

## Melioidosis

Coordinator : Howard E. Noyes, Ph.D., Chief, Department  
of Bacteriology and Mycology

Principal Investigators : Pongsom Atthasampunna, M.D.\*  
Richard A. Grossman, MAJ, MC  
Howard E. Noyes, Ph.D.

Associate Investigators : Paibul Busapathamrong, MAJ, MC, RTA\*\*  
Prayoth Tanticharoenyos, DVM  
Markpol Tingpalapong, DVM  
Saridwongsa Wongsathuaythong, LTC, MC, RTA\*\*  
William L. Wooding, MAJ, VC

Assistant Investigators : Yupa Kasemsanta, P.H.N.  
Ovath Khunphol, R.N.  
Yongyuth Raengpradub, B.S.  
Chanphen Srimunta, B.S.  
Tasna Tamaarree, Dip. Med. Tech.

**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. Prior to this study 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, attempts to find clinical melioidosis in Thai nationals, a prospective epidemiological study of melioidosis which was initiated in late August 1968 in a small village where well water and soil were found to be contaminated with Ps. pseudomallei, and determination in vitro antimicrobial sensitivities of pure cultures of Ps. pseudomallei isolated from water, soil and specimens from patients.

**PROGRESS:** 1. Survey of water and soil for the presence of Ps. pseudomallei. Specimens were obtained during field trips on other SMRL studies because soil from most provinces in Thailand have been studied in previous years. Samples from Songkla and Yala in the South and Maehongsorn in the North were positive for Ps. pseudomallei. None of 15 specimens from Vientiane, Laos were positive for this organism.

2. Case finding of clinical melioidosis in Thai Nationals and in animals. Attempts to find cases of clinical melioidosis were made in a Bangkok Hospital, a provincial hospital and by testing sera sent to this laboratory for other studies. In Bangkok, Phra Mongkutklao Hospital of the Royal Thai Army, was selected for

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\* On loan from Dept of Health, Ministry of Public Health, Thailand

\*\* Dept of Medicine, Phra Mongkutklao Hospital, Bangkok, Thailand

continuing studies because it serves civilians as well as military personnel and cases are referred there from all parts of Thailand. Those considered most likely to have melioidosis are pulmonary disease patients. Sera were tested by the indirect HA test for antibodies and sputa were collected for isolation of the organism. Ten of 448 patients tested had significant HA titers (1:80 or higher) but none of 373 sputum specimens from 140 patients were positive for Ps. pseudomallei. Five of 81 sera from patients of Songkla Provincial Hospital and sera from 4 of 234 residents of Mae Sarieng (Maehongsorn Province) had significant titers against Ps. pseudomallei. None of 67 sera submitted for febrile agglutination tests and 48 sera from Thai employees of an American military hospital in Bangkok had significant HA antibody titers. Of 160 sera from rodents trapped at Sakaerat Research Station in Nakornrojsima Province, 30 sera from Choburi Province, and 20 sera from rodents trapped in Kabinburi, Prachinburi Province, none had demonstrable HA titers against Ps. pseudomallei. However 3 of 10 cattle sera from the ranch of the Accelerated Development Center in Kanchanaburi Province and 33 of 117 other bovine sera submitted by the Department of Veterinary Medicine had HA titers, but all were 1:40 or lower.

3. 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 of that year a team returned to obtain blood specimens from the residents of the 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 had been dug only 3 years earlier so it was decided that this village offered an opportunity to study the epidemiology of melioidosis in a community where the population was continuously exposed to Ps. pseudomallei. Details of the procedures used and preliminary findings are described in the 1968-1969 Annual Report. The study is a joint project between the Department of Bacteriology and Mycology and the Department of Epidemiology.

Analyses of water sources and soil samples adjacent to water sources in the village and soil from designated sites of farming areas were analysed each month for Ps. pseudomallei. Twenty-six percent of the water and 20 percent of the soil samples were positive with one or more water specimens being positive each time samples were obtained. The organism was isolated from farm soils more frequently in the rainy than the dry season. An additional 21 soil samples taken from the habitats of domestic animals were negative for Ps. pseudomallei.

Sera from domestic animals were analysed for HA antibodies and it was found that water buffalo, cattle and dogs showed evidence of immunologic response to Ps. pseudomallei while, with one possible exception, none of 39 fowls did so. Sera from 2 of 43 water buffaloes, 2 of 12 cattle and 1 of 14 dogs were positive at significant titers. Stool specimens from 12 water buffaloes, 2 cattle, 4 pigs, 1 chicken and 1 dog were negative when cultured for Ps. pseudomallei. Fifty rodents representing 5 species were trapped in the village and rice fields for serum antibody determinations and for isolation of Ps. pseudomallei from their ectoparasites, internal organs and fecal contents. All were negative.

Attempts to produce and study water-borne melioidosis in gibbons were continued. Water from a well known to be positive for Ps. pseudomallei was the sole source of drinking water for normal and splenectomized gibbons for 19 months. None of the gibbons developed clinical symptoms suggestive of melioidosis and none had measurable serum antibodies as measured by the HA procedure.

Blood specimens were voluntarily given by the residents every three months for determination of antibodies against Ps. pseudomallei by the indirect HA test. Results shown in Table 1 show that 28 percent of the 219 residents tested had HA antibodies and 18 percent had significant titers of 1:80 or greater. It was noted that only 4 of 84 children under 10 years of age had significant titers as compared with 16 of 49 in the 10-19 year old group. Overall more males than females had significant titers. It should be noted that these titers were not static in that 7 residents had titer rises of four-fold or greater, 3 residents had similar decreases of titers and 7 residents had rising and falling titers of four-fold or greater during this reporting period. Except for the 2 patients discussed below, no clinical illnesses suggestive of melioidosis were noted in any of the residents.

Based on clinical findings, isolation of Ps. pseudomallei from sputa and serologic conversions, two brothers were diagnosed as having clinical melioidosis. The first case was a 26 year old farmer who moved into this village with his family on 27 December 1968, but he had been working on a farm in this village for 2 years at that time. He was seen first at the village clinic on 22 January 1969 with complaint of tightness in the chest. He was given two doses of 300,000 u of crystalline penicillin G and 0.5 gm of tetracycline three times daily for 3 days. On 20 February he returned with a complaint of a productive cough in the mornings in addition to tightness in the chest. His lungs were clear on examination. A sputum specimen was taken for culture and, although direct streaking of the sputum yielded no Ps. pseudomallei, the organism was isolated from heart blood of hamsters dying after inoculation with the specimen. On 6 March he complained of anorexia and abdominal discomfort. Again Ps. pseudomallei was isolated from heart blood of hamsters injected with sputum but not from the direct culture of the sputum. Indirect HA titer for Ps. pseudomallei which was less than 1:20 in January rose to 1:2,560. On 20 March he complained of fever and shaking chills in the afternoons for the previous 5-6 days but his coughing had decreased. A history of past illness indicated he had chest pains before and had been to the Prachinburi Provincial Hospital in October 1965. A physical examination at that time revealed no positive signs and he was given symptomatic treatment. No chest X-ray was taken. Since then he had experienced chest pains with or without coughing from time to time.

A physical examination carried out on 20 March 1969 revealed a 26 year old male with a temperature of 99.6 F and normal pulse and respiration rates. There was tenderness of the left anterior chest wall at there 3rd and 4th intercostal spaces. No crepitation or rhonchi were heard on auscultation. Liver and spleen were not palpable. A blood culture was negative but his serum antibody titer was 1:640. Immediate hospitalization in Bangkok was advised but the patient waited until 4 April when he was hospitalized at the Phra Mongkutklao (Thai Army) Hospital in Bangkok. A sputum specimen obtained the day before was negative for Ps. pseudomallei.

Laboratory studies were carried out on 9 April 1969. His white blood count was 7,850/cu.mm. with 28% polymorphonuclear neutrophils, 70% lymphocytes and 2% eosinophils. Hemoglobin was 11 grams %; VPC was 43 volume % and ESR was 11 mm/h. Other chemistry values and urinalysis findings were normal. A stool examination was positive for hookworm ova. Chest X-rays were normal. Sputum cultures on 9 and 10 April were negative by direct culture but positive for Ps. pseudomallei by hamster inoculation. His melioidosis HA titer on 8 April was 1:160.

While in the hospital he had no fever; the chest pain diminished; the coughing became less frequent and sputum became scanty. On 11 April he felt much better and could produce no sputum. However, he was given 0.5 g of chloramphenicol every 6 hours by mouth for a total dose of 25 g. On 16 April, another chest X-ray was taken and was normal as before. Cultures of sputum specimens obtained on 16, 17, 18 and 21 April was negative and his melioidosis HA titer on 17 April was 1:1,280. On 18 April his WBC was 7,150/cu.mm. with 42% neutrophils, 55% lymphocytes and 3% eosinophiles. Hemoglobin was 14.1 g%; VPC 44 vol.% and ESR 10 mm/h. He was discharged symptom-free on 23 April and remained free of clinical symptoms during this reporting period.\*

The second case was a 16 year old brother of the first case. He was first seen at the village clinic in January 1969 with a history of chest pains after hard work and on cold days during the last year. Physical examination revealed a young man too small for his age with normal chest findings. In May 1970 he was taken to Prachinburi Provincial Hospital for chest X-rays which were normal. His HA titer of serum taken at that time was less than 1:10. On 25 July he returned to the clinic with complaints of fever and joint pain. His HA titer had risen to more than 1:10, 240. On 14 August he was seen with the same complaints plus pain in the calves. He did not feel feverish although his body temperature was 100 F. His chest was clear and his liver and spleen were not palpable. There was slight enlargement of left submandibular

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\* In mid April 1970 this patient had a relapse as indicated by isolation of Ps. pseudomallei from his sputum.

lymph node. Hemocultures yielded no growth and blood films for malarial parasites were negative. A sputum specimen was positive for Ps. pseudomallei by hamster inoculation but not by direct culture. His HA titer vs Ps. pseudomallei was 1:2,560. On 11 September 1969 he felt weak and had a productive cough. Sputum taken then was positive by both direct culture and hamster inoculation and his HA titer was 1:20,480. Chest X-rays taken on 18 September were negative except for increased lung markings in the left upper lobe area. A CBC done on the same day revealed that he had Hgb 11 gm%, Hct 38%, WBC 7,500 with 35% PMN, 53% L and 12% E. Stool examination was positive for Taenia spp. ova. His sputum was again positive for Ps. pseudomallei. No antibiotics were given and during the next month his cough and joint pains disappeared. However his sputum was still positive and his HA titer was 1:655,360. In November he felt much better but was unable to do hard work. He had gained 5 lbs since September (from 80 to 85 lbs). He was unable to produce sputum for culture during the remainder of the study period. The result of his subsequent HA titers were as follows:

<u>Date</u>	<u>HA titer</u>
11 November 69	1:20,480
16 December 69	1:5,120
14 January 70	1:81,920
11 February 70	1:20,480
12 March 70	1:5,120

He has had no additional clinical symptoms and had returned to his regular farm duties at the time this report was prepared in April 1970.

4. Antimicrobial sensitivities of Ps. pseudomallei. The plate dilution procedure was used to determine antimicrobial sensitivities of 104 isolates of Ps. pseudomallei isolates from water, soil, and specimens from patients with suspected melioidosis. Overall these in vitro studies showed that the most effective antimicrobial studied was novobiocin followed in order of decreasing effectiveness by kanamycin, chloramphenicol, oxytetracycline, rifampin, and neomycin. Isolates from patients tended to be more resistant than those from soil or water. No isolates were sensitive to 0.78 mcg/ml or less of any of the antimicrobials tested and none from humans was sensitive to 3.12 mcg/ml or less.

SUMMARY: During this period the presence of Ps. pseudomallei was documented in Yala, Songkla, Prachinburi and Maehongsorn Provinces but was not detected in water or soil samples from Vientiane, Laos. Fifteen of 878 Thai nationals from the north, central and southern areas of Thailand had significant (1:80 or greater) HA titers against Ps. pseudomallei. Sera from water buffaloes and cattle (but not rodents) showed evidence of immunologic response to that organism. Based on clinical and laboratory studies a second melioidosis patient was diagnosed in Ban Huay Jode, a study area where 18% of the 219 residents tested had significant HA titers against Ps. pseudomallei. In vitro studies of isolates of Ps. pseudomallei showed novobiocin was the most effective of the 6 antimicrobials tested.

Table 1. Melioidosis Epidemiology Study  
 HA Antibody Titers of Ban Huay Jode Residents  
 (1 April 1969–31 March 1970)

Age groups (years)	Number of residents bled		Total with positive titers		Total with titers of 1:80 or greater	
	Male	Female	Male	Female	Male	Female
Under 5	12	12	2	0	0	0
5–9	29	31	6	2	3	1
10–19	25	24	11	8	9	7
20–29	5	11	2	6	1	2
30–39	10	18	5	6	3	4
40–49	11	12	4	6	3	2
50–59	4	5	2	1	2	0
60 and over	7	3	3	1	3	1
<b>Total</b>	<b>103</b>	<b>116</b>	<b>35</b>	<b>30</b>	<b>24</b>	<b>17</b>