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# Cytomegalovirus infections in infants in Uganda: Newborn-mother pairs, neonates with sepsis, and infants with hydrocephalus

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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.

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Disclosures

The authors declare no conflict of interest in this article.

Ethical Approval Statement

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 mapped and reported patient location information at the 0.1-degree accuracy (11 × 11 km at the equator), and we did not map patients from grid locations with populations less than 500 people.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi: 10.1016/j.ijid.2022.02.005.

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## Abstract

**Objectives:** To estimate the prevalence of cytomegalovirus (CMV) infections among newborn-mother pairs, neonates with sepsis, and infants with hydrocephalus in Uganda.

**Design and Methods:** Three populations—newborn-mother pairs, neonates with sepsis, and infants (≤3 months) with nonpostinfectious (NPIH) or postinfectious (PIH) hydrocephalus—were evaluated for CMV infection at 3 medical centers in Uganda. Quantitative PCR (qPCR) was used to characterize the prevalence of CMV.

**Results:** The overall CMV prevalence in 2498 samples across all groups was 9%. In newborn-mother pairs, there was a 3% prevalence of cord blood CMV positivity and 33% prevalence of maternal vaginal shedding. In neonates with clinical sepsis, there was a 2% CMV prevalence. Maternal HIV seropositivity (adjusted odds ratio [aOR] 25.20; 95% confidence interval [CI] 4.43–134.26; p = 0.0001), residence in eastern Uganda (aOR 11.06; 95% CI 2.30–76.18; p = 0.003), maternal age < 25 years (aOR 4.54; 95% CI 1.40–19.29; p = 0.02), and increasing neonatal age (aOR 1.08 for each day older; 95% CI 1.00–1.16; p = 0.05), were associated risk factors for CMV in neonates with clinical sepsis. We found a 2-fold higher maternal vaginal shedding in eastern (45%) vs western (22%) Uganda during parturition (n = 22/49 vs 11/50, the Fisher exact test; p = 0.02). In infants with PIH, the prevalence in blood was 24% and in infants with NPIH, it was 20%. CMV was present in the cerebrospinal fluid (CSF) of 13% of infants with PIH compared with 0.5% of infants with NPIH (n = 26/205 vs 1/194, p < 0.0001).

**Conclusions:** Our findings highlight that congenital and postnatal CMV prevalence is substantial in this African setting, and the long-term consequences are uncharacterized.

### Keywords

cytomegalovirus infection; sub-Saharan Africa; hydrocephalus; neonatal sepsis

## Introduction

Cytomegalovirus (CMV) is the most common congenital viral infection (Manicklal et al., 2013). The prevalence of congenital CMV (cCMV) is more than 3-fold higher in low- and middle-income countries (LMIC) compared with high-income countries (Ssentongo et al., 2021). Congenital CMV infection is the leading cause of nongenetic sensorineural hearing loss and is associated with cerebral palsy, neurodevelopmental impairment, microcephaly, ventriculomegaly, and secondary infections (Kenneson and Cannon, 2007; Lanzieri et al., 2017, Martinez et al., 2021; Müller et al., 2019).

An infrequently reported sequela of CMV infection is hydrocephalus (Gabrielli et al., 2012; Nickerson et al., 2012; Simeone et al., 2013). Hydrocephalus is one of the most common brain disorders of childhood (Isaacs et al., 2018) and the most common indication for childhood neurosurgery (Dewan et al., 2018). Worldwide, an estimated 400,000 yearly new cases of childhood hydrocephalus occur predominantly in LMIC (Dewan et al., 2018). Prevention of hydrocephalus is imperative because surgical management palliates but does not cure it, and neurosurgical treatments (shunts or endoscopic fenestration) require expertise, have relatively high yearly failure rates (Dewan et al., 2018), are expensive, and may produce unsatisfying long-term outcomes (Schiff et al., 2021; Warf et al., 2011). The etiologies of hydrocephalus are characterized as primary (congenital) or secondary (infection, hemorrhage, or trauma) (Kahle et al., 2016, Karimy et al., 2016). Postinfectious hydrocephalus (PIH) appears to be one of the most common causes of hydrocephalus in LMIC; and in infants, it is often preceded by clinical neonatal sepsis (NS) presumably caused by bacteria (Karimy et al., 2020).

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 in Uganda in infants less than 3 months old with

hydrocephalus (Paulson et al., 2020). Another report from Uganda found a 43% CMV prevalence in a small group of otherwise healthy infants (n =32) less than 3 months old (Gantt et al., 2016). The role CMV plays in the development of both congenital or acquired hydrocephalus in young infants is unknown.

We examined the prevalence of CMV infections in: (1) newborn-mother pairs, (2) neonates with sepsis, and (3) infants with hydrocephalus in 3 Ugandan settings using blood and CSF. In the first group, we also measured the prevalence of maternal CMV infections and shedding with blood, placenta, and vaginal specimens.

## Material and Methods

### Study Recruitment and Sample Collection

We performed a multigroup study across 3 collection sites in Uganda from 2016–2019. The 3 groups included: (1) a newborn-mother group on the day of birth, (2) a neonatal (≤28 days old) group with clinical signs of sepsis, and (3) a hydrocephalus group of infants (aged 3 months or less). The newborn-mother and NS 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, which serves as a de facto nationwide referral center for children with hydrocephalus. For all participants, mothers had to be at least 18 years old and able to give informed written consent in either English, Lumasaba, Lugwere, Luganda, Ateso, or Runyankole.

For the newborn-mother pairs, 100 women in labor were recruited, 50 from Mbarara Regional Referral Hospital and 50 from Mbale Regional Referral Hospital as previously described (Movassagh et al., 2021). For further details, see supplemental information.

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

For the hydrocephalus group, 200 infants with PIH and 200 with non-postinfectious hydrocephalus (NPIH) at CCHU (400 in total) were recruited (the first 100 of these infants were included in a report by (Paulson et al., 2020). For further details, see supplemental information.

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 NS 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 were fresh frozen in sterile cryovials using either suspension over liquid nitrogen in Dewars, or placed in an −80 °C freezer. Transfer of specimens between hospitals within Uganda and 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 (Paulson et al., 2020). Briefly, DNA was extracted from 500 μL of the sample using Zymo-BIOMICS 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 as previously described (Sanchez and Storch, 2002). The PCR conditions were based on the recommendations by Habbal and colleagues (Habbal et al., 2009). 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 copy 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. Any sample that had amplifiable DNA ($C_t < 45$) was considered CMV-positive. 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.

## Calculating Nutritional Status in Infants With Hydrocephalus

Infants with hydrocephalus have excess weight from their extra CSF; therefore, estimating the nutritional status based on unadjusted body weight is misleading. We overcame the limitation by calculating the volume of CSF in the head from CT scan data, and subtracting excess fluid weight (using 1 gm/mL CSF) for age based on normative curves (Peterson et al., 2021).

## Statistical Analysis

Unless otherwise stated, for continuous outcome variables between groups, we used the 2-tailed Student's *t* test or the Wilcoxon rank-sum test to compare means or medians of 2 groups, respectively. The Chi-squared or the Fisher exact test was used for the comparison of categorical variables. Values are expressed as mean ± standard deviation (SD), median and interquartile range (IQR) 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 (Shapiro et al., 1968). Ninety-five percent confidence intervals (95% CI) for prevalence were estimated using an exact binomial test. Univariate and multivariate binary logistic regressions were used to evaluate risk factors. We report the odds ratios (OR) and 95% CI for the odds of CMV infection. The comparison of the geographical distributions of the subjects who were CMV-positive versus CMV-negative was performed using Ripley K functions (Diggle and Chetwynd, 1991). The statistical significance level was set at $p < 0.05$, unless otherwise stated. All analyses were performed with the R statistical language (R Development Core Team 2020 version 3.0.6).

## Results

### Study Population

From 2016–2019, 1249 participants were recruited into 3 groups at 3 hospitals in Uganda (Figure 1). Samples were collected and evaluated for the presence of CMV DNA with qPCR. Participants comprised 99 (8%) mother-newborn pairs enrolled during labor, 751 (60%) infants with NS, and 399 (32%) infants with hydrocephalus (Table 1).

Of the 99 newborn-mother pairs, 49% (n = 49/99) were recruited from Mbale and 51% (n = 50/99) from Mbarara (Table 1); of these 49 were afebrile and 50 were febrile. 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 clinical sepsis within 48 hours of birth and were recruited into the NS group. Umbilical cord blood was available for 92 neonates, 51% (n = 47/92) from febrile mothers and 49% (n = 45/92) from afebrile mothers.

For the NS group, 751 neonates, with a mean age of 5 days were recruited, 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. Of the 751 cases of NS, 1.3% (10/751) developed PIH, and 3 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.

### Prevalence of CMV Detected by PCR Across the Study Population

In the newborn group, CMV was detected in 33% (n = 33/99, 95 % CI 24–44) of the maternal vaginal swabs (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.

In the NS 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 NS 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 NS group into the hydrocephalus group were positive for CMV (n = 0/3). The distribution of cases with positive blood or CSF as a function of age with NS and hydrocephalus are summarized in Figure 2. Further details on quantities of genomes across sample types are summarized in Supplemental Table 5.

## 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 (Mussi-Pinhata and Yamamoto, 2020). 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 old) in the hydrocephalus group (Table 3). All the cases identified as cCMV in the hydrocephalus group were participants with NPIH and only had CMV detected in the blood.

## 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 neonates who were CMV-positive 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, and 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 with Mbarara (n = 22/49 vs 11/50, the Fisher exact test; p = 0.02) (Figure 3A).

In the NS group, neonates who were CMV-positive were older (9 days vs 5 days, p = 0.006) and had lower average weight-for-age *z*-score (−1.67 vs −0.85, p = 0.004) compared with the CMV-negative neonates (Table 4, Supplemental Table 2). A higher CMV prevalence was detected in the blood of the NS group from Mbale compared with Mbarara, Uganda (n = 15/398 vs 2/353, the Fisher exact test; p = 0.003) (Figure 3B).

In the hydrocephalus group, clinical attributes were evaluated separately for CSF and blood CMV measurements. When stratified by the normative values for age (Adeli et al., 2015; Soldin et al., 2011), there was no significant difference between blood hemoglobin and hematocrit levels in participants who were CMV-positive (Supplemental Table 3). When blood was assessed for CMV, there was no association of CMV positivity and sex, peripheral white blood cell count, CSF protein and glucose, or maternal HIV status, season (rainy vs dry) of birth, season at biospecimen collection, or cause of hydrocephalus (PIH or NPIH). However, when CSF was assessed for CMV there was a significantly higher CSF white blood cell count per $\mu$L (mean = 69.2 vs 22.1, p < 0.0001) in patients with hydrocephalus who were CMV-positive and a higher proportion of CMV-positive cases in

the PIH compared with the NPIH group (n = 26/205 vs 1/194, p < 0.0001) (Figure 3C, Supplemental Table 4).

### Risk Factors Associated With CMV Infection

We explored potential risk factors associated with blood or CSF CMV positivity in the NS and the hydrocephalus groups. For NS cases, multivariate logistic regression analysis demonstrated that older infant age (aOR 1.08 for each day older; 95% CI 1.00–1.16; p = 0.05), maternal HIV seropositivity (aOR 25.20; 95% CI 4.43–134.26; p = 0.0001), residence in the eastern region of Uganda (aOR 11.06; 95% CI 2.30–76.18; p = 0.003), and maternal age <25 years (aOR 4.54; 95% CI 1.40–19.29; p = 0.02) were significantly associated with increased odds of CMV infection (Table 5).

For hydrocephalus cases, 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).

### Geospatial Clustering

We examined the spatial distribution of cases based on the geographical location of patient villages, but for privacy only reported the accuracy in plots within a 121 $km^2$ grid square containing that patient's village by randomly jittering the points by 5 km and using point markers that covered a 10-km diameter. Furthermore, we did not map patients from grid locations with populations less than 500 people. The geographical location of the village of newborn-mother pairs (Figure 4A), neonates (Figure 4B), or infants with hydrocephalus (Figure 4C) with respect to the CMV status was based on either maternal vaginal specimen or cord blood (for newborn-mother pairs); blood or CSF (for neonates with sepsis and infants with hydrocephalus); and used as representative of the spatial distribution of the population at risk for CMV positivity. Spatial clustering is indicated by the Riley K statistic. There was spatial clustering of CMV-positive cases in the newborn-mother and NS groups above the degree of spatial aggregation from CMV-negative, peaking at spatial scales of approximately 5 and 35 km, respectively. This was demonstrated by the positive difference between the empirical K functions for CMV-positive cases lying outside the 95% Monte Carlo confidence limits obtained under 1000 random labeling simulations of CMV-positive and -negative cases (Figure 4A and B). In contrast, the older infants with hydrocephalus group demonstrated no evidence of clustering of CMV-positive versus CMV-negative cases (Figure 4C).

## Discussion

This multicenter study identified the prevalence of CMV in infants from Uganda who were less than 3 months old in 3 populations: newborn-mother pairs, neonates with clinical sepsis, and infants with hydrocephalus. Compared with neonates, we found a nearly 10-fold higher CMV prevalence in older infants less than 3 months old and with hydrocephalus. In patients with hydrocephalus, almost all the CMV detected in CSF were from participants diagnosed with PIH. In the neonatal population, we identified maternal HIV seropositivity, older neonates, lower weight-for-age *z*-score, younger maternal age, and residence in eastern

versus western Uganda as significant risk factors of CMV infection. This geographic risk factor of CMV is also correlated with maternal shedding of CMV during parturition.

The relationship of CMV with 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 2 groups, and increasing age may be the most significant factor we identified with postnatal CMV acquisition. 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 (Gantt et al., 2016). CMV has been associated with both congenital hydrocephalus (Gabrielli et al., 2012; Nickerson et al., 2012; Simeone et al., 2013) and PIH (Paulson et al., 2020). In our study, CMV was almost always detected in CSF from infants with PIH (n = 26/27) ver sus NPIH (n = 1/27). While this observation is striking, its im plications regarding causation are uncertain, as the pathophysiology of PIH in our Ugandan setting is complex (Paulson et al., 2020). The CMV in the setting of PIH may be an opportunist infection; a marker of the incompetence of the blood-brain barrier, which allows CMV-infected leukocytes into the CSF; an indicator of immune deficiency, which leads to prolonged CMV shedding (Miles et al., 2007); a cause of ependymal and subependymal inflammation and necrosis followed by fibrosis narrowing CSF outflow channels from the brain (Griffiths et al., 2015); or provocation of autoimmune acute disseminated encephalomyelitis which induces fibrosis (Imataka and Arisaka, 2014). In the first 3 possibilities, CMV is a facilitating factor or a marker of central nervous system infection due to other agents that lead to PIH. For instance, the elevated CSF white blood cell count in PIH may be due to bacterial infection and may concurrently enable the transport of the intracellular CMV virus into the CSF. In the last 2 possibilities, CMV may, by itself, cause the inciting infectious or autoimmune process that leads to hydrocephalus. All 5 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 (Mostad et al., 1999; Pathirana et al., 2019) and younger maternal age (Fowler et al., 2003; Preece et al., 1986). Furthermore, neonates who were positive for CMV had significantly lower weight-for-age *z*-score despite being older than the neonates who were negative for CMV. We identified a 33% prevalence of vaginal shedding in pregnant women, similar to a prevalence in pregnant women reported from Brazil (Barbosa et al., 2018). 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 an increased prevalence (Figure 3A and B) and significant geospatial clustering (Figure 4A and B) of CMV-positive cases in maternal vaginal shedding or newborn cord blood (or placenta) and blood of neonates with sepsis in the eastern region in contrast to the western region. In the older hydrocephalus group, this geographical risk difference was no longer detected (Figure 4C). This implies a potentially increased risk of early exposure and acquisition of CMV in eastern Uganda compared with western Uganda, but later sampling from infants with

hydrocephalus (with presumed additional postnatal acquisition) does not reflect this earlier geospatial risk. It is unknown currently whether there is a CMV strain variability across this geographic region or whether host 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 (Boppana et al., 2010, Boppana et al., 2011; Ssentongo et al., 2021). Although we sampled the cord blood of healthy newborns, we did not have age-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, auditory, or visual assessments. These limitations highlight the importance of further investigating the risk of early exposure to CMV and the long-term consequences in this setting.

## Conclusion

Our study demonstrates that CMV is a neglected infection in sub-Saharan countries such as Uganda, consistent with previous work. 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-resource settings, cCMV testing tends to be limited to symptomatic neonates; and in LMIC settings, testing is rarely available. More widespread testing of infants and long-term follow-up of CMV infections will increase our understanding of the consequences of early infant CMV infection in LMIC settings.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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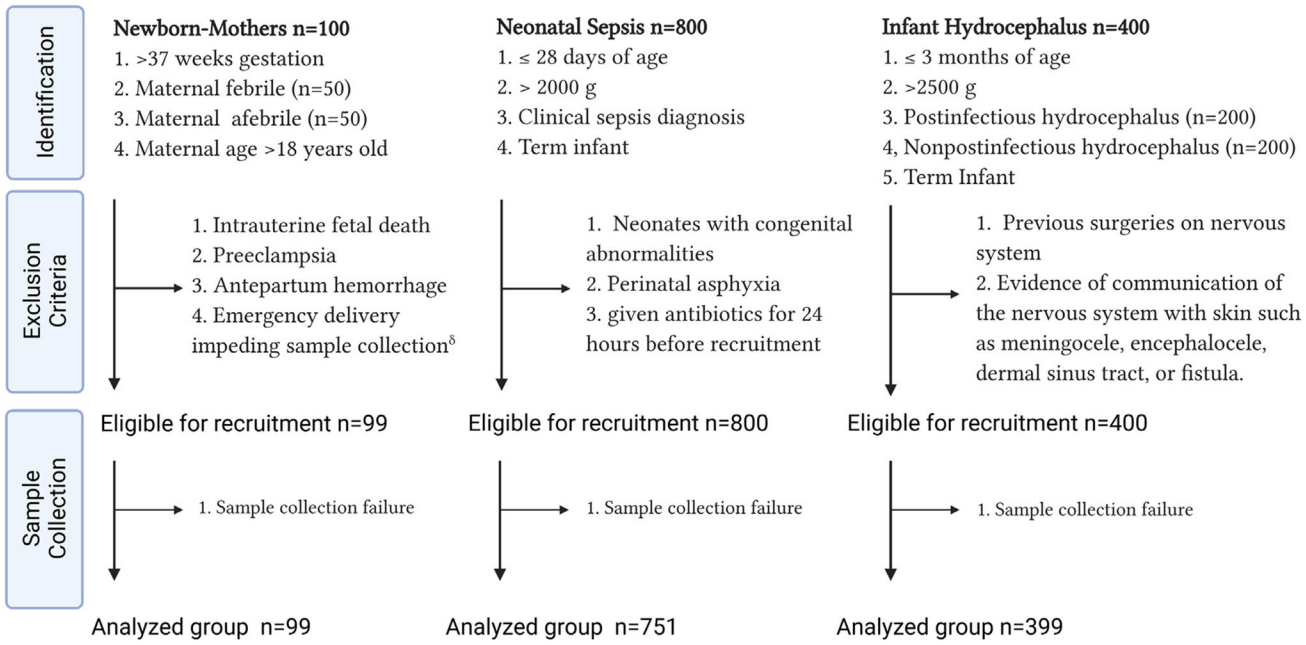


[δ]5. No follow up contact

**Figure 1.**

Group recruitment summary of inclusion and exclusion criteria. Three groups were recruited across 3 different sites in Uganda. Shown is a summary of group inclusion criteria for patient identification, exclusion criteria, and sample collection that led to our final analyzed group. Patients were excluded if their mothers could not give informed consent. In the newborn-mother group, 99 pairs were recruited based on the presence or absence of maternal fever at delivery defined by 1 temperature of 38.1 °C or 2 temperatures of 38 °C within an hour of each other. In the neonatal sepsis (NS) group, 751 neonates were recruited based on the following criteria defining clinical sepsis from a possible serious bacterial infection: (1) axillary temperature > 37.5 °C, lethargy, and poor feeding; (2) axillary temperature < 35.5 °C, lethargy, and poor feeding; or (3) full fontanelle and/or seizures, axillary temperature > 37.5 °C, and poor feeding. In the hydrocephalus group, hydrocephalus was diagnosed using computerized tomography scans following the observation of growing head circumference. Fluid build-up and increased pressure along with characteristic structural abnormalities with clinical history and surgical findings were used to diagnose and understand the etiology of hydrocephalus. Postinfectious hydrocephalus was defined as follows: 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). Nonpostinfectious hydrocephalus was defined as follows: findings of a noninfectious origin for hydrocephalus on computed tomography (CT) brain scans or at endoscopy (eg, 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 cerebrospinal fluid (CSF), and absence of findings consistent with postinfectious hydrocephalus or congenital origin of hydrocephalus. Figure created with Biorender.com.

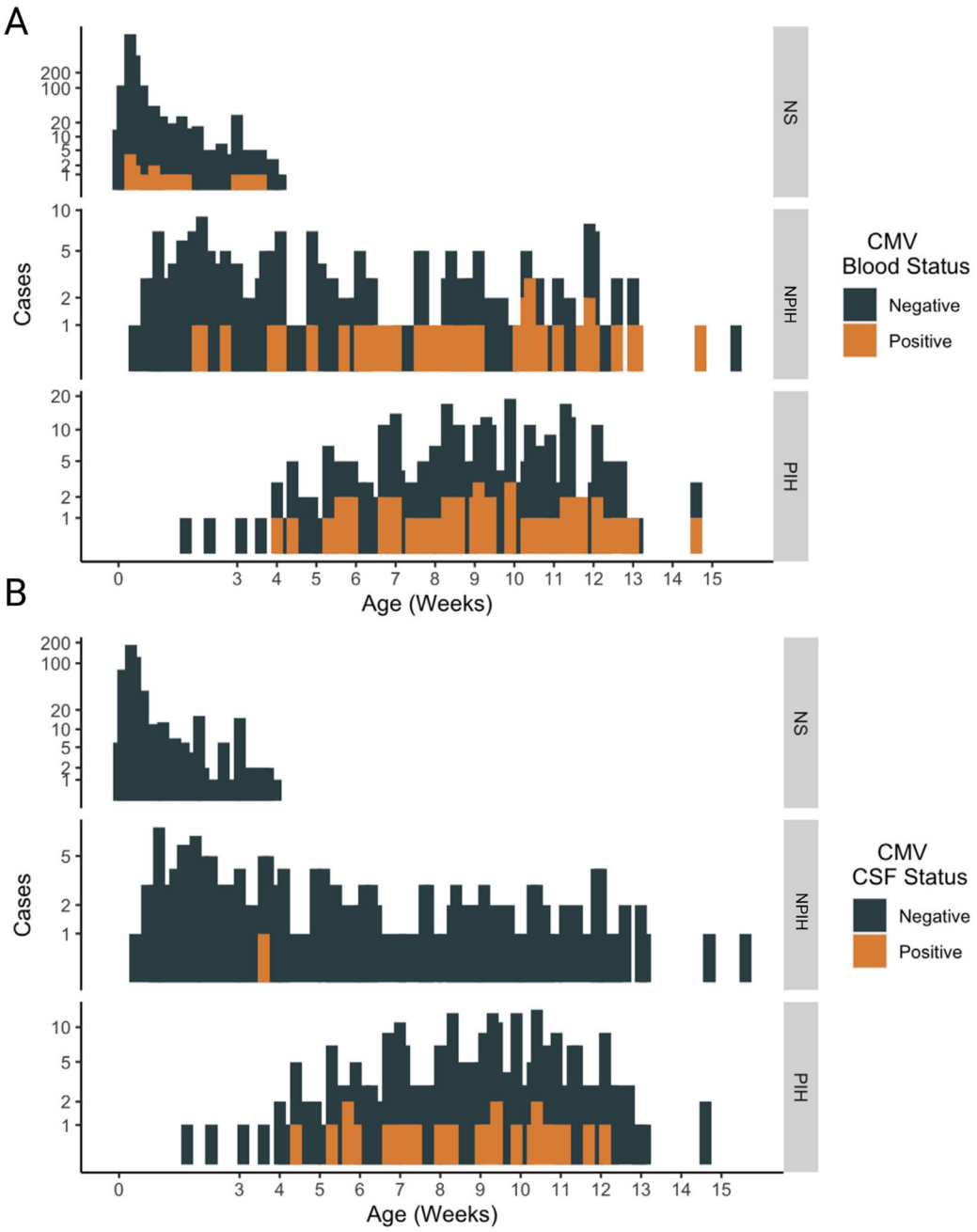


**Figure 2.**

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 CMV-negative cases from 0–15 weeks are plotted for participants recruited in the neonatal sepsis group (NS) or the hydrocephalus group (nonpostinfectious [NPIH] or postinfectious [PIH]) in blood, or (B) in cerebrospinal fluid (CSF).

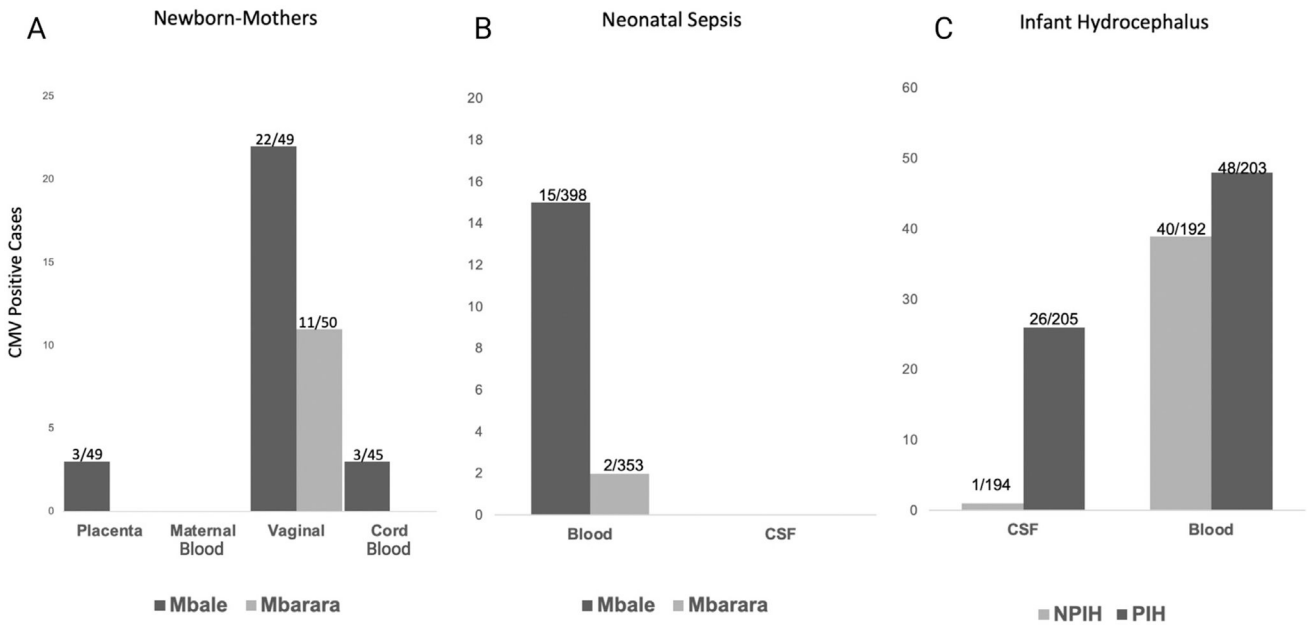


**Figure 3.**

The prevalence of CMV across regional hospitals for the neonatal sepsis (NS) and newborn cohorts and across hydrocephalus groups. In (A), all the positive cord (n = 3/92; 95% CI 1–9) and placental tissues (n = 3/99; 95% CI 1–9) were from participants from the eastern Ugandan Region (Mbale). There was also a higher prevalence of vaginal shedding in the mothers who gave birth in the eastern (Mbale) compared with the western Ugandan Region (Mbarara) (n = 22/49 vs 11/50, the Fisher exact test; p = 0.02). (B) CMV prevalence in the blood was 2% (n = 17/751; 95% CI 1–4) in the NS cohort overall and no CMV was detected in cerebrospinal fluid (CSF). There was a higher prevalence of CMV in the neonatal blood in eastern compared to western Ugandan region (n = 15/398 vs 2/353, the Fisher exact test; p = 0.003). (C) In the hydrocephalus cohort, the overall prevalence of CMV in blood was 24% (n = 95/399; 95% CI 20–28) of which 21% (n = 40/194; 95% CI 15–27) of the nonpostinfectious hydrocephalus (NPIH) and 24% (n = 48/205; 95% CI 18–30) of the postinfectious hydrocephalus (PIH) participants had cytomegalovirus (CMV) infections. CMV was detected in 7% (n = 27/399; 95% CI 5–10) of the CSF from participants with hydrocephalus of which almost all had PIH (n = 1/194 NPIH vs 26/205 PIH; p < 0.001).

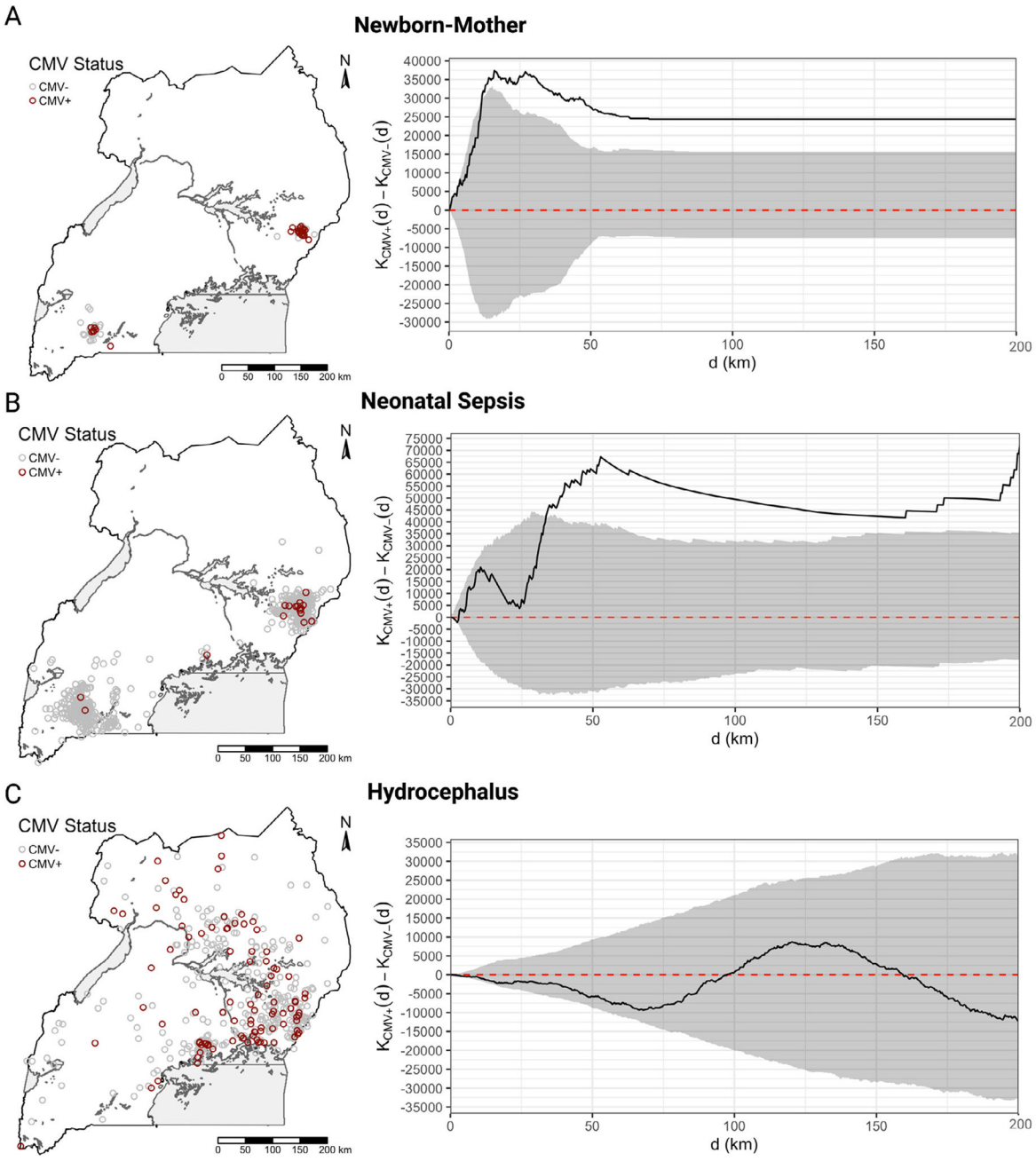


**Figure 4.**

Spatial distribution of cases. On the left of each panel is a map of Uganda showing the distribution of cytomegalovirus (CMV)-positive (red, CMV+) and CMV-negative (black, CMV–) cases and on the right a graph showing the difference between the Ripley K functions by randomly permuting the labeling of CMV status 1000 times to evaluate clustering within each group. Although we used the spatial location of the patient's village centroid in our statistical calculations, for privacy reasons (see Supplemental Methods), we randomly jittered these large plotted markers to geomask the identity of the participant's village latitude and longitude within at least 121 $km^2$. (A) For the newborn-mother group,

on the left, we plotted the geographical location of the village of the newborn-mother pair based on CMV status of the cord blood or vaginal swabs. The Ripley's K function plotted in the graph on the right shows that CMV+ cases tend to cluster above the degree of spatial aggregation from the CMV−cases by approximately 12.5 kms. (B) For the NS group, on the left we plotted the geographical location of the village of the neonates based on CMV status in blood. The Ripley's K function plotted in the graph on the right shows that CMV+ cases tend to cluster above the degree of spatial aggregation from the CMV cases by approximately 35 kms. (C) For the hydrocephalus group, on the left the geographical location of the village of the infant labeled with CMV status in blood or cerebrospinal fluid. The Ripley K function, plotted in the graph on the right, shows that in contrast to the other 2 groups, the spatial distribution of CMV+ cases lie within the 95% confidence limits showing no increased clustering compared to CMV− cases.

**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) |
| Nonpostinfectious | - | - | 194 (49) |
| **Mode of Delivery, n (%)** | | | |
| Vaginal | 60 (60) | 466 (62) | 279 (70) |
| Cesarean Section | 39 (40) | 283 (38) | 132 (30) |

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

**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) |

**Abbreviations:** CI, confidence interval; CMV, cytomegalovirus.

**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) |

**Abbreviations:** CMV, Cytomegalovirus; +, positive; −, negative.

**Table 4**

Key Demographic and Clinical Attributes in CMV Groups in the Study Population

| Variable | Newborn (n = 92) | | Neonatal Sepsis (n = 751) | | Hydrocephalus (n = 399) | |
|---|---|---|---|---|---|---|
| | 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) |
| Nonpostinfectious | | | | | 42 (44) | 152 (50) |

**Abbreviations:** HIV, human immunodeficiency virus; CMV, cytomegalovirus; SD, standard deviation; WAZ, weight-for-age *z*-score; Mbale Hospital, Eastern Region; Mbarara Hospital, Western Region.

*For the newborn group, maternal febrile illness is at delivery while for the neonatal sepsis group maternal febrile illness is throughout pregnancy.

**Table 5**

Risk Factors for 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, days | **1.08 [1.01, 1.14]** | **0.01** | **1.08 [1.00, 1.16]** | **0.05** |
| Sex | | | | |
| Female | 0.78 [0.27, 2.09] | 0.64 | 0.68 [0.21, 1.95] | 0.49 |
| Male | Reference | | Reference | |
| WAZ | **0.56 [0.37, 0.84]** | **0.005** | 0.67 [0.42, 1.02] | 0.07 |
| **Maternal Factors** | | | | |
| Mode of delivery | | | | |
| Cesarean section | 0.68 [0.21, 1.86] | 0.47 | 1.15 [0.31, 3.85] | 0.83 |
| Vaginal | Reference | | Reference | |
| Age | | | | |
| <25 years | 3.30 [1.16, 11.81] | 0.04 | **4.54 [1.40, 19.29]** | **0.02** |
| ³25 years | Reference | | Reference | |
| HIV status | | | | |
| Positive | **5.79 [1.28, 19.13]** | **0.008** | **25.20 [4.43, 134.26]** | **0.0001** |
| Negative | Reference | | Reference | |
| **Site of Study** | | | | |
| Eastern region | **6.87 [1.92, 43.81]** | **0.01** | **11.06 [2.30, 76.18]** | **0.003** |
| Western region | Reference | | Reference | |

**Abbreviations:** CI, confidence interval; CMV, cytomegalovirus; HIV, human immunodeficiency virus; OR, odds ratio; Eastern Region, Mbale; Western Region, Mbarara; WAZ, weight-for-age *z*-score.

*Adjusted for the effect of neonatal age, sex, WAZ, mode of delivery, maternal age, neonatal HIV exposure status and site of study.

**Table 6**

Risk Factors for CMV 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, days | **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 < −2 SD) | 1.88 [0.34, 35.72] | 0.56 | 1.69 [0.27, 32.59] | 0.64 |
| Normal WAZ $^{3}$ −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 | |

**Abbreviations:** CI, confidence interval; CMV, cytomegalovirus; HIV, human immunodeficiency virus; OR, odds ratio; WAZ: weight-for-age *z*-score.

*Adjusted for the effect of hydrocephalus status, malnutrition, mode of delivery, child's age and HIV exposure. Significant estimates are in bold.