Cytomegalovirus Infections in Ugandan Infants:

Newborns, Neonates with Sepsis and Infants with Hydrocephalus

Christine Hehnly, BS[1*], Paddy Ssentongo, MD, PhD, MPH[2,3*], Lisa M. Bebell, MD[4], Kathy Burgoine, MD, PhD[5,6,7], Joel Bazira, MD[8], Claudio Fronterre, PhD[9], Elias Kumbakumba, MD[10], Ronald Mulondo, MD[11], Edith Mbabazi-Kabachelor, MD, MSc[11], Sarah U. Morton, MD, PhD[12], Joseph Ngonzi, MD[13], Moses Ochora, MD[9], Peter Olupot-Olupot, MD, PhD[7,14], Justin Onen, MD[10], Drucilla J. Roberts, MD[15], Kathryn Sheldon, PhD[1], Shamim A. Sinnar, MD, PhD[16], Jasmine Smith, BS[1], Peter Ssenyonga, MD[10], Joseph N. Paulson, PhD[17,‡], Frederick A. Meier, MD[18,‡], Jessica E. Ericson, MD, MPH[19,‡], James R. Broach, PhD[1,‡], Steven J. Schiff, MD, PhD [2,20,21,‡,§]

*,‡ Equal Contribution
§To whom correspondence should be addressed:
Steven J. Schiff
W311 Millennium Science Complex
The Pennsylvania State University
University Park, PA 16802 USA
814-863-4210
steven.j.schiff@gmail.com

1 Institute for Personalized Medicine, Department of Biochemistry and Molecular Biology, The Pennsylvania State University College of Medicine
2 Center for Neural Engineering, Department of Engineering Science and Mechanics, The Pennsylvania State University
3 Department of Public Health Sciences, The Pennsylvania State University College of Medicine
4 Department of Medicine, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA
5 Neonatal Unit, Department of Paediatrics and Child Health, Mbale Regional Referral Hospital, Mbale, Uganda
6 Institute of Translational Medicine, University of Liverpool, Liverpool, United Kingdom
Division of Infectious Disease, Massachusetts General Hospital, Harvard Medical School
7 Mbale Clinical Research Institute, Mbale Regional Referral Hospital, Mbale, Uganda
8 Department of Microbiology, Mbarara University of Science and Technology
9 Centre for Health Informatics, Computing, and Statistics, Lancaster University, Lancaster, United Kingdom
10 Department of Pediatrics, Mbarara University of Science and Technology
11 CURE Children's Hospital of Uganda
12 Division of Newborn Medicine, Boston Children's Hospital and Department of Pediatrics, Harvard Medical School
13 Department of Obstetrics and Gynaecology, Mbarara University of Science and Technology
14 Busitema University, Busitema, Uganda
15 Department of Pathology, Massachusetts General Hospital, Boston, and Harvard Medical School, Boston, Massachusetts
16 Department of Medicine, The Pennsylvania State University College of Medicine
17 Department of Biostatistics, Product Development, Genentech Inc.

18 Wayne State University School of Medicine, Detroit, Michigan
19 Division of Pediatric Infectious Disease, The Pennsylvania State University College of Medicine
20 Center for Infectious Disease Dynamics, Department of Physics, The Pennsylvania State University
21 Department of Neurosurgery, The Pennsylvania State University College of Medicine

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Summary

Background: Congenital and postnatal cytomegalovirus (CMV) infections in Uganda is prevalent and may compromise the health of Ugandan children. The objective of this study is to estimate the prevalence of CMV infections in newborns, neonates with sepsis, and infants with hydrocephalus in Uganda.

Methods: Three populations: (1) newborn-mother pairs, (2) neonates with sepsis, and (3) infants (≤ 3 months) with non-postinfectious (NPIH) or postinfectious (PIH) hydrocephalus, were evaluated over four years (2016-2019) for CMV infection at three medical centers – two in the Eastern (Mbale) and one in Western (Mbarara) Uganda. To characterize the prevalence of CMV we used quantitative PCR (qPCR) analysis. In newborn-mother pairs maternal blood (n=99) and a subset of matching cord blood (n=92), placental tissue (n=99), and vaginal specimens (n=99) were tested for CMV. In neonates and infants aged 3 months or less, peripheral blood (751 with sepsis, 399 with hydrocephalus) and cerebrospinal fluid samples (560 with sepsis, 399 with hydrocephalus (205 PIH, 194 NPIH) were also tested for CMV.

Findings: The overall CMV prevalence across all groups was 9%. In newborn-mother pairs, a 3% (n=3/92; 95% CI, 1-9%) prevalence of cord blood positivity and 33% (n=33/99; 95% CI, 24-44%) prevalence of maternal vaginal shedding of CMV was estimated. In neonates with clinical sepsis, a 2% (n=17/751; 95% CI, 1-4%) CMV prevalence was estimated. Maternal HIV seropositivity (adjusted odds ratio [aOR], 21.09; 95% CI, 4-109; p= 0.0002), residence in Eastern Uganda (aOR, 11.10; 95% CI, 3-77; p=0 .003), maternal age < 25 years (aOR, 4.91; 95% CI, 2-20; p=0.012), and older neonatal age (9 days vs. 5 days; p= 0.006) were associated with CMV in neonates with clinical sepsis. In infants with PIH, the prevalence in blood was 24% (n=50/205; 95% CI, 19-31%) and in infants with NPIH it was 20% (n=39/194; 95% CI, 15-26%;

p=0.34). CMV was present in the CSF of 13% (n=26/205; 95% CI, 8-18%) of infants with PIH compared to 0.5% of infants with NPIH (n=1/194; 95% CI, 0-3%, p<0.0001).

Interpretation: Our findings highlight that congenital and postnatal CMV prevalence is high in this African setting and the associated complications may be significant. Universal testing and longitudinal studies are critical to understand the burden infant CMV has in sub-Saharan African countries.

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Introduction

Cytomegalovirus (CMV) is the most common viral congenital infection.[1] The prevalence of congenital CMV (cCMV) is more than 3-fold higher in low- and middle-income countries (LMIC) compared to high income countries.[2] Congenital CMV infection is the leading cause of non-genetic sensorineural hearing loss and is associated with cerebral palsy, neurodevelopmental impairment, microcephaly, ventriculomegaly and secondary infections.[3,4 5,65,6](*5, 6*)[5,6](Mills, 1984; Muller et al., 2019)[5,65,6]

An infrequently reported sequela to CMV infection is hydrocephalus.[7-9] There are an estimated 400,000 new yearly cases of childhood hydrocephalus worldwide predominantly in LMIC.[10] Hydrocephalus is one of the most common brain disorders of childhood[11] and the most common indication for childhood neurosurgery.[10] Prevention of hydrocephalus is imperative because surgical management does not cure it, and neurosurgical treatments (shunts or endoscopic fenestration) require expertise, have relatively high yearly failure rates,[10] are expensive, and may produce unsatisfying long-term outcomes.[12] There are a variety of etiologies of hydrocephalus. Its causes are generally characterized as primary (congenital) or secondary (infection, hemorrhage, or trauma).[13,14] One secondary cause, postinfectious hydrocephalus (PIH), is the leading cause of hydrocephalus in LMIC, and in infants it is often preceded by clinical neonatal sepsis presumably caused by bacteria.[15]

In a recent report, a CMV prevalence of 27% (n=27/100) in blood and 8% (n=8/100) in cerebrospinal fluid (CSF) was found from infants in Uganda less than three months of age with hydrocephalus.[16] Another report from Uganda found a 43% CMV prevalence in a small group of healthy infants (30) by three months of age.[17] The role CMV plays in the development of both congenital or acquired hydrocephalus in young infants is unknown.

In three Ugandan settings, we examined the prevalence of CMV infections in: (1) newborns, (2) neonates with sepsis, and (3) infants with hydrocephalus. In the first group we also measured the prevalence of maternal CMV infections and shedding with blood, placenta and vaginal specimens.

Methods

Study Recruitment and Sample Collection

We performed a multi-group study across three collection sites in Uganda from 2016-2019. The three groups included: 1) a newborn group on the day of birth, 2) a neonatal (<28 days of age) group with clinical signs of sepsis, and 3) a hydrocephalus group of infants (aged 3 months or less). The newborn and neonatal sepsis groups were recruited at Mbarara Regional Referral Hospital in Western Uganda and Mbale Regional Referral Hospital in Eastern Uganda. The hydrocephalus group was recruited at the CURE Children's Hospital of Uganda (CCHU) also in Mbale but serving as the de facto nationwide referral center for children with hydrocephalus. For all participants, mothers had to be at least 18 years of age and able to give informed written consent in either English, Lumasaba, Lugwere, Luganda, Ateso or Runyankole. The study was performed with approval from the CCHU Institutional Review Board, the Mbarara University of Science and Technology Research Ethics Committee, the Pennsylvania State University Institutional Review Board, and with oversight from the Ugandan National Council on Science and Technology. Material Transfer Agreements and a US Centers for Disease Control permit were obtained for the proper transfer and importation of samples to the Pennsylvania State University. As part of our data sharing agreement with the above ethics and oversight committees, we only map and report patient location information at the 0.1 degree accuracy (11

x 11 km at the equator), and we do not map patients from such grid locations with populations less than 500 people.

For the newborn-mother pairs, 100 women in labor were recruited, 50 from Mbarara and 50 from Mbale Regional Referral Hospitals. Half of the mothers were febrile at presentation for delivery, defined by one oral temperature above 38.1°C or two of 38.0°C taken at least an hour apart. Inclusion criteria included delivery at term (>37 weeks estimated gestational age). Mothers with known intrauterine fetal death, pre-eclampsia, antepartum hemorrhage, emergency delivery impeding sample collection, or no follow-up by telephone or post-discharge contact, or domicile > 10km from hospital were excluded. Four sample types were collected: prepartum maternal blood and vaginal swab, and postpartum cord blood and placenta.

For the neonatal sepsis group, 800 neonates, weighing >2000g, who presented with a possible serious bacterial infection defined as clinical sepsis were recruited, 400 from Mbale (Eastern Uganda) and 400 from Mbarara (Western Uganda) Regional Referral Hospitals. Clinical sepsis presumably caused by a serious bacterial infection was defined as the presence of one of the following three combinations of symptoms and signs: 1) axillary temperature >37.5°C, lethargy, poor feeding, 2) axillary temperature <35.5°C, lethargy, poor feeding, or 3) full fontanelle and/or seizures, axillary temperature >37.5°C, poor feeding. Neonates with congenital abnormalities, perinatal asphyxia, who had received antibiotics for more than 24 hours prior to recruitment, or whose mothers could not provide informed consent were excluded.

For the hydrocephalus group, 200 infants with PIH and 200 with NPIH at CCHU were recruited (400 hydrocephalic infants in total).[16] Inclusion criteria for PIH were as follows: age of 3 months or less, weight greater than 2.5 kg, no history consistent with hydrocephalus at birth and either a

history of febrile illness or seizures preceding the onset of clinically apparent hydrocephalus or alternative findings (such as brain imaging and endoscopic results indicative of previous ventriculitis including septations, loculations, or deposits of debris within the brain ventricular system). Inclusion criteria for NPIH were as follows: age of 3 months or less, weight greater than 2.5 kg, findings of a noninfectious origin for hydrocephalus on CT brain scans or at endoscopy (e.g., lesions obstructing the aqueduct of Sylvius such as a tumor or cyst, aneurysm, or cavernous malformation, Dandy-Walker cyst, or other congenital malformation of the nervous system), or evidence of hemorrhage as a cause of hydrocephalus such as bloody CSF and absence of findings consistent with PIH or congenital origin of hydrocephalus. Exclusion criteria were previous surgery on the nervous system (shunt, third ventriculostomy, or myelomeningocele closure) or evidence of communication of the nervous system with skin such as meningocele, encephalocele, dermal sinus tract, or fistula.

In the newborn-mother pairs, placental samples and vaginal swabs were placed in 1 mL DNA/RNA Shield (Zymo, CA, USA), and for maternal and cord blood, 1 mL of blood was mixed with 1 mL double-concentrated DNA/RNA Shield in DNA/RNA free sterile cryovials. Participants recruited to the neonatal sepsis or hydrocephalus group had both blood and CSF collected and added to either 1 mL double-concentrated DNA/RNA Shield in DNA/RNA free sterile cryovials or fresh frozen in sterile cryovials using either suspension over liquid nitrogen in Dewars, or placed in a -80^{o}C freezer. Transfer of specimens between hospitals within Uganda, to Penn State University was done using liquid nitrogen dry shippers to maintain cryogenic temperatures.

DNA extraction and quantitative polymerase chain reaction

Nucleic acid extraction of DNA/RNA Shield preserved samples was performed as previously described.[16] Briefly, DNA was extracted from 500 µL of the sample using ZymoBIOMICS DNA Miniprep Kit (Zymo, CA, USA) with bead lysis, and was eluted in 100 µL of heated elution buffer. The placental tissue was homogenized with DNA/RNA free disposable pestles in 1 mL of DNA/RNA Shield before extraction.

A TaqMan assay targeting the UL54 gene in CMV used primers and probes previously described.[18] The PCR conditions were based on the recommendations by Habbal et al.[19] Briefly, 2 µL of DNA was added to 8 µL of PCR mastermix containing 5 µL 2X Gene Expression PCR buffer (Applied Biosystems™, USA, CA) and 200 nM of primers and probes. A standard curve was generated using a block gene fragment (IDT, Iowa, USA) of the UL54 region from 1 million to 10 million copies. PCR was run on the QuantStudio 12K Flex Real-Time PCR instrument with the following cycling conditions and times: 60°C for 30 seconds, 95°C for 5 minutes, then 45 cycles of 95°C for 15 seconds and 60°C for 1 minute. Positive detection of CMV was considered for any sample that had amplifiable DNA ($C_t < 45$). Technical replicates were evaluated in duplicate. Inconsistent results were confirmed with a third independent replicate, and samples were considered positive only if the majority of the replicates were positive. A no template control and a CMV negative blood sample, confirmed with alternative testing (data not shown), were run as controls. Standard curve analysis was done for all PCR runs, overall efficiency was >75%, and R^2 was >0.95 for all runs.

Statistical analysis

Unless otherwise stated, for continuous outcome variables between groups we used two-tailed Student's t-test or Wilcoxon rank-sum tests to compare means or medians of two groups,

respectively. Pearson's chi-squared or Fisher exact tests were used for comparisons of categorical variables. Values are expressed as the mean ± standard deviation (SD), median and interquartile range in case of a skewed distribution, and as counts and percentages for categorical variables. The normality assumption for continuous variables was tested using the Shapiro-Wilk test. Ninety-five percent confidence intervals (95% CI) for prevalence were estimated using an exact binomial test. Univariate and multivariate binary logistic regression was used to evaluate risk factors.[20] We report the odds ratios (OR) and 95% CI for the odds of CMV infection. Comparison of the geographical distributions of PIH vs. congenital hydrocephalus, or CMV positive vs. CMV negative subjects, was performed using Ripley's K functions,[21] and spatial relative risk surfaces.[22] Statistical significance level was set at $p < 0.01$ for spatial relative risk surface and $p < 0.05$ for all other analyses. All analyses were performed with the R statistical language (R Development Core Team 2020 Version 3.0.6).

Results

Study Population

From 2016 to 2019, 1249 participants were recruited into three groups at three hospitals in Uganda. Samples were collected and evaluated for the presence of CMV DNA with qPCR (**Figure 1**). Participants comprised 99 (8%) mother-newborn pairs enrolled during labor, 751 (60%) infants with neonatal sepsis, and 399 (32%) infants with hydrocephalus (**Table 1**).

Table 1: Key Demographic and Clinical Characteristics of the Study Population

Variable	*Newborn*	*Neonatal Sepsis*	*Hydrocephalus*
Study Site, n (%)			
Mbale Hospital	49 (49)	398 (53)	-
Mbarara Hospital	50 (51)	353 (47)	-
CURE Hospital	-	-	399 (100)
Child factors			
Age in days, mean (± SD)	-	5 (6)	51 (25)
Female sex, n (%)	-	307 (41%)	172 (43)
Maternal factors at delivery, n (%)			
Febrile	50 (51)	-	-
HIV seropositive status	10 (10)	29 (4)	9 (2)
Type of hydrocephalus, n (%)			
Postinfectious	-	-	205 (51)
Non-postinfectious	-	-	194 (49)
Mode of Delivery, n (%)			
Vaginal	60 (60)	466 (62)	279 (70)
Cesarean Section	39 (40)	283 (38)	132 (30)

Abbreviation: HIV, human immunodeficiency virus; Mbale Hospital, Eastern Region; Mbarara Hospital, Western Region

For the 99 newborn-mother pairs, 49% (n=49/99) were recruited from Mbale and 51% (n=50/99) from Mbarara (**Table 1),** of whom 49 were afebrile and 50 were febrile, half from each site. Maternal blood, vaginal swab, and placental tissue samples were collected from all participants along with matched umbilical cord blood samples. Of the 99 neonates, 6.1% (n=6/99) developed possible serious bacterial infection and were recruited into the neonatal sepsis group. Umbilical

cord blood was available for 92 neonates, 51% (n=47/92) from febrile women and 49% (n=45/92) from afebrile mothers.

For the neonatal sepsis group, 751 neonates were recruited, with a mean age of 5 days, 53% from Mbale (n=398/751) and 47% from Mbarara (n=353/751) (**Table 1**). All participants had blood samples collected and 75% (n=560/751) had CSF samples collected (**Figure 1**). Of the 751 cases of neonatal sepsis, 0.4% (n=3/751) were recruited into the hydrocephalus group with PIH.

For the hydrocephalus group, 399 participants were recruited at CCHU, 49% (n=194/399) had NPIH and 51% (n=205/399) had PIH (**Table 1**). The mean age of the hydrocephalus group was 51 days (**Table 1**). Blood and CSF were collected from all infants in the hydrocephalus population (**Figure 1**).

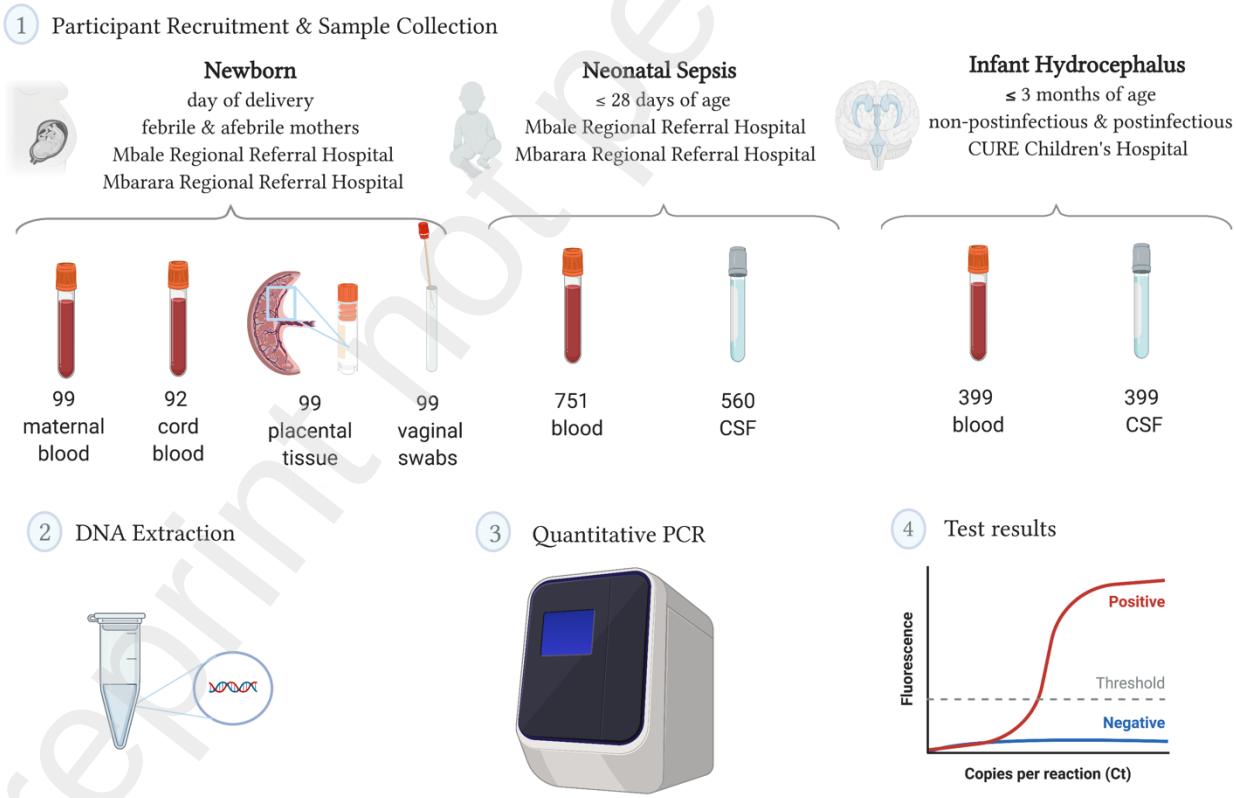

Figure 1: Group recruitment and sample processing overview. Three groups were recruited

across three different sites in Uganda. Shown is a summary of the group recruitment criteria, sample types collected, and processing overview. In the newborn group, 99 newborn-mother pairs were recruited based on the presence or absence of maternal fever at delivery. Maternal blood, placenta, vaginal swabs and cord blood were collected from these dyads. Matched blood and CSF specimens were collected in both the neonatal sepsis and hydrocephalus groups. DNA was extracted, and qPCR quantifying the UL54 CMV gene was performed in all three settings. Figure created with Biorender.com.

Prevalence of CMV detected by PCR across the study population

In the newborn group, 33% (n=33/99, 95 % CI, 24-44%) of the maternal vaginal swabs had CMV detected (**Table 2**). The CMV prevalence in paired cord blood and placenta were 3% (n=3/92, 95 % CI, 1-4%) and 3% (n=3/99; 95% CI, 1-9%), respectively (**Table 2**), with 100% concordance.

Table 2: Prevalence of CMV in the Study Population by Sample Type

Sample Type, % (95% CI)	*Newborn*	*Neonatal Sepsis*	*Hydrocephalus*
Maternal Blood	0 (0)	-	-
Vaginal Specimen	33 (24-44)	-	-
Placental Tissue	3 (1-9)	-	-
Cord or Infant Blood	3 (1-9)	2 (1-4)	22 (18-27)
Cerebrospinal Fluid	-	0 (0)	7 (5-10)

Abbreviation: CI, confidence interval; CMV, cytomegalovirus

In the neonatal sepsis group, the overall prevalence of CMV was 2% (n=17/751; 95% CI, 1-4%) in blood and 0% (n=0/560) in CSF. None of the participants recruited from the newborn group into the neonatal sepsis group were positive for CMV (n=0/6).

The overall prevalence of CMV in either blood or CSF in the combined hydrocephalus group was 24% (n=95/399; 95% CI, 20%-28%). Twenty-two percent (n=89/399; 95% CI, 18-27%) of the blood samples were positive for CMV, and 7% (n=27/399; 95% CI, 5-10%) of CSF samples were positive (**Table 2**). Eighty-one percent (n=21/27) of the participants with CMV positive CSF also had CMV-positive blood samples. None of the cases recruited from the neonatal sepsis group into the hydrocephalus group were positive for CMV (n=0/6). The distribution of cases with positive blood or CSF as a function of age with neonatal sepsis and hydrocephalus are summarized in **Figure 2**. Further, quantities across sample types are summarized in **Supplemental Table 4**.

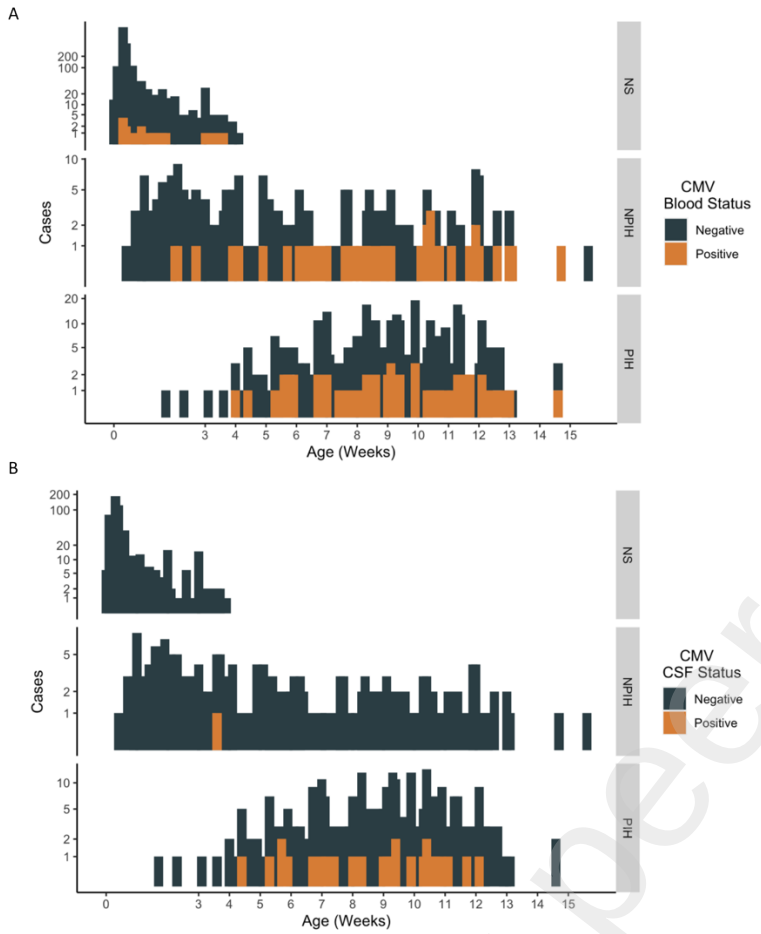

Figure 2. The CMV positivity in blood and CSF as a function of age in infants with sepsis or hydrocephalus. (A) The proportion of CMV positive and negative cases from 0-15 weeks are plotted for participants recruited in the neonatal sepsis group (NS) or the hydrocephalus group (non-postinfectious [NPIH] or postinfectious [PIH]) in blood, or (B) in CSF.

Prevalence of congenital CMV across all groups

cCMV was defined as the detection of CMV DNA in the blood or CSF of the infants within the first 21 days of life[23]. By such criteria, the prevalence of cCMV was 3% (n=3/92, 95 % CI, 1-9%) of the cord blood, 2% (n=15/729, 95 % CI, 1-3%) of neonates with sepsis, and 4% (n=3/72; 95% CI, 1-11%) of the neonates (<28 days of age) in the hydrocephalus group (**Table 3**). All of the cCMV cases in the hydrocephalus group were participants with NPIH.

Abbreviations: CMV, Cytomegalovirus; +, positive; -, negative

Table 3: Congenital CMV Prevalence Across Groups						
CMV +/-, n (%)	*Newborn (n=92)*		*Neonatal sepsis (n=751)*		*Hydrocephalus (n=399)*	
	≤ 21 days	> 21 days	≤ 21 days	> 21 days	≤ 21 days	> 21 days
CMV +	3 (3)	0 (0)	15 (2)	2 (9)	3 (4)	90 (28)
CMV -	89 (97)	0 (0)	714 (98)	20 (91)	69 (96)	237 (72)
Total	92 (100)	0 (0)	729 (97)	22 (3)	72 (18)	327 (82)

Sociodemographic and clinical attributes associated with CMV positivity

We explored participant clinical attributes and demographics associated with CMV positivity. In the newborn-mother group, all 3 CMV positive neonates were born to mothers who were febrile at delivery and delivered in Mbale. There was no significant association of neonatal clinical signs of cCMV (low birth weight, jaundice, skin rash, microcephaly, hepatosplenomegaly, seizures) with CMV positivity (**Supplemental Table 1**). In addition, maternal age, mode of delivery, and maternal HIV status were not associated with the presence of CMV in the cord blood (**Table 4**). A 33% (n=33/99) overall prevalence of maternal vaginal shedding was detected and was higher in samples from Mbale compared to Mbarara (n=22/49 vs. 11/50, Fisher's exact test, p=0.02) (**Supplemental Figure 1A**).

Table 4: Key Demographic and Clinical Attributes in CMV groups in the Study Population						
	Newborn (n=92)		*Neonatal Sepsis (n=751)*		*Hydrocephalus(n=399)*	
Variable	CMV+ n=3	CMV- n=89	CMV+ n=17	CMV- n=734	CMV+ n=95	CMV- n=304

Child factors						
Age in days, mean (±SD)	-	-	9 (8)	5 (6)	62 (22)	47 (25)
Female sex, n (%)	-	-	6 (35)	301 (41)	45 (47)	127 (42)
WAZ, mean (±SD)	0.63(12.21)	-0.64 (1.23)	-1.67 (1.55)	-0.85(1.44)	-1.66 (2.04)	-1.88 (1.98)
Maternal factors						
Age in year, mean (±SD)	26 (4)	25 (7)	23 (4)	26 (6)	-	-
Febrile illness*, n (%)	3 (100)	44 (49)	11 (69)	391(54)	-	-
Vaginal, n (%)	2 (67)	58 (67)	12 (71)	454 (62)	76 (80)	203 (67)
Cesarean section, n (%)	1 (33)	31 (33)	5 (29)	278 (38)	19 (20)	101 (33)
HIV status, n (%)	0 (0)	10 (11)	3 (17)	26 (3)	2 (2)	
Study Site, n (%)						
Mbale Hospital	3 (100)	42 (47)	15 (88)	383 (52)	-	-
Mbarara Hospital	0 (0)	48 (53)	2 (12)	351 (48)	-	-
CURE Hospital	-	-	-	-	95 (100)	304 (100)
Type of hydrocephalus, n (%)						
Postinfectious	-	-	-	-	53 (56)	152 (50)
Non-postinfectious					42 (44)	152 (50)

Abbreviation: HIV, human immunodeficiency virus; CMV, cytomegalovirus; SD, Standard Deviation; WAZ, weight-for-age Z-scores; Mbale Hospital, Eastern Region; Mbarara Hospital, Western Region

*For the Newborn group, maternal febrile illness is at delivery while Neonatal Sepsis is referring to febrile illness throughout pregnancy.

In the neonatal sepsis group, CMV positive neonates were older (9 d vs. 5 d, p=0.006) and had lower average weight-for-age z-scores (-1.67 vs. -0.85, p=0.004) compared to the CMV-negative neonates (**Table 4**, **Supplemental Table 2**). A higher CMV prevalence was detected in the blood of the neonatal sepsis group from Eastern (Mbale) compared to Western (Mbarara) Uganda (n=15/398 vs. 2/353; Fisher's exact test, p=0.003) (**Supplemental Figure 1B**).

In the hydrocephalus group, clinical attributes were evaluated separately for CSF and blood CMV measurements. When stratified by the normative values for age[24,25] there was no

significant difference in blood hemoglobin and hematocrit levels in CMV positive participants (**Supplemental Table 3**). There was no association of CMV positivity in hydrocephalic participants with infant sex, peripheral and CSF white blood cell count, CSF protein and glucose, maternal HIV status, hydrocephalus status, season (rainy vs. dry) of birth, and season at biospecimen collection or cause of hydrocephalus (PIH or NPIH). However, when stratified based on CSF CMV positivity, we observed a significantly higher proportion of CMV positive cases in the PIH compared to the NPIH group (n=26/205 vs. 1/194, p<0.0001) (**Supplemental Figure 1C)**.

Risk factors associated with CMV infection

We explored potential risk factors associated with blood or CSF CMV positivity in the neonatal sepsis and the hydrocephalus groups. In the cases of neonatal sepsis, multivariate logistic regression analysis demonstrated that older infant age (aOR, 1.09 for each day older; 95% CI, 1.01-1.16; p= 0.007), maternal HIV seropositivity (aOR, 21.09; 95% CI, 3.76-109.13; p= 0.0002), residence in Eastern region of Uganda (aOR, 11.10; 95% CI, 2.82-76.89; p=0.003) and maternal age < 25 years (aOR, 4.91; 95% CI, 1.56-19.93; p=0.012) were significantly associated with increased odds of CMV infection (**Table 5**).

Table 5: Risk factors associated with CMV prevalence in neonates with sepsis

Variable	**Univariate logistic regression OR [95% CI]**	**p-value**	**Multivariable Adjusted* logistic regression OR [95% CI]**	**p-value**
Neonatal factors				
Age, d	1.08 [1.01, 1.14]	0.01	1.09 [1.01, 1.16]	0.007
Sex				
Female	0.78 [0.27, 2.09]	0.64	0.63 [0.99, 1.80]	0.41
Male	Reference		Reference	
Maternal factors				
Mode of delivery				
Cesarean section	0.68 [0.21, 1.86]	0.47	1.10 [0.31, 3.64]	0.883
Vaginal	Reference		Reference	
Age				

< 25 years	3.30 [1.16, 11.81]	0.04	4.91[1.56, 19.93]	0.012
≥ 25 years	Reference		Reference	
HIV status				
Positive	5.79 [1.28, 19.13]	0.008	21.09 [3.76, 109.13]	0.0002
Negative	Reference		Reference	
Site of study				
Eastern Region	6.87 [1.92, 43.81]	0.01	11.10 [2.82, 76.89]	0.003
Western Region	Reference		Reference	

Abbreviation: CI, confidence interval; CMV, cytomegalovirus; HIV, human immunodeficiency virus; OR, odds ratio; Eastern Region, Mbale; Western Region, Mbarara

*Adjusted for the effect of neonatal age, maternal age, neonatal HIV exposure status and site of study

In the cases of hydrocephalus, multivariate logistic regression analysis demonstrated that CMV infection was associated with older infant age (aOR. 1.03; 95% CI, 1.02-1.05 p<0.0001) (**Table 6**).

Table 6: Risk factors associated with CMV prevalence in infants with hydrocephalus

Variable	**Univariate logistic regression OR [95% CI]**	**p-value**	**Multivariable Adjusted* logistic regression OR [95% CI]**	**p-value**
Child factors				
Age, d	1.027 [1.02, 1.04]	<0.0001	1.034 [1.02, 1.047]	<0.0001
Sex				
Male	0.80 [0.50, 1.27]	0.34	0.85 [0.53,1.37]	0.50
Female	Reference		Reference	
Hydrocephalus type				

PIH	1.33 [0.84, 2.13]	0.22	0.645 [0.34,1.18]	0.15
NPIH	Reference			
Malnutrition status				
Underweight (WAZ <-2SD)	1.88 [0.34, 35.72]	0.56	1.69 [0.27, 32.59]	0.64
Normal WAZ ≥ -2 SD	Reference			
Maternal factors				
HIV status				
Positive	0.91 [0.13, 3.85]	0.91	0.60 [0.09,2.64]	0.54
Negative	Reference		Reference	
Mode of delivery				
Vaginal	2.15 [1.24, 3.88]	0.008	1.10 [0.83, 2.23]	0.10
Cesarean section	Reference		Reference	

Abbreviation: CI, confidence interval; CMV, cytomegalovirus; HIV, human immunodeficiency virus; OR, odds ratio; WAZ: weight-for-age Z-scores

*Adjusted for the effect of hydrocephalus status, malnutrition, mode of delivery, child's age and HIV exposure

Geospatial clustering

We examined the spatial distribution of cases based upon the geographical location of patient but will only report a 11 km grid square of that patient's village in Uganda for privacy reasons (see methods). We used NPIH and CMV- locations as representative of the spatial distribution of the population at risk for PIH and CMV+ respectively. As previously shown in a smaller group[16,2616,26](*16, 26*)[16,26](!!! INVALID CITATION !!! ; Paulson et al., 2020)[16,2616], PIH cases were more clustered geographically than NPIH cases (**Figure 3A**). The evidence of this clustering is shown in **Figure 3B** by the positive difference between the empirical K functions for PIH and NPIH cases lying outside the 95% Monte Carlo confidence limits obtained under random labelling of PIH and NIPH cases. To further investigate the location of these clusters within the country we estimated the spatial relative risk obtained as the ratio of the spatial intensity of PIH and NPIH cases. **Figure 3C** shows the presence of two significant ($p < 0.01$) clusters, in Eastern and Northern region of Uganda. Although a higher prevalence of CMV was

seen at the Eastern compared to the Western collection site, analysis based on geographic location did not reveal any difference in spatial clustering for CMV positive vs. CMV negative cases (**Figure 3D-3F**).

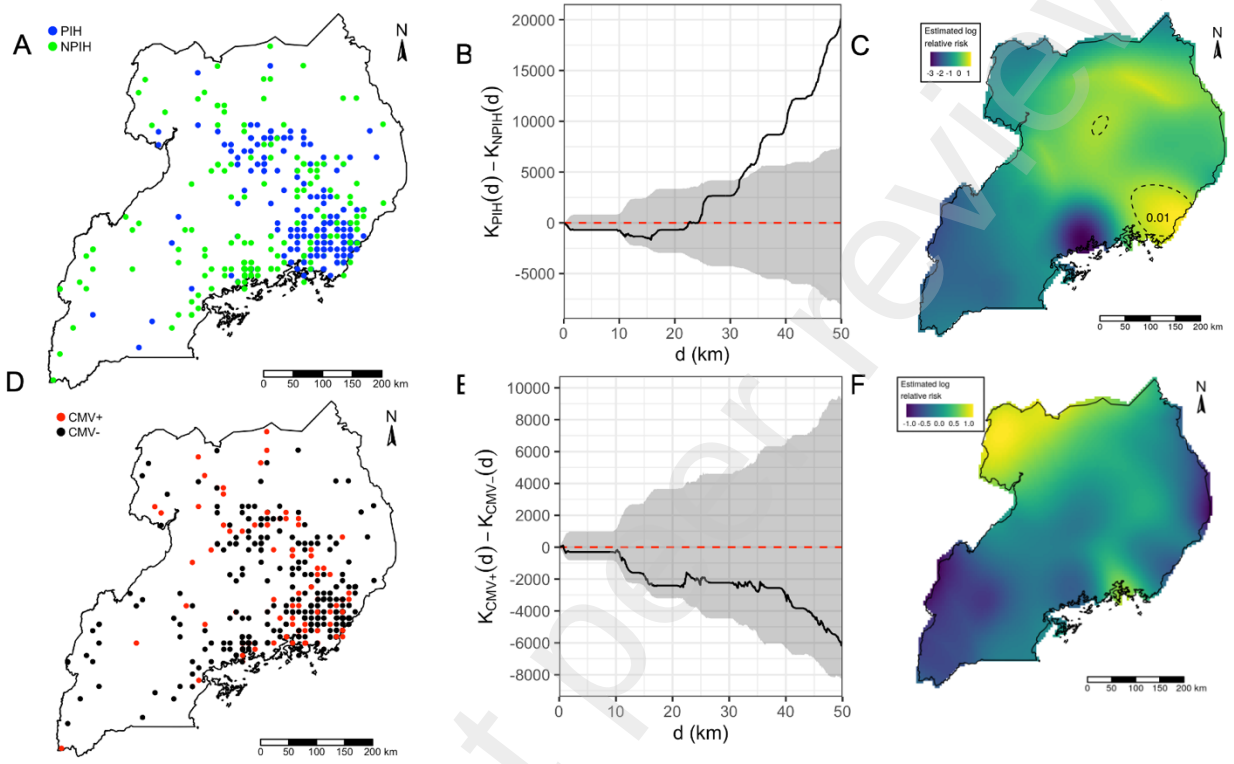

Figure 3. Spatial distribution of cases. Map of Uganda showing the distribution of PIH (blue) and NPIH (green) cases (A). Although we use the spatial location of the patient's village centroid in our statistical calculations, for privacy reasons (see methods) we only map these locations within a 11-kilometer square grid of the participant's village latitude and longitude. Utilizing the difference between the Ripley's K functions of PIH and NPIH cases (B) we see that the PIH cases tend to cluster above the degree of spatial aggregation of the NPIH cases at distances of approximately 30km as shown by randomly permuting the labeling of PIH and NPIH assignments 1,000 times. Panel C shows the estimated spatial relative risk of PIH vs. NPIH cases on the log-scale, values significantly higher than 0 ($p < 0.01$) identify geographical regions

where PIH cases are more clustered than NPIH cases and are shown by the dashed lines. The distribution of the CMV positive (+) and negative (-) cases within the outline of Uganda (D). In contrast with PIH and NPIH clustering, the CMV+ and CMV- cases all lie within the 95% confidence limits (E), and an analysis of the spatial relative risk did not identify significant geographical clusters (F).

Discussion

This multi-center study identified the prevalence of CMV in Ugandan infants less than three months old in three groups: in newborns, in neonates with clinical sepsis, and in infants with hydrocephalus. The cases of hydrocephalus were of both non-postinfectious and postinfectious origin. Our study found a CMV prevalence of 3% in newborns, 2% in neonates with clinical sepsis, and 24% in infants with hydrocephalus. Almost all of the CMV detected in cerebrospinal fluid (CSF) were in participants diagnosed with PIH (n=26/27). In the neonatal sepsis population, CMV positive had significantly lower weight despite being older than the CMV negative neonates. We identified maternal HIV seropositivity, older infant and younger maternal age, and residence in Eastern vs. Western Uganda as significant risk factors among the participants in the neonatal sepsis population with CMV infection. This geographic risk factor is also correlated with maternal shedding of CMV during parturition, with significantly higher rates in mothers delivering in Eastern (vs. Western) Uganda (n=22/49 vs. 11/50).

The relationship of CMV to perinatal disease is notoriously complex and its role in the development of hydrocephalus is unclear. In this context, it is noteworthy that the detection of CMV in our hydrocephalic group is remarkably more frequent (24%) than it is in cord blood (3%) and neonates with clinical sepsis (2%). However, infants with hydrocephalus were older than those in the other two groups, a factor that by itself increases the risk of acquiring CMV

postnatally. This later acquisition is consistent with previous reports of progressively higher infant prevalence of CMV infections in high adult seropositive populations in LMIC such as Uganda[17]. CMV has been associated with both congenital hydrocephalus[7-9] and PIH.[16] In our study, CMV was almost always detected in CSF from infants with PIH (n=26/27) vs NPIH (n=1/27). While this observation is striking, its implication regarding causation are uncertain, as the pathophysiology of PIH in our Ugandan settings is complex.[16] In PIH in this setting CMV may be: (i) an opportunist infection, (ii) a marker of incompetence of the blood-brain barrier, which allows CMV-infected leukocytes into the CSF, (iii) an indicator of immune deficiency, which leads to prolonged CMV shedding[27], (iv) a cause of ependymal and sub ependymal inflammation and necrosis followed by fibrosis narrowing CSF outflow channels from the brain[28], or (v) provocation of autoimmune acute disseminated encephalomyelitis which induces fibrosis.[29] In the first three possibilities, CMV is a facilitating factor or marker of central nervous system infection due to other agents that lead to PIH. In the last two possibilities, CMV may, by itself, cause the inciting infectious or autoimmune process that leads to hydrocephalus. All five of these possibilities are potential contributing factors in the hydrocephalus group.

Our results are consistent with previous reports on risk factors for CMV including maternal HIV seropositivity[30,31] and younger maternal age.[32,33] We identified a 33% prevalence of vaginal shedding in pregnant women, similar to a prevalence of pregnant women reported in Brazil.[34] In neonatal sepsis, vaginal delivery was a trend in the CMV positive (71%) compared to CMV negative (64%), but did not reach statistical significance (Table 5). We do not know the eventual prevalence of CMV from perinatal acquisition following vaginal exposures given the ages in our groups of participants and lack of longitudinal sampling.

Within Uganda we found a geographic variation in CMV positivity. There was a higher prevalence of CMV infection in infants and more maternal vaginal shedding appeared in the Eastern region in contrast to the Western region It is unknown currently whether there is CMV strain variability across this geographic region or whether host or viral genomic variation contributes to such differential prevalence.

Limitations

There are limitations of this study including a lack of urine or buccal sampling in the neonates, which are known to have an increased sensitivity for CMV detection.[35,36] Although we sampled cord blood of healthy newborns, we did not have aged-matched healthy controls for the older infants. Also, our definition of cCMV based on age (21 days) could have misclassified early postnatal infections or failed to recognize congenital infections that were not tested early enough. In addition, we did not have CMV-specific follow-up evaluations such as repeat viral testing and/or auditory or visual assessments. These limitations highlight the importance of further investigating the risk of early exposure to CMV and such exposures' potential long-term consequences in this setting.

Conclusion

Our study finds that CMV is a neglected infection in sub-Saharan countries such as Uganda, consistent with previous work.[1,37,38] The impact of hydrocephalus on motor and cognitive development, as well as the challenges of the lifelong management of hydrocephalus, urges further scrutiny of the role that CMV plays in the health of such children. Even in high-resourced settings cCMV testing tends to be limited to symptomatic neonates and in LMIC settings testing is rarely available. [23,31] Widespread testing of infants and long-term follow up of groups would increase our understanding of CMV in infant health in LMIC settings.

Research in context

Evidence before this study

Cytomegalovirus (CMV) is a common but neglected pathogen in low- and middle-income countries like Uganda. We performed a PubMed search up to July 7, 2021, using the terms ("Cytomegalovirus" OR "CMV" OR "Human Herpesvirus 5") AND ("infant" OR “neonates”) AND ("neonatal sepsis" OR "hydrocephalus") AND ("Uganda"). This search resulted in one of our previous reports that did not describe the risk factors of CMV in neonates with sepsis or infants with hydrocephalus. In Uganda, only one study of 30 infants defined risk factors and postnatal CMV transmission rates (59% by one year). However, this study did not define the CMV maternal exposure (via vaginal shedding or placental transmission), prevalence of congenital CMV or prevalence in the context of neonatal sepsis and hydrocephalus. In addition, the spatial distribution and relative risk of CMV in Uganda was not determined. Despite being a country with a high burden of risk factors of congenital and postnatal CMV (HIV exposure, high adult CMV seropositivity) there is a dearth of studies that have defined the early maternal exposure, prevalence and determinants of CMV in young infants in Uganda across various geographical settings.

Added value of this study

In the present study of 1242 infants less than three months old, CMV was detected at a much higher prevalence in older infants with hydrocephalus (24%) than in neonates with clinical sepsis or apparently healthy newborns (2-3%). CMV was detected in cerebrospinal fluid more commonly when hydrocephalus developed following clinical sepsis. There was a high rate of vaginal shedding of CMV (33%). Maternal HIV seropositivity, age less than 25 years, and residence in Eastern vs. Western Uganda were significant risk factors for CMV infection in neonates with clinical sepsis.

Implications of all the available evidence

We defined CMV prevalence in Ugandan newborns-mother pairs, neonates with sepsis, and infants with hydrocephalus across various geographical regions. Overall, the burden of congenital and postnatal CMV infections on the health of Ugandan children may be substantial. Universal testing and longitudinal studies are critical to understanding the burden of infant CMV infection in sub-Saharan African countries.

Table Legends

Table 1: Key Demographic and Clinical Characteristics of the Study Population: For the newborn-mother group, 100 women were recruited in labor, 50 from Mbarara and 50 from Mbale. Half of the mothers were febrile at presentation for delivery. The mode of delivery was vaginal in 60% of the sample. For the neonatal sepsis group, 400 infants less than 28 days of age were each recruited from Mbale and Mbarara (800 neonates in total). The mean age at recruitment was 5 days, 4% were HIV sero-exposed, 62% were delivered vaginally and 38% cesarean section. For the infant hydrocephalus group, 205 infants with PIH and 194 with NPIH at CCHU were recruited (399 hydrocephalic infants in total). The mean age was 51 days, 43% were female, 2% were HIV sero-exposed, and 70% were born vaginally.

Table 2 Prevalence of CMV in the Study Population by Sample Type. In the newborn group, 33% (vaginal specimen), 3% (placental tissue) and 3% (cord blood) were positive for CMV. 2% of the neonates were positive for CMV by blood and 0% in the cerebral spinal fluid (CSF). Lastly, 22% and 7% of the infants with hydrocephalus were positive for CMV by blood and CSF, respectively.

Table 3: Congenital CMV prevalence Across Groups. The birth prevalence of congenital CMV (CMV positivity in infants aged 21 days and younger) was 3% in the newborn group, 2% in the neonatal sepsis group and 4% in the hydrocephalus group. The rates were higher in: 9% and 28% in the neonatal sepsis and hydrocephalus groups.

Table 4: Key Demographic and Clinical Attributes in CMV groups in the Study Population. In the newborn group, CMV-positive cord blood samples were all from mothers

who were febrile during delivery and all lived in the eastern region of the country (Mbale). In the neonatal sepsis group, there were significant between-group differences in age of the child (p=0.006 by t-test) and mother (p=0.04 by t-test), weight-for-age z-scores (p=0.004 by t-test), HIV exposure (p=0.04 by Fisher's exact test), and region of residence (p=0.003 by Fisher's exact test). In the hydrocephalus group, there were significant between-group differences in age of the child (p<0.0001 by t-test) and mode of delivery (p=0.008 by Fisher's exact test).

Table 5: Risk factors associated with CMV prevalence in neonates with sepsis In univariate regression analysis, increased age of the infant, HIV exposure, maternal age less than 25 years and living in Eastern region of Uganda were significantly associated with CMV prevalence. These associations remained significant in the adjusted logistic regression model. Significant estimates are in bold.

Table 6: Risk factors associated with CMV prevalence in infants with hydrocephalus. In univariate regression analysis, increased age of the infant and birth by vaginal route were significantly associated with CMV prevalence. Only age remained significantly associated with CMV prevalence in the adjusted logistic regression. Significant estimates are in bold.

Article Information

Author Contributions: Dr. Schiff had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of data analysis.

Study concept and design: Schiff, Broach, Hehnly

Acquisition, analysis, or interpretation of data: Hehnly, Burgoine, Smith, Bebell, Mbabazi-Kabachelor, Kumbakumba, Mulondo, Onen, Ssentongo, Broach, Paulson, Morton, Ericson, Schiff

Drafting of the manuscript: Schiff, Broach, Hehnly, Ssentongo, Ericson, Meier

Critical revision of the manuscript for important intellectual content: All authors.

Statistical analysis: Ssentongo, Paulson, Schiff, Hehnly, Fronterre

Administrative, technical, or material support: Schiff, Broach, Sinnar, Sheldon

Study supervision: Schiff, Broach

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