# Frequency of Rubella Antibody among the Residents in Nagasaki District in Southern Japan

# Kumato MIFUNE and Sachiko MATSUO

Department of Virology, Institute for Tropical Medicine, Nagasaki University (Director : Dr. Kaoru HAYASHI)

## **Rokushi OKA**

Department of 2nd Internal Medicine, Nagasaki University School of Medicine (Director : Dr. Shiro OSAJIMA)

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#### Abstract

A seroepidemiological survey for rubella antibody was undertaken with the sera collected from the residents in Nagasaki district in southern Japan. Of 450 sera tested, 144 (32 percent) were found not to have NT antibody. From the incidences of NT antibody by age group, it was noticed that there are about 10 to 20 percent individuals susceptible to rubella virus infection in adults over 20 years old. This antibody distribution pattern by age group and the distribution pattern of antibody titers suggested that the chance of infection from rubella virus begins in childhood and is considerably frequent in the adults around 20 years old in this district.

#### Introduction

Rubella virus is known to be one of the few viruses producing a congenital malformation of human beings in addition to being an agent of rubella mostly in childhood. Congenital rubella appears to develop only when maternal rubella infection occurrs during the first few months of gestation (4). The employment of vaccine to prevent from rubella is about to be enforced in our country. At this time, it seems to be important to know the epidemiology of rubella virus.

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The present report briefly concerns a seroepidemiological survey for rubella antibodies performed among the residents of Nagasaki district in southern Japan.

# Materials and Methods

R-1 strain of rubella virus, supplied from Baylor College of Medicine, U. S. A. was grown in Vero cell monolayer cultures with Eagle's minimum essential medium (MEM) containing 2 percent calf serum and 1.4g of sodium bicarbonate per liter. Newcastle disease virus (NDV), California strain, was grown in chorioallantoic membrane of chick embryo. Viruses were stored at -70 C until used.

BSC-1 cells, a stable line of monkey kidney cells, were grown in 60 mm petri dishes with Eagle's MEM supplemented with 10 percent of bovine serum and 1.4g of sodium bicarbonate per liter in a 5 percent  $CO_2$  incubator. Maintenance medium of the cells consisted of Eagle's MEM with 2.1g of sodium bicarbonate per liter and 5 percent calf serum.

Sera were collected in Nagasaki city and its suburbs from 450 healthy individuals in 1970 and 1971 during which any sporadic rubella epidemic was not reported in this district. Sera were inactivated by heating at 56 C for 30 minutes and stored at -20 C.

For the detection of rubella antibody, the neutralization test was selected. This test was already confirmed to be as highly sensitive as hemagglutination inhibition test (1,2). The surviving virus after neutralization was assayed in BSC-1 cells by a previously

described modification of hemadsorption negative plaque test (5). This method markedly shortened the period in obtaining the results. Briefly, four-fold dilutions, in some instances, two-fold dilutions of sera were made and equal volumes of the appropriately diluted virus and serum dilutions were mixed and incubated at 37 C for 2 hours or at 4 C overnight. After neutralization, BSC-1 cells in petri dishes were inoculated with 0.2 ml of the mixture. Duplicate petri dishes were used pre dilution. The virus was allowed to adsorb for 1 hour at 37 C in a 5 percent CO2 atomosphere. The monolayers were refed with 4.5 ml of maintenance medium and incubated for 48 hours, after which 0.2 ml of 1:30 dilution of NDV virus stock (approximately 1.0 x 109 Plaque-forming Unit per ml) was added.

The monolayers were incubated for an additional 18-20 hours at 37 C. The medium was removed and 2.0 ml of a 0.5 percent sheep red blood cells in saline was added. Within 20 minutes at room temperature, hemadsorption-negative plaques were counted. Neutralizing (NT) antibody titer was expressed as the reciprocal of serum dilution which showed 50 percent plaque reduction. Sera with NT antibody titers of 10 or greater were considered positive.

#### **Results and Discussion**

Of 450 sera tested, 306 (68 percent) were found to have NT antibody, suggesting that 32 percent of individuals in this district had never been infected with rubella virus in the past.

The incidences of NT antibody classified



The incidences of rubella NT antibodyby age group and sex.



by age group and sex are shown in Fig. 1. Looking at the average of both sex, the incidences were fluctuating around 50 to 60 percent among the children under 2 years old and dropped to the minimum of 30 percent in the children of 3 years age group. The positive rate then gradually increased and reached the maximum of 96 percent in the age group of 40, although it dropped temporarily to 53 percent in the age group of 12. It should be noticed that in adults over 20 years old, there are still about 10 to 20 percent individuals susceptible to rubella virus infection and that the generation in which the antibody positive rate reaches the maximum is the age rgoup of 40. However, this antibody distribution pattern seems to be similar to those observed in various parts of southern Japan (3, 6),

but in different situation from those observed in northern Japan, where approximately 100 percent of the adults over 20 vears was found to have antibodies (6, 7). In all age groups under 20 years old, the incidences were slightly but constantly lower in female when compared by sex, however the differences disappear in adults over 20 years old. It is not certain at present whether these differences between sex are significant or not and what reasons the differences would result from.

The distribution of NT antibody titers and geometric mean titers in age groups are shown in Fig. 2. The NT antibody titers of 1:160 or greater became predominant from the age group of 6 and reached the peak in the age groups of 15 and 20, with the geometric mean titers of 59.3 and 76.2 resFrequency of Rubella Antibody among the Residents in Nagasaki District in Southern Japan 59

Fig. 2 The distribution of rubella NT antibody titers and geometric mean titers by age group.

GEOMETR MEAN TITER	ACE	1:10>	1:10	1:20	1:40	1;80	1:160
33.6	-6м		•			•	•
21,5	7-11M			•	•		• :.
36.0	1-2y				000 000 00		•
18,9	3-5				•	•	•
38,3	6-8						••• •• ••
58,6	9-11			•	•	1	•• •• ••
30.3	12-14						
59,3	15-19						
76.2	20-24			•			
63,6	25-29	•	•••	•			••• •••
47.8	30-39	ļ					** ** **
47.1	40-49	<i>₹</i> •	*** ** **				0 0 0
60,4	50-	6 8 6 6 0	••		00 04 00		*** *** **

NEUTRALIZING ANTIBODY TITER

pectively. The increase of the geometric mean titer of 20 years age group is statistically significant when compared with those of age groups of 7 months, 1, 3 and 6 years (F value: 6.67, 7.06, 14.51 and 6.49 respecti-

vely). From these results, it can be assumed that the chance of infection from rubella virus begins in childhood and is considerably frequent in the adults around 20years old in this district.

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