Consequences of congenital cytomegalovirus infection in early childhood

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Chapter 2

Cytomegalovirus infection in the Netherlands: seroprevalence, risk factors, and implications

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Abstract

Background
Cytomegalovirus (CMV) infections occur worldwide and are usually asymptomatic in healthy individuals. In fetuses and immunocompromised persons, they can cause severe disease and disabilities.

Objective
To determine the CMV seroprevalence and risk factors for CMV infection in the Netherlands.

Study design
In a cross-sectional population-based study (PIENTER-2, 2006-2007), sera and questionnaire data were collected from 6386 individuals. Sera were tested for CMV-specific IgG antibodies using enzyme-linked immunosorbent assay (ELISA).

Results
The CMV seroprevalence in the general population (6 months - 79 years) was 45.6%. Age and country of origin were the most prominent independent risk factors. The seroprevalence was significantly lower in native Dutch and Western individuals (41.5%) than in non-Western individuals (76.7%). Multivariable logistic regression analysis showed that age, lower educational level, first-generation migrancy, and among native Dutch/Western individuals, female gender and having contact with young children, were independently associated with CMV seropositivity. The geometric mean concentrations of antibodies increased with age and were higher in women than in men.

Conclusion
CMV seroprevalence in the Netherlands is relatively low compared to other countries. This is in line with our finding of a higher seroprevalence among migrants compared to the native population. The higher seroprevalence in women and individuals who have contact with young children is especially important for women of reproductive age. Preventing CMV infection in these women, through counseling on hygiene or possible future vaccination, may lead to a decrease of congenital CMV infections.

Background

Cytomegalovirus (CMV) is a common cause of infections worldwide. Like other herpes viruses, primary infection is followed by lifelong latency, with episodes of reactivation when the virus can be transmitted again. Transmission is possible via saliva, urine, blood, sexual contact, breastfeeding, and organ transplantation. In addition, CMV can be transmitted vertically through the placenta [1].

Both primary and recurrent CMV infections are usually asymptomatic in healthy individuals but can lead to serious complications in organ or stem-cell transplant recipients and other immunocompromised individuals [2].

The most important burden of disease comes from CMV infections during pregnancy that can lead to infection of the unborn child. Worldwide, between 0.2% and 1.0% of all newborns have a congenital CMV infection, which can cause severe and long-lasting disability including sensorineural hearing loss and cognitive or motor developmental delay [1].

A congenital CMV infection may be caused by a primary maternal infection or by a recurrent maternal infection due to reactivation of latent virus or reinfection with another CMV strain. Primary infection is more likely than recurrent infection to be transmitted to the fetus and to cause more severe disability. Still, recurrent infections account for almost 60% to 95% of congenital CMV infections, given population seroprevalences between 30% and 95% [3,4].

Since most CMV infections are asymptomatic in healthy individuals, serological testing is the only reliable way to estimate the prevalence of a prior infection in a population. Most seroprevalence studies have focused on selected subpopulations. A meta-analysis among women of reproductive age showed worldwide CMV seroprevalences ranging from 45% to 100%. The seroprevalences are lowest in Western Europe and the United States and highest in South America, Africa, and Asia [5].

Large nation-wide population-based seroprevalence studies are essential to reliably assess the CMV prevalence and risk factors for infection in order to direct future preventive measures and estimate their cost-effectiveness [6].

Objectives

The aim of this study was to determine CMV seroprevalence in the general population of the Netherlands using a national serum bank and furthermore to define risk factors for contracting CMV infections based on demographic and epidemiologic data.

Study design

Study population and design

This study used information from the PIENTER2 project, a cross-sectional population-based serum bank established in 2006-2007. Details of the study design have been previously published. [7] Briefly, 40 municipalities equally distributed over five geographic regions of the Netherlands were randomly selected proportional to their population size. An age-stratified sample was drawn from the population register, and the migrant population was oversampled in 12 of the 40 municipalities.

A total of 19,781 individuals, including 2558 non-Western migrants, were invited to complete a questionnaire and donate a blood sample. Serum samples were obtained from 6386 individuals (32%). Sufficient serum to test for CMV antibodies was available from 6382 participants. The study was approved by the local medical ethics committee (Almere, ISRCTN 20164309), and all participants, or their legal representatives, gave written informed consent.

Antibody assay

An ELISA, ETI-CYTOK-G PLUS (DiaSorin, Saluggia, Italy), was used to detect CMV-specific IgG antibodies. A concentration above 0.4 IU/ml was considered seropositive for a CMV infection, according to the manufacturer's instructions. Antibody concentrations were included in the analysis, and for that purpose, concentrations above the upper limit of detection (>10 IU/ml) were registered as 10 IU/ml (169 individuals).

Definition of variables

In accordance with the CBS (Dutch Statistics) definitions, a non-Western migrant is defined as a person originating from Turkey or a country in Africa, South America, or Asia (except Indonesia and Japan). A Western migrant is someone from Japan or a country in Europe (except Turkey), North America, Oceania, or Indonesia. First-generation migrants are foreign-born residents of the Netherlands who have at least one foreign-born parent. Second-generation migrants are born in the Netherlands but have at least one foreign-born parent. If both parents were foreign-born, a participant's origin was based on the mother's country of birth [8].

Educational level was classified as low (no education or only primary education), middle (junior technical school, lower general or intermediate vocational secondary education), or high (higher vocational or higher general secondary education, pre-university or university education). If the participant was a child (<15 years of age), the educational level of the mother was used.

Women of childbearing age are defined as women 20−45 years old. Household members are persons living in the same house as a participant. Contacts are non-household persons with whom a participant spoke the day before filling in the questionnaire.

Statistical analysis

Overall CMV seroprevalence and 95% confidence intervals (CI) were calculated for persons older than 6 months of age. Children younger than 6 months were excluded because their antibodies tend to be maternally derived.
The overall seroprevalence was weighted proportional to the Dutch reference population as recorded on 1 January 2007, taking gender, age, ethnic origin, and degree of urbanization into account.

Unweighted seroprevalences were calculated for three subgroups: native Dutch and other Western individuals, non-Western individuals, and women of childbearing age.
Logistic regression analysis was performed to study risk factors for CMV seropositivity in the general Dutch population. It was stratified by origin, since considerable differences were observed between Dutch/Western participants and non-Western participants. Variables included age, gender, educational level, generation of migrants (first or second), number of persons per household, a child under 4 years of age in the household (yes/no), number of contacts, and contact with children under 4 years of age (yes/no). Logistic regression analysis, adjusted for age and gender, was conducted for each risk factor separately, and multivariable analysis was performed for all risk factors together.
Both analyses were also performed for Dutch women of childbearing age and their Western migrant counterparts. The subgroup of non-Western childbearing women was too small to be included in the analysis (n = 101).

Geometric mean concentrations (GMCs) of CMV antibodies were calculated for CMV-seropositive individuals in the overall population and in the three subgroups. Multivariable linear regression analysis was limited to seropositive individuals, to study whether any variables were independent predictors of CMV antibody concentration levels. The regression coefficients of the linear regression were exponentially transformed to the GMC ratio (the ratio of GMC between two categories).

Data analyses were performed using SAS, version 9.1.3 (SAS Institute Inc., Cary, NC, USA).

Results

Study population

After exclusion of children under 6 months of age, the analysis includes 6249 persons, aged 6 months to 79 years, of whom 5205 (83.3%) were of Dutch/Western origin and 1044 (16.7%) of non-Western origin.
The Dutch/Western individuals differed from non-Western in mean age (37.6 years ± 23.6 SD vs. 24.3 years ± 24.0 SD) and educational level, with 40.6 vs. 21.9% defined as high, 50.8 vs. 40.7% as middle, 7.5% vs. 31.8% as low, and 1.1% vs. 5.6% missing.

CMV seroprevalence

Based on our study population, CMV seroprevalence in the general population of the Netherlands was 45.6%. It was lower in individuals of native Dutch origin (40.1%) than in Western migrants (57.3%) and non-Western migrants (76.7%). Among all migrants, seroprevalence was higher in the first generation than the second generation of residents in the Netherlands (Table 2.1).

Table 2.1 - CMV seroprevalence in the Dutch population, aged 6 months to 79 years, in relation to geographic origin

Geographic origin	Number	Seroprevalence	
		%	95% CI
Overall study population	6249	45.6	41.9 - 49.3
Native Dutch	4772	40.1	38.7 - 41.5
Western	433	57.3	52.6 - 61.9
1st generation migrant	152	69.7	62.4 - 77.0
2nd generation migrant	281	50.5	44.7 - 56.4
Non-Western	1044	76.7	74.2 - 79.3
1st generation migrant	664	87.2	84.7 - 89.7
2nd generation migrant	380	58.4	53.5 - 63.4
Non-Western (per country)			
Morocco / Turkey	338	84.0	80.1 - 87.9
Surinam / Aruba / Netherlands Antilles	351	69.5	64.7 - 74.3
Other non-Western countries	355	76.9	72.5 - 81.3

The seroprevalence in the general population increased steadily with age (Figure 2.1).

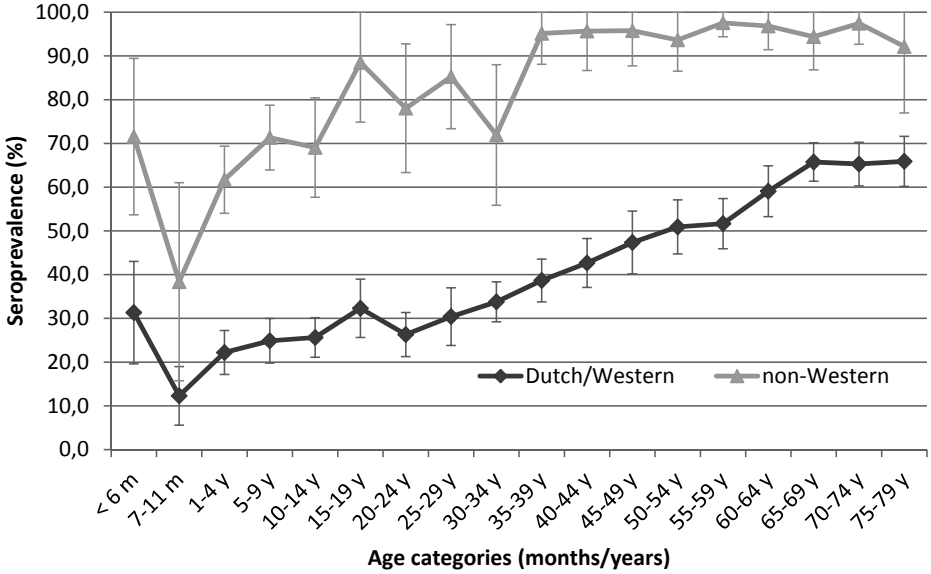

Figure 2.1 - Weighted CMV seroprevalence (with 95% CI) per age category for individuals of Dutch/Western origin (n = 5313) and non-Western origin (n = 1069), aged 0 - 79 years.

Women of reproductive age

In women of reproductive age (20-45 years), seroprevalence was much higher in non-Western individuals (85.1%; 95% CI: 78.1 - 92.2) than in Dutch/Western individuals (36.9%; 95% CI: 33.9 - 40.0).
In native Dutch women, seroprevalence increased with age from about 30% at 20-24 years of age to more than 40% at 40-44 years of age. An increase of 0.58% per year (95% CI: 0.16 - 1.01) was found based on linear regression analysis.

Risk factors for seropositivity

The odds of seropositivity, adjusted for gender and age, was 8.26 (95% CI: 6.97 - 9.80) times higher in non-Western individuals compared to Dutch/Western individuals.

- *Dutch and Western origin* (Table 2.2a)

In logistic regression analysis, adjusted for age and gender, and in multivariable analysis, the odds for CMV seropositivity was statistically significantly higher in the following groups: older age, women, persons with a low educational level, first-generation migrants, and persons who reported contact with young children (not living in the same household). Due to the high correlation between age and seropositivity, the crude seroprevalence in persons who have no contact with young children is higher compared to persons who have contact with young children, even though an inverse association is found in regression analysis. This discrepancy between crude seroprevalence and odds ratios, adjusted for age and gender, is also noticeable within subgroups of household size and persons living with or without a young child in the household.

- *Non-Western origin* (Table 2.2b)

For non-Western individuals an independent positive association for CMV seropositivity was found with older age and lower educational level. The odds of seropositivity was almost three times higher (2.97; 95% CI: 2.15-4.12) in first-generation migrants than in second-generation migrants.

- *Women of reproductive age*

The multivariable logistic regression analysis among Dutch/Western women (n = 950) revealed no statistically significant associations between any of the studied risk factors and CMV seropositivity, except for age.

Table 2.2a - Seroprevalence and risk factors for seropositivity among Dutch/Western persons

Characteristics	number	seroprev	logistic regression**		multivariable logistic regression	
		%	OR	95% CI	OR	95% CI
Overall population	5205	41.5				
Age						
6 m-9 y	830	21.8	1 (ref)		1 (ref)	
10-19 y	629	28.0	1.38*	1.08 - 1.75	1.28	0.96 - 1.69
20-29 y	643	28.9	1.41*	1.11 - 1.79	1.52*	1.16 - 1.99
30-39 y	646	36.8	2.04*	1.62 - 2.57	2.09*	1.64 - 2.66
40-49 y	603	45.3	2.91*	2.31 - 3.67	3.04*	2.33 - 3.95
50-59 y	609	52.7	3.90*	3.10 - 4.91	4.04*	3.06 - 5.32
60-69 y	703	62.2	5.82*	4.65 - 7.28	5.88*	4.40 - 7.85
70-79 y	542	64.6	6.61*	5.19 - 8.42	6.59*	4.83 - 8.97
Gender						
male	2348	38.7	1 (ref)		1 (ref)	
female	2857	43.9	1.26*	1.12 - 1.42	1.26*	1.12 - 1.42
Education	57 missing					
high	2113	36.2	1 (ref)		1 (ref)	
middle	2646	42.9	1.14*	1.01 - 1.29	1.17*	1.04 - 1.33
low	389	59.9	1.75*	1.38 - 2.22	1.85*	1.44 - 2.38
Group of migrants						
Native Dutch	4772	40.1	1 (ref)		1 (ref)	
1st generation Western	152	69.7	3.03*	2.10 - 4.35	3.22*	2.23 - 4.65
2nd generation Western	281	50.5	1.54*	1.20 - 1.99	1.57*	1.22 - 2.03
Number in household	37 missing					
1 or 2 persons	2183	52.1	1 (ref)		1 (ref)	
3 or 4 persons	2137	34.2	1.05	0.90 - 1.22	1.00	0.85 - 1.19
>4 persons	848	32.7	1.19	0.97 - 1.45	1.13	0.91 - 1.40
Child*** in household						
no	4287	44.5	1 (ref)		1 (ref)	
yes	918	27.8	1.04	0.87 - 1.24	0.97	0.78 - 1.20
Number of contacts						
< 8 persons	2252	44.4	1 (ref)		1 (ref)	
≥ 8 persons	2953	39.4	1.06	0.94 - 1.20	1.09	0.96 - 1.24
Contact with child***						
no	4050	42.4	1 (ref)		1 (ref)	
yes	1155	38.6	1.23*	1.06 - 1.42	1.25*	1.06 - 1.46

* statistically significant ($p < 0.05$); ** corrected for age and gender (age is corrected for gender and gender is corrected for age only); *** child younger than 4 years of age; seroprev: seroprevalence;

Table 2.2b - Seroprevalence and risk factors for seropositivity among non-Western persons

Characteristics	number	seroprev	logistic regression**		multivariable logistic regression	
		%	OR	95% CI	OR	95% CI
Overall population	1044	76.7				
Age						
6 m-9 y	516	65.9	1 (ref)		1 (ref)	
10-19 y	101	71.3	1.30	0.81 - 2.08	1.62	0.95 - 2.76
20-29 y	69	81.2	2.29*	1.22 - 4.32	3.66*	1.79 - 7.48
30-39 y	69	84.1	2.79*	1.43 - 5.47	2.85*	1.36 - 5.99
40-49 y	37	94.6	9.02*	2.15 - 38.0	8.18*	1.86 - 36.0
50-59 y	105	94.3	8.65*	3.72 - 20.1	6.19*	2.49 - 15.4
60-69 y	96	95.8	11.9*	4.29 - 32.9	8.62*	2.78 - 26.8
70-79 y	51	96.1	13.0*	3.11 - 54.0	11.4*	2.39 - 54.4
Gender						
male	489	77.5	1 (ref)		1 (ref)	
female	555	76.0	0.83	0.61 - 1.12	0.83	0.60 - 1.15
Education	58 missing					
high	229	62.9	1 (ref)		1 (ref)	
middle	425	76.0	2.33*	1.60 - 3.41	2.59*	1.73 - 3.87
low	332	85.8	4.07*	2.62 - 6.33	3.92*	2.45 - 6.27
Group of migrants (non-Western)						
1st generation migrant	664	87.2	1 (ref)		1 (ref)	
2nd generation migrant	380	58.4	0.34*	0.24 - 0.47	0.31*	0.22 - 0.45
Number in household	44 missing					
1 or 2 persons	243	87.2	1 (ref)		1 (ref)	
3 or 4 persons	434	72.4	1.40	0.83 - 2.36	1.22	0.68 - 2.16
>4 persons	323	74.3	1.76*	1.02 - 3.04	1.35	0.73 - 2.49
Child*** in household						
no	722	81.4	1 (ref)		1 (ref)	
yes	322	66.1	0.84	0.61 - 1.17	0.92	0.63 - 1.34
Number of contacts						
< 8 persons	667	79.0	1 (ref)		1 (ref)	
≥ 8 persons	377	72.7	0.75	0.55 - 1.03	0.93	0.65 - 1.33
Contact with child***						
no	874	79.3	1 (ref)		1 (ref)	
yes	170	63.5	0.61*	0.42 - 0.88	0.78	0.51 - 1.18

* statistically significant ($p < 0.05$); ** corrected for age and gender (age is corrected for gender and gender is corrected for age only); *** child younger than 4 years of age; seroprev: seroprevalence

Geometric Mean Concentration

The geometric mean concentration (GMC) among seropositive individuals increased with age by 0.85% per year (95% CI: 0.75-0.96). The GMCs were lower in males than females from the age of 15 to 19 years onwards (Figure 2.2). The GMC ratio, in multivariable analysis, for women compared to men was 1.24 (95% CI: 1.17 - 1.32) among Dutch/Western individuals and 1.13 (95% CI: 1.03 - 1.24) among non-Western individuals.

Across both genders, GMCs were higher among non-Western individuals compared to Dutch/Western individuals, with a GMC ratio of 1.19 (95% CI: 1.11 - 1.27).

Among Dutch /Western individuals, the GMC ratio was 1.17 (95% CI: 1.04 - 1.33) for persons who lived in a households with young children compared to those who did not.

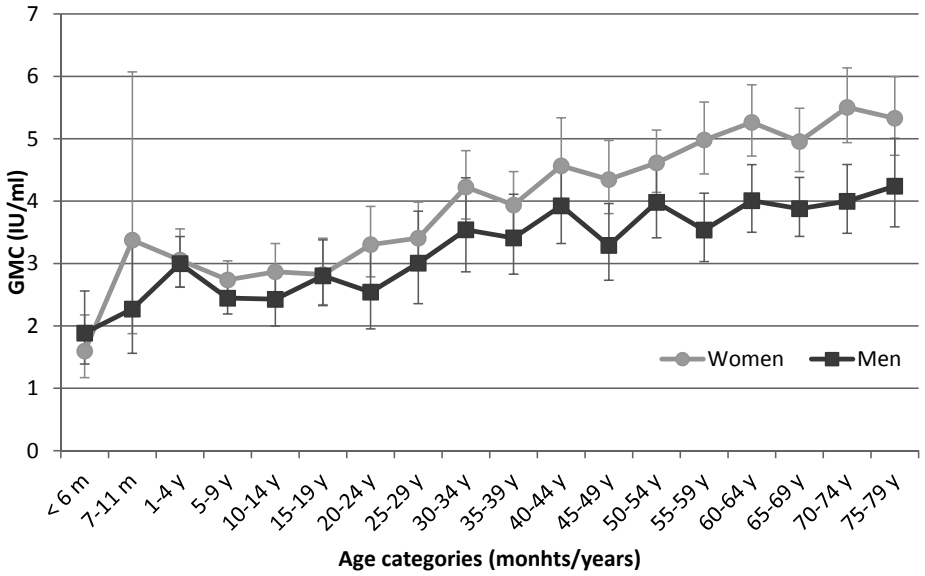

Figure 2.2 - Geometric mean concentration (with 95% CI) per age category by gender among CMV-seropositive individuals in the general population of the Netherlands (n = 2963).

Discussion

This large population-based study showed that the CMV seroprevalence in the general population of the Netherlands (2006-2007) is 45%. This is somewhat lower than that found in the USA (58.9% in 1988-1994) [9], Australia (57% in 2002) [10], and Portugal (77% in 2002-2003) [11]. These differences may be partly explained by differing study populations (e.g. different age distribution) and possible changes in seroprevalence over time.

Our large sample size and the population-wide weighted sampling ensure sufficient statistical power to enable reliable risk factor analysis. Given the relatively low response rate of 32%, the study population might not be fully representative, but data were collected from many subgroups of the general population, unlike most seroprevalence studies.

Age and country of origin were the most prominent independent risk factors for CMV seropositivity in this study. Additionally, low educational level and, among Dutch/Western individuals, female gender and contact with young children were independently associated with a higher CMV seroprevalence.

The association between non-Western country of origin and seropositivity probably reflects the higher infection rate in those countries, which also explains the higher seroprevalences among first-generation compared to second-generation migrants. Differences might be related to different lifestyles. For example, sharing the same plate or cup can facilitate CMV transmission among family members and close relatives. Also, a higher frequency of breastfeeding among Mediterranean first-generation mothers [12] and longer breastfeeding among Turkish women may contribute to increased seroprevalence in their young children [13].

Other factors aside, the increase of seroprevalence with age is well known. This may result from cumulative exposure to CMV throughout life, but seroprevalence measured in older people might also reflect CMV infections that occurred in childhood. Studies in Spain and Japan found decreased seroprevalence with age [14, 15], perhaps related to changes in wealth, socio-economic status, and hygiene. Other studies showed no changes in CMV seroprevalence over time [16, 17].

The geometric mean concentration (GMC) of CMV antibodies in seropositive individuals increases steadily with age. This phenomenon may reflect viral reactivation or reinfection with another CMV strain, boosting the humoral immunity. Yet again, the titer difference among the age categories could be caused by a higher pressure of infection during the childhood of older individuals. The cross-sectional design of our study precludes exploring these trends over time. Longitudinal data would be essential to investigate the increase in seroprevalence and antibody titers with age and to distinguish between continuous exposure over time and prior infections.

Young children constitute a well-known source of CMV because they often excrete relatively large amounts of virus in their saliva and urine for a long time [18-22]. We found that household composition (number and age of household members) had little influence on CMV seroprevalence. Yet among Dutch/Western individuals, CMV seroprevalence was associated with exposure to young children outside the household. Higher GMCs were seen in persons living with children younger than 4 years of age. Moreover, in Dutch/Western individuals, both seroprevalence and GMC were higher in females than males. This might be related to traditional role patterns, with women more involved in childcare and therefore more exposed to CMV infection and re-infection. Exposure could take place both at home and at work, as demonstrated in other studies [23, 24]. The GMC difference between genders could also result from hormonal differences in general or specific to the menstrual cycle or pregnancies, as has been suggested for other herpes viruses [25, 26].

Finally, the association between seroprevalence and educational level are in line with the 10-30% higher seroprevalences in persons with a lower socioeconomic status compared to persons with a higher socioeconomic status, as reported in a meta-analysis by Cannon et al. [5]. They suggest that this finding is related to cultural differences and differences in household crowding, child rearing, child day-care attendance, and sexual activity [5].

The identified risk factors for CMV infection, including female gender and having contact with young children are especially important for women of reproductive age. Prevention of CMV infection could be partially achieved by avoiding exposure to children's urine and saliva. For example by handwashing after changing diapers, feeding and handling children's toys; not sharing foods, drinks and eating utensils; and cleaning objects that came into contact with urine or saliva. The feasibility of behavioral intervention has been demonstrated by studies among pregnant women showing that CMV acquisition could be prevented by counseling on better hygiene [27, 28].

The relatively low seroprevalence among Dutch/Western women of reproductive age (37%) means that more than 60% are seronegative and therefore at risk of a primary CMV infection during pregnancy, putting offspring at risk for severe disability caused by congenital CMV infection. In contrast, despite approximately 85% seropositivity in non-Western women of reproductive age, they are at risk of reactivation or reinfection, which likewise can cause a congenital CMV infection and its consequences. [3, 4].

Thus preventive measures against congenital CMV infections should be aimed both at women susceptible for primary CMV infection (mostly of Dutch/Western origin) and at seropositive women (mainly of non-Western origin). Accordingly, future vaccines should be able to prevent both primary and recurrent infections. The data from this study could be used to make more detailed estimates, by modeling, of the potential effects of a vaccination program within the various subgroups. Meanwhile, influencing behavior and increasing awareness of congenital CMV infection remains the most import means for prevention.

In conclusion, this study showed that CMV seroprevalence in the Netherlands is relatively low compared to other countries. However, seroprevalence is considerably higher in the growing subpopulation of non-Western residents, especially among first generation migrants. Other independent risk factors for CMV infection are older age, lower educational

level, and among Dutch/Western individuals, female gender and contact with children. Preventive measures, including hygienic counseling, should be focused mainly at women of reproductive age, in order to prevent CMV infection and subsequently congenital CMV infection with risk of severe disabilities.

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Competing interests
None declared.

Ethical approval
Medical Ethics Committee, Almere, ISRCTN 20164309.

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