# ANNUAL PROGRESS REPORT

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| 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 146, San Francisco, California |
| | Division of Medical Research Laboratories |
| | Department of Virology |
| Period Covered by Report: | 1 April 1963 to 31 March 1964 |
| Principal Investigator: | Major Scott B. Halstead, MC |
| Associate Investigator: | Dr. Rapin Snitbhan |
| Reports Control Symbol: | MEDDH-288 |
| Security Classification: | UNCLASSIFIED |

ABSTRACT

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The objective of this study is to recover, characterize and determine the disease potentiality of enteroviruses in Thailand. During 1962 and 1963, 1400 throat and rectal swabs were processed for recovery of enteroviruses. During 1962 most specimens were collected from randomly selected in-patients and out-patients at Children's Hospital, Bangkok. In 1963 specimens were predominantly collected from American residents of Bangkok ill with a variety of febrile syndromes. Of 425 patients studied in 1962 only 20 had enteroviruses recovered from throat or rectal swabs. In 1963, of 350 persons studied only 6 had enterovirus recovery. Viruses recovered were predominantly polio 3, while many different serotypes occurred sporadically. Nearly all patients had serologic evidence that recovered viruses had produced infection during the period of symptoms. The prevalence of enteroviruses as a cause of disease in tropical Bangkok is unexpectedly low. In future investigations recovery

systems will be diversified in an attempt to increase the spectrum of viruses recovered.

An influenza outbreak occurred in Thailand September to December, 1963. Three viruses recovered have been identified as being closely related to the original 1957 A2 strains.

BODY OF REPORT

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

Description: Throat and rectal swabs are collected from patients admitted to SMRL #2 study. In addition, specimens are actively sought from patients visiting the JUSMAG Medical Unit and Bangkok Sanitarium and Hospital. Specimens are processed in primary monkey kidney cells (Macaca irus), titered against patient's acute and convalescent serum and identified with commercial enterovirus antisera. Serologic surveys for specific enterovirus antibodies are conducted. Outbreaks of disease are studied wherever they occur.

Progress: Total of 1400 throat and rectal swabs collected in 1962 and 1963 have been tested in monkey kidney cells to date. After collection, throat or rectal swabs are immersed in 2 ml of Hanks BSS containing 3% calf or bovine serum, placed on wet ice, returned to the Laboratory where they are centrifuged at 10,000 rpm for 30 minutes. The supernatent then is stored at -70° C until tested. After inoculation of cultures all specimens are observed for 14 days. Negative tubes are then challenged with polio type 1. Unchallenged controls of tubes resisting challenge are passaged further.

Whenever possible isolates are tested against acute-convalescent serum obtained from patients from whom virus was isolated. For identification virus isolates are tested against 20 units final concentration of the following antiserum pools: polio 1-3, Coxackie B1-6, ECHO 1-5, 6-10, 11-15, 16-20 and 21-25; positives are then retested against 20 units of single type hyperimmune serum.

Of 850 rectal and throat swabs obtained from 425 persons collected in 1962, 26 viruses were recovered. Three agents are low titered and have not been identified. Altogether 20 out of 425 persons had enteroviruses recovered from their enteric tract. Six viruses were duplicate recoveries of the same virus from the same individual. Of 10 persons with ample acute and convalescent 7 had a rise in antibody to the virus isolated (Table 55).

Of 700 specimens from 350 patients tested in 1963, 8 viruses were recovered from 6 persons. All persons in whom tests are complete had a diagnostic antibody response to their own virus. It should be noted that in 1962 specimens were collected from hemorrhagic fever patients (HFI), in-patients with febrile diseases (NH), surgical patients and out-patients (PUO) while in 1963, specimens collected from Children's Hospital were obtained from out-patients only. Approximately 2 patients per week were studied. In addition, specimens were collected from Americans visiting the JUSMAG Medical Unit or hospitalized at the Bangkok Sanitarium and Hospital. The 1963 specimen collection was largely from American adults as contrasted with 1962 which was almost exclusively from Thai and Chinese children.

The relative infrequency of recovery of enteroviruses especially in 1963 is in marked contrast to frequency of arbovirus disease. It is impossible, of course, to estimate the efficiency recovery of virus without sending duplicate specimens to other laboratories. The predominance of polio 3 in 1962 is of interest. No enteroviruses other than polio occurred with sufficient frequency to suggest epidemic disease. In 1962, enterovirus recoveries occurred more frequently from August through November than any other period. In neither year did enterovirus recoveries predominate during dry weather.

## Influenza in Bangkok, 1963

From August to December, 1963 a number of cases of febrile upper respiratory disease occurred among Americans and other foreign residents of Bangkok. In addition, pediatric out-patient departments and in-patient facilities reported a sharp increase in upper respiratory disease, pneumonitis and pneumonia deaths in infants and children. Statistics for upper respiratory disease for this period have not been received, thus, no estimation of the size of the outbreak can be made.

In order to document the etiology of this outbreak, throat swabs were collected from 11 children at Children's Hospital on 30 October 1963. Specimens were inoculated intra-amniotically in 10 day embryonated hens eggs. Specimens were blind passed after 7 days incubation. Amniotic fluids were tested for HA activity with 1% human O red blood cells. Three viruses were covered. Two of these isolates have been typed; first, in the SMRL virus laboratory and later by Dr. H.G. Pereira of the World Influenza Center, Mill Hill, London and by Captain Howard Weinberger, WRAIR. Results from the WIC are shown in Table 56. These data are interpreted as showing close relationship of the Thai virus to A2/Singapore/1/57 and a remote relationship to the more recent influenza strains, A2/England/1/61 and A2/Netherlands/65/63. A close antigenic relationship to original A2 influenza may explain the apparent low morbidity in Thai adults during the outbreak, many of whom were infected during the 1957 influenza pandemic.

Summary and Conclusions: During 1962 and 1963, 1400 throat and rectal swabs were processed for recovery of enteroviruses. During 1962 most specimens were collected from randomly selected in-patients and out-patients at Children's Hospital, Bangkok. In 1963 specimens were predominantly collected from American residents of Bangkok ill with a variety of febrile syndromes. Of 425 patients studied in 1962 only 20 had enteroviruses recovered from throat or rectal swabs. In 1963, of 350 persons studied only 6 had enterovirus recovery. Viruses recovered were predominantly polio (3) while many different serotypes occurred sporadically. Nearly all patients had serologic evidence that recovered viruses had produced infection during the period of symptoms. The prevalence of enteroviruses as cause of disease in tropical Bangkok is unexpectedly low. In future investigations recovery systems will be diversified in an attempt to increase the spectrum of viruses recovered.

An influenza outbreak occurred in Thailand September to December, 1963. Three viruses recovered have been identified as being closely related to the original 1957 A2 strains.

Table 55. Enteroviruses recovered from human illnesses, Bangkok, 1962 and 1963.

| Year | Study No. | Diagnosis | Date onset | Specimen from which virus was recovered | Isolate titer vs. | | Identifcation | Remarks |
|---|---|---|---|---|---|---|---|---|
| | | | | | Acute | Conval. | | |
| 1962 | 2320-62 | Poliomyelitis | 28 Feb 62 | RS | ND | ND | Polio I | |
| | 2324-62 | Poliomyelitis | 28 Feb 62 | RS | ND | ND | Polio I | |
| | 2329-62 | Poliomyelitis | 11 Mar 62 | RS | ND | ND | Polio I | |
| | NH-14 | Purulent meningitis | 20 May 62 | TS, RS | ND | - | Polio III | Pt. died |
| | HFI-28 | Hemorrhagic fever | 13 Jun 62 | RS | < 1:10 | 1:15 | Cox B5 | |
| | PUO-23 | Pharyngitis | 21 Jun 62 | TS | < 1:10 | 1:40 | ECHO 21 | |
| | PUO-48 | URI | 22 Aug 62 | TS | ND | ND | Not identified | |
| | NH-54 | Hemorrhagic fever | 18 Aug 62 | RS | ND | ND | Not identified | |
| | HFI-76 | Hemorrhagic fever | 22 Aug 62 | TS | < 1:10 | < 1:10 | Not identified | |
| | PUO-54 | PUO | 6 Sep 62 | RS | 1:10 | 1:10 | ECHO 8 | |
| | NH-64 | Influenza with febrile convulsion | 17 Sep 62 | TS, RS | QNS | QNS | Polio III | |
| | HFI-115 | Hemorrhagic fever | 22 Sep 62 | TS, RS | QNS | QNS | ECHO 11 | |
| | PUO-62 | Febrile exanthem | 27 Sep 62 | TS, RS | QNS | QNS | Polio III | |

Table 55. Enteroviruses recovered from human illnesses, Bangkok, 1962 and 1963. (Continued)

| Year | Study No. | Diagnosis | Date onset | Specimen from which virus was recovered | Isolate titer vs. | | Identification | Remarks |
|---|---|---|---|---|---|---|---|---|
| | | | | | Acute | Conval. | | |
| 1962 | PUO-65 | URI | 4 Oct 62 | RS | 1:10 | 1:15 | Polio III | |
| | S-68 | 2nd degree burn | Burned 16 Oct 62 | TS, RS | 1:15 | 1:640 | Polio III | |
| | PUO-71 | Pharyngitis | 21 Oct 62 | RS | 1:10 | 1:10 | Cox B3 | |
| | PUO-76 | PUO | 31 Oct 62 | RS | 1:10 | 1:120 | Polio III | |
| | NH-89 | Purulent meningitis | 13 Nov 62 | RS | ND | - | Polio III | Pt. died |
| | S-82 | 2nd degree burn | Burned 27 Nov 62 | RS | 1:10 | 1:40 | Cox B3 | |
| | NH-96 | Poliomyelitis | 29 Nov 62 | TS, RS | 1:10 | 1:320 | Polio III | |
| 1963 | PUO-108 | Lymphadenitis | 24 Feb 63 | RS | 1:10 | 1:120 | Not identified | |
| | CAU-46 | Aseptic meningitis | 22 Mar 63 | TS, RS | 1:10 | 1:120 | ECHO 9 | |
| | CAU-55 | Fever with headache | 10 Apr 63 | TS | 1:10 | 1:60 | Cox B2 | |
| | PUO-137 | Pharyngitis | 16 Jun 63 | RS | 1:160 | 1:2560 | Cox B5 | |
| | CAU-168 | Dengue fever | 20 Sep 63 | TS, RS | ND | ND | Cox A9 | |
| | TH-214 | Poliomyelitis | 15 Nov 63 | RS | 1:320 | 1:1280 | Polio I | |

Table 56. Haemagglutination-inhibition and complement-fixation tests of Thailand influenza viruses versus prototype influenza antisera.

Ferret sera

| Strains | A2/Singapore/1/57 | A2/England/1/61 | A2/Netherlands/65/63 |
|---|---|---|---|
| A2/Singapore/1/57 | 3,840 | 960 | 480 |
| A2/England/1/61 | 1,920 | 2,560 | 640 |
| A2/Netherlands/65/63 | 1,920 | 960 | 3,840 |
| A2/Thailand/2699/63 | 30 | <10 | <10 |
| A2/Thailand/2706/63 | 15 | <10 | <10 |

Complement-fixation tests

Strain-specific guinea pig sera

| Antigen | A2/Singapore/1/57 | A2/Netherlands/65/63 |
|---|---|---|
| A2/Singapore/1/57 | 480 | 60 |
| A2/Netherlands/65/63 | 80 | 640 |
| A2/Thailand/2699/63 | 240 | 40 |
| A2/Thailand/2706/63 | 120 | 30 |