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SHORT REPORT
Prevalence of *Burkholderia pseudomallei* in Guangxi, China

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SUMMARY

Melioidosis, an infectious disease caused by the Gram-negative bacterium *Burkholderia pseudomallei*, is now recognized as an important public health problem in Southeast Asia and tropical northern Australia. Although *B. pseudomallei* has been detected in various water and soil samples in southeast China, the enviromental distribution of *B. pseudomallei* in China is unclear. In the winter months of 2007, 154 and 130 soil and water samples, respectively, were collected from several locations in Guangxi, China. The samples were screened for *B. pseudomallei* by bacterial culture and identification and confirmed by PCR for species-specific 16S rDNA and flagellin genes. *B. pseudomallei* was detected in 8·4% of the soil samples but in none of the water samples. All positive samples were confined to a single low-lying region from rice paddy fields. Counts of *B. pseudomallei* ranged from 23 to 521 c.f.u./g soil. This is the first geographical distribution survey of *B. pseudomallei* in soil in Guangxi, China, and the data are of importance for further evaluating the impact of this pathogen on melioidosis in this region.

Key words: *Burkholderia pseudomallei*, China, detection, environment, Guangxi.

Burkholderia pseudomallei is a Gram-negative bacterium naturally occurring in rice-farming fields, rubber plantations, agricultural sites and water in endemic regions, particularly Southeast Asia and northern Australia [1]. It is the causative agent of meliodosis, a potentially acute fulminating disease in animals and humans with the clinical syndromes varying from bacteraemia, pneumonia, skin or soft tissue infection, and brain, splenic and liver abscesses; pneumonia is the most common presentation and is involved in about half of all cases [2]. Most cases of melioidosis occur within latitudes 20° N and 20° S [3], and may account for 20% of all community-acquired septicaemias and 20–40% of sepsis-related mortality in northeast Thailand and Australia [4, 5].

The first human case of melioidosis in China was reported in Hong Kong in 1983 [6], followed by five cases a year later [7]. A seroprevalence survey in Hong Kong showed 14% seroconversion in a tuberculosis sanatorium in 1987 [8], and since these cases had never travelled outside Hong Kong the infections were regarded as being acquired locally. On mainland China, probably due to unawareness of melioidosis, it was not until 1990 that the first human case was diagnosed in Hainan, a southernmost province [9], and subsequently some animal and human cases were reported in Guangdong, Guangxi and Hainan [4].

A seroprevalence study of antibody responses to *B. pseudomallei* in humans and animals undertaken in 1979 in Nanning, Guangxi showed that the

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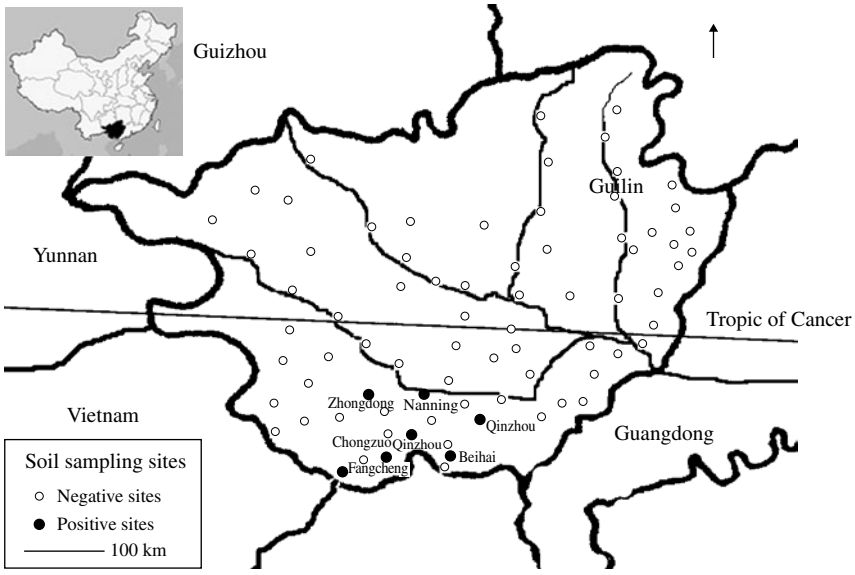

Fig. 1. Map illustrating soil sampling sites in Guangxi, China.

seropositive rate in humans was lower than that in animals (9·7% for humans *vs*. 34% in cattle and 15% in pigs). *B. pseudomallei* has routinely been isolated since 1975 from clinical samples in Guangxi, but there is limited information on the geographic distribution of this species in the region. To this end we undertook a survey of the distribution of *B. pseudomallei* in soil and water sites throughout this region.

In total, 274 soil and water samples were collected from 77 sites mainly in the rice field regions in Guangxi in the winter of 2007 (Fig. 1). About 10 g soil was sampled at a depth of 30–60 cm and placed into a sterile plastic bag; each site was sampled from two separate holes at the same time. Water samples were 10 ml volumes of pond surface water collected in sterile plastic tubes. All samples were stored in an ice box for transportation to the laboratory. About 5 g soil was shaken vigorously in 2 ml distilled water in sterile screw-capped glass vials; 1 ml water sample was added to 9 ml selective enrichment broth consisting of threonine-basal salt plus colistin (TBSS-C50 broth) [10] and incubated at 42 °C for 48 h. One drop of soil suspension or 10 μl of the 48-h water enrichment culture were spread on Ashdown's agar. The plates were incubated at 42 °C for 6 days and isolates with dry, wrinkled, violet to purple colonies were regarded as presumptive *B. pseudomallei*, and were stored in Luria–Bertani broth containing 15% glycerol at −70 °C for further identification. For quantitative culture, 5 g soil was shaken in 5 ml water and 10- and 100-μl aliquots were plated on Ashdown's agar as above. All presumptive *B. pseudomallei* isolates were identified by phenotypic tests [11], and confirmed by PCR amplification of the *fliC* and 16S rDNA genes as described by Su *et al*. [12]. The 16S rDNA amplicon was sequenced and the phylogenetic relatedness to the reference strain *B. pseudomallei* strain (CMCC 53001) was displayed by the Neighbour-Joining arithmetic method.

B. pseudomallei was detected in 13/154 (8·4%) soil samples, and seven sampling sites (9·1%) (Fig. 1). It was not found in any of the surface water samples. Counts of *B. pseudomallei* in the soil samples ranged from 23 to 521 c.f.u./g. Isolates identified by phenotypic tests were positive for *fliC* and 16S rDNA genes. Sequencing of the latter revealed high similarity to the reference strain.

There was an uneven distribution of *B. pseudomallei* in Guangxi. Four of the seven positive sites were located in coastal areas (Qinzhou, Beihai, Fangcheng) and the remainder to the south of Nanning city, a plain of rice fields <200 m above sea level (latitude 23·5° N). No positive sample was identified in mountainous regions in the north and west (Fig. 1) and no *B. pseudomallei* were detected in samples from Chongzuo where cases of melioidosis have been reported. The geographical distribution of *B. pseudomallei* is possibly affected by several factors including altitude, rainfall, and temperature and local ecological conditions; indeed 11 negative sampling sites were located close to seven positive sites.

The bacterial counts in the soil are probably related to the risk of developing melioidosis. The concentrations of *B. pseudomallei* in soil (23–521 c.f.u./g) were

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comparable to levels found in studies in Thailand (1–17 000) and Laos (10–1200) [13, 14]. Previous antibody prevalence surveys to *B. pseudomallei* in animals and humans reported rates of 9·7% in Nanning and 1·8% in Guilin [15]. This is consistent with the fact that one-third of the sites around Nanning cultured positive for *B. pseudomallei* while Guilin region yielded no positive isolations.

Recreational or occupational exposure to soil and water contaminated with *B. pseudomallei* are recognized risk factors for contracting melioidosis [16]. Although no cases have yet been reported in Guangxi, physicians in this area should consider melioidosis when patients present with an unknown fever or community-acquired pneumonia. Special attention should be given to seropositive individuals as the pathogen may persist in humans for very long periods and be reactivated leading to relapse, especially among immunocompromised individuals, in whom the disease can be fatal [12, 17].

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DECLARATION OF INTEREST

None.

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