Children infected by HHV-6B with febrile seizures are more likely to develop febrile status epilepticus: a case control study in a referral hospital in Zambia.

John Tembo[1,2]*, Kanta Chandwe[3]*, Mwila Kabwe[2,4], Moses Chilufya[2], Ornella Ciccone[3],

Evans Mpabalwani[3], Dharam Ablashi[5], Alimuddin Zumla[6,7], Tie Chen[1]*, Matthew Bates

[2,8]*

1. Department of Clinical Immunology, Tongji Hospital, Tongji Medical College, Tongji Hospital, Huazhong University of Science & Technology, Wuhan, China

2. HerpeZ, University Teaching hospital, Lusaka, Zambia (www.herpez.org)

3. Department of Paediatrics & Child Health, University Teaching Hospital, Lusaka, Zambia

4. La Trobe University, Melbourne, Australia

5. HHV-6 Foundation, Santa Barbara, California, United States

6. Division of Infection and Immunity, University College London, United Kingdom

7. NIHR Biomedical Research Centre, University College London Hospitals, London, United Kingdom

8. School of Life Sciences, University of Lincoln, United Kingdom

*Authors contributed equally

ABSTRACT

Background:

Human herpesvirus 6B (HHV-6B) is the causative agent of *Roseola infantum*, and has

also been suggested to play a role in the pathogenesis of febrile seizures in young

children, a percentage of whom go on to develop febrile status epilepticus (FSE), but existing data is conflicting and inconclusive. HHV-6A is a distinct species, rarely detected in most parts of the world, but prior studies suggest a higher prevalence in febrile African children. We describe a case control study comparing the frequency of HHV-6A and/or HHV-6B infections in children with febrile seizures (including febrile status epilepticus) and a control group of febrile children without seizures.

Methods

We recruited children aged 6-60 months admitted with a febrile illness with (cases) or without (controls) seizures presenting within 48 hours of commencement of fever. 3mls of whole blood was centrifuged and plasma stored at -80°C for pooled screening for HHV-6B and HHV-6A by Taqman Real Time PCR.

Results

102 cases and 95 controls were recruited. The prevalence of HHV-6B DNA detection did not differ significantly between cases (5.8% (6/102)) and controls (10.5% (10/95)) but HHV-6B infection was associated with febrile status epilepticus (OR 15; 95% CI, [1.99-120]; p=0.009). HHV-6A was not detected.

Conclusion

Prevalence of HHV-6B was similar among cases and controls. Within the febrile seizure group, HHV-6B infection was associated with FSE, suggesting HHV-6B infections could play a role in pathogenesis of FSE.

INTRODUCTION

Human herpesvirus 6 (HHV-6) is a lymphotropic virus first isolated from peripheral blood lymphocytes of patients suffering from lymphoproliferative disorders [1]. Whilst initially referred to as 'strain variants', HHV-6A and HHV-6B are now recognized by the International Committee for the Taxonomy of Viruses (ICTV) as distinct virus species based on genotypic and phenotypic differences (differential tropism in cell culture) and clinical distinctions [2, 3]. HHV-6B is the most common cause of *Roseola infantum*, a febrile rash that often follows primary infection which is almost ubiquitous in early childhood [4, 5] and HHV-6B is also associated with febrile illness in young children [6, 7]. Whilst older studies do not include genotype analysis, it is known from more recent studies that 99-100% of primary infections in America, Europe and Japan are with HHV-6B [2, 8]. In addition to *Roseloa infantum*, which is often mild an self-resolving, in a subset of cases there is evidence of neurological involvement, sometimes manifesting as seizures or convulsions [6, 9] and also more severe encephalitis due mostly to reactivated infections in immunocompromised adults [10-14]. HHV-6B has also been linked with mesial temporal lobe epilepsy (MTLE)[15-17]. HHV-6A is rarely detected in children in Western countries, but two Zambian studies have detected HHV-6A readily in blood or sera specimens from children [18, 19]. An *in vitro* study has also shown that both HHV-6A and HHV-6B can productively infect human fetal astrocytes, causing multinucleated

syncytia, with progeny virions maintaining T-cell tropism [20], evidence that HHV-6 is able to directly infect and cause pathology in CNS tissue.

The most common seizures suffered by children in early childhood are clinically defined as 'simple febrile seizures', lasting less than 15 minutes, comprised of generalized tonic and clonic activity without a focal component, and does not reoccur within 24 hours or within the same febrile illness. Roughly 5-8% of cases will have prolonged or recurrent seizures of sufficient severity to meet the criteria of Febrile Status Epilepticus (FSE) defined as any febrile seizure lasting more than 30 minutes or recurrent seizures lasting a total of more than 30 minutes without fully regaining consciousness [21], a condition which has been associated with an increased risk of developing MTLE [22]. There are 13 studies which have screened for HHV-6 infection in children with febrile illness or febrile seizures, comprising five case-control and eight cross-sectional studies . The most prominent of these is a large prospective study from the U.S where 9.7% (160/1653) of febrile children had primary HHV-6 infection (defined by seroconversion and PCR detection of HHV-6 virus) [6]. Among the 160 cases of primary HHV-6 infection, 21 (13.1%) were admitted to hospital with febrile seizures, with primary HHV-6 infection being detected in one third of all febrile seizure cases [6]. Comparing febrile children with and without seizures, the rates of HHV-6 infection were over 3-fold higher in those with febrile seizures [6]. However, results from 4 subsequent case control studies did not find any difference in HHV-6B prevalence between children with and without seizures [23-26]. With respect to children with complex seizures and status epilepticus a link with

HHV-6 has been suggested in three studies [9, 27, 28]. In the most recent study (FEBSTAT) the prevalence of HHV-6 infection at baseline was 32% (54/169) in children with febrile status epilepticus, but no clinical distinctions could be made between patients with and without HHV-6B infection [27]. The available data points towards a possible association between HHV-6B and both FS and/or FSE, but no studies have demonstrated a causal relationship [29].

The prevalence of epilepsy in sub-Saharan Africa is double that in developed countries [30] and as CNS infections are a major risk factor for febrile status epilepticus and epilepsy [30, 31] there is a need for studies to evaluate possible etiologies in the sub-Saharan African region, which also take into account HIV infection and exposure status in children, which may influence clinical outcomes of HHV-6 infection [32, 33]. A Zambian study in 1997 identified HHV-6 in 30% (16/53) of non-malarial febrile children (PCR on DNA extracted whole blood) and interestingly, of the 9 cases that were genotyped by single locus Sanger sequencing, four were found to be HHV-6A [19]. A recent study of people living with HIV in Burkina Faso identified HHV-6A in 88% (15/17) of cases of HHV-6 infection, although two thirds of HHV-6A cases were co-infected with HHV-6B [34]. PCR studies in Zambia on sera from healthy children also identified highly prevalent HHV-6A infections [18]. Another study also from Zambia screened for HHV-6 in lung tissue (taken at autopsy)[18] and sera specimens from children admitted with respiratory disorders, where HHV-6B was more prevalent [35].

We present here the first case-control study from the Africa region comparing HHV-6 infection rates in admitted febrile children with and without seizures, and also comparing

those with and without febrile status epilepticus, the largest study of its kind to be undertaken since the Hall study of 1994.

METHODS

Study objectives

To determine whether there is an association between HHV-6A and/or HHV-6B infection and febrile seizures in children admitted with febrile illness to the University Teaching Hospital (UTH), Lusaka, Zambia.

To determine whether there is an association between HHV-6A and/or HHV-6B infection and febrile status epilepticus (FSE), in children admitted to the UTH with febrile illness.

Ethical considerations

Ethical approval was sought from the Research Ethics Committee (ERES). Permission was obtained from the University Teaching Hospital Department of Pediatrics to conduct study. Written consent was obtained from the parents/guardians of the participants as they were aged between 6-60 months of age.

Study definitions

For the purpose of the study, fever was defined as an axillary temperature of 37.5^{o}C and above.

1. Febrile seizure (FS) - Seizures that occurred in a febrile infant or child between the ages of 6-60 months who clinically did not have an intracranial infection, metabolic disturbance, or history of febrile seizures.

2. Febrile status epilepticus (FSE) - FSE was defined as any febrile seizure lasting more than 30 minutes or recurrent seizures lasting a total of more than 30 minutes without fully regaining consciousness.

Recruitment

The study enrolled children between the ages of 6 months to 60 months from January 2015 to May 2016. The study recruited patients from the pediatric emergency room and the admissions ward the University Teaching Hospital in Lusaka, Zambia. The facility is a tertiary referral centre receiving cases from various parts of Lusaka as well as referral cases from all over the country.

Inclusion and Exclusion criteria

Inclusion criteria included:

1. Any child aged 6-60 months with first episode of FS and FSE presenting within 48 hours of a febrile seizure. These were our cases.

2. Any child aged 6-60 months with a febrile illness without seizures presenting within 48 hours of a febrile illness. These were our controls.

Exclusion criteria included:

1.Children with signs and symptoms of central nervous system (CNS) infection (eg, neck stiffness, positive Brudzinski sign, positive Kernig sign and persistent altered level of consciousness) were excluded.

2. Children with clinical evidence of CNS anomalies such as hydrocephalus, cerebral palsy and microcephaly were excluded.

3.Children younger than 6 months or older than 60 months were excluded.

4.Children with known seizure disorders were excluded.

5.Children already enrolled in other studies were excluded.

Plasma collection and DNA extraction

3mls of blood was collected from a peripheral vein and placed in labelled EDTA tubes and spun. The plasma was drawn from the tubes and stored at -81°C for later testing. Plasma samples had DNA extracted using the QIAGEN QIAamp® DNA Mini Kit according to the manufacturer's specifications.

HHV-6 PCR assay

Real Time Taqman PCR was undertaken using consensus primers detecting both HHV-6A and HHV-6B [36]: Primers (HHV6U41-F4: 5′- CGGAACATTGTTGAGCAGAAA-3′, HHV6U41-R106: 5′- AAGAAGAATCCCTTGTCTGGC-3′) and species-specific

probes (HHV-6A U41-ProbeA: FAM-CTCTAAGCACGAAATCTTCACATTCGGAAACA-TAMRA, HHV-6B U41-ProbeB: JOE-CTCTAAGCACGAAATTTTGACATTCGGAAACA-BHQ1). The thermocycling conditions were as follows: Denaturation at 95 °C for three minutes, followed by 45 cycles of denaturation at 95 °C for 15 s, annealing at 55 °C for 30 s and extension at 60 °C for 30 s. Assays were run on a Rotor-Gene™ 6000 (Qiagen, Hilde n, Germany). The fidelity of the PCR enzyme and purity of the DNA-extraction was controlled through amplification of the house-keeping gene, β- Actin, from every 12th sample. Presence and quality of extracted DNA from clinical specimens was quantified (ng/μl) using a Nanodrop (Thermo Scientific, Waltham, MA, U.S). We used a cut off of 200 copies/ml to be indicative of an active infection. Study participants were also tested for HIV and malaria according to standard hospital procedure.

Statistical Analysis

Data analysis was undertaken using SPSS version 21 (IBM, Armonk, NY, USA). For study descriptives (Table 2), binary variables were compared by Pearson chi-squared test (or Fishers exact test when the numerator was <10) and patient age was compared by Mann Whitney U. Multivariate binary logistic regression (Tables 3 and 4) was used to evaluate whether HHV-6 and other clinical/demographic factors demonstrated significant associations with either cases or controls.

RESULTS

Study recruitment, clinical and demographic characteristics

All pediatric emergency room and/or pediatric admissions aged between 6-60 months with febrile seizures were approached to participate in the study, along with a similar number of febrile controls (convenience sampling). If the child's parents consented to participate in the study and patient met inclusion criteria clinical and demographic data was recorded and 3mls of blood were collected. 47.1% (48/102) of the cases were female, median age was 27 months (IQR18-37 months), 3.4% (3/89) of children were infected with HIV and 17.4% (16/92) had confirmed malaria. The point prevalence of HIV was 2-fold higher in controls (8.8% (7/80), but HIV status, sex, median age and malaria status did not differ significantly between cases and controls (Table 2). HIV status was undetermined for 13 cases and 15 controls. HHV-6 infection was not detected in any of the 10 HIV positive children.

Comparison of Febrile Seizures with Febrile controls

DNA-extracted blood specimens from all 102 cases and 95 controls were tested and analysed for HHV-6A and HHV-6B. A total of 16 specimens were positive for HHV-6B, 5.8% (6/102) among cases and 10.5% (10/95) among controls, with no significant difference observed in HHV-6B prevalence, or clinical factors, between cases and controls except for mean temperature which was slightly higher $0.28^{\circ}C$ in controls than cases p=0.003 (Table 3). No specimens were positive for HHV-6A.

Comparison of Febrile Seizures and Febrile Status Epilepticus

We then analysed the data looking at whether amongst our cases patients with febrile status epilepticus were more likely to test positive for HHV-6B infection. We found that among children with febrile seizures, being classified as having febrile status epilepticus was associated with increased odds of testing positive for HHV-6B infection: OR15; 95% CI, [1.99-120]; p=0.009 (Table 4).

DISCUSSION

In our study HHV-6B was not associated with febrile seizures. These results are similar to four previous case control studies, in which no association was found between HHV-6B infection and febrile seizure [23-26] (Table 1), although these studies were small and had various methodological weaknesses, such as only screening by PCR in CSF or blood [24, 26] . This stands in contrast to the Hall study in which HHV-6 infection was associated with a 3-fold increase in the prevalence of febrile seizures [6] and the panel of eight cross-sectional studies where HHV-6 infection is readily detected in children with febrile seizures with a median HHV-6 prevalence of 17.4% (range 6-26.2%) (Table 1)[29]. The most important inconsistency between the different studies, is the range of diagnostic tools used, and these distinctions are important, as different PCR assay sensitivities and targets, and different serology methodologies and reagents will give different results. Importantly, HHV-6 species are latent in monocytes or macrophages [37], or CD34+ve progenitor cells [38, 39] and periodically reactivate sub-clinically, as evidenced by the ready detection of this virus in healthy children [18]. The Hall study,

and all but one of the cross sectional studies, included serological analysis to define HHV-6 primary infection. In our study we did not use serology but based on median age ranges and previous studies it is possible that a significant percentage of the HHV-6 infections in this study are reactivations, as primary infection in Zambia peaks at around 9 months of age [35]. In the FEBSTAT study (U.S) median age was 14.8 months, and of the 58 HHV-6 and HHV-7 infections, 14 (31%) were reactivations. In our study, the median age was considerably higher (29.5 months), and so we postulate that the relative contribution of primary infections could have been lower than in the FEBSTAT study. The two studies have also been undertaken in different geographical populations and could be affected by host or virus genetic factors and levels of maternal immunity in early infancy [17, 40]. A meta-analysis using a random effects model suggested 21% (95%CI: 14–30%) of children with febrile seizures could be positive for primary HHV-6 infection, but it's clear that there are other causes, and our data support their conclusions that HHV-6 may be the cause or co-factor in a limited subset of febrile seizures [29].

Within children with febrile seizures we found HHV-6B infection was associated with febrile status epilepticus (FSE)(OR 15; 95% CI, [1.99-120]; p=0.009). This is the first controlled study to observe this correlation between HHV-6B infection and FSE, adding weight to previous cross-sectional studies, which have identified a subset of cases of FSE associated with HHV-6B infection [9, 27, 41]. Whilst we only recruited 5 cases of FSE, our detection of HHV-6B infection in 40% (2/5) of FSE cases is consistent with the most recent cross-sectional study, FEBSTAT, which estimated that HHV-6B infection could account for up to 33% of FSE [27].

In poor communities in sub-Saharan Africa young infants are exposed to a high burden of infectious diseases, including neurological infections. A baseline study of MRI data from healthy Malawian teenagers found brain abnormalities in 18% (16/96) of children [42]. The observed incidental atrophy and white matter abnormalities are not seen in healthy U.S children, suggesting that the higher disease burden during early childhood may be responsible for these abnormalities [42]. Identifying the causal pathogens in this population group may inform on targets for vaccine development, that could prevent or reduce the incidence of CNS infections during infancy, impacting on acute disease burden and later developmental sequelae. With respect to epilepsy, a recent study from Poland treated epileptic children who had primary cytomegalovirus (CMV) infection with ganciclovir, achieving successful long term outcomes (up to 4 years) [43], but in the African setting, routine diagnostic testing for betaherpesviurses (CMV, HHV-6A, HHV-6B and HHV-7) is not available and drugs for the treatment of HHV-6 are not routinely administered. Ganciclovir, Foscarnet and Cidofovir are used to treat HHV-6 infection in the transplant setting in Western countries, but indications for treatment and conditions of drug administration are poorly defined [44]. Ganciclovir is used at some centres in South Africa to treat CMV pneumonia in HIV-infected/exposed children [45-47]. HHV-6 has been shown to be sensitive to Artesunate and was used for the first time in a recent case study to treat a child with HHV-6 associated myocarditis [48, 49]. Artesunate is a drug already approved for the treatment of malaria and is widely available in Africa and could present a good drug candidate for future pilot trials looking at treating HHV-6 positive FSE in this population.

None of the samples tested positive for HHV-6A, similar to results from the FEBSTAT study in 2012 and mirroring results obtained in earlier cross-sectional study of infants admitted to the hospital [27, 35]. However, this contrasts with an earlier study of healthy Zambian children that found HHV-6A to be more common [18]. A possible explanation for these inconsistencies could be the existence of novel recombinant HHV-6 viruses with components of both HHV-6A and HHV-6B, as evidence in a recent whole genome sequencing study in the U.S[50]. There are currently no full genome sequences for HHV-6A or HHV-6B from sub-Saharan Africa other than the two reference strains Z29 and U1102 [2]. As more whole genomes become available it might help us better understand the molecular epidemiology of these viruses.

Limitations: Our study was not longitudinal and so we could not compare the clinical/virological course of patients or outcomes of HHV-6B infection in cases and controls. Lacking serial specimens and serological analysis we could not differentiate primary infections from reactivations/reinfections. We did not include CSF analysis as this is unreliable for detecting active HHV-6 infection and lumbar puncture is difficult to undertake in the research setting in Zambia due to low cultural acceptance [51]. Whilst rare, detection of HHV-6B in CSF makes a stronger case for direct pathology affecting the CNS, although 11/13 previous studies analyzed blood, including the influential Hall and Epstein studies, and so our results are comparable [6, 27]. The low number of FSE cases on our study demands that the resulting odds ratio be viewed with caution and due respect for the wide confidence intervals. We did not have access to maternal HIV status.

We conclude that this is the first case-control study to suggest an association between HHV-6B and FSE, which has been associated with increased risk of developing epilepsy in children with febrile seizures. Epilepsy is highly prevalent in the Africa region and further studies should look at the virological course of HHV-6B and other candidate viral causes, in FSE patients, and correlate with long term outcomes. Artesunate is a possible affordable and accessible treatment option to be piloted for HHV-6 and other betaherpesvirus infections in the African setting.

Table 1: Previous studies of HHV-6 in febrile seizures

Author	Year	Location	Age	Study Design	HHV-6 diagnostic methodology	Is methodology accurate and sensitive for active infection?	Outcome
Case-control or internally controlled cohort studies (n = 5)							
Hall	1994	U.S	<3 yrs	Cohort of febrile	PCR and Serology on	Yes	Overall 9.7%(160/165

				children including a subset with seizures	blood		3) Febrile controls 8.7% (139/1590) Febrile Seizures 33% (21/63)
Bertolani	1996	Italy	<3 yrs	Case-control study	Isolation and Serology	Yes	35% (23/65) HHV-6 infection in cases and 50% (12/24) in controls
Hukin	1998	U.S	<6 yrs	Case Control comparing febrile seizures with febrile controls	PCR and Serology on blood and/or saliva	Yes	Febrile controls 42.9% (15/35) Febrile Seizures 45.5% (15/33)
Teach	1999	U.S.A	6m – 6 yrs	Case-control study	PCR on CSF	No (HHV-6 rarely detectable in CSF during active	No HHV-6 found in 23 cases or the 21 controls

							infection)	
Farshadmoghada m	2014	Iran	<2 yrs	Emergency room cohort of febrile children, half of whom had seizures.	PCR on blood	No (whole blood contains latent virus)	No association between HHV-6 and seizure 57% (45/78) infection in seizure patients and 43% (31/72) in those without seizure.
Cross sectional studies of febrile seizures without non-seizure controls (n = 8)							
Ward	1994			Retrospectiv e Cross sectional study	Serology IgG detection and avidity	Yes	9.7% (25/258) with primary infection of these 5 patients presented with febrile fits
Barone	1995	U.S		Cross sectional febrile convulsions	Isolation and Serology	Yes	19% (8/42) culture positive Additional 3

							cases by serology Total 26.2% (11/42) HHV-6 primary infection
Chua	1998	Malaysia		Cross sectional febrile seizures	Isolation, serology and PCR	Yes	16.1% (5/31) HHV-6 infection
Suga	2000	Japan		Cross sectional febrile seizures	Isolation and Serology	Yes	20% (21/105) HHV-6 primary infections
Pancharoen	2000	Thailand		Cross sectional febrile seizures	Serology for HHV-6 IgM on day 2-6 of fever	No (IgM takes 2-4 weeks to develop	8.5% (7/82) HHV-6 infection
Ward	2005	U.K		Cross sectional children with suspected	PCR and Serology	Yes	7.1% (11/156) HHV-6 infections

				encephalitis and/or severe illness with fever and convulsion			
Laina	2009	Greece		Cross sectional febrile seizures in first or second febrile episode	PCR and Serology	Yes	15.4% (10/65) HHV-6 infections
Mamish	2014	Iran		Cross sectional febrile convulsions	PCR CSF	No (HHV-6 rarely detectable in CSF during active infection)	6% (6/100) HHV-6 infections

Table 2: Descriptive Statistics of Case and Control groups

	Cases	Controls	P=value
n	102	95	
Female gender	48 (47.1%)[a]	50 (52.6%)[a]	.434
Median Age (months)	26.50 (17.75-37.25)[b]	20.00 (11-36)[b]	.074
Infant HIV status	3 (3/89 3.37%)[c]	7 (7/80 8.75%)[c]	.194
Malaria	16(16/92 17.39%)[a]	14(14/88 15.9%)[a]	.79

a Pearson χ2 test for binomial variables; b Mann Whitney U test for age; c Fishers Exact test

Table 3: Logistic regression analysis to identify risk factors associated with febrile seizures compared with febrile controls

	Cases n= 102		Controls n=95		OR[95%CI]	P value
Temperature (Mean)	38.48		38.76		0.482 [0.296-0.785]	0.003

Admission Diagnosis						
Tonsilitis	16	15.67%	10	10.53%		.076
Conjunctivitis	0		1	1.05%		.471
Mumps	1	0.98%	0			.431
Pneumonia	3	2.94%	10	10.53%		.432
Coryza	20	19.60%	24	25.26%		.453
UTI	2	1.96%	0			.213
Gastroenteritis	9	8.82%	10	10.53%		.994
HHV-6B prevalence as determined by PCR						
*HHV-6B	6	5.88%	10	10.53%	0.531 [.185-1.523]	.239

UTI= Urinary Tract Infection

Table 4: Logistic regression analysis to identify risk factors associated with FSE, compared with febrile seizure controls.

	FS N=97		FSE N=5		OR[95%CI]	P value
Sex (female)	45	46.39%	3	60%		.545
Age (mean)	29.13		21.80			.303
HIV +ve	3	3.09%	0	0%		.713
Malaria	15	15.46%	1	20%		.804
Temp (mean)	38.50		38.02			.102
*HHV-6B	4	4.12%	2	40%	15 [1.99-120]	.009

FS= Febrile Seizure, FSE= Febrile Status epilepticus

REFERENCES

1. Salahuddin, S.Z., et al., *Isolation of a new virus, HBLV, in patients with lymphoproliferative disorders.* Science, 1986. **234**(4776): p. 596-601.

2. Ablashi, D., et al., *Classification of HHV-6A and HHV-6B as distinct viruses.* Arch Virol, 2014. **159**(5): p. 863-70.

3. Mori, Y. and K. Yamanishi, *HHV-6A, 6B, and 7: pathogenesis, host response, and clinical disease*, in *Human Herpesviruses: Biology, Therapy, and Immunoprophylaxis*, A. Arvin, et al., Editors. 2007: Cambridge.

4. Takahashi, K., et al., *Human herpesvirus 6 and exanthem subitum.* Lancet, 1988. **1**(8600): p. 1463.

5. Yamanishi, K., et al., *Identification of human herpesvirus-6 as a causal agent for exanthem subitum.* Lancet, 1988. **1**(8594): p. 1065-7.

6. Hall, C.B., et al., *Human herpesvirus-6 infection in children. A prospective study of complications and reactivation.* N Engl J Med, 1994. **331**(7): p. 432-8.

7. Tesini, B.L., L.G. Epstein, and M.T. Caserta, *Clinical impact of primary infection with roseoloviruses.* Curr Opin Virol, 2014. **9**: p. 91-6.

8. Zerr, D.M., et al., *A population-based study of primary human herpesvirus 6 infection.* N Engl J Med, 2005. **352**(8): p. 768-76.

9. Ward, K.N., et al., *Human herpesviruses-6 and -7 each cause significant neurological morbidity in Britain and Ireland.* Arch Dis Child, 2005. **90**(6): p. 619-23.

10. Noguchi, T., et al., *CT and MRI findings of human herpesvirus 6-associated encephalopathy: comparison with findings of herpes simplex virus encephalitis.* AJR Am J Roentgenol, 2010. **194**(3): p. 754-60.

11. Provenzale, J.M., K. van Landingham, and L.E. White, *Clinical and imaging findings suggesting human herpesvirus 6 encephalitis.* Pediatr Neurol, 2010. **42**(1): p. 32-9.

12. Yao, K., et al., *Detection of human herpesvirus-6 in cerebrospinal fluid of patients with encephalitis.* Ann Neurol, 2009. **65**(3): p. 257-67.

13.   Yoshikawa, T., Y. Asano, and Y. Takahashi, *Detection of DNA of six human herpesviruses in the cerebrospinal fluid of immunocompetent non-herpetic acute limbic encephalitis patients.* Microbiol Immunol, 2010. **54**(8): p. 471-4.

14.   Virtanen, J.O., et al., *Confirmed primary HHV-6 infection in children with suspected encephalitis.* Neuropediatrics, 2007. **38**(6): p. 292-7.

15.   Fotheringham, J., et al., *Association of human herpesvirus-6B with mesial temporal lobe epilepsy.* PLoS Med, 2007. **4**(5): p. e180.

16.   Uesugi, H., et al., *Presence of human herpesvirus 6 and herpes simplex virus detected by polymerase chain reaction in surgical tissue from temporal lobe epileptic patients.* Psychiatry Clin Neurosci, 2000. **54**(5): p. 589-93.

17.   Theodore, W.H., et al., *Human herpes virus 6B: a possible role in epilepsy?* Epilepsia, 2008. **49**(11): p. 1828-37.

18.   Bates, M., 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**(5): p. 779-89.

19.   Kasolo, F.C., E. Mpabalwani, and U.A. Gompels, *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)**: p. 847-55.

20.   He, J., et al., *Infection of primary human fetal astrocytes by human herpesvirus 6.* J Virol, 1996. **70**(2): p. 1296-300.

21.   Hesdorffer, D.C., et al., *Distribution of febrile seizure duration and associations with development.* Ann Neurol, 2011. **70**(1): p. 93-100.

22.   Shinnar, S., *Febrile Seizures and Mesial Temporal Sclerosis.* Epilepsy Curr, 2003. **3**(4): p. 115-118.

23.   Bertolani, M.F., et al., *A study of childhood febrile convulsions with particular reference to HHV-6 infection: pathogenic considerations.* Childs Nerv Syst, 1996. **12**(9): p. 534-9.

24.   Farshadmoghadam, H., et al., *Human herpesvirus 6 infection in febrile children: frequency in an Iranian referral hospital.* Br J Biomed Sci, 2014. **71**(3): p. 108-10.

25.   Hukin, J., et al., *Case-control study of primary human herpesvirus 6 infection in children with febrile seizures.* Pediatrics, 1998. **101**(2): p. E3.

26.   Teach, S.J., et al., *Human herpesviruses types 6 and 7 and febrile seizures.* Pediatr Neurol, 1999. **21**(4): p. 699-703.

27. Epstein, L.G., et al., *Human herpesvirus 6 and 7 in febrile status epilepticus: the FEBSTAT study.* Epilepsia, 2012. **53**(9): p. 1481-8.

28. Suga, S., et al., *Clinical characteristics of febrile convulsions during primary HHV-6 infection.* Arch Dis Child, 2000. **82**(1): p. 62-6.

29. Mohammadpour Touserkani, F., et al., *HHV-6 and seizure: A systematic review and meta-analysis.* J Med Virol, 2017. **89**(1): p. 161-169.

30. Ba-Diop, A., et al., *Epidemiology, causes, and treatment of epilepsy in sub-Saharan Africa.* Lancet Neurol, 2014. **13**(10): p. 1029-44.

31. Preux, P.M. and M. Druet-Cabanac, *Epidemiology and aetiology of epilepsy in sub-Saharan Africa.* Lancet Neurol, 2005. **4**(1): p. 21-31.

32. Lusso, P., et al., *Human herpesvirus 6A accelerates AIDS progression in macaques.* Proc Natl Acad Sci U S A, 2007. **104**(12): p. 5067-72.

33. Matrajt, L., et al., *Virus and host-specific differences in oral human herpesvirus shedding kinetics among Ugandan women and children.* Sci Rep, 2017. **7**(1): p. 13105.

34. Traore, L., et al., *EBV and HHV-6 Circulating Subtypes in People Living with HIV in Burkina Faso, Impact on CD4 T cell count and HIV Viral Load.* Mediterr J Hematol Infect Dis, 2017. **9**(1): p. e2017049.

35. Tembo, J., et al., *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.* Clin Infect Dis, 2015. **60**(3): p. 423-31.

36. Yavarian, J., et al., *Type specific Real time PCR for detection of human herpes virus 6 in schizophrenia and bipolar patients: a case control study.* BMC Psychiatry, 2015. **15**: p. 296.

37. Kondo, K., et al., *Latent human herpesvirus 6 infection of human monocytes/macrophages.* J Gen Virol, 1991. **72 (Pt 6**): p. 1401-8.

38. Andre-Garnier, E., et al., *Reactivation of human herpesvirus 6 during ex vivo expansion of circulating CD34+ haematopoietic stem cells.* J Gen Virol, 2004. **85**(Pt 11): p. 3333-6.

39. Luppi, M., et al., *Human herpesvirus 6 latently infects early bone marrow progenitors in vivo.* J Virol, 1999. **73**(1): p. 754-9.

40. Feng, B. and Z. Chen, *Generation of Febrile Seizures and Subsequent Epileptogenesis.* Neurosci Bull, 2016. **32**(5): p. 481-92.

41. Juntunen, A., et al., *A major role of viruses in convulsive status epilepticus in children: a prospective study of 22 children.* Eur J Pediatr, 2001. **160**(1): p. 37-42.

42. Potchen, M.J., et al., *Brain imaging in normal kids: a community-based MRI study in Malawian children.* Trop Med Int Health, 2013. **18**(4): p. 398-402.

43. Dunin-Wasowicz, D., J. Kasprzyk-Obara, and S. Jozwiak, *Successful antiepileptic drug withdrawal in infants with epilepsy and cytomegalovirus neuroinfection: longitudinal study.* Epilepsia, 2010. **51**(7): p. 1212-8.

44. Agut, H., P. Bonnafous, and A. Gautheret-Dejean, *Laboratory and clinical aspects of human herpesvirus 6 infections.* Clin Microbiol Rev, 2015. **28**(2): p. 313-35.

45. Jeena, P.M., et al., *The significance of cytomegalovirus in children with pneumonia admitted for mechanical ventilation.* Int J Tuberc Lung Dis, 2017. **21**(12): p. 1230-1236.

46. Goussard, P., et al., *CMV pneumonia in HIV-infected ventilated infants.* Pediatr Pulmonol, 2010. **45**(7): p. 650-5.

47. Tembo, J.M., C.; Rojo, P.; Zumla, A.; Bates, M., *Call for ganciclovir trials for CMV pneumonia in HIV-infected and -exposed African children.* The International Journal of Tuberculosis and Lung Disease, 2018. **in press**.

48. Hakacova, N., et al., *First therapeutic use of Artesunate in treatment of human herpesvirus 6B myocarditis in a child.* J Clin Virol, 2013. **57**(2): p. 157-60.

49. Milbradt, J., et al., *Sensitivity of human herpesvirus 6 and other human herpesviruses to the broad-spectrum antiinfective drug artesunate.* J Clin Virol, 2009. **46**(1): p. 24-8.

50. Greninger, A.L., et al., *Copy number heterogeneity, large origin tandem repeats, and interspecies recombination in HHV-6A and HHV-6B reference strains.* J Virol, 2018.

51. Thakur, K.T., et al., *Lumbar puncture refusal in sub-Saharan Africa: A call for further understanding and intervention.* Neurology, 2015. **84**(19): p. 1988-90.