

# Seroprevalence to cytomegalovirus in the Portuguese population, 2002-2003

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The prevalence of cytomegalovirus (CMV) infections ranges between 50% and 85% in adults in the United States, and its epidemiology varies in different regions of the world and between socioeconomic and age groups. In Portugal, no study has been carried out to date to determine the prevalence of CMV in the general population. Under the second National Serological Survey conducted in continental Portugal in 2001–2002, we estimated the prevalence of individuals with antibodies to CMV using indirect immunofluorescence to detect virus-specific IgG. The population sample included 2,143 individuals of both sexes and different ages from all 18 districts in Portugal. The national seroprevalence of CMV was determined as 77%. We analysed the proportion of CMV IgG by sex, age group and district of residence. This was the first nationally representative study of seroprevalence of CMV in Portugal. The results of the study indicate that CMV infection is highly prevalent in the population and occurs mainly in the first years of life.

## Introduction

Cytomegalovirus (CMV) is a common virus with no known seasonal predominance and with a prevalence that ranges between 50% and 85% of adults in the United States [1-3]. The epidemiology of CMV varies in different regions of the world and in different socioeconomic and age groups [3-5].

CMV establishes a latent state following primary infection, reactivating when there are changes in immune status [6,7]. CMV infections are most often asymptomatic, but when symptomatic, can cause a syndrome similar to clinical and haematological infectious mononucleosis. The virus is excreted through body fluids, and the most common modes of transmission are via the oropharyngeal and genital tract, although transmission can also occur through breast milk, organ transplant or blood transfusions [8-11].

CMV primary infection occurs mostly in childhood and adolescence, but primary infections are also observed in adults [3,11-14]. The infection is important in certain

risk groups such as immunocompromised individuals and pregnant women. In immunocompromised individuals, CMV infection is the leading cause of morbidity and mortality, especially in connection with transplants, haemodialysis, cancer, immunosuppressive medication and infection with human immunodeficiency virus (HIV) [8]. Transplacental transmission can occur and primary infection in the first 16 weeks of pregnancy is associated with higher rate of damage in fetal development [15-20]. In primary maternal infection, the probability of transmission of CMV to the fetus is approximately 30% to 40%. In women with CMV reactivation during pregnancy the probability of fetal CMV transmission decreases to approximately 0.5% to 1.4% [21-24]. In developed countries, CMV is a major cause of congenital infection, with an incidence of 0.4% to 2.2% of total live births per year, and is responsible for neonatal morbidity and mortality [19,23,25-27]. The congenital CMV infection is asymptomatic in the neonatal period (the first 28 days of life) in approximately 85% to 90% of infants, but nearly 5% to 15% of these infants show late sequelae during the first years of life, typically hearing deficits and visual impairments [22,28-30]. Approximately 10% to 15% of infants with congenital CMV infection are symptomatic at birth, with manifestations including growth retardation, jaundice, purpura, hepatosplenomegaly, microcephaly, intracerebral calcifications, and retinitis. The risk of long-term neurodevelopmental disabilities is high in these children and include microcephaly, hearing loss, motor deficits, cerebral palsy, mental retardation, seizures, ocular abnormalities and learning disabilities [23,31-34].

In a recent review of priorities for vaccine development, the Institute of Medicine of the National Academy of Sciences in the United States concluded that in terms of healthcare costs and years of life and disability saved, a vaccine against CMV infection should be a priority [35,36].

In Portugal, the prevalence of CMV in the population and the incidence of CMV congenital infection have

not previously been determined. However, in 2003, preliminary results of a prospective study indicated that the proportion of CMV congenital infections was 0.7% in a consecutive population of newborns in the area of Lisbon, as determined using the gold standard technique, shell vial culture of urine [37]. According to a survey by Paixão et al. [38] that screened for CMV congenital infection by PCR of blood samples obtained from Guthrie cards, the proportion of congenital infection by CMV was 1.1% in Portugal and 0.7% in the region of Lisbon in the period from August 2003 to September 2004.

The second National Serological Survey conducted in continental Portugal in 2001–2002 was carried out with two objectives: firstly, to determine the prevalence of individuals with antibodies to vaccine-preventable diseases, as a means of evaluating the national immunisation programme, and secondly, to determine the prevalence of individuals with antibodies to infections deemed important in terms of public health. As part of the second National Serological Survey, we estimated the proportion of individuals with CMV antibodies in Portugal in 2002–2003 in order to assess the prevalence of CMV in the population [39].

## Methods

### Sampling

The main sample frame that was used to study the immunity to diseases included in the national immunisation programme was calculated to be nationally representative and to adequately cover all age groups, at a sample size of 3,304 individuals of both sexes, homogeneously distributed in eight age groups: 2–4, 5–9, 10–14, 15–19, 20–29, 30–44, 45–64, and ≥65 years. Each age group included individuals from each of the 18 districts of mainland Portugal, to a number proportionally representative for the population of each district. Between 2002 and 2003, the national immunisation programme recruited 3,525 participants at 38 private and public serum collections points, distributed in all 18 districts of mainland Portugal. Individuals were invited to participate until the district and age grids

prepared were completed. For the participation of individuals, a fact sheet was prepared with the objectives and benefits of the study and informed consent was obtained either from the participants themselves or from their legal representatives [39].

For our specific study on the prevalence of CMV, we used a sub-sample of 2,143 individuals. Serum samples were taken from the same batch collected during 2002–2003 and analysed in 2003–2004 for the presence of CMV-specific antibodies.

### Serological analyses

IgG antibodies specific for CMV were detected by indirect immunofluorescence, using commercial reagents (Merifluor CMV IgG, US) according to the manufacturer's instructions. Samples were added to a layer of human fibroblasts fixed on glass slides on which approximately 10% of cells are infected with CMV strain AD169, and the formation of antigen-antibody complex is viewed using a fluorescent dye. Uninfected fibroblasts on the same slide were used as an internal control of the specificity of the test. The tests were validated with negative and positive control sera. The use of reference sera ensured the reproducibility of results between batches. According to the manufacturer, the test has 97% sensitivity, 100% specificity, 100% predictive value of a positive test and 99% predictive value of a negative test.

### Statistical analysis

Statistical analysis consisted in the determination of absolute and relative frequencies (percentages). Binomial confidence intervals were calculated using the exact method that uses the relationship between the F and Binomial distributions attributed to Bliss and Brownlee as described by Zar [40].

**TABLE**

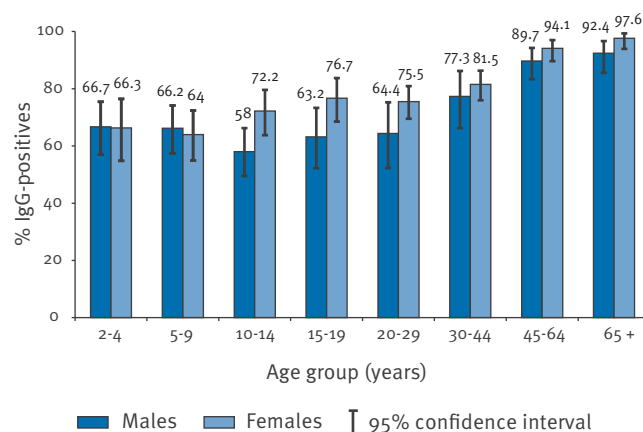
Individuals IgG-positive for cytomegalovirus, by sex, Portugal, 2002–2003 (n=2,143)

Sex	Study participants n	CMV-positive n (%)	95% confidence interval
Male	860	622 (72.3)	69.2–75.3
Female	1,283	1,029 (80.2)	77.9–82.4
<b>Total</b>	<b>2,143</b>	<b>1,651 (77%)</b>	<b>75.2–78.8</b>

CMV: cytomegalovirus.

**FIGURE 1**

Percentage of individuals IgG-positive results for cytomegalovirus, by age and sex, Portugal, 2002–2003 (n=2,143)



IgG: Immunoglobulin G.

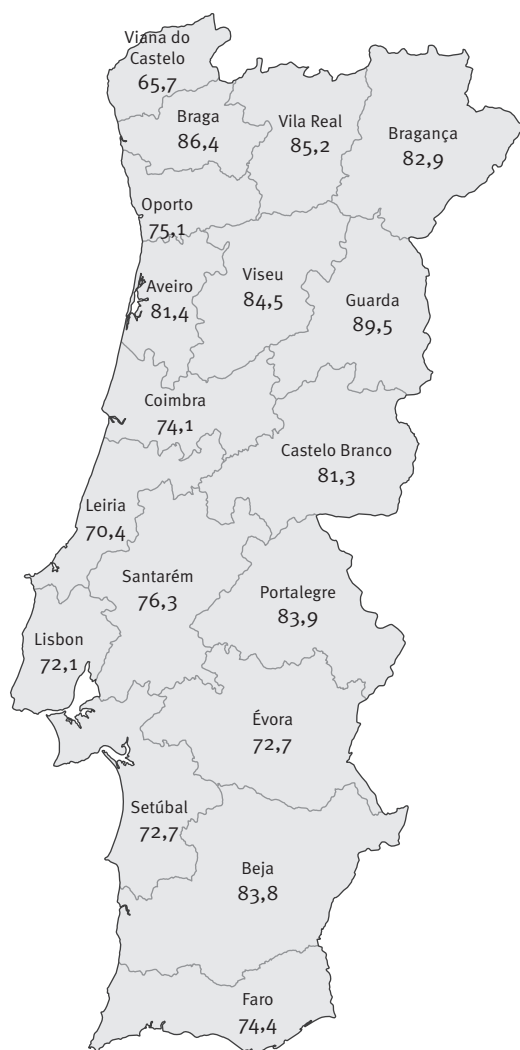
## Results

In a total of 2,143 individuals, 1,651 had IgG antibodies to CMV (77%, 95% confidence interval (CI): 75.2–78.8) and 492 were seronegative (23%, 95% CI: 21.2–24.8). Among the male participants, 622 (72.3%) were CMV-positives and among the female participants 1,029 (80.2%) were CMV-positives (Table).

When analysing the distribution of CMV IgG-positive individuals by age, 66.5% of children in the youngest age group (2–4 years) were shown to have antibodies. The percentage of seropositive individuals was similar in age group of 5–9 year-olds and 10–14 year-olds with 65.1% and 64.9%, respectively, and then increased with age, reaching 71.3% in the age group of 15–19 year-olds, 80.5% in the 30–44 year-olds and 95.6% in the oldest group ( $\geq 65$  years). The percentage of males and females with CMV IgG in each age group is shown in Figure 1.

**FIGURE 2**

Proportion of individuals IgG-positive for cytomegalovirus, by district of residence, Portugal, 2002–2003 (n=2,143)



IgG: Immunoglobulin G.

Analysis of the distribution of CMV IgG-positive individuals by district of residence showed that only one of the 18 districts of Portugal, Viana do Castelo, had a prevalence of less than 70% of seropositive individuals. The highest prevalence of CMV-positive residents was found in the districts of Guarda, Braga and Vila Real, with 89.5%, 86.4% and 85.2%, respectively (Figure 2).

## Discussion

In Portugal, the second National Serological Survey has established, for the first time, the prevalence of individuals with CMV-specific IgG. The results of this study indicated that CMV infection was highly prevalent in the population (77%), similar to what has been described for other countries, and that it occurred predominantly in the first years of life [1,3,41–46].

Seroprevalence for CMV between the age of two and four years was high, with 66.5% of IgG-positive children in this age group (95% CI: 59.3–73.2). Children younger than two years were not included in the study and conclusions on the situation at that age can therefore not be drawn. However, breastfeeding is known to be a significant source of CMV transmission to children and plays an important role in the epidemiology of CMV infection as CMV is reactivated during lactation in nearly every seropositive mother [47–49]. The proportion of infants who acquire CMV during the first year of life is directly related to the prevalence of maternal infection and to the proportion of mothers who breastfeed. In countries where breastfeeding is widely practiced and most mothers are seropositive, for example in south and south-eastern Europe, regions of Asia, Africa and Latin America, more than 50% of infants acquire CMV within the first year of life [8,50]. The seroprevalence found in the age group between two and four years (66.5%) was probably the result not only of transmission via breastmilk but also of oral transmission from other children and from seropositive adults they are in close contact with when they start attending day care centers at that age [51,52]. This was similar to the seroprevalence of 53.8% found in several studies performed in children of that age in Brazil (region of São Paulo) [53]. It was higher than that in Italy (region of Parma, 28% at two years of age) and Finland (41% at eight years of age) and lower than in Venezuela (region of Valencia; 83.3% between two and four years of age) and Turkey (region of Antalya, 82.1% in children between one and six years of age) [41,54–56].

The antibody prevalence in children at school age (age groups 5–9 years and 10–14 years) was similar to that at pre-school age, but increased further to 71.3% (95% CI: 64.8–77.2) in the age group between 15 and 19 years, which corresponds to a greater sexual exposure, in addition to close non-sexual contact [3,8,13,57]. The antibody prevalence in this age group was identical to that in the 20–29 year-olds. Studies with similar age groups conducted in other countries, such as the United States, Japan, France, England, Poland and Russia, describe seroprevalences ranging between 51.5% and 78.0% [3,44,58–62].

The prevalence of individuals with CMV IgG gradually increased further in the three oldest age groups, with values of 80.5% (95% CI: 75.6%–84.8%), 92.2% (95% CI: 88.7%–94.9%) and 95.6% (95% CI: 92.4%–97.7%), suggesting that sexual transmission was an important route of transmission of the virus in the population [8,63,64]. Another recognised source of adult CMV infection are children. Children infected with CMV shed virus in saliva and urine for years, providing an opportunity for continued spread to other children and susceptible adults (close relatives and day care workers) [8,51,65–67].

IgG-positivity was equally common in both sexes in the age groups of 2–4 and 5–9 year-olds, while in the older age groups, females were more likely to be IgG-positive than males. The statistically significant difference of 8% between males and females in the prevalence of individuals with CMV IgG could be explained by the fact that women may have more contact with children. This horizontal mode of transmission presents a risk to mothers, pregnant women and those with occupations associated with exposure to children, such as teachers and day care providers [23,63,68,69].

Nevertheless, it should be noted that in our study, 24.5% and 18.5% of women of reproductive age (from 20 to 29 years and 30 to 44 years, respectively) were susceptible to CMV, which led us to conclude that there is a considerable risk for congenital infection due to maternal primary CMV infection, which leads to fetal infection in approximately 40% of cases [11,16].

Possible approaches to preventing congenital CMV infections include improved hygiene behaviour of seronegative pregnant women, administration of CMV hyperimmune globulin (HIG) to pregnant women with primary infection, and vaccines, once available, administered to girls or women before pregnancy [70].

Several studies have been done to determine whether changing protective behaviour prevents child-to-mother transmission of CMV during pregnancy [71–74]. The United States Centers for Disease Control and Prevention recommend that seronegative pregnant women assume that children are secreting CMV in their urine or saliva. They advise on simple hygiene such as frequent hand washing, wearing gloves for specific childcare tasks and avoiding intimate contact with their child such as sharing utensils, food or towels, and kissing on or near the mouth [75–77].

Despite advances in the diagnosis of maternal-fetal CMV infection and approaches to prevent congenital CMV, an effective prenatal therapy is unavailable. A prospective, non-randomised study of pregnant women who acquired CMV infection during pregnancy and who received passive immunisation with CMV HIG, showed that this therapy was associated with a significantly reduced risk of congenital CMV disease and infection and had no adverse effects [70,78,79]. Recent case

reports supported safe administration of oral ganciclovir to mothers of CMV-infected fetuses, with no teratogenic side effects when given in the early stages of pregnancy [70,80,81]. The efficacy of ganciclovir still remains to be defined in controlled trials. Other early experience with treatment of intrauterine CMV infection using maternal oral administration of valaciclovir showed that it decreased the viral load in fetal blood significantly and could potentially also reduce the morbidity of prolonged intrauterine infection [82]. The absence of adverse effects or teratogenicity of valaciclovir is compatible with its clinical use, but a well-designed randomised controlled trial is needed.

Currently, there is no approved vaccine for CMV, but two vaccines are in phase II studies: one is a recombinant vaccine containing the major envelope glycoprotein B of the virus with the adjuvant MF59 (gB/MF59) that induces high levels of neutralising antibodies, is safe and immunogenic in adults and infants, preventing also maternal CMV infection [36,83]. The other vaccine is the live attenuated CMV Towne strain that stimulates neutralising antibodies comparable to those induced by wild type virus and protects renal transplant patients from severe CMV after transplantation [78,84].

The main interventions for the prevention of CMV infection should be aimed at women who wish to become pregnant, women who care for children and immunocompromised individuals. These individuals in whom exposure to CMV can be most detrimental will be the target groups for possible administration of a future vaccine.

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