Rubella Antibodies in Rhode Island Women of Childbearing Age

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ADVANCES in the virology, serology, and prevention of viral diseases have seldom come so rapidly as in the instance of rubella. Four different serologic techniques are now available, and prospects are excellent for licensing of the live attentuated virus vaccines currently undergoing field trials. Among these serologic techniques, the most recently developed, and perhaps the most convenient to perform, is the rubella hemagglutination-inhibition (HAI) test. Both the imminence of an effective vaccine and the convenience of the HAI test already have created considerable community and professional pressure upon health departments to make available promptly the necessary diagnostic laboratory services and to plan in advance for immunization programs. For example, at a recent meeting of State and territorial epidemiologists and laboratory directors (*1*), it was resolved that all State laboratories should perform tests for rubella antibodies. Others (*2*) have recommended that the serums now routinely obtained from couples before marriage for syphilis testing be examined for evidence of previous rubella infection as well.

In anticipation of these demands, the staff of the Rhode Island Department of Health, with the assistance of members of the department of epidemiology of Yale University, undertook an investigation to establish the feasibility of providing rubella antibody determinations as a diagnostic service and as an adjunct to the State program of premarital blood testing for syphilis. In addition, the investigation was aimed at delineating demographically the rubella-susceptible population of women of child-bearing age as an aid to the planning of any future, large-scale immunization programs.

Materials and Methods

Serums for rubella HAI determinations were obtained from blood specimens submitted for syphilis testing to the division of laboratories of the Rhode Island Department of Health. Altogether 651 specimens were collected, 537 during April and May 1967 and the remainder during September of the same year. If, upon completion of the requested test for syphilis, more than 0.5 ml. of a serum remained from a woman in the childbearing years, the specimen was stored in a screw-cap vial at −60° C. until it

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could be examined for the presence of rubella antibodies. The original laboratory request slip accompanying the specimen in most instances provided the donor's name, address, age, and the purpose for which the test for syphilis was performed. Census tracting has been completed for the entire State of Rhode Island, and social scientists at Brown University, in previous unrelated studies, have assigned an index of social rank to each tract (*3*). With the information on the laboratory request slip, it was therefore possible to classify most donors by socioeconomic status, as well as by age and place of residence.

Approximately 75 percent of the serums collected for our study had been submitted to the State laboratory for either premarital or prenatal tests for syphilis. The age of the donors ranged from 14 to 48 years; all but eight, however, fell in the range 16–45 years. Specimens were received from residents of 31 of the 39 cities and towns in Rhode Island, but most came from Providence, the major population center of the State, and adjacent communities. The location of the State laboratory in Providence may also have affected the pattern of collection.

Rubella HAI determinations were performed according to the microtechnique decribed by Stewart and associates (*4*) with but minor modifications. Disposable pipettes and microtiter plates with V-bottom wells were used. The rubella antigen and the reference serums were purchased from commercial sources, but the rest of the reagents were prepared in our laboratories. Red blood cells were obtained weekly by bleeding unfed Leghorn chicks within 24 hours of hatching. Cells were stored in Alsever's solution and washed in phosphate-buffered saline before use. No cells were used later than the 6th day after collection. Pretreatment of the serums by adsorption with chick cells, heat inactivation at 56° C., and adsorption with kaolin to remove nonspecific hemagglutinins and inhibitors was accomplished whenever convenient—even as much as 2 months before the actual HAI determination was performed. It was subsequently demonstrated that such pretreated serums when stored at −20° C. or below did not undergo any appreciable changes in titers.

All serums were tested in duplicate in serial twofold dilutions against four hemagglutinating units of antigen. Results were read following overnight storage at 4° C. In nearly all instances, the titers of the duplicate specimens were the same or within one dilution of one another. In those few instances in which greater discrepancies were observed, a second duplicate set of specimens was run, and pretreatment was repeated as necessary. Final titers recorded were the lowest in which there was good agreement between duplicate specimens. Women with HAI titers of less than 1 to 10 were regarded as susceptible to rubella, while those with higher titers, as probably immune. More than 90 percent of all women with detectable antibodies had titers of 1 to 80 or higher; more than half had titers of 1 to 640 or higher.

Results

For 71 of the 651 serums, rubella HAI titers of less than 1 to 10 were observed, an overall susceptibility rate of 10.9 percent. However, among 217 women whose blood was submitted for premarital syphilis testing, the rubella susceptibility rate was 12.9 percent while, of 271 serums submitted for prenatal testing, it was 10.7 percent. An analysis of susceptibility by ages is presented in table 1. In the age groups which accounted for 20 or more serums, the highest susceptibility rate (15.7 percent) was observed in the 21–25 year group. Lower rates were observed in both younger and older groups. By age 35, virtually all women apparently had previously experienced rubella infection. Using chi-square analysis, we found these differences were significant at $P<0.05$.

The information recorded on the laboratory

Table 1. Age distribution of donors of blood specimens who were found to be susceptible to rubella

Age (years)	Number in age group	Titer <1:10	
		Number	Percent
Under 16	7	1	14. 3
16–20	179	18	10. 1
21–25	197	31	15. 7
26–30	80	11	13. 8
31–35	44	1	2. 3
36–40	22	0	0
41–45	7	0	0
Over 45	1	0	0
Age not available	114	9	7. 9
All ages	651	71	10. 9

Table 2. Distribution by social class of donors of blood specimens who were found to be suseptible to rubella

Social class	Number in class	Mean age (years)	Rubella HAI titer<1:10	
			Number	Percent
1	15	23. 9	1	6. 7
2	45	25. 7	3	6. 7
3	84	22. 9	9	10. 7
4	140	23. 4	18	12. 8
5	115	24. 0	9	7. 8
Not available	252	24. 0	31	12. 3

request slip was adequate to permit the ranking of 399 of the specimen donors by social class. In the following table, the percentage distribution according to this variable is compared with that in the population of the entire State, class 1 being considered the highest rank and class 5 the lowest.

Social class	*Specimen donors*	*Population of Rhode Island*
1	3. 8	4. 9
2	11. 3	21. 7
3	21. 0	33. 9
4	35. 1	32. 8
5	28. 8	6. 7
All classes	100. 0	100. 0

Since the State laboratory does not charge for blood tests, it is not surprising that the less affluent classes are overrepresented in our specimen collection. The susceptibility to rubella by social class is shown in table 2. The mean ages of the groups in the five social classes were fairly similar. Classes 3 and 4 had the highest susceptibility rates, while classes 1, 2, and 5 appeared to have lower, nearly equal, rates. These class differences, however, were not statistically significant when subjected to chi-square testing.

The five largest cities in Rhode Island contain approximately 52 percent of the State's population but vary with respect to geographic location, total population, and population density. Since specimens were received from female residents of these cities at nearly equal sampling rates and since the age distribution and social classes of these donors were similar, the susceptibility rates for women 16–45 years from these cities were compared in an attempt to reveal differences related to place of residence (table 3). Although rates varied from 3.1 to 11.1 percent, the differences were not significant. It therefore appeared that neither location, total population, nor population density exerted an effect.

Discussion

The State laboratory processes approximately 50 percent of all serologic tests for syphilis in Rhode Island. Its central location in the major population focus insures convenience for those using its services. The absence of charges for laboratory services exerts a selective effect so that persons from whom specimens are submitted are likely to belong to the less affluent social classes. In addition, the most frequent reason that specimens are submitted for syphilis testing is to comply with the compulsory premarital and prenatal testing laws. The serums collected and examined in our study thus are fairly representative of the Rhode Island women of childbearing age who are the most active reproductively and who therefore constitute a suitable population upon which to base a rubella control program aimed at reducing fetal wastage and congenital defects.

Other investigators (*4–6*) have amply demonstrated both the specificity and the sensitivity of the rubella hemagglutination inhibition test. It is perhaps slightly more sensitive than the neutralization test, and the usual titers obtained are higher. However, use of either test in serologic surveys will yield similar results. Comparison of the results of our study with those of investigators who have relied mainly on the neutralization test is therefore reasonable.

Table 3. Susceptibility to rubella of Rhode Island women 16–45 years, by city of residence

City	Estimated population 1966	Persons per square mile	Estimated percent of women 16–45 susceptible
Providence	188, 170	11, 177	10. 5
Warwick	78, 097	1, 803	3. 1
Pawtucket	77, 998	9, 000	10. 0
Cranston	72, 339	2, 259	11. 1
Woonsocket	46, 955	5, 350	5. 6

The only previously reported data from Rhode Island is contained in a study by Sever and associates (*7*). Blood was obtained from 600 pregnant women who registered from January through March 1962 at 12 hospitals throughout the country which were participating in the Collaborative Study of Cerebral Palsy. Among these specimens were those of 50 white women from Providence, who had a mean age of 25.5 years. Rubella neutralizing antibody was absent in 14 percent. In the entire study group of 600 women, 17.5 percent had no antibody, and there was a progression of decreasing susceptibility with increasing age—from 25.2 percent susceptible in the 14–19 year age group to 10.9 percent in the 31–44 year group. Sever and co-workers (*8*) also examined serums from male and female residents of Maryland, collected in 1957. The donors ranged in age from 1 to 48 years. The percent with neutralizing antibody increased with age up to the adult age groups, in which the susceptibility rates remained in the 10–15 percent range. Somewhat similar results were obtained by Givan and associates (*9*), who examined serums of female residents of Ontario, Canada. They found that, while susceptibility decreased with increasing age, 20 percent of the women aged 24 and older remained susceptible.

In all of the studies mentioned, the rubella susceptibility rates were generally higher than we have found and, in contrast to our results, there was a pattern of progressively diminished susceptibility with increasing age, at least up to age 25. We believe these differences can best be explained in terms of the well-known 1964–65 U.S. rubella epidemic. The effects of this epidemic were much more drastic in the northeastern United States than elsewhere (*10*). In Rhode Island, the epidemic began in late 1963, and by the end of 1964 reported cases were 100-fold greater than the number that had been recorded in 1962. The lack of significantly different susceptibility rates for women of different social classes and for residents of different cities indicates the extent to which rubella virus had saturated the population of Rhode Island. Since children in elementary school and the early high school years represent the major group transmitting epidemic rubella (*8,9*), transmission in other age groups is most often a consequence of contact with these 5–15 year olds. During the 1964 epidemic, 3 years before our collection of serums, the present 16–20 year group—then 13–17 years old—was presumably heavily involved in transmission and, not surprisingly, now has a low susceptibility rate. The next two older groups, who at the time of the 1964 epidemic were out of high school, but either unmarried or married with no children or with only infants, would have had less opportunity for acquiring the disease from school-age children than the 13–17 year old group, and their susceptibility rates are now correspondingly higher. However, since previous experience suggests a 7-year cycle of major rubella activity, mothers who, because of the advancement of their children into the school-age bracket, are in the groups currently more susceptible will incur increased exposure about 1971 unless vaccine development makes possible an extensive immunization effort before then.

Institution of a rubella testing program aimed at the women who are reproductively active or potentially so can be an important adjunct to forestalling the harmful effects of another epidemic period. By making it possible for a woman to know her immune status, such a program would enable a susceptible woman who is pregnant or considering pregnancy to take steps to minimize an exposure to rubella. In most instances, it could easily be decided whether there was cause for concern or not since, as our results indicate, nine of 10 women are immune. Certainly, serologic confirmation of recent infection is desirable whenever termination of pregnancy is being considered. In addition, the positive knowledge of immunity to rubella provides a pregnant woman with a sense of comfort that can hardly be underestimated, especially when she is faced with inadvertent or unavoidable exposure to rubella or rubella-like disease. If precise information were available on susceptibility, immune globulin prophylaxis could be used much more judiciously than at present and with considerable savings to those health departments that make this procedure available free of charge to exposed pregnant women. We hope that it will soon be possible to couple a broad-based rubella immunization program with a premarital testing program and thereby greatly reduce the reservoir of susceptibiles and

the opportunity for rubella-induced fetal damage.

In view of the potential benefits of a rubella testing program, the cost is fairly low. If the tests are performed in the Rhode Island State Laboratory, we estimate the total cost (expendables, labor, equipment, and so forth) per test would be $1.55, or possibly even less if additional economies could be effected through large-scale operation. By comparison, in the State laboratory, the routinely used test for syphilis reactivity costs $1. We further estimate from our study and from population data (*11*) that the overall cost of identifying a rubella-susceptible Rhode Island woman of childbearing age would be approximately $15. Again, for comparison, the cost of identifying a syphilis reactive person is more than 10 times greater. There seems to be little question that a testing program is not only highly desirable but also eminently economical, particularly when we consider the ultimate cost to the general welfare of even a handful of rubella-damaged children.

Summary

Rubella hemagglutination inhibition (HAI) antibody levels were determined on the serums of 651 Rhode Island women of childbearing age whose blood specimens had been submitted to the State laboratory for testing of syphilis reactivity. Most serums represented specimens submitted either for premarital or prenatal testing. The susceptibility rate (HAI titer < 1 to 10) for the total group was 10.9 percent; among women whose blood was submitted for premarital syphilis testing, however, the rate of susceptibility to rubella was 12.9 percent. The highest susceptibility rate (15.7 percent) was observed in the age group 21–25 years; lower rates were observed in both younger and older women. This somewhat unusual age distribution of susceptibility was apparently an effect of the 1964 U.S. rubella epidemic, which heavily affected the northeastern States. Differences in susceptibility could not be related to either social class or place of residence.

Laboratory costs of the HAI test compared favorably with those of tests for determining syphilis reactivity. Provision of the rubella HAI test as a diagnostic service of the State laboratory and as an addition to the premarital testing requirements would be a fruitful and economical adjunct to a statewide rubella control program.

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