochs S, Korn K, Marschall M. Sensitivity of human herpesvirus 6 and other human herpesviruses to the broad-spectrum antiinfective drug artesunate. J Clin Virol **2009**; 46:24–8.
34. Ito T, Urushima H, Sakaue M, et al. Reduction of adverse effects by a mushroom product, active hexose correlated compound (AHCC) in patients with advanced cancer during chemotherapy—the significance of the levels of HHV-6 DNA in saliva as a surrogate biomarker during chemotherapy. Nutr Cancer **2014**; 66:377–82.
35. Bates M, O'Grady J, Maeurer M, et al. Assessment of the Xpert MTB/RIF assay for diagnosis of tuberculosis with gastric lavage aspirates in children in sub-Saharan Africa: a prospective descriptive study. Lancet Infect Dis **2013**; 13:36–42.
36. Zerr DM, Huang ML, Corey L, Erickson M, Parker HL, Frenkel LM. Sensitive method for detection of human herpesviruses 6 and 7 in saliva collected in field studies. J Clin Microbiol **2000**; 38:1981–3.
37. Khansarinejad B, Soleimanjahi H, Mirab Samiee S, Hamidieh AA, Paryan M, Sanahmadi Y. Quantitation of human cytomegalovirus DNA in plasma using an affordable in-house qPCR assay. J Virol Methods **2012**; 183:170–5.
38. Kovacs A, Schluchter M, Easley K, et al. Cytomegalovirus infection and HIV-1 disease progression in infants born to HIV-1-infected women. Pediatric Pulmonary and Cardiovascular Complications of Vertically Transmitted HIV Infection Study Group. N Engl J Med **1999**; 341: 77–84.
39. Benjamin LA, Kelly M, Cohen D, et al. Detection of herpes viruses in the cerebrospinal fluid of adults with suspected viral meningitis in Malawi. Infection **2013**; 41:27–31.
40. Kelly MJ, Benjamin LA, Cartwright K, et al. Epstein-Barr virus coinfection in cerebrospinal fluid is associated with increased mortality in Malawian adults with bacterial meningitis. J Infect Dis **2012**; 205: 106–10.
41. Siddiqi OK, Ghebremichael M, Dang X, et al. Molecular diagnosis of central nervous system opportunistic infections in HIV-infected Zambian adults. Clin Infect Dis **2014**; 58:1771–7.
42. Pelkonen T, Roine I, Anjos E, Maki M, Peltola H, Pitkaranta A. Herpesviruses in cerebrospinal fluid of children with meningitis in Luanda, Angola. Acta Paediatr **2013**; 102:e281–3.
43. Kasolo FC, Mpabalwani E, Gompels UA. Infection with AIDS-related herpesviruses in human immunodeficiency virus-negative infants and endemic childhood Kaposi's sarcoma in Africa. J Gen Virol **1997**; 78 (pt 4):847–55.
44. Gompels UA, Kasolo F. HHV-6 genome: similar and different. In: Human herpesvirus-6 : general virology, epidemiology, and clinical pathology. Krueger GRF, Ablashi DV, eds. Perspectives in medical virology. 2nd ed. London: Elsevier, **2006**:23–46.
45. Downing RG, Sewankambo N, Serwadda D, et al. Isolation of human lymphotropic herpesviruses from Uganda. Lancet **1987**; 2:390.
46. Chee MS, Lawrence GL, Barrell BG, eds. Human herpesvirus-6 (HHV-6) U1102, variant A DNA, complete virion genome. Bethesda, MD: National Center for Biotechnology Information, **2006**.
47. Salahuddin SZ, Ablashi DV, Markham PD, et al. Isolation of a new virus, HBLV, in patients with lymphoproliferative disorders. Science **1986**; 234:596–601.
48. Bhattacharjee B, Artiles AB, Renzette N, Calvo-Calle MJ, Stern LJ, Kowalik TF, eds. Human herpesvirus 6A isolate GS, complete genome. Bethesda, MD: National Center for Biotechnology Information, **2014**.
49. Pellett PE, Sanchez-Martinez D, Dominguez G, et al. Human herpesvirus 6B strain Z29, complete genome. Bethesda, MD: National Center for Biotechnology Information, **1993**.
50. Lindquester GJ, Inoue N, Allen RD, et al. Restriction endonuclease mapping and molecular cloning of the human herpesvirus 6 variant B strain Z29 genome. Arch Virol **1996**; 141:367–79.
51. Isegawa Y, Mukai T, Nakano K, et al. Comparison of the complete DNA sequences of human herpesvirus 6 variants A and B. Arch Virol **1999**; 73:8053–63.
52. Isegawa Y, Mukai T, Nakano K, et al. Human herpesvirus 6B DNA, complete genome, strain: HST. Bethesda, MD: National Center for Biotechnology Information, **2008**.
53. Pellett PE, Ablashi DV, Ambros PF, et al. Chromosomally integrated human herpesvirus 6: questions and answers. Rev Med Virol **2012**; 22:144–55.
54. Gravel A, Sinnett D, Flamand L. Frequency of chromosomally-integrated human herpesvirus 6 in children with acute lymphoblastic leukemia. PLoS One **2013**; 8:e84322.
55. Staras SA, Dollard SC, Radford KW, Flanders WD, Pass RF, Cannon MJ. Seroprevalence of cytomegalovirus infection in the United States, 1988–1994. Clin Infect Dis **2006**; 43:1143–51.
56. Oliveira SA, Turner DJ, Knowles W, Nascimento JP, Brown DW, Ward KN. Primary human herpesvirus-6 and -7 infections, often coinciding, misdiagnosed as measles in children from a tropical region of Brazil. Epidemiol Infect **2003**; 131:873–9.
57. Hall CB, Caserta MT, Schnabel KC, et al. Characteristics and acquisition of human herpesvirus (HHV) 7 infections in relation to infection with HHV-6. J Infect Dis **2006**; 193:1063–9.