

Cohort study on maternal cytomegalovirus seroprevalence and prevalence and clinical manifestations of congenital infection in China

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Abstract

Congenital cytomegalovirus (CMV) infection is the leading viral cause of birth defects and developmental disabilities in developed countries. However, CMV seroprevalence and burden of congenital CMV infection are not well defined in China.

Cohort of newborns from 5 birthing hospitals in 2 counties of Shandong Province, China, were enrolled from March 2011 to August 2013. Dried blood spots (DBS) and saliva were collected within 4 days after birth for IgG testing for maternal seroprevalence and real-time PCR testing for congenital CMV infection, respectively.

Among 5020 newborns tested for CMV IgG, 4827 were seropositive, resulting in CMV maternal seroprevalence of 96.2% (95% confidence interval [CI]:95.6%–96.7%). Of the 10,933 newborns screened for congenital CMV infection, 75 had CMV detected, resulting in an overall prevalence of 0.7% (95% CI: 0.5%–0.9%), with prevalences of 0.4% (14/3995), 0.6% (66/10,857), and 0.7% (52/7761) for DBS, wet saliva, and dried saliva specimens screened, respectively. Prevalence of congenital CMV infection decreased with increasing maternal age (0.9%, 0.6%, and 0.3% among newborns delivered from mothers aged 16–25, 26–35, and >35 years, respectively; $P=0.03$), and was higher among preterm infants than full term infants (1.3% vs 0.6%, $P=0.04$), infants with intrauterine growth restriction (IUGR) than those without (1.8% vs 0.7%, $P=0.03$), and twins or triplets than singleton pregnancies (2.8% vs 0.7%, $P=0.04$). None of the 75 newborns exhibited symptomatic congenital CMV infection, and there was no difference in clinical characteristics and newborn hearing screening results between infants with and without congenital CMV infection at birth.

Congenital CMV infection prevalence was lower and the clinical manifestations were milder in this relatively developed region of China compared to populations from other countries with similarly high maternal seroprevalence. Follow-up on children with congenital CMV infection will clarify the burden of disabilities from congenital CMV infection in China.

Abbreviations: CI = confidence interval, CMV = cytomegalovirus, DBS = dried blood spots, IUGR = intrauterine growth restriction.

Keywords: congenital infection, cytomegalovirus, seroprevalence

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1. Introduction

Congenital cytomegalovirus (CMV) infection results from vertical transmission of CMV from mother to the fetus during pregnancy. Although any maternal infection during pregnancy, primary or nonprimary (reinfection and reactivation), can result in congenital CMV infection, the risk of vertical viral transmission is lower in nonprimary than primary maternal infections.^[1] Moreover, congenital CMV infections from nonprimary maternal infection are less likely to present with symptoms at birth, and are thought to be less likely to result in long-term permanent sequelae such as hearing loss and developmental disabilities.^[2]

CMV infection is well documented as the leading viral cause of birth defects and developmental disabilities in developed countries,^[3] which typically have moderate maternal seroprevalences of 40% to 70%.^[4–7] However, the epidemiology of congenital CMV infection in developing countries with very high maternal seroprevalence (>90%) is not as well understood.^[8,9] With high CMV seroprevalence, congenital CMV infection is less attributable to maternal primary infection than in countries with lower maternal seroprevalence.^[10,11] Moreover, the likelihood of symptomatic infection and permanent sequelae among infants with congenital infection in these populations is unknown. To investigate congenital CMV prevalence and its clinical manifestations in China, where maternal CMV seroprevalence is reported to be higher than 95%,^[12] we conducted universal screening for congenital CMV infection among infants born in 2 counties of Shandong Province, China.

2. Methods

2.1. Study population and data collection

Newborn screening for congenital CMV infection was conducted from March 2011 to August 2013 in 5 birthing hospitals of Pingyin and Wendeng Counties of Shandong province (Fig. 1) which comprised more than 80% of infants delivered in the 2 counties. Wendeng County was more populous (609,737 vs 331,712 in 2010 census) and had a higher GDP per capita (96,249 Yuan, approximately 15,778 US dollars) than Pingyin County (44,128 Yuan, approximately 7234 US dollars). GDP in both of these counties was higher than the national GDP per capita (6265 US dollars in 2012). The birthrate in Pingyin County was higher than in Wendeng County (9.5‰ vs 7.4‰).

All parents were approached in the hospital about enrolling their infants before delivery, with more than 90% of infants enrolled. Demographic information on the mothers was collected by interviews by research staff. Information on delivery and outcomes of routine clinical evaluations, including newborn hearing screening, was collected from the medical record. Newborn hearing screening was typically conducted at least 48 hours after birth and before discharge in the birthing hospitals using transiently evoked otoacoustic emissions (AccuScreen, Denmark). Infant who failed were retested within 6 weeks after birth and referred for diagnostic hearing testing within 3 months of age if they failed the rescreen.

Microcephaly was defined as head circumference exceeding 2 standard deviations below the mean according to international newborn standard values,^[13] assessed for a majority of infants but not infants during the 1st year of the study. Intrauterine growth restriction (IUGR) was defined as birth weight less than the 5th percentile of the gender-specific gestational age-corrected standard reference values for Chinese infants.^[14] Symptomatic congenital CMV infection was defined

as presence of microcephaly, petechiae, or seizure detected through routine newborn care before discharge, along with congenital CMV infection which was identified by real-time PCR. Congenital CMV infection without any of these 3 symptoms at birth was defined as asymptomatic congenital CMV infection.

2.2. Specimen collection and laboratory testing

Specimens were collected from the enrolled infants within 4 days of birth. Dried blood spots (DBS) were collected using 903 Whatman filter paper (GE Healthcare, UK) only from infants enrolled during the first 12 months of the 30-month study period. Saliva specimens were collected from all infants enrolled in the study using a sterile polyester swab that was placed in the infant's mouth against the cheek and rotated for 10 seconds. To prevent potential contamination from breast milk, saliva specimens were collected at least 1 hour after breast feeding. Saliva specimens were frozen immediately, stored at -20°C and transported on ice to the testing laboratory (wet saliva). In order to compare saliva collection methods, a 2nd saliva specimen was collected from infants enrolled during the last 12 months of the study and air dried at room temperature overnight, placed in small tube, transported at room temperature, and then stored at -20°C until processing (dried saliva).

CMV serostatus of mothers was determined by CMV IgG testing on infant DBS using the SeraQuest enzyme linked immunosorbent assay (Doral, FL) since infant IgG reflects maternal IgG.

Congenital CMV infection was identified by the detection of CMV DNA in the collected saliva or DBS specimens. All laboratory testing was done in the central laboratory of Shandong provincial CDC with local staff trained by US CDC laboratory staff. DNA was eluted from swabs with Extracta (Beverly, MA) and extracted from DBS using thermal shock.^[15] Real-time PCR was performed with TaqMan-based primers and probes targeting the viral glycoprotein B gene on Mx3000P qPCR Systems (Agilent Technologies, Santa Clara, CA).^[15] For quality control, all PCR raw data were reviewed by US CDC laboratory staff, and all CMV PCR positive specimens were retested by US CDC laboratory staff with at least 100 randomly selected PCR negative specimens during annual site visits. Positive results were defined as ≥ 5 copies of CMV DNA per PCR reaction for saliva or ≥ 1 copies of CMV DNA for DBS.

2.3. Statistical analyses

The 95% confidence intervals (CIs) for the estimates of CMV seroprevalence were calculated on the assumption of a binomial distribution with normal approximation, and Poisson distribution was assumed for the prevalence of congenital CMV infection. The association of categorical or continuous factors with CMV seroprevalence and congenital CMV infection was examined using Pearson or Fisher Chi-square test, or student *t* test, as appropriate, and by logistic regression for multivariable analysis. The real-time PCR results of the DBS and dried saliva specimens were compared with those of wet saliva specimens. Sensitivity, specificity, and predictive values for the PCR assays were calculated using standard methods for proportions and their 95% CIs were calculated with the efficient-score method.^[16] All analyses were carried out with SAS V9.3 (SAS Institute, Cary, NC), and statistical significance was defined as $P < 0.05$.

China CDC Ethics Committee on Human Subjects reviewed and approved the project.



Figure 1. Geographic location of the study sites (Wendeng and Pingyin Counties) of Shandong Province in China.

3. Results

3.1. Maternal CMV seroprevalence and prevalence of congenital CMV infection

A total of 5020 infants had DBS collected for CMV IgG testing, of which 4827 were positive for a maternal seroprevalence of 96.2% (95% CI: 95.6%–96.7%). No factors were found significantly associated with maternal CMV seroprevalence except maternal county of residence; however, the absolute difference was small and unlikely to be of practical significance (97.0% vs 95.2% for Wendeng and Pingyin Counties, respectively; $P=0.001$).

CMV DNA was detected in the saliva or blood of 75 infants out of 10,933 infants screened for an overall prevalence of congenital CMV infection of 0.7% (95% CI: 0.5%–0.9%), with prevalences of 0.4% (14/3995), 0.6% (66/10,857), and 0.7% (52/7761) among DBS, wet, and dried saliva specimens screened, respectively. Prevalence of congenital CMV infection decreased with increasing maternal age (0.9%, 0.6%, and 0.3% among newborns delivered from mothers aged 16–25, 26–35, and >35 years, respectively; $P=0.03$) (Table 1). Congenital CMV infection was not associated with county of birth ($P=0.05$), or being born to a mother who had a previous live birth ($P=0.36$), lived with a child aged ≤ 6 years of age ($P=0.60$), or had occupational contact with young children ($P=0.44$).

Table 1

Association of maternal factors with congenital CMV infection among 10,933 infants tested in two counties of Shandong Province, China, 2011 to 2013.

	Congenital CMV infection		P
	Positive, n (%)	Negative, n (%)	
Overall	75 (0.7)	10,858 (99.3)	
Maternal age in years			0.03
16–25	39 (0.9)	4415 (99.1)	
26–35	34 (0.6)	5804 (99.4)	
>35	2 (0.3)	639 (99.7)	
Study site			0.05
Pingyin County	42 (0.9)	4878 (99.1)	
Wendeng County	33 (0.5)	5980 (99.5)	
Born to mother who had a previous live birth			0.36
Yes	40 (0.6)	6357 (99.4)	
No	35 (0.8)	4501 (99.2)	
Born to mother living with children ≤6 years at home			0.60
Yes	4 (0.5)	744 (99.5)	
No	71 (0.7)	10,111 (99.3)	
Born to working mothers with occupational contact with young children			0.44
Yes	1 (0.3)	301 (99.7)	
No	61 (0.7)	8581 (99.3)	
Type of residence			0.42
Urban	28 (0.6)	4549 (99.4)	
Rural	47 (0.7)	6309 (99.3)	

CMV = cytomegalovirus.

Congenital CMV infection was twice as prevalent among preterm infants as full term infants (1.3% vs 0.6%, $P=0.04$). Infants with IUGR were more likely to have congenital CMV infection than those without (1.8% vs 0.7%, $P=0.03$). Singleton pregnancies were significantly less likely to have congenital CMV infection than those pregnancies of twins or triplets (0.7% vs 2.8%, $P=0.04$) (Table 2).

3.2. Clinical manifestations of congenital CMV infection

None of the 75 newborns with CMV infection were born with symptoms associated with congenital CMV infection. Although infants with congenital CMV infection had statistically significantly shorter body lengths than uninfected infants, the absolute difference was small (49.7 vs 50.3 cm) and unlikely to be of clinical significance. There was no difference in the prevalence of jaundice between infants with and without congenital CMV infection ($P=0.99$) or in the occurrence of seizures ($P=0.87$) during the newborn hospitalization. Two (2.7%) infants with congenital CMV infection failed newborn hearing screening; both failed in both ears. However, there was no difference in the proportions of infants with and without congenital CMV infection who failed newborn hearing screening ($P=0.17$) (Table 2).

3.3. PCR results by specimen type

A total of 7720 infants had both wet and dried saliva specimens tested, and 3953 had both wet saliva and DBS tested. Compared with wet saliva, the sensitivity of the dried saliva was 93.9% (95% CI: 82.1%–98.4%) and the specificity was 99.9% (95% CI: 99.8%–100.0%). Compared with wet saliva, the sensitivity of the

Table 2

Clinical and demographic factors by congenital CMV infection status among 10,933 infants tested in 2 counties of Shandong Province, China, 2011 to 2013.

	Congenital CMV infection		P
	Positive, n (%)	Negative, n (%)	
Preterm birth (≤37 weeks)			0.04
Yes	10 (1.3)	783 (98.7)	
No	65 (0.6)	10,074 (99.4)	
Sex			0.21
Male	33 (0.6)	5558 (99.4)	
Female	42 (0.8)	5300 (99.2)	
Intrauterine growth restriction			0.03
Yes	5 (1.8)	280 (98.2)	
No	70 (0.7)	10,577 (99.3)	
Type of delivery			0.34
Vaginal delivery	25 (0.5)	4671 (99.5)	
Assisted vaginal delivery with vacuum extraction or forceps	16 (1.2)	1337 (98.8)	
C-section	34 (0.7)	4849 (99.3)	
Any injury during laboring			0.70
Yes	0 (0.0)	21 (100.0)	
No	75 (0.7)	10,837 (99.3)	
Perinatal asphyxia			0.61
Yes	0 (0.0)	37 (100.0)	
No	75 (0.7)	10,821 (99.3)	
Singleton pregnancy			0.04
Yes	70 (0.7)	10,658 (99.3)	
No	3 (2.8)	106 (97.2)	
Muscular force after birth			0.11
Normal	74 (0.7)	10,824 (99.3)	
Weak	1 (2.9)	33 (97.1)	
Microcephaly			0.62
Yes	0 (0.0)	36 (100.0)	
No	37 (0.7)	5326 (99.3)	
Jaundice at birth			0.99
Yes	2 (0.7)	292 (99.3)	
No	73 (0.7)	10,566 (99.3)	
Petechiae at birth			NA
Yes	0 (0.0)	0 (0.0)	
No	10,858 (99.3)	75 (0.7)	
Seizures at birth			0.87
Yes	0 (0.0)	4 (100.0)	
No	37 (0.7)	5358 (99.3)	
Newborn hearing screening results			0.17
Normal	73 (0.7)	10,123 (99.3)	
Abnormal	2 (0.3)	713 (99.7)	
Body length at birth, cm: mean (SD)	49.7 (2.9)	50.3 (1.8)	0.003
Chest circumference, cm: mean (SD)	34.0 (1.7)	33.62 (1.9)	0.31

CMV = cytomegalovirus, SD = standard deviation.

DBS was 39.3% (95% CI: 22.1%–59.3%) and the specificity was 99.9% (95% CI: 99.8%–100.0%) (Table 3). The mean CMV viral load in DBS was 2.7×10^3 copies/mL (interquartile range: 1.7×10^3 – 3.9×10^3), significantly lower than the DBS viral loads from a population sample of 3972 US newborns using identical lab method (1.0×10^4 copies/mL, $P < 0.001$).^[17]

4. Discussion

Our findings of high maternal CMV seroprevalence in Shandong Province are consistent with results from studies conducted within the past 2 decades across China,^[12,18–20] and suggest that high CMV seroprevalence may be ubiquitous across China. The

Table 3

Comparison of dried blood spots and dried saliva specimens with wet saliva specimens in screening for congenital cytomegalovirus infection in 2 counties of Shandong Province, China, 2011 to 2013.

Wet saliva	Dried blood spots (N=3953)		Dried saliva (N=7720)	
	Positive	Negative	Positive	Negative
Positive	11	17	46	3
Negative	3	3922	6	7665
Sensitivity (95% CI)	39.3% (22.1%–59.3%)		93.9% (82.1%–98.4%)	
Specificity (95% CI)	99.9% (99.8%–100.0%)		99.9% (99.8%–100.0%)	
Positive predictive value (95% CI)	78.6% (48.8%–94.3%)		88.5% (75.9%–95.2%)	
Negative predictive value (95% CI)	99.6% (99.4%–99.8%)		100.0% (99.9%–100.0%)	

CI = confidence interval.

prevalence of congenital CMV infection was 0.7% in Shandong Province, China, and no newborns with symptomatic congenital CMV infection were identified. Our findings of higher CMV prevalence among infants with low birth weight, IUGR, preterm birth, or nonsingleton pregnancy are consistent with reports from populations of other countries.^[11,17,21–23]

Although the prevalence of congenital CMV infection is generally higher in populations with higher maternal seroprevalence,^[9] it varies substantially across populations with high seroprevalence (0.6%–6.1%).^[8] Differences in laboratory methods, and study enrollment criteria probably account for some of these reported variations.^[8] More importantly, differences in socioeconomic status and exposure to young children likely affect chances of reinfection and transmission within populations with high seroprevalence. The 0.7% congenital CMV infection prevalence that we report from China is significantly lower than that reported in 2 other large studies with good ascertainment methods conducted in populations with high seroprevalence in Brazil (1.1%, n=8047, $P=0.003$) and Turkey (1.9%, n=944, $P<0.001$).^[11,22] The population we examined in China had much less exposure to young children (<7% in current study) than other populations as a result of China's unique 1-child policy. This is consistent with the lower IgM seroprevalence previously reported in China (0.5%) compared to Brazil (2.3%) among females of reproductive age.^[12,24] Moreover, the prevalence estimate of congenital CMV infection in the current study was similar to the recent reported prevalence (0.6%) among 4447 Brazilian newborns,^[25] and lower than 1.1% previously reported^[11] from the same investigators in the same Brazilian population. These findings echo the recent systematic review of reported variations in congenital CMV infection across developing countries.^[8]

The prevalence of symptomatic congenital CMV infection might truly be lower in China than in other populations with high maternal seroprevalence. A study conducted in Beijing also failed to detect any newborns with symptomatic congenital CMV infection.^[26] Although the Beijing study relied solely on DBS to identify infected infants, which has lower sensitivity compared to saliva,^[27] DBS testing in that study would likely have identified infants at higher risk for symptoms and sequelae.^[28]

Our finding that dried saliva is a reliable type of specimen for identifying congenital CMV infection has important public health implications in that dried saliva is much easier and more economical to store and transport than wet saliva. In populations with high maternal seroprevalence in Brazil, saliva was found to be as sensitive and specific as urine for newborn screening for congenital CMV infection and to be more easily collected than urine specimens.^[29] DBS showed relatively low sensitivity of 39% compared to saliva for the detection of congenital CMV

infection in China. Consistent with this finding, the mean CMV viral load in DBS from Chinese infants was 2.7×10^3 , far lower than reported for DBS or blood from US infants: 1×10^4 copies/mL that used identical laboratory methods^[17] and 8×10^4 copies/mL reported for asymptomatic infants (both $P<0.001$).^[30] The relatively low CMV viral loads in CMV-infected Chinese newborns may explain the absence of symptomatic congenital CMV infection in our study. Recent study has suggested that screening with DBS may enrich for infants with the high risk of developing sequelae.^[31] From a public health standpoint, screening tests for CMV that do not identify all infected infants but do identify those at higher risk for sequelae may be advantageous since 80% to 85% of infants will never develop sequelae.^[31] However, it is not yet known whether DBS testing can provide adequate sensitivity for CMV screening.

Several limitations should be considered in interpreting our findings. The prevalence of congenital CMV infection was assessed with testing on multiple specimens which might increase the detecting probability and lead to overestimation. In addition, some unenrolled newborns had been transferred to other hospitals as the results of newborn diseases who were more likely to have congenital CMV infection, though the number was small and would not make much change on the estimate. In addition, the study sites were located in the relatively developed region of China and the findings might not be generalizable to the resource-limited regions where the prevalence of congenital CMV infection was reported very high,^[32] and further studies are needed to verify the reported geographic variation and examine the associated risk factors.

In summary, we carried out the first population-based newborn screening study for congenital CMV infection in China. In this relatively developed region of China, we found lower prevalence and milder manifestations of congenital CMV infection than seen in populations from other countries with high maternal CMV seroprevalence, suggesting that disabilities from congenital CMV infection could be relatively low in China. These findings provide additional evidence that the epidemiology of congenital CMV infection varies across populations with high maternal seroprevalence. These data also suggest that while high maternal prevalence seems to be fairly consistently associated with high prevalence of congenital CMV infection, more data on congenital CMV infection in different populations with high seroprevalence are needed for developing population-specific prevention strategies in the world.

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