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# Congenital cytomegalovirus in eastern Uganda: prevalence and outcomes

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## Abstract

**Background** Cytomegalovirus (CMV) infection poses risks to both maternal and neonatal health, however there are limited comprehensive data on congenital CMV in low-resource settings where the virus is widespread, particularly among women of reproductive age. Our research in eastern Uganda aimed to assess the prevalence of congenital CMV and outcomes among infants to inform public health policies and interventions in similar settings, addressing a significant gap in current knowledge.

**Methods** We conducted a descriptive study, nested within the BabyGel Trial, across Mbale and Budaka districts in eastern Uganda, between May 2023 and January 2024. Infants underwent saliva sampling within the first week of life, which was validated through urine polymerase chain reaction testing within the first 21 days of life. At three months of age, a cranial ultrasound examination, neurological examination, developmental evaluation, and audiological assessment were conducted for all infants diagnosed with congenital CMV infection. Statistical analyses were performed using Stata 17.0.

**Results** Congenital CMV infection was found in 5 out of 1,265 newborns tested, indicating a prevalence of 0.4% (95% CI: 0.16 to 0.96). Of these 5 infected infants, two experienced febrile illness at birth and required hospitalisation within the first week of life, and three had findings on ultrasound examination consistent with congenital cytomegalovirus during the neonatal period. Audiologic follow-up until three months of age revealed that three infants had failed unilateral and bilateral hearing screening. Neurodevelopment assessments using the Malawi Development Assessment Tool fell within optimal ranges for all 5 infants; however, when evaluated using the Hammersmith Infant Neurological Examination, four infants scored below optimal levels.

**Conclusion** Our community-based study revealed a low prevalence of congenital CMV infection. Further longitudinal multi-site research is needed to assess the generalisability of these findings. Also, long-term follow-up of children is crucial to understanding the outcomes and sequelae of infected infants to inform prevention strategies, targeted interventions and scalable screening frameworks in resource-limited settings.

**Keywords** Cytomegalovirus, Congenital, Prevalence, Neurodevelopment, Hearing, Low-resource setting, Uganda

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## Introduction

Cytomegalovirus (CMV) infection is prevalent worldwide, with varying seroprevalence among different demographic groups and geographic regions. CMV infection is common in women of reproductive age, with seroprevalence ranging from 45 to 100% [1]. A high seroprevalence increases viral transmission to infants, especially during pregnancy and postnatally through breastfeeding. Primary CMV infection, reactivation, or reinfection in pregnant mothers can result in viral transmission to the developing foetus [2]. Infected infants can exhibit a wide range of symptoms at birth, including petechiae, jaundice, hepatosplenomegaly, chorioretinitis, microcephaly, and multiple organ involvement [3]. Congenital CMV infection is a significant public health concern due to its impact on neonatal health and long-term outcomes, particularly due to its association with adverse effects such as hearing loss and neurodevelopmental disability [4].

Congenital CMV is a leading cause of hearing loss in children, affecting 12.6% of congenitally infected infants [5], and estimates suggest that up to 29.6% of children affected by congenital CMV develop neurodevelopmental disabilities [6]. In the Netherlands, two separate studies were conducted on the same cohort of children up to the age of six years: their first study showed that children with congenital CMV infection were twice as likely to experience moderate to severe long-term hearing and neurodevelopmental impairments compared to those without congenital CMV infection [7]; their second study revealed a 70% increase in medical care costs during the first six years of life for children with congenital CMV infection compared to controls, with symptomatic cases accounting for the majority of these costs, based on a comprehensive analysis of healthcare resource use, including the number of consultations with various healthcare providers, length of inpatient hospital stays, and specific diagnostic procedures and interventions during both hospital admissions and outpatient visits [8]. These findings highlight the burden congenital CMV places on healthcare systems and families in terms of medical care, interventions, and support services.

There is a critical research gap in understanding congenital CMV infection, especially in low-resource settings where comprehensive studies on congenital CMV are scarce. Previous studies on congenital CMV in sub-Saharan Africa (SSA) and other low- and middle-resource countries had small sample sizes (118–593 infants), utilised diverse testing methodologies; and did not include further evaluations of infants [9–12]. Nevertheless, these limited data indicate higher rates of associated morbidities in low-resource settings compared to high-resource settings, worsened by notable disparities in healthcare access and resources [13–15]. Our research aimed to investigate the prevalence of congenital CMV infection and its outcomes among a cohort of 1,265 infants in eastern Uganda, focusing on acute clinical, neurological, and hearing outcomes. These findings have the potential to inform public health policies and interventions, such as newborn CMV screening and preventative measures addressing the specific needs of Uganda and similar settings.

## Methods

### Study design

The study was a descriptive study, nested in a cluster-randomized controlled trial, conducted in eastern Uganda.

### Study setting

The study took place between May 2023 and January 2024 in Mbale and Budaka districts, which are in eastern Uganda. Mbale district is estimated to have a population of approximately 488,960 residents, while Budaka district has 207,597; most people in both districts live in rural areas. There are approximately 166,000 women of childbearing age in Mbale district and 64,000 in Budaka district; with recorded numbers of deliveries at 15,000 and 8400 respectively every year for the two districts [16, 17]. The study was conducted at three geographical sites surrounding Busiu, Budaka and Kolonyi health centres.

### Parent study

Our study was nested within the BabyGel trial, a cluster randomized controlled trial across 72 villages randomly assigned in a 1:1 ratio to either the intervention or control arms. Details regarding the trial (Pan African Clinical Trial Registry: PACTR202004705649428) are described in the BabyGel trial protocol [18]. A cluster was eligible for inclusion if its 1–4 village(s) collectively had over 600 inhabitants and were not directly adjacent to another cluster to avoid communication and intervention contamination.

### Sample size

Our sample size was restricted to 1265 participants by the time limitations of the parent study (BabyGel trial). However, this sample size provided an absolute precision with a 95% confidence interval (CI) of 0.8 to 2.8% for values ranging from 2 to 50%, which we deemed adequate.

## Study procedures

### Subject recruitment and follow-up

Potential participants were identified by village health team members (VHTs) or midwives in the community or antenatal clinics and subsequently enrolled in the BabyGel trial during pregnancy after providing informed consent. The responsible midwife followed up each mother throughout the antenatal and perinatal period. Following delivery, each potential participant was visited at home,

assessed for eligibility, and given a detailed explanation of the cytomegalovirus study, including its purpose, procedures, potential risks, and benefits. Consent was obtained by trained study staff during the infant's first week of life. Mothers were given the opportunity to ask questions, and written informed consent was obtained before any sample collection began.

Infants were eligible for inclusion if they were born to adult or legally emancipated mothers enrolled in the BabyGel trial, delivered after an estimated gestation of 34 weeks, and resided with their parents or legal guardians in the participating villages. CMV sampling and testing was conducted using saliva samples within the first week of life, followed by urine samples within the first 21 days of life for confirmatory testing of all enrolled neonates. Both sample types were analysed using polymerase chain reaction (PCR).

The study utilised cohort data from the BabyGel trial, alongside additional information on CMV exposure and clinical outcomes. Data collection involved growth measurements and anthropometric assessments, clinical evaluations recorded in case report forms, and questionnaire-based surveys (See Supplement 1). Additional data was captured including a cranial ultrasound examination 28 days after birth, neurodevelopmental assessments using Malawi Development Assessment Tool (MDAT) and Hammersmith Infant Neurological Examination (HINE), as well as hearing screening through Otoacoustic Emission (OAE) testing at 3 months of age. All assessments, including the cranial ultrasound, MDAT, HINE, and OAE, were performed by the principal investigator (NRAO), a medical doctor with training in developmental assessment, point-of-care bedside ultrasonography, and hearing screening. The study profile for recruitment, screening and data collection is described in Fig. 1.

## Congenital CMV diagnosis

### *Sample collection*

**Saliva** Saliva sampling has demonstrated a sensitivity of >97% and specificity of 99% for CMV detection [19]. To obtain saliva samples, sterile Dacron swabs (Cat. No 20270207) were used to gently collect saliva from inside the infants' cheek for approximately 20 s until moistened. The swabs were then placed in 1 ml of universal transport media (UTM) in sterile 2 ml cryovials and immediately sealed and immersed in a double wall vacuum ice-filled receptacle within 30 s of collection.

**Urine** Urine sampling has demonstrated a sensitivity of 100% and specificity of 99% for CMV detection [20]. Urine samples were collected using McKesson® Pediatric Sterile Urine collection bags (Cat. No 42142704) before being transferred into sterile containers. These samples were temporarily stored at temperatures below −4 °C before being transported to Mbale Clinical Research Institute molecular laboratory for analysis and long-term storage at -80 °C.

### *Molecular diagnostics*

**PCR testing** CMV DNA detection was performed using **PCR**, the gold standard for diagnosing CMV infection [21]. PCR was applied to DNA extracted from both saliva and urine samples (described below).

**DNA extraction** For the extraction process, CMV DNA extraction and quantitative real-time Polymerase Chain Reaction specimens were extracted by utilising Enzymatic DNA/RNA Extraction Buffer (CHAI Biotechnologies, Cat No R05221). Enzymatic DNA/RNA extraction buffer 10x was added to the sample at 1:10 v/v buffer (20 µL of buffer to 180 µL of sample) and vortexed for 15 s to mix. The mixture was first incubated at room temperature for 15 min, followed by another incubation at 98 °C for 5 min. The lysate was centrifuged at 7000 rpm for 30 s to pellet cellular debris and obtain DNA in the supernatant which was subsequently used for the PCR reaction.

**DNA amplification** DNA was amplified using the TaqMan PCR detection kit (NORGEN Biotek Corp, Cat No TM36310) as per kit protocol along with the open QPCR real-time platform (CHAI Biotechnologies). Every test run had both a positive and negative control.

To avoid any contamination while preparing the TaqMan PCR assay, the negative control was prepared first followed by the CMV assay and positive control. In addition, PCR reagents were added in the following order to prevent contamination; MDx TaqMan 2X PCR master mix, primer and probe mix, TERT Taqman assay, nuclease-free water and the sample DNA or positive control (See Supplement 1). Diagnosis was established by the identification of CMV DNA in both the infant's saliva and urine using quantitative PCR (qPCR) within the first 21 days after birth. Between October and December 2023, laboratory analysis of saliva was delayed due to shipment delays, thus, urine sampling was consequently delayed over a 3-month period ($N = 388$).

**Quality control** Known positive and negative controls were included in each run. Thirty samples were randomly selected in three-month intervals throughout the study period and sent to an accredited laboratory, Makerere University Biomedical Research Centre for comparison of results.

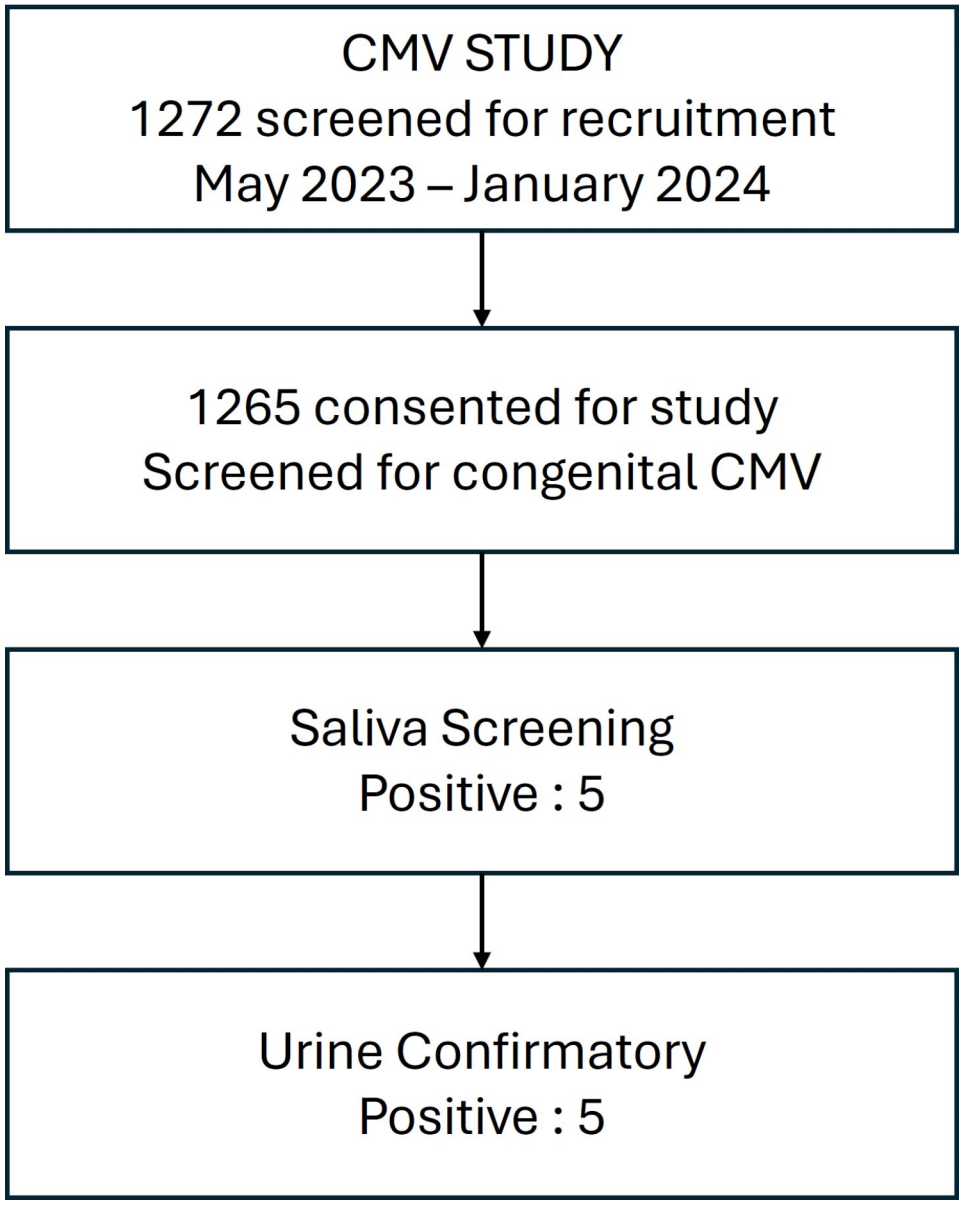


**Fig 1.** Flowchart of screening procedure for congenital CMV infection

***Clinical follow-up procedures***

**Neuroimaging** Cranial ultrasound examinations were performed using a Sonosite M-Turbo ultrasound machine with a curvi-linear probe (Cx11), and a set of standard coronal and sagittal images were recorded. Images were reviewed to identify abnormalities associated with congenital CMV infection such as intracranial calcification, ventricular dilatation, ventricular strands, lenticulostriate vasculopathy, and white matter abnormalities.

**Malawi development assessment tool (MDAT)** The MDAT is a comprehensive instrument, adapted for rural African settings, designed to assess child development across four domains: gross and fine motor coordination, language, and social skills [22]. It has been used in multiple research studies and clinical settings to evaluate neurodevelopment in children. In Uganda, it has been used in studies to evaluate neurodevelopmental recovery within wasting recovery trajectories in children hospitalised for an acute illness with malnutrition [23], assess neurodevelopmental outcomes of HIV-infected children receiving antiretroviral therapy in clinical settings [24], and follow up on infants born to mothers with obstructed labour [25]. Its validity and reliability have been tested and established through a multi-step process. It employs both binary assessment (pass/fail) and gross domain modelling to measure a child's abilities against established bench-

marks for their age group. In our study, MDAT was utilised to assess early developmental outcomes at 3 months of age. Specific passing ranges for gross motor, fine motor, language, social domains, and subsequent z-scores were provided based on typical expectations at that age.

**Hammersmith infant neurological examination (HINE)** The HINE is a comprehensive tool for assessing the neurological status and developmental milestones of infants [26, 27]. The assessment consists of three parts: examination of the nervous system, developmental milestones, and behaviour. Sections 2 and 3 of HINE assess developmental milestones and the child's behaviour during the examination. HINE scores are calculated by summing up all item scores from Sect. 1 using predefined optimal global scores for various age groups. The maximum overall score achievable is 78. Global scores are optimal if they achieve or exceed 73 at months 9 to 24, 70 at months 4–6, and 67 at months 2–3.

**Hearing assessment** OAE testing provides a non-invasive objective measure of normal cochlear function and is widely used in universal newborn hearing screening programmes [28]. Our study screened for hearing disorders among enrolled infants using an OAE device (Otoport Lite, Otodynamics Limited, Hatfield, United Kingdom, European Medical Device Directive; B-05060396170034). This device was able to automatically interpret DPOAE (Distortion product otoacoustic emission) screening test results to provide clear 'Pass' or 'Refer' indications to further assessment using Automated Auditory Brainstem Response (AABR) testing to confirm sensorineural hearing loss at Mulago National Referral Hospital (AABR testing was outside the scope of our study). The analytical functions of DPOAE are useful for characterising and monitoring peripheral auditory function during clinical investigations, making these tests applicable across all age groups to obtain objective evidence of peripheral auditory function while providing valuable information on cochlear function and potential hearing loss [29, 30]. The testing was conducted at the Ear, Nose and Throat Department at Mbale Regional Referral Hospital.

#### *Statistical analysis*

We conducted descriptive analyses that included frequencies and percentages for categorical data as well as median, inter-quartile ranges and ranges for continuous variables. Means and standard deviations (SD) and 95% confidence intervals (95% CI) were provided for normally distributed data. No test statistics were done due to the small frequency of the outcome of interest. Anthropometric indices were generated using the WHO z-scores for infants less than 24 months [31], and MDAT z-scores were generated using the MDAT scoring app [32].

#### *Ethical considerations*

The study protocol was approved by the coordinating ethics committee: Cure Children's Hospital of Uganda Research and Ethics Committee (CUREC-2022-41), Uganda National Council of Science & Technology (HS2668ES) and Regional Committee for Medical Research Ethics Western Norway (REK West 256906). The study was conducted in compliance with the Declaration of Helsinki and Good Clinical Practice guidelines to ensure ethical and governance standards were met.

## Results

### Demographic and socioeconomic characteristics of study population

Among the 1,265 study participants, 630 were male, 634 were female, and 1 had ambiguous genitalia. The median birthweight was 3.21 kg (interquartile range: 3.18 to 3.25 kg). The median household size was 3 members, with an interquartile range of 4 to 6. Most participants lived in rural areas (76.1%), while 23.9% resided in peri-urban settings. In terms of maternal education, 0.8% had no formal schooling, 63.5% completed primary school, 31.2% completed secondary school, and 4.5% attained higher education. Only 5.5% of mothers reported having a regular salary. Among husbands, 2.2% had no formal education, 48.3% completed primary school, 42.9% completed secondary school, and 6.6% attained higher education, with 17.9% reporting a regular salary (See supplement 2).

### Characteristics of the congenitally infected infants

#### *Maternal characteristics*

One of the five mothers was a primigravida, while the other four were multiparous. Three of the five mothers underwent routine antenatal ultrasound scans; one showed a malposition that later resolved, resulting in a vaginal delivery, and the remaining two scans detected no anomalies. Two mothers delivered via caesarean section, while three delivered vaginally. None of the mothers had a history of HIV infection or antenatal malaria. One mother reported a history of miscarriage, and two had previous preterm deliveries. All five mothers reported experiencing flu-like illness during pregnancy. Three mothers had frequent contact with young children, and two shared utensils during meals. Handwashing practices varied: two mothers consistently used soap and water, one reported occasional use, and two did not regularly use soap after close contact with children. None of the mothers reported having a regular household income. Antenatal care visits ranged from three to six per mother. Regarding residence, three mothers lived in peri-urban settings, and two in rural areas. Maternal education levels varied, with two mothers completing primary education and three completing secondary education. Household

water sources included boreholes and tapped water, and all households used pit latrines shared between few to multiple people (See Table 1).

### *Infant characteristics*

The congenital CMV infected infants were male and born at term, with birth weight ranging from 2.5 kg to 3.4 kg, with two out of five being small for gestational age (SGA), and two had head circumference Z-scores below − 2. None of the infants required resuscitation at birth. One infant was born with severe congenital malformation (spina bifida). Another infant presented with petechiae and required hospitalisation within the first week of life due to febrile illness. Three infants required hospitalisation during the first week of life. Febrile illness beyond the neonatal period was observed in three infants, including two who had febrile illness during the neonatal period. Two infants remained asymptomatic during both the neonatal period and follow-up (See Table 2).

### *Clinical assessment findings*

Neuroimaging findings varied among the infants (See Fig. 2; Table 3). These included mild microcalcifications for two infants, lenticulostriate vasculopathy in one infant, and normal results for two infants. Saliva and urine samples showed cycle threshold (CT) values ranging from 23.16 to 27.30 for saliva and 26.59 to 32.02 for urine. Hearing screening was normal in two out of five infants; two had unilateral failed hearing screening; and one had bilateral failed hearing screening. Developmental assessments using MDAT were within optimal ranges for all infants. However, neurological assessments using HINE, found four out of five infants scored below optimal levels (See Table 3).

## Discussion

We found the prevalence of congenital CMV among infants in eastern Uganda to be 0.4% (5/1265infants). Among these cases, the symptoms varied; some infants were asymptomatic, while others exhibited clinical manifestations such as SGA (2/5) and a severe congenital malformation (spina bifida, 1/5). Additional findings during the three-month follow-up included petechiae (1/5), febrile illness requiring hospitalisation (3/5), neuroimaging findings related to congenital CMV (3/5), and suboptimal HINE assessments (4/5). Hearing screening was abnormal in three infants (3/5), including two with unilateral and one with bilateral failed screening; however, the screening was non-specific and did not differentiate between conductive and sensorineural hearing loss.

The prevalence observed in our study is significantly lower than reported rates in other African countries. A

**Table 1** Household and maternal characteristics

| **Household and Maternal characteristics** | | | | | |
|---|---|---|---|---|---|
| Serial number | 1 | 2 | 3 | 4 | 5 |
| Age | 18 | 23 | 23 | 29 | 24 |
| Antenatal HIV status | Negative | Negative | Negative | Negative | Negative |
| Antenatal Ultrasound | No | Yes | No | Yes | Yes |
| Antenatal Ultrasound findings | Unremarkable | Malposition | Unremarkable | Unremarkable | Unremarkable |
| Antenatal maternal malaria | Negative | Negative | Negative | Negative | Negative |
| Parity | Primigravida | Multipara | Multipara | Multipara | Multipara |
| ANC Visits | 4 | 3 | 4 | 5 | 6 |
| Mode of delivery | Vaginal delivery | Vaginal delivery | Vaginal delivery | Caesarean section | Caesarean section |
| Place of delivery | Health Centre III | Health Centre IV | Private Clinic | Health Centre III | Health Centre IV |
| History of miscarriages | No | No | No | Yes | No |
| History of preterm deliveries | No | No | Yes | No | Yes |
| Flu-like illness in pregnancy | Yes | Yes | Yes | Yes | Yes |
| Rural and peri-urban status | Rural | Peri-Urban | Rural | Peri-Urban | Peri-Urban |
| Maternal education | Primary | Secondary | Primary | Secondary | Secondary |
| Maternal regular household income | No | No | No | No | No |
| Frequent contact with young children | No | Yes | Yes | Yes | No |
| Children under the age of five living in same household | 0 | 1 | 1 | 2 | 1 |
| Sharing utensils during meals | No | Yes | Yes | No | No |
| Water source in household | Borehole | Tapped water | Borehole | Tapped water | Borehole |
| Hand washing using soap and water | Yes | No | No | Yes | Yes |
| Type of toilet facility | Pit latrine | Pit latrine | Pit latrine | Pit latrine | Pit latrine |
| Number of people sharing toilet facility | 2 | 3 | 22 | 6 | 6 |

Frequent contact with children: *Mother answers yes or no regarding frequent contact with children.* Sharing utensils during meals: *Mother rates how often she shares utensils on a Likert scale from 'No, never' to 'Yes always.'* Hand washing using soap and water: *Mother rates how often she uses soap and water for hand washing on a Likert scale from 'No, never' to 'Yes, always'*

**Table 2** Infant characteristics

| **Infant characteristics** | | | | | |
|---|---|---|---|---|---|
| Sex | Male | Male | Male | Male | Male |
| Anthropometry | | | | | |
| Birth weight, kg | 3.4 | 3.1 | 2.5 | 3.4 | 2.5 |
| - Weight-for-age Z-score | 0.1 | -0.5 | -1.9 | 0.1 | -1.9 |
| - Percentile | 54.4 | 30.2 | 2.9 | 54.4 | 2.9 |
| Head circumference, cm | 34.5 | 35 | 31.8 | 34.8 | 33 |
| - Z-score | 0 | 0.4 | -2.1 | 0.3 | -1.9 |
| - Percentile | 51.2 | 66.3 | 1.8 | 60.6 | 2.6 |
| Required resuscitation at birth | No | No | No | No | No |
| Severe malformations at birth | No | No | Yes, spina bifida | No | No |
| Febrile illness in neonatal period | No | Yes | No | Yes | No |
| Other symptoms/ characteristics at birth | No | Petechiae | SGA | No | SGA |
| Hospitalised in 1st week of life | No | Yes | Yes | Yes | No |
| Clinical manifestations in first 3 months of life | - | Febrile illness | Febrile illness | Febrile illness | - |

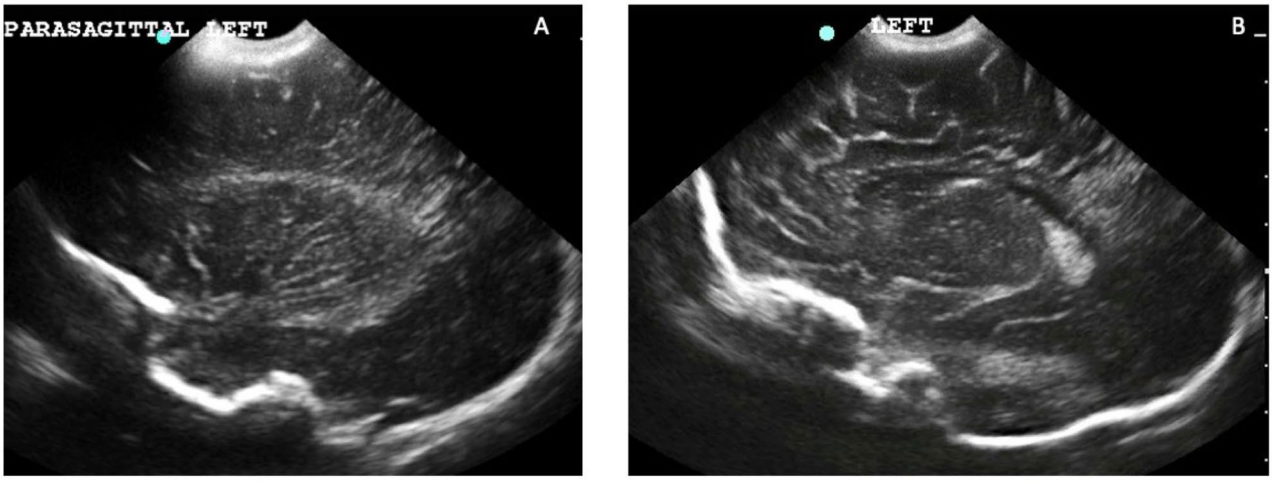


**Fig 2.** Neuroimaging findings: Cranial ultrasound images from patients with congenital cytomegalovirus at 28 days showing: A sagittal view showing the typical branching linear hyperechogenic lesions of LSV from participant 3 (**A**); a parasagittal plane over the left ventricle showing periventricular calcifications from participant 2 (**B**)

review of 21 studies conducted in 15 African countries across northern, western, eastern, and southern Africa reported a pooled prevalence of congenital CMV among newborns at 3.3% (278 out of 8401) with a range of 1.3–6.3% as a generalised estimate without stratification or description of population characteristics [33]. Our study's results are similar to the lower prevalence rates found in high-resource settings, such as Sweden (0.46%) the United Kingdom (0.32%) [34], Japan (0.4%) [35, 36], Italy (0.19%) [37], and Austria (0.1%) [38].

Although our study found a low prevalence of congenital CMV compared to other African countries, Uganda has comparable high general population seroprevalence of CMV, exceeding 90% [39–41]. This could suggest high sero-immunity among adults in eastern Uganda, however, to fully understand the acquisition mechanisms and temporal trends in congenital CMV prevalence larger longitudinal population-based multi-site studies or registries are needed. Variations in prevalence estimates across studies can be attributed to several factors, including differences in study settings, associated clinical co-factors, testing protocols and diagnostic methods, which often involve a range of biological samples such as cord blood, nasopharyngeal aspirates, cerebrospinal fluid, and saliva, tested using ELISA, PCR-based assays, or viral culture.

Our community-based study found an almost tenfold lower prevalence compared to a hospital-based study conducted in Uganda between 2016 and 2019, which reported a prevalence of 3% using cord blood and 2% using cerebrospinal fluid [42] demonstrating the influence of study setting, population characteristics and testing modalities on CMV prevalence. In recent years, public health interventions aimed at improving hygiene, nutrition, maternal and child health including enhanced access to antenatal care, facility-based deliveries, and

**Table 3** Clinical assessments

| **Clinical Assessment Findings** | | | | | |
|---|---|---|---|---|---|
| CT Values (Saliva) | 25.35 | 27.30 | 23.16 | 25.95 | 25.32 |
| CT Values (Urine) | 28.85 | 29.50 | 32.02 | 26. 59 | 27.96 |
| Cranial Ultrasonography | Micro-calcifications (Parenchymal) - Mild | Micro-calcifications (Periventricular and | Lenticulostriate vasculopathy Calcifications (Periventricular and Parenchymal) - Mild | Normal | Normal |
| Hearing screening | Unilateral failed hearing screening (Right) | Unilateral failed hearing screening (Right) | Normal hearing | Bilateral failed hearing screening | Normal hearing |
| MDAT – Zscore (DAZ) | -1.20 | -0.03 | -0.92 | -0.37 | 0.07 |
| Sub-scales | | | | | |
| - Gross motor | 9 | 6 | 6 | 6 | 4 |
| - Fine motor | 4 | 4 | 4 | 5 | 3 |
| - Language | 5 | 4 | 5 | 4 | 4 |
| - Social | 7 | 5 | 7 | 5 | 5 |
| HINE | 64 | 66 | 67 | 65 | 60 |

MDAT: Malawi Developmental Assessment Tool. HINE: Hammersmith Infant Neurological Examination. DAZ: Developmental Age Z-score. CT values corrected to 2 decimal places

HIV prevention and management, have become more widely adopted [43–46]. Notably, our study was conducted during and after the COVID-19 pandemic, within the context of a clinical trial intervention involving alcohol-based hand rub (ABHR). These combined factors may have synergistically contributed to the observed prevalence and played a role in reducing congenital CMV acquisition.

Another key observation in our study was the difference between MDAT and HINE outcomes. While MDAT classified all infants as achieving normal developmental milestones, HINE detected subtle neurological deficits. The MDAT is a broad-based assessment, evaluating multiple developmental domains, including motor, language, and social abilities, making it well-suited for capturing overall developmental progress. In contrast, HINE provides a focused assessment of motor-specific and detailed neurological function, identifying subtle differences that might not be detected through broader developmental measures. This difference in focus likely explains the observed variations in results. The MDAT, with its broad scope, may lack the sensitivity required to detect subtle neurological issues that the more targeted HINE assessment can identify. This complementary approach ensured that the strengths of each tool were leveraged, by using both tools together our study allowed for a thorough assessment of neurological and developmental outcomes, identifying potential risks early and informing the need for further monitoring and intervention in early childhood.

Universal screening for congenital CMV has been recommended as an effective method for detecting infected newborns [47–49]. However, while universal screening offers the advantage of identifying all cases at birth, it is often logistically and financially unfeasible in low-resource settings. In these settings, targeted screening methods, such as CMV testing following failed newborn hearing screenings, have been proposed as a more practical alternative. Some studies suggest that this approach could be cost-neutral or even cost-saving, allowing for more efficient resource allocation while identifying high-risk infants who require timely intervention [50, 51].

In our study, hearing screening identified only 3 of 5 congenital CMV-infected infants, illustrating its limitations as a standalone tool. Nonetheless, it remains a practical strategy in resource-limited settings due to its useful potential for integration into routine paediatric care and its accessibility as a primary method for identifying at-risk infants. Routine hearing screenings conducted during immunisation visits offer ongoing opportunities for monitoring, ensuring that cases missed at birth can be detected and referred for further evaluation. Findings from a multicentre study in Uganda among children aged 0 to 59 months revealed a high prevalence of failed hearing screening, which led to a recommendation to integrate childhood hearing screening into the services offered for all children attending immunisation clinics [52]. The study's short duration prevented the evaluation of long-term outcomes, however, previous research has established a link between ultrasound findings, hearing loss, and developmental outcomes in cases of congenital CMV [53, 54], underscoring the necessity of timely interventions during critical speech and language development.

The feasibility and cost-effectiveness of universal versus targeted hearing screening require careful evaluation, considering infrastructure, sustainability, equity, and accessibility. While these evaluations are ongoing, there

is an immediate need for practical and sustainable strategies, including preventative measures to reduce both primary and non-primary infections during pregnancy, which would help prevent vertical transmission of CMV to the foetus and reduce congenital CMV-related complications, such as hearing loss.

### Strengths and limitations

This study faced limitations stemming from its design as participants were sourced from a randomized controlled trial, potentially introducing biases related to the ABHR intervention. The ABHR intervention, aimed at reducing infections, may have influenced CMV transmission dynamics. Additionally, the timing of the study during and after the COVID-19 pandemic likely affected hygiene practices. This may have resulted in an underestimation of CMV prevalence, however analysing this is speculative due to the small number of infected infants. Secondly, the small number of congenital CMV-infected cases ($n = 5$) limited the ability to conduct meaningful comparative analyses with the larger congenital CMV-uninfected group ($n = 1{,}260$), which served as the control cohort for hearing and neurodevelopmental assessments, ultimately constraining the study to a descriptive focus and limiting the interpretation of findings. Given the rarity of the disease, a larger sample size was warranted for more accurate estimates and subgroup analyses. The short follow-up hindered investigating long-term neurodevelopmental trajectories and definitive audiological outcomes in infants with congenital CMV-related complications. Also, although the developmental and neurological testing tools used in this study are validated and widely applied, they have inherent limitations in fully capturing outcomes in very young children due to the rapidly evolving nature of early infant development. Given that each tool assesses different developmental domains, the MDAT provides a broad-based evaluation, while the HINE focuses specifically on neurological and motor function, variations in individual findings are plausible. Additionally, the scoring systems of the two tools differ, which may contribute to discrepancies in results. Therefore, while these tools are valuable, continuous and longer-term assessments are essential to accurately track developmental outcomes throughout infancy and early childhood. Finally, OAE screening, while effective for preliminary hearing loss detection, does not definitively confirm the cause of hearing loss and may reflect other acquired, genetic or congenital conditions. The absence of appropriate tools for paediatric otoscopy and AABR testing due to equipment limitation further restricted the ability to attribute hearing loss to CMV. Infants with failed OAEs were referred to higher-tier facilities for further investigation and follow-up.

The study's strengths include a community-based approach ensured that data was collected from a representative sample of the population in eastern Uganda, enhancing the generalisability and applicability of the study findings to the broader community. Additionally, rigorous methodologies were utilised for data collection and analysis, incorporating standardised diagnostic tests for identifying congenital CMV infection. The study benefited from rigorous laboratory procedures conducted at a molecular laboratory with standardised protocols, quality control measures, and advanced molecular techniques – including PCR considered as gold standard for diagnosing congenital CMV infection. Furthermore, the use of saliva and urine for sampling was based on evidence showing their high sensitivity and specificity, ensuring accurate CMV diagnosis. Finally, the comprehensive evaluation employed multidisciplinary approaches to assess infant health outcomes. Clinical evaluations, audiological screenings and neurological assessments were contributed to an overall understanding of congenital CMV's impact on various aspects of infant health and development. To ensure accuracy and minimize bias, hearing screening was conducted using a validated and calibrated OAE machine, with supervision from an ENT specialist who reviewed the findings. Neurodevelopmental assessments (MDAT and HINE) were performed by the principal investigator (NRAO), a practicing medical doctor with training in developmental assessments, under the supervision of a paediatrician and neonatal specialist who also reviewed the cranial ultrasound findings.

## Conclusion

Our community-based study revealed a low prevalence of congenital CMV infection. Further longitudinal multisite research is needed to assess the generalisability of these findings. Also, long-term follow-up of children is crucial to understanding the outcomes and sequelae of infected infants to inform prevention strategies, targeted interventions and scalable screening frameworks in resource-limited settings.

**Abbreviations**

| | |
|---|---|
| AABR | Automated auditory brainstem response |
| ABHR | Alcohol based hand rub |
| CMV | Cytomegalovirus |
| COVID 19 | Coronavirus disease 2019 |
| DNA | Deoxyribonucleic acid |
| DPOAE | Distortion product otoacoustic emissions |
| EDTCP | European & Developing Countries Clinical Trials Partnership |
| ELISA | Enzyme-linked immunosorbent assay |
| HINE | Hammersmith Infant Neurological Examination |
| HIV | Human immunodeficiency virus |
| MDAT | Malawi Development Assessment Tool |
| OAE | Otoacoustic Emission Testing |
| PCR | Polymerase chain reaction |
| RNA | Ribonucleic acid |
| SSA | Sub-Saharan Africa |
| SGA | Small for gestational age |

VHTs Village health team members
WHO World Health Organisation

## Supplementary information

The online version contains supplementary material available at https://doi.org/10.1186/s12887-025-05518-7.

Supplementary Material 1

Supplementary Material 2

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**Author contributions**
NRAO: Study design, processing of ethical approvals, data collection, project administration, data analysis, manuscript writing for publication, responding to comments from the journal. IMSE: primary mentorship and supervision, study design contribution and conceptualization, data analysis, drafting and revision of the manuscript, funding acquisition and resources. KB: mentorship and co-supervision, project administration, contributed to the study design and conceptualization, data analysis, revision of the manuscript. DM: Project administration, mentorship, co-supervision, and revision of manuscript. POO: Mentorship, co-supervision, resources, and revision of manuscript. TT: Mentorship, co-supervision, revision of manuscript, funding acquisition and resources. AW: Mentorship, revision of manuscript, funding acquisition and resources. RM, EM: Set up and management of the laboratory procedures, MC, FO: Initial manuscript draft contribution and data analysis. All authors read and approved the final manuscript.

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**Data availability**
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

## Declarations

**Ethics approval and consent to participate**
The study was approved by CURE Research and Ethics Committee (CUREC-2022-41) and registered with the Uganda National Council of Science and Technology (UNCST HS2668ES) and Regional Committee for Medical Research Ethics Western Norway (REK West 256906). Voluntary informed consent was obtained from the parents/caretakers of the infants before participating in the study after an explanation of the nature and purpose of the study, the potential benefits, and risks if any.

**Consent for publication**
Consent for publication of ultrasound scans from infants was obtained from both parents.

**Competing interests**
The authors declare no competing interests.

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