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Serological Evidence for Increased Human Exposure to *Burkholderia pseudomallei* following the Tsunami in Southern Thailand

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A serological study was performed to determine recent human exposure to *Burkholderia pseudomallei* (the cause of melioidosis) in residents of southern Thailand affected by the tsunami of 26 December 2004. The findings were suggestive of increased recent exposure in both tsunami survivors and uninjured bystanders. Survivors of the Thailand tsunami may be at increased risk of melioidosis.

Melioidosis is a severe infection caused by the gram-negative bacillus *Burkholderia pseudomallei*. The organism exists in soil and water in regions of the tropics where melioidosis is endemic. Disease is acquired through inoculation, bacterial contamination of wounds, inhalation, and aspiration, including near drowning. The clinical manifestations are extremely broad ranging. The most frequent presentation is that of a septicemic illness, often associated with bacterial dissemination to distant sites, resulting in pneumonia and hepatic and splenic abscesses. The severity of illness is variable, however, and ranges from fulminant sepsis and rapid death to a chronic illness characterized by fever, weight loss, and wasting.

The tsunami of 26 December 2004 involved four provinces in southern Thailand, the worst affected being Phangnga, where approximately 1,000 patients presented in 1 day to Takuapa General Hospital (5). This was associated with a rise in the incidence of melioidosis; six of those admitted to Takuapa Hospital developed melioidosis, whereas nine cases of melioidosis among patients presenting there had occurred during the previous 6 years (2). *B. pseudomallei* is detectable in a low proportion of soil samples in Phangnga (2). The development of melioidosis in tsunami survivors was presumed to have followed contamination of wounds and/or inhalation of bacteria suspended in aerosolized mud or water particles. Residents who were not physically involved in the tsunami were exposed to significant mud and water aerosols; we hypothesized that bystanders underwent acute exposure to *B. pseudomallei* during this event. We have performed a prospective cross-sectional serological survey to measure indirectly the exposure to *B. pseudomallei* in tsunami survivors and the general population of Phangnga. This was achieved by the indirect hemagglutination assay (IHA), currently the most common test used worldwide for quantification of the human antibody response to *B. pseudomallei*.

Unselected consecutive serum samples were collected during January 2005 and again in April 2005 from the Biochemistry Department of Takuapa Hospital. Blood samples were derived from outpatients and inpatients of all ages and from all departments. The patient's ages and sexes were recorded, and duplicate samples from the same patient in the same month were excluded. An existing hospital database of individuals treated as outpatients or inpatients for tsunami-related injuries was used by hospital staff to determine whether any samples had been taken from listed individuals. Four sample groups were then created: those collected in January and April from individuals not known to have been directly involved in the tsunami (n = 1,039 and n = 948, respectively) and samples from known tsunami survivors collected during these two periods (n = 52 and n = 4, respectively). Serum samples were subsequently tested in a blinded fashion, and the results were stored in an anonymous database. IHA was performed with pooled antigens prepared from *B. pseudomallei* clinical isolates 199a and 207a, obtained from patients with melioidosis in northeast Thailand, as described previously (1). Ethical approval for this study has been obtained from the Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand, and the Oxford Tropical Research Ethics Committee.

IHA titers were compared between three groups (the samples from the nontsunami groups collected in January and April 2005 and samples from the tsunami survivors collected in January 2005). Table 1 summarizes the data for the ages, sexes, and IHA titers. The April 2005 samples from tsunami survivors were excluded from the analysis because of their very small numbers; the IHA titers for these individuals were 1:40 (two samples), 1:320 (one sample), and 1:640 (one sample).

The titers for the two nontsunami populations sampled 3 months apart were compared. A titer of 1:10 or more has previously been used as evidence of exposure to *B. pseudomallei* (3); approximately 60% of the patient in both groups fell into this category. Although it is of low specificity, a titer of

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TABLE 1. Patient data and IHA results[a]

Characteristic	Tsunami (−)		Tsunami (+), January 2005	Historical data (1995)
	January 2005	April 2005		
No. of patients	1,039	948	52	424
% Female	63.1	64.1	51.9	55.0
Age range (yr)	<1–94	<1–105	15–80	5–94
Median (IQR) age (yr)	56 (44–67)	54 (39–67)	39.5 (30–56)	
IHA titer range	0–1:2,560	0–1:10,240	0–1:2,560	≤1:20–1:160
Median (IQR) IHA titer	1:10 (0–1:40)	1:10 (0–1:40)	1:20 (0–1:80)	1:20 (1:20–1:1:40)
No. (%) of samples:				
IHA negative	430 (41.4)	401 (42.3)	19 (36.5)	
With IHA titer ≥1:10	609 (58.6)	547 (57.7)	33 (63.5)	
With IHA titer ≥1:160	76 (7.3)	98 (10.3)	7 (13.5)	10 (2.4)

[a] Symbols and abbreviations: (+), individuals known to have been directly involved in the tsunami; (−), individuals not documented at Takuapa Hospital as having been physically injured during the tsunami; IQR, interquartile range.

≥1:160 is commonly used in Thailand to support a diagnosis of melioidosis in patients with clinical features consistent with this diagnosis. The number of individuals with a titer of ≥1:160 was significantly higher in the April group than in the January (nontsunami) group (7.3% and 10.3%, respectively; $P = 0.02$). The rise over time in the magnitude of the overall IHA titer in the positive subset is consistent with the *B. pseuduomallei* exposure of residents not thought to have been physically involved in the tsunami.

Comparison of the two nontsunami groups with 52 patients who were sampled in January 2005 and who were listed as having been injured during the tsunami demonstrated higher median and interquartile IHA results for the latter group (Table 1). The tsunami group had a lower proportion of samples with negative IHA results and a higher proportion with titers of ≥1:160 compared with the proportions in both nontsunami groups (Table 1). This finding is consistent with a greater degree of exposure to *B. pseudomallei*; the relatively early timing of samples with such titers in relation to the time of the tsunami suggests that this is related to the tsunami itself rather than a later exposure event, such as the cleanup operation.

Our data were then compared with unpublished results of IHA performed by the Microbiology Laboratory of Takuapa Hospital with samples from 424 unselected outpatients and inpatients in 1995 (Table 1). These samples were not available for retesting; the assay was performed by use of a methodology comparable to that described above, with the exception that the lowest IHA titer was ≤1:20 (thus, it was not possible to assess the number of patients with a negative IHA result). The highest titer used in the assay was 1:2,560. The maximum titer seen in 1995 was 1:160, which is lower than those for all three groups tested in 2005. The proportion of patients with titers of ≥1:160 in 1995 was significantly lower than the proportion of patients with such titers in each of the three groups in 2005 ($P < 0.0001$ for both nontsunami groups; $P = 0.001$ for the January tsunami group). This indicates an increase in population-based IHA titers between two time points a decade apart.

In conclusion, our findings support the possibility that human exposure to *B. pseudomallei* has increased in both tsunami survivors and residents who were not documented at a major referral center as having been physically injured during the tsunami event. We propose that individuals living in regions of southern Thailand affected by the 2004 tsunami are at increased risk of melioidosis. These findings are relevant to tourists who were injured or who witnessed the tsunami in Thailand. The individuals at the highest risk are likely those with predisposing factors, such as diabetes mellitus, renal insufficiency and alcohol abuse. The variable and sometimes extremely prolonged incubation period between exposure and the development of melioidosis (maximum reported period, 62 years [4]) implies that long-term clinical vigilance for new cases is necessary.

IHA data for residents of Phangnga in 1995 were presented by oral presentation to the Ministry of Public Health in 1995. We are grateful to the hospital director, Pornlert Jitpratoom, and other staff at Takuapa Hospital, Phangnga. We thank Premjit Amornchai for technical assistance and other members of the Wellcome Trust-Oxford University-Mahidol University Tropical Medicine Research Programme for their support.

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