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Randomized Soil Survey of the Distribution of *Burkholderia pseudomallei* in Rice Fields in Laos▽†

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Melioidosis is a major cause of morbidity and mortality in Southeast Asia, where the causative organism (*Burkholderia pseudomallei*) is present in the soil. In the Lao People's Democratic Republic (Laos), *B. pseudomallei* is a significant cause of sepsis around the capital, Vientiane, and has been isolated in soil near the city, adjacent to the Mekong River. We explored whether *B. pseudomallei* occurs in Lao soil distant from the Mekong River, drawing three axes across northwest, northeast, and southern Laos to create nine sampling areas in six provinces. Within each sampling area, a random rice field site containing a grid of 100 sampling points each 5 m apart was selected. Soil was obtained from a depth of 30 cm and cultured for *B. pseudomallei*. Four of nine sites (44%) were positive for *B. pseudomallei*, including all three sites in Saravane Province, southern Laos. The highest isolation frequency was in east Saravane, where 94% of soil samples were *B. pseudomallei* positive with a geometric mean concentration of 464 CFU/g soil (95% confidence interval, 372 to 579 CFU/g soil; range, 25 to 10,850 CFU/g soil). At one site in northwest Laos (Luangnamtha), only one sample (1%) was positive for *B. pseudomallei*, at a concentration of 80 CFU/g soil. Therefore, *B. pseudomallei* occurs in Lao soils beyond the immediate vicinity of the Mekong River, alerting physicians to the likelihood of melioidosis in these areas. Further studies are needed to investigate potential climatic, soil, and biological determinants of this heterogeneity.

Melioidosis is an infectious disease caused by *Burkholderia pseudomallei*, a Gram-negative saprophytic soil bacterium (2, 3, 4, 25, 26, 30). The majority of patients are reported in southeast Asia and northern Australia (3, 4, 25, 26). Risk factors include being a rice farmer and having diabetes, chronic alcoholism, renal failure, and/or thalassemia. Most patients probably contract the infection by contact with contaminated soil and water (6, 15, 22, 24, 26). The mortality rate among individuals with septicemic melioidosis, even with optimum antibiotic treatment and intensive care, is very high (19 to 68%) (3). In northeast Thailand, where *B. pseudomallei* is found in ~50% of soil samples, patients with melioidosis represent ~18% of patients admitted with community-acquired septicemia (1).

The Lao People's Democratic Republic (Lao PDR, or Laos) is a small land-linked country of considerable environmental and ethnic diversity situated between Burma (Myanmar), Thailand, Cambodia, Vietnam, and the People's Republic of China (PRC). Most of the western border is the Mekong River. Melioidosis was first identified in 1999 (17), and subsequently (to October 2010), 409 culture-positive patients have been recorded in the capital, Vientiane City (unpublished data). However, there is only one laboratory with a routine accessible diagnostic service for *B. pseudomallei* in Laos, and the disease is probably underrecognized outside the capital.

In a soil survey conducted in 1998, 36% of 110 soil samples collected in rice fields around Vientiane City contained *B. pseudomallei* (28). The reasons for the abundance of this organism in soil in adjoining northeast Thailand and around Vientiane are not understood, but factors such as the physical and chemical properties of soil and/or interaction with other organisms and plants may be important (5, 9, 12, 13, 15). In Australia, proximity to a stream and moist soil rich in roots were independently associated with the presence of *B. pseudomallei* in soil (11). The majority of Lao patients (86%; unpublished data) diagnosed with melioidosis in Vientiane have houses in Vientiane City and Vientiane Province which are close to the Mekong River. However, it is uncertain whether this represents the relative lack of diagnostic facilities elsewhere or whether *B. pseudomallei* does not occur in soil in the highlands of Laos, which cover the northwestern, northeastern, and eastern aspects of the country. We therefore ex-

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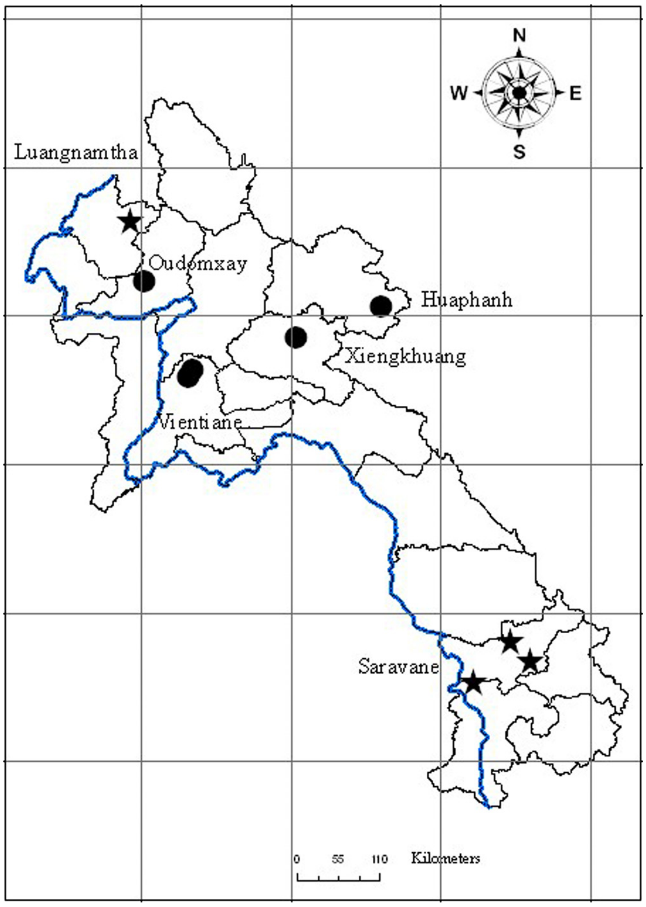

FIG. 1. Distribution of *Burkholderia pseudomallei* at nine sampling sites in Laos negative (●) and positive (★) for *B. pseudomallei* in 2009. The blue line represents the course of the Mekong River.

plored whether *B. pseudomallei* occurs in Lao soil distant from the Mekong River.

MATERIALS AND METHODS

We defined sampling sites across Laos based on three transects (Fig. 1). To facilitate this, Vientiane and Saravane Provinces were divided into two (western and eastern) and three (western, central, and eastern) areas of equal width, respectively (Fig. 1). The transects were from western Vientiane Province to Oudomxay Province to Luangnamtha Province in the northwest (toward the Chinese border), from eastern Vientiane Province to Xiengkhuang Province to Huaphanh Province in the northeast (toward the Vietnamese border), and from the Mekong River close to the Thai border in southern Laos across Saravane Province, toward the Vietnamese border, giving a total of nine sampling areas. All areas except that in Huaphanh are in the watershed of the Mekong River.

Within each sampling area, one site was randomly selected according to all of the following criteria: (i) within a rice field with an area of ≥1 ha, (ii) ≤100 m from a perennial river, (iii) ≥50 m from a known area of unexploded ordnance (UXO), and (iv) ≥50 m from buildings. The distribution of rice fields was determined from land use data (2001 to 2003; Forest Inventory and Planning Division [FIPD], Government of the Lao PDR) and photographs taken by satellite (Landsat 7 ETM; http://glovis.usgs.gov/) from 2008 to 2009. National Geographic Department (NGD; Government of Lao PDR) land use data were also used to finalize the choice to identify rice fields located ≤100 m from river edges. Data from the Ministry of Communication, Transport, and Post were used to confirm the absence of buildings in a proposed sampling site. Laos is the most heavily bombed country in the world (23), and UXO remains a major public health and economic problem. Therefore, the digging of holes in eastern Laos poses important safety risks, and to reduce the risk of detonating buried munitions, data from the Lao National Unexploded Ordnance program were checked to ensure that the sampling site was ≥50 m of a known bombing area. In addition, the local UXO clearance authorities were consulted during the field survey, and the grid area was checked by them if they considered this necessary. Soil data were provided by the National Agriculture and Forestry Research Institute (NAFRI), Government of Laos, from geographic information system (GIS) coordinates. ArcGIS (Environmental Systems Research Institute, Inc., Redlands, CA) was used to map these variables and perform the random selection of three points for each site, with the 2nd or 3rd point selected in case the 1st or 2nd point, respectively, was not accessible or appropriate. Each random point defined the location of the southeast corner of a grid of 45 by 45 m in which 100 soil samples were collected from holes dug 5 m apart. If the predetermined grid was not included in one rice field or if the grid included habitats other than rice fields, the nearest point in the nearest rice field able to include the 45- by 45-m grid was taken. The random sampling points were found using a Global Positioning System (GPS; Garmin GPS Map 60CSx). A tape measure was used to mark out the grid of 100 holes separated from each other by 5 m, and 100 g of soil was removed from a depth of 30 cm (30). Oral consent for the removal of soil samples was obtained from the farmers concerned, and they were compensated for any crop losses. The samples were maintained at ambient temperature during transport and processed within 48 h. *B. pseudomallei* was cultured; identified by colonial morphology, latex agglutination test, and resistance to colistin and susceptibility to amoxicillin-clavulanic acid; and quantitated for each soil sample as previously described (30). The 200-kDa exopolysaccharide latex agglutination test used is positive for *B. pseudomallei* but not for *B. thailandensis* (27). Lao place name spellings follow those of Sisouphanthong and Taillard (20).

RESULTS

Sampling was performed in June 2009, at the start of the rainy season. All sampling sites had to be moved to the nearest point in the nearest rice field able to include the 45- by 45-m grid, as the randomly selected rice field was too small (four sites) or other habitats, such as a cemetery, forests, or pools, were within the site (five sites). *B. pseudomallei* was isolated from four of the nine (44%) sites, including Luangnamtha Province in the northwest and all three sites in Saravane Province in the south (Fig. 1; Table 1). The most easterly sampling point in Saravane Province, farthest (99 km) from the Mekong River, had the highest number of positive soil samples (94/100), followed in frequency by the two other sampling sites in this province. In contrast, only 1/100 samples from the site in Luangnamtha Province was *B. pseudomallei* positive. The frequency of positive samples was mirrored to some extent by the quantitative count of *B. pseudomallei*, in that the sampling site in Saravane Province with the highest number of positive samples had the highest geometric mean count of 464 CFU/g soil (95% confidence interval [CI], 372 to 579 CFU/g soil; range, 25 to 10,850 CFU/g soil), exceeding that of western Saravane Province, where 61% of holes were positive with a geometric mean concentration of 82 CFU/g soil (95% CI, 47 to 141 CFU/g soil; range, 2 to 22,250 CFU/g soil), and central Saravane Province, where 42% of holes were positive with a geometric mean concentration of 122 CFU/g soil (95% CI, 65 to 227 CFU/g soil; range, 2 to 4,700 CFU/g soil). The mean concentrations of *B. pseudomallei* at the three sites in Saravane were significantly different (analysis of variance, $P < 0.001$).

The elevations of the sampling sites ranged from 140 to 581 m above mean sea level. The three sites with *B. pseudomallei* at high density were at relatively low elevations (140 to 191 m), but the site in Luangnamtha where *B. pseudomallei* was detected was at a relatively high elevation (557 m), unlike the other sites of intermediate elevation where *B. pseudomallei* was not detected (Fig. 2; Table 1). Rainfall at the provincial capi-

TABLE 1. Geographical and climatic features and distribution of *B. pseudomallei* at nine sampling sites in Laos

Province	Subdivision	Nearest village	Elevation (m)	Latitude	Longitude	Rainfall in 2009 (mm/yr[a])	Soil type	Soil description[b]	% sample holes positive	CFU/g soil	
										Geometric mean (95% CI)	Minimum-maximum
Saravane	West	Natane	140	15°26′10.1″N	105°47′21.0″E		Ferric acrisols	Not strongly humic; showing ferric properties within 125 cm of the surface; lacking plinthite within 125 cm of the surface; lacking gleyic properties within 100 cm of the surface	61	82 (47–141)	2–22,250
Saravane	Central	Nadou gnai	177	15°55′33.0″N	106°14′28.9″E	2,272	Ferric luvisols	Ferric properties within 125 cm of the surface; lacking an albic E horizon; lacking plinthite within 125 cm of the surface; lacking gleyic and stagnic properties within 100 cm of the surface	42	122 (65–227)	2–4,700
Saravane	East	Pon tong	191	15°41′31.3″N	106°29′56.5″E		Ferric alisols	Not strongly humic; showing ferric properties within 125 cm of the surface; lacking plinthite within 125 cm of the surface; lacking gleyic and stagnic properties within 100 cm of the surface	94	464 (372–579)	25–10,850
Vientiane	West	Xamfon	385	19°07′49.5″N	102°11′32.9″E	1,483	Ferric luvisols	As for Saravane central	0		
Vientiane	East	Nahong	407	19°12′46.3″N	102°14′20.6″E		Ferric alisols	As for Saravane east	0		
Luangnamtha		Thong chiatai	557	21°00′13.1″N	101°25′02.2″E	1,415	Gleyic lixisols	Gleyic properties within 100 cm of surface	1	80	
Oudomxay		Naborn	513	20°16′40.3″N	101°36′00.8″E	1,340	Calcaric cambisols	Ochric and calcareous, at least between 20 and 50 cm from the surface; lacking vertic properties; lacking gleyic properties within 100 cm of the surface	0		
Xiengkhuang		San	581	19°37′10.7″N	103°33′23.0″E	1,318	Eutric cambisols	Not strong brown to red; lacking ferralic properties in the cambic; lacking gleyic properties within 100 cm of the surface	0		
Huaphanh		Xamtai	323	19°59′31.9″N	104°37′50.5″E	1,264	Eutric cambisols	As for Xiengkhuang	0		

[a] Rainfall data, courtesy of the Department of Meteorology and Hydrology, Lao PDR, is from the provincial capital and hence is listed only once for each province sampled.
[b] Soil descriptions follow the standards described elsewhere (8).

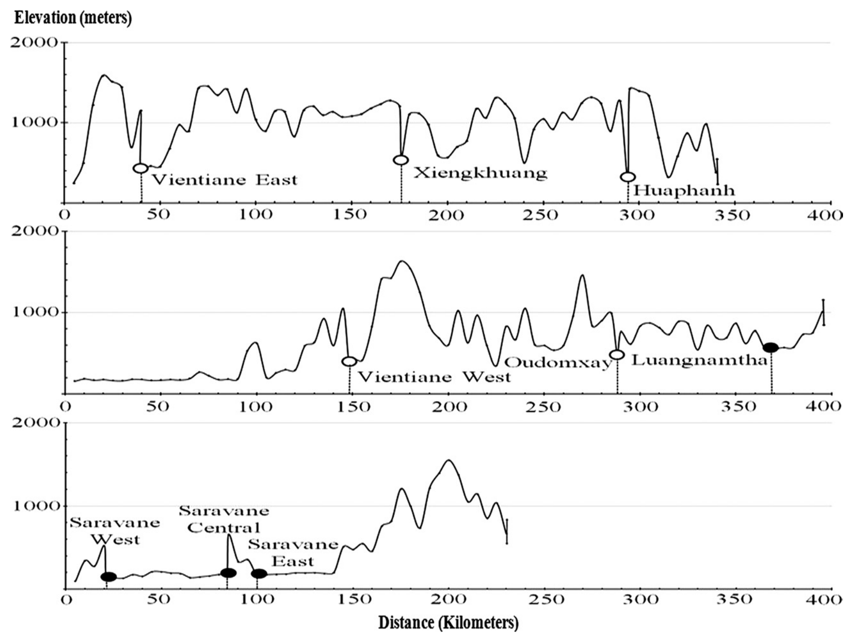

FIG. 2. Presence of *B. pseudomallei* in relation to elevation and distance from the Mekong River. (A) Northeast; (B) northwest; (C) south. ●, positive for *B. pseudomallei*; ○, negative for *B. pseudomallei*. The vertical bar at the right end of the topographical line represents the international Lao border.

tals was of similar seasonality but was higher in the south at Saravane, with a high *B. pseudomallei* density, than in the north at Luangnamtha and Huaphanh, with low or zero *B. pseudomallei* density (Table 1). The gross soil type (8) at the three Saravane sites with high *B. pseudomallei* soil densities was ferric soil, similar to those in Vientiane Province, where *B. pseudomallei* was not detected. The other four sites had markedly different soil types (Table 1).

DISCUSSION

The results from this pilot study, the first randomized survey of *B. pseudomallei* distribution in soil in Asia (10), demonstrate that the organism is common in southern Lao soil and is not confined to soil in the immediate valley of the Mekong River. Saravane east, 99 km east of the Mekong River (Fig. 2), had the highest geometric mean *B. pseudomallei* soil density described in any survey to date. Interpretation of past soil surveys is hard if *B. pseudomallei* was not distinguished from *B. thailandensis* (18, 21, 24). The *B. pseudomallei* soil concentration around the Lao capital, Vientiane, in central Laos, was relatively low, with a mean *B. pseudomallei* concentration of 90 CFU/g (range, 10 to 1,200 CFU/g) (28). Notably, unlike the previous study (28), we did not find *B. pseudomallei* in soil at either site in Vientiane Province, but as recently discussed (12), the distribution of *B. pseudomallei* is very heterogeneous and the sample size is small. There is a tendency for the frequency of *B. pseudomallei* isolation in rice fields to be lower at higher latitudes (Fig. 3). This suggestion could be confounded by different sampling and culture techniques and seasons of sampling in different studies and confusion with *B. thailandensis*. However, the northern boundary of a *B. pseudomallei* soil rice field frequency of >10% is at approximately 20°N, which has been also suggested to be the northern

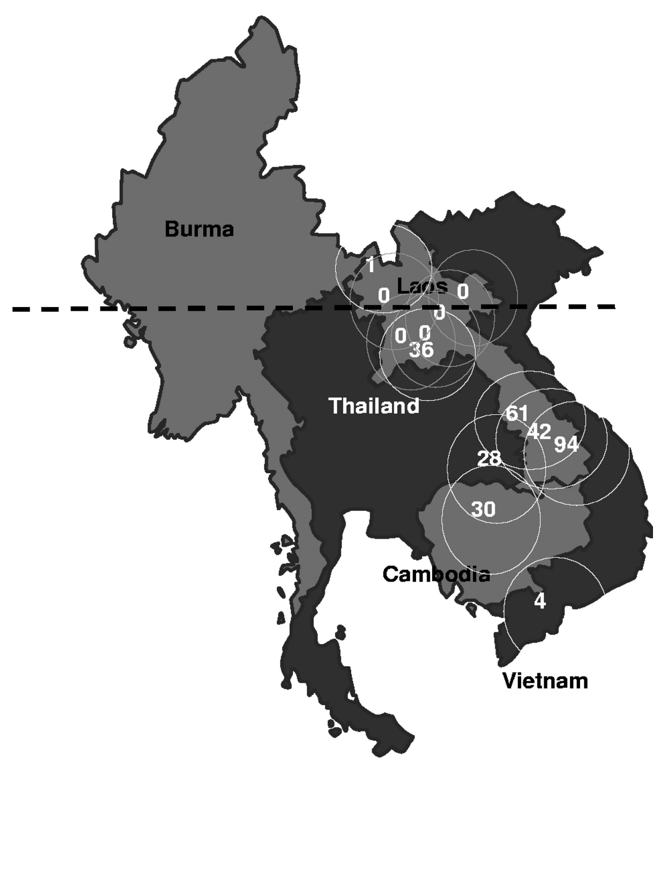

FIG. 3. Map of southeast Asia showing the percentage of soil samples positive for *B. pseudomallei* at different sampling sites in rice fields. Data are from Parry et al. (16), Wuthiekanun et al. (27), Wuthiekanun et al. (28), Wuthiekanun et al. (29) and this study. The horizontal dashed line is at 20°N. Only studies in which *B. pseudomallei* has been distinguished from *B. thailendensis* are included.

boundary for a high incidence of melioidosis (3). This pilot study has important limitations, including the fact that only nine sites were sampled (of note, the processing of 900 soil samples took 772 person-hours) and the fact that the physical, biological, and chemical characteristics of the soil samples and farming techniques were not investigated. However, these data suggest that health workers in northeastern and northwestern Laos may be able to put melioidosis lower on their differential diagnosis of sepsis than health workers in southern Laos. There is no accessible local blood culture service in Saravane Province, but these data strongly suggest that melioidosis will be an important public health problem. This has important economic implications, given the high relative cost of empirical ceftazidime therapy (17). The factors determining the geographical distribution of *B. pseudomallei* in soil are not understood. The gross soil type was not related to the distribution of *B. pseudomallei* in soil in Laos. However, there is relatively little published information on the comparative chemistry and ecology of Lao soil (7, 19). In Thailand, there is evidence that acidic rice field pH may be associated with the presence of *B. pseudomallei* in soil (14, 15). Further work investigating the distribution of this organism in relation to climatic, physical, chemical, and botanical features of farming practices in diverse parts of Laos is needed and would inform public health decisions about this severe disease.

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