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Seroprevalence of Anti-Flagellin Antibody against *Burkholderia pseudomallei* in Taiwan

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Melioidosis in Taiwan is an emerging disease that was first described in 1984 (1). The etiologic agent is *Burkholderia pseudomallei*, an environmental saprophyte found mainly in endemic areas such as Southeast Asia and northern Australia (2). Since 1994, the number of reported cases of melioidosis in Taiwan have been steadily increasing (3). In at least 11 of these patients, infection appears to have been indigenous, rather than arising from travel to endemic areas (3-5). Since its clinical manifestations are quite protean, clinical diagnosis is often difficult, so the true incidence of melioidosis may actually be higher than is currently believed (6). Previously, a truncated flagellin antigen has been used with an indirect ELISA to detect the specific antibody against *B. pseudomallei* to distinguish *B. pseudomallei* septicemia from other bacterial infections in Taiwanese patients, having a sensitivity of 93.8% and a specificity of 96.3% (7). It has been reported that highly specific antibody titers against *B. pseudomallei* in populations correlate with the incidence of melioidosis and can indicate the prevalence of inapparent infection (8,9). Whether melioidosis in Taiwan will develop into an overt and widespread disease remains unclear at present. Accordingly, the seroprevalence of melioidosis was surveyed in various geographical regions in Taiwan. A related aim was to evaluate the prevalence of patients with bacterial pulmonary disease since pulmonary melioidosis is the most common form (>83%) of *B. pseudomallei* infection in Taiwan (3). In particular, a form of melioidosis also known as "Vietnam tuberculosis" can mimic tuberculosis both clinically and pathologically (10).

Serum samples from healthy donors were collected from Northern (Cathay General Hospital, Taipei; $n = 200$), Central (Hsing-Lin Hospital, Taichung; $n = 180$), and Southern (Kaohsiung-Veterans General Hospital [KVGH], Kaohsiung; $n = 220$), Taiwan. These donors have not been documented as having history of melioidosis. Patient serum samples ($n = 152$) were collected from KVGH. The melioidosis serum group ($n = 17$) was collected from confirmed patients, whom were identified based on bacterial cultures from blood. The patients with tuberculosis ($n = 54$) had diagnoses confirmed by bacterial cultures. The patient group with pneumonia ($n = 81$) had diagnoses based on chest X-ray findings, and diagnoses of tuberculosis or melioidosis had been excluded with bacterial cultures. All sera were frozen at −70°C. Anti-*B. pseudomallei* flagellin antibodies were assayed using an indirect ELISA (7). When the average of the OD reading of the tested sample was greater than that of the negative controls plus two standard deviations, the tested sample was considered to be positive for the specific antibody. The highest dilution of the tested sample, which still gave a positive result, was defined as the endpoint titer and listed on the data sheet. The chi-square test was used to determine if a significant difference ($P < 0.05$) existed between the tested groups.

With the indirect ELISA, a titer cut-off value of 1:256 was used in accordance with a previous study (7). Healthy donor serum seropositivities to *B. pseudomallei* flagellin were 4, 2.8, and 5% in the samples collected from Northern, Central, and Southern Taiwan, respectively (Table 1). The seroprevalence of melioidosis in the surveyed areas did not differ significantly ($P > 0.5$). At a dilution of 1:256, seropositivity was expected to distinguish melioidosis from tuberculosis and pneumonia ($P < 0.001$). A total of 152 sera with pulmonary disease were examined and seropositivity was found to be 94.1% (16/17) in melioidosis, 11.1% (6/54) in tuberculosis, and 2.5% (2/81) in pneumonia (Table 2). The pneumonia group had relatively low positive rates. Compared with the sera from healthy individuals by KVGH, the seropositivity of the tuberculosis group (6/54) was not significantly increased (11/220) ($P > 0.1$).

The seroprevalence of melioidosis usually reflects geography. For example, northeast Thailand is defined as a hyperendemic area where 80% of children have specific antibodies against *B. pseudomallei*, compared with 10-29% of healthy people in other parts of Thailand (11,12). The seroprevalence of melioidosis in Southern China's Hainan Island ranges from 8.8 to 66.7%, with the highest antibody titers found in farmers in the spreading areas of Xinglong (13). In contrast, the present study failed to identify significant differences in seropositivity attributable to geographical regions in Taiwan. Seropositive rates may be expected to be higher in tuberculosis patients since melioidosis can simulate pulmonary tuberculosis clinically, but neither the tuberculosis nor the pneumonia groups found to have a greater prevalence of anti-*B. pseudomallei* antibodies in those patients. Whether the slightly increased seropositive rate in tuberculosis patients is due to their earlier acquisition of an

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Table 1. The seroprevalence of melioidosis in healthy adults in Taiwan

Group	Total cases	Anti-flagellin antibody titers							
		≤16	32	64	128	256	512	≥1024	(%)[1]
Northern Taiwan	200	72	72	34	14	5	2	1	4.0
Central Taiwan	180	36	68	49	22	3	1	1	2.8
Southern Taiwan	220	53	57	49	50	4	4	3	5.0
	600	161	197	132	86	12	7	5	4.0

[1]: The positive rate (%) of sera were calculated with the sera number of antibody titers above or equal to the 1:256 / the sera number of antibody titers below this dilution fold.

Table 2. The seropositive rate in pulmonary disease groups

Group	Total cases	Anti-flagellin antibody titers							
		≤16	32	64	128	256	512	≥1024	(%)[1]
Melioidosis	17	0	0	0	1	6	8	2	94.1
Tuberculosis	54	9	10	15	14	2	2	2	11.1
Pneumonia	81	21	18	21	19	1	0	1	2.5
	152	30	28	36	34	9	10	5	

[1]: See footnote [1] of Table 1.

inapparent infection or their concurrent infection by both tuberculosis and melioidosis requires further study. Based on comparatively low and evenly distributed seropositive rates to melioidosis, it seems reasonable to conclude that Taiwan has not developed hyperprevalent areas although melioidosis cases have increased substantially. Nevertheless, seropositive rates of 2.8 - 5% with inapparent infection in the Taiwanese population highlights the need for physicians managing patients in Taiwan to be aware of the possibility of community-acquired pneumonia and sepsis arising from *B. pseudomallei* infection.

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