

Title: Studies on Epidemic Rubella

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During January, 1968 the occurrence of cases of febrile rubelliform exanthems began reaching obvious epidemic proportions in the Bangkok area. Data is not yet accumulated as to the prevalence of such disease, but it is believed illnesses first appeared during October, 1967 with the peak incidence occurring in February–March, 1968. During February, 1968, staff physicians in the Out–patient Dept., Children's Hospital, made the clinical diagnosis of rubella on over 200 cases. Because of the implications of epidemic rubella to a community over the ensuing months, studies were initiated to identify the specific etiology of the disease. Clinically, the lack of prodromal illness, the characteristic exanthem and presence of adenopathy were compatible with the diagnosis of rubella. No reports of unduly severe illness suggesting an increased virulence have been received.

53 throat swabs were obtained from suspect patients; in addition, rectal swabs were collected from eight of the patients. Paired sera were available from 39 patients.

Attempts at virus isolation were carried out by inoculation of WI–38, LLC–MK₂ and primary cynomolgous monkey kidney cultures. No cytopathic or hemadsorbing viruses were isolated. 14 of the specimens were positive for the presence of an interfering agent. Such challenge virus resistance could be demonstrated only in BS–C–1 or LLC–MK₂ cells.

Two of the interfering agents were identified as rubella virus by neutralization with hyperimmune monkey reference antiserum. Paired sera were available from six of the individuals from whose throat swabs a CVR positive agent was isolated. Five of these showed a diagnostic rise in rubella HI titer (see below).

Commercial antigen (Microbiological Associates Inc.) was used for measurement of hemagglutination-inhibition antibody. It was found that optimal titer of the antigen was obtained by using 2–day old chick erythrocytes, and controlling the pH of the reaction mixture at pH 5.8 using the borate saline and phosphate buffer system employed in arbovirus HI serology.

For routine testing, sera were heat–inactivated and adsorbed with kaolin and chick cells. A two hour incubation time at 0°C was used, following which 0.25% red cell suspension was added.

Results of serological tests and isolation studies are combined in table 1.

Of the 39 paired sera tested, 35 showed diagnostic increases in HI antibody titer. Twelve of these pairs were selected for further study. Previous reports of optimal methods for removing non–specific inhibitors in human sera have differed so various methods were compared in parallel tests. Sera were treated by kaolin adsorption, acetone extraction or manganous chloride–heparin precipitation and compared to HI titers of untreated sera. Following treatment all 24 sera showed identical titers no matter which method had been used; untreated sera showed consistently higher titers of two–to eight–fold magnitude. The consistency of latter observation suggests that some antibody may be removed or its activity reduced by treatment methods in addition to removal of inhibitors. Immunochemical studies of these methods and their effect on rubella HI antibody are under study.

Table 1. Diagnostic studies on suspected rubella patients.

Case No.	Age	HI Titer		Interfering Agent Isolated, Throat Swab
		Acute	Conv	
1	28	0	1260	NT
2	9	0	2560	NT
3	10	0	80	NT
4	6	0	2560	NT
5	3	40	20	NT
6	7	40	640	NEG
7	16	NT	—	NEG
8	4	320	1280	NEG
9	17	20	320	NEG
10	23	NT	—	NEG
11	20	NT	—	NEG
12	28	40	640	NEG
13	2	0	320	NEG
14	6	0	1280	POS
15	40	80	1280	POS
16	8	20	1280	NEG
17	7	NT	—	NEG
18	31	40	320	NEG
19	19	0	640	POS
20	8	40	640	NEG
21	5	NT	—	POS
22	11	NT	—	POS
23	8	NT	—	POS
24	25	80	2560	NEG
25	34	40	80	POS
26	15	NT	—	POS
27	18	NT	—	POS
28	45	NT	—	NEG
29	30	0	1280	NEG
30	23	NT	—	NEG
31	12	NT	—	NEG
32	30	NT	—	NEG
33	10	80	320	NT
34	20	0	320	POS
35	19	20	320	NEG
36	31	80	2560	NT
37	23	20	1280	NEG
38	22	80	640	NT
39	22	1280	1280	NT
40	22	80	80	NEG
41	20	20	640	NEG
42	24	NT	—	POS
43	27	0	80	NEG
44	26	NT	—	NEG
45	29	NT	—	NEG
46	30	NT	—	POS
47	19	40	1280	NT
48	30	160	320	NT

Table 1. (Continued)

Case No.	Age	HI Titer		Interfering Agent Isolated, Throat Swab
		Acute	Conv	
49	14	80	1280	NT
50	27	80	1280	NT
51	12	0	320	NT
52	21	NT	—	NEG
53	28	0	1280	NT
54	21	0	2560	NT
55	27	0	320	NT
56		0	320	POS
57		0	640	NT

The results of virus isolation and serologic testing indicate that a large percentage of febrile exanthems observed in Bangkok during the period studied were due to rubella infections. They further suggest that the period of time since the most recent epidemic of disease occurred has been sufficiently long that older age groups were also infected. The implications in terms of infection of pregnant women are serious. Continued awareness and surveillance of spontaneous abortions and miscarriages, and observations for infants with congenital infection will be important. Plans to detect congenital infections by assay of fetal IGM immunoglobulins and/or rubella macroglobulin antibody are being formulated.

While the relative sensitivity of the stable monkey renal cell lines LLC-MK₂ and BS-C-1 to rubella virus are unknown, it appears that they offer a sufficiently sensitive detection system to recover virus from at least a certain percent of infections. On the other hand the rubella HI test appears to represent a means of detecting infection by relatively simple means when appropriately obtained sera are available. The magnitude of the antibody response, in some cases in the present study on the order of a thousand fold increase in titer, is superior to the low levels usually detected by the more cumbersome neutralization- and CF methods.