RUBELLA ANTIBODIES IN FEMALE APPLICANTS FOR PREMARITAL HEALTH CERTIFICATES IN MAR DEL PLATA, ARGENTINA[1]

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INTRODUCTION

It is evident that rubella, once considered a mild disease of no particular importance, can trigger a prenatal process with serious individual and social consequences *(1–3)*. At the International Symposium on Rubella Vaccine held in Washington, D.C., in 1969, Cooper *et al. (4)* reported the results of a survey of 400 children born to mothers who had contracted rubella during pregnancy. The study showed very high percentages of malformations among children delivered by mothers who were infected during the first twelve weeks of pregnancy.[4]

In Argentina, national morbidity data provided by the Epidemiologic Surveillance Area of the National Ministry of Public Health and the Environment placed the number of cases of rubella in 1981 at 15,194, or about 63 cases per 100,000 inhabitants. These figures would undoubtedly have been higher if allowances had been made for underreporting and for subclinical cases, the latter of which were said to be very common *(5)*. In addition, the total numbers of cases reported at that time, as shown in Table 1, suggested a rising morbidity trend that could further increase

TABLE 1. Rubella cases reported in Argentina, 1977–1985.

Year	No. of cases
1977	10,541
1978	7,520
1979	9,667
1980	16,421
1981	15,194
1982	13,067
1983	33,632
1984	27,645
1985[a]	5,933

[a] Up to week 43.

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[4] The specific percentages found with malformations were 84% of the children where infection occurred in the first four weeks of pregnancy, 70% of those where it occurred in weeks five through eight, and 50% of those where it occurred in weeks nine through 12. These figures were based on cases in which the diagnosis was virologically confirmed. The weeks cited were counted from the mother's last menstrual period.

the numbers of rubella-associated congenital malformations in the future. Overall, serologic studies indicated that between 10 and 20% of all Argentine women in their reproductive years lacked antibodies against the rubella virus, a situation in which susceptibility may be assumed *(6–9)*.

The study reported here examined a group of women 15 to 49 years old, all residents of the General Pueyrredón Department[5] of the city of Mar del Plata, who applied to our institute (the "Dr. Juan Héctor Jara" National Institute of Epidemiology) for a premarital health certificate in 1981. The aim of this work was to determine what share of the group was susceptible to rubella, so as to help provide a basis for considering measures to prevent infection during pregnancy.

MATERIALS AND METHODS

The study, which was performed at the institute's Laboratory Department, dealt with a sample of 781 female applicants for premarital health certificates—these constituting 36.1% of the total (2,164) applying for certificates that year. According to the Provincial Register of Vital Events (for the Province of Buenos Aires that includes Mar del Plata), the total number of marriages performed in Mar del Plata in 1981 was 2,621; and, according to the National Population Census of 1980 *(21)*, the female population of the General Pueyrredón Department between the ages of 15 and 49 was 113,769.

The size of the sample was based on the expectation that 7.5% to 12.5% of the women examined (10% ± 2.5% at the 95% confidence level) would test negatively for rubella antibodies, and that for purposes of analysis a sample of some 700 applicants was needed. Regarding selection, applications for premarital certificates are unsolicited; and so applicants were simply included in the group consecutively as their applications were received, until roughly the desired number had been attained.

Serum samples were obtained from all group members by separating sera from the blood samples taken for the venereal disease research laboratory (VDRL) test. Until tested, the sera were stored at −20°C.

The sera were then tested by hemagglutination inhibition adapted to microtiter plates, using commercially available "*Rubeokit-Pasteur*" materials manufactured by *Pasteur Production* of Paris, France. Initially, the sera were diluted 1:8 and were subjected to a treatment designed to reduce the nonspecific inhibitors found associated with beta-lipoproteins and glycoproteins. The samples were then diluted serially from 1:8 to 1:512, and the antigen was tested to obtain four hemagglutinant units. The antigen was also tested simultaneously against positive and negative control sera obtained from *Pasteur Production* and from Flow Laboratories in the United States *(10–12)*.

The levels of antibodies detected were expressed as the reciprocal of the greatest dilution capable of inhibiting hemagglutination. All sera yielding results of 8 or greater (responding positively at a dilution of 1:8 or more) were considered positive, while all those not responding positively at any dilution (i.e., those yielding values below 8) were considered negative. In all cases where a serum was found to respond positively at

[5] General Pueyrredón Department is an administrative subdivision of Buenos Aires Province.

only the lowest dilution (yielding a value of 8), the serum was retested so as to minimize the chances of a low-dilution false positive response.

Results

The age distribution of the 781 study subjects is shown in Table 2. It should be noted that most subjects were toward the low end of the 15–49 year age range, with 71% being under 25 years old and 96% being under 35.

Five of the 781 sera tested were found to yield nonspecific positive responses. These sera were eliminated from the study, leaving a total of 776. Of this remainder, a total of 92 sera responded negatively, indicating that 11.9% of the 776 study subjects lacked specific antibodies against the rubella virus.

Table 3 shows the age distribution of the subjects yielding negative responses. As the percentage of negative responders in each age group indicates, the distribution of negatives was fairly even between the 15–19, 20–24, 25–29, and 30–34 year age groups that accounted for the bulk of the sample. The percentages of negative responses within the 35–39, 40–44, and 45–49 year age groups were not considered representative because of the small numbers of women in those age groups.

The antibody titers obtained with the 684 sera found to contain rubella antibody are shown in Table 4. As may be seen, most of the positive sera (79%) yielded values of 16, 32, or 64. It also emerged (see Table 5) that all of the

TABLE 2. The age distribution of the 781 study subjects, by five-year age group.

Age group (in years)	Subjects		
	No.	%	Cumulative %
15–19	229	29.3	29.3
20–24	331	42.4	71.7
25–29	153	19.6	91.3
30–34	43	5.5	96.8
35–39	18	2.3	99.1
40–44	6	0.8	99.9
45–49	1	0.1	100.0
Total	781	100	100

TABLE 3. The age distribution of subjects whose sera yielded negative results, by five-year age group.

Age group (in years)	Subjects		
	No. tested	No. negative	% negative
15–19	228	23	10.1
20–24	329	40	12.2
25–29	151	20	13.2
30–34	43	5	11.6
35–39	18	(2)	(11.1)[a]
40–44	6	(2)	(33.3)[a]
45–49	1	(0)	(0)[a]
Total	776	92	

[a] The figures in parentheses are not considered representative because of the small numbers of test sera obtained from subjects in each of the indicated age groups

TABLE 4. Antibody levels of the positive study sera (based on the reciprocal of the highest serum dilution yielding a positive response).

Antibody level	Positive study sera		
	No.	%	Cumulative %
8	28	4.1	4.1
16	171	25.0	29.1
32	187	27.3	56.4
64	183	26.8	83.2
128	94	13.7	96.9
256	19	2.8	99.7
512	2	0.3	100.0
Total	684	100.0	100.0

TABLE 5. Antibody levels of the positive study sera in each of the study subject five-year age groups.

Age group (in years)	Sera with the indicated antibody level														Total	
	8		16		32		64		128		256		512			
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
15–19	7	3.4	60	29.3	60	29.3	44	21.5	28	13.7	6	2.9	—	—	205	100
20–24	10	3.5	72	24.9	74	25.6	81	28	41	14.2	10	3.5	1	0.3	289	100
25–29	8	6.1	29	22.1	39	29.8	31	23.7	20	15.3	3	2.3	1	0.8	131	100
30–34	2	5.3	8	21.1	10	26.3	15	39.5	3	7.9	—	—	—	—	38	100
35–39	—	—	(2)	(12.5)[a]	(4)	(25)	(9)	(56.3)	(1)	(6.3)	—	—	—	—	(16)	100
40–44	(1)	(25)	—	—	—	—	(2)	(50)	(1)	(25)	—	—	—	—	(4)	100
45–49	—	—	—	—	—	—	(1)	(100)	—	—	—	—	—	—	(1)	100
Total	28		171		187		183		94		19		2		684	

[a] The figures in parentheses are not considered representative because of the small numbers of test sera obtained from subjects in the indicated age groups.

five-year age groups studied showed this same pattern, with most of the antibody values in each group being in the 16–64 range. It is also worth noting that the 15 sera yielding very high values (256 or 512) were distributed among the subjects in the younger (15–29 year) age groups, and none were found among the relatively smaller number of older subjects.

DISCUSSION AND CONCLUSIONS

The percentage of susceptible subjects found (11.9% ± 1.16%) was within an anticipated range of values (between 10 and 20%) derived from the findings of other researchers in our country *(7–9)* and upon data reported by the United States Centers for Disease Control (CDC) and the United Kingdom Ministry of Health *(13, 14)*.

It should be noted that most of the study subjects would become pregnant at some time in the future. Therefore, considering the incidence range of rubella in pregnant women (from some eight cases per 10,000 inhabitants in nonepidemic periods to as many as 200 cases per 10,000 during epidemics—*15*), the degree of susceptibility encountered is epidemiologically significant.

In general, a titer of 1:8 (antibody value of 8) or more was taken to indicate that the serum's donor had been infected with rubella at one time or another and had developed immunity to the infection. Titers between 1:16 and 1:64 were taken to indicate the probable existence of well-established and long-standing immunity. Finally, titers of 1:128 or more (found in 115 sera from mostly younger subjects 15 to 29 years old) were considered evidence of fairly recent infection—a demonstration of rubella virus circulation in the study subjects' geographic area of residence.

Since 1969, the year when an effective rubella vaccine became available *(16)*, various preventive strategies have emerged. In the United States, mass vaccination of preschool-age and young school-age children of both sexes has been adopted; this was done in order to block the chain of transmission, thereby providing indirect protection for pregnant women by reducing the risk of infection.[6] In addition, some states (including California, Colorado, and Rhode Island) have serologically screened women applying for premarital health certificates and have vaccinated those found susceptible to rubella.

In the United Kingdom, children 10 to 14 years old are systematically tested, and susceptible girls are vaccinated before reaching childbearing age.

A study done in the U.S. and published in 1979 estimated the cost of a single case of congenital rubella in that country as being on the order of US$162,000. This cost estimate included physicians' fees, medication, and special education provided for the victim *(17)*.

Regarding prevention of rubella in Argentina, the available data indicate that rubella is endemoepidemic, and that some 80 to 90% of all women now in their childbearing years have been infected. It thus appears advisable to seek out those women who are still susceptible, through serologic screening, and to provide them with immuniza-

[6] For more information about the success attained with this approach, see the report entitled "Rubella prevention in the United States" in the Abstracts and Reports Section, *Bulletin of the Pan American Health Organization* 19(4), 1985.

tion. One way of doing this would be to screen all women who apply for premarital health certificates, since it appears that all facilities performing serologic tests for syphilis could test sera for rubella-specific antibodies and vaccinate those women whose sera yielded negative results. It would of course be necessary to expressly advise all those vaccinated about the importance of avoiding impregnation during the three months following the vaccination *(18)*.

In weighing this selective vaccination strategy against that of mass vaccination, it is important to consider the costs and benefits of both. In this regard, serologic screening of girls in the 10–14 age group would require an infrastructure that is not now available in Argentina. It should also be noted that mass vaccination of boys and girls would be somewhat costlier than the selective strategy proposed, the cost of one vaccination being greater than the cost of a screening test.[7] Furthermore, the costs of screening can be expected to drop considerably if radial hemolysis in gel is employed. This latter procedure, just recently coming in, is simpler, more specific, and more sensitive than hemagglutination inhibition testing. It is also cheaper, because the sera are merely inactivated; no special treatment for removing nonspecific inhibitors is required; and a single technician can perform between 200 and 300 tests per hour *(19, 20)*. All of these factors suggest that a selective strategy such as that described is one deserving serious consideration.

[7] As of 1985, the cost of vaccination in Argentina was about US$3 per dose, versus the cost of US$2 for a hemagglutination inhibition screening test.

Summary

In the city of Mar del Plata, Argentina, sera from a group of 781 women applying for premarital health certificates, all between 15 and 49 years old, were screened by hemagglutination inhibition tests for rubella-specific antibodies. The test results showed that 12% of the women lacked rubella-specific antibodies and were presumably susceptible to infection. Of those 88% testing positively for rubella antibody, a small share (4%) were found to have antibody levels indicating some immunity; most (79%) were found to have levels indicating a well-established, long-lasting immunity; and some (17%) were found to have high antibody levels indicating relatively recent infections.

The percentage of susceptible women was within an anticipated range (10 to 20%) derived from prior research findings. Nevertheless, existing data on rubella morbidity patterns combined with these and related findings on susceptibility among women of childbearing age underline the point that the degree of susceptibility to rubella infection in Argentina has serious public health implications. Within this context, therefore, the authors propose that consideration be given to screening and selectively vaccinating women applying for premarital health certificates in order to prevent subsequent rubella infection during pregnancy.

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