Surprisingly Low Seroprevalence of *Burkholderia pseudomallei* in Exposed Healthy Adults in the Darwin Region of Tropical Australia Where Melioidosis Is Highly Endemic

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In the Darwin region of Australia where melioidosis is highly endemic, only 11/354 (3%) healthy residents were seropositive by indirect hemagglutination assay, despite extensive exposure to *Burkholderia pseudomallei*. None developed melioidosis, but some described a prior self-limiting illness. This seropositivity rate is much lower than that seen in northeast Thailand, where melioidosis is similarly highly endemic, potentially reflecting important differences between these two locations in the epidemiology of melioidosis.

Melioidosis, which is caused by the environmental bacterium *Burkholderia pseudomallei*, is endemic in northern Australia and other tropical regions in southeast Asia (1). Serology studies from Thailand suggest that for each diagnosed case of melioidosis there are many asymptomatic infections (2). However, while seropositivity rates in northeast Thailand have been described to increase with age in children to levels of over 50% (3), a large community survey in northern Queensland, Australia, showed only 5.7% seropositivity overall, with higher rates seen in those with risk factors for melioidosis such as diabetes (8.6%) and chronic alcoholism (15%) (4). Furthermore, while the incidence of melioidosis in the tropical Top End of the Northern Territory of Australia is at least as high as in northeast Thailand (5), limited data suggest that seropositivity rates in the Top End are as low as those seen in Queensland (reference 6 and unpublished data). Melioidosis is increasingly being recognized as an opportunistic infection, with diabetics particularly at risk and healthy people rarely dying from melioidosis when early diagnosis and state-of-the-art intensive care facilities are available (7). Nevertheless, in the Darwin Prospective Melioidosis Study, 106/540 (20%) cases had no identifiable medical risk factor for melioidosis, with the majority of these cases having a history of recreational and/or occupational exposure to monsoonal wet-season soils and surface water (8). We therefore performed a serological study on healthy Darwin residents to assess both seropositivity and potential associations with asymptomatic infection with *B. pseudomallei*.

We recruited healthy adults living in the Darwin region who self-identified as being active and having exposure to wet-season soils and surface water. We excluded anyone with a known risk factor for melioidosis, including diabetes, hazardous alcohol use, chronic respiratory or renal disease, and malignancy or immunosuppressive therapy, as identified in the Darwin Prospective Melioidosis Study (8). An extensive questionnaire documented the number of years living in the region of endemicity, specific occupational and recreational activities, and estimated quantification of potential exposure events, including immersion of hands or feet in surface water, direct contact of skin with wet-season soil, and cuts and abrasions during outdoor activities. Serology was performed using the indirect hemagglutination assay (IHA) as previously described, with the IHA antigen derived from a combination of three local clinical *B. pseudomallei* strains and a positive titer being 1:40 or higher (4, 6). The study was approved by the Human Research Ethics Committee of the Northern Territory Department of Health and the Menzies School of Health Research.

A total of 354 individuals were enrolled and completed the questionnaire. Of the 354 individuals, 207 (58%) were female; the median age was 38 years (range, 18 to 61 years), and the median time living in the region of northern Australia where melioidosis is endemic was 13 years (range, 1 to 57 years). While 88% of the individuals worked predominantly indoors, 71% spent the majority of their recreational time outdoors. A total of 310 (88%) lived in urban residences in Darwin, and 321 (91%) used the chlorinated town water supply. Exposure to wet-season soil and water was estimated as extensive in 77%, moderate in 18%, and low in only 5%. A total of 334 (94%) participants had knowledge of melioidosis prior to the study, but only 21 (6%) claimed to always cover their hands and/or feet during exposure-prone outdoor activities in the wet season.

Despite extensive exposure to the Darwin environment where the presence of *B. pseudomallei* is known to be widespread in soils and surface water (9), only 11/354 (3.1%) people were seropositive (Table 1). IHA titers in those who were seropositive ranged from 1:80 to 1:2,560. None had a titer of 1:40, and the only person positive (Table 1). IHA titers in those who were seropositive ranged from 1:80 to 1:2,560. None had a titer of 1:40, and the only person with a titer of 1:20 had a repeat titer of <1:20. Of the 11 seropositive individuals, 7 were male, 10 were in the extensive-exposure group, 1 was in the moderate-exposure group, and 9 had experienced >10 wet seasons in Darwin (Table 1).

The 11 seropositive individuals were recalled for repeat testing, and all were assessed for evidence of melioidosis by full blood count, C-reactive protein analysis, urine analysis, chest X-ray, and urine culture for *Burkholderia pseudomallei*. In each case the re-
pept serology confirmed the positive result (Table 1), but all were healthy and none was found to have melioidosis, although 3 had nonspecific scarring on the chest X-ray. All but 1 (who had lived 24 years in the region of endemicity) had substantial exposure to B. pseudomallei through occupational and/or recreational activities (Table 1), but none identified a specific event more likely to be their infecting event. Four individuals described a prior prolonged (>2-week) episode of influenza-like symptoms which could have been consistent with primary infection with Burkholderia pseudomallei, but in all cases this illness resolved without any specific melioidosis treatment. Over the 3 years subsequent to the study, none has developed melioidosis.

The surprisingly very low B. pseudomallei IHA seropositivity rate in this study of heavily exposed healthy people in a region with among the highest documented incidence rates for melioidosis (5, 8) is in sharp contrast to the high seropositivity rates seen in northeast Thailand (3), where incidence rates of melioidosis are also high (10). Possible explanations include exposure to non-pathogenic cross-reacting environmental Burkholderia species in Thailand and differing epidemiologies between the regions, with possible differences in the route of infection and repeated exposure in Thai children even potentially conferring some immunity to disease (melioidosis) later in life and possible differences in virulence of B. pseudomallei between Australia and Thailand. While most cases are attributed to percutaneous inoculation, the potential roles of inhalation during severe weather events and ingestion from contaminated water supplies are receiving increasing attention (7). What is clear is that the estimate from Thailand that only approximately 1 in 4,600 antibody-producing exposures result in clinical infection (i.e., melioidosis) is not applicable to Australia (2).

In conclusion, infection with B. pseudomallei is uncommon in healthy individuals in the Darwin region where melioidosis is highly endemic, but self-limiting clinical illness from primary infections may be occasionally occurring. The proportion of seropositive individuals who harbor latent B. pseudomallei remains entirely unclear, but while activation from latency with melioidosis occurring many years after infection is well described (11), prospective studies suggest that such events are very uncommon (8).

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### TABLE 1 Summary for 11 Burkholderia pseudomallei-seropositive individuals

| Patient no. | Age (yr)/sex | Occupation | No. of yrs in tropics | Urban/rural residence | Town mains/bore water | Possible infecting event | Initial titer | Titer upon review |
|-------------|--------------|------------|----------------------|----------------------|----------------------|------------------------|--------------|------------------|
| 1           | 59/M         | Environmental scientist | 52 | Urban | Mains | Direct contact with soil through occupational activities | 1/160 | 1/160 |
| 2           | 40/M         | Public servant | 15 | Urban | Mains | Remote tourism guide or rugby | 1/80 | 1/80 |
| 3           | 39/M         | Firefighter | 20 | Urban | Mains | Remote tourism guide or football | 1/640 | 1/320 |
| 4           | 41/M         | Pharmacist | 15 | Urban | Mains | Cross-country running | 1/160 | 1/160 |
| 5           | 28/M         | Planning engineer | 4 | Urban | Mains | Work overseeing bore dig | 1/160 | 1/40 |
| 6           | 24/F         | Lawyer | 24 | Rural | Mains | None identified | 1/160 | 1/80 |
| 7           | 34/F         | Environment officer/scientist | 16 | Urban | Mains | Direct contact with soil through occupational activities | 1/160 | 1/80 |
| 8           | 47/F         | Administration manager | 29 | Rural | Bore & mains | Gardening | 1/1,280 | 1/640 |
| 9           | 25/M         | Linesman | 25 | Urban | Mains | Football, goose shooting, or direct contact | 1/640 | 1/640 |
| 10          | 47/F         | Nurse | 11 | Urban | Mains | With soil through occupational activities | 1/320 | 1/640 |
| 11          | 25/M         | Environmental scientist | 3 | Urban | Mains | Previous remote nursing or gardening | 1/2,560 | 1/2,560 |

a The result of a screen for melioidosis was negative for all 11 individuals. M, male; F, female.