

# Birth Prevalence and Natural History of Congenital Cytomegalovirus Infection in a Highly Seroimmune Population

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**Background.** The natural history of congenital cytomegalovirus (CMV) infection is scarcely known in populations with high maternal CMV seroprevalence. This study evaluated the birth prevalence, clinical findings at birth, and hearing outcome in CMV-infected children from such a population.

**Methods.** Consecutively born infants were screened for the presence of CMV in urine and/or saliva specimens during the first 2 weeks after birth. Neonatal clinical findings were recorded, and CMV-infected children were tested to document hearing function during follow-up. A subset of mothers of CMV-infected infants were prenatally tested for the presence of anti-CMV immunoglobulin G antibodies.

**Results.** Congenital CMV infection was confirmed in 87 (1.08%; 95% confidence interval [CI], 0.86%–1.33%) of 8047 infants. Seven infants (8.1%; 95% CI, 3.3%–15.9%) had at least 1 clinical finding suggestive of CMV infection, and 4 (4.6%; 95% CI, 1.3%–11.3%) had >3 findings of systemic disease. Sensorineural hearing loss was found in 5 (8.6%; 95% CI, 2.9%–19.0%) of 58 children tested at a median age of 21 months. Bilateral profound hearing loss was observed in 2 children, and the hearing threshold was >60 decibels in all 5 children with hearing loss, including 2 children born to mothers with probable nonprimary CMV infection.

**Conclusions.** The results of this large newborn screening study in a population with high CMV seroimmunity provide additional evidence that congenital CMV disease occurs in populations with high seroprevalence rates, with a similar incidence of CMV-related hearing loss to that reported in the offspring of women from populations in developed countries with lower rates of seroimmunity to CMV.

Cytomegalovirus (CMV) is a frequent cause of congenital infection in humans in all regions of the world and an important cause of neurological disease and sensorineural deafness in children in the United States [1, 2] and in other developed countries [3, 4]. In contrast to most congenital viral infections, congenital CMV infection and disease can occur in children born to women with preconceptional immunity [3, 5–8]. The factors that are associated with intrauterine transmission of CMV and the occurrence of fetal, neonatal, or

infant disease have not been well defined [9]. However, the incidence of congenital infection depends on the epidemiological characteristics of the population—in particular, the maternal CMV seroprevalence. High rates of congenital CMV infection have been consistently demonstrated in populations with a high seroprevalence [10]. Therefore, it is not possible to simply extrapolate the knowledge acquired from populations of developed countries with a low-to-intermediate CMV seroprevalence to those of developing countries with high CMV seroprevalence. In addition, it has yet to be established whether congenital CMV infection and disease represent similar public health problems in developing countries as in North America and Europe.

To our knowledge, only studies that were performed without universal screening, enrolled a small sample of selected infants [11–16], or used nonvirologic diagnostic methods [17] have been reported from Latin

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America. These studies have reported a wide range of congenital CMV infection prevalence rates (0.10%–6.8%), and the frequency of symptomatic infection ranged from 0% to 66.6% of infants [11, 14, 16].

We conducted a prospective newborn screening study in a representative sample from a low-income population with a high maternal CMV seroprevalence in the southeast region of Brazil [14] to determine the prevalence of congenital CMV infection and clinical findings in infected infants. The frequency of CMV-related hearing loss was defined.

## PATIENTS AND METHODS

Mothers and their newborns attending 2 public hospitals in Ribeirão Preto city, State of São Paulo, Brazil, were studied. Both hospitals provide care for a low-income population with no private health insurance. The first maternity (Mater) provides care for low-risk parturients. The second hospital, Clinical Hospital of Faculty of Medicine of Ribeirão Preto, University of São Paulo, Brazil (HCFMRP), serves as a referral center for high-risk parturients but also provides care for low-risk parturients. A 95.7% seroprevalence of CMV infection has been detected among pregnant women from this population [14]. Approximately 9500 babies are born every year in this city, 4200 (44%) of whom are attended at these 2 maternity hospitals. The study was approved by the Research Ethics Committee of the University Hospital (Processes 9366/2003 and 9145/2004), and written informed consent was obtained from all participants.

Babies consecutively born and present in 1 of the 2 hospitals from 7:00 AM on Monday to 6:00 PM on Friday were selected for the study on the basis of the following criteria: mother's consent to participate; any maternal condition or type of delivery, gestational age, and clinical characteristics at birth; and the possibility of obtaining a saliva or urine sample during the first week of life. Infants born at HCFMRP were screened between March 2003 and July 2007. Screening was performed in infants born at Mater during the year of 2004. A total of 85.3% of the babies born during the study period were enrolled.

Gestational age (in completed weeks) was determined either by obstetrical estimation or by pediatric newborn examination [18]. Neonates were classified as small for gestational age (less than the fifth percentile) or adequate for gestational age (the fifth percentile or greater) according to a standard reference curve [19]. Head circumference was measured by the second day after birth, and circumferences were compared with standard values for term and preterm infants [20]. For the 7 infants with head circumference <2 standard deviations from the mean who were also small for gestational age, microcephaly was defined as the head circumference *z* score adjusted for weight deficit less than  $-2$  [21].

A saliva and/or urine sample was obtained from the new-

borns within 24 hours of life and processed by polymerase chain reaction for the detection of CMV DNA [22, 23]. We have previously demonstrated that urine and saliva samples were comparable for the diagnosis of congenital CMV infection [22]. The detection of CMV DNA in the initial sample was considered to be presumptive evidence of congenital infection. Subsequent urine and saliva samples were collected from these newborns during the first 3 weeks of life, were tested for the presence of viral DNA, and were inoculated into human fibroblast cells for virus isolation for diagnostic confirmation [24]. In all newborns with a positive screening polymerase chain reaction results, CMV DNA was found and virus was isolated in subsequently obtained specimens of both urine and saliva. All infants with confirmed infection underwent complete physical examination, ophthalmologic evaluation by fundoscopy (performed by a trained ophthalmologist), and cranial computerized tomography (CT). Hearing evaluation was conducted using auditory brainstem response (ABR) during follow-up [25]. Hearing evaluations were not performed at birth. Sensorineural hearing loss was defined as an air conduction threshold of >30 decibels (dBHL) on ABR [26].

Routine maternal screening at delivery included serological tests for human immunodeficiency type 1 virus and syphilis. However, if findings suggestive of congenital infection were observed, other congenital infections were excluded, such as those caused by *Toxoplasma gondii*, rubella virus, and in selected cases parvovirus B19, enterovirus, and herpes simplex virus. Blood specimens were collected for determination of the white blood cell count, platelet count, and bilirubin level and for performance of liver function tests at the discretion of the child's physician. For a subset of 44 CMV-infected infants (50.6%), an available stored prenatal maternal sample was tested for the presence of anti-CMV immunoglobulin G (IgG; Vidas CMV IgG ELISA; bioMérieux) and CMV-IgG avidity index (Vidas CMV IgG avidity; bioMérieux).

Once other congenital infections were excluded, infants were classified as symptomatic on the basis of the presence of at least 1 of the typical findings suggestive of congenital infection, including petechiae, cholestatic jaundice (conjugated bilirubin level, >2.0 mg/dL), hepatosplenomegaly, purpura, microcephaly, seizures, chorioretinitis, or abnormal CT findings (intracranial calcifications, ventriculomegaly, cerebral atrophy, and/or malformations).

To determine the rate of congenital CMV infection among all live-born infants, the sample size was estimated on the basis of a previous smaller study that reported a ~2% prevalence of congenital infection in this population [14]. The sample size necessary for a 99% confidence level, a precision of 0.5%, and under the assumption that  $\alpha$  equals 1% was estimated to be 5176 newborns. To determine the proportion of symptomatic cases among CMV-infected infants, the sample size was in-

creased by 50%. The binomial exact 95% confidence intervals (CIs) were calculated for birth prevalence rates and proportions. Categorical variables were compared by univariate analyses using the  $\chi^2$  test with  $\alpha = 0.05$ . Continuous variables were tested with the unpaired  $t$  test. EpiInfo software, version 6.04 (Centers for Disease Control and Prevention), was used in these calculations.

## RESULTS

**Characteristics of participants and birth prevalence of CMV infection.** A total of 8047 infants (1623 from Mater and 6424 from HCFMRP) born to 7848 mothers (192 twin pairs and 7 triplets) were enrolled in the study. Most pregnant women (98.2%) received prenatal care. Overall, 87 of the 8047 live born infants had a confirmed diagnosis of congenital CMV infection, an overall prevalence of 1.08% (95% CI, 0.86%–1.33%), which was similar between infants born at Mater (16 of 1623; prevalence, 0.98%; 95% CI, 0.56%–1.59%) and those born at HCFMRP (71 of 6424; prevalence, 1.10%; 95% CI, 0.86%–1.39%). In addition to younger maternal age and lower birth weight, a higher frequency of intrauterine growth restriction (odds ratio [OR], 3.00; 95% CI, 1.8–5.0) and twin pregnancies (OR, 2.66; 95% CI, 1.3–5.4) were found among neonates with congenital CMV infection, compared with CMV-uninfected infants (Table 1). Although the data on maternal tobacco use, arterial hypertension, and/or chronic diseases were not available for mothers of all CMV-uninfected infants, these data were available in a subset of 173 mothers of CMV-uninfected infants who were selected as control subjects by individual matching according to place and week of birth and gestational age with mothers of CMV-infected children as part of a separate study. A similar frequency of the presence of at least 1 of the mentioned maternal risk factors for intrauterine growth restriction between the CMV-infected and CMV-uninfected groups was found (55.2% and 48.0%, respectively),

**Findings in infants and children with congenital CMV infection.** None of the infants had evidence of other congenital or perinatal infections. As shown in Table 2, at least 1 clinical finding suggestive of congenital infection was found in 8.1% (95% CI, 3.3%–15.9%) of CMV-infected infants. The overall prevalence of symptomatic CMV infection was 0.09% (7 of 8047 infants; 95% CI, 0.03%–0.18%). Features among 3 infants with 1 typical finding were cholestatic jaundice (1 infant), petechiae (1 infant), and abnormal cranial CT findings (1 infant). The remaining 2 infants with abnormal CT findings also revealed >1 additional typical finding. The inclusion of intrauterine growth restriction as a defining criterion for symptomatic infection increased the proportion of symptomatic infants to 26.4% (23 of 87). Four infants (4.6%; 95% CI, 1.3%–11.3%) had  $\geq 3$  typical signs consistent with CMV multisystem disease.

At the time of this analysis, 63 (72.4%) of 87 CMV-infected children underwent at least one ABR test; the median age at the time of the hearing test was 21 months (range, 3–63 months). Among these children, 5 (7.9%) had a conductive loss due to middle ear effusion; it was not possible to determine the cochlear function. Sensorineural loss was found in 5 (8.6%; 95% CI, 2.9%–19.0%) of the remaining 58 children who underwent adequate evaluation. These 5 children were tested at a median age of 24 months (range, 15–50 months). Bilateral and profound hearing loss ( $>90$  dBHL) was detected in 2 infants, and the third child had severe ( $>60$  dBHL) bilateral loss. Among 2 children with unilateral loss, one had a threshold of 60 dBHL, and the other had a threshold of 90 dBHL. The remaining 53 children (91.4%) had normal hearing function. Two (40.0%) of 5 infants with hearing loss had  $>3$  neonatal clinical signs suggestive of congenital CMV infection, 1 was small for gestational age, and the other 2 had no other abnormal clinical findings.

**Type of maternal infection and infants' features.** Among 44 mothers of congenitally infected infants whose prenatal serum specimens were available and tested for anti-CMV IgG

**Table 1. Demographic Characteristics of Mothers and Infants, According to Infant Cytomegalovirus (CMV) Infection Status at Birth**

Characteristic	CMV-uninfected group (n = 7960)	CMV-infected group (n = 87)	P
Maternal age, mean years $\pm$ SD	25.6 $\pm$ 6.6	23.9 $\pm$ 7.1	.023 <sup>a</sup>
Male sex	4072 (51.1)	54 (62.1)	.12 <sup>b</sup>
Twin infants	370 (4.6)	10 (11.5)	.007 <sup>b</sup>
Birth weight, mean g $\pm$ SD	2993 $\pm$ 709	2671 $\pm$ 724	<.001 <sup>a</sup>
Gestational age, mean weeks $\pm$ SD	37.9 $\pm$ 2.7	37.7 $\pm$ 2.7	.42 <sup>a</sup>
Gestational age <37 weeks	1442 (18.1)	23 (26.4)	.13 <sup>b</sup>
Small for gestational age	852 (10.7)	23 (26.4)	<.001 <sup>b</sup>

**NOTE.** Data are no. (%) of subjects, unless otherwise indicated.

<sup>a</sup> Determined by 2-sided unpaired  $t$  test.

<sup>b</sup> Determined by  $\chi^2$  test.

**Table 2. Clinical and Laboratory Abnormalities at Birth and the Results of Hearing Evaluations for Children with Congenital Cytomegalovirus Infection Born to a Highly Seroimmune Brazilian Maternal Population**

Finding	No. of children with finding/ total no. examined (%)
Newborn clinical findings	
Cholestatic jaundice (direct bilirubin level, >2 mg/dL)	4/87 (4.6)
Petechiae	4/87 (4.6)
Hepatosplenomegaly	4/87 (4.6)
Purpura	2/87 (2.3)
Microcephaly	0/85 (0.0)
Seizures	1/87 (1.1)
Chorioretinitis	0/78 (0.0)
Abnormal cranial CT findings	3/79 (3.8)
No. of clinical findings	
At least 1 finding (ie, child was symptomatic)	7/87 (8.1)
1	3/87 (3.4)
≥3	4/87 (4.6)
Laboratory findings for newborns	
Thrombocytopenia (thrombocyte count, <100,000 cells/mm <sup>3</sup> )	4/41 (10.0)
Elevated CSF protein level (>120 mg/dL)	3/7 (42.8)
Elevated alanine aminotransferase level (>108 IU/mL <sup>a</sup> ) and/ or aspartate aminotransferase level (>130 IU/L <sup>a</sup> )	4/20 (20.0)
Sensorineural hearing loss	5/58 (8.6)
Bilateral	3/48 (6.2)
Bilateral profound (>90 dB)	2/48 (4.2)
Unilateral (>60 dB)	2/48 (4.2)

**NOTE.** CSF, cerebrospinal fluid; CT, computed tomography.

<sup>a</sup> More than 2 times the upper limit of normal.

antibodies (median gestational age, 13 weeks; range, 2–29 weeks), 2 (4.5%) were seronegative and had a primary infection during gestation. The remaining 42 mothers were CMV IgG seropositive (95.5%). Of the mothers with CMV-seropositive samples, 1 (2.2%) had a low avidity index (13% at 9 weeks of gestation), and 41 had a high IgG avidity index (>73%). Among these, 20 samples were collected at ≤12 weeks, 19 at 13–25 weeks, and 2 at >25 weeks of gestational age. Considering that full maturation of the IgG antibodies or high avidity index can occur 12–25 weeks after primary infection [27, 28], presumed nonprimary infection was responsible for 39 (88.6%) of 44 congenital infections. It was not possible to determine the type of infection in the mother with low avidity index early during gestation or in 2 with a high avidity index at >25 weeks' gestation.

One of the neonates born to mothers with a primary infection had a single finding (petechiae), and the remaining infant was asymptomatic at birth. Hearing evaluation was not performed in one of these children, and the other had conductive loss. Among 39 infants born to mothers with presumed nonprimary infection, 2 (5.1%; 95% CI, 0.6%–17.3%) had 1 neonatal finding (cholestatic jaundice in one, and abnormal head CT findings in the other). The infant with abnormal CT find-

ings demonstrated neurodevelopment delay during follow-up. Twenty-seven infants (69.2%) born to mothers with probable nonprimary infection underwent ABR evaluation. Two (8%) were asymptomatic at birth but had hearing loss (bilateral loss in one and unilateral loss in the other) detected during follow-up (at the ages of 15 and 50 months). None of the 3 neonates born to mothers with an undetermined type of CMV infection during gestation were symptomatic. However, bilateral hearing loss was found at 24 months in the infant whose mother had low avidity early during gestation. For the 4 infants with findings of CMV multisystem disease at birth, prenatal samples were not available for testing.

## DISCUSSION

Despite the recognized importance of congenital CMV infection in the world, only limited information is available about the incidence and natural history of this infection in Brazil [11, 12, 14, 15, 17]. In this population-based newborn screening study, we found a 1.1% (95% CI, 0.86–1.33) birth prevalence of congenital CMV infection, and 8.1% (95% CI, 3.3–15.9) of infected infants exhibited at least 1 clinical finding suggestive of congenital infection in the newborn period, whereas 4.6%

(95% CI, 1.3%–11.3%) had multisystem CMV disease. Our findings are in agreement with previous studies that have demonstrated a significant rate of congenital CMV infection in infants born to seroimmune women. Although samples were not available from all 87 women who delivered CMV-infected infants, analysis of prenatal serum specimens from 44 of these women revealed that 42 (95.5%) had IgG antibodies reactive with CMV in a commercial ELISA—a rate of CMV seroprevalence that is similar to that in our previous study of this population [14]. Furthermore, CMV seroreactivity may underestimate the actual prevalence of infection [29, 30]. Thus, it is conceivable that, in this population, the rate of CMV infection is >95.5% and that nonprimary maternal infection accounts for >95% of infants with congenital CMV infection.

The rate of congenital CMV infection observed by us in infants born to Brazilian women from a predominantly low socioeconomic level is similar to that seen in other highly immune populations in Africa (0.9%–1.4%) [31, 32], Chile (1.8%) [16], Korea (1.2%) [33], Mexico (0.9%) [13], China (1.8%) [34], and India (2.1%) [35] and among low-income North American women (1.2%) [5, 36]. However, it is higher than that observed in populations with low or intermediate seroprevalence from European [37, 38] or North American [39, 40] populations in which the prevalence of congenital CMV infection ranges from 0.18% to 0.48%. The findings of our study are consistent with those of previous studies and a recent review of the literature documenting that the incidence of congenital CMV infection increases with increasing maternal CMV seroprevalence [10].

It is of interest that CMV-infected infants were significantly smaller at birth and were almost 3 times more likely to exhibit intrauterine growth restriction than infants without congenital CMV infection. Berge et al [41] previously reported that congenital CMV infection resulted in significantly lower mean birth weights in low-income populations. More recently, it has been suggested that CMV infects the uterine wall and/or the adjacent placenta, impairing critical aspects of syncytiotrophoblast and cytotrophoblast differentiation [42, 43], thus altering their capacity to provide oxygen and nutrients to the developing fetus. Even though approximately one-half (55.2%) of CMV-infected infants in our cohort were born to mothers with other chronic conditions that could result in poor placental function, oxidative stress, and intrauterine growth restriction, the effect of CMV infection on placental function may have also contributed to growth restriction. This study was not designed to test this hypothesis; therefore, complete data on maternal risk factors for intrauterine growth restriction were not obtained for mothers of CMV-uninfected infants. However, information about maternal smoking and other chronic diseases was collected from a subset of mothers of CMV-uninfected children and was compared with information from mothers of CMV-infected

infants. This comparison showed that the 2 groups are similar with respect to the presence of at least 1 maternal risk factor, suggesting that intrauterine growth restriction in CMV-infected infants is, in part, due to placental CMV infection. Although it has been reported that, in twin pregnancies, infection of only one or both twins can occur [44] independently of placenta type [45], ours is (to our knowledge) the first report suggesting an increased frequency of congenital CMV infection in twin pregnancies.

The rates of symptomatic infection vary considerably among the published reports primarily because of the lack of uniform definition that is consistently applied by different investigators [10]. We opted to classify infants as symptomatic on the basis of the presence of clinical findings that have been consistently described in larger series of more severely affected infants [1, 46, 47]. However, other authors [39] included the finding of being small for gestational age (SGA) as a typical clinical feature of congenital CMV infection. In our study, we elected not to classify infants as symptomatic on the basis of the presence of intrauterine growth restriction alone. In addition, by adjusting head circumference for weight deficit, we chose not to classify 7 infants with SGA as having microcephaly based on standard growth curves. Given that one-fourth of the infected infants studied by us were SGA, as many as 26.4% of them would have been categorized as symptomatic if this criteria was included in the definition. Significant findings of our study were the demonstration that, among infants born to mothers with probable nonprimary CMV infection, hearing loss greater than moderate severity occurred in 2 children (8%), and abnormal CT findings with neurodevelopmental delay was found in 1 child. This is, to our knowledge, the first study to demonstrate the occurrence of CMV-related hearing loss and neurological impairment in CMV-infected children following nonprimary CMV infection in a population with very high (95%) seroimmunity to CMV. Our findings clearly document the importance of congenital CMV infection as a cause of morbidity and disability, even in populations with a high maternal CMV seroprevalence. Because sensorineural hearing function was not assessed in the newborn period, and because the CMV-infected children were not monitored with periodic audiologic evaluations, the frequency of hearing loss at birth and the exact age at which hearing loss occurred could not be determined.

In conclusion, a 1% prevalence of congenital CMV infection and disease was identified in this Brazilian population of predominantly low socioeconomic level. Given the frequency of CMV disease in the newborn period and the CMV-related hearing loss during follow-up, this infection is likely to have a significant impact on the health and quality of life of these children, as demonstrated in other populations from developed countries in North America and Europe. Our results provide additional evidence that congenital CMV disease occurs in pop-

ulations with high seroprevalence rates and confirms the findings from previous reports that symptomatic infection and sequelae, such as hearing loss, also occur after nonprimary maternal CMV infection [3, 5].

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