Original Article

Shedding of Rubella Virus among Infants with Congenital Rubella Syndrome Born in Tokyo, Japan, 2013–2014

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SUMMARY: Rubella is usually a mild illness, with febrile rash being its main symptom. However, serious consequences of rubella infection can result when the infection occurs during the early stages of pregnancy. After the occurrence of a rubella outbreak in Japan that was observed from 2012 to 2013, 45 infants were reportedly born with congenital rubella syndrome (CRS). We prospectively followed the 15 CRS cases reported in Tokyo to determine the virus shedding periods by using nested reverse transcriptase-polymerase chain reaction to detect rubella virus genes. Throat swabs were used for virus detection. The virus shedding period was measured from birth until the time when the sample last tested positive followed by 2 consecutive negative samples. Kaplan-Meier method was used to estimate the proportion of cases remaining positive for rubella virus genes over time. The proportion of CRS cases shedding virus dropped steadily after birth, dropping to 33.8% at 6 months and 16.9% at 12 months. Our findings also suggested that the earlier the mother's onset of rubella during pregnancy, the longer the infant remained positive. Based on our findings, we believe that infants with CRS should be monitored for rubella virus shedding until 1 year of age.

INTRODUCTION

Rubella is a disease, which is preventable by vaccination, and in Japan, a national rubella immunization program for children was introduced in 1995. This disease has been under case-based surveillance in Japan since 2008, and from 2008 to 2011, the number of reported cases of rubella in Tokyo was below 50 (46 cases in 2008, 19 in 2009, 15 in 2010, and 39 in 2011). However, since June 2012, the number of reported cases began increasing considerably, with a total of 693 cases reported in 2012 and the number of reported cases further increased to 3,423 in 2013 (based on the date of diagnosis). The majority of cases were reported in male patients who were in their 20–40s, which was a different trend in comparison to the findings from previous years where the cases mostly occurred among children (1,2).

During this outbreak, a total of 45 cases of congenital rubella syndrome (CRS) were reported by the end of 2014 in Japan. The cases were concentrated in urban areas, and from 2013 to 2014, 16 cases were reported in Tokyo. Compared to the total number of reported cases of CRS in the 10 year span from 2003 to 2012, which was only 3, this increase from 2013 to 2014 implied the

seriousness of the situation (2).

Although infants with CRS are known to shed rubella virus after birth, and therefore, have the potential to spread the infection to others (3), very few studies have been conducted on how long the virus shedding periods continue. The local health centers and municipal health authorities started receiving inquiries on the infectiousness of infants with CRS, and in order to consider the appropriate infection prevention measures, we need to understand the extent of rubella virus shedding in the infants with CRS. Therefore, we began conducting gene tests to uncover the presence of the rubella virus in September 2013 for the purpose of evaluating the virus shedding periods of infants with CRS.

METHODS

Infants with CRS who were born January 1, 2013 onward and who were cared for as an outpatient or hospitalized at medical facilities in Tokyo were enrolled in the study after receiving consent from the parents and establishing an agreement for cooperation with their respective medical facilities. In this study, CRS was defined as stated under “The Law Concerning the Prevention of Infectious Diseases and Medical Care for Patients of Infections.” An infant with CRS is an infant who displays one or more of the following: cataracts, congenital glaucoma, congenital heart disease, hearing impairment, pigmentary retinopathy, purpura, splenomegaly, microcephaly, mental retardation, meningoencephalitis, radiolucent bone disease, jaundice within 24 hours after birth, and who has been diagnosed with a serum level of immunoglobulin (Ig)M-EIA specific to rubella using the Rubella IgM EIA kit (DENKA SEIKEN Co., Ltd., Tokyo, Japan). Antibody index value > 1.20 indicates a positive result.

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Throat swab samples were used for the virus gene detection tests, which were conducted for all of the cases. Nested reverse transcriptase-polymerase chain reaction (RT-PCR) method established by Bosma et al. was used to amplify the virus genes with a primer that specifically targets the E1 region of the rubella virus genome (4). This method has a sensitivity of approximately 2 RNA copies detected by nested RT-PCR. We began the follow-up tests in infants with CRS who had been confirmed positive for rubella virus genes by collecting samples approximately 3 months apart. In principle, we ended the follow-up for each case when 2 consecutive samples that were obtained 1 or more months apart were both negative. The virus shedding period was defined as the period from birth until the time when the sample last tested positive before the 2 consecutive negative samplings. Kaplan-Meier method was used to estimate the proportion of cases remaining positive for rubella virus genes over time, i.e. the period of virus shedding after birth.

At the beginning of follow-up, we collected information on sex, weight at birth, date of birth, gestational age, clinical manifestation(s), timing of the sample collection for testing for the rubella specific IgM, serum level of IgM-EIA specific to the rubella virus, the mother's gestational age at the time of her rubella onset, and the mother's immunization history against rubella. We asked the medical facilities for their cooperation in providing this information. At the time of application for examination, a designated form with the information we requested was filled out by the doctor, and then submitted along with the samples. Urine and blood samples were also collected when available. Ethical considerations were carefully handled through the process of obtaining informed consent in order to prevent the parents of the enrolled infants with CRS from having any disadvantages, which might occur as a result of this study. Ethical approval was given by the ethics committee at the Tokyo Metropolitan Institute of Public Health on September 9, 2013.

RESULTS

There were 15 enrolled infants with CRS in total; 8 male infants and 7 female infants. Five were born before the 37th week of gestation, and 10 were born from the 37th week up until the 42nd week. Three of the infants weighed less than 1,500 g at birth (including 1 weighing less than 1,000 g), 9 weighed between 1,500 g and 2,500 g, and 3 weighed more than 2,500 g (Table 1). The most common clinical manifestation were congenital heart disease ($n = 12$), hearing impairment ($n = 11$), and jaundice occurring within 24 hours after birth ($n = 6$). The levels of IgM-EIA specific to rubella at birth ranged from 6.87 to 11.0 (median 8.81, $n = 10$). Eight of the mothers had their rubella onset within the 6th to 10th week of gestation, 3 had within the 11th to 15th week, 2 had within the 16th to 20th week, and 2 never had any symptoms. Three of the mothers had been vaccinated against rubella once. The strain(s) of rubella virus used to vaccinate these 3 mothers was unknown. Five of the mothers were never vaccinated and 7 had an unknown history of vaccination.

From the rubella virus gene tests conducted on the 15

infants with CRS enrolled from September 2013 to January 2015, 39 out of 89 samples were found to be positive. The positive test results that were found based on the types of specimen were as follows: 50.7% (35/69) from throat swab samples, 30.0% (3/10) from urine samples, and 10.0% (1/10) from blood samples.

Examination of throat swab samples: The throat swab sample results in regards to the detection of rubella virus genes from the 15 cases of CRS are shown in Fig. 1. The first test was conducted at 6 months for 1 case (No. 1), at 3 months for 3 cases (No. 4, 14, and 15), and within 2 months from birth for the other 11 cases (No. 2, 3, and 5–13). Rubella virus genes were detected in all of the 15 cases, and 8 out of 15 cases had 2 or more positive test results. One case (No. 10) had a negative result for the 1st test at 1 month; however, this turned out to be positive at 3 months. Five cases (No. 3, 6, 8, 11, and 12) had negative result(s) that turned out to be positive at the next follow-up test. Follow-up for 11 of the cases (No. 1–5, 8, 10–12, 14, and 15) ended after confirming the validity of 2 consecutive negative test results. Four cases were lost to follow-up as 3 of the infants (No. 7, 9, and 13) died and 1 case (No. 6) discontinued follow-up before being able to confirm the 2 consecutive negative test results. The shortest duration of virus shedding was less than 1 month and the longest was 13 months.

Transition of the proportion of rubella virus shedding CRS infants based on age in months is shown in Fig. 2. The proportion of cases shedding the virus as examined through the testing of the throat swab samples was as follows: 100% at 0 month, 92.3% at 1–2 months, 84.6% at 3 months, 59.2% at 4 months, 42.3% at 5 months, 33.8% at 6–7 months, 25.4% at 8 months, and 16.9% at 9–13 months.

Among 15 cases, 13 had mothers who had rubella onset during gestation. Among these 13 cases, one case (No. 13) died at 3 months, one case (No. 7) died at 6 months and one case (No. 6) was lost to follow-up at 12 months. We plotted the virus shedding periods of the remaining 10 cases by mother's gestational age at the time of rubella onset (Fig. 3). Three out of the 5 cases in which the mother's rubella onset occurred before the 11th week of gestation had virus shedding periods of 5 months or more. In contrast, the virus shedding period for all cases where the mother's rubella onset was after the 11th week of gestation was less than 5 months. Especially for the cases where the mother's rubella onset was after the 15th week of gestation, the virus shedding period was less than 3 months.

Examination of urine samples: We were able to collect urine samples from 4 cases (No. 2, 5, 11, and 15), and of those 4 cases, rubella virus genes were detected in 2 cases (No. 5 and 11). One of them (No. 5) was found to be positive for 2 consecutive tests, at 0 month and at 3 months. In terms of the tests conducted on samples collected at 3 months, virus genes were only detected in the urine sample and not in the throat swab sample or blood sample.

Examination of blood samples: We were able to collect blood samples from 4 cases (No. 2, 5, 11, and 15), and rubella virus genes were detected in 1 case (No. 11). Virus genes were detected in all samples (blood, throat swab, and urine samples) collected at birth month of

Table 1. Demographics of each congenital rubella syndrome (CRS) case, mother's status, and details of rubella virus gene detections tests for the CRS infants born in Tokyo from 2013 to 2014 ($N = 15$)

Case No.	Demographic characteristic						Mother's status		Details of rubella virus gene detection test				
	Sex	Birth weight (g)	Date of birth (yr/mo)	Gestational age (w)	Clinical manifestation(s)	Timing of sample collection for rubella specific IgM	Level of IgM-EIA (Positive: > 1.20)	Mother's gestational age at onset (w)	Mother's history of immunization	Number of tests	Sample collection time (mo, d)	Type of specimen (Throat swab 1, Urine 2, Blood 3)	Result (Positive +, Negative -)
1	Male	1,125	2013/3	31	Cataract, congenital heart disease, hearing impairment, purpura, microcephaly, neonatal hepatitis	At birth	6.87	10	None	1st	6 m 14 d	1	+
						At 6 mo	7.8			2nd	8 m 23 d	1	+
										3rd	11 m 26 d	1	-
										4th	12 m 24 d	1	-
2	Female	2,930	2013/7	39	Congenital heart disease, pigmentary retinopathy	At 2 mo	2.92	16-18	None	1st	2 m 2 d	1	+
												2	-
										2nd	3 m 9 d	1	-
												2	-
						At 3 mo	1.03					3	-
										3rd	4 m 6 d	1	-
												2	-
3	Male	1,578	2013/8	32	Congenital heart disease, hearing impairment, splenomegaly, jaundice within 24 hrs from birth	At 6 d	10.4	No symptom	Vaccinated once at 2 yrs old	1st	2 m 10 d	1	+
										2nd	3 m 13 d	1	+
						At 2 mo	9.09			3rd	6 m 12 d	1	-
										4th	7 m 12 d	1	+
										5th	9 m 7 d	1	-
										6th	10 m 11 d	1	-
4	Male	2,483	2013/7	38	Hearing impairment, purpura	At birth	10.3	9	Unknown	1st	3 m 9 d	1	+
										2nd	5 m 25 d	1	+
										3rd	11 m 9 d	1	-
										4th	12 m 2 d	1	-
										1st	0 m 7 d	3	-
5	Female	2,082	2013/8	39	Congenital heart disease, hearing impairment, pigmentary retinopathy	At 9 d	9.13	16	None		0 m 14 d	1	+
												2	+
										2nd	0 m 15 d	3	-
											3 m 26 d	1	+
												2	+
												3	-
										3rd	6 m 24 d	1	-
												2	-
												3	-
										4th	7 m 18 d	1	-
6	Male	2,280	2013/7	39	Congenital heart disease, hearing impairment, purpura, disseminated intravascular coagulation syndrome, intraventricular hemorrhage	At birth	11.0	8	None	1st	2 m 22 d	1	+
										2nd	5 m 20 d	1	+
										3rd	7 m 25 d	1	+
										4th	10 m 11 d	1	-
										5th	11 m 11 d	1	+
										6th	12 m 5 d	1	-
7	Male	1,558	2013/10	37	Congenital heart disease, hearing impairment, pigmentary retinopathy, purpura, splenomegaly, microcephaly, brain calcification	At birth	8.09	6	Unknown	1st	0 m 27 d	1	+
										2nd	3 m 4 d	1	+
										3rd	6 m 15 d	1	-
8	Female	1,390	2013/10	32	Congenital heart disease, hearing impairment, radiolucent bone disease, jaundice within 24 hrs from birth	At birth	7.63	9	Unknown	1st	1 m 5 d	1	+
										2nd	2 m 3 d	1	+
										3rd	3 m 9 d	1	+
										4th	4 m 94 d	1	+
										5th	5 m 23 d	1	+
										6th	6 m 23 d	1	-
										7th	8 m 20 d	1	+
										8th	10 m 6 d	1	+
										9th	11 m 3 d	1	+
										10th	12 m 8 d	1	+
										11th	13 m 13 d	1	+
										12th	13 m 27 d	1	+
										13th	14 m 11 d	1	-
										14th	15 m 13 d	1	-
9	Female	650	2013/11	32	Congenital heart disease, jaundice within 24 hrs from birth	At birth	9.58	No symptom	Vaccinated once at 18 yrs old	1st	0 m 5 d	1	+
10	Female	2,664	2013/10	37	Congenital heart disease	At 4 d	3.59	14	None	1st	1 m 0 d	1	-
										2nd	3 m 2 d	1	+
										3rd	6 m 2 d	1	-
										4th	9 m 2 d	1	-
11	Male	2,474	2013/12	39	Hearing impairment, jaundice within 24 hrs from birth, external auditory canal atresia, auricle hypoplasia	At birth	8.53	12	Unknown	1st	0 m 2 d	3	+
											0 m 5 d	1	+
												2	+
										2nd	3 m 2 d	1	-
										3rd	4 m 0 d	1	+
12	Female	2,262	2014/1	35	Congenital heart disease, hearing impairment, jaundice within 24 hrs from birth	At birth	7.82	9	Unknown	4th	6 m 0 d	1	-
										5th	6 m 28 d	1	-
										1st	0 m 5 d	1	+
										2nd	1 m 23 d	1	-
						At 4 d	8.03			3rd	2 m 8 d	1	-
										4th	4 m 6 d	1	+
										5th	4 m 22 d	1	-
13	Male	1,539	2014/1	38	Congenital heart disease, purpura, radiolucent bone disease	At birth	9.44	6	Unknown	6th	5 m 7 d	1	-
										7th	7 m 3 d	1	-
										1st	0 m 7 d	1	+
14	Male	2,040	2013/6	37	Congenital heart disease, hearing impairment, microcephaly, jaundice within 24 hrs from birth	At birth	9.09	13	Unknown	1st	3 m 6 d	1	+
										2nd	7 m 13 d	1	-
										3rd	8 m 14 d	1	-
15	Female	3,100	2013/11	40	Hearing impairment	At 2 mo	8.98	6	Vaccinated once at 14 yrs old	1st	3 m 11 d	1	+
												2	-
												3	-
						At 3 mo	8.82			2nd	6 m 6 d	1	-
												2	-
												3	-
						At 7 mo	8.26			3rd	7 m 17 d	1	-
						At 10 mo	2.41			4th	10 m 9 d	1	-

Shedding of Rubella Virus in CRS

Fig. 1. Virus gene detections in throat swab samples of the congenital rubella syndrome (CRS) infants born in Tokyo from 2013 to 2014 ($N = 15$). ¹⁾: positive for urine sample.

Fig. 2. Proportion of cases of congenital rubella syndrome (CRS) remaining positive for rubella virus genes over time ($N = 15$).

Fig. 3. Relationship between the virus shedding period of the congenital rubella syndrome (CRS) infants who were born in Tokyo from 2013 to 2014 and their mother's gestational age at time of rubella onset.

this case.

DISCUSSION

The consequences of congenital rubella infection during the first 18 weeks of gestation include abortion, miscarriage, stillbirth, and a pattern of birth defects called CRS (5). The risk of CRS is related to gestational age at the time of maternal infection. When pregnant women are infected with rubella during the first 11 weeks of gestation, up to 90% of liveborn infants will have CRS

(6). The occurrence rate of CRS declines thereafter until 17–18 weeks of gestation. Rubella viruses are transmitted to 36.7% of infants born to mothers who had rubella symptoms up until 24 weeks of gestation, where 20% of these infants will develop CRS (7). World-wide, it is estimated that more than 100,000 infants with CRS from indigenous rubella transmission are born every year (8).

The CRS cases enrolled in our study all had 1 or more congenital disorder and a high level of serum IgM-EIA

specific to rubella. In addition, from their mother's onset of rubella and vaccination history, the demographics of the enrolled CRS cases did not contradict their diagnoses of CRS. None of the mothers had received 2 doses of the vaccination against rubella. Moreover, 3 mothers who were vaccinated once against rubella transmitted the rubella virus to their infant. This fact demonstrates the importance of receiving 2 doses of the rubella vaccination.

In our study, the proportion of virus shedding cases in throat swab samples was 100% at birth and 92.3% at 1–2 months of age. These figures were higher than the ones previously reported (9–11). Assuming that all of the previous studies used the same CRS definition for diagnosis of the disease, it may be highly possible that the gaps in these findings are due to differences in the sensitivities of nested RT-PCR and the virus isolation method. Although the virus isolation method has been traditionally used for proving the presence of the rubella virus, it is difficult to isolate viruses in cultured cells. The nested RT-PCR method has been found to be more useful in terms of sensitivity, time consumption, and convenience. In contrast, there is the possibility that nested RT-PCR may be detecting non-infectious RNA in a virus that was already neutralized by antibodies.

In our study, the rubella virus genes were detected in throat swab samples as well as in urine and blood samples; however, a throat swab is considered the most effective sample type because of its simple sampling method, and the detection rate for virus genes was the highest in the throat swab samples among the 3 types of sampling methods. Collection of urine samples together with throat swab samples may increase the sensitivity of detecting rubella virus because there were times when virus genes were only detected in urine samples, but were not detected in throat swab or blood samples. Blood samples may not be appropriate for evaluating the presence of the virus because the virus genes were often not detected in blood samples even during the virus shedding periods.

According to a previous study, rubella viruses were present in the throat of 9% of cases of CRS who were 10 to 13 months of age, but disappeared in cases older than 13 months (12). In another study, Katow reported that the longest period of virus shedding in the throat was 8 months (7). We observed that the detection rate for virus genes in the throat decreased as the infants with CRS advanced in age. The proportion of the CRS cases that tested positive for rubella virus genes was 84.6% at 3 months, which decreased to 33.8% at 6 months. This demonstrates that almost two-thirds of the cases stopped shedding viruses at 6 months of age. Excluding 1 case that shed viruses until 13 months of age, all of the other cases tested negative for rubella virus genes by 12 months of age. Thus, we believe that infants with CRS should be monitored for rubella virus shedding until 1 year of age.

It is also important to gather information on the mother's gestational age at the time of her rubella onset for evaluating the virus shedding periods of infants with CRS. It is possible that when the fetus is exposed to viruses at an early fetal development stage when immunity is still immature, the fetus does not recognize viruses as foreign subjects, and therefore, ends up hav-

ing prolonged virus shedding periods. Our study found that when the mother's rubella onset was before the 11th week of gestation, 60% of the cases recorded virus shedding for 5 months or longer. While one case was lost to follow-up at 12 months, the case shed virus until 11 months and his mother's onset was at 8 weeks of gestation. In contrast, when the mother's rubella onset was after the 11th week of gestation, all cases stopped shedding the virus before 5 months from birth.

The limited sample size ($n = 15$) used in this study could have influenced the results of the analysis. In addition, the virus shedding periods could have been assessed shorter because not all of the sample collections could be conducted on a periodic basis (for example, every month), and the interval of the sample collections slightly differed amongst each of the cases. Therefore, the exact time of when each CRS case stopped shedding viruses could not be determined. In addition, the reason as to why one-time negative results were obtained for several of the cases during the virus shedding periods is not clear. However, insufficient amount of virus in the samples, failure in the sample collection process, or testing errors could have led to these negative test results.

To our knowledge, the rubella viral load that shed from each of the infants with CRS has not been investigated, and its infectiousness has not been examined thus far. However, since we cannot rule out the possibility of rubella transmission from viruses shed by infants with CRS, it is important to monitor the duration of virus shedding in order to take adequate infection prevention measures at home, nursery schools, or hospitals.

Our results revealed that the virus genes were detected in all of the cases within a few months of birth; therefore, it can be speculated that the cases confirmed with CRS were already shedding the rubella viruses at birth. We believe that it is ideal to conduct repeated tests after birth to detect virus genes in order to evaluate the presence of the rubella virus. This is because we found 1 case of CRS that was initially found to be negative for virus genes but turned out to be positive during later testing.

From our findings, we recommend the first virus gene detection tests to be completed by 3 months of age, and if the infant tests positive, a follow-up test should be conducted after 6 months of age, when most infants with CRS stop shedding viruses. Two consecutive negative test results are needed to confirm the absence of viruses because it was observed in this study that some of the infants tested positive after recording one-time negative results. When it is difficult to conduct tests for virus gene detection, 1 alternative is to evaluate the virus shedding periods based on the information regarding the mother's gestational age at her rubella onset. If there is no reliable information on the mother's gestational age at her rubella onset, we believe that it is reasonable to assume that the infants with CRS may continue shedding rubella viruses until 1 year of age.

We hope that our findings will be used as a reference by public health and medical personnel who manage and support infants with CRS. We hope this will eventually contribute to the implementation of appropriate infection prevention measures against CRS and in carrying out welfare support for infants with CRS. Although there have been challenges in advocating and

educating individuals for preventing rubella in our societies, it is important to continue working towards eliminating CRS in the future. This can only be achieved by controlling rubella outbreaks by maintaining high immunization coverage.

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Conflict of interest None to declare.

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