SEROLOGIC SCREENING FOR CYTOMEGALOVIRUS, RUBELLA VIRUS, HERPES SIMPLEX VIRUS, HEPATITIS B VIRUS, AND *TOXOPLASMA GONDII* IN TWO URBAN POPULATIONS OF PREGNANT WOMEN IN CHILE[1]

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INTRODUCTION

There are only a few reports concerning the epidemiology of congenital and perinatal infections in Chile *(1-4)*. In general, these reports show that the prevalences of infection with most of the causative agents are high and that most infections are acquired at an early age. Since vaccinations against cytomegalovirus (CMV), herpes simplex virus (HSV), and *Toxoplasma gondii* have not yet been introduced, high prevalences of these agents represent naturally acquired infections.

Although the prevalence of congenital and perinatal infections is high, however, it is unclear whether their incidence during the childbearing years (and hence their potential to cause fetal disease) is different from that observed in communities such as those in developed countries where lower prevalences are the rule. Therefore, in order to help assess the importance of CMV, HSV, rubella, HBV, and *Toxoplasma gondii* as causes of

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perinatal infections, we determined the rate of susceptibility to these infections during pregnancy among women of both low and middle socioeconomic status in Santiago, Chile. The report presented here indicates the prevalences of these infections early in gestation and compares the results with other results obtained from the same population and from other populations in other countries.

MATERIALS AND METHODS

The study population consisted of 833 pregnant women residing in Santiago, Chile, who were enrolled in the study between January and March of 1983, at their first or second prenatal clinic visit. The age distribution of the study population, by income group, is shown in Table 1.

The 461 low-income women in this population, all white, were drawn from three different public health clinics operated for beneficiaries of the National Health Service in the southeast area of Santiago; their mean age was 24.5 years and their mean gestational age at enrollment was 18 weeks. This population accounted for approximately 5% of all pregnancies seen at area public health clinics in one year.

The 372 middle-income women in the study population were enrolled at the obstetrics clinic of the Hospital of the Catholic University of Chile (*Universidad Católica de Chile*). All of these women were white, their mean age was 27.2 years, and their mean gestational age at enrollment was 14 weeks. This population accounted for nearly 20% of all pregnant women seen at the obstetric clinic in one year.

Serum samples obtained at enrollment from all of the subjects were stored at −20°C until tested by one of us (P.V.) at the virology laboratory at the University of Alabama in Birmingham. IgG-class antibodies against CMV, HSV I and HSV II, rubella virus, hepatitis B virus, and *Toxoplasma gondii* were assessed using commercially available enzyme-linked immunosorbent assay (ELISA) kits. That is, serum samples were assayed on microtiter plates in accordance with the method recommended by the manufacturer of the "Cytomegalisa," "Herpelisa," "Rubelisa," and "Toxoelisa" kits (M.A. Bioproducts, Wakersville, Maryland, USA). The anti-HBc antibodies were also determined by a commercially available ELISA (Corzyme, Abbott Laboratories). The samples that were positive for anti-HBc were subsequently tested for surface antigen (HBsAg) and HBe antigen (HBeAg) by another ELISA (auszime II, Abbott HBe, Abbott Laboratories). All these assays are routinely done in our laboratory.

TABLE 1. Distribution of the study population, by income and age groups.

Age group (in years)	Middle-income group		Low-income group	
	No.	%	No.	%
< 15	—	—	21	4.6
16–20	28	7.5	122	26.5
21–25	132	35.5	119	25.8
26–30	118	31.7	96	20.8
31–35	61	16.4	68	14.7
≥ 36	33	8.9	35	7.6
Total	372	100	461	100

In each run we included appropriate positive and negative controls. Because the Herpelisa kit used to test antibodies to HSV I and II had not been licensed by the U.S. Food and Drug Administration, we felt it was necessary to verify that test's specificity. Dr. A. Nahmias of Emory University in Atlanta, Georgia, generously provided us with 30 serum specimens known to contain either type I or type II antibodies (determined by testing them against specific HSV I and II glycoproteins). We found that specimens containing HSV I antibodies had a high rate of cross-reactivity with the HSV type II antigen used in the Herpelisa test. (HSV type II antibodies did not cross-react with HSV type I antigen.) Because of this cross-reactivity, we have reported our results without distinguishing between types I and II. Statistical comparisons were analyzed by the Chi square test.

Results

Among pregnant women from the lower of the two socioeconomic groups, the rate of seropositivity was 96.5% for CMV, 97.2% for HSV, 94.8% for rubella, 68.2% for *Toxoplasma gondii*, and 1.4% for HBV. These rates differed significantly from somewhat lower rates found for the group of women with intermediate socioeconomic status in the cases of CMV, HSV, and *Toxoplasma gondii* (Table 2).

TABLE 2. Results obtained from ELISA testing of sera from two different populations of pregnant women in Chile for exposure to five agents causing congenital and perinatal disease (CMV = cytomegalovirus; HSV = herpes simplex virus; and HBV = hepatitis B virus).

Agent	Socioeconomic background of population group	No. of pregnant women in group: No. tested	No. positive	% positive
CMV	Low	456[b]	440	96.5[a]
	Middle	370[b]	321	86.8[a]
Rubella virus	Low	461	437	94.8
	Middle	372	351	94.4
HSV	Low	458[b]	445	97.2[a]
	Middle	371[b]	326	87.9[a]
Toxoplasma gondii	Low	449[b]	306	68.2[a]
	Middle	370[b]	179	48.4[a]
HBV	Low	513[c]	7	1.4
	Middle	358	5	1.4

[a] The difference between the results for the two socioeconomic groups is statistically significant ($p < 0.001$).

[b] Seven of the study subjects were not tested for CMV, four were not tested for HSV, and 14 were not tested for *Toxoplasma gondii*.

[c] In addition to sera from study subjects, sera from 52 other low-income women were tested for HBV. The serum samples tested were obtained from pregnant women coming to the three participating clinics during the study period.

Within the middle-class population group, none of five samples testing positively for anti-HBc were positive for HBsAg or HBeAg. However, of seven samples testing positively for anti-HBc in the lower-class population group, one was positive for HBsAg and two were positive for both HBsAg and HBeAg. The rate of HBsAg carriers among the total population studied thus appeared to be 0.34% (Table 3).

The age distributions of seronegative women within both populations for CMV, HSV, rubella, and *Toxoplasma gondii* are shown in Figure 1. As

TABLE 3. Indicated prevalences of three HBV markers, (anti-HBc, HBsAg, and HBeAg) among the 871 pregnant women participating in the study.

Marker	No. of subjects tested	Positive sera	
		No.	%
Anti-HBc	871	12[a]	1.4
HBsAg	871	3[b]	0.34
HBeAg	871	2[b]	0.22

[a] Five of these subjects came from the middle-class group and seven came from the lower-class group.
[b] The sera positive for HBsAg and HBeAg came from among the 12 testing positively for anti-HBc. Only these 12 sera were tested for HBsAg and HBeAg.

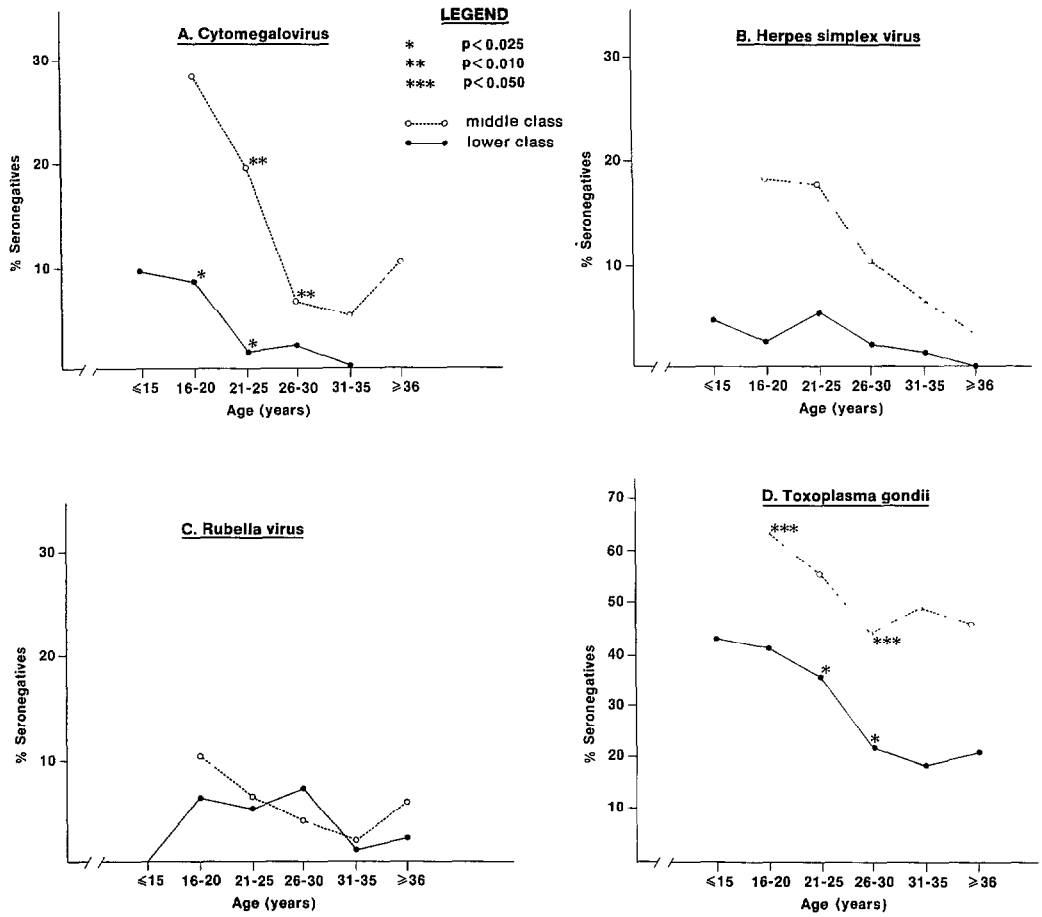

FIGURE 1. Percentages of the two Santiago study populations responding negatively to ELISA serologic testing for IgG antibodies to cytomegalovirus, herpes simplex virus, rubella virus, and *Toxoplasma gondii*, by age group.

may been seen, the number of women susceptible to CMV, HSV, and *Toxoplasma gondii* declined notably with advancing age.

DISCUSSION AND CONCLUSIONS

The results of this study support the conclusion that rubella, cytomegalovirus, herpes simplex, and toxoplasma infections are acquired at an early age in Chile, and consequently that only a small proportion of females remain susceptible by the time they reach childbearing age. Despite this high degree of immunity, however, the risk of congenital and perinatal infections with these infectious agents may still be high. That is partly because recurrent infections (due to viral reactivation or reinfection), which are common occurrences with CMV and HSV, can result in transmission of the agent from mother to baby. At the same time, the relatively few pregnant women who are still susceptible to these infectious agents are frequently exposed and hence at high risk of acquiring the infections.

Cytomegalovirus

As demonstrated previously, CMV infection in Santiago is acquired at an early age *(1)*, probably due to the common practices of breast-feeding and keeping children in day care centers, both of which have been shown to increase the incidence of CMV infections in infants and young children *(5, 6)*. Our present study shows that despite significant differences in seropositivity between the groups of low and middle socioeconomic status, both groups had high seropositive rates (97.5% and 87.5%, respectively). These rates are similar to those reported from communities in Japan *(7)*, Guatemala *(8)*, Barbados *(9)*, Saint Lucia *(10)*, and Tanzania *(11)*, and higher than those reported from the United States *(1, 15*, and *18)* and Great Britain *(12)*.

Populations highly immune to CMV are at risk of recurrent CMV infection, which can result in congenital involvement in approximately 1% of all cases *(13, 14)*. In fact, a previous study conducted in Santiago by Stagno et al. reported that 1.7% of the infants born to low-income women were congenitally infected with CMV, even though 98% of the women were seropositive *(1)*.

Rubella

Our results show that only 4% to 6% of the population escapes rubella infection during childhood and adolescence. This pattern of rubella seropositivity has been previously described for Chile, Argentina, Brazil, and Uruguay *(2)*, while considerably lower rates of seropositivity around age 20 have been found in various other countries (including Jamaica, Japan, Panama, and Trinidad and Tobago—*2, 15*). These different antibody patterns have been correlated with population density *(15)*.

Also, in the late 1960s, some countries like the United States and Great Britain showed an intermediate pattern, with rapid acquisition of infection during childhood but only 80–85% seropositivity at the end of the second decade of life *(16–17)*. This latter pattern seems to be one in which the susceptible female population experiences a relatively higher risk of acquiring rubella virus infection during pregnancy.

Regarding vaccination, in the United States routine immunization of children from 15 months to 12 years of

age, combined with selective immunization of pubertal girls and women of childbearing age, has achieved its goal of decreasing both the incidence of rubella infection in childhood and the incidence of congenital rubella syndrome *(18–21)*, although no change has been observed in the percentages of women who are seronegative. In Great Britain, where rubella immunization has been aimed only at pubertal girls and women of childbearing age, it has not had the same success in controlling congenital rubella *(18)*.

In our two Chilean populations, because of widespread infection in the community, the few women that remain susceptible may be at relatively high risk of acquiring rubella.

Herpes Simplex

There are no seroepidemiologic or virologic studies, nor any well-documented case reports, concerning genital HSV infections among pregnant women in Santiago. Infection with HSV, as shown by our serologic study and as indicated by clinical experience, is very common during childhood. The 87–90% rates of seropositivity we found are most likely a reflection of past HSV I infections. The prevalence of HSV II antibodies could not be ascertained with accuracy in our study, because the ELISA test we used has poor specificity for HSV type II antibodies.

Different rates of seropositivity, ranging from 50% to 100%, have been described in reports from Canada *(26)*, Great Britain *(27)*, and the United States *(22–25)*, suggesting that acquisition of HSV infection is related to socioeconomic conditions and age—statements that are consistent with our current data. In some parts of the world there has been an apparent decline in the incidence of herpetic infection among children, as suggested by a lower rate of seropositivity now than 10 to 20 years ago *(2, 15–17, 22–30)*. The studies providing this information, which were able to differentiate between HSV I and HSV II antibodies, showed that the increasing prevalence of HSV antibodies with age is largely related during the first 15 years of life to HSV I infections, and is principally related thereafter to HSV II infections *(23, 24, 26, 30)*.

Toxoplasma

The prevalences of toxoplasma antibodies found in our study populations are consistent with previous reports on other Chilean populations *(3, 4)*. Our data show a statistically significant difference between the prevalences among women in our low and middle socioeconomic groups, probably as a result of different sanitary conditions, different exposure to cats, and different eating habits. A previous study suggested that the most important factor leading to infection with toxoplasma in a low-income Santiago population was the practice of eating raw or undercooked meat *(4)*.

Hepatitis B

The 0.34% prevalence of HBsAg shown in our study is similar to the 0.3–0.4% prevalence of HBsAg found in Chilean blood donors. However, the 1.4% prevalence of anti-HBc differed somewhat from the 5.4% prevalence of anti-HBc among blood donors *(31–33)*. This difference could be partly explained by the fact that cohorts of pregnant women are less likely to include many prostitutes, drug addicts, and homosexuals—all groups known to be at relatively high risk of HBV infection.

What is quite remarkable (Table 4) is that the prevalence of two hepatitis B markers (HBsAg and anti-HBcAg) seems to be much lower in Chile than in

TABLE 4. Seropositivity for the markers HBsAg and anti-HBc among populations in a variety of countries and geographic areas.

Country or area and source of data	Population studied	% positive for: HBsAg	% positive for: Anti-HBcAg
Chile *(31, 32)*	Blood donors	0.4	5.3
	General population	0.3	—
	Pregnant women	0.34	1.4
Argentina *(31)*	Blood donors	0.8	9.4
Brazil *(31)*	Blood donors	2.1	27.6
Dominican Republic *(31)*	Blood donors	4.1	81.1
Latin America (average) *(31)*	Blood donors	1.6	21.3
United States			
Houston (35)	*Blood donors*	*0.9*	—
Phoenix (35)	*Blood donors*	*0.1*	—
South Atlantic states (35)	*Blood donors*	*2.7*	—
Mountain states (35)	*Blood donors*	*1.44*	—
New England (35)	*Blood donors*	*1.69*	—
New York State (35)	*Blood donors*	*0.4*	*4.9*
China *(34)*	General population	11.72	28
Tokyo *(35)*	Blood donors	2.1	—
Five South Pacific islands *(37)*	General population	1–10	—
Moscow *(35)*	Blood donors	4.2	—
Southern Africa *(36)*	Black urban population	4–9	—

other countries of comparable socioeconomic development, including some in the Americas *(31–37)*. With acute and chronic hepatitis B viral infections being infrequent during pregnancy, it appears that this infection must be very unusual among neonates in Chile.

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Summary

In seeking information about the epidemiology of congenital and perinatal infections in Chile, a serologic survey was made of 833 pregnant women residents of Santiago, 461 belonging to a "low-income" group and the remaining 372 to a "middle-income" group. Sera from these subjects were tested by ELISA for antibodies to cytomegalovirus, herpes simplex virus, rubella virus, hepatitis B virus, and *Toxoplasma gondii*.

The results indicated that over 90% of the women were seropositive for antibodies to cytomegalovirus, rubella virus, and herpes simplex virus. A lower proportion (59%) were positive for antibodies against toxoplasma, and only 1.4% tested positively for antibody against hepatitis B core antigen (anti-HBc).

These findings support the conclusion that infection with cytomegalovirus, herpes simplex virus, and rubella virus, as well as with toxoplasma to a lesser degree, tends to occur at a very early age in Chile, and so only a small proportion of females remain susceptible by the time they reach childbearing age. Despite this, however, the rate of congenital and perinatal infections may still be high, partly because of the danger of recurrent infections (common occurrences with cytomegalovirus and herpes simplex virus), and partly because those women who remain susceptible are at high risk of acquiring these infections.

It also appears that the prevalence of certain hepatitis B markers is much lower in Chile than in other countries at comparable levels of socioeconomic development. This finding, combined with data indicating that hepatitis B viral infection is very infrequent during pregnancy in Chile, gives reason to conclude that hepatitis B infections must be very unusual among Chilean newborns.

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