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Detection of *Burkholderia pseudomallei* in Soil within the Lao People's Democratic Republic

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Clinical cases of melioidosis caused by the saprophyte *Burkholderia pseudomallei* were first noted in the Lao People's Democratic Republic (PDR) in 1999. In this study, 36% of 110 soil samples in northern Lao PDR were positive for *B. pseudomallei*, providing further evidence for the presence of melioidosis in this country.

Melioidosis is caused by the saprophytic gram-negative bacterium *Burkholderia pseudomallei*, which is commonly found in wet soil and pooled surface water in areas where melioidosis is endemic (3, 6, 8, 12). Large numbers of cases are described in southeast Asia and northern Australia where disease is thought to result from inoculation of the organism through the skin and subsequent hematogenous dissemination to internal organs (11). In Thailand, the highest number of cases are described in the northeast, adjacent to the Lao People's Democratic Republic (PDR) (5). In this region, melioidosis accounts for 20% of community-acquired bacteremias and is associated with a mortality of approximately 50% (2). Patients with recreational or occupational exposure to mud and surface water, particularly rice farmers, are commonly affected (9). Melioidosis in the Lao PDR was first reported in 1999 with the description of two cases (7).

In this study, we have defined the environmental distribution of *B. pseudomallei* in soil in the region around the capital city, Vientiane. A survey of rice fields was undertaken during September 1998 (the rainy season) along roads radiating out in a 150-km radius from the centre of Vientiane. The sampling sites for quantitative and qualitative studies are depicted in Fig. 1. All sampling sites represented rice fields that had been recently ploughed or planted.

Qualitative analysis for the presence of *B. pseudomallei* in soil was performed using 110 separate soil samples from 55 geographical locations, one sample being taken from each side of the road. Approximately 5 g of soil (3 cm³) was sampled at a depth of 20 to 30 cm at each site, placed in a sterile plastic tube containing 2 ml of water, and shaken vigorously. Samples were transported to the Wellcome Unit microbiology laboratory of Sapprasitiprasong Hospital in Ubon Ratchathani, where they were processed within 24 h. Each sample was mixed well and one drop was spread onto Ashdown's agar (1) which was incubated at 42°C in air and examined daily for 6 days for colonies with an appearance consistent with *B. pseudomallei*. A

further 1 ml of the soil water sample was added to 9 ml of selective enrichment broth consisting of threonine-basal salt plus colistin (TBSS-C50 broth) (4). This was incubated at 42°C in air for 48 h, after which 10 µl of surface liquid was plated onto a second Ashdown's agar plate which was incubated and observed as before. Presumptive *B. pseudomallei* were fully identified as previously described (10).

Quantitative analysis was performed at 10 sites as shown in Fig. 1. At each site, approximately 120 to 150 g of soil was obtained from a depth of 30 cm by using a spade (cleaned and disinfected with 70% alcohol between uses) and placed in a plastic bag. Soil samples were transported to the laboratory and processed the same day. Sterile water (100 ml) was added to 100 g of soil and mixed well. After leaving overnight at room temperature to sediment, the upper layer of water was transferred to a sterile container, and 10- and 100-µl aliquots were plated onto Ashdown's agar, incubated at 42°C in air, and inspected for 6 days. An aliquot of the upper water layer (1 ml) was also added to 9 ml of TBSS-C50 broth which was incubated at 42°C in air for 48 h, after which 10 µl of surface liquid was plated onto a second Ashdown's agar plate which was incubated and observed as before.

B. pseudomallei was isolated from 40 of 110 qualitative samples (36%), encompassing 28 of 55 sites (51%) (Fig. 1). In 12 sites, samples were positive on both sides of the road; the remaining 16 isolations were from sites where only one side of the road was positive. Enrichment culture yielded *B. pseudomallei* in all of the positive soil samples, while only 23 of these were positive on direct plating onto Ashdown's medium. *B. pseudomallei* was isolated from 5 of 10 quantitative sampling sites, two of them on both sides of the road (Fig. 1); the geometric mean bacterial concentration from the seven positive samples from these sites was 39 CFU/g of soil (95% confidence interval, 23.7 to 641 CFU/g; range, 10 to 1,200 CFU/g).

The balance of factors that determine the risk of disease in a given individual is poorly understood overall, but variability in the presence of *B. pseudomallei* in soil is an important factor in determining disease incidence in a given area. For example, in northeastern Thailand where the highest rates of melioidosis are described, two thirds of rice fields yield *B. pseudomallei*

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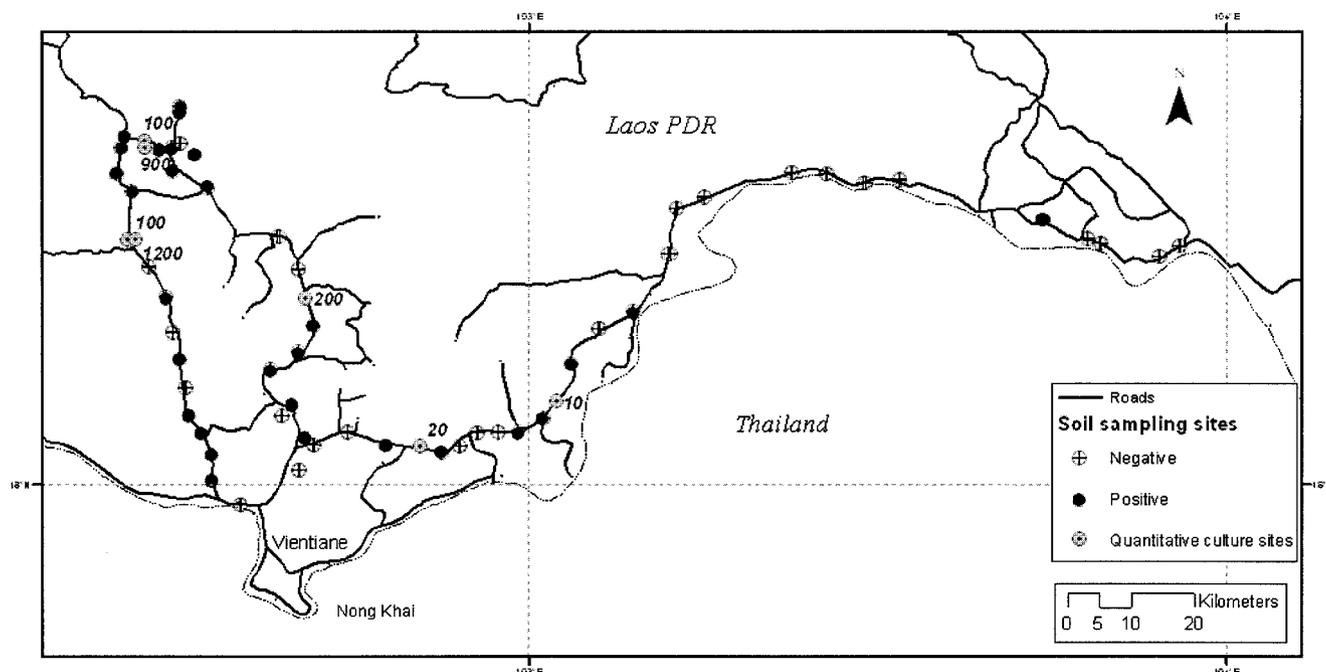


FIG. 1. Map illustrating soil sampling sites in the Vientiane region of Lao PDR. Numbers indicate bacterial concentration at positive quantitative sites (CFU/g).

(12). This contrasts with the Central Valley region of Thailand, where melioidosis is rarely reported and where the organism was not detected in the soil in two studies (8, 13). The bacterial concentrations found in this study are similar to those found previously around Udon Thani in the adjacent province (13 soil samples positive out of 49 taken from 7 sites; geometric mean, 218 CFU/g of soil [unpublished data]).

This study has demonstrated the presence of *B. pseudomallei* in soil in the Lao PDR. It provides further evidence that melioidosis may be more common than previously recognized in this country. As the ability to treat melioidosis in resource-poor settings is limited, the development of preventative strategies is timely and important. Detailed studies are required to gain a broader understanding of the soil ecology of *B. pseudomallei* and its relationship to disease.

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