Rubella Antibody Prevalence in Bangkok School Children

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OBJECTIVES:

1. To determine the pattern of distribution of rubella antibody in school children in Bangkok.

2. To develop a hemagglutination-inhibition test for rubella antibody for use in AFRIMS Laboratory.

BACKGROUND: Rubella is only one of many viral fever-rash syndromes that occur in children living in the tropics. It is generally a mild disease and tends to be discounted in the overall picture of childhood diseases. Its epidemiology in Thailand is not well defined. The importance of rubella arises from its teratogenic effects when contracted by a woman in the early stages of a pregnancy.

A survey done in Bangkok during 1969-1970 found rubella antibody in 52.7 percent of the females tested, most of whom (84%) were of child bearing age (15-45 years) (1). Of the 205 children, ages five to fourteen years, included in the earlier Bangkok survey 53.2% were seronegative. This is in contrast to similar surveys done in the U.S., Europe, and Mexico where at least 80% of females of child bearing age were found to have antibody, the seroconversions usually occurring by age 15 years (2, 3). The authors of the Bangkok report attributed the low prevalence of antibody to the absence of rubella from Thailand for several years preceding its epidemic return in September 1967.

During the decade since the 1967 epidemic, rubella has continued to be present in Thailand as evidenced by the surveillance data of the Ministry of Public Health, although there have been no major outbreaks (4).

METHODS: Phibunprachasan School in Bangkok serves a lower income and slum area population. The students range from 4 to 15 years old. An estimated 82% are Thai with the remaining 18% of Chinese or Chinese-Thai origins. Of 1987 who agreed to take part in a research project, an age stratified sample of 528 sera were selected at random to be used in this survey. Both males and females were tested.

The sera were stored at -20°C until tested. Antibody to rubella was determined by hemagglutination-inhibition test (HI) using microtechniques (5, 6, 7). Antigen and control sera were obtained from Flow Laboratories, U.S.A.

Pretreatment of sera: 0.1 ml of sera was inactivated at 56°C for 30 minutes. To remove nonspecific inhibitors, the sera was extracted with 10 ml
of cold acetone. The sample was then centrifuged (1800 rpm, 7-10 min.) and the supernate discarded. The procedure was repeated. After the second extraction the precipitate was dried in a 37°C incubator overnight. The sera was then rehydrated with 0.5 ml of borate saline, pH 9.0. This yielded a 1:5 dilution of the sera. The sera were then absorbed with 0.05 ml of a 50% suspension of baby chick red blood cells in a normal saline.

Hemagglutination (HA): Antigen activity was determined by HA. Two fold serial dilutions of antigen were made in microtiter U plates, using 0.05 ml of 0.4% bovine albumin in borate saline, pH 9.0, as the buffer. An equal volume of 0.25% baby chick red blood cells suspended in phosphate buffer, pH 6.2*, was added to each well. The plates were incubated in an ice bath for 60-90 minutes and then read. The highest dilution that yielded complete agglutination was considered to contain 1 HA unit.

The dilution that was then equivalent to 4HA units in 0.025 ml was then used in the HI test.

HI: Serial dilutions of 0.025 ml test sera (and controls) were made in microtiter V-plates. 0.025 ml of the antigen dilution (i.e. 4 HA units) was combined with the samples. Albumin borate saline, pH 9.0, was used as the buffer. After incubating the sera antigen mixtures at 40°C for 1½ hours, 0.025 ml of the 0.25% baby chick RBC suspension was added to each well. The plates were then incubated in an ice bath for 60-90 minutes. Complete inhibition of hemagglutination at a dilution of 1:10 or higher was considered evidence of prior rubella infection.

RESULTS: Of the 528 sera tested, 189(35.8%) were positive at 1:10 or greater. The antibody prevalence by age group is shown in Figure 1. The graph indicates a gradual acquisition of antibody titer with increasing age as the prevalence ranges from 0.0% positive in the youngest group to 40-60% positive in the older groups.

* Red cell suspensions at pH 6.0 and 6.2 were compared. As the suspension at pH 6.2 showed a clearer pattern it was used in the HI and HA tests.
Figure 1. Prevalence of HI Antibody to Rubella in Bangkok School Children
REFERENCES:


