RUBELLA IN GUIPUZCOA (BASQUE COUNTRY, SPAIN)
A FOUR-YEAR SEROSURVEY

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Key words: Rubella - Serosurvey - Basque country

Since the early 1980s, Spain has practiced mass vaccination of preschool children and selective vaccination of prepubertal girls. Estimated vaccination coverage in the province of Guipúzcoa (Basque Country) in recent years is about 95% (confirmed minimums of 89% for preschool children and 87.5% for 11 to 12 year-old girls). From the seroepidemiological study we could deduce that there was extensive circulation of wild rubella virus until recently (72-75% of unvaccinated girls 10 to 11 years-old had rubella antibody). More than 98% of the population at risk, represented in this study by 13,564 women (67% of all who bore children over a four-year period), possessed rubella antibodies. In the course of the study period the number of subjects (puerperal women and children) susceptible to infection declined. In spite of this favorable situation, the seroepidemiological study disclosed certain gaps that should be corrected to meet the targets for the European Region of the Expanded Programme on Immunization, and to achieve the goal of elimination of wild rubella virus in the area.

INTRODUCTION

The elimination of congenital rubella in Europe by the year 2000 is one of the goals of the European Regional Committee of the WHO in the Health for All Program (12). Abundant human and material resources are being earmarked for this purpose in Europe and in other regions of the world. In Spain, a two-pronged vaccination strategy is being used: a) mass vaccination of 12-15 month-old children using the triple viral vaccine (measles, mumps and rubella) (MMR) introduced in 1980 (2) and b) selective vaccination of 11-year-old girls, generalized in 1977 (3). In the province of Guipúzcoa (Basque country) in 1985 we introduced the vaccination during puerperium of all rubella-antibody (R-Ab) negative women using the Public Health Service for childbirth. In all occasions RA27/3 rubella vaccine were used. In this paper we report the findings of a seroepidemiological study of rubella in this area, with special reference to the evolution of susceptibility to infection in recent years.

SUBJECTS AND METHODS

Subjects

Puerperal women. These were women from all parts of Guipúzcoa province who bore children at the Hospital Nuestra Señora de Aranzazu between August 1985 and December 1988. At this time, this hospital handled almost all the deliveries assisted by the public health services. A total of 13,564 women, representing 67% of all the women in the area who gave birth (data from the Basque Institute of
Statistics), were studied. Women who were R-Ab-negative on screening were offered vaccination during their stay in hospital (first 48 hours of hospitalization): 400 women were found to be susceptible to infection, and 311 were vaccinated in this period.

**Children and adolescents.** Group I consisted of 1,416 subjects 2 to 16 years-old who were enrolled when they sought ambulatory medical care for mild situations unrelated to exanthematic disease (trauma, preoperative preparation for minor surgery, gastroenteritis, etc.); they came mainly from San Sebastian (capital of the province). Serum samples were collected during two one-year periods: 621 samples during period A: June 1986 - May 1987; 795 during period B: January - December 1988. Group II consisted of 411 female students 10-11 years old (born in 1978) who were enrolled in another study and who had not been vaccinated for rubella (group II initially consisted of 434 girls but 23, who had been vaccinated for rubella, were eliminated for purposes of analysis). This group was randomly selected by cluster sampling of 33 schools in 15 different populations, rural and urban, located in 4 geographic zones. The sample was representative of the population to 95% (z = 1.96, d = 0.05). The percentage of acceptance was 86%.

**Serologic studies**

R-Ab determination was performed within 24 hours after venipuncture by latex agglutination (LA) (Rubascan™, Becton Dickinson, USA) using 1/5 diluted serum (LA 1/5). Negative samples were stored at -40°C and later retested by ELISA (Enzygnost, Behringwerke, Marburg, FRG) using a 1/40 serum dilution, hemagglutination inhibition (Rubenosticon™, Organon Teknika, The Netherlands) at a 1/8 dilution and LA with undiluted serum (LA 1/1). Prior to this study (data not shown) the serological tests were evaluated against simulated sera containing different concentrations of R-Ab from a control serum (LA 1/1). The LA 1/5 test was capable of detecting ≥15 IU levels < 10 IU of R-Ab were detected by the LA 1/1 test.

**Estimation of vaccination coverage and incidence data**

An estimate of the minimal vaccination coverage in the area was calculated by considering the number of persons vaccinated in public health services, where each vaccination is documented. The estimate of maximum vaccination coverage was based on the number of doses of vaccine dispensed (sold or distributed without charge). The data on the annual incidence of rubella in the area were obtained from mandatory infectious disease reports in the EDO (Enfermedades de Declaración Obligatoria) System.

**Definitions**

R-Ab titers were considered “low” when the results of LA 1/5 screening were negative but two of the other tests were positive (LA 1/1 and HAI or ELISA positive). This situation was relatively common in puerperal women, but only rarely seen in children and adolescents. Nonimmune subjects, or those susceptible to infection, were those who lacked antibodies (seronegative) according to the criteria used.

**Data analysis**

Standard statistical methods were used for data analysis. The Chi square, Fisher exact and Mantel-Haenszel tests were used to compare prevalence (Statcalc program, Epiinfo 1988, CDC, Atlanta). For the cluster sampling we used the technique of sampling for unequal sizes described by Cochran (6).

**RESULTS**

**Prevalence in puerperal women**

Four hundred of the 13,564 women investigated (2.95%) failed to show R-Ab on screening (LA 1/5). The prevalence of rubella antibody (indicating prior rubella infection) with this method was 97.05%. After re-evaluation, 200 of the 400 women initially considered nonimmune revealed low-titer immunity (LA 1/5 negative but LA 1/1 positive and HAI or ELISA positive). The final prevalence was 98.5% (13,364/13,564). Throughout the study period the percentage of R-Ab positive women increased and, consequently, the susceptibility to the infection decreased. There was a significant decline in the number of seronegative and low-titer women during the study period (Table 1). Comparison of the number of seronegative women between the first and fourth years of the study (1985, 1988) yielded Chi square = 11.35, p value < 0.001; stratified analysis for comparison of the results year by year also showed statistical significance (Mantel-Haenszel summary Chi square = 4.19, p value = 0.0406).

**Prevalence in children and adolescents**

Group I. The prevalence of antibody to rubella by age in the two study periods shows that the subjects with the highest protection against rubella (presence of R-Ab as a result of previous natural infection or vaccination) were those situated at the extremes of the age range. Statistically significant differences were appreciated in the prevalences of antibody to rubella in the first and second periods among all subjects (469/621 versus 657/795 p < 0.001) and among the children of 4, 5 and 6 years age (92/120; 63/105 and 31/65 versus 57/59; 56/62 and 38/52 respectively). A drop