SEATO MEDICAL RESEARCH STUDY ON MELIOIDOSIS

Coordinator: Richard A. Finkelstein, Ph.D., Deputy Chief; Department of Bacteriology and Mycology

Principal Investigator: Richard A. Finkelstein, Ph.D.

Associate Investigators:
1. Pongsom Atthasampunna, M.D.
2. Prakai Chitrakorn, D.V.M.[1]
3. Monthree Chulasamaya, M. D.[2]
4. Dilok Kesornsombat, D.V.M.[1]
5. Udom Lexsomboon, M.D., Ph.D.[3]
6. John H. Morris, Lt. Col., VC
7. Manas Sopawanit, M.D.[4]
8. Richard O. Spertzel, Capt., VC

Assistant Investigators:
1. Pichai Charunmethee, B.S. Med. Tech.
2. Jon Goodan, PFC

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1 Research and Education Division, Livestosk Department, Ministry of Agriculture.
2 Formerly, Faculty of Graduate Studies, University of Medical Sciences, Bangkok. Presently, The University of Texas, Austin, Texas.
3 Provincial Hospital, Ubol, Thailand.
4 Central Chest Hospital, Nondhaburi, Department of Health.

Title: Investigation of Melioidosis in Thailand

Principal Investigator: Richard A. Finkelstein, Ph.D.

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Objective - This study is 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-The study is divided essentially into three phases of investigation: (1) Attempts to isolate the organism and to define its geographic distribution in the external environment in Thailand; (2) an attempt to obtain by serologic survey some information regarding the host-parasite interaction in animals and man; and (3) an attempt to associate the presence of the organism with clinical disease in human and animal populations.

Information available at the beginning of this study, in late 1964, indicated that the organism was widely distributed in the external environment in neighboring Malaysia and in Viet Nam. In those areas, melioidosis is said to be a rare and usually grave disease. A limited serologic survey had indicated that a significant percentage of Thai people, in contrast to U.S. personnel, had detectable levels of anitbody against *Ps. pseudomallei* although, to our knowledge, only three cases of melioidosis have been recorded in the medical literature of Thailand. This information suggested that subclinical infection with production of antibodies to the agent, mihgt be more common and that clinical disease is relatively rare. However, the possibility must be considered that with the relative paucity of laboratories capable of this diagrosis laboratories, actual cases of melioidosis might be missed or misdiagnosed. No information concerning the actual presence of the organism in Thailand was available at the inception of this program.

The results of this study have to date confirmed and amplified what has been found previously in other areas: namely, that the organism is widely distributed in Thailand; that the occurrence of detcetable antibody levels is quite common in that (but not U.S. personel) especially in areas where the organism may easily and frequently be found in the exteral environment; that clinical disease is apparently quite rare. These observations lead us to conclude that *Ps. pseudomallei* is essentially a saprophytic organism in nature, although when the opportunity presents (and even then perhaps only under circumstances of pre-existent low natural resistance) it may assume a pathogenic role. It may be comparable to such an organism as *Serratia marcescens*, for example, which is naturally saprophytic but can cause disease in man.

1 Research and Education Division, Livestock Department, Ministry of Agriculture.
2 Formerly, Faculty of Graduate Studies, University of Medical Sciences, Bangkok. Presently, The University of Texas, Austin, Texas.
3 Provincial Hospital, Ubol, Thailand.
4 Central Chest Hospital, Nondhaburi, Department of Health.

Our future plans call for an extensive survey for evidence of the disease in hospitals in the south of Thailand, where the isolation rate of the organism in water samples approaches or exceeds 50%, and in abattoirs in the same areas.

Progress -

1. Geographic distribution of Pseudomonas pseudomallei in Thailand.

Samples of soil and water from various areas in Thailand were inoculated intraperitoneally into hamsters. Heart blood from sick or dead hamsters was streaked on selective and non-selective media. Suspicious colonies were identified serologically and by biochemical tests according to procedures developed previously (Annual Report, 31 March 1965). The results are summarized in Table I. It can be seen that Ps. pseudomallei is rather widely distributed in Thailand although it shows a preference for certain areas, particularly in the southern peninsula. To date, no isolates have been obtained from samples taken in the Bangkok-Dhonburi area or in Chiengmai. The central plains area, from which a rather limited number of samples have been taken, seems to be relatively free. Sporadic isolates were obtained from samples taken in the northeastern provinces while in the southern peninsula (Table I) isolation rates ranging from 30 to 50% were obtained in the provinces of Narathivas, Patalung, Songkla, Pattani and Yala. If Ps. pseudomallei is indeed a naturally virulent and lethal pathogen, it is difficult to conceive of the continuing presence of man and fuana in these areas without obvious disease.

2. Distribution of Pseudomonas pseudomallei isolates in South Thailand in relation to characteristics of water sources.

In the course of one survey in South Thailand in which 793 water samples were collected and tested, information regarding the nature of the water source, whether still or flowing and the pH, was also obtained. The results are summarized in Table II. From these results, it is evident that the organism may be isolated from surface waters with a wide range of pH values, from a low of pH 2 to a high in the range of pH 8-9. There appears to be a slight tendency in favor of still rather than flowing water although the difference is not statistically significant. Caution should be exercised in drawing conclusions relating to pH preferences since these results could be markedly affected by the geographic distribution of the sites selected for testing as well as other factors. For example, the percentage figures would suggest that Ps. pseudomallei is found with lower frequency in waters of the pH range 6.0-6.99. However, this may simply be a reflection of greater numbers of samples in that pH range from areas which are relatively low in positive findings in all pH ranges.

3. Serological observations

A sensitive and specific serologic test, was developed and adapted to the microtiter apparatus. This is an indirect hemagglutination test employing chicken erythrocytes sensitized with a pool of antigen prepared by sonically disrupting heavy formalinized suspensions of Ps. Pseudomallei strains. This technique was employed to evaluate the distribution of melioidosis antibody. The results of serologic tests on 2,915 sera are summarized in Table III. First, it is evident from the results with the sera of the 809 th Engineers and Fort Dix sera, that antibodies reactive with Ps. Pseudomallei antigens in this test are rare in sera of U.S. personnel. Of the sera from the 809 th Engineers, 197 were pre-bleedings taken on their arrival in Thailand. Of these only one serum exhibited a titer (1:40). The remainder of the 809 th sera were taken during their stay in Thailand and the results were quite similar to their initial bleedings. Thus the results suggest that their exposure to melioidosis antigen during their stay in Thailand was minimal. However, the central region where these troops were stationed has not yielded Ps. Pseudomallei and, for this reason, it may that this group of specimens is not indicative of what might happen if they were stationed

in a highly infected area. Sixteen of ninetythree unselected blood donors at the Royal Thai Army Hospital exhibited some titer although the percentage of positive reactors at the blood bank of Siriraj Hospital was markedly lower. The reason for this difference is not clear. It could depend on the previous location of the donors. At Siriraj, it is more likely that were local people from the uninfected area of Bangkok and Dhonburi while at the Army Hospital many of the donors may have had service in infected areas. In Ubol, the majority of serum samples had positive antibody titers. Ubol is one of the provinces from which we have been able to isolate the organism in soil and water samples. Sera collected in Songkla also gave a significant proportion of reactions although not quite as high as one might expect based on the ease with which the organism may be isolated in the external environment there. In contrast, sera collected in Chiengmai, a Ps. pseudomallei-free area, resembled U.S. sera in the relative absence of antibody. The results on sera sent to us from USAMRU, Kuala Lumpur, are of some interest. Of the 76 sera tested, 46 were reported to have some activity in one or more of the serological tests performed at USAMRU (CF, agglutination and HA). In our laboratory, only 13 had significant reactions. Of these, 2 are especially noteworthy in that they represent consecutive sera obtained from a soldier in the British Army who was diagnosed as having active melioidosis. The two sera showed a rise in titer from 1:160 to greater than 1:320. In general, there was good agreement in the results of hemagglutination tests performed separately in the two laboratories, and it appears that this HA test is both more sensitive and more specific than the other serological tests employed. Sera obtained from Thai cattle and buffalo gave a significant proportion of positive reactions suggesting that many of these animals come in cantact with, and respond to, melioidosis antigens.

4. Attempts to isolate Pseudomonas pseudomallei from clinical specimens.

Previous information has indicated that a fertile area to look for melioidosis infection is among chronic and peracute non-tuberculous respiratory diseases patients. Accordingly, some 65 sputum samples from patients at Siriraj Hospital Chest Clinic and from the Nondhaburi Central Chest Hospital have been tested for Ps. pseudomallei by hamster inoculation and bacteriological techniques. The results have been negative. It is intended to purseu this phase of the investigation further in a region where there has been a high percentage of isolates from soil and water samples.

Summary

The results of this study indicate that Ps. Pseudomallei, the causative agent of melioidosis, is widely distributed in the external environment of Thailand. However, the ortanism exhibis a preference for certain geographic areas, notably the southern peninsula and the eastern portions of Thailand. The northwestern, central and Bangkok-Dhonburi areas appear to be free of the organism. Serologic study reveals that a significant proportion of Thai people, in contrast to U.S. personnel, have detectable levels of serum antibody against the organism. However, the proportion of serologic reactors is also dependent on the geographic area. A significant proportion of sera from people in Songkla and Ubol, areas in which the organism has been isolated from soil and water samples, had antibody titers whereas sera from people in Chiengmai resembled American sera in their lack of reactivity. Attempts to isolate the organism from cases of non-tuberculous chronic respiratory disease have, thus far, proven unsuccessful. There results lead us to conclude that Ps. Pseudomallei leads primarily a saprophytic existence. People in contact with the organism commonly respond with antibody production, but actual disease appears to be rare.

Table I

Geographic distribution of Pseudomonas pseudomallei isolates in Thailand

	No. positive/No. tested
Central	
Bangkok	0/58
Dhonburi	0/32
Nondhaburi	0/15
Pratumthani	0/28
Saraburi	0/10
Samutprakarn (Paknam)	0/6
Rajaburi	0/2
Northeastern	
Nakornrajasima (Korat)	0/72
Buriram	3*/11
Surin	0/13
Srisaket	0/3
Ubol	2/27
Nakornpanom	3*/16
Sakolnakorn	0/12
Udorn	0/16
Nongkai	0/4
Loei	2/24
Northern	
Chiengmai	0/40
Chiengrai	0/16
Southern	
Prajuab	2/37
Chumporn	6/133
Ranong	5/67
Phang-nga	4/46
Surasthani	3/27
Nakornsrithamaraj	15/113
Krabi	1/44
Phuket	5/26
Patalung	32/87
Trang	3/95
Satool	1/22
Songkla	21/79
Pattani	7/30
Yala	12/41
Narathiwas	25/47

* Questionable; dead animals inadvertently discarded before autopsy

Table II

Distribution of *Pseudomonas pseudomallei* isolates in South Thailand in relation to characteristics of water sources

pH	Type of water source: Still		Flowing		Total	
	No. positive / Total tested	%	No. positive / Total tested	%	No. positive / Total tested	%
2.0-2.99	6/8	75.0	0/15	0.0	6/23	26.1
3.0-3.99	5/11	45.5	1/5	20.0	6/16	37.5
4.0-4.99	4/7	57.1	2/7	28.6	6/14	42.9
5.0-5.99	17/50	34.0	6/31	19.4	23/81	28.4
6.0-6.99	16/234	6.8	27/261	10.3	43/495	8.7
7.0-7.99	18/68	26.5	13/53	24.5	31/121	25.6
8.0-8.99	0/1	0.0	1/1	100.0	1/2	50.0
9.0-9.99	0/1	0.0	0/0	—	0/1	0.0
Not tested	7/22	31.8	3/18	16.7	10/40	25.0
Total	73/402	18.2	53/391	13.6	126/793	15.9

Table III

Results of Indirect Hemagglutination Tests for the Presence of Melioidosis Antibody in Representative Sera

Source	No. Tested	No. with Titer of								
		20	±@ 20	20	40	80	160	320	640	640
U.S. 809 th Engineers	389	385	1	1	2					
U.S. Fort Dix	101	98	2					1		
U.S. Medical Unit Febrile agglutinin sera	6	6								
Royal Thai Army Hospital Blood Bank Donors	93	77		4	3	5	1	1	2	
Febrile agglutinin sera	42	41			1					
School of Tropical Medicine Hospital										
Febrile agglutinin sera	37	35		1						
Siriraj Hospital Blood Bank Donors	182	176	1	2		1		1	1	
Chest Clinic Attendants	93	86	3	1	3					
Out Patients	702	681	4	6	5	3	2			
In Patients	234	225	2	4	1	2				
Medical Students	70	69					1			
Ubol Provincial Hospital	117	55	11	11	9	15	6	5	2	3
Bangkok (Boy Scouts)	45	43				1	1			
Chiengmai (Children & Young Adults)	282	4								
Udorn	14	11			1	1			1	
Pakret, Nondhaburi (Socially deprived women)	59	43	1	3	4	4	4			
Songkla	159	142	3	4	1	4	3	2		
USAMRU, Kuala Lumpur	79	63	3	4	5	2	1		1	
Miscellaneous-Laboratory workers Slaughter House	7	7								
Cattle	100	76		11	7	2	2	2		
Buffalo	100	71		16	9	2	2			