

SEATO MEDICAL RESEARCH STUDY ON MELIOIDOSIS

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OBJECTIVE

This study was designed to determine the presence and distribution of Pseudomonas pseudomallei in Thailand and to evaluate its importance as the causative agent of the disease, melioidosis.

DESCRIPTION

Previous studies determined that Ps. pseudomallei was present in the water and soil of southern and northeastern Thailand, and there was an association of serological activity of Thai people with the presence of the organism in the environs. However, no clinical cases of melioidosis in man and animals indigenous to Thailand have been described for 14 years. It was concluded that Ps. pseudomallei is saprophytic in soil and water although it must still be regarded as a potential pathogen for man.

Continuing studies during this period included analyses of water and soil for isolation of Ps. pseudomallei and evaluation of techniques for recovery of the organism from water and stool specimens. Attempts to find clinical melioidosis in Thai nationals have been resumed and a prospective epidemiological study of melioidosis was initiated in late August 1968 in a small village where well water and soil were found to be contaminated with Ps. pseudomallei. In addition an attempt to produce and study water-borne melioidosis in experimental animals was initiated.

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PROGRESS

1. Survey of water and soil of Thailand for the presence of *Ps. pseudomallei*. Field trips were made to collect water and/or soil samples along the routes to Aranyapradaet in the East, Kanchanaburi in the West, Saraburi in the Central plain and from 5 southern provinces. Additional specimens were collected by Dr. Pornchai Sirisampan of Siriraj Medical School and Miss Poonsook Atthasampunna of the Applied Scientific Research Corporation of Thailand. Dried soil specimens from Udorn, Roi-et and South Laos, collected for other purposes by USOM Soil Laboratory, Bangkok, were also tested. Results are shown in Table 1. Isolates of *Ps. pseudomallei* were made from Saraburi, Nakorn Nayok and Suphanburi in central Thailand, Prachinburi in the East, Udorn in the Northeast and Pattani in the South.

2. Evaluation of techniques for recovery of *Ps. pseudomallei* from water and stool specimens. The Millipore filter technique was used in an attempt to make quantitative determinations of *Ps. pseudomallei* in well water known to be positive for this organism by hamster inoculation. Volumes of well water ranging from 5 to 40 ml were filtered through 0.45 μ Millipore membranes which were then placed on NAGCV* and MacConkey agar plates containing 10 mcg/ml colimycin. These were incubated at 37 C and examined every 24 hours for 96 hours. No colonies grown on the plates could be identified as *Ps. pseudomallei* by colonial morphology, biochemical reactions or serological tests. There was overgrowth of contaminants on all plates inoculated with membranes which had filtered more than 5 ml of well water.

An *in vitro* procedure for recovery of *Ps. pseudomallei* was evaluated to determine the minimal number of organisms that could be detected in stool specimens. Dilutions of a culture of *Ps. pseudomallei* were mixed with a 1:10 suspension of normal stool followed immediately and four hours later by streaking by means of swabs onto three MacConkey agar plates containing (1) 2 mcg/ml colimycin or (2) 10 mcg/ml colimycin or (3) 10 u/ml penicillin and 100 u/ml polymyxin B. *Ps. pseudomallei* was recovered from all plates if there were at least 10^6 organisms/ml of stool suspension but the organism could be recovered on plates containing either 10 mcg of colimycin or penicillin plus polymyxin B when the number of organisms was 10^4 /ml of stool. Overgrowth did not occur on any of these media containing antibiotics.

3. Case finding of clinical melioidosis in Thai Nationals and animals. Attempts to find cases of clinical melioidosis were made in a Bangkok hospital and in 5 southern provincial hospitals. In Bangkok, Phra Mongkutklao Hospital of the Royal Thai Army was selected for continuing studies because it serves civilians as well as military personnel and cases are referred there from all parts of the country. Those considered most likely to have melioidosis are pulmonary disease patients. Blood specimens were taken for indirect HA test for antibodies and sputum specimens were collected for isolation of the organism. Sixteen of 230 patients tested had significant HA titers (1:80 or higher) but 150 sputum specimens from 54 patients cultured for *Ps. pseudomallei* were negative. Fourteen of 235 sera obtained from patients in Satool, Songkla, Pattani, Yala, and Narathiwat Provincial Hospitals had HA titers against *Ps. pseudomallei* with one serum from Pattani and two sera from Songkla having titers of 1:80 or greater. Cultures of 25 sputa and 14 wound specimens from patients in these hospitals were negative for *Ps. pseudomallei*.

Sixty sera from horses at the Royal Thai Army Horse Breeding Farm in Kanchanaburi were negative at 1:20 for HA antibody. One of 24 buffalo sera collected from Bangkok slaughter house had a HA titer of 1:80. Thirteen rats—seven *Bandicota indica*, five *Rattus exulans* and one *R. rattus*—trapped in Srakaew and Ban Huay Jode in Prachinburi—were bled, examined and cultured. All sera were negative at 1:20 and all cultures from pathological organs, bowel contents and urine were negative for *Ps. pseudomallei*.

4. Epidemiology of melioidosis at Ban Huay Jode, Thailand. In April 1968 *Ps. pseudomallei* was isolated from a water sample obtained from a shallow roadside well in Ban Huay Jode village, Wattana Nakorn district, Prachinburi. In May a team returned to obtain blood specimens from the residents of the

* NAGCV Medium: Nutrient agar (Difco); glycerol (3%); crystal violet (1:200,000) with a final pH of 6.8–7.0.

village, most of whom used the well water for all purposes. Twelve of 114 sera tested had antibody titers of 1:20 or greater against Ps. pseudomallei and at that time the organism was isolated from neighboring wells and from soil taken near the wells. These wells were dug only 3 years earlier so it was decided that this village offered an opportunity to study the epidemiology of water-borne melioidosis. Plans were made for the study to be carried out as a joint project between the Department of Bacteriology and Mycology and the Department of Epidemiology and Special Studies.

The study was initiated in August. This village, a new settlement established after the building of the railway station 12 years ago, is situated along both sides of Route 33 between kilometer posts 265 and 266. The study area was defined; a sketch map was made and censuses were taken of human and animal populations. There were 514 people living in a cluster of 89 households. Most residents had immigrated from other parts of Prachinburi, Nakorn Nayok, Surin, Cholburi and some came from other provinces. All but seven families are farmers who grow rice, peanuts, corn and other vegetables. The nearest medical facility is a First Class Health Center at Wattana Nakorn 12 kms away. Questionnaires were completed regarding occupation, location of farms, sources of drinking and washing water, sources of food and raw meat consumption for the purpose of determining possible sources of Ps. pseudomallei infection.

Blood specimens were voluntarily given by the residents of the study area every 2-3 months for determination of antibodies against Ps. pseudomallei by the indirect HA test. Rectal swabs were taken at the same time for attempted isolation of the organism. Samples from all water sources in the village and soil samples from farming areas were taken every 6-8 weeks to determine the presence of the organism by the hamster inoculation technique. Chest X-rays (70 mm) were taken in September for a baseline determination of chest diseases in the population. Those with abnormal findings were taken for regular 14" × 17" X-ray films at Prachinburi Provincial Hospital. Sputum specimens of chest disease patients were cultured for Mycobacterium tuberculosis and Ps. pseudomallei. Initially sick calls were held at 6 week intervals and then bi-weekly starting in late November to provide clinical monitoring of illnesses and to facilitate specimen collections. Additional censuses were taken in November 1968 and in January and February 1969.

Age and sex distributions of Ban Huay Jode residents are shown in Table 2. Serum antibody titer results are summarized in Tables 3, 4 and 5. Only 347 individuals or 61% of the population participated in the study and 53 individuals or about 10% were fully cooperative by giving 3 blood specimens at 2-3 month intervals. Approximately 70% of the sera were negative at 1:20 by the indirect HA test and none of the 8.65% (30/347) with rising or falling titers had histories suggestive of clinical melioidosis. It was noted that only 2 of 48 children less than 5 years of age had measurable antibody neither had a titer of greater than 1:40. No isolates of Ps. pseudomallei was made from stools of any of the residents.

Periodical examination of water sources for the presence of Ps. pseudomallei showed that either water and/or soil taken from wells or ditches along or near the roadside were positive one or more times. However water samples from wells 100 or more meters from either side of the road were free from this organism. Isolations of Ps. pseudomallei from farming area soils were variable.

Sputa from three of the 15 residents with lung lesions were positive for Mycobacterium tuberculosis but no sputum specimens were positive for Ps. pseudomallei. In February and March 1969 isolations of Ps. pseudomallei were made by hamster inoculation from sputum specimens of a 26 year old man who had moved into the village in December 1968. He indicated a history of chest pain and a productive cough with yellow sputum for one year prior to February 1969. His general health was good and he continued to do the farm work during this year. He agreed to hospitalization in Phra Mongkutklao Hospital, Bangkok for investigation and treatment, where he remains a patient at the time of this report. The results of his HA titers and sputum cultures were as follows:

<u>Date</u>	<u>HA titer</u>	<u>Sputum culture</u>
22 Jan 69	less than 1:20	Not done
20 Feb 69		Positive for <u>Ps. pseudomallei</u>
6 March 69	1:2560	Positive for <u>Ps. pseudomallei</u>
20 Mar 69	1:640	

5. Experimental melioidosis in laboratory animals. An attempt has been made to produce and study water-borne melioidosis in gibbons. Water from a well known to be positive for Ps. pseudomallei has been the sole source of drinking water for one normal and two splenectomized gibbons with Bangkok city water being used for control animals. Stool specimens were collected before the experiment started and weekly thereafter for isolation of Ps. pseudomallei, using the method of Mariappan of the USAMRU, Kuala Lumpur, Malaysia. Blood specimens were collected before the experiment and every three weeks thereafter for determination of Ps. pseudomallei antibodies. All stool cultures have been negative for Ps. pseudomallei and all sera were negative at 1:20 by the indirect HA test. Six months after the experiment started one gibbon in the experimental group died after having diarrhea for one week. Postmortem examination revealed lobar pneumonia. Histological studies showed that the small intestines and lungs were heavily infested by filariform strongyloides. A variety of tissues cultured for Ps. pseudomallei were negative.

SUMMARY

During this period Ps. pseudomallei was found to be indigenous in additional areas of Thailand. A prospective epidemiologic study was started in Ban Huay Jode village of Prachinburi province where one case of non-fatal respiratory melioidosis was found in a recently immigrated resident.

Table 1
Isolation of Pseudomonas pseudomallei from Water and Soil Samples

<u>Area</u>	<u>Province</u>	<u>Water</u>	<u>Soil</u>
North	Chiangmai	0/6*	0/4
Central	Pathumthani	0/3	0/3
	Ayudthaya	0/9	0/9
	Saraburi	4/31	0/30
	Nakorn Nayok	1/15	2/15
	Nakorn Pathom	0/20	0/22
	Suphanburi	0/6	1/6
East	Prachinburi	3/39	4/39
Northeast	Nakorn Rajasima (Korat)	0/3	0/3
	Khonkaen	0/3	0/2
	Nongkai	0/1	0/2
	Udorn	3/13	0/12
	Udorn**	—	0/10
	Roi-et**	—	0/20
West	Kanchanaburi	0/29	0/46
South	Ko Samui	0/6	—
	Satool	0/3	0/3
	Songkla	0/3	0/2
	Pattani	0/2	1/1
	Narathiwat	0/5	0/1
Southern Laos**	Not known	—	0/30

* No. of positive/total specimens tested

** Specimens collected for other purposes by USOM Soil Laboratory

Table 2
 Melioidosis Epidemiology Study:
 Ban Huay Jode Residents by Age and Sex, February 1969

<u>Age groups</u> (year)	<u>Male</u>	<u>Female</u>	<u>Total</u>
<5	52	47	99
5-14	97	75	172
15-24	40	46	86
25-35	34	33	67
35-44	34	28	62
45-54	18	23	41
55 or more	20	14	34
Unknown	5	2	7
Total	300 (52.8%)	268 (47.2%)	568

Table 3
 Melioidosis Epidemiology Study:
 Antibody Titers of Residents Submitting Single Blood Specimen

Age groups (year)	No.			No. c titer less than 1:20			No. c titer of 1:20 or 1:40			No. c titer 1:80 or more		
	Male	Female	Total	Male	Female	Total	Male	Female	Total	Male	Female	Total
<5	15	17	32	14	16	30	1	1	2	0	0	0
5-14	32	35	67	25	31	56	3	3	6	4	1	5
15-24	15	17	32	8	7	15	2	3	5	5	7	12
25-34	8	10	18	3	6	9	1	3	4	4	1	5
35-44	9	11	20	4	6	10	3	3	6	2	2	4
45-54	5	7	12	4	5	9	1	2	3	0	0	0
55 or more	9	5	14	6	1	7	1	1	2	2	3	5
Total	93	102	195	64	72	136	12	16	28	17	14	31

Table 4
 Melioidosis Epidemiology Study:
 Antibody Titers of Residents Submitting 2 Blood Specimens

Age groups (year)	No.			No. c titer less than 1:20			No. c rising titer*			No. c falling titer*			No. c stationary titer**		
	M	F	Total	M	F	Total	M	F	Total	M	F	Total	M	F	Total
<5	4	7	11	4	7	11	0	0	0	0	0	0	0	0	0
5-14	23	12	35	21	11	32	0	0	0	1	1	2	1	0	1
15-24	3	5	8	0	3	3	1	1	2	1	1	2	1	0	1
25-34	4	9	13	1	4	5	1	2	3	0	1	1	2	2	4
35-44	9	11	20	8	5	13	0	1	1	0	2	2	1	3	4
45-54	3	2	5	1	1	2	1	1	2	0	0	0	1	0	1
55 or more	5	2	7	2	1	3	3	0	3	0	0	0	0	1	1
Total	51	48	99	37	32	69	6	5	11	2	5	7	6	6	12

* Fourfold or more change

** Less than fourfold change

Table 5
 Melioidosis Epidemiology Study:
 Antibody Titers of Residents Submitting 3 Blood Specimens

Age groups (year)	No.			No. c titer less than 1:20			No. c rising titer*			No. c falling titer*			No. c stationary titer**		
	M	F	Total	M	F	Total	M	F	Total	M	F	Total	M	F	Total
<5	4	1	5	4	1	5	0	0	0	0	0	0	0	0	0
5-14	12	12	24	9	8	17	1	2	3	0	0	0	2	2	4
15-24	1	1	2	1	1	2	0	0	0	0	0	0	0	0	0
25-34	3	5	8	2	2	4	1	3	4	0	0	0	0	0	0
35-44	2	2	4	0	1	1	1	0	1	0	0	0	1	1	2
45-54	2	4	6	1	0	1	1	2	3	0	0	0	0	2	2
55 or more	2	2	4	1	2	3	0	0	0	1	0	1	0	0	0
Total	26	27	53	18	15	33	4	7	11	1	0	1	3	5	8

* Fourfold or more change
 ** Less than fourfold change