BODY OF REPORT

SEATO Medic Study No. 11	Enteric Virus Infections in Thailand
Project No. 3A 025601 A 811	Military Medical Research Program S. E. Asia
Task 01:	Military Medical Research Program S. E. Asia
Subtask 01:	Military Medical Research Program SEASIA (Thailand)
Reporting Installation:	US Army-SEATO Medical Research Laboratory APO San Francisco 96346
	Division of Medical Research Laboratories
	Department of Virology
Period Covered by Report:	1 April 1964 to 31 March 1965
Principal Investigator:	Major Scott B. Halstead, MC
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Reports Control Symbol:	MEDDH-288
Security Classification:	UNCLASSIFIED

Objectives: To recover, characterize and determine the disease potentiality of enteroviruses in Thailand.

Description: Monolayer cell cultures of cynomolgus monkey kidney were prepared by the method of Bodian. Growth medium containing Hank's BSS with 4% bovine serum and 0.5% Lactalbumin hydrolysate. Maintainence medium contained 2% bovine serum and with 0.5% Lactalbumin. Throat and rectal swabs were obtained from randomly selected in-patients and out-patients of Children's Hospital (see SEATO Medic Study No. 2). Swabs were collected and placed in test tubes containing 2 cc of monkey kidney cell maintainence medium, and kept in an ice box until returned to the virus laboratory. After centrifugation at 10,000 rpm for 30 minutes, supernatant fluids were quick frozen and kept at -70°C in sealed vials until the isolation attempt. One tenth ml. of undiluted specimen was inoculated on monolayer cell sheet (maintainence medium removed) and incubated for 1 1/2 hours at 37° C. One ml. of maintainence medium was then added to each tube and tubes observed for CPE daily. Specimens producing

Table 1

NUMBER OF ENTEROVIRUSES RECOVERED FROM THROAT AND RECTAL SWABS FROM 1,102 THAI AND CHINESE CHILDREN STUDIED IN 1962-1964. CHILDREN'S HOSPITAL, BANGKOK, THAILAND.

Year	Viruses recovered	No. of patients	Antibody against recovered virus: Rise in titer	No rise in titer	Not done
1962	Polio 3	6	3	0	3
	Polio 1	3	-	-	3
	Coxsackie B3	2	1	1	0
	Coxsackie B5	1	0	1	0
	ECHO 8	1	0	1	0
	ECHO 11	1	-	-	1
	ECHO 21	1	1	0	0
1963	Polio 1	1	1	0	0
	Coxsackie A9	1	-	-	1
	Coxsackie B2	1	1	0	0
	Coxsackie B5	1	1	0	0
	ECHO 9	1	1	0	0
1964	Polio 2	9	6	1	0
	ECHO 9	4	4	0	0
	Polio 1	1	1	0	0
	Coxsackie B2	1	1	0	0

Table 2

AGE DISTRIBUTION AMONG STUDIED PATIENTS AND PATIENTS WITH ENTEROVIRUS RECOVERY, CHILDREN'S HOSPITAL 1962-1964

Age	Studied Cases: Number	Studied Cases: % of total cases	Enterovirus Isolations: Number	Enterovirus Isolations: % of viruses isolated
Less than 1 yr	128	11.7	8	26.0
1-2	98	9.0	11	35.5
2-3	141	12.9	5	16.0
3-4	115	10.5	4	12.9
4-5	110	9.1	2	6.4
5-6	117	10.7	0	0
6-7	92	8.4	0	0
7-8	64	5.9	0	0
8-9	79	7.2	1	3.2
9-10	43	4.0	0	0
10-11	25	2.3	0	0
11-12	30	2.8	0	0
12-13 and over	60	5.5	0	0
Total	1,102	100.0	31	100.0

Fig. I : Age specific enterovirus isolation rate from patients at Children's Hospital Bangkok, 1962-4

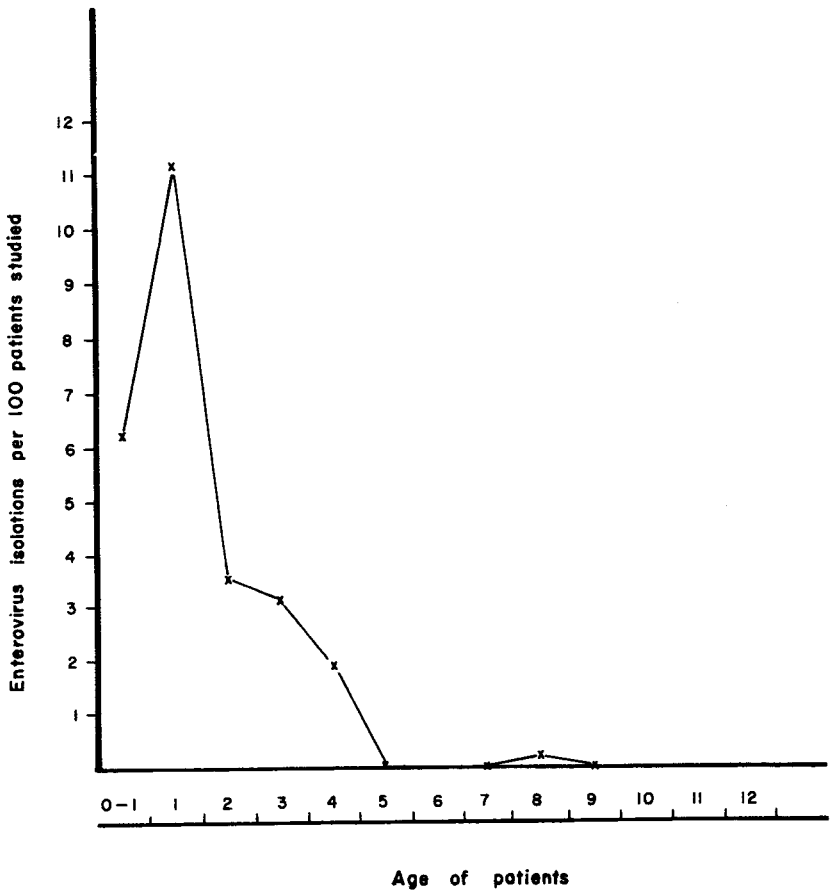

Fig. II Percentage distribution of studied patients and virus isolations by age. Children's Hospital Enterovirus study, Bangkok, 1962-4

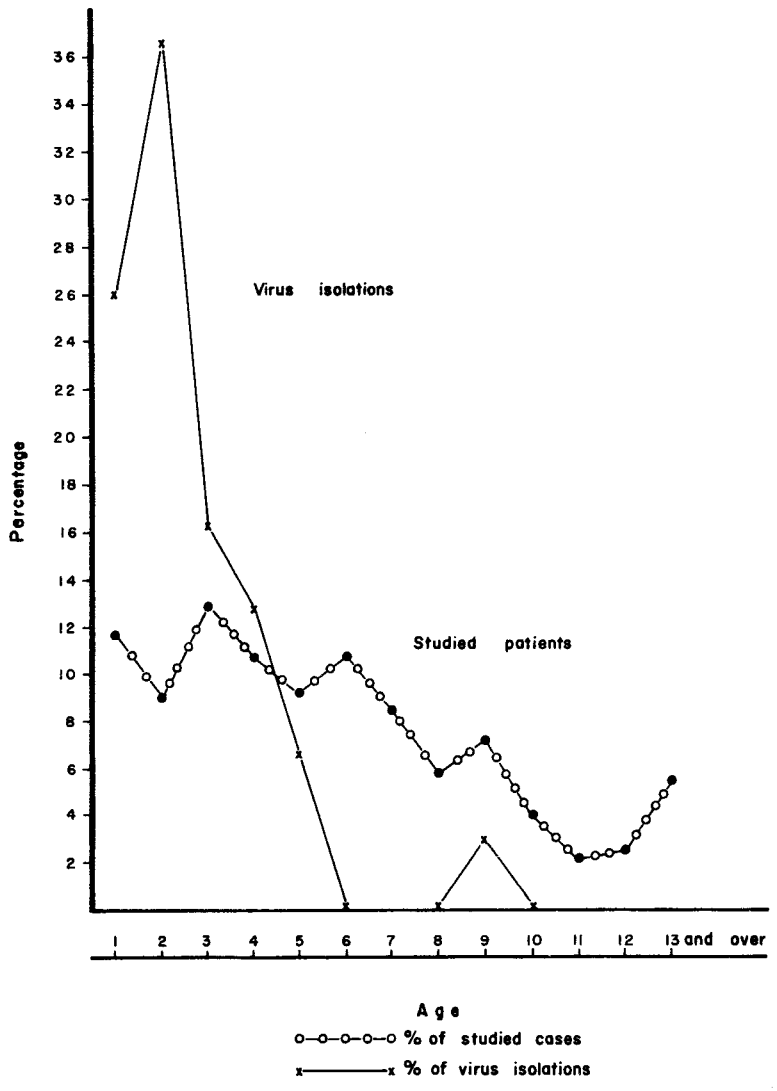

Table 3

CLINICAL DIAGNOSIS OF ENTEROVIRUS DISEASE. CHILDREN'S HOSPITAL STUDY, BANGKOK, 1962-1964. (EXCLUDING POLIOMYELITIS).

Diagnosis	Patients with virus isolated and antibody rise to recovered virus		
	Out-patients	In-patients	Total
Respiratory tract diseases			
- Upper respiratory infection	3	0	3
- Pharyngitis	2	0	2
- Bronchitis	2	0	2
- Pneumonia	0	1	1
Pyrexia unknown origin	2	0	2
Surgical cases	0	2	2
Thai hemorrhagic fever	1	0	1
Diphtheria	0	1	1
Aseptic meningitis	0	1	1
Fever with weakness of limbs	0	1	1
Febrile exanthem'	1	0	1

Table 4

CLINICAL SYMPTOMS AND SIGNS IN PATIENTS WITH ENTEROVIRUS DISEASE CONFIRMED BY ISOLATION OF VIRUS AND RISE IN ANTIBODY IN PAIRED SERA (EXCLUDING CLINICAL POLIOMYELITIS).

Virus	Cox B2	Cox B3	Cox B5	ECHO 9	ECHO 21	Polio I, II, III
No.of patients	2	2	1	5	1	6
Symptoms & Signs						
Fever	1		1	1	1	1
Headache	1			1	-	-
Nasal discharge	-		1	1	1	1
Vomiting	1			1	-	1
Pharyngo-tonsillitis	1		1	1	1	1
Myalgia	1		-	-	-	1
Diarrhea	-		-	1	1	-
Constipation	-		-	-	-	-
Paralysis	-		-	-	-	1
+ve Tourn. test	-		-	1	-	-
Change in sensorium	-		-	-	-	-
Rigidity of neck & back	-		-	1	-	-
Rash	-		-	1	-	-
Petechiae	-		1	1	-	1
Meningeal signs	-		-	1	-	1
Lungs signs	0		0	0	0	1
Heart signs	0		0	0	0	0
Liver enlargement	0		0	0	0	1
Spleen enlargement	0		0	0	0	0

CPE were identified by a serum dilution neutralization test using known enterovirus immune sera (Microbiological Associates). Paired sera from the patient were tested against his own virus. Only patients developing antibody to their own viruses were considered to have been infected systemically. No conclusion about the association of disease and virus recovered was made in the absence of rising antibody levels.

Progress: As shown in Table 1 polio type III was the most frequently recovered virus from throat and rectal swabs in the year 1962. Other viruses recovered in 1962 were Polio type 1, Coxsackie B3, Coxsackie B5, ECHO 8, ECHO 11, and ECHO 21. In 1963 and 1964 the number of different enterovirus recovered were somewhat lower than 1962.

Although the overall virus recovery rate for the study was surprisingly low (31 viruses recovered from 1102 patients, or 2.8%) when virus isolations are analysed by age (Table 2 and Figures 1 and 2), it is apparent that enterovirus recoveries were frequent in infants and young children. Children under the age of 4 constituted only 40% of the studied patients while 90% of virus recoveries were made in this age group. Virus isolations per 100 patients studied was as high as 11% in 1 to 2 year old children.

Table 3 shows the clinical diagnoses received by patients with enterovirus recoveries. Twelve illnesses were diagnoses as respiratory tract disease; four as pyrexia of unknown origin; 2 were sugical cases and 1 each were hemorrhagic fever, diphtheria, aseptic meningitis, headache, fever with weakness of limbs and febrile exanthem. It seems from the diagnoses made and the major symptoms recorded (Table 4) that enterovirus infections in the group and period studies were associate with mild and relatively undifferentiated illness for the most part.

Summary and Conclusions: From 1962 through 1964, 1102 Thai and Chinese children in Bangkok were studied for recovery of enteroviruses. Specimens were collected from patients with a variety of diseases at Children's Hospital and in a few instances from other hospitals in Bangkok. The incidence of enterovirus disease in Bangkok was relatively low for the total group studied but was high in children younger than 4 years of age. During the study an enterovirus was recovered only once from a child older than 5. These results suggest that most enterovirus infections in Bangkok occur in early infancy and childhood and resistance to enteroviruses of many types occurs early in life. This has been shown for polio viruses in earlier studies in Thailand and other tropical countries. It would appear that an enterovirus "carrier state" is not common among the children over 5 years of age. In this study enteroviruses frequently produced a disease diagnosed as upper respiratory illness. In general, the clinical patterns were non-specific and it would have been impossible to have diagnosed the specific etiology clinically. As Bangkok becomes progressively urbanized the enterovirus disease may increase as a health problem. At present, however, these viruses are a negligible cause of overt disease except, perhaps, in infants and very young children.