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Original Article

Rubella Seroprevalence among the General Population in Dongguan, China

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SUMMARY: In order to assess the immunity to rubella infection in Dongguan, China, we conducted a seroprevalence survey on rubella and used ELISA to measure rubella-specific IgG in serum samples. A total of 1,017 individuals aged 0–59 years were selected by multistage cluster sampling. Among them, 904 (88.9%) were seropositive for rubella. Two groups (20–29 and ≥40 years) had seropositivity rates of <90%. In comparison with participants aged ≥20 years, rubella immunization rates were higher in those aged <20 years (83.2% vs. 93.7%, respectively; $\chi^2 = 28.063$, $P < 0.001$). Among women aged 20–29 years, only 63.8% had antibodies above the protective level. Multivariate analysis revealed that only sex and age were significantly associated with rubella-protective antibody levels. Our results suggest that in the study area, women of childbearing age had a greater serological susceptibility to rubella. Additional vaccinations for rubella of susceptible young adults should be considered, particularly in women of childbearing age.

INTRODUCTION

Rubella is a mild self-limiting infectious disease that mainly occurs in children. However, infection with rubella virus in the first trimester of pregnancy may lead to miscarriage, stillbirth or a child born with congenital rubella syndrome (CRS). CRS is one of the greatest risks to newborn infants and may entail a variety of birth defects, such as sensor-ineural deafness, cataracts, and cardiac defects (1,2). Therefore, in 2000, the World Health Organization (WHO) recommended the use of the rubella-containing vaccine (RCV) in all national childhood immunization schedules to prevent congenital rubella infection, including CRS (3). Moreover, goals to eliminate rubella and CRS were established by the WHO in America in 2010 and will be proposed in the European and Western Pacific regions in 2015 (4,5).

Rubella has been included in the surveillance system in China since 2004. The reported incidence of rubella in China was 9.11 and 5.26 per 100,000 population in 2008 and 2009, respectively. The proportion of rubella cases aged <20 years was 82.54%. In Eastern China, where RCV was introduced earlier than in central and western areas, cases aged 15–35 years accounted for 52.35% and 55.60% of the total in the 2 study years, respectively; the proportion of cases in this age group was higher than that in central and western areas (6).

In China, a single dose of rubella vaccine was introduced in 1995, and in 2007, RCV was implemented in the national immunization program with a catch-up campaign targeting children aged 1–14 years (7,8). In Guangdong province, rubella vaccines were included in the Guangdong Expanded Program of Immunization (EPI) in 2001, but recipients were charged for the vaccine until 2008 (9). According to the national vaccination program, RCV should be administered to children at age 8 months and 18–24 months. In China, before 2008, the rubella vaccine coverage in 1-year-old children was 50%–60% (7,10).

Serological surveys can provide useful information to identify the subpopulations at risk and address possible immunization strategies for rubella. In this study, we collected serum and used ELISA to determine rubella antibodies (IgG). Our aims were to evaluate immunity to rubella following implementation of the rubella vaccination program in Dongguan, southern Guangdong province and to assess factors influencing immunity against rubella.

MATERIALS AND METHODS

Study design: A multistage sampling design was used. The 33 towns in Dongguan were stratified into 5 regions (east, south, central, west, and north) to account for geographical variations. One town in each region was sampled and 2 villages in each town were then selected. A starting household was identified from a list of households in each selected village. Simple random sampling selection was used for the towns, villages, and starting households. The household was then examined to determine if anyone belonging to the eligible age (0–59 years) was living there. Subsequently, the nearest household to the right was visited, and the procedure repeated until the desired number of people (at least 100 participants from each village) was obtained.

Sera were stratified into 7 age groups: <2, 2–4, 5–9, 10–19, 20–29, 30–39, and ≥40 years. The formula $n_i = z^2 N_i . P_i . (1 - P_i) / z^2 P_i (1 - P_i) + (N_i - 1) e^2 P_i^2$ was used to calculate the sample size for each age group (11), which estimated the age-specific seroprevalence to be

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80%–90% (12), with a precision of 0.1; the calculated sample size was between 42 and 96 in different age groups. The serological survey was conducted from March to April 2012. A total of 1,367 individuals from 414 households were eligible and they were invited to participate in the study; 1,017 (74.4%) eventually participated by filling out a questionnaire and by donating a single blood sample.

Approval for the study was obtained each year from the Medical Ethics Committee of the Guangdong Medical College (PJ2012035). Written informed consent forms were signed by individuals or by parents of children.

Data collection: Each participant was asked to fill out a questionnaire anonymously about personal information such as sex, date of birth, occupation, education status, marital status, residential status (local residents or immigrants; immigrants were defined as those who lived in Dongguan at least 3 months before sampling), and date of sampling. Information regarding RCV vaccination (routine and campaign) was collected from the China Information Management System for Immunization Programming.

Assays: Rubella antibody (IgG) level was estimated using commercially available ELISA kits (Virion Serion, Würzburg, Germany). The results were interpreted according to the manufacturer's instructions. The positive cut-off value was 20 IU/mL, and weakly positive samples (10–20 IU/mL) were considered equivocal. A value of <10 IU/mL was considered negative.

Statistical analysis: Statistical analysis was performed using SPSS for Windows version 15.0. The total seropositivity rate was calculated by adjusted sampling weight, and the formula $w_i = (\Sigma n_i)\ C_i/n_i$ was used to calculate the adjusted sampling weight, where C_i is the age group proportion, and n_i is the sample size in the age group. The age group proportion was based on the data from the 6th Chinese Population Census.

The associations between rubella antibody positivity and age, sex, occupation, education status, marital status, and residential status were analyzed using the Pearson χ^2 test or χ^2 test with continuity correction. The Kruskal–Wallis H and Mann–Whitney U tests were used for comparisons of rubella antibody titers between groups. Multivariate logistic regression was applied to determine the factors that influenced rubella antibody positivity. Variance inflation factor (VIF) was used to check for multicolinearity. A P value of <0.05 was considered statistically significant.

RESULTS

Demographics: A total of 1,017 individuals in Dongguan aged 0–60 years (502 [49.4%] males and 515 [50.6%] females) were enrolled in the seroprevalence study. There were 341 (33.5%) students, 227 (22.4%) workers, and 207 (20.4%) children. Education status was low (primary school or below) in 493 (48.5%; 420 children and 73 adults), mid-level (high school) in 413 (40.6%), and high (college or graduate school) in 111 (10.9%) individuals. Further, 601 (59.1%) individuals were unmarried, 390 (38.3%) were married, and 26 (2.6%) were of other status. There were 485 (47.7%) local residents and 532 (52.3%) immigrants.

Coverage rate of RCV: In the catch-up campaign in 2008, a total of 395,590 eligible children (aged 1–14 years) were identified in Dongguan, and the coverage rate of RCV was 100%.

In the birth cohort during November 2007 to October 2011, there were 656,627 eligible children for the first dose of RCV, and the coverage rate was 100%. There were 789,312 eligible children for the second dose of RCV; 456,985 children received the second dose, and the coverage rate was only 58.9%.

Prevalence of seropositivity and antibody against rubella: The median rubella antibody titer was 44.36 IU/mL (interquartile range: 31.21–60.71 IU/mL). The titer differed among the age groups ($\chi^2 = 78.264$, $P < 0.001$). Rubella antibody titer was significantly higher in males than in females (48.30 IU/mL, interquartile range: 36.27–75.32 IU/mL vs. 40.57 IU/mL, interquartile range: 25.06–54.54 IU/mL; $Z = 8.645$, $P < 0.001$; Table 1).

Factors associated with rubella seroprevalence: Of the 1,017 individuals, 904 (88.9%) were positive for rubella antibodies, 93 (9.1%) were equivocal, and 20 (2.0%) were negative. This corresponds to an adjusted prevalence of 86.2% in the Dongguan population. Five age groups (<2, 2–4, 5–9, 10–19, and 30–39 years) had seropositivity rates of $>90\%$, and 2 groups (20–29 and ≥ 40 years) had seropositivity rates of $<90\%$. The lowest seropositivity rate was 77.0% in the 20–29 years age group (Table 2). When considering age and sex, all 5 female age groups (≥ 5 years) had seropositivity rates of $<90\%$, and the lowest seropositivity rate was 63.8% in the 20–29 years age group (Table 2). In comparison with people aged ≥ 20 years, rubella immunization rates were higher in those aged <20 years (83.2% vs. 93.7%, respectively; $\chi^2 = 28.063$, $P < 0.001$).

In bivariate analyses, the seropositive rate among females was 81.7% (421/515), which was lower than that among males [96.2% (483/502)] [odds ratio (OR) = 0.85, 95% confidence interval (CI): 0.81, 0.89; $\chi^2 = 53.87$, $P < 0.001$]. Seropositivity differed among the age groups ($\chi^2 = 58.078$, $P < 0.001$). Education level, occupation, and marital status were associated with seropositivity ($P < 0.001$). There was no significant association between residential status and rubella antibody positivity ($P > 0.05$; Table 2).

Multiple logistic regression models were used to control potential confounders, which showed that in com-

Table 1. Rubella seroprevalence in general population in Dongguan, by age group and sex

Age (yr)	Total median (IU/mL) (QIR)	Male Seropositive (n/N[1)], %)	Female Seropositive (n/N[1)], %)
<2	36.73 (31.61, 44.86)	22/23 (95.7)	22/24 (91.7)
2–4	43.07 (33.16, 50.57)	57/60 (95.0)	57/58 (98.3)
5–9	48.14 (34.19, 60.12)	89/91 (97.8)	88/100 (88.0)
10–19	49.94 (36.33, 68.06)	97/99 (98.0)	86/98 (87.8)
20–29	36.04 (20.83, 50.59)	84/93 (90.3)	66/94 (63.8)
30–39	49.88 (37.25, 82.35)	72/72 (100)	61/69 (88.4)
≥ 40	40.50 (21.26, 97.81)	62/64 (96.9)	47/72 (65.3)

[1)]: seropositive samples/total.

Table 2. Demographic characteristic of individuals with seropositivity to rubella

Classification	Seropositive (n/N[2)], %)	95%CI (%)	Unadjusted OR (95%CI)	Adjusted OR (95%CI)
Sex[3)]				
Male[1)]	483/502 (96.2)	93.5, 97.9	1.00	1.00
Female	421/515 (81.7)	78.4, 85.0	0.85 (0.81, 0.89)	0.14 (0.08, 0.24)
Occupation				
Children[1)]	201/211 (95.3)	92.4, 98.2	1.00	
Student	310/341 (90.9)	87.9, 94.0	0.46 (0.21, 0.98)	
Worker	192/227 (84.6)	79.9, 89.3	0.25 (0.12, 0.53)	
Other	201/238 (84.5)	79.9, 89.1	0.24 (0.12, 0.52)	
Education level				
Primary school or below (Children)[1)]	398/420 (94.8)	92.7, 96.9	1.00	1.00
Primary school or below (Adults)[1)]	58/ 73 (79.5)	70.2, 88.8	0.21 (0.11, 0.42)	0.132 (0.02, 1.19)
High school	354/413 (85.7)	82.3, 89.1	0.33 (0.20, 0.55)	0.13 (0.02, 1.06)
College or graduate school	94/111 (84.7)	78.0, 91.4	0.31 (0.16, 0.60)	0.14 (0.02, 1.20)
Marital status				
Unmarried[1)]	548/601 (91.5)	89.3, 93.7	1.00	
Married	330/390 (84.6)	81.0, 88.2	0.51 (0.34, 0.76)	
Other	24/ 26 (92.3)	75.0, 99.0	1.21 (0.28, 5.24)	
Residential status				
Local	476/485 (89.5)	86.8, 92.2	1.13 (0.77, 1.67)	
Immigrants	428/532 (88.2)	85.5, 90.9	1.00	
Age groups (yr)[3)]				
<2	44/ 47 (93.6)	83.0, 98.0	4.38 (1.30, 14.81)	0.63 (0.05, 7.41)
2–4	114/118 (96.6)	93.3, 99.9	8.51 (2.97, 24.41)	1.22 (0.11, 13.09)
5–9	177/191 (92.7)	89.0, 96.4	3.78 (1.99, 7.17)	0.56 (0.06, 5.11)
10–19	183/197 (92.9)	89.3, 96.5	3.90 (2.06, 7.41)	2.82 (1.23, 6.48)
20–29	144/187 (77.0)	71.0, 83.0	1.00	1.00
30–39	133/141 (94.3)	90.5, 98.1	4.08 (2.07, 8.04)	4.37 (1.97, 9.69)
≥ 40[1)]	109/136 (80.1)	73.4, 86.8	0.99 (0.56, 1.76)	0.95 (0.48, 1.89)

[1)]: Reference category.
[2)]: seropositive samples/total.
[3)]: VIF = 1.00.

parison with people aged 20–29 years, those aged 10–19 and 30–39 years were 2.82 (95% CI: 1.23, 6.48, respectively) and 4.37 (95% CI: 1.97, 9.69, respectively) times more likely to be seropositive for rubella, respectively. In comparison with males, females had lower seropositivity to rubella virus (OR = 0.14, 95% CI: 0.08–0.24). VIF was used to check for multicolinearity. None of the VIF values reached 5, indicating that there was no colinearity in the model (Table 2).

DISCUSSION

Our study showed total seropositivity of 86.2% in the general population in Dongguan. Rubella appears in periodic epidemics in countries without routine immunization programs. Before routine administration of RCV in children, the seropositivity in the general population in Shenzhen was 68.09% in 2001 (13), and it was 73.07% in Guangzhou population during 1986–1987 (14). After the inclusion of rubella vaccine in the EPI, 2 seroepidemiology surveys were conducted in Shandong province (2009–2010) and Hefei city (2011), with seropositivity rates of 83.13% and 80.8%, respectively (12,15). The results were similar to our data, and in comparison with the pre-vaccine era, the seropositivity in the general population increased.

In our study, the seropositivity rate was high in people aged <20 years, which may have been due to routine rubella vaccination and the catch-up campaign in 2008 (which targeted children aged 1–14 years). Our study was conducted in 2012, indicating that most of the participants aged 5–19 years had received RCV in this catch-up campaign. Children aged 1–4 years were more likely to have received routine rubella vaccinations. Similarly, the seropositivity rate in children aged <15 years in Shandong province (80.9%) and Hefei city (73.2%) was higher than that observed in this age group in Guangzhou (39.9%) (12–15).

Our results were consistent with those reported by previous studies in which age was an independent predictor of anti-rubella antibody positivity (16,17). As mentioned before, the use of rubella vaccine may result in high positivity in participants aged <20 years. Most participants aged ≥ 20 years (i.e., those born before 2008) acquired immunity from natural infection. Differences in circulation of the virus and epidemics of rubella in the community may explain the variation in positivity. In addition, there is evidence that inadequate rubella vaccination coverage ($\leq 80\%$) may disturb transmission dynamics. As a result, the susceptibility age increases, and susceptibility among women of childbearing age may increase the risk of CRS (18). It has been reported that the susceptibility age increased after rubella immunization of children in Shandong province (10). In Don-

gguan, during 2007–2011, the incidence of rubella was 1.24 per 100,000 population, and the highest proportion of 36.18% of the total cases was in those aged 20–29 years (19). Routine RCV administration in our area only started in 2008; thus, the effect of the vaccination program should be investigated further, particularly the impact on age-susceptibility patterns. Our current data showed that 2 age groups (20–29 and ≥40 years) had comparatively low rates of rubella antibody positivity, and, notably, only 63.8% of women aged 20–29 years had antibodies above the protective level. The legal age for marriage is 20 years for women in China (Law of Marriage of People's Republic of China, amended on April 28, 2001). In 2010, the mean childbearing age was 28.18 years (20), indicating that the peak childbearing age of women in China is 20–29 years. Combined with the data dealing with the epidemiological characteristics of rubella in Dongguan (18), our results provide seroepidemiological evidence confirming the high disease burden in women of childbearing age during rubella outbreaks and the high risk of CRS in our area.

Similar to the results of previous study (21), our results showed that women had lower seropositivity rates than men. However, inconsistent results have been reported by previous studies. Some studies have shown that women were more likely to have high seropositivity against rubella (4), and other studies have reported no significant sex difference in seropositivity against rubella (22). This inconsistency may be partly explained by differences in rubella infection and vaccination histories between study populations.

There were some limitations to the present study. First, few of the participants reported a history of rubella vaccination, and we excluded data of vaccination history in the final analysis. Therefore, we could not identify the effect of previous vaccinations on the seroprevalence of rubella in the population. Second, the number of younger children (<2 years) was relatively small, because the parents (particularly those with children aged <1 year) were unwilling to donate a blood sample of their children. The number of samples from children aged <1 year was so small that we could not stratify this age group and analyze it independently. Third, the serology results may be biased, because the first and second sampling stages were not conducted by probability proportionate to size sampling, the number of clusters was relatively small, and the sampling design was not considered when calculating prevalence.

In conclusion, our study showed that people aged 20–29 and ≥40 years had low rates of rubella seropositivity, particularly among women. Two doses of RCV have been routinely given to children in our area since 2008. The vaccination may disturb transmission dynamics of the disease, and inadequate vaccination coverage may result in an increase in susceptibility age (18). Therefore, maintaining high vaccination coverage in children and implementing sensitive disease surveillances are necessary for controlling the disease and CRS. Women of childbearing age have high serological susceptibility to rubella in our area, suggesting that additional RCV vaccination of susceptible young adults should be considered, particularly in women of childbearing age.

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Conflict of interest None to declare.

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