High Incidence of Adults with Rubella Antibody in Northern Japan

Tsunehisa SUTO, Morihiro MORITA, Yorio HINUMA, and Nakao ISHIDA

Department of Microbiology, Akita Central Hospital; Akita Prefectural Institute of Public Health, Akita, and Department of Microbiology, Tohoku University School of Medicine, Sendai

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ABSTRACT

A seroepidemiological study of rubella in northern Japan was made by testing for hemagglutinin-inhibitory (HAI) antibodies to rubella virus using an HAI test with our modification. An epidemic of exanthematous disease, which occurred in 1966-1967, was confirmed as rubella by the age specific distribution of rubella HAI antibody. However, one of the 14 areas tested in the region, a Piedmont village, was found to have had no rubella experience during the last 15 years. In general, a higher incidence of high titered HAI antibody was observed among the younger ages, suggesting a decrease of antibody titer with an increase in age after infection. Through these studies, it is evident that 100% of adults over 20 years of age possessed HAI antibody in all age groups and all areas so far examined. This fact may possibly explain the extremely rare incidence of congenital rubella syndrome in the northern part of Japan.

The technique of hemagglutination inhibition (HAI) test for detection of rubella antibody was first described by Stewart et al. [8]. It is a most reliable, rapid and sensitive method for detecting antibodies when compared with other methods such as neutralization, complement fixation or immunofluorescence [1, 2, 8]. Thus, the HAI test seems to be the most suitable method for epidemiological studies dealing with a number of sera of either past or present rubella infection.

We have evaluated the HAI technique and improved it to give more explicit results as reported elsewhere [5]. The present report describes a seroepidemiological survey for rubella antibodies among the residents of the Akita region of Japan by this method. The results indicated an extremely high incidence of rubella antibody in adults over 20 years of age.

MATERIALS AND METHODS

The Baylor strain of rubella virus, supplied by Dr. R. Kono of National Institute of Health of Japan, was inoculated into BHK-21 cell monolayer cultures with a maintenance medium of Eagle's minimum essential medium (MEM) in Hanks' balanced salt solution without serum. The culture fluid was harvested and replaced with a fresh medium every 24 to 72 hr. The pooled harvested-fluid was treated with an equal volume of Tween 80 and ether (1:5 mixture) to increase the hemagglutinin (HA) titer and stored at -70 C.

Sera were collected in the Akita region, northern part of Honshu Island of Japan.
from 116 individuals in 1965, one year before the 1966–1967 rubella epidemic [9] in the region, and also from 857 in 1967 and 395 in early 1968. In addition, 75 sera collected in 1957 were also tested. All of the individuals from whom sera were taken had never had clinical rubella at the time of bleeding. Sera which had been stored at −20°C were adsorbed with 25% acid washed kaolin and absorbed with goose erythrocytes to remove any nonspecific HA inhibitors and normal hemagglutinins to goose erythrocytes, respectively.

The hemagglutination inhibition (HAI) test was performed according to the procedure of Stewart et al. [8], with several modifications [5]. One was that the goose erythrocytes were used as a 0.2% suspension. Another was that the diluent was modified to a phosphate buffered saline of pH 6.6 containing 0.1% bovine plasma albumin, by which a definite HA pattern could be obtained. The microtiter technique [7] was employed throughout the experiment. Twofold dilutions of pretreated serum were carried by 0.025ml dilutors onto the plate, and then one drop (0.025 ml) of HA antigen containing 4 units was added. The plate was incubated for 1 hr at room temperature (20–25°C), transferred to the cold room (4°C), and 2 drops (0.05 ml) of the chilled goose erythrocyte suspension were added. After one and a half hour incubation at 4°C, HAI patterns were read at room temperature. The reciprocal of maximal serum dilution showing complete inhibition was considered to be the HAI antibody titer of the serum. Sera with HAI titers of 8 or greater were considered positive.

RESULTS

The age-specific incidence of HAI antibody in sera collected respectively in 1957, 1965, 1967 and 1968 is shown in Fig. 1. In 1965, there were no seropositive children under 4 years of age, and the positive rate of children under 9 years of age was extremely low, suggesting that there had been no rubella epidemics for the preceding 4 years. The incidence of persons possessing antibody was as high as 85% in 10 to 11 year old children and 100% in those over 12 years of age.

The antibody distribution pattern was quite different in 1967, after a rubella epidemic which began in the spring of 1966 [9]. In 1967, none was positive among infants 7 months to 1 year of age, but the positive rate elevated abruptly to 43% in the older infants 2 to 3 years of age. Among school children of 8 and 9 years, the positive rate decreased. The rate increased again with age and reached 100% at the 20 year old age group.

In 1968, antibody distribution pattern was related to that in 1967, with the exception of children 8 to 9 years old. The pattern in 1968 is quite similar to that obtained in 1957 and it is evident from these distribution patterns that all of the adults over 20 years old have antibody against rubella virus.

The distribution of HAI antibody titers in the sera collected in 1967 and 1968 was also examined as shown in Fig. 2. The HAI titers of the cord blood were between 16 and 128. Among children from 2 to 11 years, HAI titers of 512 or greater were predominant. The antibody titer then gradually decreased with age and the geometric mean titer in those over 50 years was 51. Whereas the positive incidence at this age reached 100% as mentioned above.

The incidence of rubella infection in 1967 was further analysed with sera obtained from school children 6 to 15 years old who were the residents in 14 different areas in the Akita region. The results are shown in Table 1. Although there were...
Fig. 1. Prevalence of HAI antibody in sera from the residents in the Akita region, Japan, in 1957, 1965, 1966 and 1968.

Fig. 2. Incidence of age-specific HAI antibody titers in the 1967 and 1968 sera.
considerable differences with regard to the epidemiology from area to area, it should be noticed that in a Piedmont area, Higashi-Naruse, none of the children under 15 years of age had rubella antibodies. This suggests that there have been no rubella epidemics for the last 15 years in this village. However, 100% of the adults over 20 years was found to have antibodies, even in this area.

**DISCUSSION**

The HAI antibody titration was used to analyse the longitudinal incidence of rubella infection among residents in Akita region both before and after the 1966-1967 epidemic.

The study clearly demonstrated that all of the adults over 20 years old possessed the HAI antibody against rubella virus, irrespective of the place and period after the epidemic.

When the titer of HAI antibody was plotted against age, the highest titer was usually detected in the younger children, as observed in the convalescent sera of rubella patients [9].

Instead, the HAI titers between 64 and 256 were dominant in the age group of 12–40 years. In the age group of 50 or greater, dominant distribution of low antibody titer of 8 to 32 was demonstrated. In the cord blood, the level of antibody titer was similar to that in adults.

Looking at the results from 1957, 1965, 1967 and 1968, it can be assumed that the chance of infection from rubella virus is during childhood in this region. Recently, a similar situation has been observed in various parts of Japan (Reports by Epidemiological Study Group on Rubella, 1969). However, there has been a few reports of the incidence of congenital rubella syndrome in Japan as of this date [3, 6, ibid]. A total of 45 cases with the syndrome including suspect cases were observed from 1948 to 1969 mostly in southwestern parts of Japan. Such a very low incidence of the syndrome in northern Japan may be explained by a hypothesis that almost all of the pregnant women had had rubella either clinically or subclinically during their childhood and thus had acquired immunity before pregnancy. This hypothesis may be supported by the present studies which showed 100% of persons of 20 or greater years of age possessed HAI antibody.

**REFERENCES**


