Rubella Vaccination of Unknowingly Pregnant Women: The São Paulo Experience, 2001

Helena K. Sato,[1] Andrea Torres Sanajotta,[1] José Cássio Moraes,[3] Joelma Queiróz Andrade,[4] Geraldo Duarte,[4] Maria Célia Cervi,[4] Sueli P. Curti,[2] Cláudio Sérgio Pannuti,[6,7] Helaine Milanez,[5] Mônica Pessoto,[5] Brendan Flannery,[8] Gabriel W. Oselka,[7] and the São Paulo Study Group for Effects of Rubella Vaccination During Pregnancy[a]

[1]Immunization Division, [2]Adolfo Lutz Institute, São Paulo State Health Department; [3]Santa Casa Medical School, São Paulo; [4]University of São Paulo - Riberão Preto Medical School; [5]São Paulo State University of Campinas; [6]Tropical Medicine Institute, and [7]University of São Paulo; and [8]Pan American Health Organization, Brasília, Brazil

Background. Rubella vaccination is contraindicated during pregnancy. During mass immunization of women of childbearing age against rubella, women unknowingly pregnant may be vaccinated. To evaluate the effects of rubella vaccination during pregnancy, the Brazilian state of São Paulo conducted a follow-up study of pregnant women vaccinated during a rubella campaign in 2001.

Methods. Women vaccinated during pregnancy were reported to a national surveillance system. In the state of São Paulo, follow-up of vaccinated women included household interviews. Serum samples from vaccinated women were tested for antirubella antibodies to classify susceptibility to rubella infection. Children born to susceptible mothers were tested for evidence of congenital rubella infection and evaluated for signs of congenital rubella syndrome.

Results. The São Paulo State Health Department received 6473 notifications of women vaccinated during pregnancy. Serology performed for 5580 women identified 811 (15%) that were previously susceptible. Incidence of spontaneous abortion or stillbirth among previously susceptible vaccinated women was similar to women with prior immunity. Twenty-seven (4.7%) of 580 newborns tested had evidence of congenital rubella infection; none had congenital rubella syndrome.

Conclusions. Mass rubella vaccination of women of childbearing age was not associated with adverse birth outcomes or congenital rubella syndrome among children born to women vaccinated during pregnancy.

Wild-type rubella viruses are extremely teratogenic [1]. Women infected with wild-type rubella virus during the first 20 weeks of pregnancy have a 20% or greater risk of having a child born with congenital rubella syndrome (CRS) [2]. Infants born with CRS often have hearing impairment, cataracts, and congenital heart defects, and may have other clinical manifestations associated with CRS, including microcephaly, retinopathy, hepatosplenomegaly, purpura, meningoencephalitis, radiolucent bone disease, low birth weight, and developmental delay [3].

Occurrence of rubella infection and CRS has been dramatically reduced in countries that have implemented successful rubella vaccination programs. Rubella vaccines are live, attenuated viruses developed over 40 years ago [2]. Vaccine viruses cause viremia and may cross the placenta in pregnant women and infect the fetus [4]. Although no cases of CRS have been observed among infants born to women vaccinated during pregnancy, documentation of the safety of rubella vaccination during pregnancy in the scientific literature is limited [5]. Rubella vaccination is contraindicated during pregnancy due to a theoretical risk of CRS in infants infected in utero with rubella vaccine viruses [6].

In the Brazilian state of São Paulo, 3 actions implemented in 1992, in conjunction with accelerated

[a]São Paulo Study Group for Effects of Rubella Vaccination During Pregnancy members (in alphabetical order) are listed in the Acknowledgments section.

Potential conflicts of interest: none reported.

Presented in part: Pan American Organization ad hoc panel experts meeting on rubella and measles, Washington, DC, 27 July 2006.

Correspondence: Helena K. Sato, MD, PhD, Divisão de Imunização, Secretaria Estadual de Saude de São Paulo, Av. Dr. Arnaldo 351, sala 619, Sumarezinho 01246-902, São Paulo, Brazil (hsato@cve.saude.sp.gov.br).

The Journal of Infectious Diseases 2011;204:S737–S744

0022-1899 (print)/1537-6613 (online)/2011/204S2-0027$14.00
DOI: 10.1093/infdis/jir419

measles elimination strategies [7], led to the recognition of rubella as a public health problem: (1) the addition of rubella and CRS to the list of notifiable diseases; (2) the implementation of rubella surveillance, including testing for rubella-specific immunoglobulin M (IgM) antibodies in all suspected measles case–patients who tested IgM negative for measles; and (3) the introduction of measles-mumps-rubella (MMR) vaccine with a catch-up campaign among children 1–11 years old. Between 1992 and 2000, the incidence of rubella among adults increased dramatically, with a steady rise in detection of CRS cases. In 2000, the state of São Paulo registered 2556 confirmed rubella cases among adults aged 20–29 years (23.7 cases per 100 000 persons in this age group) with 133 confirmed rubella cases in pregnant women [8]. To reduce rubella transmission and prevent additional cases of CRS, Brazil launched a national campaign to vaccinate women of childbearing age against rubella. Recognizing the potential that some women could be vaccinated within a short interval before conception or during pregnancy, the São Paulo State Health Department commissioned a study to follow up with women vaccinated during pregnancy to determine the effects on the fetus. The objectives of the study were to estimate the incidence of congenital infection with rubella vaccine virus among infants born to susceptible women, determine the risk of CRS in infected infants, and evaluate the influence of susceptibility to rubella infection on pregnancy outcomes.

METHODS

CRS Surveillance System

Reporting of CRS has been mandatory in the state of São Paulo since 1992. Definitions for suspect, compatible, and confirmed CRS cases and congenital rubella infection are nationally standardized [9]. When health professionals notify local health departments of infants suspected of having CRS, surveillance personnel investigate to obtain blood specimens for laboratory diagnosis, clinical information, and maternal vaccination history. In addition, pregnant women with confirmed rubella infection are followed until delivery to evaluate their newborns for CRS and congenital rubella infection. In 2001, the notification rate was 4 suspect CRS cases per 10 000 live births, and 80% of suspect cases had at least 1 blood specimen for laboratory testing.

Identification and Follow-up of Vaccinated Pregnant Women

In 2001, the Brazilian state of São Paulo had a population of 40 million inhabitants, with 4.8 million women between 15 and 29 years of age in the target age range for the rubella vaccination campaign. From November 2001 to February 2002, 4 408 844 women, corresponding to 91.6% of the target population, were vaccinated at 3500 vaccination posts throughout the state during the national rubella campaign. Combined measles-rubella (MR) vaccine was purchased by the national immunization program from the Serum Institute of India and contained 1000 cell culture infectious dose 50% per dose of the 2 vaccine strains (Edmonston Zagreb and Wistar RA27/3).

In preparation for the rubella campaign, the São Paulo State Health Department discussed contraindications for rubella vaccination, including pregnancy, in training materials, press releases, and presentations to medical and professional societies. Posters at vaccination sites instructed pregnant women to defer vaccination until after giving birth, and advised women receiving MR vaccine to avoid pregnancy for 1 month [8, 10]. A reporting system was created to identify and follow up with women who were unknowingly pregnant at the time of vaccination for serologic testing. Health professionals notified municipal and state health authorities of cases of pregnant women who reported receiving MR vaccine while pregnant or within 1 month of the estimated date of conception. Case notification forms were sent to municipal health departments, and blood specimens were sent to state public health laboratories. Only women with confirmed pregnancy and documented or verbal report of rubella vaccination received follow-up.

Upon receiving serologic results, surveillance personnel conducted in-person interviews to obtain sociodemographic information (age, residence, schooling, prenatal care received in public or private health setting), vaccination date, date of last menstruation, and known gestational risk factors (maternal weight and height, parity, urinary infection, high blood pressure, diabetes, heart disease, risk of premature delivery, aggression, smoking, alcohol consumption). Women who were susceptible to rubella at the time of vaccination were informed of the theoretical risk of congenital rubella infection of the fetus. Susceptible women received sample collection kits to take to the maternity ward for collection of a blood sample from their newborn. A second household visit was conducted within 30 days postpartum to record data from the child's birth record (birth weight and length, head circumference, gestational age, Apgar score, and vaginal or caesarian delivery). No clinical examination was performed by the interviewer. Interviewers were instructed to copy information about malformations and signs of CRS recorded on the child's birth record.

Neonates testing positive for rubella IgM antibodies at birth were referred to 1 of 4 pediatric specialists for clinical assessment, radiological examination of long bones, cardiac evaluation and echocardiography, ophthalmologic evaluation (including examination of the back of the eye), auditory assessment (including otoacoustic wave emission and/or brainstem-evoked response audiometry [BERA]), neurologic assessment, and evaluation by a geneticist. Serum and urine samples were collected for detection of rubella viral DNA.

The study was approved by the Ethics Commission for Research Project Analysis of the University of São Paulo Faculty of Medicine.

Laboratory Methods

Serum samples were tested for rubella-specific immunoglobulin G (IgG) and IgM antibodies at state public health laboratories of the Adolfo Lutz Institute using commercial enzyme immunoassays (Organon Teknika). Samples collected between 30 and 70 days after vaccination that tested seronegative for IgM and seropositive for IgG antibodies were tested for IgG antibody avidity at the Tropical Medicine Institute of São Paulo using commercial kits (rubella-Enzygnost, Dade-Behring) with addition of an incubation step in 8 M urea as a dissociation agent. The avidity index, expressed as a percentage, was calculated by dividing the optical density obtained with urea by the optical density obtained without urea, multiplied by 100. An avidity index ≤30% was defined as weak avidity reflective of recent infection [11, 12].

For detection of rubella virus RNA in samples from IgM-positive infants, peripheral blood mononuclear cells were inoculated into serum institute rabbit cornea cells, and rubella viral RNA was amplified using a nested, reverse transcription polymerase chain reaction (PCR) as previously described [13, 14]. Nucleotide sequences of amplified products were compared with the RA27/3 vaccine strain.

Definitions

Pregnant women who tested positive for antirubella IgM antibodies in postvaccination serum samples were classified as having been susceptible to rubella infection at the time of vaccination, although their IgM antibodies may have resulted from past exposure to rubella virus. Women with antirubella IgG antibodies of low avidity in serum samples collected ≤70 days after vaccination were also considered susceptible to rubella infection. Women were classified as previously immune to rubella infection if serum samples collected within 30 days of vaccination tested negative for antirubella IgM antibodies but positive for IgG antibodies. Women with IgG antibodies of high avidity in serum samples collected 30–70 days after vaccination were also considered immune. Women who tested negative for both IgM and IgG antibodies in serum samples collected >30 days after vaccination were defined as nonreactive. Susceptibility to rubella at the time of vaccination was indeterminate if serum samples collected >70 days after vaccination tested negative for IgM and positive for IgG antibodies.

For all vaccinated pregnant women, spontaneous abortion was defined as the loss of a fetus <500 g in weight or prior to 22 weeks gestation, and stillbirth as death of a fetus weighing ≥500 g or at 22 weeks gestation or later [15]. For live births, prematurity was defined as gestational age <37 weeks and low birth weight as <2500 g. Complications during pregnancy (risk of premature birth, hypertension, or urinary tract infection) were defined as any or none based on self-report. Tobacco use was defined as smoking 1 or more cigarettes per day during pregnancy, and alcohol use during pregnancy was classified as any or none.

Congenital infection with rubella vaccine virus was defined as positive antirubella IgM enzyme-linked immunosorbent assay (ELISA) in a neonate born to a susceptible mother without evidence of exposure to wild-type rubella virus. Viral testing was not available. To be diagnosed with CRS, the a priori case definition (according to the World Health Organization [16]) required a positive antirubella IgM ELISA in an infant presenting with 2 major criteria of CRS (cataract or glaucoma, congenital heart disease, deafness, pigmentary retinopathy) or 1 major and 2 minor criteria (purpura, splenomegaly, jaundice, microcephaly, developmental delay, meningoencephalitis, radiolucent bone disease).

Statistical Analysis

Statistical comparisons were made to test 2 distinct hypotheses: (1) birth outcomes, including prevalence of spontaneous abortion, stillbirth, low birth weight, and prematurity, would differ between susceptible and immune women vaccinated during pregnancy; and (2) congenital infection with rubella vaccine virus would be associated with the incidence of low birth weight and prematurity among children born to women vaccinated during pregnancy. Characteristics of susceptible and immune women were compared using Fisher exact test or χ^2. Odds ratios (ORs) and exact 95% confidence intervals (CIs) were calculated for factors associated with birth outcomes or congenital rubella infection, and multivariable logistic regression was used to control for confounding variables. Variables were selected for assessment in logistic regression models for risk of spontaneous abortion, low birth weight, prematurity, or congenital rubella infection if univariate associations presented $P < .2$. Statistical significance was accepted at $P < .05$. Data were entered in EpiInfo version 6.04 (Centers for Disease Control and Prevention) and analyzed in EpiInfo version 6.04, EpiInfo version 3.4.3 (Centers for Disease Control and Prevention), and SPSS for Windows (release 11, SPSS Inc.).

RESULTS

The São Paulo State Health Department received 6473 notifications of women who received MR vaccine during pregnancy or prior to becoming pregnant (a rate of 1.47 notifications per 1000 women vaccinated). Serum samples were available for 5580 (86.2%) unknowingly vaccinated women (Figure). Based on rubella serology, 811 (14.5%) of 5580 women with serum samples submitted were considered susceptible to rubella infection at the time of vaccination, 2135 (38.3%) were previously immune, 27 (0.5%) had no detectable immune response, and 2607 (46.7%) had indeterminate serology due to collection of serum samples >70 days after vaccination. Among 1686 serum samples submitted for antirubella IgG antibody avidity testing, 118 (7.0%) demonstrated the presence of low avidity IgG antibody, suggesting prior susceptibility to rubella infection.

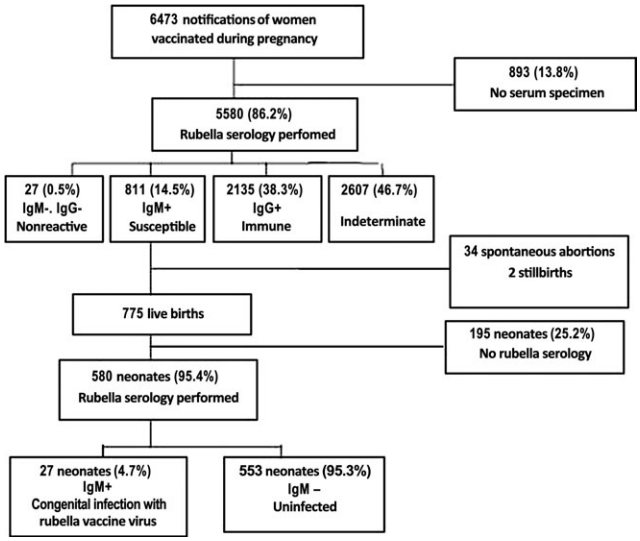

Figure. Notifications and results of follow-up of women vaccinated with measles-rubella vaccine during pregnancy in the state of São Paulo during the national rubella campaign in Brazil, 2001–2002.

Household interviews and follow-up to determine birth outcomes were conducted for 644 (79.4%) of 811 susceptible and 1433 (70.5%) of 2135 previously immune women. Table 1 compares characteristics and birth outcomes of susceptible and previously immune women. Vaccination occurred predominantly in the first trimester of gestation. Among 2077 women interviewed, 1644 (79.2%) were aware that vaccination was contraindicated during pregnancy, and 2033 (97.9%) reported being unaware of their pregnancy at the time of vaccination. Blood samples were taken for serology processing at an average of 40–50 days after vaccination for both groups. Women <20 years of age at the time of vaccination were more likely to have prior immunity to rubella than older age groups, reflecting the effects of immunization strategies in the past that focused on children and adolescents. Differences in proportions of spontaneous abortion, stillbirth, premature births, or births weighing <2500 g were not significant.

In multivariable analyses, prevaccination susceptibility to rubella was not associated with spontaneous abortion (OR, 0.68; 95% CI, .44–1.06), premature delivery (OR, 1.3; 95% CI, .9–1.8), or having an infant weighing <2500 g (OR, 0.7; 95% CI, .5–1.1). Spontaneous abortion was independently associated with lack of private insurance for prenatal care (OR, 2.7; 95% CI, 1.2–6.2) and complications during pregnancy (OR, 2.2; 95% CI, 1.2–4.0). Independent risk factors for premature birth included primiparity (OR, 1.6; 95% CI, 1.1–2.2), complications during pregnancy (OR, 3.6; 95% CI, 2.2–5.8), and smoking during pregnancy (OR, 1.8; 95% CI, 1.2–2.8). Similarly, having an infant weighing <2500 g was strongly associated with premature delivery (OR, 15.2; 95% CI, 9.9–23.3), in addition to maternal weight <50 kg prior to becoming pregnant (OR, 1.6; 95% CI, 1.0–2.5), hypertension (OR, 2.3; 95% CI, 1.3–3.8), and complications during pregnancy (OR, 2.4; 95% CI, 1.4–4.1).

Follow-up of children born to susceptible women included testing newborns for rubella-specific IgM antibodies. Serum samples were tested for 580 newborns of susceptible women, including 541 live-born infants with complete follow-up (Table 2). Serum samples were not collected from newborns of women classified as immune or with indeterminate serology. Antirubella IgM antibody was detected in 27 (4.7%) of 580 infants tested. Rubella RA27/3 vaccine virus was isolated and confirmed by reverse transcription PCR from 6 of 10 IgM-positive infants tested. Assuming that all 27 infants were congenitally infected suggests a rate of 4.7 congenital infections (95% CI, 3.1–6.7) per 100 live-born infants among previously susceptible women. Compared to IgM-negative children born to previously susceptible women, IgM-positive children were more likely to be premature, weigh <2500 g at birth, and have mothers who used tobacco during pregnancy (Table 2). In multivariable analysis, only tobacco use during pregnancy remained significantly associated with the presence of rubella IgM in the child. Results were consistent when infants born with evidence of congenital rubella infection were compared with infants of previously immune mothers.

For the 27 infants with evidence of congenital infection with rubella vaccine virus, the average gestational age at the time of mother's vaccination was 3 weeks; 1 mother's last menstrual period was 8 days prior to vaccination, and another mother was vaccinated 2 days after her last menstrual period. Among 9 infected infants born prematurely and/or weighing <2500 g, only 1 infant had an extended nursery stay for antibiotic therapy to treat a presumed bacterial infection. Although complications during pregnancy were not associated with congenital rubella infection in the final model, 3 (43%) of 7 mothers whose infected infants were born prematurely and 3 (30%) of 6 mothers whose infected infants were born weighing <2500 g reported complications during pregnancy (hypertension [n = 2], vaginal bleeding [n = 1], and urinary tract infection [n = 2]).

None of the 27 neonates presented clinical manifestations compatible with CRS (Table 3). All 27 infants with congenital rubella infection were normal upon clinical examination; 18 (67%) completed all tests included in the study protocol without evidence of CRS, while the remaining 9 had no evidence of CRS in the evaluations completed. Among 21 neonates tested for hearing impairments, none presented hearing deficits. Among 23 infants who underwent echocardiograms, 1 child presented an interatrial communication ~0.5 cm in diameter that spontaneously closed within the first year of life. None of the 23 infants examined by ophthalmologists presented cataracts or glaucoma. Of the 27 neonates followed during the first 2 years of life, none presented compromised psychomotor development.

During 2001–2002, a total of 440 infants with suspected CRS were reported to the state health department; none of the 56 confirmed or compatible CRS cases (including 40 infants

Table 1. Characteristics and Outcomes of Women Vaccinated During Pregnancy, by Classification of Rubella Susceptibility, São Paulo State, 2001–2002

Variable	Susceptible[a] (n = 644)	Immune[a] (n = 1433)	*P* value
Timing of vaccination[b]			
Prior to conception	170 (26.6)	244 (17.3)	<.001
0–4 weeks post conception	347 (54.4)	780 (55.4)	Ref[c]
5–12 weeks post conception	99 (15.5)	311 (22.1)	.01
>12 weeks post conception	22 (3.4)	73 (5.2)	.15
Unaware of pregnancy at vaccination	634 (98.9)	1399 (97.9)	.09
Age (years)			
<20	99 (15.4)	447 (31.2)	<.001
20–29	494 (76.8)	883 (61.7)	Ref
≥30	50 (7.8)	102 (7.1)	.5
White race	427 (67.3)	977 (68.9)	.3
Private health insurance	75 (11.8)	172 (12.2)	.9
Primiparity	293 (45.5)	697 (48.6)	.2
Any tobacco use during pregnancy	80 (12.4)	213 (14.9)	.4
Any alcohol use during pregnancy	56 (8.7)	101 (7.0)	.5
Any complications of pregnancy	283 (44.0)	670 (46.8)	.2
Risk of premature labor	28 (4.4)	116 (8.1)	.003
Hypertension	44 (6.9)	159 (11.1)	.01
Urinary tract infection	128 (19.9)	284 (19.8)	.5
Outcomes of pregnancy			
Live birth	608 (94.4)	1320 (92.1)	Ref
Spontaneous abortion	34 (5.3)	103 (7.2)	.12
Stillbirth	2 (0.3)	10 (0.7)	.4
Gestational age of live births			
<37 weeks	55 (9.1)	102 (7.8)	.4
≥37 weeks	550 (90.9)	1205 (92.2)	Ref
Birth weight for live births			
<1500 g	1 (0.2)	17 (1.3)	.03
1500–2500 g	37 (6.1)	99 (7.5)	.3
>2500 g	570 (93.8)	1209 (91.2)	Ref

NOTE. Percentages may not sum to 100% due to missing responses. Ref, referent group.

[a] Data are no. (%).

[b] Timing of vaccination was defined based on last reported menstrual period.

[c] For variables with 3 categories, 2 tests for significance were performed.

investigated in 2001 and 16 infants in 2002) were born to vaccinated mothers. The surveillance system also investigated 1 report of a child born with cleft palate and congenital cataracts to a previously immune vaccinated woman who associated her child's malformation with rubella vaccine. The neonate was followed to rule out a possible association with rubella vaccine. Serum samples collected 10 days after birth were antirubella IgM seronegative and IgG seropositive. Three months after birth, both IgM and IgG were seronegative. A urine specimen collected at 3 months was negative by PCR for rubella viral DNA.

DISCUSSION

The results of this study contribute to the scientific literature available on the safety of rubella vaccines when unknowingly pregnant women are vaccinated. Data compiled by the U.S. Centers for Disease Control and Prevention on 680 neonates born to women vaccinated during pregnancy reported asymptomatic infection in 2 neonates and none with clinical evidence of CRS [5]. The present study assessed 580 children of mothers known to be susceptible to rubella who were vaccinated shortly before or in the first few weeks after conception; 27 (4.7%) neonates had evidence of congenital rubella infection, and RA27/3 vaccine virus was recovered from 6 of 10 infants tested. None of the 27 congenitally infected infants presented clinical manifestations compatible with CRS. In addition, among 18 infected children who completed an extensive clinical follow-up protocol through the first 2 years of life, no birth defects compatible with CRS were identified. Although pregnancy continues to be a contraindication for receipt of rubella vaccine, this study

Table 2. Comparison of Gestational Age, Birth Weight, and Maternal Characteristics of Newborns Congenitally Infected With Rubella Vaccine Virus Versus Uninfected Newborns of Susceptible Mothers, São Paulo State, 2001–2002

Variable	Live births to susceptible mothers		Odds ratio (95% CI)	Adjusted odds ratio[a] (95% CI)
	Antirubella IgM seropositive[b] (n = 27)	Antirubella IgM seronegative[b,c] (n = 514)		
Timing of vaccination[d]				
Prior to conception	2 (7.4)	124 (24.1)	Ref	…
0–4 weeks post conception	20 (74.1)	318 (61.9)	3.9 (.9–34.8)	…
5–12 weeks post conception	3 (11.1)	56 (10.9)	3.3 (.4–40.5)	
>12 weeks post conception	0	14 (2.7)	0 (0–49.2)	
Age (years)[a]				
<20	0	75 (14.6)	0 (0–1.1)	…
20–29	21 (80.8)	402 (78.2)	Ref	…
≥30	5 (19.2)	37 (7.2)	2.6 (.7–7.6)	…
Birth weight				
<2500 g	6 (22.2)	25 (4.9)	5.6 (1.7–16.0)	3.2 (.9–11.7)
>2500 g	21 (77.8)	489 (95.1)	Ref	Ref
Gestational age				
<37 weeks	7 (25.9)	42 (8.2)	3.9 (1.3–10.3)	2.1 (.6–7.0)
≥37 weeks	20 (74.1)	470 (91.8)	Ref	Ref
Any complications of pregnancy[a]	11 (42.3)	232 (45.1)	0.9 (.4–2.1)	…
Risk of premature labor[a]	2 (7.7)	21 (4.1)	2.0 (.2–8.8)	…
Hypertension[a]	2 (7.7)	18 (3.5)	2.3 (.2–10.5)	…
Urinary tract infection[a]	5 (19.2)	106 (20.6)	0.9 (.3–2.6)	…
Any maternal tobacco use during gestation	8 (29.0)	63 (12.3)	3.0 (1.1–7.6) Ref	2.5 (1.0–6.3)[e] Ref
Alcohol use[a]	3 (11.1)	45 (8.8)	1.4 (.3–4.8)	…

NOTE. Final logistic regression model for factors associated with congenital rubella infection included birth weight <2500 g, gestational age <37 weeks, and maternal smoking. Percentages may not sum to 100% due to missing responses. Ref, referent group; CI, confidence interval; IgM, immunoglobulin M.

[a] Missing data for 1 mother-child pair with congenital rubella infection.

[b] Data are no. (%).

[c] Data from household visit not available for 39 IgM-negative live-born infants to susceptible mothers.

[d] Missing data for 4 mother-child pairs, 2 with congenital rubella infection and 2 without infection.

[e] $P = .04$

contributes evidence from follow-up of a large number of women vaccinated during pregnancy to the global evidence for the safety of rubella vaccine. Because there continue to be reports of vaccinated pregnant women counseled to terminate their pregnancy, health providers should be informed of the absence of scientific evidence for an association between rubella vaccination and CRS risk.

Most of the women identified by the surveillance system had been vaccinated during the first trimester of pregnancy, the period of embryogenesis and fetal development. The incidence

Table 3. Results of Clinical Evaluation of Children With Congenital Infection of Rubella Vaccine Virus

Specialty	Number of children evaluated (n = 27)	Tests performed	Comments
Pediatrics	27	Postnatal examination	No observation of hepatosplenomegaly, purpura, acute meningoencephalitis, or jaundice
Audiology	21	otoacoustic wave emission and/or BERA	Normal ranges
Cardiology	23	Echocardiogram	1 child with interatrial communication 0.5 cm diameter; self-resolved at 1 year of age
Ophthalmology	23	Back of the eye	Normal; no children identified with cataracts or glaucoma
Neurology	27	Neuropsychomotor development	Followed during 2 years of life

NOTE. BERA, brainstem-evoked response audiometry.

of congenital infection with RA27/3 rubella vaccine in the state of São Paulo was consistent with estimates from 0% to 6.7% in similar follow-up studies of unknowingly pregnant women vaccinated during mass campaigns [17–20], and higher than reported in earlier studies [21–23]. This suggests that congenital infection following vaccination is more common than previously thought [1, 6], although minimal compared with the estimated 80% risk of congenital infection with wild-type rubella virus when maternal infection occurs in the first trimester of pregnancy [1]. Improved detection of congenital infection may also be due to widespread use of antirubella IgM immunoassays (with reported sensitivities of 90%–99% and specificities of 98%–100% [24]), which are more accurate than hemagglutination tests used in the past [1].

The inclusion of the IgG avidity test in the screening protocol for women vaccinated during pregnancy helped identify susceptible women with collection of serum samples for rubella serology up to 70 days after vaccination. The cutoff at 70 days was based on an investigation of serologic response to measles vaccination in young children, in which 99% of vaccinated children tested within 70 days of immunization had low-affinity antimeasles IgG antibody, versus none of the children more than 70 days after measles vaccination [25]. Among vaccinated pregnant women tested in the state of São Paulo, a low percentage (7%) had evidence of low-affinity antirubella IgG antibodies suggestive of recent infection. Therefore, these women had been susceptible to rubella infection at the time of vaccination. They were informed of the theoretical risk of infection in their infant and included in clinical follow-up. For every 7 susceptible women identified using IgM ELISA, avidity testing identified 1 additional susceptible woman.

One limitation of this analysis is the incompleteness of notification of women vaccinated during pregnancy and serological testing of infants born to susceptible women. However, it is unlikely that a child born with CRS to a mother not enrolled in the study would have remained undetected by surveillance in the state of São Paulo. Attention to cases of CRS was heightened during the period following the rubella campaign independent of the present study. We did not consider the occurrence of 1 case of interatrial communication, which self-resolved, as a heart defect compatible with CRS because it was not consistent with clinical manifestations of CRS described in the literature [3, 26, 27]. A second limitation was the use of previously immune women as the control group for pregnancy outcomes, which may not have been representative of births among unvaccinated women in the state of São Paulo. However, we hypothesized that rubella vaccination of immune pregnant women should not result in viremia and therefore these women would have a pregnancy outcome comparable to that of unvaccinated women. Further, rates of low birth weight and prematurity observed in the present study are similar to those previously reported in the state of São Paulo [28, 29]. Susceptibility to rubella was not associated with occurrence of spontaneous abortion, incidence of premature infants, or low birth weight.

Although we identified a relatively large number of infants born with congenital rubella infection compared with previous studies, the sample size was too small to investigate the independent association between congenital infection with rubella vaccine virus and low birth weight or prematurity. Higher proportions of congenitally infected infants were born at <37 weeks gestation or weighing <2500 g compared with uninfected infants of susceptible mothers or infants born to previously immune mothers. However, associations were not significant when controlling for the mother's reported smoking during pregnancy. We identified no reports in the literature of an association between congenital infection with rubella vaccine virus and low birth weight or preterm births. It is biologically plausible that infection with rubella vaccine virus, albeit attenuated, could increase the incidence of preterm or low-birth-weight births. We recommend that follow-up of women vaccinated during pregnancy collect information on birth outcomes as well as possible confounding factors so that available data can be combined for analysis.

In order to assess possible effects of congenital infection with rubella vaccine virus, implementation of a reporting system for vaccinated pregnant mothers and epidemiological follow-up are required. These investigations are fundamental to rule out other possible causes of congenital malformations, including infections with cytomegalovirus, herpes virus, or *Toxoplasma*, in addition to genetic diseases. Approximately 2%–3% of neonates present malformations without any apparent cause [2]. Laboratory testing for the presence of rubella virus in serum or urine samples was not conducted for all IgM-positive infants. Especially during the circulation of wild-type rubella virus, investigation of CRS cases and collection of specimens for viral isolation from infants born to vaccinated women are essential to determine if the child was infected with wild-type or vaccine virus.

In 2008, Brazil conducted a national vaccination campaign to accelerate the elimination of rubella and CRS in which more than 67 million adult men and women 12–39 years of age were vaccinated against rubella. The vaccination of women of childbearing age involves the inherent risk of vaccinating unknowingly pregnant women despite implementing safeguards and publicizing contraindications. Identification and follow-up of vaccination of unknowingly pregnant women is vital to maintain public confidence in the immunization program. The findings of this study were important in preparation for the national campaign to speed up the elimination of rubella transmission in Brazil.

Funding

This research was supported by the São Paulo State Health Department and the Pan American Health Organization.

Acknowledgments

The authors recognize the work of many persons in municipal health departments, public health clinics, hospitals, and laboratories throughout the state of São Paulo who made this study possible. We thank A. Stangali, H. H. S. Marques, M. Z. Aquino, P. T. Sakane, L. Y. Wecks, L. Moron, B. A. F. Arantes, M. I. B. Pavão, M. Izumi, I. A. A. Andrade, N. V. A. L. Araújo, M. A. F. Góes, T. R. F. Zanni, E. A. Waldman, and the Council of Municipal Health Departments for the state of São Paulo for their contributions.

São Paulo Study Group for Effects of Rubella Vaccination During Pregnancy members: T. R. Almeida, K. J. F. Alves, C. P. Andrade, C. M. S. S. Aranda, L. M. R. Araujo, M. C. A. Araújo, S. M. N. Baba, M. T. Barbieri, E. L. L. Brunera, V. Bunduki, M. S. Carli, W. E. Carneiro, T. R. M. P. Carvalhanas, M. I. C. E. Cerezine, F. H. T. Ciccone, G. G. C. Ciciliato, J. M. De Navarro, V. M. F. Del Guercio, E. F. Del Tedesco, J. M. Dias, R. M. G. Dias, W. Ebner Filho, F. A. Faria, C. Figueiredo, E. S. P. Foltran, H. S. Freitas, A. M. S. F. Gelsi, T. M. Guerschman, E. K. K. Gushiken, M. T. Jahnel, N. M. Kashiwagi, C. A. Kim, G. N. Leal, C. Leone, L. A. Levy, E. M. G. Licursi, H. Litchcajer, S. M. M. Lotsi, K. C. G. B. Luna, L. B. Mello, M. I. Miguel, C. V. Montero, E. O. Morais, M. M. Moreno, C. Nakagawa, A. C. S. Nogueira, M. M. Nogueira, E. M. L. Nunes, A. Oda, M. I. C. G. Okai, D. M. S. P. Oliveira, E. R. Oliveira, V. P. Z. Oliveira, L. M. T. Osaki, M. R. Pacola, N. H. Paula, G. E. C. Perecin, M. F. Pereira, J. M. P. Pintya, C. M. R. Prado, M. C. F. P. Reina, M. R. Resende, L. C. F. Sá, O. H. Sakamoto, M. F. Salgado, M. C. Santos, M. M. T. Santos, S. A. J. Santos, A. Sardinha, D. Schout, J. M. Silva, M. C. C. Silva, M. H. Simonetti, A. Solera, E. Suzuki, M. H. Tamimoto, R. C. Tavares, M. Theobaldo, C. Toscano, E. N. Tsuguta, M. A. Valin, A. C. Vanzeli, V. A. Ueda, M. A. Zaparoli, and M. Zugaib.

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