

# Congenital Cytomegalovirus Infection in the Netherlands: Birth Prevalence and Risk Factors

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Congenital cytomegalovirus (CMV) infection is the most common congenital viral infection worldwide. The sequela encountered most frequently is hearing impairment, affecting approximately one out of five infants congenitally infected. Data on the birth prevalence and risk factors of congenital CMV infection in the Netherlands are scarce. The aim of this study was to determine the birth prevalence of congenital CMV in the Netherlands. A sample of 6,500 dried blood spots (DBS) from infants born in the Netherlands was tested anonymously for CMV DNA. The sample was stratified by the number of live births in different regions of the Netherlands of the year 2007. Additionally, on a regional level, risk factors for congenital CMV were analyzed. The birth prevalence of congenital CMV in the Netherlands was 0.54% (35/6,433, 95%CI 0.36–0.72). Congenital CMV infection was significantly higher in regions with more than 15% young children (0–5 years) compared with regions with a lower proportion of young children (OR 5.9, 95%CI 1.4–25.2). Congenital CMV infection was significantly higher in regions with more than 30% immigrants compared with regions with a lower proportion of immigrants (OR 2.2, 95%CI 1.1–4.6). This association was strongest for regions with more than 30% non-Western immigrants (OR 3.3, 95%CI 1.5–7.5). Based on the knowledge of the natural history of congenital CMV infection, approximately 1,000 children are born with congenital CMV infection in the Netherlands annually, of whom eventually approximately 180 children (0.1% of all newborns) will be affected by long term sequelae, with hearing loss being the symptom encountered most frequently. **J. Med. Virol. 83: 1777–1782, 2011.** © 2011 Wiley-Liss, Inc.

**KEY WORDS:** congenital CMV; birth prevalence; risk factors; dried blood spots

## INTRODUCTION

Cytomegalovirus (CMV) infection is the most common congenital viral infection worldwide. The symptom of congenital CMV infection encountered most frequently is sensorineural hearing loss, which will affect approximately one out of five congenitally infected newborns [Dollard et al., 2007; Kenneson and Cannon, 2007]. About 10% of the live-born infants with congenital CMV infection are symptomatic at birth [Dollard et al., 2007; Kenneson and Cannon, 2007], whereas an additional 10% of the infected newborns will develop permanent sequelae in the following years [Pass, 2005; Dollard et al., 2007; Kenneson and Cannon, 2007]. Among children with bilateral profound sensorineural hearing loss, the hearing disability is attributable to congenital CMV infection in one out of five patients [Barbi et al., 2003; Grosse et al., 2008; Korver et al., 2009]. This makes CMV the leading cause of non-genetic congenital hearing impairment.

The overall birth prevalence of congenital CMV was estimated at 0.64% [Knesson and Cannon, 2007], with significant variety among different countries and

Abbreviations: CMV, cytomegalovirus; DBS, dried blood spots.

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Accepted 21 June 2011

DOI 10.1002/jmv.22181

Published online in Wiley Online Library (wileyonlinelibrary.com).

populations. The birth prevalence has been shown to be correlated positively with maternal CMV seroprevalence in the population [Kenneson and Cannon, 2007]. Established risk factors for congenital CMV infection include preschool children in the household, household size more than three people, non-white race, low socioeconomic status, preconception maternal seronegative status, and maternal age below 25 years [Fowler et al., 1993; Fowler et al., 2003; Fowler and Pass, 2006; Kenneson and Cannon, 2007].

Data on the birth prevalence and risk factors for congenital CMV infection in the Netherlands are scarce. Only one study has been published on the birth prevalence of congenital CMV in the Netherlands, estimating a prevalence of congenital CMV of 0.09% [Gaytant et al., 2005]. This estimate is low when compared to the birth prevalence estimates from other northern European countries, ranging from 0.18 to 2.0% [Granstrom et al., 1977; Andersen et al., 1979; Peckham et al., 1983; Griffiths et al., 1991; Ahlfors et al., 1999; Halwachs-Baumann et al., 2000]. Furthermore, it is not in accordance with the maternal seroprevalence of CMV in the Netherlands of 41–73% [Gaytant et al., 2005; Stelma et al., 2009]. Maternal seroprevalence rates of CMV of 50–70% in other countries have been associated with birth prevalence rates of approximately 0.3–0.6% [Kenneson and Cannon, 2007].

The aim of this study was to determine the birth prevalence of congenital CMV in the Netherlands, in order to estimate the disease burden. A large, random sample of dried blood spots (DBS) was selected from all infants born in the Netherlands in 2007 and analyzed for the presence of CMV DNA. Additionally, the contribution of risk factors for congenital CMV infection was analyzed on a regional level by comparing the birth prevalence of congenital CMV with the demographic characteristics and socioeconomic status parameters of the regions.

## MATERIALS AND METHODS

### Study Design

Of all live newborns in the Netherlands in 2007 ( $n = 182,765$ ), 99.8% participated in the nationwide metabolic and endocrine screening program for which DBS are collected routinely within a few days after birth [Lanting et al., 2010] and stored at the National Institute for Public Health and the Environment (RIVM, Bilthoven, the Netherlands) (room temperature). Of those DBS, a total of 6,500 DBS were selected for this study, stratified for the number of live births per region and the month of birth. Fourteen different regions were identified (12 provinces + Amsterdam + Rotterdam). Testing of the DBS was performed anonymously, thus no information was available on the clinical outcome of these children. The study was approved by the Program Committee Neonatal Screening (RIVM, the Netherlands), and the

Medical Ethics Committee (CME) of the Leiden University Medical Center (LUMC, the Netherlands).

### CMV DNA Detection in DBS

**DNA extraction.** DNA was extracted from DBS using the protocol described by Barbi et al. [2000] (details obtained by personal communication), as evaluated previously [De Vries et al., 2009]. In short, one punch of 3.2 mm per tube (approximately 3  $\mu$ l dried blood), in triplicate, was incubated overnight in 35  $\mu$ l Minimum Essential Medium (Gibco/Life Technologies, Breda, The Netherlands), with a fixed aliquot of phocine herpes virus (PhHV, kindly provided by Bert Niesters, University Medical Center Groningen, the Netherlands) to monitor nucleic acid isolation and PCR inhibition. Incubation was followed by heating for 60 min at 55°C, and for 7 min at 100°C. After cooling, the sample was centrifuged and the supernatant was frozen. After thawing, the DNA extract was used for CMV DNA amplification.

**CMV DNA amplification.** CMV DNA amplification was performed by means of an internally controlled quantitative real-time PCR as described previously [Kalpoe et al., 2004], with minor modifications [De Vries et al., 2009]. Briefly, 10  $\mu$ l of DNA extract was added to 40  $\mu$ l PCR pre-mixture containing CMV and PhHV primers, CMV and PhHV TaqMan probes, MgCl<sub>2</sub>, and HotStar Master mix (QIAGEN, Hilden, Germany), followed by amplification of a 126-bp DNA fragment of the CMV immediate-early antigen region. Quantification was performed using a dilution series of titrated CMV (Advanced Biotechnologies Inc., Columbia MD, USA) as an external standard.

**Interpretation of triplicate PCR results.** Interpretation of triplicate PCR results was performed using the flow diagram as proposed by Barbi et al. [2006], in which every positive result was confirmed with at least one other positive result. In cases where in the initial test procedure a single positive result was found, a confirmatory PCR procedure including DNA extraction was performed.

**Demographic and socioeconomic status characteristics.** For analysis of the contribution of risk factors for congenital CMV infection, the postal code numbers (four-digit) of the DBS tested for CMV DNA were retrieved. Demographic and socioeconomic status parameters of the postal code areas of the DBS tested (for the year 2007) were retrieved from Statistics Netherlands (Centraal Bureau voor de Statistiek (CBS), www.CBS.nl/en). Characteristics of the postal code areas of the CMV-positive DBS were compared to the characteristics of the postal code areas of the CMV-negative DBS (analogue to a comparison of the birth prevalence of congenital CMV between regions with and without these characteristics). Demographic characteristics analyzed were (non-Western) immigrants, young children (0–5 years) in the population,

and household size. Socioeconomic status characteristics analyzed were income and educational level.

**Sample size and statistical analysis.** The sample size calculation was based on an estimated birth prevalence of 0.4%, a significance level of 5%, and 80% power. For risk factor analysis, sub-population numbers were expressed as proportions of the total populations in that area (e.g., the number of non-Western immigrants was divided by the total number inhabitants in that area), and categorized. Category cut-offs were based on the distribution of the characteristics in the community, while maintaining sufficient numbers in the contingency table in order to achieve reasonable power. Differences in categorical data were compared with the Chi-square test and the Fisher's exact test (for expected frequencies below 5), and odds ratios (ORs) with 95% confidence intervals (CIs) were calculated. Data were analyzed using SPSS version 17.0 (SPSS Inc., Chicago, IL) with *P*-values < 0.05 (two-sided) considered to be statistically significant.

## RESULTS

### Birth Prevalence of Congenital CMV in The Netherlands

Out of 6,500 selected DBS, 6,433 DBS were available for CMV detection. The 67 DBS were not available for testing since parents had not given permission for storage of the DBS at the time of metabolic and endocrine screening. Out of the 6,433 DBS tested, 35 DBS were positive for CMV DNA. This corresponds with a birth prevalence of congenital CMV in the Netherlands of 0.54% (95%CI 0.36–0.72%). The median CMV DNA load was 3.7 log<sub>10</sub> (5,012) copies/ml whole blood, with a median cycle threshold of 40 (range 37–44). Fifteen, 7, and 13 of the 35 confirmed CMV positive DBS were initially positive in respectively one, two, and three of the triplicates.

### Risk Factors for Congenital CMV

Postal code numbers were retrievable for 6,022 of the 6,433 DBS, resulting in 2,180 different postal code areas. On average, a postal code area contained 8,261 inhabitants (range 30–27,030). Demographic characteristics of the postal code areas of the 6,022 DBS were retrieved, and data on households/immigrants, income, and education were available for the 5,930, 5,424, and 4,589 DBS, respectively. Demographic characteristics and socioeconomic status parameters of the postal code areas and the prevalence of congenital CMV in those areas are shown in Table I. Two out of 32 CMV positive DBS were from regions with a population with more than 15% young children (0–5 years), whereas 66 out of 5,898 CMV negative DBS were from regions with a population with more than 15% young children. This is analogous to a birth prevalence of 2/68 (2.94%) in regions with more than

15% young children, whereas the birth prevalence was 5.9 times less prevalent in regions with a lower proportion of young children (30/5,862 (0.51%), OR 5.89, 95%CI 1.38–25.16). Furthermore, congenital CMV was significantly more prevalent in regions with more than 30% immigrants compared to regions with a lower proportion of immigrants (OR 2.20, 95%CI 1.06–4.57). This association was strongest in regions with more than 30% non-Western immigrants (OR 3.33, 95% CI 1.49–7.46), but remained significant when the category cut-off was lowered to more than 20% non-Western immigrants. When analyzed in a multivariate logistic regression model, the variables more than 15% young children in the population (OR 4.46, *P* = 0.048) and more than 30% non-Western immigrants (OR 3.08, *P* = 0.007) remained significantly associated with congenital CMV infection. No significant association was found between the prevalence of congenital CMV and a mean household size of more than 3.0 persons. Additionally, congenital CMV infection was not found to be significantly associated with regions with a high proportion of households with lower income, a low proportion of households with higher income, or with lower education (Table I).

## DISCUSSION

This study shows that the birth prevalence of congenital CMV in the Netherlands is approximately 0.54%. It is the first report on the birth prevalence of congenital CMV testing a large selection of DBS covering all regions of the Netherlands. Given the large sample size and the stratification by the number of births in the different regions, the birth prevalence determined is expected to be valid and representative for the Netherlands as a whole. The birth prevalence found in this study corresponds with the birth prevalence of congenital CMV reported in other northern-European countries (0.18–2.0%) [Granstrom et al., 1977; Andersen et al., 1979; Peckham et al., 1983; Griffiths et al., 1991; Ahlfors et al., 1999; Halwachs-Baumann et al., 2000], where significant differences are found among different (sub)populations. Furthermore, the birth prevalence calculated in this study is in line with the maternal seroprevalence of CMV in the Netherlands of 41–73% [Gaytant et al., 2005; Stelma et al., 2009], which has been shown to be correlated positively with the birth prevalence of congenital CMV in a population [Kenneson and Cannon, 2007]. Previously, Gaytant et al. [2005] described a birth prevalence of 0.09% in the Netherlands. The major drawback of that prospective study was that the newborns studied were born in the south-eastern part of the Netherlands with a probable under-representation of newborns from non-native parents. They found that the seroprevalence of CMV was significantly lower in this area than in the metropolitan area of Amsterdam and Rotterdam. Also, though several studies have shown reasonable sensitivities of 87–100% [Balcarek et al., 1993; Yamamoto

TABLE I. Demographic and Socioeconomic Status Characteristics of the Postal Code Areas, and the Birth Prevalence of Congenital CMV

Characteristic	Birth prevalence of congenital CMV when characteristic		OR (95% CI) <sup>a</sup>	P-value <sup>ab</sup>
	Present	Absent		
Young children >15% of population children <5 years	2.94% (2/68)	0.51% (30/5,862)	5.89 (1.38–25.16)	0.007
Immigrants				
>30% Total immigrants	0.96% (11/1,146)	0.44% (21/4,784)	2.20 (1.06–4.57)	0.03
>20% Non-Western immigrants	0.98% (10/1,016)	0.45% (22/4,914)	2.21 (1.04–4.68)	0.03
>30% Non-Western immigrants	1.47% (8/544)	0.45% (24/5,386)	3.34 (1.49–7.46)	0.002
Household size				
Mean household size >3.0 persons	0% (0/132)	0.55% (32/5,798)	0 (-)	0.39 <sup>c</sup>
Socioeconomic status				
Income				
>40% Households with income <16 700€	0.66% (5/758)	0.49% (23/4,666)	1.34 (0.51–3.54)	0.55
<20% Households with income ≥31 900€	1.80% (2/111)	0.49% (26/5,313)	3.73 (0.87–15.92)	0.06 <sup>c</sup>
Education				
>45% Households with lower education	0.96% (2/209)	0.43% (19/4,380)	2.22 (0.51–9.58)	0.27 <sup>c</sup>

CMV, cytomegalovirus; OR, odds ratio; CI, confidence interval.

<sup>a</sup>Univariate analysis.

<sup>b</sup>Chi-square test unless stated otherwise.

<sup>c</sup>Fisher's exact test.

et al., 2006] of saliva sampling, the diagnostic approach used by Gaytant et al., consisting of cord blood serology followed by throat swab PCR and subsequently urine culture, may not have been optimal technically. Thus, the birth prevalence number reported by Gaytant et al. [2005] is not likely to be representative of the birth prevalence of congenital CMV in the Netherlands.

It is likely that the actual birth prevalence of congenital CMV in the Netherlands is even higher than the number calculated in our study, due to possible suboptimal sensitivity of DBS testing. Analytical and clinical sensitivities of CMV DNA detection reported previously using DBS vary within a wide range from 34% up to 100% [Johansson et al., 1997; Fischler et al., 1999; Binda et al., 2004; Barbi et al., 2006, 2008; Scanga et al., 2006; Vauloup-Fellous et al., 2007; Soetens et al., 2008; De Vries et al., 2009, 2010; Boppana et al., 2010; Gohring et al., 2010; Kharrazi et al., 2010]. A small number of prospective studies have analyzed the sensitivity of CMV DNA detection in DBS, testing a large population of unselected newborns in comparison with the gold standard, i.e., urine CMV culture or PCR at 2–3 weeks after birth, and reported sensitivities of 34–83% [Johansson et al., 1997; Yamamoto et al., 2001; Soetens et al., 2008; Boppana et al., 2010; De Vries et al., 2010]. Using these sensitivities, the actual birth prevalence of congenital CMV in the Netherlands could be as high as 0.65–1.59%. We and others have shown that optimizing DNA extraction protocols, PCR techniques and testing algorithms, e.g., by means of performing independent triplicate testing, increases analytical sensitivity significantly [Soetens et al., 2008; De Vries et al., 2009; Gohring et al., 2010], and the DBS assay used in this study was optimized previously [De Vries et al., 2009]. Besides above mentioned technical

aspects, CMV load in blood has been described to be significantly lower than that in urine [Halwachs-Baumann et al., 2002], which may have affected the detection of CMV in DBS in our study. In addition to the possible suboptimal sensitivity, the CMV status of the 67 DBS without parental permission for storage was not known. These DBS originated from a rural region of the Netherlands referred to as the Bible Belt, containing a low proportion of immigrants, rendering it unlikely that a high number of congenital CMV cases were among these unavailable DBS. The specificity of CMV PCR assays for DBS has been reported to range between 99.3 and 100% [Barbi et al., 1996, 2000, 2006]. In the current study, the possibility of false positive test results was minimized by using an optimized test strategy including confirmatory testing of (initial single) positive test results, resulting in a specificity approaching 100%.

This study illustrates that congenital CMV infection is approximately six times more prevalent in those areas in the Netherlands with more than 15% young children in the population compared with areas with a lower proportion of young children. Additionally, we show that congenital CMV infection was more prevalent in areas with a higher proportion of immigrants, with the birth prevalence being three times higher in areas with more than 30% non-Western immigrants compared with areas with a lower proportion of non-Western immigrants. The findings correspond with results from studies assessing risk factors for congenital CMV infection in other countries [Fowler et al., 1993; Fowler et al., 2003; Fowler and Pass, 2006; Kenneson and Cannon, 2007]. The proportion of young children and immigrants in a population are demographic markers for environmental factors and behaviors that facilitate CMV transmission. Young children shed CMV in their body fluids and

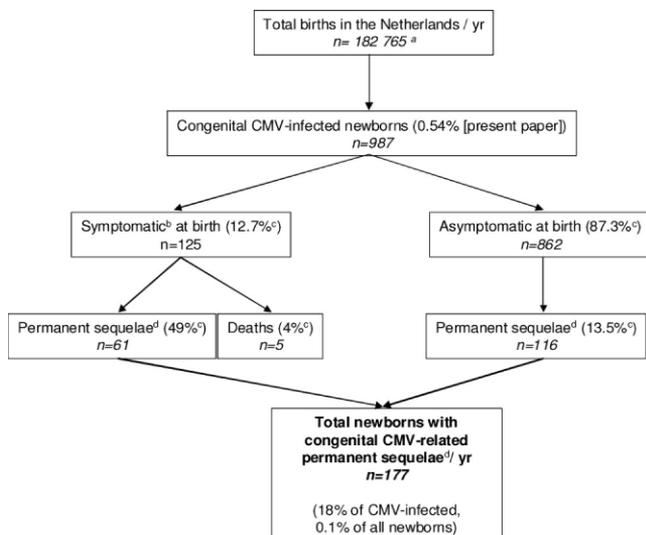


Fig. 1. Birth prevalence and implications for disease burden of congenital CMV in the Netherlands. <sup>a</sup>[Lanting et al., 2010]. <sup>b</sup>Symptomatic: Petechiae, jaundice, hepatosplenomegaly, thrombocytopenia, chorioretinitis, seizures, microcephaly, intracranial calcifications, or fetal hydrops [Dollard et al., 2007]. <sup>c</sup>[Dollard et al., 2007]. <sup>d</sup>Sequelae: Sensorineural hearing loss (uni- and bilateral), cognitive deficit (mental retardation, neurological impairment and developmental delay), and motor deficit [Dollard et al., 2007].

are, therefore, a common source for CMV. A CMV shedding child is a known risk factor for maternal CMV infection [Adler et al., 2004]. Among immigrants, maternal CMV seroprevalence has been shown to be higher than among native mothers in the Netherlands [Gaytant et al., 2005]. A higher maternal seroprevalence implies a more frequent exposure to CMV, which may be related to cultural differences in childcare practices (with frequent contact with children's saliva, urine, and maternal breast milk) and/or sexual activities. Previous studies suggest a positive correlation of congenital CMV with a household size more than three persons and low socioeconomic status [Fowler et al., 1993; Fowler et al., 2003; Fowler and Pass, 2006; Kenneson and Cannon, 2007]. However, in the present study no significant association between a big household size and congenital CMV infection was found. Due to the anonymized data of the DBS tested, no information was available on the clinical outcome of the children and risk factors could not be assessed at the individual level. Despite the latter limitation, risk factors were analyzed at regional level. Since the sample size was calculated to assess a reliable estimate of the birth prevalence of congenital CMV in the Netherlands, the risk factor analysis had limited power due to relatively low numbers. It is possible that parameters lacking a significant association in the study, might in fact be significantly associated when analyzed with a larger sample size. Finally, the limited availability of demographic and socioeconomic status variables made that not all risk factors important for congenital CMV infection could be studied.

Based on the knowledge of the natural history of congenital CMV infection [Dollard et al., 2007], a birth prevalence of congenital CMV of 0.54% implicates that approximately 1,000 children are born with congenital CMV infection in the Netherlands annually, of whom approximately 180 children (0.1% of all newborns) will develop long term sequelae (Fig. 1). These long term sequelae include hearing loss, cognitive and/or motor deficits and have significant impact on the lives of patients and their families, rendering congenital CMV infection an important public health problem. The number of children with sequelae due to congenital CMV infection is the same order of magnitude as the total number of newborns detected annually with the newborn hearing screening and metabolic screening programs in the Netherlands [Lanting et al., 2010]. CMV infection is, therefore, an important public health issue warranting further research to assess which preventive measures are most cost-effective [De Vries et al., 2011].

## ACKNOWLEDGMENTS

We thank Bert Elvers (National Institute for Public Health and the Environment, Laboratory for Infectious Diseases and Perinatal Screening, Bilthoven, the Netherlands) for facilitating the retrieval of the DBS, and Petra Oomen (National Institute for Public Health and the Environment, Bilthoven, the Netherlands) for providing the postal code numbers of the DBS. Additionally, they thank Eric van Zwet (Dept. of Medical Statistics and Bioinformatics, Leiden University Medical Center, the Netherlands) for statistical advice.

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