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Prevalence of cytomegalovirus infection in Italy

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SUMMARY

Between 1987 and 1989, the prevalence of antibody to cytomegalovirus (CMV) was determined, by the ELISA method, in serum samples from 1494 apparently healthy subjects, 3–18 years old. Subjects were selected by a systematic cluster sampling from five geographical areas in Italy. The overall prevalence of antibody was 64·2%, increasing from 54·4% in 4–6-year-olds to 73·3% in subjects 17–18 years old ($P < 0·01$). Prevalence of antibody was significantly higher in females ($P < 0·05$) and in subjects residing in the South of Italy ($P < 0·01$). A significant association was found with sociodemographic factors. Subjects belonging to a household with six or more persons had a 1·5-fold risk (C.I. 95% = 1·11–2·04) and subjects whose fathers had less than 6 years of schooling had a 1·4-fold risk (C.I. 95% = 1·1–1·87) of previous exposure to CMV infection. The high prevalence (74·4%) of young women who are naturally immune when entering childbearing years does not guarantee that there will be a low risk of fetal infection.

INTRODUCTION

Cytomegalovirus (CMV) has a worldwide distribution. Epidemiological data from antibody prevalence studies have shown that age-related prevalence of CMV infection varies with the socioeconomic status of the surveyed population. The age at which primary CMV infection occurs is much lower in developing countries, whereas in industrialized countries the infection is acquired later in life, and the total proportion of the population infected is lower [1–3]. Adults of high socioeconomic status in industrialized societies may have an antibody seroprevalence as low as 40%, while nearly 100% of subjects in lower socioeconomic groups in these societies, or in developing countries, may have evidence of past infection with CMV [1].

The aim of the present study was to assess the current status of CMV infection among children and teenagers in Italy. The effects, if any, of sociodemographic

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factors were also considered. Family size and father's years of schooling were used as surrogate markers for sociodemographic status.

MATERIAL AND METHODS

Study population

The study population consisted of apparently healthy subjects, 4–18 years old, residing in five different geographical areas of Italy: two middle-sized northern cities (Padua and Udine, c200000 inhabitants), two large southern cities (Bari and Palermo c600000–800000 inhabitants), and the entire island of Sardinia (1500000 inhabitants) (Fig. 1).

Subjects were stratified into five age-groups: 4–6 years, 7–8 years, 11–13 years, 14–16 years, and 17–18 years. In the northern areas subjects were selected beginning at 7 years of age, and in the other areas they were selected beginning at 4 years of age. The study period was May 1987 through November 1989.

Sampling procedures

This study was designed in such a way as to reduce sample bias and provide reliable representation of the community.

Subjects in the age-groups 4–6, 6–8, 11–13 and 14–16 years were recruited from kindergartens, primary schools, junior schools, and high schools, respectively, using systematic cluster sampling [4] in the following way: all private and public schools were identified within each area and then arranged progressively in four different lists, each list corresponding to the age rage of the pupils. The number of subjects was approximately the same in each class, and a single class in each list was considered a cluster. In each list, the first cluster was randomly chosen while the others were selected with probability proportional to required size of each age-group (see *Sample size*) at systematic intervals.

Subjects in the age-group 17–18 years were recruited from those consecutively going to the public health offices of the various studied districts to ask for a certificate of good health for admission to the final examination of high-school. Because serum samples were routinely taken at this time for syphilis serology, they were also tested, after informed consent, for anti-CMV IgG.

Sample size

In each stratum of age, the sample size was calculated on the basis of the expected prevalences of anti-CMV, with the purpose of achieving similar precision of our estimates in different age-groups. Confidence limits of 95% and a precision of 3% were considered to be acceptable [5]. In order to safeguard the desired level of precision and confidence of our estimate from possible refusals, we increased the calculated sample size in each stratum by about 10%. The resulting total sample size was 1600 subjects.

Laboratory tests

After informed consent of parents, blood samples from the subjects were taken. Sera were stored at −20 °C and subsequently tested for CMV IgG antibody by an ELISA method (CytoTEK G, Flow Laboratories) in a single laboratory. Samples

Fig. 1. Italy. ▨, Study area.

were considered positive when their absorbance values, at 450 nm, were higher or equal to that of a 1·25 IU/ml standard (cut-off control). The standard was calibrated against the Paul Ehrlich Sankt Gallen reference preparation.

Each blood sample was accompanied by a precoded questionnaire, recording age, sex, residence, family size, and father's years of schooling.

Statistical analysis

Differences in proportion were compared using the two-tailed chi square test. A probability value of $P < 0{\cdot}05$ was considered significant. Odds ratios (O.R.) were calculated considering as 'not being exposed' the category at the most favourable level of exposure (lowest family size and highest number of years of father's education). Confidence limits of 95% on O.R. were calculated according to the Woolf method [6].

RESULTS

We tested serum samples from 1494 subjects (53·3% male, 46·7% female). The number of refusals was thus 106 (6·6%) out of the 1600 selected.

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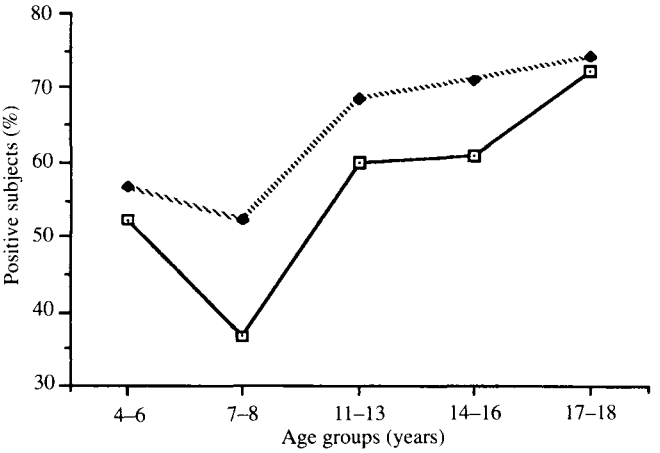

Fig. 2. Age 2. Age specific prevalence of anti-CMV among children and teenagers by sex in Italy, 1987–9. ···◆···, females; —⊡—, males.

The overall prevalence of CMV antibody was 64·2% (960/1494), increasing from 54·4% in 4–6-year-olds to 73·3% in subjects 17–18 years old ($P < 0·01$). By the age of 4–6 years, 54·4% of subjects had been exposed; the prevalence further increased during primary school age and more likely during adolescence. A female predominance was observed (67·2% versus 61·7%, $P < 0·05$) (Fig. 2). Subjects residing in the south of Italy had a significantly higher prevalence of antibody than did subjects residing in the north (71·1% versus 56·3%, $P < 0·01$) (Fig. 3). A significant association was found with sociodemographic factors. Subjects belonging to a household with six or more persons had a 1·5-fold risk (C.I. 95% = 1·11–2·04) and subjects whose fathers had less than 6 years of schooling had 1·4-fold risk (C.I. 95% = 1·1–1·87) of previous exposure to CMV infection (Tables 1 and 2).

DISCUSSION

In this nationwide survey, rather than obtaining specimens from biased populations such as volunteers or those attending a clinic, a population-based study was performed. This allows greater accuracy of the results.

CMV can spread vertically as well as horizontally. More recent serological and virological studies indicate that the most rapid rate of infections occurs in early childhood [7–9]. Babies and children become infected in a number of ways. One source of infection is passage through a contaminated uterine cervix during birth. A second mechanism is transmission from human milk by breast feeding [7]. A third mechanism of infection is transmission from other children in day-care centres [8]. A second period of increased acquisition, particularly in countries with more susceptible subjects, occurs in adolescence and young adulthood because this is a time of courtship and intimate physical contact [10].

The present study shows that the prevalence of CMV antibody in Italy increases with age. The rather high lower age limit of our sample population (4–6 years) does not appear to restrict the extent to which our results follow the above described pattern. In fact, the high prevalence (54·4%) observed at the beginning of primary

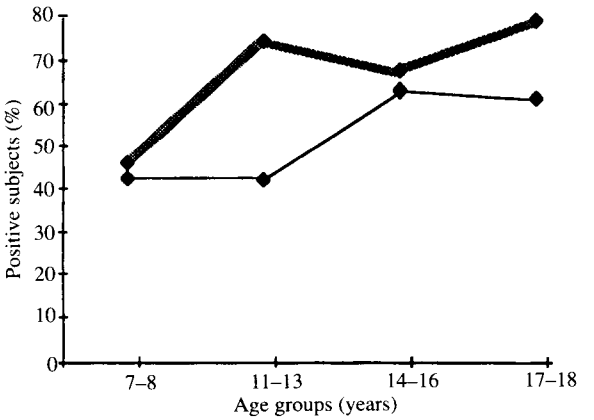

Fig. 3. Age specific prevalence of anti-CMV among children and teenagers by geographical area in Italy, 1987–9. —◆—, South; —◆—, North.

Table 1. *Prevalence of anti-CMV in children and in teenagers in Italy, by household size**

Household size (including the subject)	No. positive/ No. tested (%)	O.R. (C.I. 95%)
2–4 persons	497/823 (60·4)	1
5 persons	245/370 (66·2)	1·28 (0·98–1·67)
> 5 persons	191/274 (69·4)	1·5 (1·11–2·04)

* This information was missing in 27 subjects.

Table 2. *Prevalence of anti-CMV among children and teenagers in Italy, by father's years of schooling**

Year of father's schooling	No. positive/ No. tested (%)	O.R. (C.I. 95%)
> 12	256/447 (57·2)	1
6–12	315/466 (67·6)	1·55 (1·17–2·05)
< 6	370/562 (65·8)	1·43 (1·1–1·87)

* This information was missing in 19 subjects

school age, as a consequence of infections that occurred in early childhood, and the further increase observed during adolescence are typical of the described age-pattern of CMV infection.

The high figures observed in our study are similar to those reported in countries other than North America and Europe [1]. In fact, our findings agree closely with those recently reported from China, where, using an ELISA method, CMV prevalence was found to be 60·3% among children 4–7 years old [11]; by contrast, a much lower prevalence was recently observed in USA among subjects 6–22 years old [12].

Because CMV is unstable in the environment, close or intimate contact is required for its spread. It has been suggested that crowding is the key factor for transmission [8]. Our data clearly show that the degree of crowding as well as poor lifestyle factors, as indicated by the lowest number of father's years of schooling,

are associated with an increased risk of CMV infection (O.R. 1·5 and 1·4, respectively). Further support comes from the higher prevalence found in southern areas, where a high degree of crowding and poor lifestyle are generally more common than in the northern areas. This population-based survey shows that CMV infection is widely spread in Italy; age, female sex, residence in the south, crowding, and low number of father's years of schooling are factors associated with this infection.

Unlike rubella and toxoplasma infections in which vertical transmission occurs only when primary infection is present during gestation, congenital CMV infections may occur in the neonates of mothers with CMV antibody prior to pregnancy [13]. Despite the fact that maternal immunity does not prevent transmission of either intrauterine or perinatal CMV infections, primary CMV infection acquired before a woman enters her childbearing years would appear to be beneficial. There is evidence to suggest that, except in very few cases [14–15], infants congenitally infected as a result of their mother's primary infection with CMV are more likely to have significant sequelae than are those infants infected from reactivation or reinfection of the mother [16]. Thus, the undesirable consequences of congenitally acquired CMV infection should be very uncommon in countries such as Italy, where nearly 75% of the women are seropositive by the time they begin childbearing. However, large prospective studies elsewhere of pregnant women demonstrated a high risk of primary infection for those few seronegatives who remain within the community [17–18], particularly for those from low-income social classes [18]. Therefore the high prevalence (74·4%) of young women who are naturally immune in Italy does not garantee that there will be a low risk of fetal infection.

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